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In search for regulatory genes involved in the control of embryogenic induction in *Arabidopsis*

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Most plant cells, in contrast to animal cells, express amazing developmental plasticity allowing their reprogramming and manifestation of totiptency/pluripotency. Advances in our understanding of genetic mechanisms controlling plant totipotency are essentially based on studies on somatic embryogenesis (SE), the process in which already differentiated cells can reverse their developmental program and become embryogenic, giving rise to formation of somatic embryos and then, complete plants. Thus, deciphering of molecular determinants of the unique SE process can directly contribute to our understanding of genetic programme underlying the phenomenon of cell totipotency. Recently, molecular analysis of SE induced in *Arabidopsis thaliana*, a model species of plant genomics, have provided new data on genetic factors involved in embryogenic induction.

Among factors essential for SE induction, the regulatory genes, especially transcription factors (TF), are believed to play a key role. Beside TFs, miRNAs involved in TF genes regulation seem to be of special consideration for elucidation of a regulatory mechanism governing the induction of embryogenic development.

Thus, in search for genes essential for SE induction we focused on regulatory genes, mainly TFs and miRNA. Among TF genes of decisive function in SE-induction *LEAFY COTYLEDON2* (*LEC2*), a master regulator of zygotic embryogenesis was indicated (Gaj et al., 2005; Ladwoń and Gaj, 2009). The experimental evidence suggests that the molecular mechanism involved in *LEC2*–mediated control of embryogenic transition in somatic cells is related to auxin biosynthesis and signaling.

To identify novel SE-regulatory genes, different experimental approaches will be described with special consideration of gene expression profiling and mutant/transgenic lines analysis.

With the use of multi-qPCR platforms TF and miRNA transcriptomes of embryogenic vs non-embryogenic cultures were contrasted and TF genes of SE-specific expression were indicated. Moreover, it was found that the embryo-induction stage of SE is associated with the robust and significant changes in TF transcriptome involving over half of the TF genes active in embryogenic culture. A drastic up-regulation of a great majority (over 80%) of TF genes active in culture was noted and a significant difference in dynamics and pattern of TF gene expression between embryo-induction and embryo-formation stages was described. In contrast to SE induction, an advanced embryo-formation stage was found related to the attenuation and stabilization of TF transcript levels. Within TF genes significantly modulated under SE induction the genes involved in plant development, stress and hormone responses were found predominated. The genes of SE-specific expression pattern are being subjected to further functional analysis.

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Phytohormones in *Medicago* spp. somatic embryogenesis

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Somatic embryogenesis (SE), a multi-step *in vitro* regeneration process, is not only an efficient method of propagation of a number of biotechnologically important plant species including *Medicago*, but it also provides an ideal system for studies of early plant development. The sequence of events for SE as a morphogenic phenomenon is frequently expressed as distinct phases. Each of these phases is regulated by various intrinsic as well as extrinsic factors, and, therefore, a number of critical physical and chemical treatments are applied with appropriate schedules for this complex_regeneration system to be efficient. From of all these factors, plant growth hormones, particularly the growth stimulants (auxins and cytokinins) appear to play the most crucial role in SE. Most success achieved so far in understanding the mechanism involved in induction and expression SE in plants has been accomplished with model plant species such as carrot, white spruce and alfalfa. However, the knowledge on the role of other plant growth stimulators as gibberellins (GA_n) and, particularly, plant growth inhibitors, namely abscisic acid (ABA), jasmonates (JA_s) and ethylene (E), during SE is far from satisfactory. Concerning GA_s there are contrasting reports on their involvement in SE. In our model, plants *Medicago sativa* and *M. truncatula*. the exogenous and endogenous $GA_{s,}$ play a stimulatory role during the induction and differentiation of somatic embryos (Ruduś et al. 2002, data unpublished). ABA, jasmonates and ethylene, stress-related phytohormones, are synthesized in tissues of *Medicago sativa* L. cultured *in vitro* during the whole process of indirect SE, and the biosynthetic capacity changes substantially in distinct SE phases (Kępczyńska et al. 2009, Ruduś et al. 2006). The ability to ABA and JA synthesis is very low in callus and embryogenic suspension, whereas in differentiating somatic embryos it increases considerably. Another decrease in ABA and JA synthesis occurs in fully developed late-cotyledonary embryos. Surprisingly, low indole-3-acetic acid (IAA) contents in comparison to stress-related compounds [ABA, JA and its precursor 12-oxophytodineoic acid (OPDA)] were detected in petiole explants, embryogenic tissues and somatic embryos (Ruduś et al. 2006).

In the case of ethylene, its production by callus was the highest, but later during the proliferation phase declined (Kępczyńska et al. 2009). Globular, torpedo and cotyledonary embryos produced ethylene at a similar level, but this was only close to half of that generated by callus.

Any alternations in the contents of ABA, jasmonates and ethylene during distinct phases of SE proved to be unfavorable for subsequent somatic embryo production, suggesting that the level of biosynthetic activity in tissues is optimal for sustaining an appropriate course of this developmental process. And what is more, in the case of ethylene, it was demonstrated that not only its biosynthesis, but also its action is involved in the control of individual phases of SE in *Medicago sativa* L. (Kępczyńska and Zielińska 2011) Moreover, disturbance in ethylene biosynthesis and binding during proliferation of the embryogenic suspension and embryo development negatively impacted germination and conversion of coytyledonary embryos i.e. their vigor. Endogenous ethylene may also be involved in the regulation of *Medicago sativa* L. embryo germination and conversion by controlling starch hydrolysis through the influence on α-amylase activity and soluble carbohydrates metabolism.

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Functional analysis of bHLH109 transcription factor differentially expressed during somatic embryogenesis in *Arabidopsis*

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Among genes involved in somatic embryogenesis (SE), those encoding transcription factors (TFs) play a key role in the activation of an embryogenic program of development in somatic cells. Thus, to identify genes crucial for SE induction we analyzed the expression pattern of almost 1900 TF genes in embryogenic culture of *Arabidopsis* (Gliwicka et al. 2009). Within the genes of modulated expression in embryogenic culture, *bHLH109* displayed an SE-specific expression pattern. The gene belongs to a large TF family, whose members are involved in developmental processes in plants, including cell division and differentiation. However, the exact molecular function of the *bHLH109* gene is largely unknown.

The aim of the study was to reveal the function of *bHLH109* in SE. Immature zygotic embryos at a late cotyledonary stage of development were used as explants and alternative morphogenic pathways were induced *in vitro*, including somatic embryogenesis (SE), shoot organogenesis (Kraut et al. 2011) and seedling development. Real-Time qPCR was performed to evaluate *bHLH109* expression pattern during the studied morphogenic pathways. The obtained data suggest the involvement of *bHLH109* in SE considering: 1) differential and auxin-dependent pattern of expression during embryogenic culture; 2) drastically reduced level of transcription in non-embryogenic callus tissue; 3) a low activity of the gene in tissue induced towards shoot regeneration via organogenesis.

Moreover, the embryogenic capacity of a transgenic line with estradiol-induced *bHLH109* overexpression and an insertional mutant were evaluated *in vitro.*

In order to identify the targets of the *bHLH109* gene we used the Expression Angler tool and the candidate genes co-expressed with *bHLH109* and possessing DNA sequence bound by bHLH protein family were selected. The selected genes were evaluated in terms of their expression profile under various *in vitro* culture conditions. As a result, *MSL7* (Mechanosensitive channel of large conductance 7) encoding ion channel and *ECP63* (Embryogenic Cell Protein 63) of *LEA* (Late Embryogensis Abundant) family, were chosen for further functional analysis. To confirm the interaction between bHLH109 and the candidate target genes, expression patterns of the potential targets were analyzed in seedlings overexpressing *bHLH109.*

The study helps to decipher the gene network controlling the onset of embryogenic development in somatic cells cultured *in vitro*.

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The effect of GA_3 and paclobutrazol on somatic embryogenesis induction in *Medicago truncatula* Gaertn.

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Somatic embryogenesis (SE) is a process during which non-zygotic cells pass through cell dedifferentiation, without the gametes fusion, are able to produce embryos. Due to the ease modification of experimental systems, SE is a common model in a study of the process of embryogenesis in plants. Somatic embryogenesis is also used for clonal propagation, synthetic seed production and, increasingly, during *Agrobacterium* mediated transformation.

Medicago truncatula Gaertn. belonging to the legume is currently one of the most widely studied model plants for this taxonomic group. A sequenced genome (Young et al. 2011), an increasing number of genetic information and database resources make the translation of the processes on the physiological and molecular levels easier. The transferability of results to close relative of alfalfa, is of high agronomic value and is a highly desirable goal of many research teams.

Previous studies (Ruduś et al. 2002) showed that somatic embryogenesis of *Medicago sativa* L. requires the presence of endogenous gibberellins and its exogenous application during the induction has an inhibitory effect on the process. It also blocks its biosynthesis by inhibitor.

The effect of gibberellins (GA) and paclobutrazol (PBZ) added to the medium during the induction of SE was investigated. Leaf explants from embryogenic line M9-10a (Doque et al. 2006), *Medicago truncatula* were maintained for 21 days in a culture medium Shenk-Hildebrandt (SH) supplemented with 2,4-D (0.5 μM) and zeatin (1 μM). To study the influence of the gibberellins, the medium was enriched with gibberellin GA_3 (0.05-50 μM) and paclobutrazol (0.05-50 μM). At the next stage the callus tissue was transferred to MS medium containing no hormones and was maintained for another 21 days until the embryos differentiated. The application of GA_3 at all concentrations during induction led to a reduction in the quantity of the produced callus tissue. A similar trend was maintained in a number of somatic embryos. Paclobutrazol caused an increase in callus tissue mass, as well as somatic embryo production only at concentration of 0.5 μM. At concentrations 5 μM and higher, PBZ significantly inhibited both, the callus growth and the number of somatic embryos.

The results suggest that for somatic embryogenesis it is crucial to maintain a hormonal balance of endogenous gibberellins, and their abundance as well as their lack have an inhibitory effect on the induction of this process.

In the examined *Medicago trncatula* explants the amount of endogenous gibberellins was enough for SE but its reduction by PBZ improved the efficiency of this process.

The results of the induction stage are similar to the results of our previous studies on alfalfa, suggesting that there might be a similarity between the course of somatic embryogenesis in these plants. However, the differences occurring during the differentiation stage require a more detailed analysis.

In order to confirm our suppositions about the importance of the hormonal balance to the process, it is necessary to examine the content of endogenous gibberellins in our experimental systems Furthermore, in order to better understand the mechanism of the gibberellins action during somatic embryogenesis it is necessary to conduct analyses at the molecular level.

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Hormonal regulation of the process of tulip bulb formation *in vitro*

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In tulip micropropagation, the stage of bulb formation is prerequisite for acclimatization of micropropagules to *ex vitro* conditions. In our micropropagation system, the bulbing process is induced in shoots multiplied *in vitro* cyclically in the presence of thidiazuron (TDZ). In tulip, the process of storage organ formation (tuberization) proceeds similarly *in vitro* and in nature. There is, however, a general difference in the source of sugars needed for induction (sugar signal molecules) and filling storage organs (sugars as carbon source). In nature, abundance in plant tissue of the photosynthesis-derived sugars together with the inducing environmental factors trigger a sequence of biochemical, physiological, and finally morphological events leading to bulb formation. In the *in vitro* conditions, however, due to insufficient light intensity, sugars have to be provided exogenously. Thus, sugars in nature exist as multifunctional internal factors while *in vitro*, they are both internal and external factors. This can lead to certain disturbances in the *in vitro* tuberization. Therefore, success in storage organ formation in majority of geophytes often requires providing the proper growth regulators whose endogenous production is insufficient or too high (ABA, jasmonates, auxins, polyamines and inhibitors of gibberellin biosynthesis). Various geophytes have their own specific external inducing factors, being the requisites for storage organ formation. In micropropagated tulip, the low temperature and the high sucrose concentration in a medium constitute such storage organ inducing stimuli. The low level of endogenous gibberellins is another common factor which induces this process in the majority of geophytes, including tulip.

In the tulip micropropagation method, the stage of bulb development includes three phases and takes 9-10 months. The first phase, preparing shoots for bulb formation is performed through modifying and prolonging the last multiplication subculture prior to cooling for up to 14 weeks. The shoots develop dormancy and become more sensitive to the low temperature treatment. It was shown that this phase is associated with an increase in endogenous abscisic acid (ABA), as well as decrease in the endogenous levels of 1-indoleacetic acid (IAA) and active cytokinins in shoots. This phase proceeds at high (summer) temperature of 20°C. The second phase is the induction of bulb development by cooling shoots; it is associated with the release of shoot dormancy under low (winter) temperature treatment of 4-5°C for 12-14 weeks which is linked to a decrease in endogenous ABA and increase in both IAA and active cytokinins. In the third phase, shoots form bulbs on the sucrose-rich medium at high (spring) temperature of 20°C, that usually takes 2-4 months. The analysis of endogenous cytokinins revealed that high efficiency of bulb formation was associated with a transient increase in active cytokinin contents in the response to the cold treatment, and a subsequent decrease in their concentrations at the time when shoot bases began to swell. In such conditions, tulip shoots of majority of genotypes developed bulbs with efficiency higher than 80%. However, about 30-50% of these bulbs were irregular, not covered with tunic, and liable to desiccation. Therefore our attempts aimed at increasing bulbing efficiency through precise timing of treatments with some PGRs based on the analysis of endogenous hormones during the succeeding tuberization phases. Thus, improvement of bulb formation, was obtained by the applications of the following PGR treatments: 1) with paclobutrazol or ancymidol given before cooling of tulip shoots, 2) replacing TDZ with isopentenyl adenine in a medium during the last multiplication subculture prior to cooling, and 3) the treatment with MeJA used at the stage of shoot swelling commencement, six-eight weeks after cooling.

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Effect of various levels of sucrose and nitrogen salts on the growth and development of plants *in vitro*

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Among many environmental factors, carbon (C) and nitrogen (N) are crucial for the growth and development of plants. Both C and N nutrients are essential for building blocks of the cell and for various cellular functions. Current knowledge indicates that plants possess an interactive regulatory machinery that coordinates the capacity of nitrogen assimilation with carbon metabolism, nutrient availability and other environmental factors. Also, carbon and nitrogen are two signals that influence plant growth and development (Coruzzi and Bush, 2001, Coruzzi and Zhou, 2001, Starck, 2006).

The influence of sucrose (5, 10, 20, 30, 40, 60, 80 g l^{-1}), nitrogen salts – KNO_3, NH_4NO_3 (25%, 50%, 100% of standard MS strength) and temperature (15°C, 20°C and 25°C) on the growth and development of *Syringa, Clematis* and *Lilium in vitro* were investigated.

We have found that in *Syringa vulgaris* and *Clematis pitcheri* the axillary shoot outgrowth depended on the sucrose and nitrogen salts strength in the medium. For *S. vulgaris*, the highest number of axillary shoots was in the plantlets growing at a temperature of 20°C, on a medium with a low level of sucrose – 5 g l^{-1} and 100% strength of KNO_3 and NH_4NO_3 (Gabryszewska, 2011). Similar results were obtained for an apical stem culture of *C. pitcheri* in which sucrose in a concentration 10 g l^{-1} and 50% of KNO_3 and NH_4NO_3 strength stimulated axillary branching (Gabryszewska et al. 2008). Increased sucrose contents in the medium significantly reduced the axillary bud development in both species. By contrast, the high nitrogen salts strength in the medium appeared to counteract at least partially the inhibitory effect of high sucrose level and stimulated axillary bud outgrowth. There was clearly an interaction between sucrose and nitrogen salts such that a medium with a low sucrose-to-nitrogen ratio promoted axillary branching, whereas a medium with a high sucrose to nitrogen ratio inhibited the growth of axillary shoots.

In *Lilium* “Robina” and *L. martagon* var. album, increased sucrose levels and strength of nitrogen salts significantly enhanced the average fresh weight of lily bulblets (Gabryszewska and Sochacki, 2012). The highest fresh weight of bulblets was found on the media with high sucrose levels (60 and 80 g l^{-1}) and with 100% (for cultivar “Robina”) or 50% (for *L. martagon* var. album) strength of KNO_3 and NH_4NO_3. Increasing the concentration of sucrose in the medium led to a significant reduction in the number of leaves in lily bulblets. On the other hand, a high concentration of nitrogen salts can partially overcome the sugar-derived inhibition and stimulate leaf growth.

The results reported here show that different sucrose/nitrogen salts ratios in the MS medium exerted pronounced physiological effects that were reflected in the morphology of developing shoots (*Syringa, Clematis*) or bulblets (*Lilium*) *in vitro*.

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Influence of the media on the differentiation of grasspea (*Lathyrus sativus* L.) callus tissue

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Global warming, constantly diminishing water resources all around the world and a growing human population enforce the search of new strategies for sustainable agriculture. Consequently, scientists, breeders and farmers have turned their attention to the forgotten and underestimated crops that could improve food security (Polignano et al., 2005). One of such plant is grasspea (*Lathyrus sativus* L.) which exhibits, unusually among other legumes, resistance to abiotic and biotic stresses. Due to a high protein content in seeds, grasspea may become a valuable source of fodder protein in near future. However, negligence of its breeding in Poland causes that to expand its cultivation it is necessary to improve some of its unfavourable features. Because the reasons for incompatibility of interspecific crosses in *Lathyrus* genus are not known, its breeding is being hampered, making it impossible to obtain hybrids and introduce new desirable features to commercial varieties. Therefore conventional methods of genetic manipulations are inefficient and enforce application of biotechnological techniques including *in vitro* cultures. Tissue cultures, especially callus culture, generate a wide range of genetic variations that could be exploited in breeding programs. The potential of somaclonal variability should be fully exploited by breeders (Jain, 2001), especially in species with a limited gene pool to which grasspea also belongs. However, the development of indirect plant regeneration through callus tissue is often long and inefficient. This results, among others, from the changes occurring in the cells, such as point mutations, chromosomal rearrangements, methylation of DNA or activation of transposons (Jain, 2001).

In our stuies Polish varieties of grasspea were used as plant material. Experiments conducted in our department revealed that induction and proliferation of grasspea callus tissue are readily occurring processes. However, obtainment of indirect organogenesis or somatic embryogenesis has been much more difficult. Despite the application of many various media and combination of growth regulators as well diverse types of explant we were not successful in achieving shoot regeneration from callus. Notwithstanding the above, we frequently observed the formation of roots. A histological examination also revealed intensive differentiation of the callus tissue in which numerous meristematic centers and vessels were observed. The most important achievement of our research, until now, is obtaining the line of embryogenic callus in which we observed somatic-embryo like structures (ELSs).

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Culture of trichoblast derived stem cells

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When *Agrobacterium rhizogenes* Conn., a gram-negative soil bacterium infects a plant, the T-DNA between the TR and TL regions of the Ri-plasmid in the bacterium is transferred and integrated into the nuclear genome of the host plant. Hairy roots grow rapidly, show plagiotropic growth, and are highly branched on the growth regulator-free medium. The transformed root is highly differentiated and can cause a stable and extensive production of secondary metabolites. It is commonly known that other plant cell cultures have a strong tendency to be genetically and biochemically unstable and often synthesize very low levels of useful metabolites. Several decades have passed since the hairy root culture system, also called transformed root culture, was first established. However, the system has not been utilized globally in production. Many attempts have been made to develop economical large-scale bioreactors containing air-lifting, bubble column, mist, dual, wave reactors and others. Unfortunately, the currently established biotechnologically valuable culture systems continue to be based on flasks or small-scale bioreactors (Hu and Du 2006).

To resolve the bottleneck of the application of hairy roots, our research focused on the establishment of a more effective and economical, scaled-up new generation root culture system – a culture of trichoblast-derived stem cells (T-dSCs) that can reduce the technical problems and costs but obtain/provide the biggest benefits. We have demonstrated that a large-scale production of T-dSCs is feasible with the current state-of-the-art culture technologies (by utilizing scalable stirred-suspension bioreactors).

It is commonly accepted that in plants SCs are located at distinct regions called meristems. SCs are defined by their developmental capabilities, namely, self-renewal and multitype differentiation, but the biology of stem cells and their inherent features both in plants and animals has just begun to be elucidated (Grafi et al. 2011). On the other hand, cellular plasticity has evolved in plants as an adaptive trait providing many somatic cells with developmental flexibilities and with the capacity for dedifferentiation and acquisition of a pluripotent or even totipotent state.

Our study has revealed that root hair-bearing cells can be reprogrammed, following exposure to some external signals, resulting in dedifferentiation and acquisition of a transient, stem cell-like state. In a series of optimization experiments highly favorable in *in vitro* culture conditions were selected. As a result, culture of T-dSCs was established.

T-dSCs retain a clearly stable "epigenetic memory" of their cells of origin, which is demonstrated by their ability to overproduce riboflavin and differentiation (morphogenetic) capacity. Interestingly, it has been recently shown that induced pluripotent stem cells produced from different cell types retain, at least at early passages, a transient epigenetic memory of their somatic cells of origin demonstrated by their differential gene expression and differentiation capacity; at late passages these differences are attenuated (Kim et al., 2010).

Depending on the type of stimulus, T-dSCs can be induced to re-differentiate, trans-differentiate, re-enter the cell cycle or undergo a programmed cell death.

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LEAFY COTYLEDON2 overexpression and embryogenic capacity of *Arabidopsis* explants under treatment with polar auxin transport inhibitors (TIBA and NPA) and antiauxin (PCIB)

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LEAFY COTYLEDON2 (LEC2) gene encodes a transcription factor of a key role in the regulation of embryogenic development *in vivo* and *in vitro*. Overexpression of LEC2 induces a spontaneous formation of somatic embryos without exogenous auxin while auxin treatment impairs explant capacity for somatic embryogenesis (SE) induction (Stone et al., 2001; Ledwoń and Gaj, 2009). Hence, LEC2 is postulated to influence embryogenic induction in somatic tissue, possibly, via control of endogenous auxin level.

The aim of the study was to further elucidate the auxin-related mechanism of the *LEC2* function in SE induction. The experiments were conducted to determine the capacity for SE of explants overexpressing LEC2, in the presence of plant growth regulators (PGRs) disturbing auxin polar transport (TIBA and NPA) and auxin activity (antiauxin PCIB). The explants, immature zygotic embryos, were induced on auxin E5 medium supplemented with various concentrations of PGRs, including 5 and 10 μM of NPA or TIBA and 25, 50, 75 μM of PCIB. The capacity of the derived culture for SE was analyzed after 3 weeks. The efficiency of SE i.e. the frequency of somatic embryo-forming explants was evaluated in relation to the type and concentration of PGR used in the induction medium.

Plants of Arabidopsis transgenic line with dexamethasone(DEX)-induced overexpression of LEC2 (35S::LEC2-GR) were used as the explant source.

It was found that LEC2 overexpression drastically modified culture response under PGR treatment. Accordingly, TIBA, NPA and PCIB inhibited the embryogenic capacity of the control culture, while in contrast, SE efficiency of the explants overexpressing LEC2 was significantly increased.

The presented results provide further evidence that *LEC2* controls the embryogenic induction in a cultured somatic tissue via an auxin-related mechanism involving a positive regulation of auxin biosynthesis. This hypothesis was supported by the fact that LEC2-control activity of *YUCCA* genes involved in auxin biosynthesis was observed in the embryogenic culture, and also by the observed increase in auxin content in the tissue overexpressing LEC2 (Wójcikowska et al., 2011).

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Auxin-related control of somatic embryogenesis in *Arabidopsis* by *LEAFY COTYLEDON2*

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Somatic embryogenesis (SE) induced *in vitro* results in efficient plant regeneration and provides a valuable system in plant biotechnology applied in micropropagation and genetic transformation of different plant species. Hence, isolation and characterization of genes controlling efficient SE induction is important for practical applications. Moreover, identification of genetic factors involved in SE induction contributes to the knowledge on molecular mechanisms controlling the developmental plasticity of somatic cells.

Among genetic factors involved in SE induction, the genes encoding transcription factors, including the LEAFY COTYLEDON2 (LEC2) have been reported. *LEC2* is a central regulator of zygotic embryogenesis (ZE) and it also plays a key role in SE induction in *Arabidopsis* (Gaj et al. 2005; Ledwoń and Gaj, 2009). Numerous observations confirmed that *LEC2* is able to promote embryogenic induction in somatic cells, however the molecular mechanism involved in this developmental switch is known only fragmentary. We hypothesized that *LEC2* activity is associated with auxin biosynthesis and auxin signaling and our preliminary analysis indicated that *LEC2* may regulate *YUCCA* (*YUC*) genes encoding flavin monooxydases involved in tryptophan-dependent pathway of auxin biosynthesis (Wójcikowska et al. 2011).

The aim of the study was to analyze expression profiles of eleven *YUC* genes in Arabidopsis embryogenic culture. Moreover, considering the auxin-dependent *LEC2* expression (Ledwoń and Gaj, 2009) we selected *AUXIN/INDOLE-3-ACETIC ACID* (*AUX/IAA*) genes of *LEC2*-dependent expression. AUX/IAA proteins are repressors of auxin-responsive genes and six genes (*IAA1*, *IAA16*, *IAA17, IAA29*, *IAA31* and *IAA30)* were selected for an analysis based on the literature and experimental data. Three of them (*IAA1*, *IAA17* and *IAA30)* were found to be up-regulated during SE induced *in planta* by *LEC2* overexpression while the others (*IAA16*, *IAA29* and *IAA31)* exhibited SE-specific expression profile and insertional mutations in these genes decreased the embryogenic capacity of explants under *in vitro* culture.

The expression levels of the *YUC* and selected *AUX/IAA* genes were determined in relation to *LEC2* expression level. To this end, a transgenic line 35S::LEC2-GR with DEX-induced overexpression of LEC2 was used in the study, The results indicated that *LEC2* positively controls *YUC1*, *YUC4* and *YUC10* activity. Beside auxin-biosynthesis, also auxin-signaling genes, *IAA30* and *IAA31* were found to be LEC2-controlled in the embryogenic culture. Collectively, the analysis provided further evidences that LEC2 promotes SE induction in *Arabidopsis* culture via up-regulation of genes involved in auxin biosynthesis and signaling.

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The study on *MIRNA393* genes in somatic embryogenesis of *Arabidopsis thaliana*

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Somatic embryogenesis (SE) giving rise to efficient production of somatic embryo-derived regenerants has become a powerful method in plant biotechnology, and is widely applied in plant clonal propagation and genetic transformation. Beside transcription factors, microRNAs are believed to play a crucial regulatory role in developmental processes. Currently there are 291 *MIR* genes known in Arabidopsis, grouped into 186 families. Following the recent report on a key role of miRNAs in zygotic embryogenesis in Arabidopsis (Nodine and Bartel 2010), the central function of *MIRNA* genes in embryogenic transition *in vitro* can be assumed. However, the data on miRNA involvement in SE are very limited. The suggested in our study involvement of miRNAs in the SE regulation of *dcl1-9* mutant carrying an insert in *DICER-LIKE1* (*DCL1*) gene encoding an RNaseIII domain-protein required for processing of primary miRNA transcripts into mature miRNAs. The *dcl1-9* was found incapable of SE-induction *in vitro* and in auxin-induced culture of mutant explants only callus was formed. We also analyzed the involvement of *MIR393A* and *MIR393B* genes in SE. These genes were selected based on their differential expression during SE for functional analysis (Szyrajew *et al.* 2012). In support of our hypothesis of mi393 involvement in SE, the target of miR393, *AT1G63130,* was reported to be involved in zygotic embryogenesis of *Arabidopsis* (Nodine and Bartel 2010) and miR393 was implicated in signaling of auxin, a main inducer of SE process (Mockaitis and Estelle, 2008). The functional analysis of *MIR393* involved gene expression profiling with Real-Time RT-qPCR during a time course of SE (0, 5th and 10th days of the culture) induced in two genotypes of extremely different capacity for SE. The *tanmei/emb2757* mutant incapable of SE-induction and Col-0 ecotype highly efficient in SE induction were analyzed. Moreover, the embryogenic capacity of miRNA393 insertional mutants was evaluated *in vitro*. The obtained results provided several lines of evidence suggesting the involvement of *MIR393* in SE: 1) *MIR393A* and *MIR39*3B genes were found up-regulated in SE induction stage (53 and 27 folds, respectively); 2) both *MIR393* genes were down-regulated (about 2-folds) in *tanmei* mutant, defected in SE-induction; 3) miR393a, miR393b and miR393ab insertional mutants were found impaired in capacity for SE in terms of SE efficiency and productivity; 4) Expression of miR393 target – *AFB3* gene – in SE process was found to be auxin stimulated. Further analyses are needed to identify the targets of miRNA393 during the embryogenic transition *in vitro*.

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Localization of PIN1 proteins during somatic embryogenesis of *Arabidopsis thaliana*

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The aim of the research was to assay callus and somatic embryo formation ability and to determine localization of auxin gradient and expression of PIN1 proteins during somatic embryogenesis of *Arabidopsis thaliana.*

In zygotic embryogenesis, after zygote division auxin is first accumulated in the apical cell. After the 32-cell stage, the maximum of auxin concentration shifts basally into the uppermost suspensor cells, including hypophysis. At the transition stage, auxin is accumulated at the flanks of the apical domain where the cotyledons initiate (1). After three rounds of cell division of zygote, all eight proembryo cells express PIN1 without apparent polarity. At the globular stage, basal PIN1 polarity is established in the central lower cells of the zygotic proembryo, whereas PIN1 localizes apically in outer protoderm cells. PIN1 becomes subsequently polarly localized to the basal membranes in the provascular cells, next to the hypophysis. During the transition stage, PIN1 protein is localized at the flanks of apical embryo domain, which results in the presence of auxin maxima at these sites (1).

The plant strains used in this study include wild-type Columbia (WT Col), transgenic strains *DR5::GFP, DR5::GUS, PIN1:GFP, PIN1::GUS* and *35S::PIN1* (gain-of-function) of *Arabidopsis thaliana*. Zygotic embryos in the cotyledonary stage of the plants mentioned above were used as explants sources. They were cultured on induction medium (E5) containing basal B5 medium supplemented with 5 μM 2.4-D, 20 $g \cdot dm^{-3}$ sucrose and 3.5 $g \cdot dm^{-3}$ Phytagel; pH 5.8. After 3 weeks on E5 medium, explants were transferred to MS10 medium (MS salts and vitamins, 20 $g \cdot dm^{-3}$ sucrose and 3,5 $g \cdot dm^{-3}$ Phytagel; pH 5,8). The cultures were cultivated at 24°C under continuous light for 6 weeks.

Callus formation ability of zygotic embryos was investigated. Among the transgenic strains analysed, zygotic embryos of *PIN1::GUS* (51,36%) showed the greatest callus formation ability, whereas *PIN1:GFP* zygotic embryos (38,05%) showed the poorest, comparable with WT Col (45,35%). Almost all zygotic embryos of the transgenic plants considered, were able to indirect somatic embryogenesis (ISE). The greatest capability to ISE showed *PIN1:GFP* (26.52%) and the least capacity to ISE exhibited *35S::PIN1* (7.84%) in comparison to WT Col (21.37%). However, zygotic embryos of *35S::PIN1* plants showed the highest direct somatic embryogenesis ability. The highest average number of somatic embryos produced per one zygotic embryo was found in *PIN1:GFP* explants (11.32 ± 1.02). The lowest productivity was found in *35S::PIN1* (4.41 ± 0.53), compared to WT Col (8.24 ± 1.25).

During the following stage, the aim of the research was to localize the gradient of auxin and PIN1 expression. To achieve this, the transgenic strains *DR5::GFP*, *DR5::GUS* and *PIN1:GFP, PIN1::GUS* were used, respectively. In an embryogenic callus the gradient of auxin was observed in the outer layers of cells, but was not detected in the vascular tissue of the callus. At the torpedo stage of somatic embryos the auxin maxima was located in the initial cells of cotyledons. At torpedo and cotyledonary stages of somatic embryos the auxin was detected at the tips of cotyledons cells, in the outer layer of cotyledons cells on the abaxial side and at the root apex. In the embryogenic callus PIN1 were expressed in the internal layers of cells. That localization might cause auxin transport to the outer layers of callus cells. PIN1 were expressed in the central layers of globular somatic embryos cells. At the later stages of somatic embryos development PIN1 were expressed in cells adjacent to the vascular tissue.

On the basis on the results we can conclude that PIN1 proteins are involved in the transport of auxin during embryogenic callus and somatic embryos' vascular tissue formation.

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Spatio-temporal localization of pectic epitopes in *Arabidopsis thaliana* somatic and zygotic embryos

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The role of cell wall components, especially pectins, in somatic embryogenesis (SE) has been an interest over the recent years since dynamic changes of its occurrence and structure are believed to be correlated with the status of cell differentiation.

The aims of the presented studies were: 1) to define the temporal and spatial distribution of three pectic epitopes (recognized by JIM7, LM19 and LM5 antibodies) in *Arabidopsis thaliana* somatic embryos to answer the question if there is any correlation between the stages of development and the pectic epitopes distribution and 2) to compare the distribution of these epitopes between somatic and zygotic embryos in order to define if SE is equivalent to ZE in cell wall components aspect. Immature zygotic embryos in late cotyledonary stage (IZEs) of *Arabidopsis thaliana* (L.) Heynh Columbia ecotype were cultured on a liquid medium containing 5 μM 2,4-D to induce SE process, and after 10 d they were transferred on phytohormone-free MS liquid medium. For histochemical analysis 8-21 d old explants were fixed, dehydrated in ethanol series, embedded in Steedman's wax and cut into a series of 5 μm thick sections. Fixation and immunolabelling were performed according to Chen and Baldwin (2007, Plant Molecular Biology Reporter).

In the early globular somatic embryos, high methyl-esterified HG domains (JIM7 epitope) were detected in PEGs of protodermal outer walls and in some walls of inner cells. In contrast, low methyl-esterified HG (LM19 epitope) occurred most abundantly in the outer periclinal cell walls of embryo protodermis. LM19 epitope was also detected in walls of inner cells and in cell cytoplasm. The pattern distribution of high and low methyl-esterified HG was similar in the more advanced developmental stages of somatic embryos. A comparison of somatic embryos and their zygotic counterparts showed significant differences between the compositions of outer protodermal cell walls, JIM7 epitope occurred in outer periclinal walls of L1 layer of the SAM and in hypocotyl, and LM19 epitope was present in almost all outer protodermal walls. This was not observed in zygotic embryos.

The first occurrence of (1→4)-β-D-galactan (LM5 epitope) was observed in the globular stage of embryo development in the apical part of the embryo, in some proto- and subprotodermal cell walls. In more advanced developmental stages of somatic embryos, LM5 epitope was present in walls of cells from different tissues with most abundant occurrence in walls of dividing cells. Sometimes this epitope was detected in cytoplasm. The same LM5 epitope distribution was observed in zygotic embryos.

In summary, high and low methyl-esterified HG domains are developmentally regulated during SE. Different distribution patterns of JIM7 and LM19 epitopes point out to their distinct functions. (1→4)-β-D-galactan marks *de novo* synthesized walls and cytoplasmic localization could presumably indicate cells which are about to divide.

Secondary somatic embryogenesis in *Hepatica nobilis* Schreb. *in vitro* cultures

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Hepatica nobilis Schreb. is an evergreen hemicryptophyte found in deciduous forests for horticulture and landscape architecture due to shade-tolerance, early-spring flowering and decorative evergreen leaves. Its mass overharvesting in the wild had significantly limited its population and caused its extinction in some habitats. Consequently, *Hepatica* was included into the list of endangered species in Poland. Horticulturalists, however, are being reluctant from expressing a wider interest in *Hepatic*a mostly due to difficulties in its propagation. The low multiplication rate obtained when traditional vegetative methods are used has triggered off tests with *in vitro* techniques, particularly the induction of somatic embryogenesis, as the most efficient propagation method.

In our investigations, embryogenesis was induced on seedlings in *in vitro* cultures of hepatica. We observed differentiation of somatic embryos, conversion of somatic embryos and formation of secondary somatic embryos.

After surface disinfection (70% ethanol for 60 seconds, 15% Domestos for 10 minutes) the seeds of hepatica were placed on Murashige and Skoog medium without growth regulators, enriched with 30 g dm^{-3} sucrose and 0.7% agar, pH 5.7. The seeds were cultivated in a phytotron at 20°C/18°C (day/night), relative humidity 80%, 16 h day, PPFD 30 μM $m^{-2}s^{-1}$. After germination, the seedlings with radicles, hypocotyls and cotyledons were used for the induction of somatic embryogenesis. Three media containing 100% MS macro and microelements were used: 0.1 μM BA and 0.5 μM NAA; 0.5 μM BA and 0.5 μM NAA and growth-regulator-free medium. The culture conditions were the same as during *in vitro* germination. Direct formation of somatic embryos was observed mainly on the hypocotyl, and the highest efficiency (on 68% of seedlings) was noted on the media containing 0.5 μM NAA and 0.1 μM BA.

Somatic embryos originated from that medium were collected at different developmental stages: globular, torpedo and mature (late torpedo with distinct cotyledons). For further development, the embryos were placed on different MS media: without growth regulators (MS0) and enriched with: 0.5 μM NAA and 5 μM BA; 0.2 μM NAA and 2 μM BA; 0.1 μM NAA and 1 μM BA; 1 μM NAA and 1 μM BA. Growth conditions were the same as for the earlier induction of somatic embryogenesis.

Numerous deformations and vitrification of the embryos were observed when explants were grown on media with 0.5 μM NAA and 5 μM BA, independently of the developmental stage of the embryo. An appropriate conversion and growth of embryos was noted when a matured cotyledonary stage was used and the embryo was cultivated on two media: 0.2 μM NAA and 2 μM BA (78.25%); 0.1 μM NAA and 1 μM BA (65%).

All the studied stages of embryos cultivated on a growth-regulator-free medium (MS0) and with 1 μM NAA and 1 μM BA formed secondary somatic embryos. Numerous secondary embryos were noted on the globular stage (91.6% explants) and the torpedo stage (74% explants) cultivated on MS0 medium. The average number of secondary somatic embryos formed per one embryo at the globular stage was 5.4, torpedo 3.2 and cotyledonary 1.7, respectively. The obtained embryos were transferred to the same medium without growth regulators every 6th week and secondary embryos were constantly formed. A conversion of embryos into plantlets was possible on MS media with 0.2 μM NAA and 2 μM BA.

Somatic embryogenesis of cultivated lupin species

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The interspecific hybridization in genus *Lupinus* is assessed as very difficult approache, however obtaining single hybrid seeds is possible (Brejdak and Sawicka 1997). So, it seems necessary to develop an efficient micropropagation *in vitro* technique of such valuable materials. Unfortunately, the lupin is also regarded as one of the difficult species to establish in tissue culture (Rybczyński, 2001). Therefore, an important part of this study was to develop tissue culture micropropagation for hybrids: *Lupinus albus/ termis* x *Lupinus mutabilis* Mut-628, *Lupinus albus/vavilovi* x *L. mutabilis* Mut.45 and their parents and to determine the reaction of meristems and the embryonic axis to the applied culture conditions.

Apical meristems were used for tissue culture initiation. Sterilized explants (in 30% commercial bleach solution) were placed on the medium B_5 supplemented by growth regulators (in mg/l): B_5 + 1.5 BA, B_5 + 1.0 NAA, B_5 + 3.0 IBA, B_5 + 1.0 NAA, B_5 + 1.5BA + 3.0 IBA.

The most favorable conditions allowed the culture to regenerate plants from meristems *L. albus/ termis, L. mutabilis* MUT-628 and the hybrid (*L. albus /termis* x *L. mutabilis* MUT-628). Full regeneration of plants *L. albus / termis* followed on B5 medium + 3.0 mg/l IBA in the case of long-term culture. Flowering plants were also observed on the basic B5 medium. Initial explants originated of *L. mutabilis* MUT-628 were characterized by the best regeneration. Plantles rooted on media: B_5; B_5 + 1,5 mg/l BA; B_5 + 3.0 mg/l IBA. An extensive callus tissue was observed. Regenerated hybrid plants (*L. albus /termis* x *L. mutabilis* MUT-628) were obtained on medium B5 1.5 m/lg lBA + 3.0 mg/l IBA. However, they formed no roots.

Numerous experiments with immature and mature embryos were performed in order to elaborate methods for the most suitable regeneration. The stage of mature embryos was found to be the best for such explants. Mature embryos the length of 1 cm were isolated from immature seeds after removing the seed coat and cotyledons. Then they were sterilized by 2-5 minutes in a solution of grapefruit seed extract and then placed in modified B5 medium. Full regeneration of hybrids and their parental forms was observed. All explants derived from mature embryos showed potential of roots morphogenesis but only some of them showed shoot growth (25%). The highest number of leaves was observed in *L. albus /termis* whereas explants developed four leaves on an average. Explants formed about 3 lateral shoots with the exception of (*L. albus /termis* x *L. mutabilis* Mut-628) and *L. vavilovi.* The *L. mutabilis* Mut-628, *L. albus /termis* and hybrid (*L. vavilovi* x *L. mutabilis* Mut-45) showed the best regeneration ability. The explants *L. termis, L. vavilovi* and hybrid (*L. vavilovi* x *L. mutabilis* Mut-45) in tissue culture formed flowers and even set pods. Pollen fertility was evaluated and ranged from 57% of *L. vavilovii* to 99% for *L. albus /termis* and 80% for *L. vavilovi* x *L. mutabilis* Mut-45. In addition, the process of self-pollination in *L. termis* was observed (with the help of fluorescence microscope). The pollen tubes grew on the stigma and through the style and also into the ovary.

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Somatic embryogenesis in naranjilla (*Solanum quitoenese* Lam.)

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Plant propagation in cultures *in vitro* via somatic embryogenesis does not only allow producing of a large number of plants irrespective of the seasons, but it is also a promising biotechnological tool with a potential for breeding works and crop improvement.

Solanum quitoenese Lam., known as lulo, naranjilla, or Quito orange is a subtropical perennial plant and it is an essential crop in the countries of South America, especially in Ecuador. Its yield is made up of globular, orange-green fruits with yellow-green, tasty and juicy pulp. Although due to its high thermal requirements Quito orange does not produce edible fruit in the regions of cooler climate, it can be nevertheless enjoyed as an original and attractive ornamental plant, with large, heart-shaped leaves.

The experiment investigated the regeneration capacity via somatic embryogenesis in two forms of naranjilla: *Solanum quitoense jurassica* and *Solanum quitoense*. The former is a more primary form and its stems and leaves, despite delicate velvet hairs, are covered with numerous sharp spikes. The other form has heavily-haired stems and leaves, without spines, and vascular bundles of the leaves are purple-discoloured.

The first tested form of naranjilla was bought in a floricultural market as an ornamental plant, and the other in the internet horticultural shop. The mother plants were propagated in cultures *in vitro* by nodal explants on the MS medium without growth regulators, containing 30 $g \cdot dm^{-3}$ of sucrose, 8 $g \cdot dm^{-3}$ of Duchefa Biochemie agar, as well as a 50% increased content of calcium and iron.

Internode fragments about 1 cm in length were sampled from the middle zone of five-week mother plants and placed horizontally onto the MS media with the basic composition identical to the medium used for mother plants propagation, containing 4.0 $mg \cdot dm^{-3}$ 2,4-D and cytokinins-kinetin or BAP in the following amounts: 0; 1.0; 2.0 and 4.0 $mg \cdot dm^{-3}$. After 10 weeks the number of somatic embryos produced was determined, considering the following development stages: globular embryos, early heart stage, heart stage, early torpedo, torpedo and the cotyledonary stage.

All the explants, irrespective of the medium applied, created a vast callus. However, the presence of somatic embryos was observed only on the explants of the spiked form. We found that on the medium containing only 2,4-D globular embryos (average about 25 per explant) were very numerous, embryos at a more advanced development stages were less numerous, but there were no embryos in the cotyledonary stage. The media supplemented with cytokinins did not enhance induction of the somatic embryogenesis. The spiky form, considered to be more primitive, enjoys much more capacity for producing somatic embryos. In case of newer non spiky form, the tendency to produce somatic embryos is much lower.

Different kinds of cytokinin effect on *Narcissus* L. "Actaea" somatic embryo development in solid and liquid/solid culture systems

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Narcissus species are bulbous plants of extensive use in ornamental horticulture, grown on a large scale in Poland and worldwide. Because of the valuable compounds they contain (such as galanthamine and lectin), they are also a potential raw material for the pharmaceutical industry. The naturally low rate of narcissus propagation by adventitious bulbs, being 1.6 bulbs/year (Rees, 1969), is a serious problem hindering the introduction into production of new cultivars and the elite reproductive material. Tissue culture systems, and especially those based on the process of somatic embryogenesis, can significantly increase the propagation rate. Another possibility for enlarging the production scale is the use of liquid media. Liquid cultures enable relatively easier manipulation of successive embryo development stages (Lilien-Kipnis et al., 1994).

The objective of the experiment was to investigate the effects of the kind and concentratin of cytokinin on somatic embryo development (formation, maturation and conversion) in solid and liquid/solid culture systems.

The studies were conducted on the globular stage somatic embryos derived from an embryogenic callus of *Narcissus* L. "Actaea". The embryogenic tissue was initiated on ovaries explants isolated from bulbs (12 cm in circumference) chilled for 6 weeks at 5°C. The callus was obtained and multiplicated on Murashige and Skoog (1962) medium with 3% sucrose and growth regulators: 25 μM Picloram and 5 μM BA. Globular stage somatic embryos developed in an embryogenic callus were transferred on media supplemented with cytokinin: BA, zeatin or TDZ at 5 or 2.5 μM, and auxin NAA at 0.5 or 0.25 μM, respectively. The cultures were grown on agar-solidified media or liquid media using two systems: continuous cultivation for 12 weeks on solid media and sequential cultivation for 6 weeks in a liquid medium and 6 weeks on a solid medium. The media containing agar were distributed into Plastic Petri dishes and liquid media were poured into 100-ml Erlenmeyer flasks. The cultures were maintained at 20°C in the dark. After 12 weeks the following elements of the culture were determined: the number of explants developing secondary embryos, the regenerants fresh mass and dry matter content.

After transferred to a medium containing 5 or 2.5 μM cytokinin (BA, zeatin or TDZ) and auxin (NAA), somatic embryos in globular stage derived from the cultures carried out with of Picloram and BA, did not pass the stage. On their surface, secondary embryogenesis was observed. The frequency of secondary embryogenesis was cytokinin kind- and culture system- independent. Media (liquid and solid) containing 5 μM TDZ and 0.5 μM NAA stimulated growth of regenerants. The highest number of secondary embryos was obtained on media: with BA regardless of the cytokinin and auxin concentrations as well as culture system, with ZEA using continuous cultivation system on solid medium and with TDZ using liquid/solid culture system. Culture system affected the development of secondary embryos. The use of a liquid medium slowed down the secondary embryo development.

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Symplasmic communication and cell fate changes in *Arabidopsis thaliana* explants and seedlings in *in vitro* conditions

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Cell-to-cell communication is of fundamental importance during plant development and occurs through specialized channels called plasmodesmata (PD) which mediate the long and short-distance transport of ions, metabolites, proteins, and RNAs. Furthermore, plasmodesmata are not passive channels. There are number of mechanisms controlling intercellular transport of molecules. One of the most critical and unique functions of PD during plant development is the formation of symplasmic domains, the distribution of which corresponds to organ and tissue arrangement. It appears that symplasmic communication/isolation of cells or groups of cells is necessary for their proper differentiation within the plant body.

In vitro cultures seems to be a good model system for analysis of cell-to-cell transport during cell differentiation, dedifferentiation and redifferentiation. Therefore, to check if there is any correlation between symplasmic communication and changes in cell fate we used *Arabidopsis thaliana* culture system of immature zygotic embryos (IZEs) where somatic embryos are produced and *A. thaliana 35S::BBM* transgenic seedlings forming embryos at cotyledons. In the used model systems apart from somatic embryogenesis, organogenesis occurred. An analysis of symplasmic communication was carried out using various fluorescent tracers with different molecular weights and cytoplasm soluble GFP being expressed under control of *STM* promoter. The studies were supplemented with a morphological and histological analysis. The IZEs were cultured on a solid induction medium for at least 10 days. On every day of the culture IZEs and explants were taken for microscopic analysis. Transgenic seedlings were grown on a selective solid medium and were analyzed between the 5th and the 14th day of the culture.

An analysis of symplasmic communication showed that at the beginning of the culture PD permeability between cells of explants increased in comparison to IZEs. Further changes in symplasmic communication were observed after the next 3-5 days of the culture and were correlated with the cells divisions of explants. The changes manifested themselves as a restriction of symplasmic communication on the borders between the dividing and non-dividing cells, which indicated that parts of explants engaged in morphological processes were isolated from those not involved in cell fate changes. Moreover, symplasmic isolation between the somatic embryos and explants was detected.

Isolation of dividing cells from the rest of the plant body was also observed in the case of *35S::BBM* transgenic seedlings. Meristematic cells at the cotyledons were isolated from the remaining cells. Furthermore, small groups of dividing cells which give rise to somatic embryos were symplasmically isolated from the neighborhood. These results suggest that symplasmic connection of embryogenic competent cells is restricted at this stage of embryogenesis. However, somatic embryos were symplasmically connected with the cotyledons.

The obtained results showed a correlation between the decrease in symplasmic communication and changes in cell fate.

The genome-wide overview of genes connected with flower morphogenesis in cucumber

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Rapid whole genome sequencing tools together with computational comparative analysis gave great opportunity to study genes and proteins involved in basic cellular processes connected with organogenesis. Here we present a research based on the identification and characterization of genes in a complete cucumber genome (*C. sativus* L. cv. Borszczagowski) [Wóycicki et al. PLOS One 2011] connected to flower organogenesis. The elucidation of the plant sex determination mechanism and the flower morphogenesis leading to genetic diversity is of great importance and is the subject of interest of many research groups. The evolution of flowers covers a variety of processes that are carried out in accordance with the genetic background and are regulated at multiple levels of the organization also by endogenous and environmental factors. Clarifying which genes and how they determine the process of flower organogenesis is the purpose of basic research carried out in many species. So far there is no complete picture of the genetic regulation of these processes. The basic research presented herein is intended to clarify the phenomena of flower morphogenesis in cucumber. In the investigation performed by us we combined four methods (RAPD, AFLP, DSC, GD-DSC) to screen the difference between genomes of four isogenic lines (two pairs of nils) different regard to the sex of the flowers and two methods for transcriptome (DH, cDNA-DSC) of 1-2 mm length floral buds (male, female and hermaphrodite) (Przybecki et al., 2003, 2004). We also confirmed their differential expression by macroarray analysis. Altogether 1395 clones (290 from genomic and 1095 from transcriptome libraries) there were spotted onto nylon membranes and were screened to verify the correctness of the applied techniques. The other approach was to find genes which display different expression patterns not only in 1-2 mm long floral buds but also in leaves and in the differentiating shoot apex. Afterwards the results were normalized and genes with significant difference in expression were obtained. In order to identify the differentially expressed sequences connected with flower organogenesis in cucumber genome the blast analysis were used. To investigate genes structure we analyzed introns and exons localization with GenMark.hmm software. For promoters studies the PlantCare software was applied. For the final studies the following analysis were used: analysis of protein domains structure with ExpasyProsite Proteomic Server, Pfam, InterProScan, transmembrane helices prediction TMHMM, signal peptide and localization with SignalP, TargetP and WolfPsort. Such bioinformatics analyses enabled us to fully characterize the genes and predict proteins connected with flower organogenesis. A functional analysis and gene ontology classification revealed many processes connected with flower formation and sex determination such us: hormone pathways, Programmed Cell Death, ubiquitination, cell organization (including cell wall, chromatin, microtubule) binding, signal transduction. Such a detailed description at the structural and expressional levels allows to provide an easy plan of future genome manipulation in order to provide knock out of genes under special interest to check their role in organogenesis.

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The effect of growth regulators on the regeneration ability of flax (*Linum usitatissimum* L.) hypocotyl explants in *in vitro* culture

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Linum genus, belonging to the *Linaceae* botanical family, includes approximately 300 species, most of which are wild species, a few are grown as ornamental plants, some fibrous forms have become of local significance, but only one species of flax i.e. *L. usitatissimum* is commonly cultivated (Zając, 2004). Flax was, and still is, an economically very valuable plant. What is more, each part of the plant can be useful: the stem is the source of fibers and shives, whereas from seed capsules, seeds and chaffs are gained. It is commonly used in research in plant biotechnology, involving biochemistry and plant regeneration in *in vitro* conditions. Genetic modifications are being successfully entered into this species by way of genetic engineering, getting transgenic plants about favourably changed features (Wróbel-Kwiatkowska et al., 2009, Miśta et al., 2011).

Regeneration in *in vitro* conditions depends on various external and internal factors, alteration of which can lead to culture efficiency improvement. Not only the implementation of different media and various culture conditions, but also the kind of explant, gives an opportunity of gaining the optimal regenerating configuration. Therefore, the aim of the present study was to determine the influence of different concentrations of plant growth regulators on hypocotyl explants of two flax cultivars (*Linum usitatissimum* L.) regeneration abilities.

The plant material used for the research consisted of two flax cultivars: Modran and Selena, of which from 6-days old sterile seedlings, hypocotyl explants were taken. The hypocotyl explants were excised out from the seedlings and placed on MS basal medium and MS supplemented with different concentration of 6-benzylaminopurine (BAP) or naftalene acetic acid (NAA). Both regulators, BAP and NAA, were applied in the same concentrations: 0.2; 0.5; 1.0; 1.5 and 2.0 mg l^{-1}. The definitive evaluation of callus, shoot and root regeneration was made after 28 days from the time of setting the experiment.

Both analyzed cultivars showed different regeneration abilities. The percentage of explants forming shoots and the number of shoots formed by one explant were modified by growth regulators added to the medium. Callus was formed in explants only in the appearance of injured tissue. Shoot regeneration effectiveness, defined by the percentage of hypocotyls explants forming shoots was different in investigated cultivars and varied from 37% (cv. Selena – MS + 2.0 mg l^{-1} NAA) to 92.6% (cv. Modran – MS + 1.0 mg l^{-1} BAP).

Based on the obtained data it can be concluded that plant growth regulators that were applied in this experiment had significant influence on the succeeding *in vitro* culture.

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Effect of growth regulators on micropropagation of selected genotypes *Rhododendron sp.*

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Rhododendrons are propagated by seeds mainly for breeding purpose and the production of rooted cuttings, as well as for using the mother plants for transmission of traits to progeny. Unfortunately, many varieties that are propagated generatively not only “repeat” mother plant characters, but also the percentage of viable seeds of some species is limited. Valuable ornamental traits and, consequently, the continuous requirement for such individual copy of rhododendron plant is the reason why the methods of vegetative propagation are still improved for large-scale. The micropropagation is considered for their mass multiplication.

The aim of research was to compare the morphogenetic capacity of the analyzed genotypes, depending on the applied growth regulators in tissue culture conditions.

Flower buds from five genotypes were collected for the *in vitro* culture initiation. The material was gathered during winter seasons in 2008 and 2009 in the Forest Arboretum in Ślizów. Excised buds were washed in the detergent solution, next sterilized in 70% ethanol and washed in mixture of 30% Domestos and HgCl2 0.1%. Finally buds were washed three times in sterile distilled water with antioxidants. The culture was established after six months of culture. The experiment was conducted in three replications (10 explants in each replication). The explant was approximately 1.0 cm long, with 4-5 leaves. The basic nutrient medium was used according to Anderson, with modifications (ALo) proposed by Bojarczuk (1995). The basic medium was supplemented with various plant growth regulators at different concentrations mg/dcm^3 (8.0 2iP + 1.0 IAA; 4.0 2iP + 0.2 IAA + 0.1 TDZ, 4.0 ZEA + 1.0 IAA; 4.0 2iP + 1.0 IAA). The explants were subcultured three times. Every month the culture growth was evaluated. The plant height was measured and the number of new developing adventitious and lateral shoots was evaluated. Every time after evaluation of data, explants were subcultured. The multiplication coefficients were determined after three consecutive passages. For acclimatization, plants were subcultured twice on a hormone-free medium with reduced sucrose concentration and with addition of activated carbon.

Statistical analysis allow us to predict the best combination of hormones to achieve a favorable multiplication coefficient and growth of plants during three months of culture. A significant increase in the number of plants is noteworthy. With 30 initial plants in each version of the media, from tens to hundreds of new plants were obtained after 3 months of the culture. The highest multiplication coefficient was found for genotype no four (13-26) and five (9-13), at the presence of ALo medium + 4.0 mg/l 2iP + 1.0 mg/l IAA. The lowest rate of proliferation was found for genotype 1, but almost twice as many explants were recorded on a medium ALo + 8.0 mg/l + 2iP 1.0 mg/l IAA. Genotype 2 was characterized by the most favorable rate of multiplication – 6 – for ALo medium + 4.0 mg/l + 1.0 mg/l IAA and 2iP on media supplemented with zeatine and TDZ (average total multiplication factor of 4). The mean total multiplication factor for the three genotypes was 3, but the most useful media were: ALo + 4.0 mg/l 2iP + 0.2 mg/l IAA + 0.1 mg/l TDZ. In greenhouse acclimatization of plants was carried out after obtaining a sufficient number of plants (1000).

Rooting plants proceeded *in vivo* were found to maintain a high level of humidity, and the acclimation efficiency was above 70% for genotype 4 and 5, and for 2 and 3 below 50%. This last step was the most difficult.

The effect of type and concentration of gelling agents on micropropagation of selected varieties and species of clematis (*Clematis* sp.)

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The objective of the present study was to investigate the effect of different types and concentrations of gelling agents on micropropagation of *Clematis integrifolia* (L.), *Clematis viticella* (L.) and *Clematis integrifolia* "Petit Faucon". Shoot tips derived from aseptically grown tissue cultures were put into the media containing 2 mg 2iP·dm^{-3} and 0.5 mg IAA·dm^{-3} and gelled with one of the following: agar Sigma, Bacto-Difco agar Biocorp, agar BioMerieux, agar INC in the concentration of 6.5 g·dm^{-3} or Gelrite Kelco at the concentration of 2 g·dm^{-3}. In the second part of the experiment shoot tips were put into the media gelled with 3.25, 6.5 and 13 g·dm^{-3} of agar or into the perlite immersed in the liquid media.

On the basis of the obtained results it was noted that elongation of shoots, fresh weight and callusing of *Clematis integrifolia* were similar in all combinations. It was observed that 94% of plants formed axillary shoots on the media gelled with agar Sigma or Bacto-Difco in comparison to 63% on the media gelled with agar BioMerieux. The number and fresh weight of the axillary shoots were similar in all combinations. The number of axillary shoots per plant varied from 1.8 (agar Merinoux) to 2.3 (agar Sigma, Gelrite). On the basis of visual observations it was found that the best quality plants seemed to grow in the presence of Gelrite, but some of them were vitrified. In the case of *Clematis* "Petit Faucon", elongation, the number of nodes and the callusing of shoots were similar in all combinations. The most plants formed axillary shoots in the presence of Gelrite (40%) in comparison to agar Merinoux, Bacto-Difco (8%) and Sigma (11%). The most axillary shoots per plant appeared in the presence of Gelrite (1.7) and agar Merinoux (1.5 per plant), while the least on the media with agar Bacto-Difco and Sigma (1.0 per plant). Visually, the best quality shoots were on the media with agar Bacto-Difco. The type of gelling agent had a significant effect on *Clematis viticella* propagation *in vitro*. Shoots cultivated on the media containing agar Sigma were longer (10.9 mm) than those on the media with Gelrite (4.6 mm). The number of nodes was also the highest in the presence of agar Sigma (3.3 per plant). There were no differences in the callusing of shoots. It was observed that 33% of shoots formed on the average 1.1 axillary shoots on the media with agar Sigma. There was no branching in the presence of agar Merinoux. The number and length of axillary shoots were similar in all combinations. Visually, the best quality shoots were obtained when Sigma agar was added to the media while the worst ones were in the presence of Gelrite. In the first cycle some of the shoots of *Clematis integrifolia* and *Clematis* "Petit Faucon" were vitrified. Therefore the shoots were put on the media containing agar and Gelrite for the second cycle. *Clematis integrifolia* shoots were longer and formed more leaves on the media containing Gelrite but many of them were deformed or vitrified. In the case of *Clematis* "Petit Faucon" there were no significant differences in the height of the shoots, or the number of leaves and branching depending on the gelling agent. It was observed that many shoots cultivated on Gelrite were vitrified and unsuitable for further cultivation.

The setting of the media had no significant effect on elongation and branching of *Clematis integrifolia* and *Clematis* "Petit Faucon". However, a statistical analysis showed significant differences in the growth of *Clematis viticella* shoots depending on the media setting. The longest shoots with the highest number of nodes were obtained on the media gelled with 6.5 g·dm^{-3} of agar. The most axillary shoots were formed on the media gelled with 13 g·dm^{-3} of agar (1.7) in comparison to the liquid one (1.0 per plant per plant).

Effect of cytokinins on micropropagation of *Pulsatilla vulgaris* Mill.

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Pulsatilla vulgaris Mill. (commonly known as pasque flower) is a perennial ornamental and medicinal plant belonging to buttercup family (Ranunculaceae). *P. vulgaris* occurs throughout western and central Europe – from southern Sweden to the Bordeaux region of France and as far as Poland in the east. This species is considered to be extinct in its natural habitats in Poland and it is enlisted in "Red List of Plants and Fungi in Poland".

The aim of the study was to establish an *in vitro* regeneration system for the endangered species *Pulsatilla vulgaris* Mill. The experimental material was sterile seedlings of *P. vulgaris*. *Pulsatilla*'s seeds were obtained from: the Botanical Garden in Łódź, the Botanical Garden of the Adam Mickiewicz University in Poznań, the Botanical Garden of the Polish Academy of Sciences – Center for Biological Diversity Conservation in Powsin and from foreign botanical gardens: Alpine Garden Mt. Patscherkofel of the University of Innsbruck in Austria and Lystigarđur Akureyrar in Iceland. Surface-sterilized seeds were germinated on the half-strength MS medium or MS medium supplemented with 1.0 mg/dm^3 GA_3. Shoot tips, fragments of hypocotyls, roots and cotyledons were isolated from seedlings with well-developed cotyledons. The explants were transferred to MS medium with an addition of BAP, KIN or 2iP (1.0 or 3.0 mg/dm^3) in combination with NAA (0.1 mg/dm^3).

The morphogenetic response was observed on shoot tips and fragments of hypocotyls on a proliferation medium supplemented with all the types and concentrations of the cytokinins mentioned above. The root explants regenerated only shoots on MS medium with KIN or 2iP in both concentrations which were used. However, cotyledons did not regenerate shoots on any of the proliferation media used. The highest frequency of shoot organogenesis (100%) was observed in the hypocotyl explants cultured on the MS medium with an addition of 1.0 mg/dm^3 BAP and in the root explants cultured on the MS medium with 1.0 mg/dm^3 KIN or 3.0 mg/dm^3 2iP. The highest shoot formation rate (average of 5,3 shoots per explant) was obtained from root explants on a medium with 1.0 mg/dm^3 2iP.

The properly developed individual shoots were rooted on: MS, the half-strength MS or the half-strength MS medium with an addition of one of auxin IBA or IAA (1.0 mg/dm^3 or 10.0 mg/dm^3). The decreasing the amount of mineral salts in the rooting medium stimulated the rooting process. The highest frequency of rooting (85%) was achieved by culturing microshoots on the half-strength MS medium containing 10.0 mg/dm^3 IBA for 7 days, then transferring to the same medium without IBA. Shoot tips micropropagated on MS medium supplemented with KIN rooted on the half-strength MS with addition of either IBA or IAA in both concentrations which were used. The presence of BAP in the proliferation medium inhibited the rhizogenesis. Shoot tips multiplicated on MS medium containing 2iP were capable of rooting only on the half-strength MS or the half-strength MS medium with addition of IAA (1.0 or 10.0 mg/dm^3).

Of the various substrates tested, peat mixed with coconut fiber, perlite and styrofoam (5.5:1.5:2:1 v/v) were best for plant acclimatization. Regenerated plant lets which survived acclimatization were developing properly and they displayed normal leaves morphology.

The results of this study provide the first successful report of *in vitro* regeneration of *Pulsatilla vulgaris*.

Effect of different factors on bud explants response in tulip *in vitro* culture

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Tulip is a highly valued spring ornamental species. The naturally low rate of tulip propagation is a serious problem hindering its introduction into production of new cultivars. Propagation *in vitro*, especially that based on the process of somatic embryogenesis, can significantly increase the propagation rate (Gude and Dijkema 1997). To initiate the method, explants like stems or ovaries isolated from chilled bulbs were usually used (Ptak and Bach, 2007).

The experiments aimed to investigate the primary response of vegetative and generative buds under the influence of chemical and physical factors.

The studies were conducted on the tulip of the cultivar "Apeldoorn". The tissue cultures were initiated from buds isolated from unchilled bulbs of circumference of 11-12 cm. The vegetative buds were taken in July and September, and the generative buds were isolated only in September. Before isolation all the bulbs were treated with 20°C or 34°C for one week. The explants were placed on media containing mineral salts according to Murashige and Skoog (1962), 3% sucrose, auxins (50 μM picloram or 5 μM NAA) and cytokinins (0.5 μM BAP or 5 μM TDZ). The cultures were maintained in the dark at 20°C for 12 weeks. Within this time the callus tissue formation and shoot differentiation were observed.

During the first weeks of cultivation the explants had a swollen appearance. Then all the vegetative buds started to grow (forming shoots), but only under the influence of media containing 5 μM NAA and 5 μM TDZ. The temperature 34°C had a positive impact on growth, because the shoot length were almost 30% longer than the length of explants from 20°C, both isolated in July and September. The vegetative buds treated with 50 μM picloram and 0.5 μM BAP started callusing. In case of July explants the temperature also influenced the level of undifferentiated white callus formation. Significantly more callus formation (measured by the covering surface of the explants), near 100%, were observed at 20°C bulb treatment. A temperature of 34°C caused callus tissue formation at the level of 25%. There were no significant differences in callusing between the bulb temperature treated, under picloram and BAP influence, in the case of vegetative buds isolated in September. The vegetative buds, treated with NAA and TDZ also formed callus tissue maximum in 25%.

The response of generative buds was dependent on the previous bulb temperature treatment. After 20°C longer shoots were observed (27 mm) than after 34°C (11 mm). Also picloram and BAP stimulated shoots growth from generative buds, which were even twice longer in comparison with the media supplemented with NAA and TDZ.

Significant differences were noticed in the rate of callus formation on the surface of generative buds, depending on temperature. 34°C caused 100% covering of the explants, while 20°C inhibited the callusing process to 50%.

A great majority of generative buds after 12 weeks of cultivation were getting brown. Such an reaction was observed mainly among the explants excised from the bulbs previously treated with 34°C.

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In vitro regeneration of *Scutellaria altissima* plants through callus organogenesis and genetic stability assessment of obtained shoots

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The genus *Scutellaria* consist of 300 species, many of which have biological activity. So far one species, *S. baicalensis* officially listed in Chinese and Japanese Pharmacopeia, has been investigated in detail, also using the tissue from *in vitro* cultures of the plant. The high pharmacological activity of the *Scutellariae* radix is a consequence of the presence in the resource of above 50 flavonoids. Among them the most important and abundant are: baicalin, baicalein, wogonin, wogonoside, scutellarin and oroxylin. These compounds in the traditional Chinese medicine are widely used for the treatment of inflammatory diseases, tumors, hepatitis and bronchitis. Numerous current papers have reported that scullcap flavonoids have multiple biological properties including antioxidant, anticarcinogenic, antiviral, antibacterial, antiallergic, vasodilatory, anti-inflammatory and anxiolytic effect (Li et al., 2004). Many other *Scutellaria* species have been defined as a resource of these compounds which have pharmacological properties. In our study we focused on *S. altissima in vitro* culture.

Plant tissue culture techniques can be used as alternative methods for plant mass production. We reported effective regeneration of *S. altissima* through indirect organogenesis. The shoots were initiated from two morphologically different kinds of callus grown on Murashige and Skoog (MS) agar medium containing IAA (0.1 mg/l) and TDZ (0.2 mg/l) and Schenk-Hildebrandt) SH agar medium supplemented with NAA (0.1 mg/l), BAP (0.2 mg/l) and 2,4-D (0.5 mg/l). The culture on SH medium produced about 12, and on MS medium – about 5 shoots per culture. The obtained shoots were transferred to soil, where they rooted, and after acclimatization they grew in a greenhouse. After 10 weeks about 95% of the plants survived the acclimatization procedure.

The regenerated shoots as well as the plants from both kinds of callus did not exhibit visible phenotypic variations in morphological traits as compared with the donor plant. It is widely known that especially in the *in vitro* obtained plants, those callus-derived may differ from the mother plants. Such variations generated by *in vitro* conditions are termed somaclonal variations (Larkin and Scowcroft 1981). This type of variation may involve a chromosome number and structure, gene mutation, an altered sequence copy number, a sister chromatide exchange, somatic crossing over, DNA amplification and a deletion or change in the methylation pattern. Among the factors that cause the somaclonal variation, plant genotype is probably the most important determinant. What is more, these changes depend on such factors as: growth regulator content in the culture medium, explant type and culture duration.

In the present research the genetic stability of callus-derived shoots of *S. altissima* was evaluated. Genomic DNA was extracted from fresh leaves of a hypocotyl-derived shoot, using a modified method of Khan et al. (2007). An inter simple sequence repeat analysis was employed to estimate the obtained plant material using 8 ISSR primers. The PCR (polymerase chain reaction) products were checked by separation on 2% agarose gel strained with ethidium bromide and visualized under ultraviolet.

Based on the ISSR data no genetic variation was detected for shoots from both examined calli. The results from this report provide important evidence of genetic stability of *S. altissima* before a large-scale commercial production of this plant.

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In vitro regeneration system of *Huperzia selago*, a club moss which is a source of huperzine A

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The fir clubmoss *Huperzia selago* (L.) Bernh. ex Schrank et Mart. (*Lycopodium selago* L.) is the only European lycopod of the Huperziaceae family and is commonly found in a widespread territory stretching from Scandinavia to the northern Mediterranean countries. It also grows in the boreal and temperate regions of Asia and North America. Up to now, a number of alkaloids have been isolated from *H. selago* plant. One of these, Huperzine A (selagine, HupA), is an effective, reversible and selective acetylcholinesterase (AChE) inhibitor, now undergoing clinical trials as a treatment for Alzheimer disease and schizophrenia. Other important medicinal properties of HupA have been confirmed, including its neuroprotective activity. HupA is obtained from the Chinese clubmoss *Huperzia serrata* plant and can be synthesized. *H. selago* is the only European species which contains HupA. Studies by Szypuła et al. (2005, 2011) have shown that the plant is a rich source of HupA, much more abundant than *H. serrata.* However, procurement of the raw material from naturally growing plants is impossible or difficult. They are legally protected or have an endangered species status. Considering recent increasing interest in alternative methods of HupA procurement for its uses in the pharmaceutical industry *in vitro* micropropagation seems one of the methods of obtaining sufficient amounts of the raw material to isolate alkaloids. *In vitro* techniques have been intensively used in *H. selago* in recent years and several regeneration systems applicable to mass propagation have been described by Szypuła et al. (2005, 2006, 2011). Propagation of *H. selago* was successfully achieved on different sporophytic explants (shoots, vegetative propagules, somatic embryo). The *in vitro* culture was initiated from shoots of wild plants. The highest growth frequency was achieved on the MS medium at half-strength mineral salt content (1/2MS). The same medium was used for the induction of somatic embryogenesis. Cells of the callus, which developed from the apical meristem after 3 months of incubation, transformed into somatic embryos. A quick and efficient system to regenerate *H. selago* sporophytes using vegetative propagules (bulbils) as explants has been developed. It allows obtaining an axenic culture in 2 to 3 months, which may serve as a source of huperzine A or other alkaloids. The optimum results were achieved using Moor (Mr) medium without growth regulators or supplemented with 0.015 mg/l IBA and 0.3 mg/l kinetin. Vigorous growth of adventitious roots, especially on Mr medium with the addition of 0.25 mg/l NAA, and callus formation on shoot apices were observed. At 6 months of culture, some sporophytes obtained from the bulbils were used as the initiating material for shoot subcultures which developed best on Mr medium with 0.015 mg/l IBA and 0.3 mg/l kinetin.

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Micropropagation of *Taraxacum pieninicum* Pawł., a rare and endangered plant

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Taraxacum pieninicum Pawł. (Asteraceae), an endemic species of the Pieniny Mountains, is a declining-critically endangered species (E). It occurs only in the Pieniny in the upper parts of the Central Massif of the Three Crowns, at Okrąglica, as a component of the rock grasslands *Dendranthemo-Seslerietum. Protecting T. pieninicum* involves constant monitoring of its natural habitat (passive protection). Moreover, the seeds of this species have been placed in the Seed Bank in the Polish Academy of Sciences Botanical Garden – CBDC in Powsin, in order to protect the gene pool. Since 2007, *T. pieninicum* has also been kept in the collections of the Polish Flora of the Garden. The aim of the study was to obtain an efficient plant regeneration system and acclimatization of *Taraxacum pieninicum.*

The experimental material comprised a few-day old sterile seedlings, from which shoot tips and fragments of cotyledons, hypocotyl and roots were isolated. The seeds were surface sterilized and germinated on MS medium supplemented with 1 $mg{\cdot}l^{-1} GA_3$. Explants were exposed on MS medium containing BA at a concentration of 0.25, 0.5 and 1 $mg{\cdot}l^{-1}$ in combination with NAA in the ratio 10:1. After 4 weeks of culture, shoots were isolated and transferred to fresh proliferative media (4 passages), and then placed on a rooting medium with full- and half-strength salt MS. Microcuttings were hardened in pots filled with a sterile mixture of vermiculite and sand (1:1), a sterile mixture of soil and sand (1:2), a non-sterile mixture of soil and sand (1:2) and in hydroponic culture, in tubes with quarter-strength salt MS solution. The regenerated plants were planted in the Botanical Garden in Powsin and the Botanical Garden UMCS (Lublin). All types of the tested explants showed shoot induction on all proliferation media used, except for the roots on the medium with 1 $mg{\cdot}l^{-1}$ BA. Lower concentrations of BA stimulated both the percentage of explants capable of organogenesis, and the number of shoots and their length (except for the shoots from the hypocotyl). The highest number of axillary shoots was recorded on shoot tips 12.3 shoots per explant on a medium with 0.5 $mg{\cdot}l^{-1}$ BA. In subsequent passages, a statistically significant increase in the number of shoots on a medium containing 0.25 and 0.5 $mg{\cdot}l^{-1}$ BA was observed. The shoots were rooted on MS and 1/2MS medium, and the reduction of mineral content in the rooting medium did not significantly affect either the percentage of the rooted shoots or the number of roots, but only slightly stimulated their growth. During the 8 weeks of acclimatization of microcuttings, high levels of survival were recorded, which was over 82% when using a non-sterile mixture of soil – sand and 100% in hydroponic culture. Plantlets planted in the collection of both gardens in the first year of life produced only rosette leaves and did not enter the generative stage. Moreover, in the Botanical Garden in Powsin, strong grazing of leaves of this species by snails of the genus *Limax and Arion rufus*, was observed, resulting in low survival rates (10%, after 6 months of acclimatization). In contrast, in the Botanical Garden in Lublin all planted individuals survived. In the second year of cultivation the plants were capable of flowering.

The described method of plant regeneration allowed huge numbers of regenerants to be obtained. This confirms that the proposed method can be used for the propagation of this species.

Plant regeneration of *Beta maritima*

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Wild beet – *Beta maritima* (*Beta vulgaris ssp. maritima* (L.) Arcang.) well known as a sea beet is important as a source of resistance traits to main beet diseases (Rhizomania, Cercospora, Rhizoctonia) and tolerance to stress environment like a prolonged drought and salinity of soil (Asher et al., 2001).

The plants of this wild beet are characterized by a large morphologic and genotypic diversity both between and within population from annual open-pollinated forms to biennial cytoplasmic male-sterile (CMS) ecotypes. *Beta maritima* is a subspecies where different from the Owen type CMS system has been described.

Successful plant regeneration in sugar beet related species has been reported from various explants by a number of authors (Gośka, 2004). Plant regeneration of the most wild beet species has not been well known. The purpose of this investigation was to detect the ability to plant regeneration in *in vitro* cultures of biennial CMS ecotype of *Beta maritima*.

The plant material was originated from the field gene bank collection of the Plant Breeding and Acclimatization Institute in Bydgoszcz, part of the National Centre for Plant Genetic Resources in Radzików. The initial explants were 0.1-0.3 cm long tips of inflorescence shoots of *Beta maritime*. For surface sterilization the tips were treated with 70% ethanol for 30s, 5% calcium hypochlorite for 20 minutes and washed in a sterile distilled water four times. Sterilized tips were cultured on MS basal medium (Murashige and Skoog, 1962) supplemented with 0.2 mg/l 6-benzyloaminopurine (BAP) prior adjusting the pH to 5.8.

The investigation was carried out in a growth chamber at 25°C under artificial daylight conditions with 16-h photoperiod. Explants were transferred to a fresh medium every four weeks. The sugar beet was used as a control plant. For more effective plant regeneration of *Beta maritima* various concentrations and combinations of growth regulators such as 6-benzyloaminopurine (BAP), thidiazuron (TDZ), kinetin (KIN) were used. For rooting, multiplied shoots were transferred to 1/2 MS rooting medium with 3.0, 6.0 or 9.0 mg/l indole-3-butric acid (IBA) modified with regard to different type and level of vitamins, sucrose, aluminium, calcium and indole-3-acetic acid (IAA) and 1-naftalenoacetic acid (NAA).

After four weeks more than 90% explants of sea beet and 100% of sugar beet started to regenerate shoots or shoot-like structures. MS medium with 0.2 mg/l BAP was the best for effective regeneration. The coefficient of reproduction was high and dependent on the components of the used medium. Explants derived from the tips of inflorescence shoots were characterized by high genetic stability and high morphogenetic potential. Some of the obtained shoots occasionally developed roots on the MS basal medium but on almost all used combinations of the rooting medium *Beta maritima* showed a high ability to developed roots. The *in vitro* derived plantlets were successfully transferred to the soil. The percentage of survival was higher than 95%. All the obtained plants were healthy and male-sterile.

In *in vitro* culture direct organogenesis of *Beta maritima* was very close to that of sugar beet in contrast to another wild beet species. Similar morphogenetic responses confirm their close relationship. Because sugar beet has a relatively narrow genetic base, germplasm of *Beta maritima* can become the most important genetic resource for the enhancement of breeding programmes.

Regeneration ability of wild sea beet is used effectively for in *in vitro* cultures multiplication of original CMS plants at the gene bank collection in Bydgoszcz.

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Caulogenic capability of *Linum usitatissimum* L. hypocotyl and cotyledon explants *in vitro*

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Linum usitatissimum L. is a significant crop plant in many regions of the world, particularly in cool temperate environments. It is a dual-purpose crop, a source of oil and fibers. In addition to the commercial aspects, this species has been widely used for a range of plant biotechnological studies. *In vitro* regeneration response in terms of the number of shoots produced is an important factor determining the success of transformation experiments.

In this study, the caulogenic ability of cotyledon and hypocotyl explants of oil flax was investigated in relation to their physiological age and the effect of growth regulators in the medium. To find/lepiej: determine if any morphogenic gradients existed along the hypocotyls and cotyledons, different parts of these organs were tested as explants for shoot organogenesis.

Hypocotyl and cotyledon explants of 5- and 7-day old seedlings of oil flax (*Linum usitatissimum* L., cv Szafir) were cultured *in vitro* in 90 mm Petri dishes on two basic media: i) Murashige and Skoog (MS); ii) medium containing macronutrients after MS and micronutrients and vitamins after Gamborg (MS + B5). The media were supplemented with: i) 6-benzyladenine (BA, 1 $mg \cdot l^{-1}$) and 2,4-dichloro-phenoxyacetic acid (2,4-D, 0.05 $mg \cdot l^{-1}$); ii) tidiazuron (TDZ, 1 $mg \cdot l^{-1}$) and 2,4-D (0.05 $mg \cdot l^{-1}$); iii) 6(γ,γ-dimethylalliloamino)purine (2iP, 1 $mg \cdot l^{-1}$) and 2,4-D (0.05 $mg \cdot l^{-1}$).

Hypocotyls were cut into three pieces and segments from the same hypocotyl arranged according to their positions, from the roots to the apex (I, II, III). Cotyledons were divided in basal (B), medial (M) and apical (A) segments. Explants (parts of hypocotyl and cotyledon) were cultured for 4 weeks in a growth chamber under the following conditions: 16-h light photoperiod, light intensity 50 μmol m^{-2} s^{-1}, light/dark temperature 25/20°C.

Hypocotyls were much more responsive explants than cotyledons for shoot organogenesis. A high shoot regeneration frequency (95-100%) was obtained with hypocotyl segments derived from 5- and 7-day old seedlings. BA was more effective than TDZ in caulogenesis of these explants. In the presence of 2iP, the regenerated shoots were greater than that in BA and TDZ. However, they were flaccid, pale green and yellowish, and aged quickly. The efficiency of shoot organogenesis from three different parts of hypocotyl was similar.

Shoot organogenesis from cotyledon explants was obtained only on MS+B5 medium. A high frequency of shoot regeneration from basal segments of cotyledons was observed (50-80%, depending on the cytokinin used). However, B segments without parts which adhered to the axis showed no caulogenic capability. A low frequency of shoot organogenesis (13%) was also obtained in some cultures derived from medial parts of cotyledons. BA was more effective than TDZ in shoot organogenesis from basal segments of flax cotyledons.

The effect of media composition on propagation of dahlia (*Dalia x cultorum* Thorsr. Et Reis.) *in vitro* and adaptation to *ex vitro* conditions

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Dahlias are a group of very popular and valued plants used in flowerbeds in urban areas and home gardens. In the Netherlands around 20 million plants are produced every year.

For dahlia micropropagation different authors recommend MS media with addition of 0.25 to 3 mg·dm^{-3} BA and 0.1 to 10 mg·dm^{-3} GA_3. Because of many discrepancies in media compositions recommended for micropropagation of dahlia, studies were undertaken to establish the effect of three cytokinins: BA, 2iP and kinetin in concentrations of 0.0, 0.25, 0.5, 1.0, 2.0 and 4.0 mg·dm^{-3}. Explants for the initiation of aseptic cultures were *Dahlia* x *cultorum* "Pirat" stem nodes without leaves which were disinfected for 30 minutes in 1% sodium hypochlorite. Dahlia's growth in tissue cultures starts very quickly. The optimal length of one cycle is 4 weeks. Exceeding the cycle to over 6 weeks causes excessive elongation and tips necrosis.

Dahlia explants formed most of the good quality axillary shoots on MS media supplemented with 1 mg·dm^{-3} BA. It was observed that a good method for dahlia micropropagation is dividing stems to one-node parts. Kinetin in a concentration of 1 mg·dm^{-3} promoted elongation. Explants of dahlia reacted intensely to a high concentration of cytokinins with inhibition of growth and reduction of leaf area. In order to estimate the effect of GA_3 on the elongation of axillary shoots, this PGR was used in concentrations of 0.1, 0.5, 1.0, 2.0 and 5.0 mg·dm^{-3} and added to the media supplemented with 1.0, 2.0 and 4.0 mg·dm^{-3} BA. An addition of GA_3 in a concentration of 1 mg·dm^{-3}·to the media supplemented with 2 mg·dm^{-3} BA promoted elongation of axillary shoots.

The type and concentration of sugar added to the media is important in micropropagation of bulbous plants. In order to estimate the effect of this factor on the growth of dahlia, three different sugars were added to the media: sucrose, fructose and glucose in concentrations of 15, 30, 45 and 60 g·dm^{-3}. It was noted that low a concentration of sugar, no matter the type, increased the number of axillary shoots but inhibited elongation. The longest shoots with the highest fresh weight were obtained in the presence of glucose in concentrations of 30 and 45 g·dm^{-3}. Dahlias cultivated on media with 15 g of sugar formed thin and limp shoots with small light green leaves. A faster shoot tip necrosis was also observed.

Dahlia explants did not need roots inducing in tissue culture. In order to check a subsequent effect of cytokinins on the rooting and growth of *Dalia* x *cultorum* plants in *ex vitro* conditions, BA, 2iP and kinetin in a concentration of 1 mg·dm^{-3} were added to the media in the last cycle. After 6 weeks of growth on the media, dahlias were rooted in peat and perlite. Plants propagated in the presence of kinetin and 2iP rooted in 100% four weeks after planting, while in the presence of BA there was 60% of rooted plants and the rooting lasted 7 weeks. Plants planted into the ground flowered after 11 weeks. Dahlias propagated in the presence of kinetin formed the highest above ground part, had the biggest weight of the above ground part and the crown, 95% of the plants flowered. While 2iP was used 100% of flowering plants were obtained. The type of cytokinin did not influence the flower head diameter or the length of the flower stems of dahlia.

Dahlias propagated in the presence of BA formed the most primary shoots and did not need pinching, but their growth was slower. After 3 months of cultivation in the ground 50% of plants flowered and crowns had more bulbous roots. The crown weight was significantly lower in comparison to plants propagated in the presence of kinetin and 2iP.

Effect of culture medium on the effectiveness of multiplication of horseradish (*Armoracia rusticana* L.) *in vitro*

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Horseradish (*Armoracia rusticana*) is a perennial crop plant of the Brassicaceae family. In the Polish climate, the horseradish plant blooms profusely, but seeds are never formed. For this reason, it is only propagated vegetatively by root cuttings, which contributes to the transmission of viral diseases. The most important virus affecting horseradish crops is the *Turnip Mosaic Virus* (TuMV). Infection of young horseradish plants with this virus causes a significant reduction in yield of up to 40%. Using *in vitro* cultures it is possible to obtain healthy plants from pathogen-infected starting material. The objective of our research is to free horseradish plants from viruses, especially the turnip mosaic virus, and to develop an effective method for the multiplication of healthy plant material *in vitro*.

In the present study, the ability to regenerate horseradish on culture media containing different amounts of plant growth hormones was assessed, and the effectiveness of this process evaluated. Rosettes were chosen as explants. The starting explants were laid out onto culture media based on MS (Murashige and Skoog) supplemented with 30 g/l sucrose, enriched with growth hormones – cytokinins and auxins: Kin (kinetin) 0.2 mg/l and 1 mg/l IAA (indolyl-3-acetic acid), 2iP (2-isopentyladenine) at concentrations of 20, 30, 40 mg/l in combination with 0.01 mg/l IAA, TDZ (thidiazuron) at concentrations of 2, 5, 10 mg/l in combination with 1 mg/l NAA (α-naphthyl-1-acetic acid), and also BA (6-benzylaminopurine) 0.2 mg/l, NAA 1 mg/l with the addition of 0.5 mg/l putrescine.

The largest number of well developed plants was obtained by laying out the starting explants on MS medium supplemented with BA (0.2 mg/l), NAA (1 mg/l) and putrescine (0.5 mg/l). The largest number of non-rooted rosettes was obtained on MS medium supplemented with TDZ at a conc. of 10 mg/l in combination with 1 mg/l NAA.

A positive result in the process of micropropagation is when substantial multiplication in the form of incipient rosettes has been achieved. This type of material makes it then possible to obtain in subsequent passages. The highest multiplication rate, in the form of incipient rosettes, was obtained by laying out the rosette on MS medium enriched with 30 mg/ l 2iP in combination with 0.01 mg/l IAA. A large numbers of rosetts was also obtained on MS media supplemented with 2 mg/l TDZ in combination with 1 mg/l NAA or 40 mg/l 2iP in combination with 0.01 mg/l IAA.

On the basis of the obtained results it was decided to check the effect of increased concentrations of two cytokinins: TDZ (20 mg/l) and 2iP (50 mg/l), and of the polyamine putrescine (1 mg/l). The results showed that increased levels of both TDZ and 2iP did not cause a significant increase in the effectiveness of regeneration of horseradish. However, positive results in the process of regeneration, in the form of an increased number of well-developed plants, were produced by the addition to the medium of an increased concentration of the putrescine.

Factors modifying propagation and growth of *Arnica montana* in culture *in vitro*

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Arnica montana is a known and valued medicinal plant. Its populations usually amount to a few dozen individuals and their habitats in Europe shrink rapidly. The main reason for that is intensive harvesting of raw material from wild stands for the needs of pharmaceutical and cosmetic industries. The flower heads are collected, but more recently also the rhizomes, which is synonymous with the destruction of both the plants and their stands. In the last decade legal measures of *Arnica montana* protection have been implemented. The species is strictly protected in France, Germany, Hungary, part of Switzerland and Poland. The plant is also listed in EC Habitats, Fauna and Flora Directive. Its international trade is regulated by CITES. Attempts to grow *Arnica on Polish* territory failed for unknown reasons.

Arnica montana contains a number of secondary metabolites but it is sequiterpene lactones – helenalin and its esters – as well as flavonoids, phenolic acids and polyacetylenes that are primarily responsible for the therapeutic effects.

The aim of this study was to develop efficient methods of *Arnica* micropropagation, to learn the environmental requirements and to define the principles and methods of cultivation of this species.

An important objective was also enrichment of the natural habitat in the National Park of the Stołowe Mountains and creation of supplementary stands in the Botanical Garden of Wroclaw University.

Seeds of *A. montana* collected from the natural habitat were sown *in vitro* following surface disinfection. We tested two concentrations of macronutrients of MS medium (1962) on growth, proliferation and rooting. Although the plant naturally inhabits poor habitats, it grows and reproduces well on a medium with high salt concentration.

The influence of light intensity on the plant weight gains, reproduction and acclimatization was determined. Light of high intensity causes shortening of shoots and reduces the number of the buds that develop later, but significantly improves plant acclimatization. The roots developed under these conditions have the highest fresh weight.

Many authors have reported a positive effect of cytokinins, especially BA on vegetative reproduction of *Arnica*. In our experiments the enrichment of the medium with cytokinins increased the propagation coefficient to 2-3, but the plants very quickly became necrotic.

The reason behind rapid aging of the culture after addition of cytokines is not known. Therefore, the use of cytokines must be cautious and other factors contributing to the spread of the plant should be explored. Similar but intensified symptoms were noted after application of TDZ.

It was shown that the light conditions under which the plants were cultivated had an enormous impact on the ability to acclimatize. Plants exposed to the light intensity of 58 μmol $m^2 s^{-1}$ acclimatized easily following transfer to the greenhouse as opposed to those grown under lower light conditions. Of little significance was treatment of roots with Vaxi-root preparation containing ectotrophic mycorrhiza fungi *Pisolithus tinctorius*, *Rhizopogon*, *Laccaria* and helpful bacteria.

Visually, positive impact had ericoid mycorrhiza (ERM) vaccine with inoculum containing vegetative mycelia of *Hymenoscyphus sp.* and *Oidiodendron sp.* with bacteria of MHB type (Mykoflor).

The propagation *in vitro* of *Biscutella laevigata* L. (Brassicaceae)

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This presentation concerns *Biscutella laevigata* (Brassicaceae), the species which has developed different ecotypes, recorded on European alpine stands, metal enriched soils, and anthropogenic substrates polluted with heavy metals. In the present study a survey is conducted on the suitability of an *in vitro* culture technique for vegetative propagation of a calamine ecotype from the Olkusz ore-bearing region located in the south-eastern part of the Śląsko-Krakowska Upland. In the first stage of the experiments seed germination ability in *in vitro* conditions was determined. An effortless way to start cultures of that species can be by obtaining primary explants from seedlings previously acquired in aseptic conditions, remembering, however, that the term of seed sampling is crucial for such undertaking. The greatest number of properly shaped seedlings which could easily develop to plantlets were obtained as a result of seed germination. They were sampled on 27th June and germinated in 65.5%. As primary explants, seedlings deprived of roots were used. They were implanted into a modified MS medium, supplemented with 25 $g \cdot l^{-1}$ sucrose, 0.6 $g \cdot l^{-1}$ calcium gluconate, 0.6 $g \cdot l^{-1}$ activated charcoal, 1.0 $mg \cdot l^{-1}$ 2iP, 0.1 $mg \cdot l^{-1}$ NAA, and solidified with 0.7 $g\ l^{-1}$ agar. The pH = 5.8. Organic additives of different kinds were tested as medium supplements: squash obtained from fleshy *Ananas comosus* (Stickm.) Merr. (Bromeliaceae) fructification, liquid endosperm from *Cocos nucifera* L. (Arecales) drupe, and spent medium after culture of *Desmodesmus subspicatus* (R. Chodat) E. Hegewald & A. Schmidt (*Chlorophyceae*), that is a conditioned medium. A positive influence of the organic supplements applied on *B. laevigata* micropropagation was observed in the media containing plant growth regulators. The exploited plant-derived supplements, rich in organic compounds, concurrently with growth regulators included in the propagation medium, improved the multiplication *in vitro* of the tested plant material. The highest number of adventitious rosettes was obtained on a medium enriched with 10 $ml \cdot l^{-1}$ pineapple pulp, where the mean micropropagation coefficient (MC) reached 3.3 during a 6-week-long culture. A slightly lower efficiency of rosette multiplication was noted on the media containing a conditioned medium in both tested concentrations (MC = 2.8), and coconut water (MC = 2.6). Surprisingly, on the media without plant growth regulators, but supplemented with natural ingredients tested, the formation of adventitious rosettes was equal to that on control medium, containing plant growth regulators. This indicates a possibility of reducing the use of synthetic auxins and cytokinins in clonal propagation of the examined species. The micro-rosettes multiplied *in vitro* were also rooted in *in vitro* conditions, on hormone-free, modified propagation medium, with macro- and microelements amounting to 1/3 of the initial concentration. Plant material obtained in such a way can be effectively acclimatized to *ex vitro* conditions in a growth chamber. Rooted rosettes were transplanted to ceramic flowerpots, 90 mm in diameter, filled with autoclaved potting mixture composed of perlite, sand and horticultural substrate (1 : 1 : 1 v/v), and were kept for 28 days in a controlled growth chamber at the same conditions as during the micropropagation stage, with the exception of relative humidity, maintained during this phase at the 50% level. Afterwards young plants were placed in a greenhouse, where they were gradually transplanted to bigger containers, containing mixture of perlite, sand, and post-flotation wastes obtained in the process of zinc-lead ores enrichment (1 : 1 : 3 v/v). The cultivation of plants obtained as a result of micropropagation until flowering, fruit setting, and fruiting, can be successfully conducted in greenhouse conditions.

Callogenesis in *Chenopodium quinoa* – cytogenetics, histology and SEM analysis

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Over 90% of angiosperms have been recognized as polysomatic plants. In these plants the endopolyploidisation is a developmental process and it was found in species like *A. thaliana, Brassica napus, Cucumis sativus* and *Beta vulgaris.* In these species, named polysomatic, occurs an organ and tissue specific pattern of endopolyploidisation.

Chenopodium quinoa, a pseudograin which originated from South America, has been analysed in the context of organ specific endopolyploidy patterns. The DNA content in the analysed tissues ranged from 2 to 16C DNA. Cells with 8C DNA occurred already in the embryos radicle. During the next developmental stages cells with 8C and 16C DNA content were observed in the root and in the hypocotyl. However, cotyledons and leaves had only 2C and 4C nuclei (Kolano et al., 2008). Polysomaty is one of the most important factors in the context of variation during *in vitro* culture, so the level of explant endopolyploidy should be taken into account while making a selection of an explant for *in vitro* culture.

In our experiment roots, hypocotyls and cotyledons of one-week-old seedlings and leaves of two-week-old plants were used as explants. As in the previous estimation, the highest level of endopolyploidy was observed in the roots, and the lowest in the cotyledons and leaves. The endopolyploidy pattern of hypocotyl differed along the organ. It was the lowest near the apical meristem and the highest near the root. For this reason the middle part of the hypocotyl was chosen for the *in vitro* culture.

Three composition of media containing auxin in high and low concentrations, and auxin with kinetin, were tested in order to determine callus induction. A high efficiency of callogenesis was observed with no significant differences between the media used. Also the ability to callogenesis of all explants was very high, with a little lower results for root explants. For subsequent analyses media with lower concentration of auxin and without kinetin were chosen.

Analyses of the nuclear DNA content during 10 weeks of *in vitro* culture showed very interesting results. After two weeks of culture the level of polyploid cells decreased in root and hypocotyl explants and increased in cotyledon and leaf explants. The level of polyploidisation in explants and growing callus tissue changed with the time of the culture. After 10 weeks similar histograms were obtained for all types of cultured tissues. Two peaks (2C and 4C DNA) were dominating and a small number of 8C nuclei was observed.

The histological analyses were conducted with the aim to explain the results obtained from the flow cytometry estimations. In root and hypocotyl explants diving cells around the vascular cylinder were observed after two weeks of culture. In such cells usually only nuclei with 2C and 4C DNA content occur. On the other hand, in the cotyledon and leaf explants many giant cells were observed in which probably the endopolyploidy occurred.

After 6 and 10 weeks of culture a small population of nuclei between 4C and 8C DNA was noted, which suggested that there were numerical chromosome aberrations. This was confirmed by chromosome counting where cells with a aneuploid chromosome number occurred.

Histological and scanning microscopy analysis was also extended on the process of callogenesis and possible regeneration. Explant tissues involved in callus formation had been recognized and formation of adventitious roots occurred.

Cytomolecular analysis of epigenetic modifications during the cell dedifferentiation and callus induction of *Brassica* species

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The development of living organisms is modulated by the influences of internal and external environment in which a given organism exists. Epigenetic regulation of gene expression in response to these factors is essential for the maintenance of homeostasis during *in vivo* and *in vitro* morphogenesis. Proper gene expression is regulated by changes in the pattern of chromatin remodeling. It involves DNA methylation, histone posttranslational modifications and other factors that cause changes in chromatin condensation which influences its accessibility to transcription factors and gene expression. The epigenetic modifications in the developmental process and plant morphogenesis *in vivo* are relatively well known, both at the molecular and cytogenetic level. But little is known about the chromatin rearrangements and epigenetic modification at the cytogenetic level during dedifferentiation, proliferation and re-differentiation *in vitro*.

The aim of the presented research was to identify changes in the plant nuclear genome and pattern of epigenetic modification during cell dedifferentiation and callus induction *in vitro*. The analysis of plant organs with a defined function and development program lead to the characterization of chromatin structure and epigenetic modifications in differentiated cells. A comparison with cells that: underwent dedifferentiation to the meristematic state during the callus tissue induction allowed to trace the changes in the spatial organization of chromatin as well as the epigenetic modification pattern.

In the present study on diploid species *B. oleracea* and allotetraploid *B. napus* the immunostaining with antibodies against specific histone modification (H4K5ac, H3K4me2, H3K9me2 and H3K9me3) were used to analyze the effect of *in vitro* culture on epigenetic pattern in an isolated interphase nuclei. Cotyledons from 6-day old seedlings were chosen for an *in vitro* experiment. The types and morphology of the nuclei isolated from the explant and callus were determined using an image cytometer. Based on fluorescence intensity, the nuclei were classified to phases of cell cycle – G1, G2 or endoreduplication. For each class of nuclei the pattern of epigenetic modifications (localization in chromatin and fluorescence intensity after immunostaining) was analyzed.

The results showed differences in nuclei morphology and the pattern of epigenetic modification, especially acetylation of H4 histone and methylation of histone H3, between the cotyledons and cells undergoing the dedifferentiation process. During the *in vitro* culture, massive changes in chromatin structure were observed. The nuclei were characterized by chromatin disorganization, decondensation of chromocenters and occurrence of large nucleoli. The process of cell dedifferentiation and proliferation was associated with an increasing level of histone acetylation and methylation. In addition, significant differences were observed in the localization of acetylated histone H4 and methylated histone H3. In the cell nuclei of cotyledons the presence of these modifications was mainly detected in the euchromatin. However, in the callus tissue a significant fraction of nuclei had strong immunosignals also in chromocenters. These results demonstrate the dynamic nature of changes in the conformation of nuclear chromatin and epigenetic modifications associated with the process of dedifferentiation to the meristematic state and proliferation of callus tissue cells.

Morphogenetic potential of *Gentiana cruciata* root explants in *in vitro* culture

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Plants from *Gentiana* genus are the source of many pharmacologically active secondary metabolites and a typical place where they are accumulated is the root. Moreover, they are popular ornamental plants. Many *Gentiana* species are scarce or in danger of extinction and are, therefore, protected by law. The protection extends on *inter alia* all Polish native species, including *G. cruciata*. The root is a relatively scarcely used type of explant, also in *Gentiana* genus. This work aimed to describe the morphogenetic potential of *G. cruciata* root explants *in vitro*.

Surface-sterilized seeds of *G. cruciata* obtained from our Botanical Garden were germinated on MS medium. All the media used in this study were based on MS medium. Root explants were excised from seedlings with developed cotyledons and with a distinct terminal bud. Depending on the experiment (A – C) explants were placed on: A) solid induction medium supplemented with a combination of three NAA concentrations (0.0; 0.1; 1.0 mg/l) and four BAP, kinetin or TDZ concentrations (0.0; 0.1; 1.0; 10.0 mg/l). After three months of culture in the light or in the dark the type and intensity of the morphogenetic reaction were evaluated. In the case of B experiment solid medium supplemented with 1.0 mg/l TDZ+ 0.1 mg/l NAA or 5.0 mg/l TDZ + 0.1 mg/l NAA was employed. Later, after 2, 5, 10 and 15 days of culture (or permanent culture), the explants were transferred onto a medium without growth regulators. Cultures were conducted in the light. The evaluation of morphogenic potential of the culture was done after 70 days. For experiment C, 25 ml of liquid induction medium completed with three combinations of NAA and BAP concentrations, respectively 2.0 mg/l+ 0.0, 0.02 and 0.2 mg/l and in the fourth variant with 2,4-D (0.5mg/l) and kinetin (1 mg/l) were used. Conical flasks with cultures were hold on a rotory shaker at 100 rpm in the light. Within a few months the type and intensity of the morphogenetic response were observed. The resultant somatic embryos and aggregates with embryos were transferred to two media for conversion into plants (the first one without growth regulators and with decreased sucrose level i.e. 10 g/l, the other one with kinetin, GA3 and NAA).

The main morphogenetic reactions in the two first experiments (A, B) on solid media were rhizogenesis and callus formation with intensity depending more or less on the variant. Regeneration, mainly SE, occurred sporadically.

In experiment C, in liquid cultures on media with NAA and BAP the first intensive rhizogenesis occurred on root explants and a small callus tissue formed. However after 2-3 months, despite some differences, all three media showed massive regeneration, mainly by SE. Regenerating structures appeared as fragments of aggregates or individually, but their growth was limited to the stage similar to mature somatic embryos. In the experiments conducted so far frequency of regeneration has been 66-100% for medium with 2.0 mg/l (NABA13), 100% for 2.0 mg/l + 0.02 mg/l BAP (NABA14) and 58-100% for 2.0 mg/l NAA + 0.2mg/l BAP (NABA15). Thus, so far, the highest recurrence with high efficiency (massive regeneration in each flask) has been achieved for NABA14 medium. In the fourth variant i.e. on medium with 2,4-D and kinetin, rhizogenesis did not take place, and SE stopped at the stage of often malformed, early stages of somatic embryos. Preliminarily, the possibility of conversion of particular embryos in aggregates into plants was observed on two media: without growth regulators, with decreased sucrose level and with regulators (GA3, NAA and kinetin). The quality of regenerated plants will be examined by measuring DNA content using flow cytometry.

Conditioned medium of algae in cultures of *Nicotiana tabacum*

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The term "conditioned medium" (CM) denotes a medium in which cells have already been cultivated for a period of time. CM contains unused mineral compounds as well as extracellular organic compounds produced and secreted into the medium by the cultured cells. CMs have been widely used by plant biotechnologist for many years as supplements supporting the growth of plant cells, tissues or organs. Such an approach appeared to be a very efficient and cost-effective method of obtaining large amounts of plant biomass. The possible explanation of growth stimulation is the presence in the medium of an unknown growth promoting substance(s) with universal properties able to influence cell proliferation.

Conditioned media obtained from algal cells are very effective in improving growth cells of different plants. It is interesting that algal exudates from one species can have influence on the other ones. CM from *Desmodesmus subspicatus* is active towards closely related organisms (*Desmodesmus armatus, Desmodesmus microspina*), other algal taxons (*Chlamydomonas reinhardtii*), as well as to more evolutionarily distant species like higher plants (*Silene vulgaris*).

In this study we describe the effect of a conditioned medium obtained from green alga *Desmodesmus subspicatus*, on the growth and photosynthetic activity of dicotyledonous plant *Nicotiana tabacum* cultured *in vitro*. Tobacco plants grown on a conditioned medium diluted 5-, 2-, and 1.25-fold (CM/5, CM/2, CM/1.25) were significantly greater than those cultured on other media without CMs supplementation. There was observed an increase both in the number of leaves and leaf area. In addition, plants grown on the CM supplemented medium had a better developed root system and the most effective ones appeared a medium containing the highest concentration of CM (CM/1.25). Morphological observations were confirmed by determination of fresh weight and dry weight of the plant. The largest increase in fresh weight was observed for tobacco plants cultured on medium CM/2 and CM/1.25, and the latter medium also caused an increase in dry weight of tobacco roots. A similar trend was found for chlorophyll *a*, *b* and total carotenoid contents. Chlorophyll *a* fluorescence parameters, measured by OJIP test, were applied to explain the effect of a conditioned medium on the photosynthetic apparatus efficiency. The fresh weight as well as the values of "vitality" (PI) parameter were equally enhanced by the conditioned medium. The maximum yield of electron transport, as well as the fraction of active reaction centers were also significantly higher in plants grown on CMs. The significant differences in biomass production of plants grown on different media suggest that the growth improvement was caused by the presence of a conditioned medium. Some compounds produced by *Desmodesmus subspicatus* and secreted to the culture medium seem to have versatile properties and the fluorescence induction study suggests that the stimulation of the growth of *Nicotiana tabacum* by CMs is tightly connected with the stimulation of photosynthesis.

Effects of inbreeding on seed germination and seedling vigor of strawberry (*Fragaria x ananassa Duch.)* in the *in vitro* conditions

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Since the strawberry is a vegetatively propagated crop and thus highly heterozygous in nature, inbreeding should theoretically reveal undesirable combinations of genes which, once eliminated, should leave plants with a high concentration of desirable characters. The remaining inbred lines could be valuable as parents both in crosses between each other and with cultivars, producing progenies with a high proportion of elite selections. The value of inbreeding in strawberries has never been fully resolved.

Cultures *in vitro* are a perfect method for observation of seed germination and also the influence of inbreeding on these processes.

Experiments leading to the obtainment of subsequent inbred generations were conducted between 2004 and 2012 at the Felin Research Plantation of the University of Life Sciences in Lublin. Ten strawberry cultivars including "Kent", "Selva", "Elkat", "Elsanta", "Paula", "Ostara", "Teresa", "Senga Sengana", "Chandler" and the breeding clone 1387 were examined and self-pollinated but only 5 of them developed the second, third and fourth inbreeding generation. These genotypes derived from free pollination and different levels of self-pollination were evaluated for their tolerance to strong inbreeding under *in vitro* culture conditions. The percentage of germinating seeds and seedling viability were estimated. The germination of seeds was observed for 10 weeks. Inbreeding depression coefficients ($\delta = 1 - ws/wo$, where ws and wo were the fitness estimates of selfed and outcrossed progenies respectively) for each progeny were calculated.

"Teresa" cultivar derived from the free pollination and "Kent 7" S_4 population showed the highest germination of seeds (82% and 78%, respectively). The lowest percentage of germinability revealed seeds derived from self-pollination - the average of 16.8%. Generally, seed germination was significantly lower for selfed fruits for five S_1 offspring whose depression was 0.62 in comparison with the S_4 seedlings whose depression was 0.31. The highest seedling vigor, expressed as the average weight of a single seedling was estimated for plants obtained by free pollination (average weight 0.0138 g). Inbred offspring showed depression in relation to those plants which was in the case S_1 progeny 0.34 whereas in the case of S_4 progeny – 0.22. The highest germination energy, or the number of seeds capable of rapid germination revealed seeds of "Kent 7" S_4 – 54% and "Teresa" cultivar derived from the free pollination – 40%. Whereas the seeds obtained simultaneously from self-pollination germinated an average of 0.6%.

The obtained results indicate that in the case of strawberries, inbreeding depression for germination of seeds decreased in successive inbreeding generations and was lower in the fourth generation compared to the S_1 generation. These findings are consistent with data presented by other authors. Moreover, a significant correlation exist between inbreeding depression and selfing/outcrossing species, namely repeated selfing reduces the magnitude of depression. In contrast, outcrossing usually increases heterozygosity that tends to produce genotypes better adapted to different environmental conditions.

Improvement of the quality of anthurium microcuttings

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Anthurium is one of the most important ornamental plants, grown both as a cut flower and as a potted plant. Anthurium is mainly propagated by the *in vitro* culture but the growth of microplants after weaning is usually very slow – plants achieve 8-12 cm after about 4 months. The long acclimatization period and the time to speed up the growth of young plants can be shortened by improving the quality of microcuttings owing to the optimization of rooting and acclimatization. Microcuttings for the experiment were obtained from 8 week old cultures grown on the multiplication medium containing MS salts and vitamins, 1 mg/l BAP, 80 mg/l adenine and 20 g/l sucrose. Shoots of a length greater than 3 cm, with 3-4 leaves can be used for rooting or directly to acclimatize. Anthurium shoots produce aerial roots on a medium that stimulates their growth and propagation. To obtain more uniform material, of better quality, or in the case of varieties which root harder, it is recommended to root the shoots *in vitro*.

In our experiment root formation was conducted as a one or two-step procedure. In a one-step procedure, the rooting microcuttings were incubated in a medium containing auxin. The following experimental treatments were performed: 1 – agar medium with 0.5 mg/l NAA, 2-5 – agar medium with 0, 0.5, 1.0, 2.0 mg/l NAA with the addition of activated charcoal in the amount of 4 g/l, 6 – agar medium with 0.5 mg/l NAA with the addition of 5 mg/l of vitamin B2, 7 – vermiculite watered in the liquid MS medium supplemented with 0.5 mg/l NAA. The best results were obtained by placing shoots in the sterile vermiculite watered with a liquid medium containing MS salts and vitamins, 0.5 mg/l NAA, 80 mg/l adenine, 20 g/l sucrose, pH 5.6. In a two-step rooting, the composition of the first medium was such as the above, except that it was solidified with Gelrite 2.2 g/l. After 5 days in the dark, the microcuttings were transferred to the similar conditions, but without auxin, and placed in the light. The next experimental treatments were as follows: 1 – agar medium without NAA, 2 – medium with activated charcoal 4 g/l, 3 – vermiculite soaked with liquid MS medium, 4 – perlite soaked with liquid MS medium. The best quality microplants were obtained in the treatments 2 and 3. After 8 weeks, the rooted shoots were transferred into the greenhouse and planted in various substrates: vermiculite, Klasmann TS2, Klasmann TS2 + coconut chips (1:1), Klasmann TS1, TS1 + coconut chips (1:1), peat + perlite (1:1), peat + perlite (4:1). The best results were obtained by using vermiculite watered with MS medium, Klasmann TS1 and TS1 + coconut chips. In those substrates, microplants grew rapidly, and after two months the fresh weight, the leaf area and the root system increased by 80%. During the acclimatization, high humidity, shading of young plants and preventive protections using fungicides Previcur 607 SL 0.1% and Rovral Flo 255 SC 0.1% were applied. After 10 days, a compound fertilizer appropriate for young plants of anthurium was used in a manner recommended by the manufacturer.

Acclimatization of lachenalia (*Lachenalia* sp.) regenerants propagated *in vitro* in different light conditions

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Lachenalia J.F. Jacq ex Murray (*Hyacinthaceae*) is a flowering bulbous plant endemic to South Africa and Namibia, new to the international flower market. An increasing number of species can be recommended as a pot plant or a cut flower. They are admired for attractive flowers, ranging in color from red, yellow to purple and leaves which are frequently spotted. In the 1960s at the Vegetable and Ornamental Plant Institute in Pretoria a breeding program was initiated in order to develop new hybrids for commercial pot plant production (Coertze et al. 1992). Currently a group of most popular lachenalia cultivars is sold under the trade name "African Beauty" series.

In the present paper an acclimatization of lachenalia "Rupert" (violet blue flowers) and "Ronina" (yellow flowers), both from the "African Beauty" series has been described. Light treatments during *in vitro* culture, including white, blue, red lights and dark conditions (Bach and Kapczyńska, 2011), were used in order to determine their influence on plants in *ex vitro* conditions. After the plants were obtained by regeneration of initial explants under *in vitro* different light conditions, rooted shoots of "Rupert" and "Ronina" were transferred from the tissue culture to a greenhouse for acclimatization. The morphological features of plants, as well as the quality of bulbs obtained after the first season of cultivation, were evaluated. Additionally, after the *ex vitro* transfer, chlorophyll fluorescence was measured using a Mini PAM fluorometer.

The rate of plants acclimatization depended on the wavelength of light applied and the cultivar but generally the plants had a high percentage of survival which ranged from 90 to 100 in "Rupert" and from 70 to 86 in "Ronina".

A significant influence of light treatment on the number of leaves of "Ronina" was observed – most leaves (2.4 per plants) were obtained from regenerants transferred from blue light. About 1.7 leaves were formed per one plant of "Rupert" independently of the light factor. Regardless the light condition during *in vitro* culture, "Ronina" formed shorter leaves (16-18 cm) compared to "Rupert" (21-25 cm). It was observed that "Rupert" has a great potential, especially under white light, to form flowers even during the acclimatization process – 30-50% of plants produced inflorescence stalks. The flowering of "Ronina" was noticed occasionally and only on regenerants moved from white and red light. The results indicated that lachenalia develops distinct bulbs depending on the cultivar. The weight of bulbs (established at the beginning of the rest period) was different significantly among cultivars: "Rupert" (2,1-2,6 g) and "Ronina" (1,3-1,6 g).

The optimal level of the ratio of variable to maximal chlorophyll fluorescence (Fv/Fm) for the great majority of vascular plants should not be lower than 0.8 (Bjorkman and Demmig, 1987). Our results indicated the differences in Fv/Fm ratio between the tested genotypes. "Ronina" plants had lower Fv/Fm values (0.78-0.79) in comparison with 'Rupert' plants (0,8-0,82).

In conclusion, the experiment showed that *in vitro* light conditions prior acclimatization, as well as genotypes, are important factors for *ex vitro* growth of lachenalia.

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In vitro rooting of carrot (*Daucus carota* L.)

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Introduction

Carrot (*Daucus carota* L.) is one of the most economically important vegetable crops in the world. Currently the research in this species is focused on improving the existing, or creating new cultivars that would meet the requirements of breeders and consumers. In addition to classical breeding methods those studies frequently involve tissue cultures. Unfortunately, the use of *in vitro* techniques often entails significant losses of plant material at the stage of root formation. Therefore to limit such losses it seems advisable to determine the optimal composition of the carrot rooting medium.

Aim of the study

The aim of the study was to investigate the influence of different growth regulators (IAA, IBA and NAA) on the rooting of carrot in *in vitro* cultures.

Materials and methods

The experiment was conducted on three carrot accessions including two cultivars: Scarla and Valor F_1 and DH line no. 726. After sterilization the seeds were cultured in Petri dishes on MS (Murashige and Skoog, 1962) medium. After four weeks of germination, seedling shoot stumps with three youngest leaves were isolated and transferred to three different media containing growth regulators: MS + 2 mg l^{-1} IAA, MS + 1 mg l^{-1} IAA + 1 mg l^{-1} NAA and MS + 6 mg l^{-1} IBA + 0.5 mg l^{-1} NAA. MS medium without growth regulators was used as a control. Forty-five shoot stumps from each carrot accession were grown on each tested medium.

The number and morphology of shoots formed from explants was recorded after one month. A statistical analysis of the obtained results was performed by two-way analysis of variance ($P = 0.05$).

Results

The results indicated that both the medium and the accession had an influence on the formation of a large number (> 10 per plant) of carrot roots in *in vitro* cultures. MS+IAA and MS+IBA+NAA media proved to be the best and supported the root induction with the mean frequency of 25.3% and 22.4%, respectively. On the control medium and MS+IAA+NAA medium the mean percentage of root induction reached only 7.8% and 6.4%, respectively. The results also indicated that the highest percentage of rooted plants, reaching 24.9% and 21.3% was observed for cultivars Valor F_1 and DH line no. 726, respectively. Scarla cultivar also stimulated the root induction, but with statistically lowest frequency of only 13.3%.

Morphological observations revealed that the roots formed on MS and on MS+IAA+NAA media were similar and had a medium length and thickness. The roots formed on MS+IAA medium were the longest and thinnest, while those formed on MS+IBA+NAA medium were the shortest and thickest.

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In vitro conditions induce changes in DNA methylation and autonomous endosperm development in wild genotype and mutants of *Arabidopsis thaliana*

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Double fertilization is usually required for full seed development. This event is circumvented in apomictic plants that reproduce clonally through seeds, sporadically in sexual Angiosperms and under experimental conditions. The molecular research of mechanisms controlling autonomous endosperm (AE) development in *fie* and *met1* mutants of *Arabidopsis* strongly suggests, that changes in genomic imprinting and DNA methylation are essential for autonomous/apomictic development (Curtis and Grossniklaus, 2008; Köhler and Weinhofer-Molisch, 2009). *In vivo*, partly-developed AE occurs only in *FIE/fie* genotype; the combination of *fie* and *met1* mutations results in full AE development. On the basis of this knowledge we formulated two hypotheses: (1) *met1* mutation combined with *in vitro* condition induce AE development (2) *fie* mutation + *in vitro* condition induce full AE development.

Arabidopsis thaliana genotypes (wild, *met1* and *fie* mutants) were chosen to investigate the development of autonomous endosperm in unfertilized ovules cultured *in vitro* and to understand the mechanism controlling AE induction. Unpollinated pistils were cultured on hormone-free Murashige and Skoog medium (MS) with an addition of 6% sucrose and supplemented with combinations of phytohormones, mammalian sex hormones or a demethylating substance.

AE was induced and developed in wild and mutant genotypes on all media used with the frequency in ovaries reaching 35%. AE originated from the secondary nucleus of the central cell. Multinuclear autonomous endosperm created several developmental patterns; most of these patterns resembled developmental stages that are characteristic for *Arabidospis* endosperm after fertilization (the presence of micropylar, central and chalazal chambers in AE; NCDs; chalazal nodules and cyst, cellular AE).

AE induction was strongly depended on genotype but not on the type of medium. High frequency of AE induction and advanced development in homozygous mutant (*met1/met1*) were probably caused by accumulation of global demethylation (hypomethylation as the effect of *met1* mutation and *in vitro* condition). The most advanced autonomous endosperm development (with cellular AE) was observed in *FIE/fie* mutants.

In conclusion: the changes in DNA methylation (in one or several genes), caused by *in vitro* conditions and resulting in AE induction and/or further AE development, suggest that *Arabidopsis thaliana*, a model plant, is capapble of developing AE. The development of fertilization independent endosperm in *in vitro* conditions and in unpollinated and emasculated flower buds of *Arabidopsis* wild type (AE *in vivo*) may be treated as the first step toward apomixis.

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