POSTERS

Micropropagation of *Plantago coronopus* L.

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Buckhorn plantain *Plantago coronopus* L. (Plantaginaceae) is a vanishing species in Poland, belonging to the "critically endangered" category in Polish Red Data Book of Plants, and subject to an active protection (Ministry of Environment's Directive, 9 July 2004). To our knowledge there is no information in literature about *in vitro* culture of this taxon. However, some other species in the genus *Plantago* were studied in *in vitro* culture (Andrzejewska-Golec and Makowczyńska, 2008; Budzianowska et al., 2004; Makowczyńska and Andrzejewska-Golec, 2003; 2009).

We propagated *Plantago coronopus* from different explants of 4-week-old seedlings. Murashige and Skoog basal medium, supplemented with indole-3-acetic acid with various concentrations of cytokinin 6-benzyladenine (0.2; 1; 2 or 5 mg/dm^3) was used. After 6 weeks of culture, micropropagation rates were calculated. The plants obtained as a result of micropropagation were not phenotypically changed.

Our study proved that *Plantago coronopus* was amenable to propagation *in vitro*. This method may have significance for protection of this plantain.

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In vitro cultures of embryonic axes of *Lupinus mutabilis* and *L. albus*

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Lupine plants are difficult to cultivate under *in vitro* conditions. The aim of this study was to evaluate the regeneration ability of embryonic axes of two *L. mutabilis* genotypes (KW 7/01/05 and KW 10/01/05) and one *L. albus* cultivar Butan. Seeds were surface sterilized in a commercial bleach (Domestos). Having removed the seed coat, embryonic axes with cotyledons were additionally disinfected in Citrosept solution. Explants were divided into two groups: embryonic axes with cotyledon fragments and axes without cotyledons. All explants were placed on B5 medium supplemented with different types and combinations of growth regulators. The following concentrations and types of growth regulators were used: 3.0 mg/L BA, 3.0 mg/L 2iP, 3.0 mg/L BA + 0.2 mg/L NAA and 3.0 mg/L 2iP + 0.2 mg/L IAA.

After six weeks of culture, length of roots, hypocotyls and stem height of regenerated plantlets were measured. The number of leaves was also counted. The highest mean stem length (5.01 cm) was observed in Butan on B5 medium containing 3.0 mg/L 2iP + 0.2 mg/L IAA. The largest leaf number was found in KW 10/01/05 (6.25) on B5 medium containing 3.0 mg/L BA + 0.2 mg/L NAA. The above results were recorded on explants with cotyledon fragments.

The effect of the exogenous phenolic compound, caffeic acid on organogenesis in *Galanthus elwesii* Hook. cultured *in vitro*

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Phenolic compounds belong to secondary metabolites whose function in plants is unknown. Caffeic acid is a simple phenolic compound, a phenylpropene derivative, with two -OH groups, that inhibits IAA oxidase and, therefore, is considered to be an auxin cofactor. The aim of the present experiments was to determine the effect of exogenous caffeic acid on organogenesis (formation of roots, bulbs, leaves) in giant snowdrop in *in vitro* cultures. Bulb explants obtained from plants multiplied by adventitious buds were placed on MS (1962) media containing 3% sucrose, growth regulators BA and IAA (0.1–1.0 μM) and caffeic acid at 0, 100 and 500 mg/dm^3. The cultures were maintained in the dark at 23/25°C. Observation of the regeneration process revealed that exogenous caffeic acid at the optimal concentration (500 mg/dm^3) completely inhibited shoot regeneration and leaf and roof formation by significantly decreasing the number of the organs formed and their browning. It also reduced bulb fresh mass growth. A five-fold lower dose of exogenous caffeic acid in the medium inhibited only adventitious root elongation and caused explant browning. Analysis of the content of phenolic compounds in tissues and organs of giant snowdrop showed that the contents were diverse and depended on developmental stage and reproduction conditions. It was demonstrated that exogenous caffeic acid reduced the total content of phenolic compounds in regenerating plants from *in vitro* cultures, independently of cytokinin and auxin concentration in the medium. The highest content of phenolic compounds was observed in the control bulbs originated from plants growing in the open air, in basal plate (1723 μg/g fresh weight). Bulbs formed *in vitro* on control media were characterized by a similar content of phenolic compounds in inner and middle scales (247 μg/g fresh weight) though it was lower than in bulbs grown in the open air. The level of phenolic compounds was significantly lower in bulbs formed in the presence of 100 and 500 mg/dm^3 caffeic acid (80 and 30 μg/g fresh weight, respectively). It is suggested that the content of phenolic compounds can be a biological quality marker in cultures of plants reproduced by *in vitro* techniques.

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Changes in terpenoid level after *Fusarium* treatment in flax

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Terpenoids are a group of both primary and secondary plant metabolites playing important roles in plant growth and survival (gibberellic acid, chlorophyll, carotenoids). They are involved in responses to stress situations such as pathogen infection (tocopherol). But these compounds are also very important in the human diet because they can protect against many civilization-related diseases, such as cancer (lycopen, squalene). The understanding of synthesis pathway is important for genetic manipulation of levels of terpenoids in plants. Even though the pathway is well known in model plants, in *Linum* those genes have not been identified. Using degenerated primers we found 16 sequences of major terpenoid synthesis genes in flax (*Linum usitatissimum* var. Nike). We used those sequences to verify the level of expression of terpenoid genes after a *Fusarium* treatment. This knowledge will be used for creation of plants with terpenoid levels beneficial for human health.

Expression of HBV core antigen (HBcAg) in transgenic tobacco and lettuce for purposes of therapeutic vaccine against hepatitis B

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Hepatitis B is the most common infectious liver disease in the world, with it's prevalence at 2 billion people infected worldwide and 400 million chronically infected. Hepatitis B is the main factor inducing hepatocellular carcinoma and one of the most important factors causing the cirrhosis. For the reasons of inefficiency and difficulty of today's expensive anti-viral therapies using INF-α or antiviral drugs such as Lamivudin, Adefovir, etc., many scientists work on new therapies against Hepatitis B Virus (HBV), such as therapeutic vaccines, consisting of HBcAg (Hepatitis B core Antigen).

The aim of the study was to analyze the expression of HBcAg in transgenic plants of tobacco and lettuce for purposes of defining appropriate conditions for efficient expression and selecting plants with high HBcAg level for further work on therapeutic vaccine for chronic HBV carriers, and also finding the best method of isolation of HBcAg from plant tissue.

The transgenic plants revealed 1–5 copies of T-DNA integrated into genomic DNA, which were stably inherited. Expression of native HBcAg, probably assembled into highly immunogenic Capsid-Like Particles (CLPs), was confirmed by ELISA assay and western-blot. Observed level of HBcAg – up to 400 μg/g FW, displayed a relative stability during plant development and in sequential generations. Selected plants can be used for further research on HBcAg assembly into CLPs in plant cells and animal immunization studies with plant-associated HBcAg. In the outlook, the plants obtained by us can be exploited as an initial material to produce therapeutic vaccine for chronic HBV carriers, orally administrated when produced in lettuce, and injective, when produced in tobacco.

Phenolic compounds in shoot and callus cultures of *Plantago ovata* Forssk.

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Plantago ovata Forssk. (*Plantaginaceae*) is native to flora of S.E. Spain, N. Africa, S.W. Asia. It is cultivated for seeds and seed husks, which are commercially important laxative remedies due to the high content of mucilage. The species is propagated from seeds and does not have the ability for vegetative propagation.

In vitro regeneration of *P. ovata* through induction of axillary shoots, indirect organogenesis and somatic embryogenesis have been described (Fons et al., 2008). So far, only seeds of *P. ovata* have been studied with respect to phenolic metabolites demonstrating the presence of two flavonoids and two phenethyl glycosides (Nishibe et al., 2001).

The aim of the present work was to establish shoot and callus cultures of *P. ovata* and to investigate their phenolic compound pattern.

Micropropagation from shoot-tips of seedlings was carried out on MS medium supplemented with NAA/KIN or IAA/KIN and resulted in only 2–3 axillary shoots per explant. No shoots developed through direct organogenesis on leaf and root fragments, in contrast to *Plantago lanceolata*. Callus initiation was readily achieved on roots, leaves and hypocotyls on MS medium supplemented with 2,4-D/Kin or NAA/BAP, however, growth of callus cultures was poor. Therefore, a reduction of NH_4NO_3 content in the medium was tested, which appeared useful in the case of callus cultures of *P. lanceolata* (Budzianowska et al., 2004).

Extracts from shoot and callus cultures and from seeds of *P.ovata* were prepared and analysed by 1D-TLC and 2D-TLC. The shoots and callus exhibited the presence of phenethyl glycosides (similarly to seeds), while shoots additionally contained flavonoids different from those of the seeds and leaves of *P. lanceolata* (Budzianowski and Budzianowska, 2008).

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The use of pollen tube *in vitro* cultures for determination of nuclei positions in angiosperm pollen tubes

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Appropriate sperm cell delivery to the embryo sac is one of the crucial step of fertilization in flowering plants, and it determines reproductive success of a plant. Mechanisms of sperm cell transport during pollen tube growth are not fully understood. The concept of male germ unit (MGU) is a very attractive hypothesis on sperm cell transport within the pollen tube (Mogensen, 1992). However, MGU has been described for sperm cells or generative cells and the vegetative nucleus only for ten or so angiosperms, and in some cases the spatial relationship between sperms or generative cell and the vegetative nucleus were temporal only and found either in pollen grain or pollen tube. Here we claimed to determine the position of the vegetative and generative nuclei in the pollen tubes of lily, hyacinth, and common snowdrop. Pollen grains germinated on Brewbaker-Kwack media optimized for each species and pollen tubes were growing *in vitro* for 24 or 48 hours. The vegetative and generative nuclei were visualized by various dyes, and the ethidium bromide staining of fixed pollen tubes was chosen for major analyses. Nuclear position related to the pollen tube tip showed great variability in three species studied. No general model on vegetative nucleus guidance could be established for each species, and only in one culture variant per species the vegetative nucleus was ahead of the generative nucleus in the pollen tube (statistically significant). Also the distance between both nuclei in pollen tubes was variable within each species. This suggests that MGU could only be temporal in the pollen tubes growing *in vitro* or culture conditions affected the MGU formation in many pollen tubes. Based on nuclear position analyses, the approximate region of nuclei appearance was approx. 50% or 35% length of 48-hour-old pollen tubes. *In vitro* cultures of pollen tubes can be used for MGU investigation in plants, however, the results should be compared to the *in planta* situation.

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Molecular analysis of interspecific *Allium cepa* × *A. roylei* hybrids

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To achieve introgression of resistance to downy mildew (*Peronospora destructor*) into the bulb onion, interspecific crosses between *Allium cepa* and *Allium roylei* were conducted. F_1 *A. cepa* × *A. roylei* plants were obtained via embryo rescue technique (Chuda and Adamus, 2005). Verification of the hybrid character of the F_1 regenerants was based on three different molecular markers. SIR-F/SIR-R and ACS-F/ACS-R primers were based on AY753557 and AY585678 onion cDNA clones, respectively, while A-F/A-R primers were designed on the basis on *Allium cepa* alliinase gene according to van Heusden et al. (2000). Analysis of the electrophoretic profiles of the examined accessions revealed that 97.6% of the tested F_1 plants were interspecific *Allium cepa* × *Allium roylei* hybrids.

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Two year survey of health status of *Salix* × *Populus* hybrids in the Warmia region

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Recently willows have become increasingly popular as crop plants used for renewable energy (providing an alternative to fossil fuels). However, they require abundant water supply during the whole growth period. To avoid this problem, hybrids between willows and poplars have been produced at the Department of Botany, Faculty of Biology of Adam Mickiewicz University in Poznań.

Leaf rust is the most common and serious disease of both willows and poplars. The disease is caused by several species of the genus *Melampsora*. Willows and poplars are infected by different ranges of *Melampsora* species and also by various special forms and pathotypes, each capable of infecting a certain range of species and genotypes. The aim of this study was to investigate the resistance of hybrids obtained from the crossings between *Salix viminalis* and *Populus tremula*.

Field experiment was located in Bałdy, near Olsztyn in Warmia region (north-east Poland). Observations were conducted over a two year period (2008–2009). Each year, the first observation took place in July/August and the second in September/October. All studied hybrids (six genotypes) were infested with willow rust, similarly to parental plants. Identification of *Melampsora* spp. isolates obtained from the infected plants is under way using pathogenicity tests and molecular tools.

Albino regenerants from isolated microspores of wheat

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Significant progress has been observed in recent years in the development of culture techniques of isolated cereal microspores. One can expect a considerable improvement in the efficiency of haploid plant regeneration due to the highly embryogenic nature of microspores and a short path of the culture.

The purposes of the experiments were: to induce the androgenic development of microspores isolated using a blender, to produce embryos and to regenerate plants.

Microspores of wheat spring variety Pitic 62 were isolated using the method described by Zheng (Zheng et al., 2001, 2002) and then grown in the dark at 26°C. Isolated ovaries of spring wheat variety Orofen were used as a nurse culture. Microspore divisions and further embryo development were observed. As a result of the experiment, five albino plants have been regenerated so far.

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Optimization of the regeneration method of *Miscanthus* × *giganteus* in *in vitro* culture

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Miscanthus × *giganteus* Greef & Deuter is a triploid hybrid species belonging to the family *Poaceae*. This species is characterized by a fast biomass increase, because its shoots can growth up to 3 meters in height in the second year of cultivation. Therefore miscanthus can be used as a source of biomass for the energy industry, in phytoremediation and also in the paper industry. Under our climatic conditions *Miscanthus x giganteus* does not set seeds and the best methods for its propagation are multiple division of the rhizome and propagation *in vitro*.

The objective of the present experiment was to develop a method of obtaining regeneration of *Miscanthus* × *giganteus* plants under *in vitro* conditions. Inflorescence explants (0.5–2.5 cm in length), internodes, fragments of leaves and rhizomes were the research material. They were sterilized using 10% hydrogen peroxide. Three basic media were used: MG1 (LS medium containing 5 mg l^{-1} 2,4 D and 0.1 mg l^{-1} BAP), MG2 (LS medium with 2.5 mg l^{-1} 2,4 D and 0.5 mg l^{-1} BAP) and MG3 (WPM medium enriched with 2.5 mg l^{-1} 2,4 D and 0.5 mg l^{-1} BAP). The experiment was conducted both in the light and in the dark. Regenerated plants were transferred to LS medium supplemented with 0.2 mg l^{-1} IAA and 0.2 mg l^{-1} NAA.

Callus was not observed on explants maintained in the dark or on fragments of leaves and rhizomes kept in the light. The most suitable explants for callus induction and plant regeneration were fragments of inflorescences. Less abundant callus tissue was observed on internodes, from which only a few plants regenerated. On the remaining explants neither callus formation nor organogenesis were noticed. The largest numbers of regenerated plants were obtained on MG1 and MG3 media. They were rooted and acclimated to greenhouse conditions.

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Changes in sulfur amino acid contents in flax caused by overexpression of yeast Met25 gene lead to an increase in antioxidant capacity and *Fusarium* resistance

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Cysteine synthesis in plants is the crucial point in sulfate assimilation. It introduces inorganic sulfide into the carbon skeleton. In plants and bacteria, this reaction is catalyzed by O-acetyloserine sulfhydrylase (OASTL) with free sulfide and O-acetyloserine (OAS) as the substrates. In the present study we use *Sacharomyces cerevisiae* gene Met25 encoding O-acetyloseine-O-acetylohomoserine-sulphydrylase, yeasts analogue of plant OASTL protein in flax.

The yeast enzyme utilizes both O-acetylhomoserine and, as discovered in *in vitro* experiments, O-acetylserine as substrates, while the plant enzyme uses only O-acetylserine as its substrate in cysteine biosynthesis (Matityahu et al., 2006). An important difference between the plant OASTL and its yeast analogue is that the plant enzyme has an additional activity: it is able to degrade cysteine to ammonia, H2S and pyruvate (Riemenschneider et al., 2005). The yeast enzyme does not seem to be able to do this (Matityahu et al., 2006; Harms et al., 2000; Sirko et al., 2004), thus we expect to have an increased production of cysteine and it's derivatives in flax. Overexpressing the yeast Met25 in flax resulted in a significant increase in cysteine and methionine biosynthesis. This overproduction of sulfur amino acids also increases the synthesis of glutathione, a tripeptide containing cysteine. The increase in glutathione content in the transgenic plants increases their antioxidative potential. We also observed improvements in plants' protection against *Fusarium* infection.

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Phytosulfokine stimulates development of sugar beet (*Beta vulgaris* L.) mesophyll protoplasts

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Phytosulfokine (PSK) is a peptide plant growth factor that was originally isolated from a conditioned medium derived from asparagus mesophyll cell culture (Matsubayashi and Sakagami, 1996). In some plant species PSK was found to stimulate cell proliferation (Matsubayashi, 2003). On the other hand, so called "recalcitrant species" are known, that are difficult either to regenerate using tissue culture methods and/or to transform with foreign DNA. Sugar beet is such a recalcitrant crop, particularly with respect to protoplast-based techniques. The aim of this work was to determine phytosulfokine influence on the division activity of sugar beet protoplasts. We tested 3 clones of sugar beet male-sterile line growing in 2 different culture media (CPP with 8 mg/l putrescine and 2.12 mg/l propyl gallate; CPP with 8 mg/l putrescine, 2.12 mg/l propyl gallate, Kao and Michayluk vitamins and 2 % coconut water) with 2 concentrations of phytosulfokine (10^{-7} and 10^{-8} M). Viability of protoplasts was determined by fluorescein diacetate (FDA) staining on the day of isolation as well as 24 and 48 hours after isolation. Cell proliferation activity was evaluated on 7th, 14th and 21st days of culture. There were no differences in viability of protoplasts between media and PSK concentrations. Cell division activity was dependent on both medium composition and PSK concentration. The highest division frequency was observed in CPP containing 8 mg/l putrescine, 2.12 mg/l propyl gallate and 10^{-7} M PSK. It varied from 13.9 to 29.1% on 21st day of culture whereas in the same medium containing 10^{-8} M PSK, proliferation efficiency reached approximately 6%. In no-PSK control, proliferation efficiency varied between 0.1 and 2.6%. This result indicates that PSK strongly stimulates the division of sugar beet mesophyll protoplasts under cell culture conditions.

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Gentiana cachemirica Decne in *in vitro* culture

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Most species from genus *Gentiana* described so far are protected by law in Poland. They are valued because of important secondary metabolites and attractive flowers. They are also well known for possessing a high morphogenetic potential *in vitro*. The above characteristics have made this genus very attractive for breeding. Micropropagation through somatic embryogenesis is an alternative pathway to the conventional breeding methods for the propagation of gentians. Up to now it has been described for only a few species of the genus.

In the present study, extensive experiments with *G. cachemirica*, a new *in vitro Gentiana* species are reported. Callus tissue derived from seedling explants was used as a source for the cell suspension. The morphological potential of the culture expressed by its embryogenic capacity was assessed by counting the number of somatic embryos on agar regeneration medium and by their ability to become converted into plants.

Long-term culture brings about the risk of somaclonal variation. Two techniques were evaluated to perform cytological analysis of the regenerants. Chromosome counting was carried out on well spread metaphase plates. DNA content was determined using a flow cytometer.

Rooting *in vitro* and acclimation to the greenhouse of herbaceous peony plantlets

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The influence of sucrose concentration (10 and 30 g l^{-1}), low level of nitrogen salts (1/8 KNO_3, 1/8 NH_3NO_4), auxins (1 mg l^{-1} IBA + 1 mg l^{-1} IAA + 0.01 mg l^{-1} NAA) and temperature (15°C, 20°C) on the rooting *in vitro* and acclimation to the greenhouse conditions of two cultivars ('Jadwiga' and 'Profesor Wójcicki') of *Paeonia lactiflora* was investigated. The level of endogenous carbohydrates in the peony shoots and roots during the rooting and acclimation phases were analysed.

Rooting and acclimation success rates were higher for cultivar 'Profesor Wójcicki' than for cultivar 'Jadwiga'. Also more roots were produced by shoots of cultivar 'Profesor Wójcicki'. The presence of auxins in the medium and the higher concentration of sucrose (30 g l^{-1}) stimulated a greater number of roots/shoot. On the other hand, a higher rooting rate was found on the auxin-free medium in the presence of high level of sucrose. The shoots of 'Profesor Wojcicki' rooted best when cultured at 20°C, but shoots of 'Jadwiga' rooted best at the lower temperature (15°C). During the rooting stage, the major sugars detected in the peony microplants were sucrose, glucose and fructose. After four weeks of acclimation, plantlets accumulated starch and showed a strong inhibition of shoot growth, and dormant buds were developed.

Expression pattern of *ABH1*, *CBP20* and *HYL1* genes during somatic embryogenesis in *Arabidopsis*

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Genetic dissection of somatic embryogenesis (SE) is of great interest since the process is commonly applied for plant regeneration in transgenesis and micropropagation. Identification of genes involved in embryogenic transition of somatic cells is of interest for the improvement of the existing protocols on plant regeneration, including those for recalcitrant plants. *Arabidopsis* mutants impaired in SE provide valuable material in the search for SE-specific genes (Gaj et al., 2006). Among such SE-defective genotypes, the ABA-response (*abh1*, *cbp20* and *hyl1*) and auxin-response (*axr4-1*) mutants were found, implicating the involvement of the mutated genes in the process of somatic embryo formation. The *ABH1* and *CBP20* genes encode the subunits of the cap binding complex (CBC) involved in mRNA metabolism while the protein product of *HYL1* gene takes part in miRNA biogenesis.

To provide insight into the role of the *ABH1, CBP20* and *HYL1* genes in SE, Real-time qPCR analysis was applied to monitor the expression level of these genes at various time points of embryogenic cultures derived from the highly embryogenic (Col-0) and the SE defective mutant (*axr4-1* and *cbp20*) genotypes. The level of *ABH1, CBP20* and *HYL1* activity was evaluated between 0 and 30d of embryogenic cultures. It was observed that the genes were expressed at the same level during the whole monitored period of embryogenic cultures, implicating their basic role in cell growth and differentiation, rather than SE specificity. Moreover, the expression pattern of the *ABH1, CBP20* and *HYL1* genes found in cultures derived from the *axr4-1* and *cbp20* mutants were found to be similar to control culture (Col-0), implicating that the defects in embryogenic response observed in these mutants did not result from changes in expression of the analysed genes.

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Obtaining plants from gametic embryos of red beet-preliminary investigations

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Red beet ranks second (after carrots) in statistics of root vegetable consumption in Poland. Red beet hybrids characterised by high yields and size uniformity are becoming more and more important. Using traditional methods to obtain homozygotic parental forms to create hybrids is difficult and time-consuming. Making use of biotechnological methods (androgenesis and gynogenesis *in vitro*) could shorten the time needed to obtain F1 cultivars.

In experiments conducted at the Research Institute of Vegetable Crops in Skierniewice success was achieved in obtaining the first embryos by gynogenesis and calluses in anther cultures of red beet cv. 'Opolski'. Attempts at plant regeneration from the obtained structures were made on the media used by Barański (1996). Multiplication of shoots was obtained on MS regeneration medium (Muraschige and Skoog, 1962) containing cytokinin and a lower level of sucrose. The next step involved rooting of the obtained rosettes. They were transferred onto the same medium supplemented with IAA, and after about 6 weeks rooted on a medium containing 1/2 of the MS macroelements and IBA.

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Yacon – *in vitro* propagation trials

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The yacon (*Polymnia sonchifolia*) is a native Andean plant, cultivated mainly for its tubers, which have a particularly high fructan content. The whole plant is edible. The leaves are used in folk medicine. Extracts from yacon leaves demonstrate antioxidant activity. The plant can be cultivated in the European climate. Natural methods of propagation through tubers or cuttings are not very efficient because of the transmission of bacterial and viral pathogens (Zardini, 1990). Micropropagation *in vitro* is the best alternative for the propagation of this species (Mogor et al., 2003).

The aim of our work was to carry out trials on *in vitro* propagation of yacon: multiplication of shoots, their rooting and plant weaning.

In our preliminary experiments, we obtained the best shoot multiplication on MS medium (Muraschige and Skoog, 1962) supplemented with BAP and 3% sucrose. A medium containing 1/2 MS macroelements, IBA and 3% sucrose proved to be the best for rooting of shoots. Up to 90% of rooted plants became adapted to *ex vitro* conditions.

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The effect of cefotaxime and carbenicillin on carrot protoplast cultures

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In vitro culture techniques, especially protoplast cultures, are expensive, time consuming, and laborious. Therefore exogenous (accidental) and endogenous microbial contamination is one of the most critical points in the research relying on *in vitro* cultures. Application of antibiotics may help to solve this problem. The ideal antibiotic should efficiently affect a broad spectrum of bacteria and be non-toxic to plant cells.

In the present study we examined the toxicity of two antibiotics, i.e. cefotaxime and carbenicillin, to carrot protoplasts. The antibiotics belong to the betalactam and cephalosporin group, respectively. Protoplasts were isolated from the mesophyll tissue of cv. 'Dolanka' according to Dirks et al. (1996) protocol with modifications and cultured at five concentrations (0.4, 0.8, 1.2, 1.6, 2.0 mg ml^{-1}) of cefotaxime or carbenicillin. Protoplast viability, their mitotic activity and regeneration capacity were analyzed to characterize the toxic effect of the antibiotics on carrot cells. Reduction of protoplast viability was observed after 24 hours of culture for carbenicillin and after 48 hours for cefotaxime. The inhibitory effect of antibiotics on cell divisions was visible after 10 days of the culture. The concentrations of cefotaxime and carbenicillin equal or higher than 1.2 mg ml^{-1} in culture medium decreased the division frequency of protoplasts. However, carbenicillin showed a more dramatic negative effect on carrot cells, lowering their mitotic activity two- to ten-fold, as compared to cefotaxime. Despite the different reactions of cells at the beginning of the culture, callus tissue and somatic embryos were obtained later, both in the system with cefotaxime and carbenicillin. Owing to the fact that a higher level of plant regeneration was observed in the presence of cefotaxime, this antibiotic should be recommended to protect carrot cell or tissue cultures from bacterial contamination.

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Cytological and molecular variation of carrot regenerants obtained through *in vitro* selection of carrot protoplasts against *Alternaria radicina*

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A population of carrot regenerants was produced from protoplasts isolated from three accessions (cvs. 'Dolanka', 'Amsterdamska', and a breeding line 9304B). The protoplasts were cultured in the presence of different concentrations of crude culture filtrate from *Alternaria radicina*, the causal agent of black rot.

We examined the ploidy level of regenerants by flow cytometry and chromosome counting, the level of somaclonal variation by RAPD-PCR, and pollen viability. Cytometric analysis was performed on ca. 500 plants regenerated after *in vitro* selection and on ca. 260 control plants. 18% regenerants obtained under selection stress and 9% control plants were tetraploids, the rest of regenerants being diploids. Chromosomes were counted for ca. 110 plants, of which 72 represented plants regenerated in the presence of the *A. radicina* culture filtrate. The results of chromosome counting were consistent with the cytometric data, indicating that no aneuploids were produced in the course of protoplast cultures. Also, no major differences were generally observed on the RAPD-PCR profiles. Only sporadically additional amplicons were present in the profiles of individual regenerants. Pollen viability was the most variable trait, both for the regenerants from *in vitro* selection and for the control plants. Individuals could be divided into three groups of pollen viability: (1) up to 20%, (2) from 40 to 70%, and (3) from 80 to 90%. The results suggest that the level of variability induced in the process of *in vitro* selection was only slightly higher than that observed for control plants.

Micropropagation of *Scutellaria altissima* L.

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Genus *Scutellaria* includes over 300 species of perennial plants which show multiple pharmacological activities. Their main secondary metabolites are flavonoids (baicalin, wogonin and scutelarein derivatives) and ditepenoids (clerodane and neoclerodane derivatives). So far only one species *Scutellaria baicalensis*, listed in Chinese and Japanese Pharmacopeias, has been investigated using *in vitro* methods, but other *Scutellaria* species, such as *Scutellaria altissima* could also become sources of compounds with pharmacological properties.

The aim of the present study was to establish optimal conditions for micropropagation of *Scutellaria altissima*. Shoot tips and stem fragments with single node from five-week-old shoots grown in *in vitro* culture were used as explants. The influence of various cytokinins (6-benzylaminopurine-BAP, zeatin, thidiazuron-TDZ, kinetin,

6-dimethylallylaminopurine-2iP) on shoot proliferation was investigated. The best results were obtained when BAP and zeatin at a high concentration were used. In our work we obtained also the valuable shoots from callus tissue. The callus tissue was obtained from sterile seedlings (fragment of hypocotyls, roots and cotyledons). This investigation is concerned with selection of the most suitable medium for organogenic callus formation. The best conditions for differentiated callus tissue development were on MS medium with a low concentration of thidiazuron. Higher TDZ concentration caused hyperhydricity syndrome and shoot deformation.

Induction and proliferation of embryogenic tissues of Serbian spruce (*Picea omorika*) and their maintenance in liquid nitrogen

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Picea omorika is an endemic species threatened with extinction in the wild. Besides, it is a valuable species for nursery production. Hence it is necessary to protect its genetic resources *ex situ*.

Somatic embryogenesis is a very beneficial micropropagation method, because of the potentially high regeneration rate and possibilities to store the embryogenic tissue and somatic embryos in liquid nitrogen (LN). The aim of this study was to induce embryogenic culture of *P. omorika* and develop a protocol for cryopreservation of embryogenic tissues.

Embryogenic tissues of Serbian spruce were induced from mature zygotic embryos. They were dissected from seeds of *P. omorika* originating from the Kórnik Arboretum (Poland). Explants were cultured in two media: BM-3 (Gupta and Durzan, 1986) and 1/2 LM (Litvay et al., 1985), supplemented with 9 μM auxin (2,4-D, NAA or Picloram) and 4.5 μM cytokinin (BA). Embryogenic tissues were proliferated in 1/2 LM medium with the addition of 9 μM 2,4-D or NAA or Picloram and 4.5 μM BA.

Embryogenic tissue of *P. omorika* was cryopreserved using two methods (E-LN and E+LN). In the first method (E-LN), microfuge tubes containing only embryogenic tissue were immersed in LN for 24 h and then rapidly thawed in warm water (42°C) for 2 min. In the second method (E+LN), microfuge tubes containing LN and embryogenic tissue were immersed in LN for 24 h and then embryogenic tissues were taken out of the tubes and rapidly thawed in a warm 1.2 M sucrose solution (42°C) for 2 min.

Induction rate of embryogenic tissue of *P. omorika* was the highest (23.75%) on 1/2 LM medium supplemented with 9 μM Picloram and 4.5 μM BA. The growth of the embryogenic tissue *in vitro* was best on the same medium. Cryopreservation in microfuge tubes containing only embryogenic tissue (thawed in water inside the tubes) was more appropriate than in microtubes containing LN and embryogenic tissue (thawed directly in sucrose solution).

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Identification of European and Japanese larches and their hybrids based on genetic markers

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Larix decidua Mill. is a native species, and *Larix kaempferi* Sarg. is the most common of foreign larch species. Morphological differences between pure *Larix decidua* and *Larix kaempferi* and their interspecific hybrid (*Larix x eurolepis*) are difficult to assess. Hybrids between European and Japanese larches combine the properties of both parental species (Scheepers, 2000).

The interspecific hybrids combine traits of both parental species and contribute to their morphological variability. Molecular markers are needed to identify larch hybrids and their parental species.

The identification strategy involved a combination of maternally inherited markers from the mitochondrial genome (mtDNA) and paternally inherited markers from the chloroplast genome (cpDNA). Hybrids were identified by the presence of a mitochondrial sequence inherited from one parental species and a chloroplast sequence inherited from the other parental species.

For the identification of diagnostic markers 96 larch individuals were sampled. Sequences of cpDNA and mtDNA were amplified by polymerase chain reaction using specific primers (Acheré, 2004). The chloroplast (*ll*-Taq I) and mitochondrial (*f13*) diagnostic markers were used to identify pure species of larches and their hybrids.

Marker *ll*-Taq I revealed an interspecific polymorphism. This marker discriminated between all individuals of *L. decidua* and *L. kaempferi*. All *L. kaempferi* individuals possessed a specific 601 bp fragment. This polymorphism is due to a single nucleotide substitution in the restriction site: TCGA recognised by Taq I in *L. decidua* and TCAA in *L. kaempferi*.

Marker *f13* of approximately 1,300 bp, was amplified only in *L. kaempferi.* The test based on the *f13* and *ll*-TaqI markers is easy to perform and can constitute a very effective instrument for identification of European and Japanese larches and their hybrids.

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In vitro cultures of *Drosera binata* as a source of compounds with antimicrobial activity

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Extracts of plants *Drosera binata* (Droseraceae) is of potential medicinal use due to the content of naphthoquinones and flavonoids. The aim of this work was to establish the most efficient method for the production of secondary metabolites in the tissue of *D. binata* grown on 1/2 MS medium, 2% sucrose, pH 5.6 in order to study their antibacterial activity and ability to inhibit bacterial quorum-sensing (QS). For increasing the accumulation of secondary metabolites a series of elicitors has been used: autoclaved overnight suspension of *Agrobacterium rhizogenes*, jasmonic acid (JA), additional nitrogen salts and nitrogen deprivation in culture medium. Quantitative and qualitative determination of naphthoquinones and flavonoids in chloroform ($CHCl_3$) and methanol extracts from *D. binata* was performed using NP – HPLC/UV-DAD. JA occurred to be the most efficient elicitor, which increased the accumulation of plumbagin in D. binata tissue about 1.4 fold in comparison to control. Bactericidal activity of extracts was tested against 4 species of human pathogens: *Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae* and *Staphylococcus aureus*. To measure antibacterial activity minimal bactericidal concentrations (MBC) of extracts were determined. *S. aureus* strains occurred to be the most sensitive among human pathogens tested (MBC for $CHCl_3$ extract was 70 μg of dry weight of extract/ml). Indicator strains of *Agrobacterium tumefaciens* and *E. coli* expressing β-galactosidase or lux genes in the presence of *N*-acyl homserine lactones (AHLs) were used to study QS inhibition. It was shown that extract from *D. binata* significantly inhibited AHL-induced response of bioassay strains with IC_{50} values ranging from 14 to 27 μg/ml depending on the indicator strain used.

Acknowledgements: This research was supported by Foundation for Polish Science & by the Grant B051–5–0293–9.

Genetic diversity within population of the endangered species *Cypripedium calceolus*

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Cypripedium calceolus (L.) (Lady's Slipper) is a 15–60 cm tall terrestrial herb. It grows best in partially shady areas under hazel, oak, conifer or ash. Flowering period depends on geographical location; in Northern Poland, it occurs between May and July. According to the Polish Red List of Threatened Plants this species belongs to the vulnerable group. The aim of the study was to check the suitability of RAPD-PCR to determine the genetic diversity of Lady's Slipper population growing in the nature reserve 'Ostrzycki las'. The results should provide information on Lady's Slipper's manner of reproduction. Fresh leaves of the Lady`s Slipper taxa were collected from the hereby listed sites in the nature reserve and deep (–80°C) frozen. Four DNA isolation methods were tested: PVP isolation described in Pirtilla et al. (2001), CTAB isolation described in Bekesiova et al. (1999), DNA isolation by Fermentas Genomic DNA Purification Kit and A&A Biotechnology Genomic mini AX Plant Kit. RAPD-PCR reaction was carried out in 25 µl volume containing reaction buffer, $MgCl_2$, dNTP`s, primers [10 pmol/µl], all prepared as 'master mix', then aliquoted into reaction tubes. Template DNA (5 ng) was added to the reaction mixture after first adding 1 unit of *Taq* DNA polymerase (all from Fermentas). Amplification products were separated electrophoretically on a 2% agarose gel and afterwards stained with ethidium bromide. After examining yield and quality of the DNA, the method of DNA isolation described by Bekesiova et al. (1999) with slight modifications was chosen. Approximately 0.05–0.15 g of leaf material were used for DNA extraction experiment. 28 arbitrary 10-mer primers were tested, and seven that gave polymorphic and scorable amplified products, ranging between 100–1200 bp, were used for RAPD profiling. Preliminary experiments proved that RAPD-PCR can be used to determine genetic diversity of Lady`s Slipper.

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Morphological evaluation of interspecific F1 (*Allium galanthum* × *A. cepa*) hybrids

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The interspecific F1 hybrids (*Allium galanthum* × *A. cepa*) were obtained by natural seed forming process and via embryo rescue technique. For hybrids and parental plants (*A.galanthum* and *A.cepa*) evaluation of the morphological traits in vegetative and generative phases was conducted. In the vegetative stage the number of bulbs, the number, length and diameter of leaves, length and diameter of the stem were evaluated. In the generative phase the number and length of the flower stems, and the length and diameter of the inflorescence were evaluated. In tested populations, the majority of the investigated features of vegetative as well as generative organs showed an intermediate (V% = 21–50%) or lower variability.

The hybrids were similar to the maternal form with regard to only one feature (number of bulbils), while leaf and stem characters were similar to the paternal form. The remaining morphological features of interspecific hybrids were intermediate in relation to maternal and paternal plants.

Characteristic of the cell suspension culture of *Eryngium planum* L.

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Eryngium planum L. (*Apiaceae*) is used in folk medicine in Europe. The presence of active constituents found in majority of Sea Holly species, including triterpene saponins, flavonoids, phenolic acids, essential oils and coumarins, determines their multidirectional pharmacological activity: diuretic, expectorant and antimycotic (Duke J.A.et al.,2002).

Numerous studies have been carried out using plant cell suspension cultures as potential sources of valuable constituents. The main problem is that the desired compound usually occurs at a high concentration during a short period of culture growth. For that reason, the shake-flask suspensions have been analyzed in terms of biomass concentration suitable for elicitor addition over the course of a typical growth cycle.

This work reports the initiation, maintenance, measurement of cell culture growth and the influence of MeJA on secondary metabolites in the liquid culture of Flat Sea Holly. Methyl jasmonate (100 μM) was employed to enhance the accumulation of the secondary metabolites – triterpene saponins, flavonoids and phenolic acids.

Liquid suspension cultures of petiole-derived, stabilized callus of *E. planum* were conducted by shaking at 110 rpm in MS medium supplemented with 0.5 mg/l 2,4-D in the darkness. Subculturing was conducted periodically (every 8–10 days) by inoculating 5 ml of old culture into 50 ml of fresh medium to maintain cell vitality. Cell suspension growth was measured by cell fresh and dry weights. In order to quantify growth, a stabilized suspension culture was used. From this suspension aliquots of 5 ml were inoculated into 9 flasks. The experiment was set up in a completely randomized design with 9 treatments. Evaluations were made at 3-day intervals up to the 24^{th} day.

Suspension culture followed a typical growth curve with the complete cycle showing a lag phase, followed by exponential, linear and stationary phases.

The ethanolic extracts of suspension cultures were phytochemically analyzed by TLC for presence of complex triterpene saponins, flavonoids and phenolic acids. The data demonstrated that the addition of MeJA on the 12^{th} day of culture caused an increased secondary metabolite accumulation.

Acknowledgements: This work was supported by the Ministry of Science and Higher Education, Warsaw, Poland (grant no NN 05 065334)

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In vitro androgenic response of some pepper (*Capsicum*) genotypes to different levels of kinetin

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In the reported experiment, the effect of culture media composition on the effectiveness of androgenesis in anther culture of selected pepper (*Capsicum*) breeding lines and hybrids was tested. Anther culture was conducted according to Dumas de Vaulx et al. (1981), with slight modifications concerning the level of kinetin in R_1 medium (0.1, 0.2, 0.3, 0.5 mg·dm^{-3} KIN). The efficiency of gametophytic embryogenesis varied significantly and depended both on the genotype and on the kinetin level tested. In case of annual pepper hybrids, the effectiveness of androgenesis was relatively high and ranged from 2.6% to 15.3%. The highest effectiveness of androgenic embryo development was observed for (ATZ1 CDT) F_1 form, in the medium supplemented with 0.1 mg·dm^{-3} KIN. The effectiveness of anther culture of the lines originated from interspecific hybrid *C. annuum* L. × *C. frutescens* L. was considerably lower and very rarely exceeded 2%. However, increase of kinetin concentration in R_1 medium visibly improved the androgenic response of most of these lines. According to the cytometric analyses, there were haploid, diploid and some mixoploid plants among the androgenic pepper regenerants.

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Influence of the ploidy level on growth and organogenesis of sugar beet (*Beta vulgaris* L.) *in vitro*

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Tissue culture techniques are utilized in breeding of new sugar beet (*Beta vulgaris* L.) cultivars. They allow the production of homozygous clones after diploidization of haploid plants developed from unfertilized ovules. However, after haploidization and treatment with antimitotic agents, material with various ploidy level may develop.

The aim of the present work was to evaluate the influence of the ploidy level on the susceptibility of sugar beet tissues to culture *in vitro* and their ability for shoot development.

Biological material used in this study was derived from 24 clones of haploids obtained from developing unfertilized ovules *in vitro*, which were later treated with colchicine. In consequence, groups of plants of common origin were obtained; each group comprised plants not responding to colchicine (1n) and plants with a multiple number of chromosomes (2n and 4n). These materials were assessed for shoot morphology, necrotic changes during the culture and the ability for shoot development.

Considerable variation in shoot and leaf morphology was observed among the used genotypes. Usually, 4n plants had shortened shoots, hardened petioles and much wider leaf blades in comparison to 1n and 2n plants. Additionally, they produced fewer shoots during micropropagation. Regeneration ability of leaf explants placed on MS medium supplemented with 1 mg/l BAP was assessed after 3 and 6 weeks of the culture. Preliminary observations indicate that the reaction of explants strongly depended on the genotype of donor plant and, additionally, on the ploidy level. Usually, 1n plants exhibited a higher potential for organogenesis than did 2n and 4n plants. However, there was no difference between explants of different ploidy levels in their survival rates.

A study of the influence of humic substances on *in vitro* potato growth and tuberization

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It has long been recognized that humic substances (HS) have many beneficial effects both on the soil and on plant growth. These heterogeneous and complex molecules, ubiquitous in the environment, may produce various morphological, physiological and biochemical effects on higher plants (Chen and Aviad, 1990). The influence of the humic material on plant growth have been investigated with various measurement methods and numerous studies have shown that humic substances not only enhance the root, leaf and shoot growth but also stimulate germination of various crop species (Piccolo et al., 1993). These positive effects are explained by the direct interaction of HS with the physiological and metabolic processes (Nardi et al., 2002). When added to the soil or medium, the HS increase nutrient uptake and cell permeability and they modify the mechanisms involved in plant growth stimulation.

We examined the influence of three different HS concentrations (1%, 2% and 3%) on growth of potato plants. These substances were added to the MS medium on which single node cuttings of potato plants were grown in order to observe what influence these substances might exert on the length of the shoots and the number of leaves and roots as well as on the tuberization process. When added to the medium, the HS stopped the growth of the shoots and reduced the number of the leaves and roots in comparison to the control sample. However, the tuberization process began earlier in case of the plants grown on the medium with the HA. This paper discusses the influence of HS on the above mentioned processes.

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Genes involved in flax pathogenesis

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Fusarium is the most common flax pathogen causing serious plant diseases and in most cases leading to plant death. It is estimated that over 20% of damages in flax cultivation is caused by *Fusarium* infection. Plant response to pathogen attack is a complex mechanism, and involves multiple defense strategies and different biochemical pathways (Dixon and Harrison, 1990). The identification of the plant genes that respond to infection, and generation of suitable transgenic plants is the approach that has been used in our study. Fifty four flax genes have been identified by means of cDNAs subtraction method as those that respond to pathogen infection. Subtracted genes were classified into several classes, and the prevalence of the genes involved in the broad spectrum of antioxidant biosynthesis has been noticed. Compounds known for their antioxidative properties, like terpenoids, phenylpropanoids, catecholamines, play a well established role in the plant pathogen response. Phenylpropanoids are of special interest among them, as they are connected with cell wall biosynthesis. The phenolic alcohols are constituents of lignins and lignans, which reinforce the cell wall, the first barrier against pathogens. In addition, phenolic acids form amides with catecholamines and settle in the cell wall, making it more resistant to pathogens (Zakarés et al., 2007). By means of semi-quantitative PCR, the involvement of subtracted genes controlling phenylpropanoid pathway in flax upon infection was positively verified. Moreover, the metabolite profiling obtained from the GCMS analysis constitutes a supplementary verification. The data from analysis of the transgenic flax plants overexpressing genes of the phenylpropanoid pathway showed and confirmed the protective effect of phenolic compounds against *Fusarium* infection.

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Induced androgenesis in anther culture of *Lupinus angustifolius*

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The aim of this work was to evaluate the reaction of seven blue lupine (*Lupinus angustifolius*) genotypes – cvs. 'Graf', 'Emir', breeding line 'LAE-1' and four hybrids originated from crossing 'LAE-1 × Graf' (204, 205) and 'Emir × LAE-1' (207, 209) – after inducing androgenesis in tissue culture. Some effort was made to increase the efficiency of androgenesis through optimization of conditions, such as: developmental stage of microspores, method of pretreatment and medium composition. A cytological analysis of developmental stage of microspores was performed on anthers *in vivo* and on explants sampled during the *in vitro* culture. Microscopic observations of all materials revealed the occurrence of multicellular microspores, which indicate the induction of divisions. The tendencies of the analyzed genotypes to produce multicellular structures resembling embryos at the globular stage and to induce callus formation were observed. To confirm the microspore origin of the developed embryos, chromosome counts of root tip meristematic cells were also made.

Enhancing of salidroside and rosavin production in *Rhodiola kirilowii* callus cultures

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Rhodiola kirilowii (Regl.) Maxim (Crassulaceae) is a traditional medicinal plant used in North Asia and China. Animal studies have shown a protective effect of the root extract in cardiopulmonary disorders in the hypoxic conditions induced by high altitude. Callus tissues of *R. kirilowii* synthesize only trace amounts of salidroside and rosavins – the supposed biologically active compounds of *R. rosea*, a related medicinal plant from genus *Rhodiola*. The aim of this study was to enhance the production of salidroside and rosavins in *R. kirilowii* callus tissues by exogenous addition of precursors to the medium. Callus tissue (line derived from cotyledons) cultured on solid MS medium was used in the experiments. The supplementation with p-tyrosol (at 3–5 mM/L) resulted in a significant increase of salidroside content – up to 1106 mg/100g dry weight (110-fold increase compared to control). The addition of cinnamyl alcohol (at 2.5 mM/L) induced rosavins and especially rosin production (1193 mg/100g d.w.) in the treated tissues. The supplemented callus tissues synthesized also: p-tyrosol, epigallatecatechine gallate, tannins and polyphenolic acids. These results demonstrated that supplemented *R. kirilowii* callus was able to synthesize high amounts of salidroside and rosavins (in contrast to intact plants where traces amounts of rosavins have been found).

Acknowledgment: The project was supported by a grant of the Ministry of Science and Higher Education, project No: N 405 025 32/1687.

Comparison of micropropagation methods of Polish cultivars of barley and oat

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Obtaining transgenic plants in cereal cultivars of agronomic value requires an efficient method of micropropagation which does not cause an additional variation. Recently suggested protocols consist in inducing regeneration of multiple shoots from apical meristems of seedlings derived *in vitro* from dry mature seeds. We compared the efficiency of three protocols: after Zhang et al. (1996), Sharma et al. (2004) and Geneshan et al. (2006) in several Polish cultivars of barley and oat. In a preliminary experiment, two procedures, after Zhang et al. and Sharma et al., were compared for oat cv. Chwat. We showed that numbers of shoots obtained from one explant in the same period of culture did not differ significantly. The procedure after Zhang et al. was found to be more laborious (dehusking of grains, cutting of shoots), so in the next experiment, involving two oat and two barley cultivars, we compared protocols by Sharma et al. and Geneshan et al. In the examined cultivars, the protocol after Geneshan et al. proved to be more efficient, less laborious and faster. After 10–11 weeks of culture we obtained, on average, in barley cv. Basza – 6.3, cv. Stratus – 17.1 shoots per plated explant; in oat cv. Bohun – 28.1, cv. Szakal – 54.2 shoots per plated explant. The lesser efficiency of the protocol after Sharma et al. in the same period of culture resulted mainly from a number of nonproliferating explants in barley and from slower development and elongation of shoots.

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Shoot regeneration of perennial wild beet species *in vitro*

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Perennial wild beet species are characterized by the high level of resistance to main beet diseases and tolerance to environmental stresses such as low temperature and drought (Asher et al., 2001). *In vitro* multiplication ability of most wild beet species is not sufficiently known. The purpose of this investigation was to examine the possibility of *in vitro* shoot regeneration of perennial wild beet species.

Seeds without hard coat of *B. macrorhiza, B. trigyna, B. lomatogona, B. corolliflora* and *B. vulgaris as* a control were used in this study. For surface sterilisation, seeds were treated with 70% ethanol for 30 s, 5% sodium hypochlorite for 25 minutes and washed four times in a sterile distilled water. Sterilized seeds were incubated in darkness for 4–8 days at 25°C on sterile moist blotting-paper in glass Petri dishes to obtain germination which was observed three days later. For each species, 36 shoot tips from 7 or 9 day old seedlings with 5 mm hypocotyls were cultured at 25°C on MS basal medium (Murashige and Skoog, 1962) containing different concentrations and combinations of growth regulators (BAP, TDZ, KIN, 2iP) under artificial daylight conditions. Explants were transferred to fresh medium every four weeks. Aseptic leaf and petiole segments were also cultured.

After 10 days, 16–55% of explants from seedlings of perennial species and 99% of explants of *B. vulgaris* started to regenerate shoots or shoot-like structures. Leaf and petiole explants showed no morphogenetic responses. MS medium emriched with 1 mg l^{-1} BAP was the best for an efficient plant regeneration of perennial wild beet species. Shoot regeneration ability was the best for seedlings incubated in darkness for 8 days. Some of regenerated plants of perennial wild beet species occasionally developed root-like structures but majority of them did not form roots on the basal medium.

Acknowledgement: This work was financed by the research project No. 2 PO6A 021 30.

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Biotransformation of p-hydroxybenzoic acid in *in vitro* cultures of *Ruta graveolens* ssp. *divaricata*

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The cells of *Ruta graveolens* ssp. *divaricata** cultured *in vitro* have been shown to transform the exogenous precursor, hydroquinone into its β-D-glucoside, arbutin (Skrzypczak et al., 2005). The maximal obtained contents of this product (12–13% d.w.) were very interesting from practical point of view (Zubek et al., 2009).

p-Hydroxybenzoic acid has been reported to be a good precursor of arbutin in *in vitro* cultures of many different plant species (Dušková et al., 1999). The aim of the present studies was to test the usefulness of this compound as a precursor of arbutin.

The agitated cultures of *R. g.* ssp. *divaricata* were maintained using Linsmaier-Skoog medium (Linsmaier and Skoog, 1965), supplemented with 2 mg/l NAA and 2 mg/l BAP. Different concentrations of the p-hydroxybenzoic acid (96–384 mg/l of medium) were administered into the culture flasks in one, two or three portions. The products of the biotransformation were qualitatively determined in methanol extracts from dry biomass and lyophilized media by HPLC method.

The main product of biotransformation in the extracts from biomass had retention time (t_R= 12.20 min) different from arbutin. Unknown compound was found also in the media when the higher dose of the precursor was administered. Identification of this compound is under way. Additionally, in the biomass and the media extracts we detected hydroquinone, which is the evidence of decarboxylation of the p-hydroxybenzoic acid.

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* The cultures were obtained thanks to cooperation of our Department with Institute of Biosciences, Würzburg University (Germany) – Prof. F. Ch. Czygan, Dr. A. Abou-Mandour

Studies of the biotransformation of p-hydroxybenzoic acid in *in vitro* cultures of *Ruta graveolens*

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Arbutin, β-D-glucoside of hydroquinone, is a well-known plant metabolite possessing both therapeutic and cosmetic values. Shoot cultures of *Ruta graveolens* maintained at the Chair of Pharmaceutical Botany JU CM are a good source of this compound.

The cells of the *Ruta graveolens* shoots cultured *in vitro* successfully transformed the exogenous hydroquinone into arbutin. The maximal content of this product, obtained so far is 7.8 % d.w. (Zubek et al., 2009).

In order to obtain higher biotransformation efficiency, the usefulness of p-hydroxybenzoic acid as a potential arbutin precursor was tested. p-Hydroxybenzoic acid has been shown to be a good precursor of arbutin in *in vitro* cultures of many different plant species (Dušková et al., 1999).

The agitated cultures of *R. graveolens* were maintained using Linsmaier-Skoog medium (Linsmaier and Skoog, 1965), supplemented with 2 mg/l NAA and 2 mg/l BAP. Different concentrations of the p-hydroxybenzoic acid (96 – 384 mg/l of medium) were administered into the culture flasks in one, two or three portions. The products of the biotransformation were qualitatively determined in methanol extracts from dry biomass and in lyophilized media by HPLC method.

The main product of biotransformation in the extracts from biomass had retention time (t_R= 12.20 min) different from arbutin. Identification of this compound is under way. This unknown compound was detected also in the media when the higher dose of the precursor was administered. Additionally, in the biomass extracts we detected hydroquinone, which is the evidence of decarboxylation of the p-hydroxybenzoic acid.

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Artificial seeds of *Linum usitatissimum* L. and *Morus alba* L. in long-term storage under slow growth conditions

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Techniques of *in vitro* culture are used not only for micropropagation but also long-term storage of plant material in gene banks. They are an effective tool for maintaining gene resources of crop plants, rare, extinct and elite plants, collections of botanic gardens, as well as plants incapable of generative propagation or plants with modified features. Fragments of tissues or entire organs of such plants can be stored under slow growth conditions and in the form of artificial seeds. The aim of this study was to create artificial seeds of flax (*Linum usitatissimum* L.) as an example of a crop plant and white mulberry (*Morus alba* L. cv. Żólwińska Wielkolistna) as a genotype of a rare tree. The vitality of plants was assessed after creating artificial seeds and after three-month storage. The results proved the effectiveness of storing plant material in the form of artificial seeds on medium that conditions slow growth.

Regeneration of *Gentiana cruciata* from *in vitro* shoot tip culture

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Different explants and types of culture have been used for studies of somatic embryogenesis and organogenesis in genus *Gentiana*. Shoot tips and liquid medium have been used relatively rarely, and not at all in *G. cruciata*. Such a combination should be conducive to a low variation and an increased regeneration efficiency.

The study aimed to achieve an efficient regeneration protocol for the legally protected *G. cruciata*. Explants, i.e. shoot tips (< 1 mm) containing 2–4 leaf primordia, were dissected from 1–1.5-month-old seedlings growing under *in vitro* conditions. Single explants were placed in 100 ml Erlenmeyer flasks containing 20 ml of MS medium (half-strength macroelements) supplemented with different levels of growth regulators: cytokinin BAP and auxin NAA. Two ways of medium mixing were applied. Cultures were maintained on an orbital shaker at 100 rpm and on a rotary device at 12 rpm. Temperature was 25°C and photoperiod was 16/8 h (day/night).

Among hormone combinations and concentrations studied, the best results were obtained on media supplemented with 0.2 mg/L NAA, or with 2.0 mg/L NAA and 0.2 mg/L BAP. After 9–10 weeks of culture, more or less developed regenerants (from green points on tissue aggregates to 2–4-leaf shoots) were the main part of the biomass. It was possible to obtain 86 shoots, each with several leaflets, from one shoot tip after 3 months of culture. The type of equipment used (rotary or shaker device) did not influence the development of explants and the results were similar. Multiplied shoots transferred onto agar media with or without growth regulators were easily rooted at a high rate.

Acknowledgement: Supported by project no 39/N-COST/2007/0 of the Polish Ministry of Science and Higher Education

Cryobank of gametophytes of tropical woody fern species

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Maintaining representative collections of biological diversity of woody ferns outside of their natural habitats is very difficult, because of their large size. Mainly vegetative propagation of tropical ferns leads to constraining of their gene pool. Ferns need special procedures to introduce them into gene banks. Now, it is possible to develop new cryopreservation methods.

The aims of this study were: 1) to establish the most efficient cryopreservation conditions for gametophytes of seven woody fern species and 2) to create their gene bank in liquid nitrogen (LN). Experiments were carried out on the following fern gametophyte cultures: *Cyathea australis, Cyathea dealbata, Cyathea smithii, Cyathea schan-chin, Cibotium schiedei, Cibotium glaucum, Dicksonia fibrosa*. Encapsulation-dehydration method was applied. The influence of time of preculture, ABA addition and type of preculture 1/2 MS medium (solid/liquid) were tested.

Encapsulated gametophytes of *D. fibrosa* and *C. schan-chin* survived at 100% rate after the NL treatment, independently of preculture type and time. Gametophytes of other five species survived at 60–80% rates. Application of two-week preculture with ABA gave better results than preculture without ABA for these species.

Cryopreservation by encapsulation-dehydration allows to create a cryobank of tropical woody fern gametophytes with the possibility of a high level viability conservation.

Acknowledgement: The research was supported by project no 39/N-COST/2007/0 of the Polish Ministry of Science and Higher Education.

Somatic embryo formation in callus cultures of *Narcissus* L. 'Carlton' multiplied in liquid media

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Until now only a few studies have reported propagation of *Narcissus* L. based on the process of somatic embryogenesis in liquid and liquid/solid culture systems (Malik, 2008; Malik and Molenda, 2008; Sage and Schroeder, 2002). These studies imply that cultivation in liquid media has a positive effect on somatic embryogenesis. The objective of this study was to investigate the effects of an 8-week exposure of callus to the liquid medium as well as that of growth regulator contents in the media on biomass growth and somatic embryo formation.

Seven lines (LC1-7) of embryogenic calluses of different origins (initial explant, initial medium, culture system) were used for the experiment. Calluses were cultured for 8 weeks in liquid proliferation media with Picloram or 2,4-D (10 or 25 μM) and BA (1 or 5 μM) and then for 24 weeks on solid proliferation or regeneration medium containing 0.5 μM NAA and 5 μM BA. The control cultures were maintained for 32 weeks on solid media.

The highest biomass growth was observed in the LC7 callus line culture obtained on ovary explants under the influence of 25 μM Picloram and 5 μM BA. The highest number of somatic embryos was noted in the LC5 callus line culture initiated from ovary explants and cultured on medium containing 25 μM 2,4-D and 5 μM BA in the liquid/solid culture system (Malik, 2008). The 8-week liquid medium treatment did not result in a biomass increase but promoted somatic embryo formation. Proliferation media stimulated the process of callus culture multiplication. In turn, the regeneration medium promoted somatic embryo formation.

Acknowledgement: The research were supported by Ministry of Education and Science (No. 2P06R12129).

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The analysis of cell ploidy level in the explant tissue culture of hemp (*Cannabis sativa* L.) and flax (*Linum usitatissimum* L.)

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The influence of ploidy of hemp and flax explants on their capacity for regeneration during *in vitro* culture makes the determination of the cell ploidy necessary at the beginning of breeding and genetic studies.

The aim of the study was to evaluate the suitability of different hemp and flax explants for organogenesis by monitoring the ploidy level by the means of flow cytometry. Stems, leaves, roots and cotyledons of hemp (Beniko) and flax (Nike, Alba, Aleksim) were used. The analysis was performed for the explants at different stages of plant growth: cotyledons of swollen seeds, young seedlings (five days after germination), two week and five week old plants. Additionally, at the beginning of flowering, flax anthers containing microspores with one nucleus were isolated from flowers. The determination of ploidy level was conducted using flow cytometer PARTEC CA II. The samples were fragmented directly in analysis buffer and then isolated nuclei were filtered through polyamide filter (50 μm). The released nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI). The fluorescence of individual nuclei was evaluated. The experiments were carried out in three replicates and 3500–6000 nuclei per sample were analysed. Histograms from different samples of DNA were compared with standard with known DNA content (erythrocytes of trout).

The results with respect to hemp demonstrated that young seedling and their cotyledons were mixoploid (2n+4n) which makes them unsuitable for *in vitro* culture. A clear decrease of DNA content was observed in leaves and in upper part of shoots from five week old plants. Two week old hemp was found to be the best source of explants due to their stable 2n DNA content. The experiment conducted on flax revealed that explants obtained from five day and two week old plants were the most suitable for *in vitro* culture. In the leaves of five week old flax there was a decrease in DNA content. Based on the results of this research, it may be concluded that the best explants for regeneration purposes originated from two week old hemp, as well as five and fourteen day old flax.

Does a relationship between the frequency of androgenesis and vernalization requirement exist in wheat?

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Androgenesis was achieved in Polish spring and winter wheat cultivars, which were all derived from F1 hybrids. Anthers were incubated on C17 medium and embryo-like structures on 190-2 regenerating medium. The relationship between anther culture traits and selected phenotypic traits of donor plants were evaluated by correlation, regression and variance analysis. It was found that the frequency of production of embryo-like structures and the number of regenerated green and albino plants were significantly different for winter and spring genotypes. The effectiveness of androgenesis was as much as two times higher in winter cultivars compared to spring cultivars. Is it possible that vernalization stimulated this process? The exposure of germinating seeds to a prolonged period of low temperature promoted flowering in adult plants of wheat (Chaudhary et al., 2003). It is possible that genes involved in vernalization, can also be involved in the molecular mechanism of androgenesis, including the role of DNA methylation. Androgenesis was also related to some morphological traits of the main shoot and yield structure independently of the kind of genotype within the winter and spring cultivars. Such relationships may be due to the fact that these traits are influenced by the genetic basis of hybrid parental lines (Dodig et al., 2008). These results suggest that it is possible to screen genotypes with good anther culture traits directly by morphological markers, which are practical and simple to use for breeders, to select highly productive wheat lines in anther culture. The advantages of double haploid technology for accelerating breeding programs include reduction of costs of cultivar development, *via* the greater efficiency, with which the homozygous double haploid lines can be evaluated for selected desired traits.

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Analysis of indole compounds in *Calocera viscosa* mycelium cultured on liquid medium and in its fruiting bodies

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In Poland *Calocera viscosa* (Pers.; Fr.) Fr. (*Macromycetes*) is a widespread species of mushroom. The aim of this study was to investigate the contents of indole compounds in fruiting bodies of this species and in its mycelium cultured *in vitro*.

Fruiting bodies were collected in mixed forests in southern Poland. *In vitro* cultures was initialized from fruiting bodies taken from the natural state. The optimal medium composition for a submerged culture was determined. Fresh material: fruiting bodies (61.5 g) and mycelium (170 g) was frozen and then dried by lyophilization. The crushed dry biomass was extracted with petroleum ether to remove the oil fraction which was discarded. The remaining biomass was extracted with methanol.

Analysis of indole compounds was performed in methanol extracts using chromatographic methods: TLC, PTLC and HPLC.

The HPLC method allowed to estimate the contents of the following metabolites (mg/100g d.w.): tryptophan (0.92), skatol (0.37), 5-methoxytryptamine (0.14), melatonin (0.79) in fuiting bodies and tryptophan (0.89), skatol (0.31), 5-hydroxytryptophan (0.23) and melatonin (0.45) in mycelium from *in vitro* cultures.

It was demonstrated that *in vitro* cultures of *Calocera viscosa* and its fruiting bodies contain comparable amounts of indole derivatives. *In vitro* cultures will make a good model for studies of accumulation of this group of metabolites.

Conversion – the critical point of induced embryogenesis in *Capsicum* spp.

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The creation of new, useful genetic variation is the main aim of *in vitro* gametic embryogenesis, while the somatic embryogenesis provide an opportunity for multiplication of heterozygotic genotypes. In both these processes, the conversion of the embryos into plantlets is the limiting factor for their practical exploitation. From this point of view the effectiveness of induced embryogenesis should be presented as the relationship of obtained plantlets to explant number. The success rate of converting haploid embryos to plants in *Capsicum annuum* L. ranged between zero to over 50%, according to genotype and conditions of anther culture. In the study on individual reaction of *Capsicum* F2 plants (Nowaczyk et al., 2009), interspecific hybrids showed a higher conversion coefficient (84%) than did *C. annuum* L. (52%). In both of these genotype groups the share of haploids was lower than that of diploid plants. Therefore, spontaneous diploidization of haploid embryos derived from anther cultures seems to be a very interesting phenomenon. The lines obtained as a result of distant hybrid progeny selection showed that the conversion efficiency depends mainly on the genotype characteristics. Similar effectiveness has been observed when the microspores were used as explants (Supena et al., 2006). In some of hot pepper genotypes 15–33% of embryos converted to complete plants. This information is not good for pepper breeders because conversion is the limiting factor in the rapid use of the new genes and character arrangements in hybrid recombinants. In addition, research on somatic embryogenesis has not supplied satisfactory results concerning this way of propagation, however the maximum conversion effectiveness currently described for one cultivar has reached 80% (Khan et al., 2006).

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Shoot induction potential of nine *Brassica napus* varieties and two types of explants

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Rapeseed is recognized as the most important source of vegetable oil and protein-rich meal worldwide. It is well known that the improvement of plant breeding methods is slow, time consuming and labor-intensive. Non-conventional genetic improvement programs based on tissue culture and molecular genetics are essential as a complement to standard breeding. Efficient micropropagation and transformation methods require a reliable and efficient callus induction and plantlet regeneration protocols therefore *B. napus* has become an object of extensive tissue culture studies (Moghaieb et al., 2005). A wide range of target explants of *B. napus* have been studied, including hypocotyls, petioles, thin cell layers, stem segments and protoplasts. The effects of culture media and genotypes on shoot regeneration in oilseed *Brassica* species have also been examined (Tang et al., 2003). Due to the highly variable and genotype specific regeneration in *B. napus*, it is especially important to determine the varieties and types of explants with particularly high potential for tissue culture micropropagation among Polish accessions. In our research we tested nine rapeseed varieties registered in Poland and two types of explants to detect the variation in frequency of callus induction, shoot induction and shoot induction effectiveness. We cultured hypocotyl and petiole explants of each variety on MS medium supplemented with hormones (0.15 mg/l NAA and 3 mg/l BAP) and $AgNO_3$ (2.5 mg/l). By measuring the shoot induction frequency and effectiveness parameters we showed that the differences in shoot regeneration ability of various explants were significant within the same variety as well as in different genotypes. Thus, the shoot regeneration from rapeseed explants highly depended on the genotype and kind of explant.

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Is it possible to select drought-resistant *Rubus* genotypes from *in vitro* populations of seedlings or adventitious shoots?

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Raspberry is an important fruit in the Polish horticulture, fresh produce and food industries. Irrigation during spring and summer is essential in some years and on some plantations, but this increases the production costs. Therefore, drought stress tolerance should be one of the breeding goals. We would like to develop a reliable system enabling the preliminary screening of seedlings and adventitious regenerants at the *in vitro* stage.

We compared the reactions of 6 raspberry and 2 blackberry cultivars propagated *in vitro* to drought stress simulated by the addition of polyethylene glycol 6000 (PEG) to the media. We measured the contents of osmoprotectants (proline and soluble carbohydrates) and of the percentage dry mass at different PEG concentrations and after different culture times. We also observed the reaction to water deficit of one-year old plants grown in pots in the greenhouse using the same genotypes, by measuring: leaf water potential, the intensity of gas exchange and chlorophyll fluorescence.

A metabolic reaction *in vitro* was already observable after one week, but the patterns of reactions differed between genotypes depending on the stressor concentration and the length of the stress period. Differences in the osmoprotectant contents were also found in the control shoots (non-stressed). The water deficit in the soil limited the intensity of gas exchange and the water potential in the leaves, and decreased the ETR coefficient, but differences between genotypes were also observed. All the parameters, whether *in vitro* or *in vivo*, indicated cv. Latham as the most resistant to water stress.

The effect of cytokinins on *in vitro* morphogenesis of *Passiflora caerulea* L.

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The development of an effective method of *in vitro* regeneration of *Passiflora caerulea* L. (blue passion flower) is essential for the improvement of cultivation and rapid micropropagation of the plant, whose expected medicinal properties include anxiolytic, sedative, analgesic, antiepileptic activities and utility as a remedy for opiate withdrawal.

The aim of the research was to estimate the effect of cytokinins on the production of morphogenic callus and shoot induction from different explants of *P. caerulea*. Several concentrations of 6-benzylaminopurine (BAP), alone or in combination with indole-3-acetic acid (IAA) or gibberelic acid (GA_3), were used *in vitro* to induce indirect morphogenesis and to achieve micropropagation. Three types of explants obtained from *in vitro* seed germination: leaf discs, root segments and shoot tips (30-day seedling) were used in experiments.

The frequency of callus and shoot formation was dependent on the origin of the explants, concentration of cytokinin and medium supplementation. The culture medium containing 0.5 mg/l BAP was the most suitable for all the explants. The highest rate of induction of indirect morphogenesis was observed in leaf discs. Direct morphogenesis was observed on root segments. The best results for shoot regeneration, elongation and propagation from node explants were obtained on media containing 1.0 mg/l BAP or 1.0 mg/l BAP and 1.0 mg/l IAA, without a statistical difference.

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Cryopreservation of *in vitro* grown shoot buds of rose 'New Dawn' using encapsulation-dehydration method

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In the present study, a protocol for preservation in liquid nitrogen by encapsulation-dehydration method of shoot meristems of the park rose 'New Dawn' has been developed. Shoot apical and axillary meristems about 2 mm in diameter were collected from plants propagated *in vitro* on the mineral medium according to Quoirin et al. (1977) supplemented with 5 μM BA, 0.3 μM GA_3, 0.5 μM NAA and 0.06 M sucrose. Moreover, some explants for cryopreservation were pre-cultured on the medium containing a higher sucrose level (0.25 M) for 8 weeks. Before encapsulation some explants were cultivated on medium supplemented with 2.5 g dm^{-3} activated charcoal for 3 days. After encapsulation plant material was dehydrated by the quick method (capsules were placed in liquid medium containing 0.75 M sucrose for 18 h) or by the gradual method (capsules were transferred to liquid solutions of media with sucrose concentrations increasing from 0.3 M to 1 M for 7 consecutive days).

Results indicate that the survival rate of plant tissue after freezing in liquid nitrogen depended on plant material: apical meristems of roses regenerated callus and shoots but axillary meristems did not survive the freezing. Cutting capsules after freezing did not influence the regeneration of the cryopreserved plant explants. It was shown that the slow dehydration method used in preparation of plant material for cryopreservation allowed to obtain viable tissues of Rosa 'New Dawn'. After cryopreservation, 34% of explants incubated on the pre-culture medium supplemented with a high level of sucrose (0.25M) and activated charcoal developed shoots.

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Optimization of medium for callus induction and plant regeneration of *Miscanthus* × *giganteus*

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Miscanthus × *giganteus* is a sterile triploid, which reproduces only vegetatively from rootstocks (Lewandowski, 2006). The result of vegetative reproduction is a small range of genetic variability within this species. The *in vitro* culture can be used as a method to increase its genetic variability via somaclonal embryogenesis. *Miscanthus* produces a lot of phenolics, which, when oxidized and secreted into the medium, show toxicity to tissue development. The aim of this work was to optimize composition of medium for callus induction and plant regeneration. The medium inducing callus was supplemented with compounds that inhibit oxidization of phenolics.

Callus was induced from immature inflorescences on MS (Murashige and Skoog, 1962) medium containing 6.5 mg dm^{-3} 2.4 D, 0.25 mg dm^{-3} BAP, 500 mg dm^{-3} of casein hydrolysate and 30 g dm^{-3} of sucrose as a control and on the same medium supplemented with 1) 200 mg dm^{-3} of chitosane; 2) 65 g dm^{-3} of banana pulp; 3) 100 mg dm^{-3} of cysteine; 4) 30 g dm^{-3} of honey instead of sucrose. Callus was induced at 25°C in the dark. After 2 months, callus was transferred onto regeneration MS medium supplemented with 1) 0.2 mg dm^{-3} of BAP or 2) 0.2 mg dm^{-3} of kinetin. Regeneration was performed at 20°C in the light (PPFD=300 mol m^{-2} s^{-1}).

The best initiation of callus with numerous somatic embryos was observed on MS medium containing honey (84% of all explants) and banana pulp (79%), while on control medium such callus tissue was obtained with 54% frequency. On the regeneration medium with 0.2 mg of BAP no plants were obtained, while on medium containing 0.2 mg dm^{-3} of kinetin, 52 plants were obtained but only from calli induced on medium supplemented with banana pulp.

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Initiation of an *in vitro* culture of elder (*Sambucus nigra* L.) meristems with the use of various sterilizers

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Elder (*Sambucus nigra* L.) is a shrub from the family *Caprifoliaceae*. It is used in phytotherapy, food processing industry and as a decorative plant. The objective of this study was to develop an effective method for surface sterilization of initial material for *in vitro* cultures of elder *var.* Aurea. The initial material consisted of axillary and apical buds sampled from the shoots of shrubs grown at the Experimental Station of the University of Warmia and Mazury in Olsztyn. Shoot samples were collected at the beginning of the growing season in 2007 and 2008. Buds were isolated from the shoots, rinsed under running water for 1 hour and then immersed in water with the addition of a detergent for 5 minutes. Prior to disinfection, buds were immersed in 70% ethanol for 30 seconds. The material was disinfected with various combinations of disinfectants applied over different periods of time. Treatment 1 involved the use of 0.1% mercury chloride ($HgCl_2$) for 60, 120 and 240 seconds. In treatment 2, buds were disinfected with 3% sodium hypochlorite (NaOCl) for 5, 10, 15 and 30 minutes. In treatment 3, the samples were disinfected with 0.1% mercury chloride ($HgCl_2$) for 60, 120 and 240 seconds, after which the incisions were immersed in silver nitrate ($AgNO_3$). In the last treatment, plant material was immersed in a calcium hypochlorite [$Ca(OCl)_2$] solution for 5, 10 and 15 minutes. After sterilization, buds were rinsed three times in sterile distilled water. Explants of tissue fragments containing the meristem were isolated from the disinfected material. Every explant was placed in a 10 ml test tube containing 1.5 ml MS medium (Murashige and Skoog, 1962) whose macronutrient and micronutrient contents were reduced by half. Culture media were additionally enriched with 8 $g \cdot dm^{-3}$ agar and 30 $g \cdot dm^{-3}$ saccharose. The medium pH was set at 5.5 before autoclaving. Every tratment was represented by 20 explants. Incubation was carried out in a phytotron at 23°C with 16 h of light exposure. The highest percentage share of sterile plants occurred in cultures where the initial material was disinfected with calcium hypochlorite. In the remaining treatments infections were more prevalent and led to the death of explants within a short time.

The effect of kinetin content in the culture medium on micropropagation of elder (*Sambucus nigra* L.)

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Elder *var.* Aurea is an attractive decorative shrub with golden yellow leaves that maintain their color throughout the growing period. Elder shrubs of this variety reach 2 meters in height, and they create a highly attractive combination when set against plants with dark foliage. As an additional advantage, this elder variety has low soil requirements. This study investigated the effect of the kinetin content in the culture medium on *in vitro* micropropagation of elder var. Aurea. The experiment was carried out on a medium (Murashige and Skoog, 1962) whose micronutrient and macronutrient contents were reduced by half, enriched with 8 $g \cdot dm^{-3}$ agar and 30 $g \cdot dm^{-3}$ saccharose. The medium pH was adjusted to 5.5 before autoclaving. Plant material was obtained from a sterile and stable culture. Explants consisting of two nodes were placed individually in 10 ml test tubes containing 1.5 ml medium each. The kinetin (KIN) concentrations used in the experiment were: 2, 4 and 6 $mg \cdot dm^{-3}$. Each treatment was represented by 40 explants. After an 18-week propagation cycle (3 subcultures), the propagation ratio was computed for every treatment, and the morphological characters of the plants were assessed.

The highest propagation ratio occurred in the treatment with 4 $mg \cdot dm^{-3}$ kinetin. Most plants growing in this medium were not affected by any developmental anomalies. Lower propagation ratios were noted in culture media with the lower kinetin content, while higher concentrations of this phytohormone resulted in similar or lower propagation ratios. The resulting plants were characterized by short internodes, fewer and smaller leaves and lower root mass.

ISSR analysis of genetic stability of Polish tulip cultivars propagated *in vitro*

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The aim of the study was an early detection of somaclonal variation (SV) which could occur within the micropropagated plant material. Shoot cultures of nine Polish cultivars were used. They were propagated *in vitro* for one or two years. Plant material was propagated *in vitro* by the means of adventitious shoot regeneration in the presence of thidiazuron (Podwyszyńska and Marasek, 2003). The SV was detected with the inter-simple sequence repeat (ISSR) markers. For ISSR analysis, leaf samples were taken from *in vitro* propagated plant material and from conventionally propagated true-to-type plants which served as standards. Ten ISSR primers were used (TIBMOLBIOL, Poznań and IBB, Warszawa): 824, 843, 844, 845, 846, 852, 853, 857, 973 and 895. The DNA extraction was performed using the DNA purification kits Genomic Mini AX PLANT (A&A Biotechnology). The molecular markers were first used for cultivar identification and then for the detection of SV. The degree of genetic similarity between the micropropagated plants and the standard of each cultivar was calculated based on the number of monomorphic and polymorphic bands. The Cafe-Jaccard coefficient was used to plot a dendrogram using the UPGMA method. The statistical analyses were performed in XLSTAT software environment (Addinsoft, 2006). The UPGMA dendrogram of genetic similarity showed nine clusters grouping cultivars, their standards and micropropagated plants. For the six studied genotypes, the ISSR analysis, performed with ten primers, did not reveal polymorphisms between the standard and the micropropagated plants. Analysis of the other three cultivars showed that some of the plants, micropropagated for either one or two years, differed slightly from standards (in cultivar A, three of the five analyzed plants; in cultivar B, two of the four plants); whereas in cultivar C, all plant showed a high (96%) similarity. These results indicate that the changes in DNA structure may occur during the first year of the plant micropropagating process.

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Evaluation of somaclonal variation in micropropagated *Hemerocallis* sp. plants using phenotype and ISSR markers

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The genetic fidelity of micropropagated daylily plants was evaluated by phenotypic observation and inter-simple sequence repeat (ISSR) techniques. Plants of nine daylily cultivars were used in this study. The plants which were propagated *in vitro* for the period of one to five years, were then grown outdoors in the years 2002 to 2006. The observations comprised 1600 plants and concerned the number and quality of flowering plants. The colour, the size and the shape of flowers were evaluated and compared to the published cultivar descriptions in accordance with American Hemerocallis Society (AHS). The nine selected somaclones were analized with ISSR markers in order to determine the character of variation. The first micropropagated plants flowered sporadically in the first growing season. In the second season, the highest percentage of flowering plants was found for the cultivar 'Moonlit Masquerade' (84%) and the lowest for 'Pink Debutante' (20%). The latter cultivar had the flowering rate of 70–100% in the third season. In general, the length of flower stems of all studied cultivars was similar to the lengths given in the AHS cultivar descriptions.

Somaclonal variants occurred within the micropropagated plants of the five genotypes. The somaclones differed from the true-to-type plants in terms of the colour and the shape of flowers. The frequency of SV ranged from 1.4% to 100%. In 'Moonlit Masquerade', 62.6% of plants developed flowers with narrower petals and a smaller brown eye compared to the true-to-type. In 2006, for 'Ruby Moon', the colour of the eye was changed from ruby-red to a lighter colour in all of the plants, while in 2007 this was observed only for 25% of the plants. For 'Pink Debutante', only one plant was found with a malformed flower in the first growing season, while in the second season, the SV frequency was 11.1%. The observation performed during 2004–2006 revealed that for 'Amethyst Jewel' the colour of all the flowers changed from light amethyst-violet to light grey pink. However, in 2007, all plants of this cultivar had flowers with colour similar to the type. Four cultivars appeared to be genetically stable.

The ISSR analyses of the selected somaclones revealed that the phenotypic changes observed had the characteristics of a mutation.

To summarize: 1) the SV frequency depends on the genotype, 2) a reliable information on the SV, introduced during the *in vitro* propagation, can be obtain when more than 70% of the plants have flowered; this can occur as early as in the second or third growing season.

Frequencies of spontaneous doubled haploids of winter triticale plants obtained by anther culture

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The haploids and spontaneous doubled haploids are obtained with varying efficiency in the process of cereal androgenesis. The aim of this study was to evaluate the production frequency of spontaneous doubled haploids in winter triticale plants derived from anther culture.

Anthers from ten F1 hybrids of winter triticale 5953 were plated on a C17 medium containing 0.5 mg/l KIN + 2.0 mg/l 2,4-D + 90 g/l maltose. 5412 (average of 91.2%) embryo-like structures were obtained (29.7 to 150.4%, depending on the genotype), from which 463 green plants were regenerated (average of 7.8% in relation to anthers plated and from 2.2 to 26.7% depending on the genotype) on a 190-2 medium supplemented with 0.5 mg/l KIN + 0.5 mg/l NAA + 30g/l sucrose.

The ploidy levels of green plants were determined by flow cytometry of the DNA content of DAPI – stained nuclei from leaves. Among the 463 green regenerants analyzed, 239 (average of 51.6%) were spontaneous double haploid, with frequency varying from 32.1 to 85.7%, depending on the genotypes. The identified haploid plants were subjected to colchicine treatment (0.1% aqueous solution of colchicine was used together with 4% DMSO and 25 mg/l GA3 for 6 hours in the light at 25°C). All plants were potted and vernalized for 8 weeks at 4°C, and then moved to the greenhouse where they were grown to maturity. The efficiency of chromosome doubling was determined based on the fertility of spontaneous doubled haploids as well as individuals subjected to colchicine treatment.

Isolation and *in vitro* culture of endosperm tissues in selected monocot and dicot species

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The competence of isolated endosperms of selected important crop plants for proliferation and differentiation *in vitro* was assessed on culture media supplemented with the plant growth regulators. Mature endosperm tissues were excised from seeds of kiwifruit (*Actinidia deliciosa* cv. Hayward). Ovules of eight cultivars of winter, spring and durum wheat (*Triticum aestivum* and *T. durum*) and two lines of *T. monococcum* were used as sources of immature endosperm.

The basal medium consisting of MS salts and vitamins was supplemented with 3% (w/v) sucrose, auxin (2,4-D), cytokinins (kinetine, thidiazuron – TDZ) and 5-azacytidine (5-azaC). Culture were incubated in the dark or exposed to a 16 h photoperiod provided by cool-white fluorescent tubes (60–90 µmol photons m^{-2} s^{-1}). The material for sectioning (freshly isolated and cultured endosperm) was prepared by embedding tissues in Technovit 7100 (Heraeus Kulzer) and stained with toluidine blue or auramine O.

In kiwifruit the long-term culture of endosperm-derived callus, organogenesis induction and histological events have been described previously (Popielarska et al., 2006; Popielarska-Konieczna et al., 2008). In the present work localization of cutine on callus and meristematic protuberances were examined.

Studies on proliferation and histological analysis of isolated endosperm of heksaploid plants *T. aestivum*, tetraploid *T. durum* and diploid *T. monococcum* cultured *in vitro* have been described recently (Popielarska-Konieczna et al., in press). The present experiments were conducted on immature endosperm tissues isolated from 6–8 DAP-old grains of *T. aestivum*. Flowers and/or isolated endosperm were treated with 5-azaC. The aim of the experiment was to induce hypomethylation and expression of silencing genes.

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Micropropagation of *Leucojum aestivum* in a temporary immersion bioreactor system (RITA)

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Leucojum aestivum belongs to the family Amaryllidaceae, the alkaloids of which are known to exhibit a wide range of biological activities. Galanthamine, an acetylocholinesterase inhibitor, is used for symptomatic treatment of Alzheimer's disease (Heinrih and Lee Teoh, 2004). Our previously published results have shown that *in vitro* cultures of *L. aestivum* are a source of galanthamine and other Amaryllidaceae alkaloids (Ptak et al., 2009). In order to optimize somatic embryogenesis of L. aestivum we used RITA temporary immersion bioreactor systems (TIBS). Embryogenic callus obtained by the method described previously was transferred to solid and liquid MS media (Ptak et al., 2009). Liquid cultures were carried out in RITA vessels, and every 2 hours 5 or 15-minute flushing with the medium was applied. The medium was supplemented with: picloram (2, 5, 10, 25 μM) and BAP (0.5 μM). Callus growth on the solid medium enriched with 2 or 5 μM of picloram and 0.5 μM of BAP was characterized by a higher multiplication index. However, the greatest number of somatic embryos was observed on the callus multiplied on the liquid medium containing 2 μM of picloram and 0.5 μM of BAP, while using 5-minute flushing with the medium every 2 hours. Globular embryos developed into torpedo-stage embryos under the influence of NAA (0.5 μM) and zeatin (5 μM). In our experiment, the TIBS also promoted the development of *L. aestivum* somatic embryos. Torpedo embryos derived from RITA vessels were characterized by a greater increase in fresh weight compared with the embryos cultivated on the solid medium. The RITA derived embryos converted into normal plantlets on the medium enriched with NAA (0.5 μM) and zeatin (5 μM). The analysis of the ploidy level of the regenerated *L. aestivum* plantlets from the solid medium and the RITA TIBS did not reveal any changes.

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Preliminary analysis of polyphenolic fraction from intact plant and *in vitro* cultures of *Securinega suffruticosa*

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Securinega suffruticosa, Phyllanthaceae, is one of the basic Chinese folk medicines used in the treatment of various diseases, e.g. rheumatoid disease or quadriplegia (Yuan et al., 2005). Many studies have been conducted on the alkaloid fraction of the plant, however there are very few reports on other types of compounds, especially on the polyphenolic constituents (Lee, 1994; 1996). To better understand the complex mode of action of plant medicines, we need to detect all the physiologically active compounds. Considering the well known health promoting properties of polyphenolics, it seemed advisable to analyze flavonoids, tannins and phenolic acids from *S. suffruticosa*.

Plant tissue cultures are considered to be an interesting source of attractive secondary metabolites, allowing the production of biologically active compounds in a controlled way, independent of climatic conditions and seasonality. Considering the potential ability of some plant cell cultures to accumulate high amounts of polyphenolic compounds, it seemed pertinent to investigate *in vitro* cell cultures of this plant.

The aim of this work was to determine qualitatively and quantitatively the main compounds of the polyphenolic fraction from *S. suffruticosa in vitro* cultures and compare them with results from intact plant grown under Polish conditions. To this end, callus cultures, shoot cultures and tissues of intact plants were analyzed with respect to their polyphenolic fraction. Moreover, all plant matrices were compared to each other to determine "the best producer" of the analyzed compounds. Calli were grown on the Schenk – Hildebrandt (SH) medium and SH medium modified with plant growth regulators; shoots were grown on the Murashige for *Lilium* (ML) medium. TLC was the method of choice for the preliminary qualitative analyses. For the quantitative analyses of polyphenols HPLC with DAD UV detection was chosen.

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Agrobacterium rhizogenes serves cytogenetics

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Transformation using *Agrobacterium rhizogenes* has become a very popular and widely used method in plant biotechnology. *A. rhizogenes* is a Gram negative soil bacterium, a natural pathogen of many plants. In the process of plant tissue infection, a fragment of the bacterial Ri plasmid, the T-DNA, is transferred and integrated with the plant genome. Expression of specific bacterial genes from T-DNA cause the production of hairy roots. This natural process has been used to produce hairy roots from many different species. Hairy roots have several characteristic features which makes them noteworthy and easy to work with.

The hairy roots culture is a valuable source of material in different areas of studies, such as bioproduct and secondary metabolite production or phytoremediation. In this work we present the use of hairy roots in cytogenetics studies. A hairy root culture is a very good tool for maintaining rare genotypes for further analysis, especially if species are not self-fertilizing. Hairy root cultures are usually cytogenetically stable for a long time, but in some species, such as *Brassica*, chromosome elimination or chromosomal rearrangements may occur.

A large number of root tips with many dividing cells in a hairy roots culture, makes cytogenetic analysis much easier than using seedlings. Additionally, chemical treatment e.g. with hydroxyurea can increase the metaphase index even to 50%. Such a high metaphase index together with a large number of root tips in the culture create the possibility for chromosome isolation using flow cytometer equipped with sorter.

We discuss the use of hairy roots from long and short term cultures in cytogenetic analysis of individual chromosomes and interphase nuclei with different numbers of B chromosomes.

Also we have used *C. capillaris* hairy roots for evaluation of genotoxicity. Comparison of cytogenetic effects in hairy roots and roots of seedlings showed a much higher sensitivity of hairy roots, which makes them a convenient material for monitoring the DNA damage after mutagenic treatment.

Use of wide crossing and *in vitro* culture for the induction of haploid embryogenesis in three cereal species

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Wide crossing is an important tool for expansion of variability and for haploid production. The bulbosum method is one of the oldest approaches, in which crosses of cultivated barley with wild *Hordeum bulbosum* are made to help produce haploid plants. In other cereal species crosses with maize are more popular. There is evidence in the literature showing that pollination of cereals with maize pollen results in haploids production (Lauriea and Benett, 1986; Wędzony, 1999). However, there are also doubts concerning the actual fertilization. Therefore, the aim of this work was to explain whether there is a complete conjunction of maize sperm with cereal egg cell or whether the presence of maize sperm is sufficient for the induction of embryogenesis from an unpollinated cereal egg cell.

Crosses of three cereal species (wheat, triticale and rye) with maize were carried out in a greenhouse belonging to the Department of Genetics and Plant Breeding, Poznań University of Natural Sciences. Spikes of these species were castrated shortly before earing. The pollination was conducted from 2 to 6 days after castration. Next day after pollination the ears were sprayed with 50 mg l^{-1} solutions of 2,4-D and picloram. Embryos or ovules from wide pollinations were isolated and placed on White or MS medium depending on the embryo developmental stage. For the observation of pollen grain germination and pollen tube growth, the pollinated pistils were fixed in the Carnoy solution and stained with aniline blue (Martin, 1959).

Based on our results it can be concluded that there were differences in the intensity of pollen tube growth and the development of embryos between particular cross-combinations.

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Phenolic acids in *in vitro* cultures of *Exacum affine* Balf. f.

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Plant tissue cultures can be the source of many various secondary metabolites (Malepszy, 2001). *Exacum affine* Balf. f. (*Gentianaceae*) grows on Socotra Island (Aden Bay). In Poland it is known as a decorative pot plant. Secondary metabolites such as secoiridoid glucosides, flavonoids, phenolic acids and saponins were found in plants of the genus *Exacum* (Das et al., 1984). Chemical composition of *Exacum affine* has been only partly analysed (Kuwajima et al., 1996). The only known phenolic acid that has been reported from this plant is *p*-coumaric acid. We found no literature information on chemical analysis of *in vitro* cultures of *Exacum affine*.

The aim of the present investigation was chemical analysis of phenolic acids in *in vitro* cultures of *Exacum affine* Balf. f. and their comparison with constituents of pot plants of this species. Free and bound phenolic acids were determined.

In vitro cultures of *Exacum affine* (shoot cultures) were maintained on the MS medium supplemented with BAP (1 mg/l), NAA (0.5 mg/l), GA_3 (0.25 mg/l). Dried plant materials (shoots from *in vitro* cultures and aerial parts of pot plants) were extracted with methanol. Additional samples of plant materials were hydrolysed with 2 M HCL before extraction. Methanolic extracts were qualitatively and quantitatively analysed by HPLC. Compounds were detected at 254 nm. The identification of phenolic acids was accomplished by the comparison of their retention times with standards and followed by the internal standard method. Some phenolic acids were identified in the investigated extracts (protocatechic, *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric and ferulic acid). Free phenolic acids were usually present in small or even trace amounts. More compounds were found after hydrolysis.

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Induction of androgenesis in oat (*Avena sativa* L.) depending on kind of culture medium

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The production of doubled haploids under laboratory conditions allows to obtain a high level of homozygotic DH lines, to shorten the breeding time and to increase the selection effectiveness of required genotypes. However androgenic response of plants highly depends on the species and even genotype. An extremely low efficiency of androgenesis is observed in oat in comparison to wheat or triticale.

The aim of the study was to compare the effect of type and physical properties of the medium on anther vitality and induction of embryo like structures (ELS).

Five F1 generations, nine F2 generations and two cultivars of oat were used. Donor plants were grown in the greenhouse. Anthers were isolated after a cold pretreatment and cultured on solid media: C17 (Wang and Chen, 1983), W14 (Ouyang et al., 1989) and modified W14 (Kiviharju et al., 2005) or on a liquid and semi-solid modified W14. The analyses of results showed that anther vitality and ELS formation mostly depended on oat genotype and kind of media but less on media fluidity. Forty one ELS were obtained from eleven genotypes. The ELS from five genotypes regenerated eight plants. Only two plants survived colchicine treatment used for chromosome doubling.

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Evaluation of phenotypic trueness-to-type for selected cultivars of narcissus propagated by *in vitro* cultures

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Micropropagation of 13 narcissus genotypes was carried out in 2001. After acclimation, the plants were cultivated in an insect-proof mesh tunnel. Single plants of two cultivars ('Tete-a-Tete' and 'Golden Ducat') produced first flowers in 2004. The following year, eight cultivars were in full blossom after 4 years of *ex vitro* cultivation. All blooming plants of 11 cultivars were evaluated according to UPOV descriptors during the next two growing seasons – 2006 and 2007. Nineteen morphological characteristics, including the shape and colour of perianth segments, and the type, shape and colour of corona, were observed and measured. The observations of plants propagated *in vitro* were compared with the observations of the same cultivars growing in the field and propagated traditionally, or with the descriptions available in the literature.

Narcissi obtained via *in vitro* propagation, showed no phenotypic differences in comparison with the initial genotypes. Small differences in the colour gradation of flowers or the length of flower stems were a result of growing conditions. The lower colour intensity in the case of plants cultivated in the mesh tunnel was probably caused by extensive shading due to the very dense mesh. However, in the case of one cultivar – 'Marie-Jose' from the split-corona group, significant modifications in the shape and colour of perianth segments were observed. A considerable fraction of plants (22%) showed no creamy-white stripes on the orange split corona. In addition, changes in the corona (from split, via partly split, to completely connate, like in small-cupped narcissi) were noticed.

Methyl jasmonate and fosmidomycin affect mono- and sesquiterpenoid production in root cultures of *Inula royleana* DC. and *Inula macrocephala* Boiss. & Kotschy ex Boiss.

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Root cultures of *Inula macrocephala* and *Inula royleana* were obtained as described by Stojakowska et al. (2006) and cultivated either in WP (Lloyd and McCown, 1980) or in 1/2 GB5 (Gamborg et al., 1968) liquid media, respectively, at 25°C in the dark. The cultures produced monoterpenoids (thymol derivatives) and sesquiterpene lactones of eudesmane type. Methyl jasmonate or fosmidomycin solution was added to the flasks containing 20 day old cultures, to reach the final concentration of 100 μM in the nutrient medium. Roots from both treated and control cultures were harvested 72 h after the beginning of experiment, frozen and lyophilized. Contents of selected mono- and sesquiterpenoids were measured by means of analytical RP-HPLC (Stojakowska et al., 2006).

Jasmonates are elicitor signal transducers for the production of secondary metabolites in plants and plant tissue cultures. Methyl jasmonate added to *I. macrocephala* and *I. royleana* root cultures caused an enhanced accumulation of eudesmanolides and thymol derivatives in the roots. The eudesmanolide content in the treated cultures was up to four fold higher than that in the controls. The C_5 unit (IPP) for isoprenoid biosynthesis in plants is generated either through the plastidic (GAP, DXP or MEP) or the cytoplasmic (mevalonate) pathway. Mono- and diterpene biosynthesis is generally attributed to the plastidic compartment of the plant cell. In contrast, the enzyme responsible for the C_{15} isoprenoid chain (FPP) synthesis seems to be bound to the cytoplasmic compartment. Fosmidomycin, an inhibitor of the plastidic pathway of terpenoid biosynthesis, decreased monoterpenoid production in the *Inula* roots, as expected. The eudesmanolide content remained unaffected.

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Mycelial cultures of some *Aphyllophorales* (Basidiomycetes): optimization of *in vitro* culture conditions

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Sarcodon imbricatus (L.) P. Karst., *Sparassis crispa* Wulf.: Fr. and *Hydnum repandum* L.: Fr. are species of fungi belonging to Aphyllophorales (Basidiomycetes). *S. imbricatus* and *S. crispa* are under strict legal protection in Poland.

In vitro cultures were established from fruit bodies growing naturally in the forests of southern Poland. The aim of this study was to optimize the conditions of submerged culture for mycelial biomass increments (MBI) during 4 week cycles of culture. The optimal medium composition for submerged culture was determined. To investigate the effect of carbon and nitrogen source on hyphal growth, the mycelium was cultivated on the medium containing various carbon (fructose, glucose, maltose, lactose, sucrose) and nitrogen (amonium nitrate, sodium nitrate, casein hydrolyzate of, malt extract, yeast extract) sources. Additionally the optimum initial value of pH and optimal temperature for mycelial increment were determined. The optimal medium composition for biomass increment of *Sarcodon imbricatus* was 5% fructose (MBI=8.0 g d.w/dm^3) and 1% casein hydrolyzate (MBI=9.6 g d.w/dm^3). Maximal growth of biomass was observed at initial pH 6.0 (MBI=4.8 g d.w/dm^3) and the optimal temperature of incubation was 30°C (MBI=9.4 g d.w/dm^3). The optimal medium composition for biomass increments of *Sparassis crispa* was 5% glucose (MBI=13.7 g d.w/dm^3) and 1% casein hydrolyzate (MBI=7.95 g d.w/dm^3). Maximal growth of biomass was observed at initial pH 6.0 (MBI=9.92 g d.w/dm^3) and optimal temperature of incubation was 30°C (MBI=7.95 g d.w/dm^3). The optimal medium composition for biomass increments of *Hydnum repandum* was 5% glucose (MBI=11.0 g d.w/dm^3) and 1% casein hydrolyzate (MBI=14.7 g d.w/dm^3). Maximal growth of biomass was observed at initial pH=6.0 (MBI=9.8g d.w/dm^3) and optimal temperature of incubation was 25°C (MBI=9.6 g d.w/dm^3). Preliminary investigation of chemical compounds from these species showed the presence of endo- and egzogenic amino acids, fatty acids and phenolic acids. All these compounds were determined by chromatographic methods (TLC, HPLC).

These results show that submerged cultures of investigated fungi species could make a useful subject for future chemical analysis.

Molecular characterization of resynthesized oilseed rape (*Brassica napus* L.)

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Oilseed rape (*Brassica napus* L; genome AACC, 2n =38) is a relatively young species that originated through a spontaneous hybridization between turnip rape (*Brassica rapa* L.; genome AA, 2n=20) and cabbage (*Brassica oleracea* L., genome CC, 2n=18). Today oilseed rape is one of the most important oilseed crops in the world. However, its limited geographical range has led to a comparatively narrow genetic basis currently available for breeding. Resynthesized (RS) rapeseed genotypes developed through interspecific crosses between different *B. rapa* and *B. oleracea* genotypes have the potential to increase significantly the available gene pool and provide important germplasm base for further improvement of seed quality and resistance to biotic and abiotic stress.

Embryo rescue techniques considerably assist in obtaining distant hybrids in cases when hybrid embryos abort at early stages of development.

This work focuses on the development of interspecific hybrids between *Brassica rapa* L. subsp. *pekinensis* and *Brassica oleracea* L. var. *acephala* subsp. *lanciniata* through embryo rescue culture *in vitro*. The objectives of this research were: to develop an *in vitro* culture system for hybridization and to analyze the regenerated resynthesized plants using RAPD molecular markers.

Anti-inflammatory properties of transgenic flax fiber extract

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Flax (*Linum usitatissimum* L.) is a very important source of natural fibers used by the textile industry and also a source of valuable oil. Also seed cakes left after oil pressing contain many valuable components. Flax oil and seed cake meal is widely used for its health promoting properties. Many health-promoting components have been identified to date, including lignans, phenylopropanoids and Omega 3 fatty acids. However, most data concern compounds found in flax seeds.

We observed the anti-inflammatory and wound healing properties of wound dressing made of transgenic flax fibers and so we decided to identify compounds present in flax fibers responsible for those properties. Several components of fiber extract were identified by UPLC method, including phenolic acids and terpenoids. One of the most interesting compounds found were cannabinoid derivatives, not reported previously from flax fibers,. Therefore we concentrated our research on cannabinoid-like compounds know for their anti-inflammatory and analgesic properties. The effects of flax fiber extract on the expression levels of cannabinoid receptor and several pro- and anti -inflammatory genes were tested in mouse 3T3 Balb cell line in which the inflammation state was mimicked by LPS or TNFa treatment. The gene expression was estimated by means of semi-quantitative PCR. The most interesting finding was the induction of anti-inflammatory SOCS-1 (suppressor of cytokine signaling) gene and a decrease in the expression of Interleukin 6 and MCP-1 (monocyte chemotactic protein1) genes responsible for propagation of inflammation. Thus anti-inflammatory effects of flax fiber extracts were confirmed and it was shown that at least some of the effects are caused by activation of endocannabinoid signaling pathway.

Effect of 5-azacitidine on DNA methylation pattern and somatic embryogenesis in *Arabidopsis*

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Methylation of DNA is an epigenetic feature, controlling gene expression and maintaining proper functioning of cells. The aim of this work was to evaluate the pattern of DNA methylation in embryogenic culture of *Arabidopsis thaliana* (L.) Heynh. To evaluate DNA methylation level Methylation Sensitive Amplified Polymorphism (M-SAP) method was used. The method is a modification of AFLP technique taking advantage of enzymes sensitive to DNA methylation: *MspI* and *HpaII*. Moreover, the influence of 5-azacitidine (5-AzaC) on embryogenic capacity of the culture was evaluated. 5-Aza-C is an analogue of cytosine nucleoside and thus changes the level of methylated cytosine in DNA.

Embryogenic cultures of *Arabidopsis* were derived from immature zygotic embryos of Col-0, the ecotype possessing a high capacity for somatic embryogenesis (SE). The standard conditions for induction of embryogenic culture in *Arabidopsis* were used (Gaj, 2001) and in addition to the basal induction medium (E5), media supplemented with 5-Aza-C (8 μM and 10 μM) were used. A significant reduction in SE efficiency (64.7%) and productivity (61.5%) was observed on E5+8 μM 5-AzaC medium while 10 μM of 5-AzaC resulted in a total inhibition of the embryogenic response. M-SAP-based experiments involved 26 primer combinations analyzed at various time points (0, 5, 15 and 30th day) of embryogenic cultures induced on E5 and E5+8μM 5-AzaC media. The results confirmed that 5-AzaC influences the level of DNA demethylation as 142 new short DNA fragments in 5-Aza-C treated tissue were observed. This indicated that the observed negative impact of 5-AzaC on embryogenic capacity of cultured tissue resulted from a reduced level of DNA methylation. Moreover, DNA methylation patterns at various time points of SE culture were compared to identify the loci differentially methylated during the time course of SE. In total 1108 loci were analyses but none of them were found to be polymorphic in the standard embryogenic culture. The analysis of culture induced on E5+5-AzaC medium revealed one polymorphic DNA fragment which indicated a demethylation event on 5th day of culture.

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Identification of culturable and non-culturable endophytic fungi isolated from shoots of *Huperzia selago* (L.) Bernh. ex Schrank & Mart. (*Lycopodium selago* L.)

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Gametophytes as well as sporophytes of *H. selago* are colonized by endophytic fungi (Higgins et al., 2007; Winther and Friedman 2008). The class Dothideomycetes is the main group of fungi mentioned among the endophytes of club-moss sporophytes (Higgins et al., 2007). Some representatives of this group are known as dark septate endophytes -DSE (Schmid and Oberwinkler, 1993).

In recent years, the pharmaceutical industry has been becoming increasingly interested in huperzine alkaloids found in some club-mosses (genus *Huperzia*). Excessive harvesting of sporophytes as a source of huperzine has resulted in a marked decline of these plants in some countries. Studies are now being conducted that focus on the use of *in vitro* cultures for propagation of plant material for pharmaceutical industry. *In vitro* cultures of club mosses may also contribute to *ex situ* protection of these plants. However fungal contamination is a serious problem that causes severe loss of *in vitro* grown cultures of a number of plants. This problem becomes even more acute if the fungal contamination is of endophytic origin. In such cases, identification and characterization of the contaminants is essential for achieving specific control of the contaminants through selective use of antibiotic agents, especially if the routinely used contamination control methods practiced elsewhere in tissue culture studies are ineffective (Kulkarni et al., 2007). Such is the case with the fungal contamination observed in the present study.

The aim of the present study was to detect the presence of endophytic fungi in *in vitro* grown shoots of *H. selago* originating from different European sites (Szypuła et al., 2005, 2006). From 420 tissue fragments, 132 isolates representing 13 species of endophytic fungi were recovered in culture. The isolated endophytes belonged to the Zygomycota (1 species) and Ascomycota (12 species). The representatives of the Ascomycota phylum belonged to 3 classes: Dothideomycetes, Soradriomycetes and Eurotiomycetes. The most abundant taxon were members of Sordariomycetes. Representatives of Dothideomycetes were detected most often in shoots originating from mountain sites.

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Plant regeneration from liquid root culture of *Rumex acetosa* L. Analysis of genetic variability: preliminary studies

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Rumex acetosa L. is one of the few dioecious species that have sex chromosomes (2n = XX + 12 in females, 2n = XY_1Y_2 + 12 in males). *In vitro* culture of *R. acetosa* cv. Lionski adventitious roots was initiated and maintained on a hormone – free, liquid MS medium, containing 1/2 strength macronutrients (Mosiołek et al., 2005). This long – term root culture was genetically stable. Recently, we were successful in obtaining a method of high efficiency plant regeneration (via indirect organogenesis) *in vitro* from roots of *Rumex acetosa*. Root fragments (approx. 5 mm.) derived from liquid male root culture (RAY1 and RAY2 lines) were placed on MS medium (solidified with agar) supplemented with growth regulators: 2,4-D, NAA, kinetin, BAP and TDZ at different concentrations and combinations. The highest frequency of callus induction was found on MS + 0.5 mg/l TDZ medium after 5–6 weeks of culture. Callus culture maintained on MS medium with 0.5 mg/l TDZ resulted in a high frequency (90%) of shoot formation after 6 weeks. Regenerated shoots were isolated and inoculated onto a rooting medium (1/2 MS + 2% sucrose + 0.5 mg/l IBA) that had been proved to be optimal for root induction among the tested media. Complete plants with well developed root systems (after 2 weeks of culture on the rooting medium) were transferred to soil and acclimated to *in vivo* conditions in a phytotron chamber and then in the field. *In vitro* culture method of *Rumex acetosa* with a high frequency of plant regeneration was used for a preliminary analysis of genetic variation. Genomic DNA was extracted (using CTAB buffer) from plant material representing each of the following culture steps: roots from liquid culture, callus, plants regenerated *in vitro* from callus and plants acclimated *in vivo*, and analyzed using RAPD markers.

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Cytogenetic characteristics of the interspecific somatic hybrids of *Solanum villosum* (+) *S. tuberosum*

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Solanum villosum, a wild tetraploid (2n=2x=48) non-tuber bearing species resistant to *Phytophthora infestans* could be a potential source of resistance genes for cultivated potato. Since *S. villosum* is genetically and therefore reproductively isolated from *S. tuberosum*, the fusion of leaf mesophyll protoplasts was used to produce interspecific somatic hybrids between *S. villosum* and the diploid *S. tuberosum*. The objective was to transfer the late blight resistance genes from *S. villosum* into the susceptible potato. The expected ploidy level of the hybrids was 6x, but the real ploidy of the regenerants varied from 4x+ to 6x. The ploidy level was estimated by the direct method of counting chromosomes in metaphase plates of the root tip meristem cells. Analyses showed that the most vigorously growing hybrid plants were heksaploid, although all hybrids were quite similar morphologically and resembled the dominating wild tetraploid species. Selected euploid hybrids will be assessed for their resistance to *P. infestans.*

Acknowledgement: The research was supported by the Polish Ministry of Science and Higher Education as project PBZ-MNiSW-2/3/2006/33.

Bioactive secondary metabolites in *in vitro* cultures of *Eryngium alpinum* L.

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Eryngium alpinum L. (Apiaceae), an endangered subalpine perennial is easily recognizable by its blue amethyst coloured flowers heads. This taxon is protected all over Europe and cultivated mainly for decorative purposes. Rosmarinic acid with antioxidant activity has been detected in this species (Le Claire et al., 2005). *E. alpinum* in natural populations produces very few seeds that become dormant soon after harvest, so their germination is poor (Gaudeul and Till-Bottraud, 2004).

Plant material regenerated from *in vitro* cultures offers an opportunity to carry out phytochemical investigations of rare or vulnerable plants without collecting them from natural sites. Moreover, *in vitro* techniques could be used to complement *ex situ* conservation of endangered species.

The aim of our study was to establish *in vitro* cultures of *E. alpinum* and to conduct a preliminary phytochemical study. Seedlings obtained from seeds that had been isolated from fruits after their stratification and scarification, were used for initiation of *in vitro* cultures. Shoot culture was developed via the induction of axillary buds from shoot tips and established on MS medium supplemented with BAP (1.0 mgl^{-1}) and IAA (0.1 mgl^{-1}). The shoots were rooted on MS medium containing IAA, IBA or NAA (0.1 mgl^{-1}). Root cultures were obtained from root tips placed on MS liquid medium enriched with auxins (0.5 mgl^{-1}) and were grown in 300 ml flasks on a rotary shaker, in darkness.

To detect the presence of selected secondary metabolites, ethanol extracts from shoot and root cultures were analyzed chromatographically (TLC) on cellulose and silica gel F_{254} in different mobile phases. Spray specific reagents were employed for detection of phenolics, flavonoids and saponins. We showed that both types of *in vitro* material were able to produce those bioactive compounds.

The study was also undertaken to develop some *in vitro* techniques for conservation of *E. alpinum*.

Acknowledgements: This work was supported by the Ministry of Science and Higher Education, Warsaw, Poland (Grant NN 405 065334)

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The regeneration of sugar beet (*Beta vulgaris* L.) plants from unfertilized ovules cultured *in vitro*

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The aim of the experiment was to improve the effectiveness of regeneration of sugar beet (*Beta vulgaris* L.) plants from unfertilized ovules cultured *in vitro*. A two-step method was applied: (1) culture in liquid medium that initiated meristem differentiation, followed by (2) transfer of explants onto Murashige and Skoog media solidified with agar or gelrite, which stimulated shoot development and growth.

During the experiments 30 media were tested in terms of their influence on shoot formation. The media differed in the composition and content of growth regulators, carbohydrates and gelling compounds. The best efficiency of organogenesis was observed when 4.4 μM BAP, 0.09 M glucose and 0.7% agar were used. Root formation was induced after addition of 14.8 μM IBA and 0.049 μM 2iP, and the rooting rate ranged between 0.0 and 65.2% depending on the experimental treatment. After planting into pots and several weeks of acclimation in the greenhouse, the plants were transferred to the field, where their morphological characteristics were observed.

The ploidy level analysis of 413 regenerants performed by flow cytometry showed that 32.7% of plants were haploids, 45.8% diploids and 21.5% mixoploids. Haploid plants had narrower and more extended leaves, longer petioles and smaller roots in comparison to diploids. It also turned out that in some plants the chromosome number doubled spontaneously.

Detailed DNA study with the RAPD method revealed large differences among the regenerants. There were 13 polymorphic sequences identified in total, and 11 of them typified only the diploids' DNA.

The effect of cytokinins on morphogenetic responses of *Polemonium caeruleum* seedling explants

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The aim of this study was to estimate the morphogenetic response of *Polemonium caeruleum* explants. The donor material were 10-day-old seedlings. Surface sterilized seeds were germinated on MS medium (Murashige and Skoog, 1962) supplemented with GA_3 (1 mg·dm^{-3}). Seedling explants (shoot tips, fragments of cotyledons, hipocotyls and roots) were isolated and transferred onto solidified MS medium supplemented with different types of cytokinins (BA, Kn, Zea, 2iP) at concentrations of 1.0, 3.0 and 5.0 mg·dm^{-3} in combination with NAA (0.1 mg·dm^{-3}).

All explant types produced callus proliferation. It was observed that calli developed on the entire surface of hipocotyl and root fragments. On the other hand, shoot tips and cotyledonary petioles formed callus tissue at the cut ends, and petioles only at abaxial ends. The growth of calli on all explant types was strongly stimulated by Zea. Among the explants tested, only shoot tips exhibited shoot organogenesis. The highest frequency of shoot organogenesis was observed if the explants were cultured on medium supplemented with 5.0 mg·dm^{-3} BA (100%) or 5.0 mg·dm^{-3} Zea (97%). The highest shoot number per explant (8.4 on average) was obtained in the presence of 5 mg·dm^{-3} Zea. The presence of BA or Zea in the proliferation medium inhibited rhizogenesis and the elongation growth of shoots. However, root organogenesis was supported by exposure to Kn.

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Capacity for shoot organogenesis in cultures of *Arabidopsis* hormone-response mutants

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Plant hormones are considered to be the key factors involved in plant morphogenesis *in vitro*. Mutants featuring altered hormonal responses are useful in studies of molecular mechanisms involved in *in vitro* induction of plant morphogenesis.

In *Arabidopsis*, adventitious shoots can be efficiently regenerated via indirect organogenesis induced in the culture of root explants. The objective of this work was to evaluate the shoot organogenic (ORG) capacity of root explant cultures of 32 mutants defective in responses to plant hormones. The studied genotypes included auxin (7), ABA (12), cytokinin (2) and gibberellin (11) mutants and their parental genotypes (Columbia, Landsberg erecta, Wassilewskija). Explants isolated from 14-day old sterile plants were preincubated on an auxin-rich liquid callus induction medium (CIM), and then transferred to a cytokinin-rich shoot solid induction medium (SIM) according to Feldman and Marks (1986) method. The capacity for ORG was evaluated in 4-week old cultures and the ORG productivity (average number of shoots produced per 1 cm of explant) was evaluated.

The majority of the analyzed mutants (84%) displayed ORG productivity at the level similar to their parental genotypes. However, 5 of the analyzed mutants displayed distinct defects in the ORG response. Three of these mutants: *aba1-3*, *abi4-1* (ABA) and *axr4-1* (auxin) showed a significant decrease in organogenic response, while in two other mutants: *hyl1* (ABA) and *pkl1* (gibberelin) shoot regeneration was completely inhibited.

Except for the *abi4-1*, all mutants impaired in ORG (*aba1-3, hyl1, axr4-1* and *pkl1*) were previously reported to display significant defects in their capacity for somatic embryogenesis (Gaj et al., 2006) implicating the involvement of the mutated genes in basic cellular processes governing plant cell growth and differentiation.

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Evaluation of regeneration efficiency of selected double haploid lines of rape (*Brassica napus* ssp. *oleifera*)

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Regeneration, determined by the genotype and environment, is the most important process leading to production of plants under *in vitro* conditions. Genus *Brassica* belongs to the family *Brassicaceae* whose representative species regenerate *in vitro* with a high efficiency (Wojciechowski, 1998).

The aim of this research was to characterize the regeneration efficiency of 14 doubled haploid lines of rape (*Brassica napus* ssp. *oleifera*) that were expected to show a range of different tissue response *in vitro*.

Plant material consisted of cotyledon and hypocotyl explants gathered from 6-day old seedlings. Explants were placed on basic MS medium (Murashige and Skoog 1962) with the addition of 1 mg/l or 10 mg/l BAP. Evaluation of regeneration efficiency was conducted after 28 days of culture and was based on the number of explants regenerating shoots in relation to the total number of explants used. The examined genotypes varied in their regeneration ability.

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Efficient shoot organogenesis induced in the culture of immature zygotic embryos of *Arabidopsis*

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To study the genetic determination of somatic embryogenesis induced in *in vitro* cultures, the model plant *Arabidopsis thaliana* is widely used because of its advanced genomics and the availability of numerous mutants. In the model system for SE analysis, immature zygotic embryos (IZEs) of *Arabidopsis* provide an efficient explant source (Gaj, 2001). The aim of this study was to establish *in vitro* conditions for IZE culture that would promote adventitious shoot organogenesis as an alternative to the SE mode of plant regeneration. The IZE explants of commonly used genotypes, Columbia (Col-0) and Wassilewskija (Ws), were analysed and four methods differing in media sequence and composition (ZOR1 – ZOR4) were tested. Efficient shoot regeneration was observed when cotyledons of IZEs were cultured on MS medium supplemented with B5 vitamins and plant growth regulators: 1 mg/l BAP and 0.1 mg/l NAA (ZOR3); however the method was found to be genotype-specific. Explants of Col-0 cultured with the use of ZOR3 media developed shoots at 65% rate while the protocol was much less efficient for Ws culture (15% of responding explants). An improvement of Ws explant response was achieved when 7 day pre-culture of the explants in CIM (0.5 mg/l 2,4-D; 0.1 mg/l kinetin) medium was employed (ZOR4). Additionally, ZOR4 media resulted in an increase of average culture productivity and more rapid shoot formation. The regenerants developed into fertile plants upon culture on hormone free medium.

Moreover, the expression of embryogenesis-specific *LEC* genes (*LEC1*, *LEC2* and *FUS3*) was monitored with the use of RT-PCR. The analysis was performed at various time points of the culture induced by the ZOR4 method. In contrast to IZE-derived embryogenic culture, in which activity of the *LEC* genes was detected up to 30d of culture (Ledwoń, 2008), in IZE culture induced for shoot ORG, the expression of *LEC1* and *FUS3* genes disappeared gradually and vanished by 7 and 21d, respectively.

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In vitro culture of *Cordyline australis* (G. Forst) Endl.

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Cordyline australis "Red Star" with it's beautiful and attractive red decorative foliage is one of the most important ornamental house plant. Since the growth of plants in genus *Cordyline* is very slow, the interest in using *in vitro* propagation is increasing (DeMason and Wilson, 1985).

Shoot apices of about 5 cm length used for this study were excised from plants growing under greenhouse conditions. After removing the apical sheathing leaves, explants were washed thoroughly and surface sterilized for 20 min with 20% Domestos, followed by three rinses in sterile distilled water each for 5 min and a second sterilization with 2% Domestos for 5 min. Explants (about 1 cm long) were isolated and transferred to culture vessels. Full strength MS (1962) medium containing 3% sucrose and 0.7% agar was used as the basal medium.

Two different cytokinins: BA (5 μM i 17 μM) and zeatin (5 μM) were tested for shoot bud induction in combination with 0.5 μM NAA. For the development of shoot buds from the shoot apex, positive responses were observed only in the culture media that were supplemented with the lower concentration (5 μM) of BA. Shoot buds began to appear after 12 weeks in culture and the highest frequency of explants forming shoots was 55%. The effect of BA was quite inhibitory when used at the higher concentration (17 μM). Small clusters of shoots (10–15 shoots in each cluster) were subcultured to the proliferation medium containing four different cytokinins: BA, Zeatin, meta-Topolin and 2iP. Maximum increase in shoot number was 15.80 after four weeks in culture. It occurred in media containing a combination of 5 μM zeatin and 0.5 μM NAA. Tiny shoots, when transferred to a fresh medium continued to produce adventitious shoots.

Individual shoots were cultured separately in the rooting medium enriched with IAA or IBA and containing 0.01% activated charcoal. Most of the plants showed normal and healthy growth after they were transferred to pots.

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In vitro culture of *Dracocephalum moldavica*

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Dracocephalum moldavica L. (Lamiaceae) is an annual plant native to Central Asia and naturalized in Central and Western Europe. *D. moldavica* has been reported to be used as a food ingredient as well as tea and a herbal drug for the treatment of stomach, kidney and liver disorder in folk medicine. The spectrum of *D. moldavica* effects is ascribable principally to the presence of an essential oil, phenolic acids, flavonoids and tannins.

In the investigation reported here, we attempt to induce callus and suspension cultures. Callus cultures of *D. moldavica* were initiated from hypocotyls and roots of 4-week-old seedlings. The explants were incubated on Murashige and Skoog agar (0.7%) medium with 2,4-D or NAA at various concentrations (0.1, 0.5 or 1.0 mg/l). The most abundant callus proliferation was observed on root explants, after a 3-week culture on MS medium with 2,4-D at 0.5 mg/l. In addition to callus proliferation, somatic embryo formation was also observed. Suspension cultures were initiated by transfer of the callus tissues into MS liquid medium supplemented with 0.1 or 0.5 mg/l 2,4-D and 0.1 mg/l BAP. Suspension cultures with high biomass production were established on medium with 2,4-D at 0.5 mg/l. Cell suspension growth curves were obtained.

Influence of growth regulators and environmental factors on shoot multiplication of *Camellia japonica in vitro*

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Camellia japonica is known to be relatively recalcitrant to tissue culture. Strong apical dominance, shoot-tip necrosis, leaf browning and difficulties with rooting are the main problems, on which this study was focused.

Shoot cultures were initiated from axillary buds and shoot tips collected from a juvenile plant of *Camellia japonica*. MS medium supplemented with 0.1 mg l^{-1} BAP i 0.5 mg l^{-1} GA_3 was used. The aim of this investigations was to determine the influence of growth regulators (MemT, TDZ, GA_3) and environmental factors – temperature (15°C, 20°C) and sucrose (5, 10, 20, 30 g l^{-1}) on the growth and development of *Camellia japonica* shoots. The experiments were carried out with cooled (15°C) and non-cooled shoots (20°C). The highest multiplication rate and high quality of shoots were noted on the WPM supplemented with MemT (0.5 mg l^{-1}), TDZ (0.01 mg l^{-1}), GA_3 (1.0 mg l^{-1}) and sucrose at concentrations of 10–20 g l^{-1}. The higher sucrose concentration stimulated elongation and strong lignification of shoots. Low sucrose concentration, as well as the 15°C temperature regime decreased growth of *Camellia* shoots. The addition of GA_3 to the medium together with cytokinins and 10–20 g^{-1} sucrose activated axillary shoot development and leaf formation of both cooled and non-cooled shoots.

Pectin metabolism in *Fusarium*-infected flax seedlings

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Flax (*Linum usitatissimum* L.) is a common crop, highly valued as a source of fibre, oil and linseed. The greatest losses in flax crops are caused by fungal diseases. *Fusarium culmorum* and *Fusarium oxysporum* are fungal pathogens that cause the most severe and imminent infections of flax. Two subtractive cDNA libraries were constructed from flax seedlings treated with salicylic acid or infected by *F. oxysporum*. Further analyses revealed two sets of gene sequences, which are potentially involved in flax defence responses. In order to ascertain the level of expression of these genes, 9-day old flax seedlings infected by *Fusarium culmorum* and *Fusarium oxysporum* were subjected to semi-quantitative reverse transcription PCR analysis. Metabolite profiling was performed by means of GS-MS and UPLC analysis and revealed major changes in several metabolite groups such as phenolic acids, amino acids and sugars. Among genes found in the subtractive libraries, two (UDP-D-glucuronate 4-epimerase and formate dehydrogenase) are of particular interest to us, as they are connected with cell wall sugar polymers in plant. UDP-D-glucuronate 4-epimerase delivers the main substrate for pectin polysaccharide biosynthesis. We have determined that the expression level of UDP-D-glucuronate 4-epimerase gene was decreased after the *Fusarium* infection. However, the content of pectins remained unchanged. Formate dehydrogenase participates in the metabolism of formic acid, a toxic side-product of pectin demethylation. The expression level of formate dehydrogenase gene increased after the *Fusarium* treatment, which can be due to formate release from pectins under the influence of fungi. This observation is supported by research on transgenic plants overexpressing pectin degrading enzymes, where the level of formic acid is elevated. Our research point to early changes in flax plants, especially in the cell wall, occurring after the *Fusarium* treatment.

Regeneration of shoots from root explants in *Mammillaria carmenae* Castañeda (Cactaceae)

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Micropropagation of cacti from adult tissues has been difficult because of the low organogenic potential and heavy microbial contamination of potential explants, such as areoles or stem segments. Another problem is the damage to the stock specimen associated with collecting explants from these stem-succulent plants. Two alternative sources of explants are flowers and roots. Whereas a complete cycle of micropropagation from flowers has been demonstrated for three *Mammillaria* species (Wyka et al., 2006, 2009), the only report of micropropagation from roots concerns root explants harvested from *in vitro* culture of *Coryphantha elephantidens* (Bhau 1999).

We attempted to use root tips of pot-grown *M. carmenae* rinsed with 70% ethanol followed by disinfection in NaOCl (0.05–0.1%), CaOCl (2–5%), or $HgCl_2$ (0.1%). In no case was there a viable root culture obtained because of persistent heavy contamination and the resulting mortality of explants. Irrigation of the plants with fungicide did not improve the outcome.

In contrast, when 10 mm long root tips were harvested from existing long-term perianth-derived shoot cultures of *M. carmenae* and transferred to MS medium containing 30 g l^{-1} sucrose, 1 mg l^{-1} kinetine and 0.5 or 2.0 mg l^{-1} 2,4-D, they became hypertrophic and produced white calli that soon greened up. When calli were subdivided and transferred to regeneration medium (MS containing 30 g l^{-1} sucrose, 0.1 mg l^{-1} NAA and 5 or 10 mg l^{-1} BAP or kinetin), within 12 weeks shoot organogensis was recorded. At 24 weeks after transfer, the greatest efficiency of shoot formation (1.7 shoots per callus) was on medium containing 0.1 mg l^{-1} NAA and 5.0 mg l^{-1} BAP. Root-derived shoots were then used to re-establish a proliferating shoot culture. New shoots were rooted in perlite saturated with MS medium containing 0.1 mg l^{-1} NAA and later acclimated to *ex vitro* conditions.

Our results confirm the earlier finding by Bhau (1999) that roots of Cactaceae are a viable source of explants and point to the need to develop novel disinfection methods.

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Intergeneric hybridization between *Salix fragilis* and *Populus* spp. *in vivo* and *in vitro*

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As a result of experimental pollinations *in vivo* and *in vitro* between species of *Salix* and *Populus*, embryos and plantlets were obtained from crosses of *Salix fragilis* × *Populus tremula* and *Salix fragilis* × *Populus simonii*. Fully developed stigmas were pollinated with pollen grains of six poplar species: *Populus tremula, Populus simonii, Populus alba, Populus trichocarpa, Populus violascens* and *Populus nigra*. Under in vivo conditions, pollen grains were placed on stigmas using pencil tip. For pollinations *in vitro*, basal catkin segments were disinfected for 10 s in 70% ethanol, 4–5 min in chlorine water and washed three times in sterile water. After pollination, catkins were placed on basal MS medium. At 24 hours after pollination pollen germination occurred and 3 DAP pollen tube growth as well as style and ovule penetration were observed. Globular embryos were formed 5–6 days after pollination. After 18–24 days fully developed embryos were transferred aseptically to MS-based medium modified by the addition of l mg l^{-1} KIN. Three week old plantlets were transferred on MS-based medium supplemented with 0.2 mg l^{-1} NAA. Both media contained 3% sucrose and were solidified with 8 mg l^{-1} agar and their pH had been adjusted to 5.8 before autoclaving. Plantlets that possessed 3–4 leaves were transferred into pots containing a mixture of sand and peatmoss. Fully developed embryos (18–21 days after in vivo pollination) died after 2–4 weeks from transfer to MS medium.

The influence of iron source in red raspberry cultures on the chlorophyll contents and histology of the leaves

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Iron is an essential nutrient for plants and is involved in fundamental metabolic processes: photosynthesis and respiration, growth regulator synthesis and many enzymatic reactions. Interveinal chlorosis of leaves is the most visible symptom of iron deficiency. Such chlorosis was observed in raspberry shoot cultures when a typical MS formula containing FeEDTA was used. The most chlorotic cultivars were also the most recalcitrant to adventitious regeneration. Supplementing the medium with FeEDDHA (i.e. a more photostable iron chelate) resulted in the elimination of chlorosis, an increase in the rate of shoot multiplication and rooting and an increase in the efficiency of adventitious leaf regeneration.

We compared the effects of FeEDDHA and FeEDTA on the chlorophyll contents and leaf histology of five raspberry cultivars: Beskid, Canby, Malling Seedling, Norna and Veten. Leaf samples were obtained after 4 weeks on shoot multiplication medium, after 4 weeks on rooting medium, and after 2 and 4 weeks of acclimation in the greenhouse. The chlorophyll content was measured spectrophotometrically. For histological observations leaf sections of 10-μm were stained with safranin and fast green.

FeEDDHA significantly increased the chlorophyll content at each stage of propagation (shoot multiplication, rooting and microplant acclimatization) in all cultivars, but the degree of increase was genotype dependent. Differences in leaf histology depended on the source of iron in the medium, and the stage of propagation. In leaves produced on the medium containing FeEDDHA, the parenchyma and palisade cell layers were thicker and the cells more abundant and condensed at the multiplication stage, whereas on the medium containing FeEDTA, such leaf structure was not observed until the rooting stage.

In vitro reproduction of *Drosera rotundifolia* and conservation plant genetic resources of this species

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All carnivorous plants that can be found in Poland are under legal protection. Recently, the number of their natural stands has been dramatically decreasing. The principal causes of their reduction include draining, exploiting and eutrophication of peat bogs. All possible measures to prevent populations of these plants from further decline should be taken.

The objective of our study was to develop a reproduction method of *Drosera rotundifolia* using *in vitro* cultures. Results of our experiments indicate the possibility of effective generative reproduction of *Drosera rotundifolia in vitro*, involving the production of small amounts of vital seeds. Our data confirmed earlier reports that seeds of this species require a stratification period for plants to develop normally. Moreover, it has been confirmed that 100% of plants survive in temperature 0–2°C, however, their growth is inhibited and also formation of winter buds can be observed. After transfer to a fresh medium and moving from refrigerator to a phytotron with temperature of 25°C, the plants resume growth. Storing *Drosera rotundifolia* at a reduced temperature is useful for preservation of their germplasm, maintenance of long-term cultures and development of a gene bank of this species *in vitro*. Examination of fertilization in *Drosera rotundifolia* confirmed other reports of self-fertilization in this species.

The contents of phenolic compounds in water collected from natural stands of *Drosera rotundifolia* did not show any relationship with the type of stand for a given population. Moreover, the use of liquid medium indicated that *Drosera rotundifolia* does not tolerate excessive amount of water, as the plants became curled and reddening of the plants could be observed, which is characteristic of mineral deficiency.

Chemical analysis of water taken from individual stands of populations that are developing dynamically or disappearing, did not show any significant differences in the contents of mineral compounds. This indicates the possibility that there could be other critical factors, not connected with the contents of nutrients in the water at the given stand.

Propagation of *Chamaedaphne calyculata* (L.) Moench *via* indirect somatic embryogenesis

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Leather leaf *Chamaedaphne calyculata* (L.) Moench is a wintergreen dwarf shrub belonging to family Ericaceae. In Poland it is a rare species and is under full legal protection. Because of the limited number of existing stands of this plant (presently 9 remaining of the 13 historically known stands), it is important to determine the possibility of *Chamaedaphne calyculata* propagation for the purpose of ex situ protection.

Somatic embryogenesis may be used for propagation of non-seed producing plants, sterile male or female plants and plants that do not respond to conventional propagation techniques. It can also be used for germplasm preservation of valuable genotypes or critically endangered species. According to current literature, there are only a few reports concerning somatic embryogenesis in the *Ericaceae* (eg. Vejsadova and Pretova, 2003; Antony et al., 2004). We tried to find if indirect somatic embryogenesis could be used for an effective propagation of *Chamaedaphne calyculata*. This study may have significant implications in the management of leather leaf in restoration and conservation programs.

Leaves removed from shoots derived from *in vitro* cultures were used as explants. Leaves were cut transversely and placed with the abaxial surface in contact with the medium.

Embryogenic callus production and initiation of somatic embryos was achieved using Gamborg's B5 (Gamborg et al., 1968) medium (with pH adjusted to 5.6) and supplemented with 3% sucrose, 0.7% agar, 10 μM TDZ and 5 μM IAA. Somatic embryos were removed from the parent tissue and transferred to Anderson's (1980) medium containing 2.28 μM zeatin for elongation. Root development occurred on hormone-free Anderson's (1980) medium. After transfer to a mixture of sphagnum-peat and perlite (3:1), approximately 65% of plantlets survived.

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