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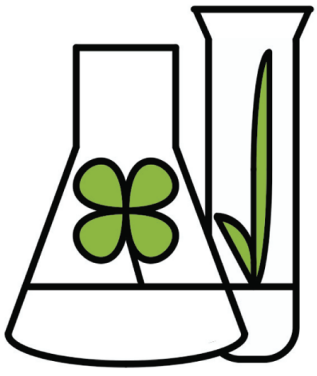

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ORAL PRESENTATIONS

Factors affecting the efficiency of oat (*Avena sativa* L.) doubled haploid production

Ilona Czyczyło-Mysza, Edyta Skrzypek, Izabela Marcińska, Katarzyna Juzoń, Katarzyna Cyganek, Agata Nowakowska, Kinga Dziurka, Marzena Warchoł

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Most oat genotypes proved to be susceptible to androgenesis. The aim of the investigation was to study the conditions for obtaining haploid plants of oat (*Avena sativa* L.) by androgenesis in anther culture and by wide crosses (pollination of oat by maize). Oat plants (generations $F_1 – F_3$) were obtained from DANKO Plant Breeding Sp. z o. o., Plant Breeding Strzelce Sp. z o. o. and Małopolska Plant Breeding – HBP Sp. z o. o.

The method compares the effect of cold treatment, density of anther culture and type of medium for the production of embryo like structures and regeneration of haploid plants. Among the studied factors the most important were: density of anthers (the best was 51–80 anthers per plate) and the type of induction medium embryogenic structures were formed more efficiently on the C17 medium than on the W14 one, regardless of their solidification).

In the wide crossing method, conditions of donor plants vegetation, type of pollinator, duration of the individual steps of the method (emasculation, pollination, auxin application), embryo pretreatment, type of regeneration medium and concentration of sugar in the medium were studied. More haploid embryos formed if the donor plants were grown in a greenhouse with natural light, and not in a vegetation chamber with artificial lighting (sodium lamps). The most effective pollinator was maize (*Zea mays* L.) as compared to sugar sorghum (*Sorghum bicolor* L.) and pearl millet (*Panicum miliaceum* L.). It has been shown that many embryos were formed when pollination occured three days after flowers emasculation and if ovaries were treated with a solution of auxin two days after pollination. It was found that cooling (4°C) of embryos does not stimulate them to germination. Haploid embryos remained alive longer and produce more plant on 190-2 medium with maltose than on the same medium with sucrose. Efficiency of haploid embryos formation depended on the plant genotype, and did not correlate with their ability to germination and the number of regenerated plants. Efficiency of oat pollination by maize pollen was 3.7% of the embryos, 1% of haploid plants and 0.3% DH lines per emasculated flowers. Within the four years experiments 136 oat DH lines were obtained.

ACKNOWLEDGEMENT: The research is supported by the Ministry of Agriculture and Rural Development, project no. HORhn-801-1/13.

Caryopteris incana, a plant of ornamental and medicinal value – *in vitro* propagation and essential oil analysis

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Caryopteris incana is a small, woody shrub, native to China, Japan and Korea (Miller, 2007). Although in Europe this species is valued mainly as the ornamental plant with the fragrant leaves and blue flowers, in Asia it has been used in traditional medicine due to its anti-inflammatory properties, connected with the high content of essential oil (Chu et al., 2011). Therefore, in the Department of Pharmacognosy GUMed it was decided to determine this volatile fraction composition. However, in the temperate climate the cultivation of *C. incana* is difficult. To provide the continuous maintenance of the examined species gene pool, *in vitro* shoot cultures were initiated from the seeds and established on a solid Schenk-Hildebrandt (SH) medium, supplemented with 2 mg/l 6-benzylaminopurine (BAP) and 0.22 mg/l thidiazuron (TDZ).

The obtained *in vitro* biomasses as well as the aerial parts of *C. incana* ground plants were dried and after extraction in the Deryng apparatus analyzed phytochemically for the volatile fraction, using GC-MS. It was determined that the shoot cultures essential oil (0.57%) can be a source of many valuable compounds, e.g. 1,8-cineol (34.2%), a terpenoid oxide with the significant anti-inflammatory activity (Santos et al., 2004).

Parallely, for the first time the micropropagation protocol of *C. incana* was specified. SH medium supplemented with 2 mg/l BAP and 0.22 mg/l TDZ was found to be the most suitable for the microshoots multiplication from all 30 examined media, varied in terms of mineral and phytohormone composition. The highest level of rooting was observed on SH medium with addition of indole-3-butyric acid. The most satisfactory result of acclimatization of the rooted cuttings was achieved after the stage of their strengthening on the hormone-free SH medium in *in vitro* conditions.

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Santos FA, Silva RM, Campos AR, de Araujo RP, Lima RCP, Rao VSN. 2004. 1,8-cineole (eucalyptol), a monoterpene oxide attenuates the colonic damage in rats on acute TNBS-colitis. *Food and Chemical Toxicology* 42: 579–584.

Endogenous auxin- and ABA-mediated microspore embryogenesis in *Brassica napus* L.

Ewa Dubas[1], Eva Benkova[2], Franciszek Janowiak[1], Piotr Waligórski[1], Michał Dziurka[1], Monika Krzewska[1], Iwona Żur[1]

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Under stress-inducible *in vitro* conditions, auxin and abscisic acid (ABA) are involved in microspore embryogenesis (ME). A better understanding of endogenous auxin/ABA role in the regulation of ME could bring significant progress to the utilization of doubled haploid (DH) technology. *Brassica napus* microspore suspensions are considered to be a perfect model for such a study.

In the present study, two oilseed rape genotypes with different embryogenic capability were used. Endogenous auxin was purified and measured by common chromatographic technique. ABA content was evaluated by ELISA. Both hormones were extracted from microspores (mcs) isolated from plants growing at different temperatures (18°C or 10°C) and collected from the subsequent *in vitro* culture conditions (isolation day, 1d and 5d at 18°C, 1d and 5d at 32°C).

Auxin concentration (ng mg^{-1} protein) and ABA content (fmol *per* 10^4 mcs or pmol g^{-1} FW) depended significantly on the genotype and the treatment. Auxin derivate IBA (indole-3-butyric acid) was the main auxin form that prevailed in isolated mcs. 24h of heat shock significantly increased IBA concentration (5-fold) and ABA content (2-fold) in mcs of the highly embryogenic line. Assuming a mean mcs radius of 10 μm, ABA content corresponded to ABA concentration of 2.1 μM. Extended treatment of mcs at high temperature (32°C) was required for IBA increase in mcs of the non-embryogenic cultivar. Prolonged heat shock significantly improved mcs embryogenesis in the non-responsive genotype. We have found a positive correlation between auxin level and efficient ME induction. Heat-induced increase of ABA content in mcs had no clear-cut impact on ME.

Our findings point to possible importance of endogenous auxin and ABA in ME induction. Presented results suggest a more complex mechanism of embryogenesis process initiation.

ACKNOWLEDGMENT: This work was supported by an agreement with the PAS-FWO in frame of the joint Polish-Belgian project and the national project 2011/01/D/NZ9/02547.

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Nanosilver: safe or dangerous plant protection?

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Biocidal properties of nanosilver are already used practically in numerous fields. It is considered that it will be possible to use nanosilver in plants protection against diseases. Currently, the number of patent applications related to nanopesticides exceeds 4000. However, it is still a controversial issue. The aim of the experiment performed was an assessment of an influence of water nanosilver colloid manufactured using physical method of high voltage arch discharge (Kasprowicz et al., 2010) on the seedlings of *Triticum aestivum* L. Tybalt cultivar in water culture with a contribution of *Fusarium culmorum* spores in a concentration of $2 \cdot 10^6 \cdot 1\ m^{l-1}$. The research objects were seed boxes containing 100 caryopses. The experiment was performed in 4 replications. Morphological evaluation, an assessment of infestation with fusarium foot rot in 4° scale as well as an assessment of silver content in roots and leaves using ASA method with wave length of 328.1 nm and detection limit of 0.012 $mg \cdot dm^{-3}$, were performed after 7 days of plants growth in controlled atmosphere. Nanosilver present in water culture very strongly disturbed the growth of wheat seedling. An average length of seedling root in the control object and one with *F. culmorum* contribution only was 70.4 and 73.5 mm, respectively. Statistically significantly shorter root was noted in seedlings in the objects with nanosilver and nanosilver with fungus spores, and these values were 24.6 and 23.0 mm, respectively. In case of the leaves, the longest ones were observed in the control seedlings (119.1 mm on average). The leaves formed in other objects were statistically significantly shorter: 99.8 mm in case of water suspension of fungus spores, 93.8 mm for water nanosilver colloid, 89.3 mm for nanosilver combined with spores suspension (all differences significant statistically). The lowest index of plant infestation with *F. culmorum* was observed in the object with nanosilver (9.2%) and the control one (12.4%), with an average in the scale of 3.6 and 3.5, respectively. In the culture with fungus spores contribution, as much as 28.8% of the seedlings were subjected to a moderate degree infection, and an average infestation scale was 2.9. Nanosilver presence limited the process of seedlings infection by *F. culmorum*. In this case infestation index was 15.6%, and an average in the scale was 3.4. Silver content in roots and leaves on the control object and this with fungus spores contribution was below detection limit for applied analytical method. An accumulation of the examined metal in plants roots for the culture with nanosilver was high and reached 30.83 $mg \cdot kg^{-1}$, while in the object with nanosilver and fungus spores in was even higher (nearly of 20%). Translocation of silver cannot be evaluated as high. Silver contents in leaves were low, on a level of 1.718 and 1.657 $mg \cdot kg^{-1}$ for the culture with nanosilver and nanosilver combined with fungus spores, respectively. Summing up, nanosilver caused a considerable decrease in plants infestation with *Fusarium*, but in turn fungal infection contributed to a significantly higher silver accumulation in seedlings roots tissues.

ACKNOWLEDGEMENT: the study financed from DS3109/KOŚR/2013

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Presence and activity of mammalian hormone progesterone in plants

Anna Janeczko

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A lecture is devoted to the issue of distribution and physiological activity of mammalian sex hormone – progesterone – in plants. Progesterone is a C-21 steroid (pregn-4-ene-3,20-dione). Progesterone was discovered in the corpus luteum of mammals and chemically synthesised in the beginning of XX century. In humans, this steroid regulates the course of pregnancy and menstruation. It is a precursor in androgen and estrogen biosynthesis and neurosteroid important for brain functioning. Presence of progesterone-like compounds in plants was first reported by Gawienowski and Gibbs in 1968. In 2007, in a model plant *Arabidopsis thaliana* L. progesterone was found in shoot and inflorescence in amount 160 and 400 ng/kg fresh weight, respectively. Progesterone, in dependency on applied concentration, promotes or inhibits growth of shoots and roots of sunflower, and it stimulates tube growth of an *in vitro* matured tobacco pollen. Progesterone also induces plant flowering in winter wheat and *Arabidopsis thaliana* L.. Recently molecular studies reviled presence of progesterone receptors in plant cell: in membrane and in the cytosolic fraction.

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Quantitative trait loci associated with androgenic responsiveness in triticale (×*Triticosecale* Wittm.) anther culture.

Monika Krzewska[1], Ewa Dubas[1], Ilona Czyczyło-Mysza[1], Gabriela Gołębiowska-Pikania[2], Stefan Stojałowski[3], Iwona Żur[1]

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Quantitative trait loci (QTLs) associated with androgenic responsiveness in triticale were analyzed using a population of 90 DH lines derived from the F1 cross between inbred line 'Saka 3006' and cv. 'Modus', which was used in a number of earlier studies on molecular mapping in this crop. Using Windows QTL Cartographer and MapQTL 5.0, composite interval mapping (CIM) and association studies (Kruskal-Wallis test; K-W) for five androgenesis parameters (androgenic embryo induction, total regeneration and green plant regeneration ability, and two characteristics describing final androgenesis efficiency) were conducted. For the studied components of androgenic response, CIM detected in total 28 QTLs which were localized on 5 chromosomes from A and R genomes. Effects of all QTLs that were identified at 2.0 or above of the LOD score explained 5.1–21.7% of the phenotypic variation. Androgenesis induction was associated with seven QTLs (LOD between 2.0 and 5.8) detected on chromosomes 5A, 4R, 5R and 7R, all of them confirmed by K-W test as regions containing the markers significantly linked to the studied trait. What is more, K-W test revealed additional markers on chromosomes: 5A, 2BL, 7B and 5R. Both total and green regeneration ability were controlled by genes localized on chromosome 4A. Some of the QTLs that affected final androgenesis efficiency were identical with those associated with androgenic embryo induction efficiency, suggesting that the observed correlation may be either due to tight linkage or to pleiotropy.

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Positive and negative sides of double haploids production in wheat

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The production of double haploid (DH) plants from cultivars and F1 crosses provides for wheat breeders the source of true homozygotic lines and to accelerate the release of new varieties. Commonly DH lines are obtained by two methods: androgenesis or pollination with maize. Theses techniques can thus complement the conventional breeding programs. The success of anther culture ability in wheat was found to be influenced by genotype, donor plant growth conditions and the developmental stage of microspores, pre-culture treatments, and media components. Pollination with maize additionally needs the synchronization of corn pollen production with wheat flowering time.

Our investigations were performed during 2008–2013 years. Material derived from Polish Plant Breeding Ltd. comprising tens F1 wheat (*Triticum aestivum* L.) genotypes was used for the anther culture and as female plants in making crosses with mixture of Waza and Dobosz sweet corn (*Zea mays* L.). Wheat and maize plants were grown in controlled conditions in a greenhouse (16h photoperiod, 21/17ºC day/night). Induction of androgenesis was maintained on C17 (Wang and Chen, 1983) or W14 (Wang and Chen, 1983) media in the dark and at 30ºC. Emasculated and pollinated florets were treated by drops of 100 $mg \cdot dm^{-3}$ 2,4-D solution. Green haploid plants obtained by both methods were regenerated on 190-2 (Zhuang and Jia 1983) or MS (Murashige and Skoog, 1962) media et 25ºC. DH lines were obtained by colchicine treatment at the least 3–4 tillers stage.

It was shown, that both methods have positive and negative sides. An efficient doubled-haploid production technology that induces homozygosity can greatly reduce the time and cost of cultivar development. Moreover, DH techniques has advantages that base on gamete selection, developing of immediate homozygosity, providing greater efficiency of selection in plant breeding, improving the precision of genetic and mapping studies, accelerating gene pyramiding and improving efficacy and efficiency in screening for resistance. DH techniques via anther culture is however very much genotype dependent in comparison to wheat x? hybridization that shows less genotype-dependent response, has 2–3 times greater efficacy than anther culture, save 4–6 weeks in obtaining the same age haploid green plants. Androgenesis method produce greater number of albino than green plants, as compared to pollination of wheat by maize method, where all regenerated haploid plants are green. We prefer obtaining of DH wheat lines by pollination with maize.

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Fern somatic embryogenesis – new phenomenon for plant experimental biology

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Somatic embryogenesis (SE) is the phenomenon which has fascinated plant biologists for more than half century. This process was described for many angio- and gymnospermous species, but it is unknown to plants belonging to *Monilophyta* clad which includes ferns. The regeneration by somatic embryogenesis, except spermatophytes, has been induced for only two club-moss species: *Lycopodiella inundata* and *Huperzia selago*. Currently, we would like to bring ferns on the investigation on the somatic embryogenesis process. Ferns are plant group that directly precedes an evolution of *Spermatophyta* plants.

Our study showed that the induction of somatic embryogenesis of tree fern *Cyathea delgadii* and development of somatic embryos are very unique and different from any other system described for seed plant. Among factors essential for induction of somatic embryogenesis, the plant growth regulators play a key role. Our investigation provides evidences that fern SE needs any plant growth regulators.

The discovery open the door for exploration of somatic embryogenesis in cryptogamic plants and comparison the developmental and genetic circumstances with those in spermatophytes. From a practical point of view, unlimited propagation of ferns by somatic embryo production can bring in benefits for protection of fern natural genetic resources, for instance a tree fern species suffering from horticultural business.

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Possibility of using *Burkholderia phytofirmans* for *in vitro* rooting *Helleborus niger*

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Helleborus niger is a valuable ornamental plant. According to the data from literature and our experience, it seems that the main obstacle for effective micropropagation is the initialization of cultures free from contaminating bacteria, and the necessity for multiplication, rooting and acclimatization in temperature arrouding 15°C. This condition can be a problem due to the cost of cooling. The knowledge obtained so far shows that inoculation of cultures with bacteria *Burkholderia phytofirmans* can help in achieving greater tolerance to the non-optimal growth temperature (Dhooghe and van Labeke, 2007). Firstly, we would like to prove whether the bacterium, which is known as producing relatively high amount of IAA (Weilharter et al., 2011) is able to stimulate Helleborus root formation. Microshoots were inoculated with bacterium obtained from a 24h culture on the liquid KING B medium. Microshoots were first induced to root for 5 days on the agar medium (MS mineral salts, WPM vitamins and 30 g/sucrose) containing auxins (3 mg/l IBA + 1 mg/l NAA) or on the medium without auxins, and then transferred to perlite filled with liquid medium without auxins either with the addition of bacteria at 10^8/l or without them. The following experimental treatments were applied: only the basic medium for the induction and root growth, induction for 5 days on the medium with auxins, induction on the auxin free medium with bacteria and induction on the medium with auxins and inoculated with bacteria. After 4 weeks the result of rooting was assessed and the root number, shoot and root length were scored.

The microshoots cultured without auxins or bacteria rooted in 83% with 1.7 roots of 0.6 cm long. The microshoots, which shoots were induced for rooting with auxins were rooted in 94% with 2.0 roots of 1.1 cm long. The microshoots inoculated with bacteria were rooted in 95% with 2.3 roots of 1.2 mm long. The microshoots that were induced for rooting on the auxin medium and then inoculated with bacteria were rooted in 100% with 6.9 roots of 2.1 cm long. The best results of rooting (three times more roots and the longest roots) were obtained in the treatment where the induction medium contained auxins and then microshoots were inoculated with bacteria.

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Structural and molecular features of somatic embryogenesis in *Trifolium nigrescens*

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During somatic embryogenesis (SE) the explant somatic cells reverse their state of differentiation and acquire meristematic identity. The developmental fate of such cells can then be redirected to form somatic embryos. The formation of an extracellular matrix surface network (ECMSN), distribution of cell wall epitopes and expression of specific genes were often associated with somatic embryo formation in many species cultured *in vitro* (Šamaj et al., 2006; Elhiti et al., 2013).

In *Trifolium nigrescens* Viv. direct SE and embryogenic and non-regenerative callus were initiated from cotyledonary-staged zygotic embryos (CsZE) on medium containing 0.5 mg/l NAA and 2.0mg/l 2iP. Direct and indirect SE was preceded by high accumulation of *somatic embryogenesis receptor-like kinase 1* (*SERK1*) transcript in SE-competent cells. High level of *TnSERK1* expression was observed in cells of somatic embryos throughout their development as well as in cambium-like cells of xylogenic nodules. Conversely, the non-dividing cells of explants lacked *TnSERK1* hybridization signal. Accumulation of *TnSERK1* was also found to decrease with development of aberrations in embryoid morphology. RT-PCR analysis revealed that the embryogenic explants displayed about one-third higher rate of *TnSERK1* expression than non-regenerative ones.

In contrast to previous reports on different species ECMSN was not confirmed as structural marker of SE and it was found on somatic embryos and non-embryogenic cells. Pectins containing low-methylesterified homogalacturonan were identified as the main component of strands and membranous structures over explant surface.

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Application of mutagens in the breeding of new crop cultivars

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Induction of mutagenesis is an important tool still used in the breeding of new cultivars of crop plants. Up today more than 3000 mutant cultivars belonging to 200 species are registered. Most mutagenesis works were performed on wheat, rice, barley, oat, potato and sunflower. To the most often used physical mutagens belong X-rays, gamma rays, and fast neutrons, while to chemical ones ethyl methane-sulphonate (EMS) and methyl- or ethyl-nitroso urea (MNU or ENU). In the presentation some examples of the physical and chemical mutagens have been described, as well as their application in order to obtain improved cultivars for higher quality and quantity of yield or plant resistance to abiotic and biotic factors was discussed. In our experiments MNU was used in order to increase genetic variability of triploid *Miscanthus* × *giganteus* plants, especially for better frost resistance. This species is a sterile hybrid between *Miscanthus sinensis* and *M. sacchariflorus*, and in recent years it becomes important industrial plant. Unfortunately, due to its sterility and only vegetative propagation (mainly from rhizomes or by micropropagation) classic breeding works are impossible. We applied MNU in various concentrations and time treatment on *M.* × *giganteus* immature inflorescences or rhizomes. The single genomic changes we observed only in regenerants obtained in *in vitro* culture. These plants demonstrated higher chlorophyll a fluorescence (Fv/Fm) compared to control plants. The changes in this parameter we have observed also in plant regenerated from rhizomes treated with different MNU concentrations, however no regenerated plant was more frost resistant.

In vitro cultures give also possibility to obtain new plant forms due to phenomenon called somaclonal variability. This genome variability occurs mainly in callus tissue and is a result of point or chromosome mutations. Source and age of explantats, culture duration, number of sub-cultures, culture environment, chemical additives or growth regulators and media composition are the main factors inducing variability *in vitro*. Also the level of ploidy of single cells or whole regenerants may be changed, however an increase of chromosome number is mainly induced by anti-mitotic factors such as colchicine or oryzaline. These compounds are used mainly to obtain of double haploids or fertile forms of sterile plants. Such experiment we have conducted on *M.* × *giganteus* and we have obtained one hexaploid and some mixoploids after treatment with 12.5 mM colchicine for 18h. Our earlier study confirmed also that plants of M. x giganteus regenerated in tissue culture are more frost tolerant in field cultivation compared to plants propagated from rhizomes, but only during the first winter. Up today we cannot explain this phenomenon. We have also demonstrated that regenerants obtained in *in vitro* conditions are characterized by different chemical content (lignin, cellulose, hemicellulose) than *in vivo* propagated plants.

In vitro culture as a tool for modification of endosperm development

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The seed of the most angiosperms is composed of three major components: the seed coat, the embryo and the endosperm – a unique tissue, that is finally consumed by the embryo. There are no reports concerning *in vivo* endosperm differentiation resulted in plant regeneration. But it was proved that endosperm under *in vitro* cultures revealed proliferation, differentiation and even plant regeneration ability. Experiments conducted on isolated endosperm have opened new possibility to investigate this specific plant tissue. Results are important as well for basic knowledge as for agriculture practice. The regulation of cereal endosperm development is still far from understood. One of the advantages of endosperm culture can be manipulation of the development of this tissue. Endosperm development of cereals is considerably connected with the unequal expression of maternal and paternal genes, starch synthesis and programmed cell death (PCD). Recently (Popielarska-Konieczna et al., 2013) we conducted detailed histological and ultrastructural studies on isolated endosperm of bread wheat, durum wheat and triticale under *in vitro* conditions. *In vivo* the accumulation of starch in endosperm cells is connected with PCD. There was reported that PCD in cereals is irreversible and in triticale occurs in 16 days post anthesis. However our data (not published) revealed that cultured endosperm tissue of triticale, which accumulated starch granules under *in vitro* conditions showed the viability during 3–4 months of the culture. Recent reports (Carciofi et al., 2012; Li and Berger, 2012) pointed that experimental research concerning endosperm-tissue are still needed.

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Present status of species consisting family Gentianaceae in bioscience

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The lecture will be dedicated the group of species, which have been playing important role in traditional exploration of the natural resources of flora of various countries over the world, traditionally call gentians. The name consists about 1700 species and over 90 genera. However, the tropics are the biosphere in which new species have been describing in tropical forests. Plants are known to biosynthesize rare substances which are responsible for the beauty of flower and exploration for the healthcare.

The limits of natural material and strong pharmaceutical demands on plant material (secondary metabolites) are the main reasons for development and establishment of very efficient *in vitro* plant regeneration system. However, horticultural value of some species included into family Gentianaceae stays behind the genetic progress with the exploration of transformation and mutagenesis.

Among subjects undertaken, the presentation will show the development of plant tissue culture and biotechnology of gentians during at least thirty years. Majority of 30 species studied belong to taxa Gentiana. Biochemical studies on Gentianaceae aimed secondary metabolites production are important for humane healthcare not only with the gastrointestinal tract, especially of gastric juice, but with the protection against malaria, fevers and symptoms related to malaria. In our climatic zone the most popular is gentian root (Gentianae radix), which consists of the dried rhizome and roots of *Gentiana lutea* L. (Gentianaceae). The raw material contains gentiopicroside (also known as gentiopicrin), swertiamarin and sweroside and a very small amount of amarogentin, which causes the bitter taste. Also present are xanthones (gentisin, isogentisin, gentioside), phytosterols, phenolic acid, trisaccharides (gentianose), polysaccharides (pectin), and essential oil. The bitterness of the raw material stimulates secretions in the gastrointestinal tract, especially of gastric juice.

Explant originated from earliest stages of development have been used for culture initiation that seedling were most frequently applied. It was improved that the differences between particular organs of a few day-old seedling are significantly different in the presence of MS medium. Leaves originating from *in vitro* culture plantlets were used for description of their morphogenic potential and as the source of the protoplasts for somatic hybridization and transformation. The culture of flower parts help to get interspecies hybrids and haploids to improve floriculture gentian breeding programs.

Localization expression of AtPIN4 and role AtPIN4 during indirect somatic embryogenesis in *Arabidopsis thaliana*

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Somatic embryogenesis is the process in which embryos are formed from somatic cells of the plant body. Polar auxin transport plays an important role in zygotic embryogenesis (Friml et al., 2003). The aim of the study was to determine the localization of auxin gradient and expression of AtPIN4 proteins during indirect somatic embryogenesis in *A. thaliana*. PIN proteins transport IAA from the cytoplasm to the apoplast. In *A. thaliana* has identified eight PIN proteins. AtPIN4 are one of the four expressed during zygotic embryogenesis (Friml et al., 2003).

As explant sources zygotic embryos in cotyledonary stage were used. To achieve the aim of the study the wild-type Columbia (WT Col) and the transgenic strains: *DR5rev::GFP; DR5rev::GUS* and *AtPIN4:GFP, AtPIN4::GUS* of *A. thaliana* were used in all experiments. Zygotic embryos were cultured on induction medium (E5) containing basal B5 medium supplemented with 5 μM 2,4-D, 20 $g \cdot dm^{-3}$ sucrose and 3.5 $g \cdot dm^{-3}$ Phytagel; pH 5.8. After 3 weeks on E5 medium, explants were transferred to MS10 medium (MS salts and vitamins, 20 $g \cdot dm^{-3}$ sucrose and 3.5 $g \cdot dm^{-3}$ Phytagel; pH 5.8) (Gaj et al., 2005). Cultures were cultivated at 24°C under continuous light.

Ability to callus formation of zygotic embryos was similar among studied transgenic plants and comparable with wild type plants. Almost all zygotic embryos of considered transgenic plants were able to indirect somatic embryogenesis (ISE). The average number of somatic embryos produced per one zygotic embryo was 8.24±1.25.

Indirectly, using reporter genes GUS and GFP localization of auxin gradient and expression of AtPIN4 in embryogenic callus and somatic embryos were possible. To achieve this, the transgenic strains *DR5rev::GFP; DR5rev::GUS and AtPIN4:GFP, AtPIN4: :GUS* were used, respectively. Auxin was accumulated in the cells of the outer layer of callus clumps. The localization of AtPIN4expression were also observed in these cells. AtPIN4 gene expression was not identified in either the early or in late globular stage of somatic embryos. The presence of gene transcripts AtPIN4 were found in heart- and torpedo-stage somatic embryos of *A. thaliana*. It was located in the cells forming the root meristem cells and epidermis. The localization of AtPIN4 expression might suggest that AtPIN4 proteins transport auxin into the cells of the outer layer of callus clumps and in heart- and torpedo-stage somatic embryos of *A. thaliana* into the root meristem cells and epidermis.

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Methods for obtaining doubled haploids of oat (*Avena sativa* L.)

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Obtaining new cultivars of oat based primarily on several years of outputting a high degree of plant homozygous inbred crosses and selection of individuals with desired characteristics. Production of haploid plants and doubled haploids (DH) allows to reduce several years of crosses. DH of oat are produced in anthers or isolated microspores cultures and by pollination with pollen of genetically distant species such as maize, millet and sorghum. The most common problem in anther culture of oat is the low percentage of formed embryo like structures and regenerated plants. The promising method seems to be oat pollination by maize pollen, wherein the frequency of haploid embryos is greater in comparison with androgenesis and it does not cause the formation of albino plants.

High impact on the method of distant crossing, despite the selection of appropriate genotypes for crosses, has environmental conditions, especially temperature and light intensity. As a result of such crossing frequently occurs unstable chromosomes, which are eliminated during the embryo development. Obtained plants contain only nuclear genetic material of one parent. Such homozygous lines are often used in the elaboration of new cultivars, because it is easier to predict the outcome of their crossing compared with heterozygous plants. Obtaining haploid oat plants is very difficult compared with other cereals. So far, not only in Poland but also in other countries has not been developed yet a commercial method of obtaining oat haploids. For oat crossing maize is most often used because of the large amount of pollen produced. The maize chromosomes during cell division, a few days after pollination are eliminated, so that the haploid oat embryos develop. After oat pollination by maize chromosomes are usually completely eliminated in the early stages of embryogenesis, and resulting F_1 plants are allohaploids. However, unlike other cereals, there is possible that one or more maize chromosomes are integrated into the genome of oat. Such oat plants can produce stable and fertile partial hybrids. Among the distant crosses of maize with cereals only oat produces embryos with integrated maize chromosomes capable for germination and growth. Depending on which of the maize chromosomes has been integrated into the genome of oat, in the F_1 generation various morphological changes, like different shape of plant or panicle, different leaf color, etc. can be observed.

DH lines are used not only to shorten the time for obtaining new cultivars, but also for determining the characteristics of the desired combinations and are used to study molecular markers and to create mapping populations.

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Schisandra chinensis (Turcz.) Baill. *in vitro* cultures as an attractive source of dibenzocyclooctadiene lignans

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Schisandra chinensis (Turcz.) Baill. is a medicinal plant species which is known mostly for adaptogenic, hepatoprotective, antioxidant and anticarcinogenic actions. Biological activity of the plant is attributed mostly to lignans, dibenzocyclooctadiene derivatives, which include among others schizandrin, gomisin A, γ-schizandrin and deoxyschizandrin (WHO Monographs on Selected Medicinal Plants, 2007).

In the last years (2011–2012) contents of dibenzocyclooctadiene lignans in biomass extracts from *Schisandra chinensis* shoot-differentiating callus cultured on six variants of solid Murashige and Skoog (MS) medium (1962) supplemented with differing contents of cytokinin (BAP) and auxin (NAA), ranging from 0.1 to 3.0 mg/l were examined. In addition, the extracts of undifferentiating callus cultured on two of the MS solid medium variants (2 mg/l BAP and 2 mg/l NAA, and 3 mg/l BAP and 1 mg/l NAA) were investigated. The compounds were assayed by the use of HPLC method (Zhang et al., 2009).

The concentrations of plant growth regulators influenced the production of metabolites. The maximum contents of lignans in shoot differentiating callus equaling 308.51 mg% for deoxyschizandrin, 86.41 mg% for gomisin A, 75.54 mg% for schizandrin and 22.09 mg% for γ-schizandrin. In extracts from the undifferentiating callus, the maximum contents of these compounds amounted to 18.75 mg%, 2.66 mg%, 2.16 mg% and 1.03 mg%, respectively. The maximum total contents of lignans (486.76 mg%) were obtained on MS medium containing 3 mg/l BAP and 1 mg/l NAA and was 1.3- and 3.8-fold higher than in extracts from fruits and leaves of native plant, respectively, analyzed for comparison (Szopa and Ekiert, 2011; 2013).

The studies demonstrated that shoot-differentiating callus cultures could be a potential biotechnological source of four lignans under study, especially deoxyschizandrin.

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Plant *in vitro* cultures: A potential rich source of therapeutically important phenolic compounds

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The research conducted in the Department of Pharmaceutical Botany CMJU in the last two years has continued exploration of potential of plant *in vitro* cultures for production of phenolic acids and the phenolic glucoside, arbutin. These compounds occupy an important place both in phytotherapy and cosmetology.

The effect of concentrations of plant growth regulators (PGRs), in the Linsmaier & Skoog (L-S) (1965) and Murashige & Skoog, (M-S) (1962) medium on the accumulation of free and bound phenolic acids and cinnamic acid was investigated in shoot cultures of *Aronia melanocarpa*. The PGR concentrations showed a distinct effect on accumulation of the metabolites under study. Salicylic acid (SA), 51.96–84.82 mg% and p-hydroxybenzoic acid (p-HBA), 18.42–56.65 mg% dominated in shoot extracts from the L-S media, while SA (41.28–95.54 mg%), p-HBA (5.03–51.90 mg%), and also p-coumaric acid (p-CA), 22.21–65.24 mg% where the main metabolites in shoot extracts from M-S media, respectively (Szopa and Ekiert, 2013 a).

Analogical studies were conducted also on callus cultures of *Anethum graveolens*. In extracts from biomass cultured on the L-S media, the contents of both main metabolites were similar and amounted to 22.93–25.80 mg% for p-HBA and 11.04–11.57 mg% for SA. In biomass from M-S media, production depended on PGR concentrations and ranging from 33.92 to 61.46 mg% for SA, and from 22.33 to 44.55 mg% for p-HBA (Szopa and Ekiert, 2013b).

Repeatability of results of hydroquinone biotransformation into arbutin was tested in agitating shoot cultures of *A. melanocarpa* maintained on the chosen variant of the M-S medium (BAP – 2mg/l, NAA – 2 mg/l). The maximum content of the product was 8.27 g% (Kwiecień et al., 2013). Most of the obtained results have a potential of being practically applicable.

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Flow cytometry – an easy way to detect somaclonal variation

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Flow cytometry (FCM) is a fast, convenient and accurate method for estimation of nuclear DNA content, and therefore can be an alternative to classical microscopic chromosome counting. Genome size or ploidy can be established in any plant material that contains living cells, such as the seed, leaf, root, hypocotyl, callus, plantlet, or somatic embryo; the presence of mitotically active cells is not required (Sliwinska, 2002). It is especially convenient for in-vitro-produced plant material, which is usually characterized by a low frequency of mitotic cells. Preparation of FCM samples is simple and rapid. It involves chopping a small amount of tissue in nuclei isolation buffer containing a DNA-specific fluorochrome. Within a few minutes the fluorescence (correlated linearly with DNA content) of several thousands nuclei can be measured. Since somaclonal variation, often observed in plant tissue cultures, can be an effect of a change in DNA content, FCM is widely used as a basis for controlling the stability of genome size of micropropagated plant material (Rewers et al., 2012). Detection of endopolyploidy, which is easily achieved using FCM, can be helpful in selecting the plant part most suitable to be an explant for *in vitro* cloning (Sliwinska and Łukaszewska, 2005). The method is also recommended for checking the ploidy of haploids and doubled haploids or for screening for novel ploidy levels (e.g. in polyploid breeding). It can be used for identification of somatic hybrids obtained by protoplast fusion (Szczerbakowa et al., 2011). Heterokaryons can be sorted under sterile conditions, cultured *in vitro* and hybrid plants regenerated. Additionally, FCM can be applied to count cells in suspension and to establish cell cycle activity.

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Influence of auxins on callus induction in *Cordyline australis* G. Fostr. (Endle.)

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Among other cordylines, australian cordyline 'Red Star' is the most valuable decorative pot plant. The genus *Cordyline* is propagated by cuttings of its stem or the tip of its rhizome. This is a slow method of propagation, and it tooks about 50 years to increase the stock of the variegated C. australis 'Alberti' to provide sufficient plants for commercial release (DeMason and Wilson, 1985; Harris 2001).

The plants of *Cordyline australis* G. Fostr. (Endle.) 'Red Star' used for this study were grown under greenhouse conditions. Shoot slices (about 1-mm-thick) were obtained and transferred to growing vessels. Explants were cultured on initial MS medium (Murashige and Skoog, 1962), consisted of 3% sucrose and 0.7% agar, supplemented with various growth regulators.

In the experiment three different auxins: Picloram, 2,4-D and Dicamba (25 and 50 μM) combined with 0.5 μM BA or TDZ were tested for callus induction. The frequency of nodular callus formation was determined by counting explants forming nodular callus from the total number of the cultured explants. The highest percentage of explants forming callus was observed in medium containing 25 μM Picloram and 0.5 μM BAP and the lowest in those with 50 μM Dicamba and 0.5 μM BAP. On the medium supplemented with 25 μM 2,4-D and 0.5 μM BA recorded not only the lowest percentage of explants forming callus, either explants necrosis. The developed callus proliferated on initial media the and the optimal callus formation was noted in the presence of Picloram (for both 25 and 50 μM).

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Factors important for successful induction of androgenesis in triticale (× *Triticosecale* Wittm.)

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The continuing growth of the economic importance of triticale (× *Triticosecale* Wittm.) generates a strong interest in biotechnological tools which can be used for its further improvement. Among others, the process termed androgenesis enabling instant production of totally homozygotic, doubled haploid (DH) lines can significantly accelerate the breeding progress. Unfortunately, relatively low effectiveness of this process in triticale continues to limit its wide application. In order to gain a better understanding of the mechanisms controlling androgenesis in triticale, endogenous plant hormones concentration (auxins, cytokinins, and abscisic acid) as well as the generation of reactive oxygen species and the efficiency of antioxidative defense (the activity of superoxide dismutase, catalase, peroxidase, and non-enzymatic antioxidants) were analyzed in ten triticale DH lines significantly different in androgenesis responsiveness. The selection of five highly responsive and five recalcitrant DH lines from the population of 102 DH lines 'Saka 3006'×'Modus' was based on the anther culture method (Krzewska et al., 2012). The analyses were performed in anthers excised from freshly cut tillers and then in anthers excised from tillers after low temperature pre-treatment (3 weeks at 4°C) used as a trigger for androgenesis induction. The results obtained allowed for the identification of the factors which significantly influenced androgenesis induction and which were critical for high effectiveness of the process.

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POSTERS

In vitro bud and shoot formation on different explant type of some *Lachenalia* cultivars

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Lachenalia (Hyacinthaceae) is a new bulbous ornamental plant, native to Africa, increasingly used by the horticultural industry both as a cut and pot plant, with a trade name "Cape Hyacinth". *Lachenalia* is considered to be difficult in propagation by offsets (Kleynhans, 2006), thus micropropagation through plant tissue culture creates possibilities for multiplication of this genus during commercial plant production. The aim of the study was to determine the effect of the cultivar and explant type on the adventitious and axillary buds and shoots formation in *in vitro* cultures of *Lachenalia*. The material for research consisted of three cultivars: 'Rupert', 'Namakwa' and 'Ronina'. Three explant types (apical bud, top scale part, basal scale part) were cultivated on MS basic medium solidified with agar (0.7%), containing 3% of sucrose and supplemented with growth substances: BA and 2,4-D or NAA (1–10 μM). After four weeks of culture initiation the axillary buds and next the axillary shoots were observed on apical bud explants and the adventitious buds and next the adventitious shoots on scale explants.

Irrespectively of the cultivar, type of explant affected the forming of buds. The largest percentage of apical bud explants formed axillary buds. There were no significant differences in forming of adventitious buds between the top scale parts and the basal scale parts. Analyzing the development of axillary shoots from apical bud explants and adventitious shoots from scale explants no difference was noticed in the percentage of rate of explants forming shoots. Irrespectively of the explant type, all tested cultivars responded similar to the forming of buds but differences were observed in the stage of shoots development. All explants of *Lachenalia* 'Ronina' and 'Rupert' had higher ability to form shoots in comparison to explants of *Lachenalia* 'Namakwa'.

The results of the present investigation show the existence of a an inter-species variability in *in vitro* culture of *Lachenalia* at the stage of shoots formation and the influence of explant type on the axillary and adventitious buds appearance.

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The cytotoxic effect of zearalenone on wheat (*Triticum aestivum* L.) callus cells

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Zearalenone (ZEA; 6-[10-hydroxy-6-oxo-trans-1-undecentyl]-B-resorcyclic acid lactone) is a no-steroidal estrogenic *Fusarium* mycotoxin known as a regular contaminants of cereal crops worldwide (Duca et al., 2006). ZEA, previously described as F-2 toxin, implicates reproductive disorders and hyperesterogenic syndromes in animals and humans. The mechanism underlying the toxicity of the ZEA is still not well understood. Plant cell culture serves an useful model to determine the influence of ZEA on the single cell. The aim of this study was to find the threshold concentration of this substance, which will be safe for cell vitality and membrane stability.

To perform the experiment, wheat (*Triticum aestivum* L.) callus were produced from the immature embryos on Murashige and Skoog medium supplemented with 2,4-D. After 3 months of culture, callus was transferred into MS medium containing ZEA in subsequent concentrations (0.5 and 10 $mg \cdot l^{-1}$). After 3 days of *in vitro* culture, cytological and biochemical analyses were made. The fluorescein diacetate (FDA) was used in cell viability test under the fluorescent microscope Nikon Eclipse E-600 equipped with a digital camera DXM 1200F. Lipid plasma membranes oxidation was assumed as the increase of the malondialdehyde (MDA) content by spectrophotometric analysis.

Statistical analysis revealed that ZEA at the concentration of 5 $mg \cdot l^{-1}$ did not trigger cell vitality and membrane oxidation in comparison to the control. Non destructive effect of that ZEA dozes was suggested earlier by Biesaga-Kościelniak and Filek (2010). Contrary, ZEA at the concentration of 10 $mg \cdot l^{-1}$ significantly induced cell damage. Cell vitality drastically decreased to about 68%. Moreover, the stressogenic ZEA effect was confirmed by the increase of MDA content (26%) in relation to the control.

We could conclude that 5 $mg \cdot l^{-1}$ of ZEA is the threshold concentration, above which the toxic effect of this substance induces cells damage.

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Effect of different medium on diosgenin production in *Trigonella foenum-graecum* L.

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Fenugreek (*Trigonella foenum-graecum*) is widely distributed throughout the world and belongs to the Fabacecae family (Mehrafarin et al., 2011). Fenugreek is a rich source of steroidal sapogenins, mainly diosgenin. Diosgenin is of great interest to the pharmaceutical industry because of its oestrogenic effect on the mammary gland. It also plays an important role in the control of cholesterol metabolism. Diosgenin is mainly used as starting material for partial synthesis of oral contraceptives, sex hormones and other steroids (Oncina et al., 2000).

The aim of work was the induction on diosgenin production by different medium such as Murashige and Skoog medium (MS) supplemented with 1% sucrose (Murashige and Skoog, 1062), McCown's woody plant medium (WPM) with 1% sucrose and half-strength WPM + 1% sucrose (Lloyd and McCown, 1980). Leaves were harvested from seedlings every 4–5 days. Diosgenin was identified by UPLC coupled with mass spectrometry with triple quadrupole. UPLC analyses were performed using a Shimadzu apparatus on a Kinetex C-18 RP column (50 mm × 2.1 mm I.D., 1.7 μm). AB Sciex QTRAP 4500 was equipped with an electrospray ion source operating in positive ion. For targeted metabolites we used very sensitive multiple reaction monitoring (MRM) method.

The result of analysis showed that the most effective production of diosgenin taken place on the half-strength WPM (about 1038 μg diosgenin/g dry weight). The level of diosgenin on the other media was similar. We observed increase of diosgenin content in the time of plant growth on all media. Content of diosgenin raised on each media up to 38th day of experiment.

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The effect of selected fungicides on *Trichoderma* spp. antagonistic fungi under *in vitro* conditions

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Trichoderma spp. fungi are powerful antagonists of parasitic soil fungi of the following genera: *Pythium, Verticillium, Gaeumannomyces, Sclerotinia, Rhizoctonia* and *Fusarium* inflicting plants with root-rots of seedlings, root rot and wilt which lead to plant withering. Some strains of *Trichoderma* promote plant growth, increase nutrient availability, improve crop production and enhance disease resistance (Druzhinina et al., 2011; Harman et al., 2004)

Integrated plant protection systems strive to use antagonistic fungi together with fungicides. It is important to find the forms of microorganisms antagonistic towards pathogens of plants resistant to plant protection chemicals because chemical plant protection will undoubtedly remain an important element of plant protection programs for a long period of time. Microorganisms used in biopreparations will be successful in their protective roles in the environment on condition that they become insensitive to chemicals used in agriculture (Dłużniewska, 2005).

Investigations were carried out to study the effect of fungicides against *Botrytis cinerea* (Bravo Plus 500 SC – chlorotalonil, Euparen Multi 50 WG – tolytfluanide, Folicur BT 225 EC – tebuconazole and triadimefone, Sumilex 500 SC – procemidone) on a development and biological activity of antagonistic fungi *Trichoderma harzianum* Rifai, *Trichoderma pseudokoningii* Rifai and *Trichoderma viride* Pers. ex Gray.

The work aimed to determine the effect of the fungicides in various concentrations on the below-mentioned features of studied fungi: the linear growth rate and inhibition of mycelium development; morphology of mycelium and sporulation; spore germination and biological activity of *Trichoderma* fungi towards three soil pathogens, i.e. *Botrytis cinerea* Pers., *Fusarium solani* Sacc. and *Rhizoctonia solani* Kühn. based on laboratory tests.

In vitro experiments revealed strong fungistatic properties of Bravo Plus towards the *Trichoderma* spp. isolates tested. Fungicides deteriorated the antagonistic properties of *Trichoderma* genus fungi against *B. cinerea, F. solani* and *R. solani* pathogenic fungi.

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Cold storage of *Tussilago farfara* L. nodal cultures

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Coltsfoot (*Tussilago farfara* L.) is a valuable medicinal plant used in the upper respiratory tracts disorders. The main active compounds of coltsfoot are: mucopolysaccharides, flavonoids, sterols, phenolic acids and also hepatoxic pyrrolizidine alkaloids – senkirkine and senecionine. Storage of plant cultures under cold condition reduces the growth and allows avoiding frequent passages thus reducing the labour costs. The aim of this study was to evaluate possibility of long term storage of coltsfoot *in vitro* cultures. The effect of temperature (10°C) on growth and content of pyrrolizidine alkaloids after 6 months of storage has been determined. Control group was cultivated at standard conditions (23°C temperature). Cold storage influenced on length of shots, dry weight of biomass and content of pyrrolizidine alkaloids. Dry weight of shots was higher compared to the control group. Survival and multiplication rates did not differ significantly. Content of pyrrolizidine alkaloid (3.83 mg/100 g d.w.) decreased 2.4-fold versus control (9.33 mg/100 g d.w.). Therefore, it can be concluded that cold storage of *in vitro* coltsfoot is possible for up 6 months without passages and allows preserving plants in good condition.

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Effect of yeast elicitor on *Salvia przewalskii* Maxim. suspension culture

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Salvia przewalskii is a herbaceous perennial plant originated from north-western China. Roots of the plants contain pharmacological active compounds: diterpenoids – tanshinones (I, II-A, II-B), miltirone, cryptotanshinone, przewalskins (A-G), przewaquinone A, and triterpenoids: przewanoic acid (A, B), oleanolic and urosolic acid, as well as phenolic deratives. So far no studies on *S. przewalskii* callus cultures have been reported. The aim of the study was to determine the content of tanshinones and phenolic acids in *S. przewalskii* elicited callus tissues. Callus suspension culture was initiated from axilary buds of young plants, in MS (Murashige and Skoog, 1962) liquid medium supplemented with 2,4-D (2.0 mg/L) and BA (2.0 mg/L). Elicitor (precipitated yeast extract) was administrated at concentration range: 30 – 100 mg/L on the 7th day of culture. After 7 days of incubation, biomass of elicited and non-treated cells (control) was collected and dried. Content of tanshinones and phenolic acid was determined using HPLC method. Yeast extract induced accumulation of cryptotanshinone and tanshinone II A in elicited callus. The side effect – decrease in biomass and content of rosmarinic acid was noted. The results demonstrated effect of yeast elicitor on *S. przewalskii* suspension culture and content of bioactive compounds for the first time.

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Induction of cell suspension culture from isolated roots of rock soapwort (*Saponaria ocymoides* L.)

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Saponaria ocymoides L. is a small perennial native to Southern Europe. It produces secondary metabolites generally called saponins that have antibacterial, antifungal, cytostatic, and antiviral properties (Hu et al., 2012). Saponins in *S. ocymoides* occur in both, in roots and shoots. Cell suspension culture is one of the possible ways of saponins production in controlled conditions. Our aim was to obtain an *in vitro* cell suspension culture from isolated roots. A five-step procedure was developed to reach this goal. 1) Obtaining of sterile seedlings. 2) Optimization of the solid medium composition for growth of microplants. 3) Induction of root culture in liquid medium. 4) Induction of cell suspension from root culture. 5) Optimization of media composition and culture conditions for rapid growth of cell suspension biomass. Seeds of *S. ocymoides* proved to be very difficult for surface sterilization. A six-step procedure proved to be effective: 1) 1 h 30 min. in 50% H_2SO_4, 2) 5 × washing in sterile water, 3) 5 min. in 70% EtOH, 4) 1 × washing in sterile water, 5) 1 h 30 min. in 2% sodium hypochlorite, 6) 5 × washing in sterile water. For the growth of seedlings solid medium based on Murashige and Skoog (1962) was developed. The major alteration was elevation of the pH value to 6.5. Plants growing on this medium were used as a source of roots for culture in liquid medium. For sustainable growth of roots 1mg of BAP and 0.5 mg of IAA per 1 liter was optimal combination of hormones. Induction of cell suspension culture was obtained by switching to medium with 2 mg/l of BAP. For long-term maintenance of cell suspension culture two media proved to be effective: 1) 1 mg/l BAP plus 0.5 mg/l IAA, 2) 2 mg/l BAP.

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Morphogenesis in *Rumex in vitro* culture – sex ratio among regenerated plantlets

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Rumex thyrsiflorus is one of the model dioecious plant species in studies on sex chromosomes (with an XX/XY_1Y_2 system). The aim of the present experiment was to define the type of morphogenetic events during *in vitro* culture of *R. thyrsiflorus* using histological and scanning electron microscope (SEM) analysis and to examine sex ratio among seedlings and *in vitro* regenerated plantlets using PCR – based method, involving DNA markers located on Y chromosomes.

In vitro cultures were established using fragments (ap. 5 mm) of roots, hypocotyls and cotyledons isolated from 11 – day – old seedlings. They were cultured on MS basal medium supplemented with 0.5 mg/l TDZ. Callus induction was observed after 11 days from the beginning of the culture. The first signs of organogenetic response were visible after 21 days of culture.

Indirect organogenesis (adventitious shoots formation via callus) was confirmed using histological analysis of tissue stained with periodic acid Schiff/naphtol blue black (PAS/NBB). The regenerated shoots derived from meristemoids located under surface or deep inside callus. Both, histological and SEM analysis, revealed secondary organogenesis. Among 20 randomly selected seedlings, 11 showed female – biased sex ratio, whereas in case of 16 plantlets regenerated *in vitro*, 14 revealed to be females, indicating a higher regenerative potential of female seedlings.

Electrophysiological and structural properties of natural and model membranes as a tool for measurement of lipids/polyamines interaction

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Studies of membranes in model systems are important for understanding the mechanisms of action of biologically active substances with both plasmalemma and membranes of selected cell organelles such as chloroplasts (Peetla et al., 2009). The effect of spontaneous formation of mono- and bi-layers (liposomes) by the lipids (major components of all membranes), when placed in an aqueous medium (a model system for the cytoplasm) is exploited (Hąc-Wydro and Dynarowicz-Łątka, 2008). In the presented experiments polyamines, group of compounds of the hormone character was chosen as biologically active substances, whose role both in development and stress is not fully recognized (Rudolphi-Skórska et al., 2013). The studies were focused on comparing the influence of polyamines on the properties of the membranes of chloroplasts of wheat with effects observed for single lipids (1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) and 1,2-dioleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol), which represent components of these membranes. Analyses of the surface pressure as a function of area per lipid molecule (obtained from Langmuir trough) give information about the structural effects lipid/polyamines interactions, and electrokinetic potential measurements – about the changes of the electrical properties of the membranes.

It has been found that the presence of polyamines in an aqueous environment leads to stabilization of chloroplast membranes and reduces the negative charge on both liposomes and chloroplasts. The range of changes was estimated and compared with the results obtained for the known inorganic (calcium ions) 'stabilizers' of membranes. Effects of divalent putrescine and calcium on the electrical properties of the membranes were comparable and consisted in the compensation of the negative charge of the lipid by these cations, whereas noticeable differences were found in the action of these substances on the mechanical properties of lipid monolayers.

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Plant tissue cultures in pharmaceuticals

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Plant cell and tissue cultures hold great promise for controlled production of myriad of useful secondary metabolites. In the search for alternatives to production of medicinal compounds from plants, biotechnological approaches, specifically plant tissue cultures, are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites (Vijayasree et al., 2010). Exploration of the biosynthetic capabilities of various cell cultures has been carried out by a group of plant scientists and microbiologists in several countries during the last decade (Siahsar et al., 2011). A number of medicinally important alkaloids, anticancer drugs, recombinant proteins and food additives are produced in various cultures of plant cell and tissues. Advances in the area of cell cultures for the production of medicinal compounds has made possible the production of a wide variety of pharmaceuticals like alkaloids, terpenoids, steroids, saponins, phenolics, flavanoids and amino acids. Some of these are now available commercially in the market for example shikonin and paclitaxel (Taxol). Until now 20 different recombinant proteins have been produced in plant cell culture, including antibodies, enzymes, edible vaccines, growth factors and cytokines. Advances in scale-up approaches and immobilization techniques contribute to a considerable increase in the number of applications of plant cell cultures for the production of compounds with a high added value. Some of the secondary plant products obtained from cell suspension culture of various plants. Cell suspension culture systems are used now days for large scale culturing of plant cells from which secondary metabolites could be extracted. A suspension culture is developed by transferring the relatively friable portion of the callus into liquid medium and is maintained under suitable conditions of aeration, agitation, light, temperature and other physical parameters. Cell cultures cannot only yield defined standard phytochemicals in large volumes but also eliminate the presence of interfering compounds that occur in the field-grown plants. The advantage of this method is that it can ultimately provide a continuous, reliable source of natural products. The major advantage of the cell cultures include synthesis of bioactive secondary metabolites, running in controlled environment, independently from climate and soil conditions (Yesil-Celiktas et al., 2010). A number of different types of bioreactors have been used for mass cultivation of plant cells. The first commercial application of large scale cultivation of plant cells was carried out in stirred tank reactors to produce shikonin by cell culture of *Lithospermume rythrorhizon*. Cell of *Catharanthus roseus*, *Dioscorea deltoidea*, *Digitalis lanata*, *Panaxnoto ginseng*, *Taxus wallichiana* and *Podophyllum hexandrum* have been cultured in various bioreactors for the production of secondary plant products. Plant tissue culture represents the most promising areas of application at present time and giving an out look into the future (Karuppusamy, 2009).

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Role of plant tissue culture technology for production and conservation of species

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The *in vitro* culture has a unique role in sustainable and competitive agriculture and forestry and has been successfully applied in plant breeding for rapid introduction of improved plants. Plant tissue culture has become an integral part of plant physiology and breeding. As an emerging technology, the plant tissue culture has a great impact on both agriculture and industry, through providing plants needed to meet the ever increasing world demand. It has made significant contributions to the advancement of agricultural sciences in recent times and today they constitute an indispensable tool in modern agriculture (Thorpe, 2007). Interventions of biotechnological approaches for *in vitro* regeneration, mass micropropagation techniques and gene transfer studies in tree species have been encouraging. *In vitro* cell and organ culture offers an alternative source for the conservation of endangered genotypes. Germplasm conservation worldwide is increasingly becoming an essential activity due to the high rate of disappearance of plant species and the increased need for safeguarding the floristic patrimony of the countries (Sengar et al., 2010). Tissue culture protocols can be used for preservation of vegetative tissues when the targets for conservation are clones instead of seeds, to keep the genetic background of a crop and to avoid the loss of the conserved patrimony due to natural disasters, whether biotic or abiotic stress (Tyagi et al., 2007). The plant species which do not produce seeds – sterile plants or recalcitrant seeds that cannot be stored for long period of time can succesfully be preserved via *in vitro* techniques for the maintenance of gene banks. Cryopreservation plays a vital role in the long-term *in vitro* conservation of essential biological material and genetic resources. It involves the storage of *in vitro* cells or tissues in liquid nitrogen that results incryo-injury on the exposure of tissues tophysical andchemical stresses. Successful cryopreservation is often ascertained by cell and tissue survival and the ability to re-grow or regenerate into complete plants or form new colonies (Filho et al., 2005). It is desirable to assess the genetic integrity of recovered germplasm to determine whether it is 'true-to-type' following cryopreservation (Corredoira et al., 2004). The fidelity of recovered plants can be assessed at phenotypic, histological, cytological, biochemical and molecular levels, although, there are advantages and limitations of the various approaches used to assess genetic stability. Cryobionomics is a new approach to study genetic stability in the cryopreserved plant materials. The embryonic tissues can be cryopreserved for future use or for germplasm conservation.

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The effect of selected foliar fertilizers on *Phoma exigua* and *Sclerotinia sclerotiorum* phytopathogenic fungi under *in vitro* conditions

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Foliar nutrition is the main element of plant cultivation technology. It enables fast supply of nutrients crucial for plants in the case of their deficiency in soil or difficulties in their uptake (Michałojć and Szewczuk, 2003). Properly fed plants do not reveal any symptoms of physiological disorders and are more stress resistant. Some authors (Sawicka 2003; Gleń 2008) also observed that during foliar fertilizer application, the intensity of some plant diseases diminishes. Foliar fertilizers may therefore improve plant immunity to infections. On the other hand, decreasing the degree of infection by phytopathogens may result from the direct impact of foliar fertilizers on these pathogenic microorganisms. The research conducted so far revealed that under *in vitro* conditions foliar fertilizers limit development of some pathogens (Gleń, 2008, 2011).

The effect of foliar fertilizers, such as Mikrovit Fe and Mikrovit Zn and mixtures of these fertilizers with urea and magnesium sulphate under *in vitro* conditions reveal fungistatic effect towards dangerous phytopathogens – fungi of *Phoma exiqua* Desm. and *Sclerotinia sclerotiorum* (Lib.) de Bary species. Reduction of surface growth of the fungi colony and biomass increment was stronger on media containing field doses (1.0 mm/cm) of foliar fertilizers in comparison with lower concentrations. Irrespectively of the concentration used, Microvit Zn inhibited the increment of P. exiqua biomass in 100%. Moreover, higher concentration of this fertilizer in the medium inhibited sporulation process in the test fungi and favoured sclerotia production by *S. sclerotinium*. However, *P. exiqua* sporulation was most strongly inhibited on the media with added Mikrovit Fe mixture with urea and magnesium sulphate and with Mikrovit Fe added separately. On the other hand, urea, particularly in 1.0 mm^3/cm^3 concentration most intensively inhibited axospore generation by *S. sclerotiorum*. Irrespective of applied concentration, magnesium sulphate stimulated surface growth of *S. sclerotiorum* and *P. exiqua* colony biomass and limited their sporulation. Fungicidal effect of foliar fertilizers registered in the experiments *in vitro* is an optimistic premise for agricultural practice. Foliar fertilization of plants may serve to protect plants against diseases or reduce development of pathogens.

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Immobilization technique of pollen tubes on microscope slide

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In experimental praxis, it is important to analyze (in real time) not only statistically significant group of cells but also single pollen tube to changes in the extracellular osmotic potential. To perform such experiments, we had to develop a technique to immobilize the cells so they cannot move during the experiment. We developed method to grow the cells in an agarose gel medium directly on microscope slides. We measured and recorded growth in populations of pollen tubes using different concentrations of agarose. Pollen was germinated and grown in culture chambers in standard germination medium (6% [w/v] sucrose, 1.6 mM H_3BO_3, 200 μM $CaCl_2$, and 25 μM MES [pH 5.5]) where it was assembled on microscope slides using silicone isolators for 3 h at 22.5°C before performing experiments on slabs cons containing 0.3% (w/v) low gelling temperature agarose (plant cell culture grade, Type VII, Sigma). The results showed that the concentration of 0.3% agarose gave the best growth results but still kept the cells immobilized. Next we tested how long it would take for the osmotic treatments to penetrate through the agarose medium. It was found that the osmotic treatments diffused through the agarose medium within 12–15 seconds. That was rapid enough for the live cell studies.

We report on our results concerning growth rate and oscillation modes of the individual pollen tube apex. The observed volumetric growth and growth rate periodicity in the longitudinal (axial) direction turned out to be accompanied by transverse oscillations with similar frequencies but higher energies than the axial modes. Examination of the time-domain coherence between oscillations in mutually perpendicular directions revealed minimal energy dissipation in the unperturbed (isotonic) case, opposite to the two remaining cases (hypertonic, hypotonic) with notable correlations. We conjecture that the minimal energy loss is therefore optimal in the natural growth conditions. The longitudinal growth velocity is also found to be the fastest in the unperturbed case. As a result, the isolated system (pollen tube tip) is conserving energy by transforming it from elastic potential energy of extending apical wall to the kinetic energy of periodical motion (Haduch-Sendecka et al., 2013).

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Effect of colchicine on polyploidization of *Miscanthus × giganteus*

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Miscanthus × giganteus is a natural, sterile triploid hybrid (2n = 3x = 57) between the tetraploid *M. sacchariflorus* (2n = 4x = 76) and the diploid *M. sinensis* (2n = 2x = 38). *M. × giganteus* is a C_4 plant and produces a large quantity of biomass under relatively low input levels. It is cultivated mainly as an energy crop, however, due to its high cellulose content, it may be used in the paper industry or in 'small architecture'. Miscanthus as a non-forage plant is planted on industrial waste areas contaminated with heavy metal ions or polycyclic aromatic hydrocarbons. Miscanthus may be grown also on soil with elevated salinity. Apart from long-term cultivation of Miscanthus enhances soil carbon sequestration.

The sterility of this species makes breeding works impossible. The aim of this study was to obtain polyploids of *M. × giganteus* using colchicine, as an antimitotic agent. The experiment was performed on immature inflorescences and *in vitro* propagated plants. In the study two concentrations of colchicine (12.5 or 25 mM) and two exposure times (9 or 18 h) were tested. Callus tissue was obtained from inflorescences treated with colchicine on the MS (Murashige and Skoog, 1962) medium supplemented with 5 mg dm^{-3} of 2,4-D, 0.2 mg dm^{-3} of BAP, 500 mg dm^{-3} of caseine hydrolysate and 30 g dm^{-3} of honey instead of sucrose. Plants were regenerated on MS medium with 0.05 mg dm^{-3} of kinetin and 30 g dm^{-3} of sucrose. *In vitro* propagated plants treated with colchicine were planted into pots with perlite and grown in glasshouse conditions. Plants obtained after treatment with colchicine were analyzed by flow cytometry. Only one plant obtained after 18 h-treatment of *in vitro* propagated plant with 12.5 mM colchicine was identified as hexaploid, while twelve *in vitro* propagated plants, after exposure to colchicine were mixoploids. All plants regenerated from colchicine-treated immature inflorescence did not demonstrate any changes in their ploidy levels.

Applicability of 2-DE to assess changes in the protein profile after androgenesis-inducing treatment in winter triticale (× *Triticosecale* Wittm.).

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Upon stress treatment, the microspores are directed towards sporophytic development by initiation of the process called androgenesis. As a result, instead of pollen grains, embryo-like structures (ELS) are formed. ELS can regenerate into haploid (n) plants and then after genome doubling, doubled-haploid plants (DHs, 2n) could be produced. The potential effectiveness of the process is enormous as each of thousands of microspores which develop inside the anther could regenerate into DHs plant. However, usually only a few percent of microspore population enter embryogenic program. The identification of proteins specific for androgenic cultures under low temperature inductive conditions could be helpful in better understanding of the mechanism.

The protein expression patterns were examined in four DH lines of triticale (× *Triticosecale* Wittm.) selected from the 'Saka 3006'×'Modus'mapping population. The chosen DH lines significantly differ in androgenic efficiency. The protein analysis was conducted on anthers collected from freshly cut (control) and low temperature-treated tillers (3 weeks at 4°C). The proteins were isolated according to the phenol-based procedure (Hajduch et al., 2005). The protein expression patterns were examined by using two-dimensional polyacrylamide gel electrophoresis (2-DE), the pH 5–8 linear IPG strips and Coomassie Brilliant Blue staining. Protein expression was evaluated by PDQuest 2-D software version 8.0. As low temperature effectively triggers androgenesis induction in triticale, the evaluation of its effectiveness was performed by anther culture method.

Our research revealed that cold-treatment caused some changes in protein expression profiles in anthers prerequisite for androgenesis induction. It could be supposed that those differences are connected with androgenesis induction efficiency. Interestingly, low temperature treatment also significantly increased (2-fold) the number of ELS in anther cultures of triticale (ELS per 100 anthers).

Based on data obtained so far, we are going to identify the differentially expressed proteins possibly connected with androgenic responsiveness in triticale.

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Collecting and storage of the genus *Beta* genetic resources *in vitro*

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Collecting and storage of the genus Beta genetic resources *in vitro* was initiated in 1990s of the last age in the Division of the Plant Breeding and Acclimatization Institute in Bydgoszcz. The most of accessions kept in tissue cultures is represented by wild species of beet because they are important as a source of resistant genes to diseases, pests and stress abiotic factors (Asher et al., 2001). The ability to regeneration of whole plants of the most wild beet species has not been well-known. At present in *in vitro* cultures on MS medium (Murashige and Skoog, 1962) supplemented with 0.2-1 mg l^{-1} BAP (6-benzyloaminopurine) is kept and multiplied biennial male-sterile genotype of *B. maritima* (Kużdowicz, 2012) and B. *macrocarpa*, *B. patula* and *B. patellaris* species. The plant material was originated from field or greenhouse gene bank collection of the National Centre for Plant Genetic Resources. The initial explants were the 0.1–0.3 cm long tips of inflorescence shoots. Sterilized tips were cultured at 25°C on MS basal medium under artificial daylight conditions with 16 h photoperiod. Explants were transferred to fresh medium every four weeks. Coefficient of reproduction was high and dependent on species and components of the used medium. Explants were characterized by a high genetic stability and high morphogenetic potential. Some of obtained shoots occasionally developed roots on the MS basal medium. For rooting, regenerated shoots are transferred to 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 more than 75%. Because there are no wild species of beet in the natural flora of Poland germplasm of stored genotypes is used for study of the *Beta* genome, for plant breeding and for molecular biology research.

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The effect of liquid culture on differentiation of *Narcissus* L. 'Carlton' somatic embryos

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Narcissus species are bulbous plants of extensive use in ornamental horticulture. The naturally low rate of *narcissus* propagation by adventitious bulbs is a problem delaying the production of new cultivars and the elite reproductive material. Tissue culture systems based on the process of somatic embryogenesis and use of liquid media can significantly increase the propagation rate (Chen and Ziv, 2001; Sage et al., 2000; Selles et al., 1997; Malik, 2008). The objective of the study was to investigate the effect of liquid culture on somatic embryos formation and development in embryogenic callus cultures of *Narcissus* L. 'Carlton'.

The embryogenic callus obtained on ovary explants under the influence of Picloram and BA multiplicated for 6 weeks in liquid media shaken at speed 100 or 60 rpm, in temporary immersion system – Rita (15 min every 24 h – immersion time) as well as on solid media (control) were used for the experiment. For differentiation of somatic embryos calluses were cultivated for 6 weeks in liquid – shaken at 100 or 60 rpm and on solid media containing 5 μM BA and 0.5 μM NAA an then on solid medium for 14 weeks.

Process of *narcissus* somatic embryos formation was not affected by physical state of medium during regeneration stage, and depended on culture conditions during multiplication. The highest number of somatic embryos was noted in callus propagated in Rita vessels and regenerated on solid medium. Maturation and conversion of somatic embryos were stimulated by cultivation on solid media, shaking at low speed or slowing down the shaking during regeneration stage.

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The activity of photosystem II in *Rumex tianschanicus* × *Rumex patientia* – a perspective energetic plant

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Rumex tianschanicus × *Rumex patientia*, a hybrid of English spinach (*R. patientia* L.) and Tien Shan sorrel (*R. tianschanicus* A. Los.) is a perspective plant as a source of renewable energy. The efficiency of photosynthetic electron transport in the proximity of PSII was studied in *R. patientia, R. tianschanicus* and hybrid under *in vivo* and *in vitro* conditions. Fragments of hypocotyls of 7-day-old seedlings were used as explants. The induction of adventitious shoots was observed on MS medium supplied with 2% sucrose, 2,2 mg/l BAP and 0,17 mg/l IAA. Histological analysis showed, that regenerated plantlets were obtained by direct organogenesis mainly from pericycle cells and secondary organogenesis was also observed. Moreover histological and SEM studies revealed the presence of structure similar to ECM (Extracellular Matrix). Higher biomass production for *Rumex tianschanicus* × *Rumex patientia* in comparison to parental lines suggested enhanced intensity of photosynthesis in hybrid form. Determination of selected chlorophyll fluorescence parameters from photosystem II (PSII), such as: maximum quantum efficiency of PSII photochemistry (Fv/Fm), PSII operating efficiency (ΦPSII) and non-photochemical quenching (NPQ) did not show any significant changes in all analysed *Rumex* plants.

The obtained results suggest that the efficiency of photosynthetic electron transport in the proximity of PSII is not a key factor responsible for growth differences between parental lines *R. patientia* and *R. tianschanicus* and hybrid *Rumex tianschanicus* × *Rumex patientia*.

The influence of iron sources on the efficiency of micropropagation of sage

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Due to the totipotency and the capability to regeneration micropropagation allows obtaining a large number of genetically and phenotypically homogeneous plants and therefore is commonly used for the clonal propagation of plants on an industrial scale. The aim of the research was to analyze different sources of iron in a micropropagation process of sage. Iron is one of the most important microelements essential for the synthesis of chlorophyll and the proper functioning of chloroplasts as well as an element of proteins. Symptoms of iron deficiency can be observed in explants used for micropropagation (the youngest leaves and meristems), hence the interest in this element. The literature data show that the iron has a very significant impact on the quantity and quality of yield. The problem is its facility to switch from easily assimilable forms to the forms not available for plants. Due to the weak assimilation of iron in *in vitro* culture, it is commonly applied in the form of chelates, which facilitates the administration of that element within the plant tissue in a broad range of pH and prevents precipitation of the Fe ions in the form of iron oxide. The use of chelated forms promotes a gradual release of iron cations to the medium or their absorption in the form of complexes. However, the durability and supply of iron for plants are dependent on the properties of the ligand (Chohura et al., 2007). In the present study the influence of three different chelates in the process of micropropagation of sage was examined. The ANOVA analysis of variance showed that the Fe source had statistically significant effect on the plants growth and development. In this study FeEDDHA, FeEDTA, $FeSO_4$ with Na_2EDTA were tested. The best results were obtained when using ferrous sulfate combined with Na_2EDTA prior to addition to the medium. It can be assumed that the use of such complexes allowed slow and continuous release of iron and resulted in the best multiplication rate of plants. Micropropagation protocol of Polish only variety of sage (Bona) described in the study turned out very efficient and can be applied for fast obtainment of herbal raw material and secondary metabolites.

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The contents of macro and micro-elements in embryos of grains as a marker of different tolerance of wheat genotypes to drought stress

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The water deficit is the main environmental stress limiting cereal yield worldwide. Plants respond to drought through morphological, physiological and metabolic modifications occurring in all organs. The investigations of the drought tolerance conducted in the natural conditions are difficult because of the large variance in the genotype-environment interacting. One of the biotechnological techniques useful in these studies is the plant tissue culture as an alternative way of plant vegetative propagation (Shah et al., 2009). *In vitro* cultures are widely applied in biochemical and physiological investigations of plant response to stress at the cellular level (Liu et al., 2006). In seeds of four wheat genotypes the contents of macro- and microelements in terms of the possible application of these parameters to the evaluation of the susceptibility of these varieties to drought stress were estimated. Seeds were sterilized for 15 min with a 10% commercial bleach (Domestos) and opened with needles under a dissecting microscope. The contents of macro and microelements were determined by inductively coupled plasma (ICP) optical emission or mass spectrometry – OES/MS, which could quantitatively measure the elemental content from the ppt to the percentage range. It was found that the amount of Mg in embryos differentiated sensitive genotypes from the tolerant ones and was higher in the former. The same dependence was observed for phosphorous content. On the other hand, the content of transition metal ions: Cu, Fe, Zn and Mo did not classified the genotypes, whereas the amount of Mn varied, but its level was not connected with the genotype stress tolerance.

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Analysis of tryptophan and its derivatives content in the biomass of *in vitro* *Bacopa monniera* cultures

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Bacopa monniera L. Pennell (Brahmi) a plant belonging to Scrophulariaceae family is one of the most important plant from Ayurveda system. It can be found in wet areas, especially in South India. The most important active compounds of this plant are triterpenoid saponins called bacosides, alkaloids such as nicotine, herpestine and steroid compounds. Their main action is to increase blood flow in the brain, improving concentration, it also have antidepressant, antioxidant, antiinflammatory, antibacterial, antitumor effect and it is also used as a support in the treatment of neurodegenerative diseases. The aim of actually research was to maintain *in vitro* culture of *B. monniera* and greenhouse crops of this species. The next step was to obtain methanol extracts from biomass, in which the analysis of L-tryptophan and their derivatives was made by HPLC method. These compounds were analyzed due to their effect on the CNS, as they are neurotransmitters or their precursors. L-Tryptophan and 5-hydroxytryptophan cross the blood-brain barrier and are metabolized into serotonin showing antidepressant action. This analysis (of: L-tryptophan, 5-hydroxytryptophan, 5-methyltryptophan, serotonin, melatonin, tryptamine, 5-methyltryptamine, kynurenine, indoleacetic acid, indoleacetonitrile, indole, indoleacetamide) was performed according to the procedure developed by Kysilka and Wurst with some modifications. HPLC analyses were performed using a Hitachi apparatus with a pump: L-7100; column: Purospher® RP-18 (4 × 200 mm, 5 μm) thermostated at 25°C. The solvent system used for the analysis was composed of ethanol/water/acetic acid (25:99:1 v/v/v); flow rate: 1 ml/min. Detection was carried out with a UV detector, using $\lambda = 280$ nm. The identification of the indole compounds was made by comparing the retention times of sample peaks with the standards. The presence of the tested metabolites in the sample was corroborated by an increase in peak height at the appropriate retention time. The results were expressed as mg/100 g of dry matter, calculated on the basis of the area under the peak.

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Mono- and sesquiterpenoids from leaves of *Telekia speciosa*: *in vivo* versus *in vitro* plant

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Telekia speciosa (Schreb.) Baumg. (basionym – *Buphtalmum speciosum* Schreb.) is the only species included into the genus *Telekia* Baumg. (Asteraceae). This perennial plant, native to Southeastern Europe and Asia Minor is closely related to a group of resiniferous taxa of Inula L. The best known secondary metabolites of the plant are sesquiterpene lactones which have shown marked antiproliferative activity against human cancer cell lines *in vitro*. Moreover, some of the compounds are inhibitors of lipopolisaccharide induced nitric oxide synthesis and moderate inhibitors of transcription factor NF-κB activation.

Our previous studies led to the isolation of terpenoid metabolites from *T. speciosa* multiple shoot culture (Stojakowska et al., 2011) and to the identification and quantification (GC/MS/FID) of over one hundred volatile compounds (mainly mono- and sesquiterpenes) in essential oils obtained from different organs of the plant and *in vitro* grown shoots (Wajs-Bonikowska et al., 2012). Surprisingly, the essential oil from *in vitro* grown multiple shoots shared more similarities in composition with the oil from inflorescences than with the essential oil from leaves and stems. The analysis of essential oils, however, did not tell us much about the content of pharmacologically active sesquiterpene lactones present in the plant material, due to low volatility and termolability of the compounds. The only sesquiterpene lactone isolated from multiple shoots of *T. speciosa* was 2,3-dihydroaromaticin, whereas subsequent GC/MS/FID analysis revealed the presence of isoalantolactone in the multiple shoots.

The present work was aimed at understanding the differences in monoterpenoid (thymol derivatives) and sesquiterpene lactone production in aerial parts of field-grown plants (leaves and ligulate flowers) and in aseptically grown multiple shoots of *T. speciosa*. Apart from being exceptionally rich in thymol derivatives, the multiple shoots, showed numerous differences in sesquiterpene lactone pattern in reference to leaves and flowers of the plant. The most striking were high contents of 2,3-dihydroaromaticin and isotelekin, and only traces of isoalantolactone.

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Secondary metabolites in *in vitro* culture of Virginia mallow (*Sida hermaphrodita* Rusby)

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One of the most important areas in biotechnology is *in vitro* production of secondary metabolites. This covers a wide range of organic substances often with a complex structure, which are synthesized in complex and mutually correlated metabolic pathways.

Secondary metabolites are unique for a certain species, tissues and show specific biological activity. They are not crucial in basic metabolism but they affect the plant adaptive mechanisms for particular environment. The most often reason for collecting secondary metabolites are disorders in metabolic cycles such as metabolic pathway enzyme deficiency connected with basic metabolism. They lead to collecting intermediate reaction products and activating secondary metabolic pathways producing characteristic metabolites (Malepszy, 2005). Production of biologically active substances using plant *in vitro* cultures allows for optimization and better control over the biosynthesis of these substances.

The phytochemical analysis were conducted to qualitatively and quantitatively identify biologically active substances contained in the leaves of Virginia mallow. The trials to identify flavonoids, tannins, phenolic acids, scopolamine and atropine were conducted.

Flavonoids were determined by spectrophotometric and thin layer chromatographic methods. The concentrations found were in the range of 1.240–1.197% in relation to dry matter while the content of flavonoids in liquorice is 0.16–1.17% and in peppermint 1.5–2.2% (Matławska, 2008).

The dry matter concentration range of identified tannins was 0.940–1.280%. In comparison with other commercially used raw materials like green tea (3.28–15.40%) or beach family plants (68–70%), the obtained results show only trace amounts of these compounds.

The HPLC profile of organic acids performed for ultrasound homogenized raw material showed the presence of numerous phenolic acids, among others: caffeic, ferulic, chlorogenic, p-coumaric and salicylic.

No presence of scopolamine nor atropine was found in tested material, although the detection limit for these substances, as shown on the calibration curves, was 0.0004 and 0.0005 mg/ml.

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Cryopreservation of *Tulipa tarda* Stapf. apical meristems by droplet vitrification

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Tulipa tarda Stapf., belongs to botanical tulips, a native of Central Asia. Among its advantages are low height, multi-flower stem and the capacity to remain in one place for several years (Botschantzeva, 1982). It is very important to save germplasm collection of *T. tarda* becouse field collections are threatened by pests and diseases.

A protocol for the cryopreservation of apical meristems involves excision of about 0.5 mm^3 explants from *in vitro* grown bulblets. The bulblets were cultured on MS medium, supplemented with 60 g/l sucrose and incubated at 20°C in the dark. The isolated apical meristems were exposed to a loading solution (LS) for 20 min., then dehydrated with a highly concentrated vitrification solution (PVS2) for 10–30 min. at 0°C and quickly immersed in liquid nitrogen. After rapid rewarming the explants were transferred to a MS medium with 0.3 M sucrose for 1 day. Recovery and regrowth was on MS medium, containing 60 g/l sucrose, in the dark.

The survival rate of the meristems was determined 2 weeks after rewarming, and ranging from 65% (10 min. of the PVS2 activity) to 90% (20 and 30 min. of the PVS2 activity). Viability of control meristems (not cooled) reached 100% in case of the shortest vitrification solution treatment. Visible growth of the apices (increase in length and enlargement of leaf primordium) appeared 4 weeks after rewarming. The regrowth rate does not exceed 40%, irrespectively of time of exposure to PVS2. Control meristems showed a regrowth rate at a level of at least 50%. Future experiments may be performed to improve regeneration after cooling and to develop an efficient cryopreservation protocol for *T. tarda*.

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Bud culture of grasspea (*Lathyrus sativus* L.) – effect of some factors on shoot regeneration and rooting

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Grasspea (*Lathyrus sativus* L.) is one of the least known grain legume species in Europe. Its unique features: resistance to flooding, drought, salinity, low soil fertility, and high protein content in seeds, cause that *L. sativus* could become the cheapest source of protein component of animal feed. However, traditional breeding (of this crop) are still dealing with problems with interspecific hybridization among different *Lathyrus* species. The best solution of this problem would be to benefit from other methods including accession of biotechnological techniques exploiting *in vitro* cultures.

Notwithstanding grain legume plants are considered to be recalcitrant to cell and tissue culture. Very often the most difficult step is regeneration of whole plants due to problems with root induction on newly regenerated shoots. Development of rooting protocols reliable in different regeneration systems is a prerequisite for the effective use of micropropagation by plant breeders.

In presented studies we have evaluated the effect of different factors, inter alia: origin and the position of explants, applied growth regulators and their concentrations on shoot regeneration efficiency. We have also examined influence of aforementioned factors and additionally different auxin concentrations in rooting media and type of substratums on rooting of regenerated shoots.

The addition of growth regulators was essential to optimize shoot regeneration in the culture of apical and lateral buds of grasspea. Frequency of shoot regeneration (76–100%) was not affected by different concentrations of applied auxin and cytokinin, whereas it was affected by the origin of explants. The highest number of shoots per explant (45.0) was noted from ex vitro lateral buds on the medium with addition of 0.02 mg/dm^3 NAA and 4.4 mg/dm^3 BAP. Rooting of newly regenerated grasspea shoots was completely ineffective on the media solidified with agar. In turn, rooting efficiency increased significantly when it was carried out in perlite substrate soaking with liquid media. Rooting frequency amounted more than 75% for shoots regenerated mostly on media with either the least content of cytokinin or the highest of auxin from ex vitro explants. The average number of roots per shoot reached more than 3.8 for shoots mostly regenerated on the multiplication medium with the lowest growth regulator content from apical ex vitro buds.

Growth of mycelium of *Microdochium nivale* on medium containing 24-epibrassinolide and 28-homobrassinolide – verification of fungistatic effect

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Brassinosteroids (BR) are natural plant steroid regulators which may be used in agriculture to avoid synthetic chemicals. Especially interesting from agricultural point of view is their high activity in plant protection against different stressors including inducing disease resistance. Action of BR is accompanied with stimulation growth and crop of plants. However little is known about influence of BR on growth of plant pathogens.

Mycelium of six isolates collected from rye and perennial ryegrass in Poland were cultured on medium containing 0.25 $mg \cdot dm^{-3}$ of 24-epibrassinolide (EBR) or 28-homobrassinolide (HBR). The mycelium grew up equidistantly around the fungus agar plug at 2°C and 18°C. The last day of culture the mycelium of control and cultured with EBR was scraped and homogenised. Next activity of beta-D-glucosidase and alfa-D-galactosidase was measured spectrophotometricaly as described in Pociecha et al. (2013). The impact on growth of mycelium has been studied for EBR and HBR while enzymes activity of was evaluated only for EBR treatment.

Experiment showed that at 2°C the growth of all isolates from perennial ryegrass were slightly inhibited especially by HBR while in isolates from rye by EBR. At 18°C mycelium grew faster and inhibiting effect was visible only in one isolate from perennial ryegrass and one from rye. In the case of ryegrass isolates enzyme activity was strongly inhibited by EBR at both temperatures. In turn in rye isolates galactosidase activity increased at 18°C while glucosidase activity was inhibited only in one isolate at 2°C.

To conclude the slowing down of growth of mycelium by BR was more pronounced in isolates from perennial ryegrass. In thus isolates EBR decreased activity of enzymes involved in cell wall reorganisation by releasing glycosidase-bound phenolics. In contrary in isolates from winter rye enzyme activity was on the control level or even increased. Summarizing the effect of BR depended on isolate origin and temperature of mycelium growth.

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Effect of explant and chemico-physiological properties of culture conditions on the somatic embryogenesis induction of tree fern *Cyathea delgadii*

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Pteridophyte is very old group of vascular plants important because of ecological, medicinal and ornamental reasons. Ferns are micropropagated by diverse systems. For initiation of axenic culture mainly spore – derived gametophytes are used. To investigate the regeneration ability rhizomes, stolons, stipes, fronds or adventitious buds excised from sporophytes as explant are also used. Up to now it was possible to *in vitro* multiply the gametophytes *via* gemma or by branching, and receive the sporophytes via sexual or apogamic reproduction. The somatic embryogenesis (SE) process, that for Spermatophytes is common issue, for ferns have not been examined yet.

Almost every research in field of induction and maintenance of SE process is based on usage of exogenous plant growth regulators (PGRs). Little work is done to describe PGRs-free SE induction systems. Generally, the induction of somatic embryos without PGRs can be caused by stress treatments (pH, heavy metal ions, osmotic shock, culture medium dehydration, mechanical wounding of explant).

The study was undertaken to describe the somatic embryogenesis induction for tree fern *Cyathea delgadii*. Primary results of SE of ferns we would like to present.

Hormon-free system of SE induction is presented. Stipes excised from young sporophyte are the primary explant. To establish optimal conditions for this process an effect of explant size and leaf age were studied. Moreover the effect of mineral salt and sucrose concentration in initial culture medium on SE was examined. Our attention was paid for light/darkness and liquid/agar medium, too.

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The level of Mn*Sod* gene transcripts *in vitro* cultures of *Triticum aestivum* L. and *Vicia faba* ssp. *minor* L. under osmotic stress

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Many factors in plant *in vitro* cultures can induce stress in their cells. The plant response to the stress factors can be activated by dismutases (Szechyńska-Hebda et al., 2012), catalases or peroxidases genes (Dąbrowska et al., 2007a), and metallothioneins (Dąbrowska et al., 2012). Skrzypek et al. (2008) indicated that the oxidative stress and the osmotic potential play an important role in the regulation of regeneration processes.

The aim of this study was to analyze the level of Mn*Sod* gene transcripts in different conditions of osmotic stress (impact of sucrose/mannitol) at explants and callus of wheat (*Triticum aestivum* L.) and field bean (*Vicia faba* ssp. *minor* L.) of a certain regeneration ability. cDNA sequences of genes encoding Mn*Sod* were radioisotope-labeled and used to perform Northern hybridization. Molecular probes after hybridization detected transcripts of approximately 1000 bp in both wheat and field bean.

The studies found an increased amount of Mn*Sod* transcript at regenerating callus of *T. aestivum* induced from immature embryos compared with callus induced from mature embryos, both in culture on media supplemented with 6% sucrose and in the presence of 7% mannitol. In the case of field bean, a higher accumulation of Mn*Sod* was observed in the nonregenerating callus than regenerating one. Most likely, this reaction was caused by the callus response to prolonged *in vitro* culture time as an inverse correlation was noted at control conditions (3% sucrose) at day zero. The results seem to indicate a lack of differentiation of field bean Mn*Sod* gene expression in response to osmotic or trophic stress conditions.

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In vitro cultures of *Phyllanthus glaucus* as a source of securinega-type alkaloids

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The securinega-type alkaloids comprise a group of approximately 40 compounds, which have been isolated from the family *Euphorbiaceae* and were found i.a. in *Phyllanthus* and *Securinega* genera. The most known compound of this group is securinine which is stereospecific GABAA receptor antagonist with a significant *in vivo* CNS stimulating activity (Zhang et al., 2011). Some studies suggested that securinine can significantly decrease acetylocholiesterase activity and reduce glial inflammatory responses induced by β-amyloid proteins and it may be helpful in the treatment of Alzheimer's disease (Lin et al., 2004).

In our studies securinega alkaloids were investigated in the obtained *in vitro* shoot cultures of 5 *Phyllanthus* species – *P. grandifolius*, *P. juglandifolius*, *P. multiflorus*, *P. glaucus* and *P. amarus*. Using chromatographic methods the presence of these compounds was revealed only in the shoot culture of *P. glaucus* harvested on MS medium supplemented with IBA 0.5 mg/L and BAP 0.5 mg/L.

The aim of the study was to investigate the influence of the plant growth regulators from the group of auxins (IBA, IAA) and cytokinins (BAP, K, 2iP, TDZ) on the shoot development and roots formation to establish micropropagation method for *P. glaucus*. The highest number of shoot per explant (11,5) was obtained on MS medium with the addition of BAP 1.0 mg/L, IBA 0.5 mg/L and as the optimal auxins for root induction the IBA was chosen. Additionally, the influence of plant growth regulators on the accumulation of securinega-type alkaloids in *P. glaucus* biomasses was studied as well as the influence of ex vitro conditions. The studies revealed that concentration of these compounds varied from 1.7 to 5.8 mg/g d.w. depending on the composition of harvesting media and was the highest in biomasses obtained on MS medium with BAP 0.5 mg/L. After two months in *ex vitro* conditions the concentration of alkaloids was approximately 3 times higher than on the rooting medium and amount to 14.12 mg/g d.w.

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In vitro selection of *Sida hermaphrodita* under cadmium stress

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Taking into account the highly toxic effects of cadmium on human health it is essential to eliminate this element from the human environment. Virginia mallow (*Sida hermaphrodita*) belongs to a group of high biomass energy crops which are tolerant to heavy metals, including the highly toxic cadmium (Antonkiewicz and Jasiewicz, 2005). This feature may be useful in the phytoremediation process of contaminated soils. The aim of this study was to characterize the protein profiles of virginia mallow under stress due to the presence of cadmium ions, thus to define the effect of cadmium on synthesis of heavy metal binding proteins in virginia mallow. Apical buds and lateral buds obtained from mother plants were the source of explants for *in vitro* cultures. The plants were grown on media containing 0 (control), 25, 75, 150 and 300 mg Cd × dm/kg^{-3}. Observations and plant vitality assessment showed a significant impact of cadmium on the development of *Sida* grown *in vitro*. Plants grown on contaminated culture media were characterized by the presence of chlorosis and necrosis. Additionally, the inhibition of root development was observed. Effect of cadmium was also observed at the molecular level by analyzing the changes in the profiles of proteins isolated from test and standard plants. Unlike the material from standard plants, the test plant extracts revealed the presence of cysteine-containing proteins during spectrophotometric analysis of the fractions obtained by the use of ion exchange column (fractions of 0.4, 0.5 and 0.6 M NaCl). The differences in the protein profiles of plants grown on contaminated culture media in relation to plants grown on the control media were also demonstrated by electrophoretic separation. Increased synthesis of proteins containing cysteine residues capable of heavy metal binding presents a defense reaction of plants under stress induced by the presence of cadmium in the culture medium.

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Plant regeneration in the *in vitro* cultures of the selected species of *Miscanthus*

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Miscanthus is a perennial grass, considered to be one of the leading energy crops. It constitutes a significant source of biomass, which can be used directly in combustion, as a resource for chemical industry or for producing fuels containing bio-ethanol. Yield efficiency of *Miscanthus* can be augmented using genetic-breeding and biotechnological methods, including transformation. Developing an effective regeneration system is a requirement for undertaking the research of plant transformation.

The aim of the experiments was to determine the efficiency of obtaining callus and plants in the *Miscanthus in vitro* cultures. The object of research was two species of *Miscanthus*: *M. sinensis* (line no. 1, 16 and 17) and *M. giganteus* (line D-116 from Germany and one line from Denmark). Explants material was a fragment of young spikelets, spike axles and nodes were placed on medium MS + 5.0 mg/l 2,4-D + 0.5 mg/l BAP for the induction of callus. They were placed on MS Medium + 2.0 mg/l BAP was applied for the purpose of plant regeneration. The callus tissue was obtained after 2–3 weeks of culture and after 2 months the regeneration of the plants from all the tested explants and forms was observed. The efficiency of obtaining both callus and plants depended on the type of the explant and the genotype. In all the tested forms the highest frequency of callus induction and plant regeneration was obtained from the fragments of young spikelets – from 1.5 to more than 10 times higher than from the spike axles. The highest regeneration rate was observer in *M. sinensis* (no. 17) and *M.* × *giganteus*. In these genotypes respectively 659.0 and 517.9 calluses/100 explants and 2454.4 and 1811.9 plants/100 explants (young spikelets) were obtained. In the other *Miscanthus* forms the efficiency of attaining callus from the young spikelets varied between 82.9 and 241.6, while the effectiveness of acquiring plants ranged from 353.3 to 380.3. 95% of the regenerated plants adapted to the ex vitro conditions after being replanted in the soil.

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In vitro propagation of *Lychnis flos-cuculi* L., a plant with potential medicinal value

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Lychnis flos-cuculi L. (Ragged Robin) is a perennial herb belonging to Caryophyllaceae family. It is known to contain several groups of biologically active compounds, most notably phytoecdysteroids (polypodines A and B and their further derivatives), triterpenoid saponins (glycosides of hederagenin and gypsogenin), phenolic acids (caffeic acid) and flavonoids (Tomczyk, 2008).

The aim of present study was to develop the method of *in vitro* propagation of *L. flos-cuculi* multiplication of shoots and their rooting and to compare chemical composition of aerial parts from both wild plants and plantlets from *in vitro* cultures. Propagation protocols for this species *in vitro* have not yet been reported. However, cultures of other species belonging to the genus *Lychnis* L. (*Lychnis senno* Siebold et Zucc.) have been successfully established (Chen et al., 2006a).

A successful micropropagation protocol through axillary bud formation has been developed. The highest plant regeneration efficiency (95%), with over 12 shoots per explant was induced on a MS basal medium supplemented with BAP (2.0 mg L^{-1}) and NAA (0.2 mg L^{-1}). The multiplied shoots were rooted on MS medium with NAA (0.5–1.0 mg L^{-1}) and plantlets were transferred to pots. Callus cultures from root explants were also established on MS medium with 2,4-D and BAP (1.0 mg/mL each). Cytological, morphological and phytochemical characterization of *in vitro*-derived plantlets of *L. flos-cuculi* will be carried out. This study provided a shoot culture that will be used for *in vitro* induction of tetraploids in liquid medium using colchicine (Chen et al., 2006b). Tetraploid plants may be valuable source of plant material with higher content of secondary metabolites.

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Influence type of container and sugar contain in medium on *in vitro* storage of *Senecio macrophyllus* shoot

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Senecio macrophyllus M. Bieb. (Astreaceae) is an extremely rare species and is listed in the Polish Red Data Book. Cultures *in vitro* used to micropropagation of *Senecio* could help in its conservation, because its seeds completely lose their ability to sprout after one year, making it unfeasible to set up a seed bank. The aim of the study is to determine the conditions for storage of shoots of *S. macrophyllus* in the slow-growing cultures.

The study material were axillary shoots of *S. macrophyllus* obtained from the shoot tips culture (Trejgell et al., 2010). Isolated shoots were stored in glass jars and magenta boxes that contained 50 ml 1/2MS medium (Murashige and Skoog, 1962) supplemented with 0.5 mg·L^{-1} BA and 0.05 mg·L^{-1} NAA. The three carbohydrate treatments were tested: (I) 3% sucrose, (II) 1.5% sucrose and 1.5% sorbitol and (III) 3% sucrose and 3% sorbitol. Cultures were conducted in the darkness at reduced temperature (10°C) for 9 months. The plant material was evaluated at 3 month intervals. Analysis of visual rating (Reed, 1992), viability and proliferation rate of shoots were evaluated directly and after 4 weeks transfer to optimal growth conditions, respectively. The obtained shoots were rooted on medium without auxin and adapted to *ex vitro* conditions.

Magenta boxes were better type of containers for storage of *S. macrophyllus* shoots. Data recorded after 3 months of storage were quite similar to results obtained during multiplication of shoots in subculture under optimal condition (Trejgell et al., 2010). Extension of the storage to 9 months significantly reduced analysed parameters. The best responses were observed with treatment II, where percentage of survival after 6 and 9 months was 56.3 and 37.5, respectively, while multiplication rate was 2.3 and 1.0. ANOVA indicated significant interaction between type of container and carbohydrate treatment during shoots multiplication.

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Transformation of anthracene in callus and cell suspension cultures of *Daucus carota* L.

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Compounds from the group of persistent organic pollutants undergo transformation in cell suspension cultures of selected plant species (Kucerova et al., 2001; Chroma et al., 2002). Anthracene is a highly hydrophobic compound, and in nature, its uptake by plants from wet and dry atmospheric deposition and soil solutions is significantly limited.

The degree of anthracene transformation in a system and its bioaccumulation was determined in a study of callus and cell suspension cultures. The cells were incubated with anthracene at concentrations of 0, 75, 250 and 1800 μg/L for 10 days in the dark at 24°C. Callus tissue was cultured for 21 days on solid agar containing anthracene at concentrations of 0, 100, 500, 2500 and 10000 μg/kg. In cell suspension cultures (with increasing anthracene concentrations), the post-culture liquid contained 0.3%, 0.6% and 7.3% of the initial anthracene dose, and the cells contained 1.3%, 1.8% and 62.6% of the initial dose, respectively. In carrot cells from cultures with low anthracene concentrations, the analyzed compound was degraded in up to 99%, and similar results were obtained in tomato, soybean and wheat cells (Kucerova et al., 2001). The values of the bioconcentration factor were similar to those noted in aqueous organisms. The above suggests that the degree of contamination is more likely to be determined by the physicochemical properties of an aqueous environment than the differences between species.

In callus cultures, the contact area between the callus and the solid medium was small, and after incubation, anthracene was not determined in tissues from two cultures with low concentrations. In tissues from cultures with higher concentrations (2500 and 10000 μg/kg), the compound was determined at 80 and 485 μg/kg, respectively. A comparison of callus and cell suspension cultures with similar anthracene concentrations revealed that anthracene concentrations in callus tissue were approximately 7000 lower than in cells.

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Amberlite resins induced flavone production in *Scutellaria barbata* normal root culture

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The "wild-type" root cultures of *Scutellaria barbata*, in comparison to the transformed root culture of *Scutellaria lateriflora*, accumulate in higher concentrations biologically active flavones (baicelin, baicalin, wogonin) and acteoside. *In vitro* organ cultures of both species were obtained as a result of earlier research in the Department of Pharmacognosy (Wilczańska-Barska et al., 2011, 2012). The normal roots of *S. barbata* were cultivated on B_5 IBA (1.0 mg/L) and SH IBA (1.0 mg/mL) media. However, unknown phenolic compounds were released into used both culture media causing difficulties in a maintenance of the *S. barbata* "wild-type" root culture (Wilczańska-Barska et al., 2011).

Several experiments with an addition into the culture media resins: Amberlite XAD-4, Amberlite XAD-7 and charcoal, directly or in a form of "mini-bags", were performed. As a result, quantitative changes in metabolic profiles of the *in vitro* obtained biomasses were observed. An analysis of secondary metabolites was performed using HPLC-DAD-ESI/MS method (Wilczańska-Barska et al., 2012). The wogonin concentration, in a presence of resins, significantly increased. Moreover, the resins introduced to culture medium in a form of "mini-bags" additionally stimulated the release of flavones into the media.

Due to the different rate of flavones release into medium and also observed intra- and extracellular metabolites accumulation, 3-fold increase of wogonin production (154.8 mg/L) and 4-fold increase of chrysin production (7.7 mg/L) was determined on B5 IBA (1.0 mg/L) medium with the presence of "mini-bags" containing XAD-4 resin at concentration of 50 mg/50mL. Furthermore, on SH IBA (1.0 mg/L) medium containing "mini-bags" with XAD-7 resin at concentration of 50 mg/50mL – only 1.8-fold increase of wogonin production (75.6 mg/L), comparing to control, was showed.

The results of research confirm the significance of wogonin as phytoalexin – compound which biosynthesis in *Scutellaria* plants is growing up under stress conditions (Wilczańska-Barska et al., 2012).

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Factors affecting shoot formation in *Magnolia* × 'Spectrum' *in vitro*

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Magnolia × 'Spectrum' is a hybrid of *M. liliiflora* 'Nigra' and *M. sprengeri* 'Diva' (*Magnoliaceae*). It is a single-stemmed, pyramidal tree-form magnolia, valuable for its very large, deep red-purple flowers appearing before the leaves and high resistance to frost. Because of difficulties in propagating magnolias from cuttings, tissue culture techniques seem to be a quicker method of multiplication.

Shoot cultures were initiated from apical and axillary bud explants, collected from two-year-old field-grown plants, on MS medium containing 1.5 mg l^{-1} BAP. To obtain cyclic shoot formation in M. × 'Spectrum', the effects of different concentrations of BAP (0.0, 0.2, 0.5, 1.0, 1.5 mg l^{-1}), GA_3 (0.0, 0.1, 0.5, 1.0 mg l^{-1}), nitrogen salts (KNO_3/NH_4NO_3 100/100 and 75/50% in relation to MS medium), and sucrose (20 and 30 g l^{-1}) were investigated.

The results showed that shoot formation in *M.* × 'Spectrum' was regulated by the interaction between cytokinin, nitrogen and sucrose. The highest multiplication rate (4.7 shoots/explant) and good quality shoots were obtained on full strength MS medium containing 20 g l^{-1} sucrose and 0.5 mg l^{-1} BAP. In the presence of 20 g l^{-1} sucrose, higher BAP concentrations caused inhibition or stimulation of shoot formation depending on the nitrogen level (KNO_3/NH_4NO_3 100/100 and 75/50%, respectively). Significant inhibition in the axillary bud activity and formation of mature shoots was caused by sucrose at a concentration of 30 g l^{-1}. These symptoms intensified at the lower nitrogen level. The presence of BAP (0.2–1.5 mg l^{-1}) partly reversed the inhibitory effect of sucrose on the axillary bud activity, but simultaneously induced leaf yellowing and necrosis. Addition of GA_3 to BAP-medium inhibited shoot formation and strongly induced leaf necrosis.

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Interaction of ethylene and ABA in the regulation of shoot formation in *Pelargonium* × *hortorum* 'Bergpalais' *in vitro*

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Ethylene is known to interact with ABA in many aspects of plant growth and development at many levels, including both positive and negative reciprocal effects on synthesis and interactions between signaling pathways. Among the various *Pelargonium* genotypes cultured *in vitro*, *P.* × *hortorum* 'Bergpalais' is distinguished by low susceptibility to early leaf senescence and increased shoot formation in the presence of cytokinin (meta-Topolin) and a precursor of ethylene biosynthesis – ACC. By contrast, application of $AgNO_3$ – an inhibitor of ethylene action – significantly inhibited shoot formation. Addition of exogenous ABA to *m*T-medium had no influence on leaf yellowing, but strongly inhibited shoot and leaf formation to the extent dependent on concentration. On the other hand, the use of nordihydroguaiaretic acid (NDGA) – an ABA biosynthesis inhibitor – resulted in increased shoot formation and inhibition of leaf yellowing. Moreover, application of NDGA partly reversed the inhibitory effect of $AgNO_3$ on shoot formation. Both ABA and NDGA inhibited ethylene production by *P.* × *hortorum* shoots, but only NDGA enhanced shoot formation. It was previously found that mT-stimulation of shoot formation was associated with an increase in ethylene levels at the beginning of the culture period. The highest multiplication rate of *P.* × *hortorum* 'Bergpalais' shoots was obtained on the medium supplemented with mT, NDGA and ACC.

The results suggest that ethylene and ABA interaction plays an important role in the regulation of shoot formation in *P.* × *hortorum* 'Bergpalais' *in vitro*. In normal growth conditions, endogenous ABA inhibits the ethylene-induced shoot formation by lowering the sensitivity to ethylene.

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Sugar beet breeding: efficiency in gynogenic DHs production

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For breeding purposes handling the procedures which can replace a highly time-consuming and costly inbred lines production is very important. In case of sugar beet, establishment of homozygous lines achieved by traditional way can take 5–8 years. Meanwhile, a well-developed doubled haploid technology can shorten the time needed to produce sugar beet homozygous lines up to 2 years and significantly speed up the whole breeding process.

Early attempts to obtain homozygous DH-lines in sugar beet involved both androgenesis and gynogenesis. Some trials were performed to explore the most common techniques of plant haploidization. Androgenesis is not routinely used for sugar beet plants. Sporophytic development from the unfertilized egg cell proved to be an alternative method. The first results of *in vitro* gynogenesis in sugar beet were announced nearly 30 years ago by Bossoutrot and Hosemans (1985) and Gośka (1985). The basic protocol derived since that time has been successfully deployed to KHBC laboratory in Straszków and with some modifications is being applied up to this day.

A serious problem for the overall success of haploid plant production from unfertilized ovules of sugar beet is genotypic variation. To study importance of that factor plants obtained in 2012 were separated into three groups: N-type (Normal), Z-type (Zucker) and ZN-type (Normal/Zuker). The percentage of plants obtained from ovules derived from all analyzed types varied between 4.5% to 6.2%. The highest ability of plant regeneration was observed in ZN-type. Flow cytometric and isozymeanalysis reveals the participation of 5.3% of spontaneous DHs. After colchicine treatment number of DH plants ranged between 29% in type Z up to 36% in type N. Produced doubled haploid plants have been already effectively incorporated into our breeding programs.

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