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Plenary lecture

PL3.1

Cellular and regulatory basis of early floral organ growth

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Much has been revealed about how homeotic genes direct the formation of each of the four types of floral organ (sepals, petals, stamens and carpels). However, we still know very little about how regulatory genes control of the location, rate, and direction of cell growth and cell division to produce the shape and size of each organ type. Our work in this area has focused on genes such as JAGGED (JAG), which is a direct target of floral homeotic genes in Arabidopsis and has a general role in organ growth. To understand what cellular processes are targeted by JAG, we combined quantitative, 3D analysis of cell geometry with imaging of cell cycle progression. JAG promoted a transition from isotropic to anisotropic growth, enhanced cell growth and division and caused a loss of coordination between cell volume and DNA synthesis. The de-coupling of cell size from cell cycle induced by JAG was associated with increased cell size heterogeneity, which may be related to accelerated growth and the onset of cell differentiation. Current work indicates that JAG modulates cell size homeostasis by repressing expression of CDK inhibitors of the KRP family. In addition, we found that JAG regulates multiple genes involved in auxin transport and in cell wall modification, which may be required for the shift to anisotropic growth during early organogenesis. Our work highlights the power of quantitative, three-dimensional image analysis to reveal how cell behavior is linked to the gene activities that control floral morphogenesis.

PL3.2

Measuring the mechanics of morphogenesis

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Unlike animal tissues, growth in plants is symplastic, i.e. cells do not migrate nor slide with respect to each other. This puts limitations on the mechanisms available to the plant to generate its form and shape, with morphogenesis relying on the careful coordination of growth at the cellular level. Plant cells are like small balloons, and are under considerable turgor pressure (typically 3-10 atm) resulting from the difference in osmotic potential inside and outside the cell. Containing this turgor pressure is the plant cell wall, a connected extracellular matrix mainly composed of polysaccharides. In order for a plant to grow, stiff cell walls increase in size by several orders of magnitude while withstanding tension in the range of 100-1000 atmospheres. While many of the upstream molecular and signaling pathways driving plant morphogenesis have been uncovered, mechanical aspects of cell wall plasticity and growth coordination remain relatively poorly understood. In order to elucidate these mechanisms, temporal and spatial variations of cell wall properties (visco-elasticity) and turgor pressure have to be assessed in growing organs. However, these kinds of measurements in living cells are a technical challenge. To address this gap, we have developed Cellular Force Microscopy (CFM), a micro-indentation technique for in-vivo stiffness measurements of individual cells. Depending on experimental conditions, the stiffness measured by CFM can give an insight into cell turgor, local wall elasticity as well as cell wall strength. We use physical based simulations to untangle these different contributions to the measured stiffness. Another approach to quantify cell wall elasticity is to track deformation in respon-

se to changes in turgor pressure. Using osmotic treatments to modify turgor, we have shown that the shoot apical meristem, which produces new leaves, is divided into zones of distinct elastic properties. To precisely quantify cell expansion, we have developed software called MorphoGraphX to extract cell geometry from 3D confocal stacks. Using our software we could also track growth at the cellular level. Our results show that the slow growing central zone, containing the stem cell niche, is substantially strained-stiffened compared with the fast growing surrounding peripheral zone. Thus, wall mechanical properties do not only result from gene action and cell history, but feedback on morphogenesis.

Oral presentation

O3.1

Analysis of geometrical features of a developing lateral root by means of biophysical tools

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The process of the lateral root formation involves dramatic changes in its geometry. Experimental data revealed that the cross-sectional shape of the lateral root in *Arabidopsis* changed from its basal to its apical region. The base of the lateral root in the location where it is attached to the parental root is elongated along the parental root axis, which results in its oval shape in the front view, while the circumference of the apical part is usually a circle. This means that the lateral root undergoes radialization along its own axis. The radialization is associated with oblique cell divisions occurring at early stages and leading to breaking the symmetry of the cell pattern. The oval (ellipse-like) shape of the organ base may be a consequence of the origin of the founder cells of the lateral root primordium; namely the group of the two or three xylem-pole pericycle cells, that occupy the area elongated longitudinally. From the mechanical point of view the lateral root formation may be considered as a buckling of the cell wall and the geometrical change demonstrated by a developing lateral root may be initiated and maintained by a local loss of stability of structure of the material, here – walls of the pericycle cells of the parental root giving rise to the lateral root. There are two main objectives of this study: 1) to analyze a character of the growth field of the basal region of the lateral root and 2) to describe a change of form of the lateral root in mechanical aspect. On the basis of measurements of the long and short diameters (DL and DS, respectively) of the ellipse-like figure representing the bases of particular lateral roots the asymmetry ratio (DL/DS) was determined, then the cases of the maximal, minimal and mean value of the ratio were taken into further consideration. For the three mentioned asymmetrical cases as well as for the hypothetical case of symmetrical base (asymmetry ratio equal to 1) the rates of growth at selected points on the surface of the lateral root were determined with the application of the growth tensor (GT). On the basis of this method maps showing distribution of growth rates in lateral roots of various shapes of the basal part were obtained and compared. In description of the lateral root formation in mechanical aspect only the surface of the lateral root was taken into consideration. First the critical value of turgor causing the local loss of mechanical stability was estimated. The local buckling of the cell walls resulted from the loss of stability was considered as a deformation of the external surface representing developing lateral root. Under specific assumptions concerning mechanical properties of the cell wall, growth of the organ at early stages was analyzed. The form of the virtual lateral root was fitted to the form of a real organ on the basis of data from literature.

O3.2

Light-triggered protochlorophyllide to chlorophyllide reduction – new insights into enzyme-substrate interactions

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Biosynthesis of chlorophyll, the main photosynthetic pigment, is entirely light-dependent in Angiosperms. Protochlorophyllide oxidoreductase (POR) catalyzes reduction of one of double bonds in the tetrapyrolle ring of protochlorophyllide (Pchlide) molecule. To convert the substrate into the product, ternary Pchlide:POR:NADPH complexes have to be formed, and the pigment molecule has to be in electronically excited state. In the present studies, we have obtained recombinant PORA, PORB and PORC isoforms from *A. thaliana* in *E. coli* expression system. All the POR isoforms spontaneously formed photoactive complexes with Pchlide and NADPH in lipid-free reaction system. Only slight differences in fluorescence emission spectra measured at 77K were noticed among the investigated POR isoforms. A fluorescence maximum was observed between 650-655 nm, which is characteristic for aggregates of Pchlide:POR:NADPH complexes *in vivo* [reviewed by Schoefs 2005]. We have also shown that POR molecules spontaneously organized into oligomers that are able to bind substrates forming photoactive complexes. Using site-directed mutagenesis and bioinformatics tools, we have identified a histidine residue involved in a Pchlide binding and a few other amino acid residues that stabilize Pchlide – binding pocket in PORA. These results allowed us to distinguish several steps of substrates binding to POR oligomer and to propose the mechanism of this process. This work was supported by Grant 2011/01/B/NZ1/00119 from the National Center of Science of Poland (NCN).

O3.3

Interaction between meristem tissue layers controls phyllotaxis

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Phyllotaxis and vein formation are the most conspicuous patterning processes in plants, and are thought to occur via feedback of the plant hormone auxin on its own transport. In Arabidopsis, the expression and polarization of the auxin efflux carrier PIN1 is the earliest known marker for both processes, with patterning models based on the hypothesis that PIN1 can respond to auxin gradients and/or auxin flux. Using cell-layer specific PIN1 knock-outs and partial complementation of auxin transport mutants, we examined the interaction between phyllotaxis patterning, which occurs primarily in the L1 surface layer of the meristem, and midvein specification in inner tissue. We show that PIN1 expression in the L1 is both sufficient and necessary for correct organ positioning. Thus, differentiation of inner tissues can proceed without PIN1 or any of the known polar transporters. We also demonstrate that auxin is concentrated in the L1 layer via the activity of L1-specific auxin influx carriers and PIN1 expressed in the inner tissue. Our results demonstrate that correct phyllotaxis requires a spacially coordinated action of both auxin exporters and importers.

O3.4

Functional analysis of ABI5 and RGL2 in dormancy breaking of *Acer platanoides* L. seeds

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Dormancy is the temporary failure of a seed to complete germination under favourable condition. It is an innate seed property that defines the environmental conditions in which the seed is able to germinate. It prevents germination in environments generally unfavorable for subsequent vegetative plant growth and reproduction of the next generation. The breaking of dormancy and the germination is governed by environmental cues, including temperature, light, nitrate, and some smoke components. Seeds of Norway maple (*Acer platanoides* L.), characterized by deep physiological dormancy located in embryo, require for germination a cold stratification lasting around 15 weeks. Seed germination is antagonistically controlled by the phytohormones gibberellic acid (GA) and abscisic acid (ABA). GA promotes seed germination by enhancing the proteasome-mediated destruction of RGL2 (RGA-LIKE2), a key DELLA factor repressing germination. By contrast, ABA blocks germination by inducing ABI5 (ABA-INSENSITIVE5), a basic domain/leucine zipper transcription factor repressing germination. *ABI5* expression is strongest during the later stages of embryogenesis (Finkelstein and Lynch, 2000). The studies carried out on *Acer platanoides* seeds dormancy breaking showed that in this processes proteins of different classes are involved, which activity depends on antagonistic influence of ABA and GA. Goal of this study was to estimate the role of ABI5 and RGL2 in regulation of deep physiological dormancy in *Acer platanoides* seeds. Using immunodetection Western blot and immunolocalisation methods we estimated involvement of transcription factors of signal transduction pathways of ABA and GA in dormancy breaking in embryo axes of *Acer platanoides* seeds. The work was supported by the National Science Centre, Poland, grant number 2011/01/B/NZ9/02868.

O3.5

The beginnings of sexual land plant reproduction – regulation of gene expression involved in archegonia development in dioecious liverwort *Pellia endiviifolia* sp B

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The migration of aquatic algae to terrestrial environment and the subsequent evolution of land plants required many morphological and reproductive adaptations which enabled the effective optimization of the vegetative and reproductive activity on land. Among early diverging lineage of land plants (bryophytes) liverworts are considered the most basal one, what puts this group in critical evolutionary position to investigate the genetic basis of key innovations which allowed the adaptation to stresses of the terrestrial environment. *Pellia endiviifolia* species B is a dioecious liverwort belonging to class *Jungermaniopsida*, which comprise over 80% of today living liverworts. The representatives from genus *Pellia* are recognized as the one of the most basal lineage of the simple thalloid liverworts. The dominant generation of liverworts lifecycle is haploid gametophyte producing egg- and sperm-forming gametangia, in many species on separate male and female individuals. Presently there is almost no data about the mechanisms regulating the sexual reproduction in the case of liverworts from class *Jungermanniopsida*. The utility of RDA-cDNA technique allowed us to identify three genes specifically expressed in the female individuals of *P. endiviifolia*. These are *PenB_CYSP* coding for cysteine protease, *PenB_MT2* and *PenB_MT3* coding for *Mysterious Trans-*

cripts1 and *2* containing ORFs of 143 and 177 amino acid residues in length, respectively. All three genes are expressed in the female thalli, regardless of whether they were cultured *in vitro* or were collected from natural habitat. To learn about the exon-intron structure of these genes and their transcript ends genome walking and RACE experiments were performed. Interestingly the comparison of 5′RACE and genome walking results revealed five mRNA isoforms produced from *PenB_MT2* gene, which are result of alternative splicing within the second and third exon. All observed splicing events take place within the 5′UTR and do not interfere with the coding sequence. What is the biological implication of the alternative splicing event needs to be further investigated. Our present and previous studies show for the first time the specific contribution of several identified genes in the male or female gametophyte development of the representative species from genus *Pellia*. Now with the use of next generation sequencing technology we are performing in-depth analysis of genes engaged in antheridia and archegonia development in *P. endiviifolia* sp B. RNA-seq was performed on four different developmental stages: the male thalli i) producing or ii) without antheridia, the female thalli iii) producing or iv) without archegonia. As first set 62 differentially expressed genes were selected with 10-fold higher expression in the female gametophytes producing archegonia in comparison to the male ones. To confirm whether the selected genes show indeed the archegonia specific expression qRT-PCR experiment will be performed.

Posters

P3.1

Stress distribution in cell wall – inferring stress anisotropy from the pattern of buckling in the innermost cell wall layer

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In a plant cell mechanical stresses at the subcellular level are transmitted mainly by cell walls. The stresses at the organ level can be very different from those at the cellular level, because of the impact from organ geometry and tissue stresses. In the case of cell in the intact organ, tissue stresses can modify the stress pattern which would occur if a cell would be isolated from the organ. For example, in a cylindrical isolated cell (*Nitelopsis* internode) the maximal tensile stress is in the transverse direction with respect to the cell axis, but in the case of a cell in organ (an epidermal cell in the stem), it is in the longitudinal direction. Previous experiments showed that isolation of stem epidermis from the organ abolishes tissue stresses and application of hyperosmotic solution to detached epidermis brings the turgor to zero. In consequence of stress removal we can observe the pattern of transverse bands differing in paleness on the inner surface of the outer wall of epidermis. The striation represents the pattern of transverse folds on the inner surface of the wall. They result from buckling of the inner wall layer under longitudinal compressive force exerted on this layer by the outer layer, when it shrinks elastically after abolition of turgor-caused stress. The adaxial epidermis of mature onion bulb scales adheres weakly to the underlying parenchyma. Therefore, no tissue stress occurs in the epidermis and tensile stress in cell wall is only that directly caused by turgor. Thus the distribution of tensile stress in cell wall is a function of cell geometry. The epidermal cell monolayer exhibits rather uniform cell geometry (elongated cells), although at the higher organization level than *Nitelopsis*. Theoretically, in elongated cells of onion epidermis the pattern of bands should be longitudinal i.e. parallel with respect to cell axis and perpendicular to the direction of maximal tensile stress (which is in the transverse direction with respect to the cell axis). However, our observations show that three main types of bands pattern could be recognised after plasmolysis: oblique (the most frequent), longitudinal, and transverse (the rarest) to the longitudinal cell axis. This phenomenon is reversible, it means that after deplasmolysis bands disappear. The TEM micrographs of cross-sections through the outer wall of the epidermis showed that as in case of sunflower epidermis, waviness of inner cell wall surface results from buckling of the inner wall layer. Additionally, we observed different bands pattern near the cell ends (which can be wedge or rectangular shape). Because bands pattern in onion epidermis is usually different from theoretically expected, the question arises, what determines bands arrangement in the wall: cell shape, different stress distribution and cellulose microfibril arrangement at the ends of cell from that in the linear part of cell, forces exerted from neighbouring cells? MAESTRO grant No 2011/02/A/NZ3/00079.

P3.2

Protochlorophyllide photoreduction in angiosperms: focus on protochlorophyllide – surroundings interaction in model systems

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Protochlorophyllide (Pchlide) is an intermediate in chlorophyll biosynthesis, providing the main pigment involved in light-photosynthetic reactions. In angiosperms, Pchlide is a substrate of light-dependent protochlorophyllide oxidoreductase (POR, E.C. 1.3.1.33) which catalyses its reduction to chlorophyllide. This reaction is triggered by light and plays a key regulatory role in angiosperm development, being the first event in deetiolation. Pchlide that accumulates in darkness in etioplast inner membranes shows high spectral heterogeneity (reviewed in [1-3]), which reflects interactions of a Pchlide molecule with its molecular environment, including Pchlide and POR molecules in an aquous/lipid environment. To elucidate the nature of the interactions between the components of Pchlide:LPOR:NADPH complexes *in vivo*, the mechanism of their aggregation, as well as Pchlide localization in etioplast inner membranes and interactions with the lipid moiety, we have performed a set of experiments in various well-defined model systems (organic solvents, liposomes and micelles) that simulate *in vivo* conditions [4-7]. The present study, which is a continuation of that research, was focussed on examination of Pchlide aggregation in aqueous solutions analyzed by absorption and fluorescence study. At room temperature, we have observed a concentration-dependent broadening of the fluorescence band and red-shift of the maximum from 640 nm to 660-680 nm. The shift was accompanied by a decrease of the fluorescence intensity. In the presence of imidazole, some changes of the shape of the spectrum were noticed. The spectrum consisted of two bands, at 637 and 657 nm, which were stable both at room temperature and at 77K, and resembled low-temperature spectrum of etioplast membranes *in vivo*. The relative intensity of the bands depended on imidazole, as well as Pchlide concentration. These results will be discussed in light of the mechanism of Pchlide aggregate formation *in vivo*. This work was supported by Grant 2011/01/B/NZ1/00119 from the National Center of Science of Poland (NCN).

P3.3

Differentiation of circular vessels in inflorescence stems of *Arabidopsis thaliana*

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One of the most intriguing type of vessels are circular vessels composed of vessel members connected with each other by perforations and arranged in a close ring. In presented studies, we investigated events underlying development of circular vessels from the vascular cambium on the example of Arabidopsis stems with the secondary growth. Analysis includes histology, auxin response distribution (with the use of *DR5::GUS Arabidopsis* transgenic line), localization of PIN1 auxin transporter and *Athb8* vascular marker gene expression in the cells differentiated into this type of vessels. The first event was the change in auxin distribution in cambium, where elevated activity of the *DR5::GUS* auxin response reporter in small group of cambial initials (group of 2- or 3-cells) predicted for circular vessels development was observed. Localization of PIN1 was rearranged and transiently moved from basal to apical and lateral plasma membranes of the cells. The position of PIN1 indicates a change in cell polarity, and thereafter marks the prospective perforation plates of the differentiated circular vessels. Such localization of PIN1 fitted strictly the concept of auxin canalization as the basis for the pattern of vessel differentiation. Our studies concerned also

detail analysis of *Athb8* gene expression, which is known as a marker for early stages of vascular tissue differentiation. High expression of the gene in the differentiated groups of cells marked the very early stages of circular vessels development in the wounded regions of Arabidopsis stems.

P3.4

The regulatory role of AtDeg5 chloroplast protease in chronological progression of principal growth stages in *Arabidopsis thaliana* plants

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AtDeg5 is a serine-type protease (chymotrypsin family) peripherally attached to luminal side of thylakoid membrane. There is experimental evidence that AtDeg5 is involved in degradation of a few photosystem II proteins in response to stresses. Much less is known, however concerning a role of this protease in the progression of growth and development processes during the entire life of the plant under non-stressing conditions. Publicly available transcryptomic data (TAIR database, eFPbrowser tool) indicates that *AtDEG5* is expressed in almost all organs, growth and development stages of *A. thaliana* ontogenesis and this is why we could expect that AtDeg5 is in fact engaged in the regulation of the majority of growth and development stages in *A. thaliana*. In order to have a more detailed insight into the regulatory role of AtDeg5 phenotypic differences have been identified and interpreted among two *Arabidopsis thaliana* mutants: *deg5-1* and *deg5-2* in which AtDeg5 was down regulated to various extent (100% and 23%, respectively) and wild type plants. The set of principal and secondary stages of *A. thaliana* growth and development have been defined and coded based on a system prepared for the description of the ontogenesis of crop plants and weeds (BBCH). We have established that AtDeg5 is involved in regulation of emergence and growing of defined rosette leaves, flowers opening, ripening of siliques and the setting of relevant morphological features of the leaf blade such as length, width, perimeter, area and shape coefficient.

P3.5

Aggregation of LS Rubisco in response to exposure of plants to low irradiance

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Rubisco is a bifunctional enzyme that catalyzes two competing reactions, namely photosynthetic CO_2 assimilation and photorespiratory carbon oxidation; both reactions take place in the stroma of the chloroplasts. In terrestrial plants, Rubisco exists as a holoenzyme composed of eight large (LS) and eight small subunits (SS). Its abundance is highly regulated in response to short and long term fluctuations in the environment as well as in response to ontogenetical context. It has been demonstrated that aggregation and/or degradation is an important regulatory factor influencing LS Rubisco level in leaves. Some results indirectly suggest that reactive oxygen species (ROS) generated as a result of an exposition of plants to adverse environmental conditions may induce oxidative modifications of LS Rubisco molecules rendering them more susceptible to aggregation/ degradation phenomena. A new experimental model was used to study the mechanisms of regulation of LS Rubisco amount in response to changes in environmental factors i.e. the exposure to low irradiance (25 or 50 μmol quanta $\times m^{-2} \times s^{-1}$, 5 min to 96 h) of *Arabidopsis thaliana* plants previously grown under moderate irradiance (250 μmol quanta $\times m^{-2} \times s^{-1}$). Acclimation to low irradiance was found to be accompanied by a clear decrease in LS Rubisco abundance, maximally to about 60% of its initial level in leaves of plants exposed for 24 h to 50 μmol quanta $\times m^{-2} \times s^{-1}$. We have shown that a massive

accumulation of LS Rubisco homo- and heteroaggregates took place during the exposure to low irradiance with no signs of a degradation of aggregated molecules. The aggregates were found to have arisen due to both disulphide bridges as well as non-disulphide bonds. A prominent increase in MDA amount (a lipid peroxidation product) was demonstrated to occur after 6 h of exposure to low irradiance and this suggests that the exposure is accompanied by a rapid accumulation of ROS which may induce oxidative modification of LS Rubisco. To fully confirm the involvement ROS in the LS Rubisco aggregation, levels of $^1O_2$, $O_2^{\bullet -}$ and H_2O_2 were followed in leaves during the course of exposure of plants to low irradiance.

P3.6

Arginine metabolism during NO- and polyamines-induced dormancy removal and germination of apple embryos

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Deep dormancy of apple embryos (*Malus domestica* Borkh.) is manifested by block of germination and/or development of morphologically atypical seedlings. Transition of embryos from dormant into non-dormant stage is observed after their short term pre-treatment with nitric oxide (NO) or imbibition in water solution of polyamines (PAs) such as putrescine (Put) and spermidine (Spd). Spermine (Spm) acts as inhibitor of embryo germination. Biosynthetic pathways of both NO and PAs depend on arginine (Arg) availability. In plant tissues, NOS-like activity leads to formation of NO and citrulline from Arg. On the other hand, Put may be synthesized from Arg in reaction catalyzed by arginase or Arg decarboxylase. Put is a major diamine and a direct substrate for triamine – Spd and tetraamine – Spm formation. Moreover, it was proposed that PAs stimulate NO generation in plant cells. Therefore, the close relationship between PAs and NO metabolism may directly impact seed dormancy and germination. The aim of our study was to investigate the influence of NO fumigation and PAs treatment on concentration of Arg and activity of arginase in germinating apple embryos after 2 days of culture and at the stage of termination of *sensu stricto* germination. We determined also NOS-like activity as the putative pathway of NO formation depending on Arg consumption. The decrease in free Arg content was characteristic for cotyledons of germinating apple embryos and developing seedlings. It correlated well with fluctuation of arginase and NOS-like activity. In NO pre-treated embryos activity of arginase increased just 2 days after starting the experiment both in cotyledons and embryonic axes. In Put and Spd treated embryos activity of NOS-like enzyme in axes increased more than twice just after the beginning of the culture. In contrast, Spm led to decrease in NOS-like activity in embryonic axes after 2 days of imbibition and then an increase of the activity was observed at the termination of germination *sensu stricto*. Our data suggest involvement of Arg-dependent generation of NO in germination stimulated by PAs, and beneficial role of Arg in dormancy allelviation of apple embryos. The part of the work was financed by grant NN303821840 founded by NSC.

P3.7

Hydrotime model analysis of cornflower achene germination

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Studies on the biology of weeds, including ecophysiology of dormancy and germination of their seeds, contribute to better weed control and in addition the limitation of environmental pollution. Still knowledge about the environmental regulation of seed dormancy in weed species with varied life cycles is very poor. Examples of such weeds can be winter and summer annuals, biennials or short-lived perennials, such as Centaurea cyanus L. A better under-

standing of the mechanisms regulating the level and depth of seed dormancy during such processes as stratification or after-ripening in the seeds of species other than annual was finally possible through creating the population-threshold hydrotime model. Mature seeds of *Centaurea cyanus* were buried in wet light loam in a plastic pot and stored at 5, 12, 19 or 26°C for 150 days. Other seed portions were dry stored at 12, 19, 26 or 33°C for 150 days. Before the experiment and after 10, 30 and 150 days' storage, seeds representing each temperature and experimental variant were tested for germination. For this purpose the seeds were placed on top of two layers of filter papers in 9-cm Petri dishes moistened with 5 ml of water or PEG solution of indicated (0, −0.3 and −0.6 MPa), in four replications of 25 seeds each, at 12°C for 10 days. The repeated probit analysis technique and the computational procedure suggested by Bradford (1990, 1995) and Gołaszewski and Bochenek (2008) were used to estimate hydrotime model parameters and the germination time-course curves. An increase in the germination percentage of *C. cyanus* achenes after short stratification resulted from a narrower distribution of the average sensitivity of the seed population to water stress rather than the lowering of this sensitivity. The reduction of the total hydrotime required for a seed to complete its germination is also of importance. Dormancy development resulting from a long stratification period resulted from a shift of towards positive values. The lowering of the dormancy level in *C. cyanus* seeds during a short after-ripening period and its induction after long dry storage were also mainly connected with changes of this hydrotime model parameter. The analysis of parameter changes in the hydrotime model is very important for a better understanding of the physiological basis of dormancy development and release. This analysis is also important to find relationships between weather conditions, soil, or also artificial treatments which can help break dormancy and physiologically significant parameters of the model.

P3.8

The 3D simulation model for growth and cell divisions applied to the surface layer of cells of the shoot apex

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Simulation model of growth and cell divisions in a superficial layer of cells for the paraboloidally shaped shoot apex in 3D is shown. It is assumed that growth at the surface is isotropic, and cell pattern is derived from three idealized initials that are joined together at the summit. A field of growth rates of the apex is of a tensor type and cells divide in accordance with the Errera's rule, i.e., along the shortest path dividing the cell into two more or less equally sized daughters. A division wall passes either by the geometric cell center (variant I), or through the randomly determined point located near the center (variant II). In both variants, cell pattern of the growing apex, visualized from the side and at the top view, develops realistically. Statistical analysis of orientation of new walls was carried out (1700 divisions). The results indicated that in variant I none orientation is distinguished while in variant II two maxima appear, indicating a small preference for the radial and tangential orientations. Such preference is observed not at once, but after when cell pattern in the superficial layer is fully generated. It does not depend on the distance from the summit of the apex as well as the surface curvature because a similar preference takes place in the computer-made apex which is completely flat. Such a result is rather surprising due to growth rate isotropy at the organ surface. However, it may be related to principal growth directions which correspond to these two orientations.

P3.9

Phytotoxic effects of *m*-tyrosine on tomato (*Solanum lycopersicum* L.) root growth – alterations in ROS production and hormonal balance

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m-Tyrosine (*m*-Tyr) is a non-protein amino acid, identified in root exudates of chewing fescue (*Festuca rubra* L. ssp. *commutata*) and donkey-tail spurge (*Euphorbia myrsinites*) and is considered as phytotoxin. *m*-Tyr mode of action is poorly understood although it's use as a bioherbicide in weed management strategy is proposed (Bertin et al., 2007; Huang et al., 2012). The aim of this study was to investigate some aspects of phytotoxic effect of *m*-Tyr on root growth of tomato (*Solanum lycopersicum* L.) seedlings. Tomato seeds were germinated and after radical protrusion transferred to water or *m*-Tyr solution (50-250 μM). The root length, ROS (H_2O_2) production, auxin concentration and emission of ethylene were determined after 1-3 day of *m*-Tyr treatment. Inhibition of root growth of tomato seedlings by *m*-Tyr was accompanied by disturbances in root gravitropism. It correlated well with enhancement of ethylene emission and overaccumulation of auxin. Furthermore, increased ROS (H_2O_2) concentration in the tomato seedling roots and in the surrounding medium was observed. Our data suggest that *m*-Tyr leads to disorder of regulatory complex (axin-ethylene-ROS) responsible for management of root growth.

P3.10

The activity of DGAT and PDAT enzymes in the developing seeds of *Brassica napus*

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Triacylglicerols (TAGs) are major storage lipids found in seeds, flower petals, pollen grains and fruits of a number of plant species, including *Brassica napus*, a highly important oilseed crop. Triacylglycerol biosynthesis is catalyzed by membrane-bound enzymes operating in the endoplasmic reticulum – the Kennedy Pathway, in which 3-phosphoglycerol is changed into triacylglycerol. In the final step of this reaction, TAG is formed with addition of the final acyl group from acyl-CoA with the help of acyl-CoA:diacyloglycerol acyltransferase (DGAT). There is, however, an alternative pathway in which the third acyl group is transferred not from acyl-CoA, but from phosphatidylcholine or other phospholipid, by an enzyme called phospholipid:diacyloglycerol acyltransferase (PDAT). The study aimed at characterizing the activity of DGAT and PDAT enzymes in the developing seeds of *Brassica napus* by relatively defining the participation level of those enzymes in accumulating triacylglycerols (TAGs) in those crops' seeds, which were grouped into four developmental stages based on their colour, shape, size and age. After harvesting and decoating them, the seeds were homogenized and microsomal fractions were isolated from the homogenate. The DGAT and PDAT activities were measured using those seeds' microsomal fractions in a number of reactions with different radioactive substrates resulting in de novo TAG synthesis. After terminating the reaction, and separating the lipids with TLC, electronic autoradiography was used to read the results. Reactions using di-6:0-DAG and [14C]labeled acyl-CoA proved to be more successful than those with radioactive di-18:1-DAG and non-radioactive acyl-CoA. 12:0-CoA proved to be the best donor of the fatty acyl. DGAT activity was usually the highest in the second developmental stage, while PDAT's activity peaked at the third stage. DGAT's activity is also higher than that of PDAT. The experiments show, that the enzymes activities vary depending on the stage of development and different acyl-group-donating substrate.

P3.11

Molecular characterization of early stages of ovule embryogenesis in sugar beet (*Beta vulgaris* L.)

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During recent years, effective production of haploids via ovule embryogenesis has become an indispensable element of creating donor materials and improving varieties in breeding of sugar beet (*Beta vulgaris* L.). Efficiency of *in vitro* response of unpollinated ovules and ovaries cultures depends on many factors, from which the donor plant genotype is the most important. Currently, despite many published reports regarding the genes involved in haploid induction by androgenesis, still little is known about the molecular mechanisms involved in gynogenesis induction. The identification of large number of genes that might serve as stage-specific markers of ovary embryogenesis would help in further understanding of that process. To gain insight into the molecular events occurring in the early stages of sugar beet ovary embryogenesis, mRNAs from different stages of ovary development were isolated and characterized with the use of PCR technique. The aim of this study was to identify a number of cDNAs which are expressed in the particular stages of ovary *in vitro* culture and are not expressed in natural conditions. Any progress in understanding ovary embryogenesis should help to improve the use of haploids and further doubled haploids in genetic studies and breeding programs.

P3.12

Apoplastic markers of cell differentiation status during somatic embryogenesis in *Arabidopsis thaliana*

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Induction of somatic embryogenesis in plant tissues cultured *in vitro* implicates dedifferentiation of cells and establishment of the undifferentiated totipotent state. Knowledge of the underlying mechanisms controlling the developmental switch from the somatic to embryogenic cell pathway, *de facto* meaning the change of cell fate, has increased dramatically in recent years, but we are still far from a full understanding of the phenomenon of cellular totipotency. The aim of the study was to compare the occurrence and localization of the major cell wall components (pectins, arabinogalactan proteins, lipid transfer proteins, lipids) in explant cells exhibiting various cytological features and to identify potential markers of cell differentiation status. Embryogenic cultures were initiated from immature zygotic embryos at the late cotyledonary stage of development. Explants were sampled daily during the culture period, fixed and further processed for histochemical and immunocytochemical procedures. Three types of cells of the explant cotyledons were taken into consideration: embryogenic cells, meristematic cells and ground tissue cells. Low and high methyl-esterified pectins (JIM5 and JIM7 epitopes) occurred in all types of examined cells. However, both the intensity and the pattern of labelling were different. Arabinogalactan protein (AGP) epitopes recognized by monoclonal antibodies JIM4, JIM8, JIM13, JIM16 and LM2 were mainly detected in highly vacuolated cells of ground tissue. Labelling with the antibody against lipid transfer protein 1 (LTP1) epitopes resulted in a strong apoplastic signal in the majority of embryogenic cells, while in meristematic-like cells no signal or exclusively cytoplasmic signal was observed. Staining with lipophilic dyes (Sudan Black B, Nile Red) revealed a co-localization of LTP1 epitopes with lipids. The obtained results showed a large variation in cell wall composition in different cell types and allowed for indicating the possible apoplastic markers of cell differentiation status.

P3.13

The degradation of storage materials in germinating tomato seeds after red and far red light irradiation

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Starch is the main storage material in plants and its degradation in seeds is one of the key metabolic processes, determining the course of germination and seedling development. Until now, not much information has been gathered about storage starch degradation pathways in dicotyledonous seeds. Numerous plant species also accumulate lipids apart from starch. In many seeds, triacylglycerols are the main storage material. However, in order to become a source of energy for the growing seedling, fat must be converted into carbohydrates via the glyoxylate cycle. Fat degradation has been studied mostly in oil-storing seeds and information about the interplay between starch and lipid metabolism in seeds containing both types of storage material is very scarce. The aim of this study was to analyze the degradation of starch and lipids in germinating wild type tomato (*Solanum lycopersicum*) seeds, which contain both types of storage materials, and to check whether these processes are regulated by light via phytochromes. Starch and lipids were quantified using biochemical assays in germinating seeds and seedlings during the first 5 days of growth. In order to investigate the process of fat-carbohydrate transformation, the levels of sucrose were also measured. It is well known that the germination of many plant species, including tomato, is regulated by phytochromes, photoreceptors sensible to red and far-red light. However, phytochromes also control many other physiological processes, acting through several different pathways. To investigate the role of phytochromes in storage material degradation imbibed seeds were subjected to red or far red light treatment, which respectively induces or inhibits germination.

P3.14

Molecular cloning and expression analysis of *ABA 8′-hydroxylase* genes during germination of triticale (X *Triticosecale* Wittm.) seeds

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Pre-harvest sprouting (PHS) refers to germination of mature seeds while they are still on mother plants. This phenomenon usually occurs under heavy rainfall and high humidity during maturation of grains in spikes, shortly before harvest. PHS is a serious problem in cereals, especially in wheat, rye, and triticale. It leads to reduced grain yield and affects the quality of end-products. Abscisic acid (ABA) is a plant hormone that participates in induction and maintenance of seed dormancy. The decrease in ABA level in the imbibed seeds is correlated with the release of dormancy, which consequently leads to germination. It is assumed that the decrease in ABA during germination may be the result of increased catabolism of this phytohormone. ABA catabolism can occur via two pathways: oxydation or production of inactive conjugates with glucose. According to recent studies hydroxylation of abscisic acid at 8′-position, catalyzed by ABA 8′-hydroxylase, is the key step in ABA inactivation. Two triticale *ABA 8′-hydroxylase* genes were cloned and designated as *TsOH1* and *TsOH2*. Deduced amino acid sequences of the cloned *TsOHs* showed a high percentage of identity with ABA 8′-hydroxylases from other monocots. When mature triticale grains were subjected to imbibition, the expression of both triticale *TsOHs* was higher in embryos of cultivar more sensitive to pre-harvest sprouting (Leontino) than of cultivar more resistant to pre-harvest sprouting (Fredro). In Leontino cultivar, the highest *TsOH1* mRNA level was observed 4 hours after the start of imbibition and preceded grains germination, while the highest expression of *TsOH2* was observed 8 and 16 hours after the start of imbibition and was correlated with germination of grains. In Fredro cultivar, the expression of *TsOH1* was observed at slightly higher

level during first 8 hours of imbibition than in the subsequent hours, while the expression of *TsOH2* remained at constant low level. The obtained results suggest that reduced ABA catabolism might result in higher resistance to pre-harvest sprouting.

P3.15

The correlation between size of *Beta vulgaris* L. flower buds and the stadium of pollen grains development

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Androgenesis is an *in vitro* method for conducting haploid embryos derived from male line of gametophyte without fertilization. It is an alternative to conventional plant breeding which allows fixation of new gene combination in homozygote homozygoteous plants. The precise moment of induction of this process is very important. Therefore the aim of our research was to correlate the size of flower buds with the stadium of pollen grain development. Inflorescences of *Beta vulgaris* L. (Opolski) obtained from Research Institute of Horticulture Laboratory of Tissue Culture in Skierniewice were used in the investigation. After fixation of the inflorescences with Carnoy'a mixture the flower buds were isolated, measured in CX21 light microscope (OLYMPUS) with accuracy to 0.01 mm. Subsequently squashed specimens made with acetocarmin were analyzed in light microscope ECLIPSE 50i (NIKON) equipped with PowerShot A640 camera (CANON). All stages of pollen grain development were observed: archespores, I meiotic division, II meiotic division, tetrad of microspores, mature microspore, immature and mature pollen grains (microgametophyte). The analysis of 260 buds from 8 independent inflorescences reveled that the above mentioned stadiums were observed when bud mean lengths were: 0.93 mm, 1.12 mm, 1.20 mm, 1.26 mm, 1.36 mm, 1.42 mm and 1.60 mm, respectively. Moreover, on the basis of the distribution of particular stadia of pollen development within 15 bud length classes, we delimited the size of buds in which the specific phases of microsporegenesis or microgametophytogenesis occurred with the highest probability ($p < 0.05$): below 1.0 mm for the archespores; 1.1-1.2 mm – I meiotic division; 1.1-1.3 mm – II meiotic division; 1.2-1.3 mm – tetrad of microspores; 1.3-1.4 mm – mature microspore; 1.3-1.5 mm – immature pollen grain; above 1.5 mm – mature pollen grain. Reassuming, the flower bud length could be a useful parameter for determining the degree of *Beta vulgaris* pollen grain development.

P3.16

ERF022 controls somatic embryogenesis in *Arabidopsis* via ethylene-related mechanism

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Genes encoding transcription factors (TFs) provide essential role in activation of new developmental programs in somatic cells. Thus, to identify the regulatory genes controlling induction of somatic embryogenesis (SE) in culture of *Arabidopsis* explants, the expression pattern of 1880 TF genes were analysed and among the TFs of SE-modulated expression *ERF022* was identified (Gliwicka et al., 2013). The gene belongs to a large TF family involved in plant stress responses regulated by ethylene. Several experimental data support a hypothesis on a key role of the *ERF022* gene in SE, including auxin-dependent down-regulation of its expression in embryogenic culture and a significantly decreased capacity for SE observed in *erf022* mutant. Moreover, in agreement with a predicted stress-related function of *ERF022*, the expression of this gene was found to be modulated in response to ACC, a precursor of ethylene biosynthesis. Thus, the present study was conducted to reveal the genetic mechanism of ethylene-related *ERF022* function during SE induction. To indicate the potential targets of ERF022, the genes co-expressed with

ERF022 and associated with ethylene responses were selected, including *ACS7, ACS8, ERF1* and *ERF5.* The candidate *ACS* genes encode ACC synthases and operate in ethylene biosynthesis while the *ERFs* encoding transcription factors are involved in ethylene signal transduction. The regulatory relations between the ERF022 and the candidate targets were verified. Accordingly, the embryogenic cultures differing in *ERF022* transcript level were analysed in terms of gene expression profiles and thus, the cultures derived from Col-0, *erf022* mutant and pER8-ER022 line were evaluated. It was observed that during SE the transcription levels of *ACS7* and *ERF1* are modified by the level of *ERF022* activity. A lack of *ERF022* expression leads to up-regulation of *ACS7* and *ERF1* while overexpression of *ERF022* results in the reduced level of *ACS7* and inhibition of *ERF1* expression. The study indicated a regulatory function of ERF022 during SE induction in Arabidopsis. The gene seems to operate in relation with ethylene as a negative regulator of genes involved in biosynthesis (*ACS7*) and signaling of ethylene (*ERF1*). Thus, it was found that apart from auxin, also ethylene plays an essential role in the molecular mechanisms operating in SE induction in Arabidopsis.

P3.17

Contribution of phytocystatins to the processes of seed development and germination

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At the seed development process, storage proteins are being accumulated and then, after the dormancy, they are subject to hydrolysis. Cysteine proteinases play a major role in these processes. Therefore, the precise control of their activity is of vital importance. One of many activity control mechanisms which protect the cell against proteolysis is the synthesis of specific inhibitors. In the case of cysteine proteinases this role is played by proteins belonging to the superfamily of cystatins named phytocystatins. Till date many members of phytocystatin family have been found and investigated. Nevertheless, different inhibitory specificity for various cysteine proteinases was established. This suggests the existence of a number of specific inhibitors for their target enzymes. From 7 phytocystatins identified in triticale (× *Triticosecale* Wittm.) in The Department of Biochemistry (WULS-SGGW) only two have been proved to inhibit cysteine proteinase activity so far. Therefore, it is necessary to examine more triticale phytocystatins and select those which indeed may share the largest part in the control of grain development and germination processes. The goal of the presented project was to examine new phytocystatins in triticale. We have identified a gene encoding new phytocystatin TrcC7. Full gene sequence with 5′ and 3′ UTR have been obtained. Coding sequence of TrcC7 gene constitutes of 369 bp which encodes 123 aa and the gene lacks introns. Phylogenetic analysis showed that TrcC7 belongs to C1 cluster, described by Martinez et al. (2009) whereas 6 previously identified triticale phytocystatins belongs to A cluster and one to C2 cluster. Calculated molecular mass of TrcC7 is 13 010,7 Da. The protein is expressed with signal peptide targeting it to ER which indicates its secretory destination. Amino acid sequence analysis showed that TrcC7 lacks putative glycosylation sites as all known phytocystatins. TrcC7 sequence possesses three conserved regions present in all cystatins: QXVXG where X stands for any amino acid, in the central region of the polypeptide, G residue in the N-terminal region and W residue in the C-terminal region. It also has characteristic motif of plant inhibitors, which distinguishes them from the others from the superfamily of cystatins, which is the LARFAVXEHN amino acid sequence. It is located in the central part of the amino acid chain and its function still remains unclear. We have found transcripts of this new phytocystatin in developing and germinating triticale grains with various expression patterns. The presence of phytocystatins in crop grains aims at possible regulation of cysteine proteinase activity which in consequence may influence the control of seed development and germination processes. Nevertheless, further analysis of TrcC7 inhibitory activity against particular cysteine proteinases is required. This work was supported by the National Science Centre (Poland) grant No 2011/03/N/NZ9/04115.

P3.18

High activity of *AUXIN RESPONSE FACTORS (ARF5, ARF6, ARF10* and *ARF16)* in somatic embryogenesis of *Arabidopsis*

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The *AUXIN RESPONSE FACTORS* (*ARFs*) along with *AUXIN/INDOLE-3-ACETIC ACID* (*AUX/IAA*) genes are the main elements of auxin signaling controlling auxin-dependent gene expression. The presented study aimed at identification of the *ARF* genes involved in somatic embryogenesis (SE) process induced with auxin in Arabidopsis somatic cells cultured *in vitro*. The essential role of *ARF* genes in SE induction was hypothesized considering the engagement of these regulators in many plant developmental processes controlled by auxin, including zygotic embryogenesis (ZE). In this study, a model for plant genomics, *Arabidopsis thaliana* was applied and with the use of versatile experimental approaches including expression, mutant and reporter line analysis, *ARF* genes involved in SE were identified. The results of qRT-PCR analysis indicated that expression of *ARF5, ARF6, ARF10* and *ARF16* seem to positively control embryogenic transition in cultured explants as these genes displayed a distinct up-regulation and auxin-dependent activity in SE. The involvement of *ARF5, ARF6, ARF10* and *ARF16* in SE was further confirmed in spatiotemporal analysis of *ARF* expression conducted with the use of GFP reporter lines. Expression of *ARF5, 6, 10* and *16* was observed in explant regions involved in SE induction (cotyledons and shoot apical meristem) as well as in developing somatic embryos. Moreover, the analysis of *arf* insertional mutants revealed that two of them, *arf5* and *arf6,* showed a decreased embryogenic response. Altogether, the results suggest that *ARF5, ARF6, ARF10* and *ARF16* seem to positively control the embryogenic transition in somatic cells of Arabidopsis. However, the target genes operating in ARF-controlled molecular mechanism involved in SE induction need to be identified.

P3.19

Identification of cell cycle proteins interacting with *Arabidopsis thaliana* Proliferating Cell Nuclear Antigen using bimolecular fluorescence complementation assay

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Proliferating cell nuclear antigen (PCNA) – a DNA sliding clamp was described for the first time over thirty years ago as protein recognized by sera of patients with autoimmune disease called systemic lupus erythrematosus. The studies on human, yeast and plant PCNA demonstrated that this protein forms homotrimeric ring structure. The detailed analysis of PCNA showed that during DNA replication it is loaded onto DNA by RF-C complex and stimulates the processivity and activity of DNA polymerase delta. Beyond the basic function in DNA replication, PCNA was also shown to be involved in other processes such as DNA repair and cell cycle control. The previous studies on mammalian proteins showed direct interaction of PCNA with several proteins responsible for cell cycle control including: p21 (cyclin dependent kinase inhibitor), cyclin D, CDK2 (cyclin dependent kinase 2), CDK4 and CDK6. In contrast to these data in plants only A-type cyclin dependent kinases within the group of cell cycle proteins were indicated to interact with PCNA. To characterise plant cell cycle proteins interacting with PCNA, the bimolecular fluorescence assay was used. Our studies enabled the selection of potential candidates which may form complex with plant PCNA during cell cycle. The identified interactions will be verified using other in vitro and in vivo approaches for better understanding of PCNA role in plant cells. The project was supported by Polish Ministry of Science and Higher Education project Iuventus Plus, No. IP2011 052571.

P3.20

Changes of peroxiredoxins in beech seeds (*Fagus sylvatica* L.) during development and maturation

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Peroxiredoxins (Prxs) are thiol-specific antioxidants. Prxs catalyse the detoxification of hydrogen peroxide and alkyl hydroperoxides and reduce peroxinitrite, a reactive nitrogen species that occurs in cells by nitric oxide reacting with anion superoxide ($O_2^{\bullet -}$). Prx play crucial role in metabolic processes, modulate redox signal during plant development and adaptation to environment conditions. They can be classified into 4 classes of closely related proteins: 1-Cys peroxiredoxine (1-Cys Prx), 2-Cys peroxiredoxine (2-Cys Prx), peroxiredoxines type II (PrxIIF, PrxIIE, PrxIIC) and proteins migrating with bacterioferrytine (PrxIIQ). The aim of the study was the detection and measurement of Prxs expression in beech seeds that differ in desiccation tolerance during development and maturation. Investigation of beech seeds (*Fagus sylvatica* L.) with the use of Western blot and specific antibodies resulted in 1Cys Prx, PrxIIF, PrxIIE, PrxIIC, and PrxIIQ identification. Densitometry analyses showed that the amounts of Prxs differed. The most interesting changes at the protein level were found for PrxIIF – the main mitochondrial antioxidant that regulates the redox state. During seed development metabolism activity is shifting and consequently reactive oxygen species (ROS) are generated, therefore the levels of $O_2^{\bullet -}$ and hydrogen peroxide were measured. In beech seeds a change of the $O_2^{\bullet -}$ level significantly increased at 16^{th} week after flowering. We present and discuss the relations between ROS and peroxiredoxins in the process of seeds maturation.

P3.21

Efficient *in vitro* callus induction and regeneration of different Polish cultivars of tomato

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In this study we established a stable regeneration system of polish cultivars of tomato in order to lay the foundation for future genetic transformation of tomato. The regeneration capacities of two types of explants (segments from hypocotyls and cotyledons) were compared in three cultivars of tomato (*Lycopersicon esculentum* L.). Explants were cultured on 10 regeneration media (basal medium MS or B5, and supplemented with BAP and IAA). The regeneration capacity was significantly influenced by cultivars and explants types. The best explants for bud induction are cotyledon segments followed by the hypocotyls. All regenerated plantlets positively responded when passaged to 1/2 MS and 1/2 MS supplemented with 0.1 mg/l IAA media for rooting. However, addition of IAA was found to be essential for fast generation of healthy roots.

P3.22

Brassica oleracea var. botrytis: evaluation of *in vitro* potential of two commercial cultivars

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Two commercial varieties of *Brassica oleracea var. botrytis*, Pionier and Rober, were evaluated as a future target for genetic transformation. The regeneration potential of both hypocotyls and cotyledons was tested on five medium variants: standard Murashige and Skoog medium (MS) supplemented with 1% sucrose and 0.8% agar as a control (*A*) and four combinations of the MS medium supplemented with different concentrations of growth regulators (*B* – MS + 1 mg/l BAP, *C* – MS + 0.5 mg/l BAP + 0.1 mg/l NAA, *D* – MS + 1 mg/l BAP + 0.1 mg/l NAA, *E* – MS + 2 mg/l BAP + 0.1 mg/l NAA). It has been shown that among the five tested media, the D medium has induced the strongest *in vitro* response in the case of Rober hypocotyls, while the Pionier hypocotyls have demonstrated the greatest regeneration potential when grown on the E medium. Here we present also the impact of different root inducing media on the effectiveness of root formation and on the variation of morphology of both roots and whole plants as well.