POSTERS

Plant stem cells in terms of embryology

Tatiana B. Batygina, G. E. Titova, I. I. Shamrov, E. A. Bragina, V.E.Vasilyeva, I. V. Rudsky

Department of Embryology and Reproductive Biology, V. L. Komarov Botanical Institute, Prof. Popov str., 2, 197376, Saint-Petersburg, Russia, e-mail: batygina@tb1390.spb.edu

Analysis of approaches to the study of human and animal stem cells and of data on embryology of flowering plants allowed to firstly manifest that the formation of stem cells occurs in all organs (flower, stem, leaf, root), at all stages of life cycle (sporophyte, gametophyte). Moreover their functioning first of all depends on their localization and destination. Zygote, the initial cell of flowering plant sexual embryo, is a unique stem cell – "grandparent" of not only apical root and shoot meristem, but also stem cells of other orders (hypophyseal and epiphyseal in sexual embryo, cambium in stem, "dormant" meristem in leaves, apical part of primordia of ovule and anther (microsporangium) and also the initial cells of somatic embryos of different origin – phenomenon of embryoidogeny). The original characteristics of main features of plant stem cells are given: 1) the property of toti- or pluripotency, i.e. the capacity to form not only different types of tissues and organs, but also new individual – throughout various pathways of morphogenesis (embryo-, embryoido-, hemmorhizogenesis); 2) the feature of self maintenance, i.e. the formation of cell pull generally due to the symmetrical divisions and to the system of intercellular interactions; 3) the capacity to proliferation and formation of cells – precursors of different tissue types – due to asymmetrical divisions under the effect of certain signals; 4) the condition of relatively dormancy and rare divisions in compare with surrounding cells ("niche"); 5) pulsated and multistage character of formation in the tissue or organ and capacity to switching over developmental program, that is provided by various molecular-genetic mechanisms. The differences of stem cells in plants and animals and their functioning in the system of reliability at the various ontogenesis stages are discussed.

A general approach to uncover the origin of tissue culture-induced variation in barley (*Hordeum vulgare* L.)

Piotr Tomasz Bednarek[1], Renata Orłowska[2], Robert M.D. Koebner[3], Janusz Zimny[1]

[1]*Plant Breeding & Acclimatization Institute, 05–870 Błonie, Radzików, Poland, e-mail: p.bednarek@ihar.edu.pl*
[2]*University of Warmia and Mazury in Olsztyn, Poland*
[3]*John Innes Centre, Colney Lane, Norwich NR4 7UH, UK*

It is a well established phenomenon that, following plant regeneration from non-differentiated tissue, the resulting population of clonal individuals is not phenotypically or genetically homogeneous. Both somaclonal and gametoclonal events contribute to this tissue culture induced variation. The term somaclonal variation was introduced by Larkin and Scowcroft (1981) to describe variation arising during somatic cell culture, while gametoclonal variation represents heritable variation arising during the culture of cells of gametic origin.

Genetic (changes at the DNA sequence level) and epigenetic (localized alteration in DNA methylation state) events provide a molecular route to induction somaclonal variation. DNA fingerprinting technology has the potential to detect DNA changes with growing precision and efficiency. However, neither RFLP- nor RAPD-based approaches have been particularly successful in recognizing somaclonal variants and seem to be not effective in identification of changes at the methylation level. The AFLP technique is potentially more appropriate as a detection platform, since it both identifies a relatively large number of amplified fragments and can be easily adopted for studies of methylation-induced variation. The development of such a technique was the main purpose of the given study.

Presented variant of methylation sensitive AFLP, based on the isoschizomeric combinations *Acc65*I/*Mse*I and *Kpn*I/*Mse*I was applied to analyze, at both sequence and methylation level, the outcomes of regeneration from tissue culture in barley. Two sets of progeny of the single regenerated plant were compared – one derived via androgenesis, and the other from somatic embryos developed from cultured immature embryos. Of the overall 5% level of variation detected, almost 2% was accounted for by sequence alteration, and the remainder by changes in methylation state. Although the sequence mutation rates were similar between the two sets, the androgenesis induced a higher rate of methylation state alteration. About 95% of restriction fragments visualised were stably transferred to the progeny independently of the way plant material was regenerated. Tissue culture-induced variation was generally randomly distributed between progeny but there was evidence that some sites represent "hotspots", where changes of methylation state prevail over variation in DNA sequence. To the best of our knowledge, the analytical system we propose is the first dedicated to a quantitative study of somaclonal variation.

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Nuclear endosperm and endosperm haustoria in *Linum usitatissimum* L.

Anita Biskup and Romana Izmaiłow

Department of Plant Cytology and Embryology, Jagiellonian University, Grodzka 52, 31–044 Kraków, Poland, e-mail: izmailow@iphils.uj.edu.pl

The pattern of endosperm development in *L. usitatissimum* has been recognized as the Nuclear type, whereas many publications follow opinion of Schürhoff (1924) who reported Helobial type for this species. Endosperm development starts before division of the zygote. The primary endosperm nucleus and its daughter nuclei undergo free nuclear divisions; cellularization is initiated at the stage of one layer of endosperm nuclei around the older globular proembryo and proceeds to the chalaza.

A peculiar feature in the endosperm development in *L. usitatissimum* is the presence of haustoria. The chalazal haustoria are formed in two ways: (1) the nucellar cells near the chalazal part of the embryo sac undergo lysis already before fertilization, and next at the stage of 4–8 endosperm nuclei one of them migrates into the formed chamber. Haustorium increases its volume and becomes half-length of the embryo sac; after free-nuclear divisions it reaches the stage of 32–64 nuclei; (2) in several ovules the chalazal haustorium was formed after four-nucleate stage of endosperm as result of formation of pectin wall, which is progressively built below the zygote and the endosperm nuclei grouped in its vicinity. The wall grew in centripetal direction and before this process was finished, one of endosperm nuclei migrated into the forming chalazal chamber. Several nuclear divisions in endosperm proper and in the haustorium were synchronous and not followed by cytokinesis. However, further developmental stages could not be observed. Moreover, apart of chalazal haustorium also micropylar one has been formed in a few ovules; one or two nuclei from the endosperm proper have migrated into its cavity.

Micropylar haustorium as well as development of chalazal haustorium starting in nucellus before fertilization have been detected in the genus *Linum* for the first time.

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Dihaploid production via anther culture in Czech breeding lines of caraway (*Carum carvi* L.)

Iva Smykalova[1], Prokop Smirous jr.[2], Michaela Kubosiova[3], Nikol Gasmanova[4], and Miroslav Griga[1]

AGRITEC Ltd., [1]Plant Biotechnology Department & [2]Department of Technical Crops, Zemedelska 16, CZ-787 01 Sumperk, Czech Republic, e-mail: smykalova@agritec.cz
[3]Department of Crop Science, Faculty of Agronomy, Mendel University of Agriculture and Forestry, Zemedelska 1, Brno, CZ-61300, Czech Republic
[4]Department of Botany, Faculty of Science, Palacky University, Slechtitelu 11, CZ-783 71, Olomouc-Holice, Czech Republic

Caraway (*Carum carvi* L.) is umbelliferous plant known through its medicinal and nutritive properties. The species is allogamous, cultured as annual (winter) or biennial (spring) form (Nemeth, 1998). Low level of autogamy, long vegetation period, low genetic variability of available germplasm and sensitivity to diseases (Evenhuis *et al.*, 1995) represent main obstacles limiting caraway breeding. Research of caraway at Agritec is aimed to create varieties with stable seed yield and enhanced content of essential seed oil (carvone). In this study, we examined the possibility of rapid induction of homozygosity by production of doubled haploids through *in vit*ro androgenesis. The donor plants represented third generation of enforced self-pollination of winter type caraway. 17036 anthers (stage of uninucleate microspores) from 17 genotypes were cultured based on protocol for *in vitro* production of haploids in carrot (Andersen *et al.*, 1990 ; Adamus and Michalik, 2003). The effect of cold pretreatment of donor plants, and 15 formulations of induction media at different culture conditions were tested. Cream-coloured embryo-like structures (ELS) developed directly from cultured anthers of responsive genotypes in a low frequency (0.3 ELS/100 anthers). Regenerated R0 plants displayed both haploid and diploid cytological status as revealed by flow-cytometry. Diploid (probable anther wall origin), spontaneous and colchicine-induced dihaploid R0 plants were fertile.

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Pollen development in *Rumex acetosa* L., a plant with XX/XY$_1$Y$_2$ sex chromosome system and female-biased sex ratio

Magdalena Błocka-Wandas[1], Krzysztofa Odrzywół[2], and Andrzej J. Joachimiak[1]

[1]*Department of Plant Cytology and Embriology, Institute of Botany, Jagiellonian University, Grodzka 52, 31–044 Kraków, Poland, e-mail: a.joachimiak@iphils.uj.edu.pl*
[2]*Department of Plant Breeding and Seed Science, The Agricultural University of Cracow, Łobzowska 24, 31–140 Kraków, Poland*

The problem of predominance of females in natural *Rumex* populations was investigated for over eighty years. Because female-biased sex ratios are also reported in other plants with heteromorphic sex chromosomes, it has been suggested that this phenomenon is a consequence of Y-chromosome degeneration (the poorer performance of Y-bearing gametophyte and/or sporophyte). It is still not clear, however, when the female-biased sex ratio is established in *Rumex*: before or after fertilization (Korpelainen, 2002; Stehlik and Barrett, 2005). Some results suggested that disturbed sex ratio is the result of slower pollen-tube growth of male- versus female-determining pollen grains (Correns 'certation' hypothesis), the other that it reflects differences in mortality between male and female plants in a population. Some other mechanisms could also influence sex ratios in *Rumex* populations, e.g. disturbances in the development of Y-bearing gametophytes. To solve the problem we examined the pollen development in *R. acetosa*.

It was found that pollen development in *R. acetosa* is very regular. Cytological disturbances (e.g., meiotic/mitotic disturbances, micronuclei, irregular nuclei, pollen size difference etc.) were observed only exceptionally. At the first pollen mitosis the X- and Y$_1$Y$_2$-bearing pollen grains occurred with similar frequency within anthers. The mature pollen grains were regular and contained two sperms with normal morphology. All gametophytes at this stage were well equipped with starch granules. The regular course of pollen development was confirmed by Alexander test, which proved viability of 98% pollen grains. The development of the anther tapetum was also undisturbed. It was accomplished with inhibited first cytokinesis and further concerted polyploidization of nuclei within binucleate cells. Densitometric DNA measurements revealed the occurrence of four classes of nuclei (2n, 4n, 8n, 16n) in terminally differentiated cells. Some tapetal cells reach ploidy level 32n (2 × 16n).

Measurement of relative DNA amount in Feulgen-stained sperm nuclei revealed that distinguishing of the male- and female-determining mature pollen grains is not possible on this way. The two separate peaks expected from large (17%) DNA difference between X- and Y$_1$Y$_2$-bearing sperms were not evidenced. It suggests that before anthesis the sperm nuclei were most probably not in G1 phase but during DNA replication.

To resolve the controversial issue of biased sex ratio of seeds in *Rumex* we begin molecular analysis of seeds with the pair of PCR primers (RAY-f and RAY-r) developed by Korpelainen (2002) as highly specific to *R. acetosa* Y chromosomes. It turned out that these primers, although useful to designed studies, generate also short (~80 bp) product with the female DNA.

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Obtaining interspecific hybrids of *Allium cepa* and *Allium roylei* via embryo rescue techniques

Alicja Chuda, Dorota Madej, Adela Adamus

Department of Genetics, Plant Breeding and Seed Science, Agricultural University, Al. 29 Listopada 54, 31–425 Kraków, Poland, e-mail: kghn@ogr.ar.krakow.pl

Embryo rescue techniques are among the earliest and the most successful applications of in vitro procedures used with plant species. Embryo rescue refers to a number of techniques that promote development of the immature embryo into the viable plant. These procedures are often used for producing plants from hybridization, in cases when proper seed setting is impossible.

The study was performed to obtain interspecific hybrids of *Allium cepa* and its wild relative, *Allium roylei*. The experiment was carried out using five maternal accessions: NOE-M3, O/P/1, ZIF 401, S–400 and Kutnowska. Flowering plants from maternal stocks were subsequently castrated and manually pollinated with *Allium roylei* pollen. Seven to 13 days after pollination, ovaries were collected and sterilized. Ovules were excised in sterile conditions, plated onto two agar media: K3 and BDS, and kept in the dark. In total, over 13,000 ovules were plated on regeneration media. At the stage of leaf and root formation, 407 embryos were removed from the dark to the 16 h day photoperiod and transferred onto the MS medium. Completely developed plants were put in the soil and acclimatized to the ex vitro conditions in a chamber with controlled temperature and humidity. Development of interspecific embryos depended on the breeding stock. From all 407 embryos, 176 and 137 were obtained from S–400 and O/P/1 lines, respectively. On average, maternal lines formed from 0.1 to 5% hybrid embryos. Embryo development also depended on the medium composition. Depending on the genotype, the development of embryos occurred with a frequency of 0 to 5.9% on the K3 medium, while on the BDS medium it reached 0.2 to 4.7%. In the experiment, 196 hybrid plants of *Allium cepa* × *Allium roylei* were obtained through embryo rescue techniques. Currently, the hybrid character of obtained plants is being confirmed by using molecular markers.

Association of stored messenger ribonucleoprotein particles with the cytoskeleton in tobacco male gametophyte

Jana Feciková[1], David Reňák[1,2], David Honys[1,3], Věra Čapková[1,3]

[1]*Laboratory of Pollen Biology, Institute of Experimental Botany ASCR, Rozvojová 135, 165 02, Prague 6, Czech Republic, e-mail: honys@ueb.cas.cz*
[2]*Department of Plant Physiology and Anatomy, Faculty of Biological Sciences, University of South Bohemia, Branišovská 31, 370 05, České Budéjovice, Czech Republic*
[3]*Department of Plant Physiology, Faculty of Sciences, Charles University, Viničná 5, 128 44, Prague 2, Czech Republic*

Rapid formation of cell wall in growing pollen tubes requires expression of new set of proteins. Among them, preferentially synthesized glycoprotein 69kDa forms gradually the main fraction of non-covalently bound pollen tube wall proteins. However, transcription of corresponding gene *ntp303* occurs much earlier, during pollen grains maturation. Translationally inactive *ntp303* mRNAs are stored in compact high molecular stable complexes co-sedimenting with polysomes (EPPs; Honys et al., 2000). High resistance of EPPs to polysome-destabilizing drugs prevented their separation for further analyses.

Presence of puromycin, EDTA and high concentrations of KCl together with absence of polysome-stabilizing cycloheximide and Mg^{++} ions from the extraction media led to the disintegration of polysomal complexes, to their re-distribution into the post-polysomal supernatant and preservation of only EPP complexes in the pelleted fraction. Here, further characterization of stored repressed mRNP complexes containing pollen-specific transcripts is presented. Our analyses led to the identification of their tight association with cytoskeleton, presence of both small and large ribosomal subunits and specific protein spectra.

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Preliminary results on ex-vitro conversion of encapsulated somatic embryos of *Citrus reticulata* Blanco (cv. Mandarino Tardivo di Ciaculli)

M.A. Germanà[1], M. Micheli[2], A. Standardi[2]

[1]*SENFIMIZO Department, University of Palermo – Viale delle Scienze, 90128 Palermo, Italy, e-mail: agermana@unipa.it*
[2]*Department of Agricultural and Environmental Sciences, University of Perugia – Borgo XX Giugno 74, 06121 Perugia, Italy, e-mail: asep@unipg.it*

In *Citrus*, highly embryogenic somatic calli are valuable for propagation or genetic improvement. Although anther culture is the most commonly used method to produce haploids, doubled-haploids and homozygous pants in *Citrus* (Germanà, 2003a), it has been also employed to obtain somatic embryos and for the regeneration of many woody plants. By anther culture, not only homozygous haploid, diploid and triploid calli and plantlets have been recovered, but also heterozygous regenerants have been obtained. The production of somatic embryogenic callus through in vitro culture of anthers of *C. aurantium*, *C. sinensis*, *C. aurantifolia*, *C. madurensis*, *C. reticulata*, *Poncirus trifoliata*, the hybrid No. 14 of *C. ichangensis* × *C. reticulata* and *C. paradisi* is described. Anther culture could be regarded as a method of obtaining somatic embryogenesis (Germanà, 2003b). Long-term somatic embryogenic callus has been obtained from *in vitro* anther culture of the cultivar Mandarino Tardivo di Ciaculli (MTC) N.L. 19 of *Citrus reticulata* Blanco. Somatic embryos obtained from these calli were employed for encapsulation in sodium alginate matrix, enriched of artificial endosperm (Redenbaugh et al., 1987; Standardi et al., 1999). In order to evaluate the conversion ability in ex vitro conditions of the synthetic seeds, Plant Preservative Mixture (PPM^{TM}) and Thiophanate-methyl (TM), alone or in combination, were added to alginate matrix and to sowing substrate during conversion. The preliminary results showed that the fungicide TM and the germicide PPM allowed the conversion in ex vitro conditions at level ranging from 20.0 to 37.5% (respectively for TM and PPM in combination and TM alone), while synthetic seeds sown in sterile conditions and no submitted to treatment with TM and PPM (control) showed 35.0% of conversion. Although preliminary, the results reported conversion of MTC synthetic seeds in non-sterile conditions, showing interesting applications of the encapsulation technology in the nursery activity.

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Chromosome arrangement in meiotic and mitotic cells of the ring-forming varieties of *Rhoeo spathacea* (Swartz) Stearn.

Hieronim Golczyk[1], Robert Hasterok[2], and Andrzej Joachimiak[1]

[1]*Department of Plant Cytology and Embryology, Institute of Botany, Jagiellonian University, Grodzka 52, 31–044 Kraków, Poland, e-mail: h.golczyk@iphils.uj.edu.pl; a.joachimiak@iphils.uj.edu.pl*
[2]*Department of Plant Anatomy and Cytology, University of Silesia, Jagiellońska 28, 40–032 Katowice, Poland, e-mail: hasterok@us.edu.pl*

The assembling of a complete (composed of all chromosomes) ring/chain at diakinesis/metaphase I is the most intriguing feature of meiosis in *Rhoeo spathacea* (Sax 1931, Lin and Paddock 1973). It was postulated, that similarly to *Oenothera* and some other plant genera, a series of reciprocal translocations must have occurred in the past (Belling 1927). Nevertheless, cellular mechanisms conditioning this unusual mode of meiosis remain obscure (Coleman 1941, Natarajan and Natarajan 1972; Golczyk and Joachimiak 1999, 2003; Golczyk et al., 2005). Here the chromosomal organization of meiotic and somatic cells of *Rhoeo* was studied with the special attention paid to the behaviour of the centromeric heterochromatin, telomeres, NOR sites/nucleoli. FISH (with rDNA and telomeric repeats as probes), silver staining, and fluorescent staining were performed on the two hetorozygote varieties of *Rhoeo spathacea* (*R. spathacea* and *R. spathacea* var. *vittata*). Centromere clustering contributed to the preservation of highly polarized Rabl arrangement of mitotic and meiotic cells. Similarly to the situation found in *Drosophila*, centromeric heterochromatin domains show a strong tendency for self adherence that can reach its extreme in the form of one large collective chromocenter. Ectopic pairing of the heterochromatin domains showed to be a universal feature of the architecture of somatic (meristematic and differentiated) and meiotic cells (prophase meiocytes) in *Rhoeo*. Furthermore, large collective chromocentres were also observed in the premeiotic interphases, and in the haploid microspore nuclei (tetrads). The spatial organization of *Rhoeo* chromatin in meiotic and mitotic cells was also strongly stabilized by clustering of distal NOR sites in common nucleolar domains (collective nucleoli). This could be facilitated by the unusual excess of transcriptionally active rDNA sites (10 sites per diploid complement, 2n=12) and telomere associations revealed by FISH study. It was concluded, that meiotic and somatic nuclei in *Rhoeo* share the same principal scheme of chromosomal organization, which is highly prescribed and non random, characterized by the strong, Rabl-like centromere-telomere polarization, heterochromatin stickness, and telomere association/fusion. These results were discussed in relation to meiotic catenation in *Rhoeo*.

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Organogenesis in in vitro culture of *Actinidia deliciosa* L. cv. Hayward endosperm

Grzegorz Góralski[1], Marzena Popielarska[1], Halina Ślesak[1], Dorota Siwińska[2], Mariola Batycka[1]

[1]*Department of Plant Cytology and Embryology, Jagiellonian University, 52 Grodzka str., 31–044 Cracow, Poland, e-mail: g.goralski@iphils.uj.edu.pl*
[2]*Department of Plant Anatomy and Cytology, Silesian University, 28 Jagiellońska str., 40–032 Katowice, Poland*

Kiwifruit is an important commercial plant. The efficient procedure of shoot regeneration was obtained in the *Actinidia deliciosa* cv. Hayward endosperm culture. Isolated mature endosperm was cultured on MS medium with 5 mg/l kinetine and 2 mg/l 2,4-D in darkness. Approximately 80% of endosperm explants formed callus. Callus was transferred on regeneration media based on MS medium containing different variants of plant growth regulators: 2,4-D, TDZ, IAA, NAA, BAP, KIN and 2iP. Striking differences in regeneration response were observed between media supplied with TDZ and other hormones. Only media containing TDZ as the sole plant growth regulator stimulated shoot induction. The highest average number of shoots per culture (6.2) was noticed on the medium supplied with 0.5 mg/l TDZ. Lower efficiency of shoots formation was observed on media supplied with 1 or 2.5 mg/l TDZ, achieving 4.2 and 3.8 shoots per culture, respectively. The highest amount of root formation was noticed on the MS media supplied with 1 mg/l IAA + 1 mg/l BAP and 1 mg/l IAA + 0.1 mg/l 2iP (15.3 roots per culture and 11.3 roots per culture, respectively). Flow cytometry confirmed the endospermal origin of callus, roots and shoots obtained in the culture. Histological analysis of callus and roots regenerated during the culture was performed.

Male and female genome size in two *Humulus* species

Aleksandra Grabowska-Joachimiak[1], Elwira Śliwińska[2], Urszula Skomra[3]

[1]*Department of Plant Breeding and Seed Science, The Agricultural University of Cracow, Łobzowska 24, 31–140 Kraków, Poland, e-mail: rrjoachi@cyf-kr.edu.pl*
[2]*Department of Genetics and Plant Breeding, University of Technology and Agriculture, Kaliskiego 7, 85–796 Bydgoszcz, Poland*
[3]*Department of Breeding and Agriculture of Special Crops, Institute of Soil Science and Plant Cultivation, Czartoryskich 8, 24–100 Puławy, Poland*

Angiosperms have evolved various mechanisms to avoid deleterious effects of inbreeding and to promote cross-pollination (allogamy). The most effective mechanism is the production of unisexual flowers on separate male and female individuals by dioecious species. Dioecy is estimated to appear in about 6% of 240,000 flowering plants (Renner and Ricklefs, 1995), but only in a very small number of species is accompanied by sex chromosome heteromorphism. In the family Cannabaceae all three species: *Humulus lupulus*, *H. japonicus* and *Cannabis sativa* are dioecious, possess sex chromosomes and sex determination based on the X/autosome balance (Parker, 1990; Ainsworth, 2000). Two *Humulus* species differ in chromosome number and sex chromosome system. In polytypic *H. lupulus* (2n=20), the simplest sex chromosome system is female XX/male XY, with the Y smaller than the X, while in *H. japonicus* females have 2n=14+XX and males 2n=14+XY_1Y_2.

The knowledge about the genome size in *Humulus* should be helpful for recognition of existing differences between basal diploid taxa belonging to this interesting genus, for breeding and germ-plasm characterization.

To attain the knowledge on this field we analysed chromosome lengths, karyotype structure, and nuclear DNA content (flow cytometry) in diploid European *Humulus lupulus* var. *lupulus*, American *H. lupulus* var. *neomexicanus* and *H. japonicus*. Diploid female representatives of *H. lupulus* var. *lupulus* and *H. l.* var. *neomexicanus* varied in total genome length (23.16 µm and 25.99 µm, respectively) and nuclear 2C DNA amount (5.598 pg and 6.064 pg) despite of similar karyotype structure. *H. japonicus* showed different karyotype structure, smaller basal chromosome set (female/18.04 µm and male/20.66 µm) and lower nuclear DNA amount (female/3.208 pg and male/3.522 pg). There are first evaluations of absolute nuclear genome size and intersexual differences in DNA content in hops.

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Self incompatibility in different *Crocus* species and in *Hermodactylus tuberosus (Iridaceae)*

Caiola M. Grilli, R. Zanier

Department of Biology, University of Rome Tor Vergata, V. Ricerca Scientifica, 00133 Roma, Italia, e-mail: grilli@uniroma2.it

Crocus sativus L.(Iridaceae) is a triploid geophyte, autumnal flowering, cultivated for millennia for production of the saffron spice (Grilli, 2004). It is considered autotriploid unfertile, mainly because of male sterility. However when pollinated with pollen of diploid species it seed sets and matures capsules. It is unknown in wild state and also its progenitor are discussed although recent molecular studies (Grilli et al., 2004) have indicated *C. cartwrightianus* as most probable progenitor of saffron. But also *C. thomasii* is considered candidate progenitor of the species. Both *C. thomasii* and *C. cartwrightianus* are diploid, self incompatible but out compatible (Zanier and Grilli, 2001).

To compare the sexual reproduction in *Crocus* genus and other Iridaceae a set of experiments were prompted in vitro and in planta on *C. thomasii*, *C. cartwrightianus*, *C. hadriaticus*, *C. biflorus*, *C. imperati* and *Hermodactylus tuberosus*. For the research several flowers of each species were emasculated two days before anthesis. At anthesis the stygma of flowers were unpollinated, self-pollinated, out-pollinated, cross-pollinated with pollen of other species. After pollination flowers were covered in a bag and labelled.

The results confirmed that *C. sativus* is self-and out-sterile, mainly male sterile. Its pollen does not fertilize pistil of the other *Crocus* species. *C. thomasii* and *C.cartwrightianus* are self sterile but out-fertile; *C. hadriaticus* and *C. biflorus* are self- and out- compatible; *Hermodactylus tuberosus* is mainly self incompatible but out compatible. Capsules with seeds were obtained from *C. sativus* pollinated with pollen of *C. cartwrightianus*, *C. thomasii* and *C. hadriaticus*, *in vitro* and in fields. All the other species produced capsules and viable seeds after out pollination. The hybrid seeds from *C.sativus* were germinated and produced a green leaf at the base of which a small corm formed. This corm after a dormancy period sprout giving rise a new long gren leaf forming a new small corm at its base. Seed and corm morphology and structure were similar in *C. sativus*, *C. thomasii*, *C. cartwrightianus*.

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Micropropagation of *Picea abies* (L.) Karst and *P. omorika* (Pancić) Purk. by somatic embryogenesis

Teresa Hazubska[1], Krystyna Bojarczuk[2]

Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62–035 Kórnik, Poland,
[1]e-mail: hazubska@o2.pl; [2]e-mail: bojark@man.poznan.pl

Somatic embryogenesis, thanks to its many advantages, is used for micropropagation of many plant species, including some conifers. To produce embryogenic tissue of *Picea abies* (Norway spruce) and *P. omorika* (Serbian spruce), mature zygotic embryos were cultured in the BM–3 medium (Gupta and Durzan, 1986) with various concentrations of sucrose and in the 1/2 LM medium (Litvay et al., 1985), supplemented with various hormones. In both species, the frequency of initiation of embryogenic tissue was the highest in BM–3 with sucrose at a concentration of 20 g/l. The frequency was also high in 1/2 LM with 9 μM Pikloram. For initiation of embryogenic tissue, also somatic embryos were used as secondary explants; the frequency of production of embryogenic tissue was then higher in *P. abies*. The use of the BM–3x medium for propagation of embryogenic tissue caused a greater weight gain of this tissue in both spruce species, as compared to the 1/2 LMx medium. Abscisic acid (ABA) concentration in the maturation medium had a significant effect on the total number of somatic embryos in *P. omorika*, which was the highest at a concentration of 60 μM. ABA concentration affected also the number of produced somatic embryos with cotyledons in *P. abies*, and this number was the highest at a concentration of 20 μM. At the stage of germination, somatic embryos formed hypocotyls more often than radicles, irrespective of the ABA concentration applied during embryo maturation.

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New tools for the manipulation of microspore gene expression

David Honys[1,2,5], Sung-Aeong Oh[3,5], Blanka Šolcová[4], James Andrew Johnson[3], Rita Boudová[1], Jiří Douda[1,2], David Twell[3]

[1]Laboratory of Pollen Biology, Institute of Experimental Botany ASCR, Rozvojová 135, 165 02, Prague 6, Czech Republic, e-mail: honys@ueb.cas.cz
[2]Department of Plant Physiology, Faculty of Sciences, Charles University, Viničná 5, 128 44, Prague 2, Czech Republic
[3]Department of Biology, University of Leicester, Leicester LE1 7RH, U.K.
[4]Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany ASCR, Rozvojová 135, 165 02, Prague 6, Czech Republic
[5]The contribution of both authors was equal

Ongoing characterisation of male gametophyte gene functions requires the introduction of tools enabling spatially and temporally targeted expression of both marker and modified genes of interest. Compared to the limited current resources, promoters driving the expression at earlier developmental stages are required. Available transcriptomic datasets covering four stages of male gametophyte development in Arabidopsis (Honys and Twell, 2004) were used to identify candidates showing microspore-specific gene expression. The putative specific expression profiles were verified by RT-PCR analysis. Verified candidate promoters were cloned into pKGWFS7 vectors (GATEWAY) and their specificity was tested by in situ GUS detection in transformed plants. Two promoters (MSP1, MSP2) showing early expression profiles were found to be specifically expressed in the male gametophyte and tapetum. To demonstrate their effective application, the MSP1 and MSP2 promoters were both used successfully to complement a cytokinesis defective mutant, which provides the first example of the application of microspore expression tools in *Arabidopsis*.

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Identification of interspecific barriers in the genus *Lotus*

Barbara Jungmannová[1], Jana Řepková[2]

[1]*Research Institute for Fodder Crops, Ltd. Troubsko, Zahradní 1, 664 41 Troubsko, Czech Republic, e-mail: jungmannova@vupt.cz*
[2]*Department of Genetics and Molecular Biology, Masaryk University Brno, Kotlářská 2, 611 37 Brno, Czech Republic*

The genus *Lotus* is a large group comprising approximately 200 species. *Lotus corniculatus* is a dehiscent species with an indeterminated growth habit. It is a valuable forage species exhibiting several advantageous characteristics, such as high nutritive value, wide adaptation to soil, pH, and moisture, and low bloat hazard. However, its use has been somewhat restricted because of high seed costs. Pod shattering has been a major problem as seed loss is high due to the continuous flowering and time of pod maturity. Shattering resistance is a character of high heritability and in *Lotus* is considered to be controlled by more than one gene. Classical breeding approaches to reduce shattering through recurrent selection were unsuccessful (Grant, 1996).

Some species of *Lotus* reported to be indehiscent, *L. conimbricensis*, *L. uliginosus* and *L. ornithopodioides*, could be potential sources of genes conferred pod indehiscence. Natural interspecific hybridization does not appear in the genus *Lotus*. There are pre- and post-fertilization barriers of crossability. One of the pre-fertilization barriers is the incapability of pollen to germinate on the foreign stigma and the incapability of pollen tubes to grow through the style. Post-fertilization barriers cover defects in the endosperm and embryo development. Interspecific hybridization has been carried out successfully in this genus using embryo culture procedures (O'Donoughue and Grant, 1988; Sharma et al., 1996).

The aim of our research study is determination of barriers of crossability after interspecific hybridization. An interspecific hybridization programme was undertaken involving the nonshattering species *Lotus conimbricensis* (2n = 12), *L. uliginosus* (2n = 12, 14), *L. ornithopodioides* (2n = 14), and they were crossed with *Lotus corniculatus* (2n = 24). To study the pre-fertilization barriers, callose in growing pollen tubes were stained by aniline blue. To investigate the role of post-fertilization barriers, hybrid embryo viability was traced by a clearing treatment of immature seed using chloral hydrate (Mayer et al., 1993) after modification.

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Female gametophyte development of *Leucanthemum ircutianum* DC in unpolinated ovules cultured in vitro

Iwona Karcz and Elżbieta Kuta

Department of Plant Cytology and Embryology, Jagiellonian University, 52 Grodzka str., 31–044 Cracow, Poland, e-mail: e .kuta@iphils.uj.edu.pl

In vitro culture of unpollinated ovules could result in gynogenic embryos formation and/or autonomous endosperm development but also could be a very promising model to investigate embryo sac development in controlled conditions (Lobanova and Enaleeva, 1998; Mól, 1993).

In *Leucanthemum ircutianum* DC (=*Leucanthemum vulgare* Lam. s.str.) female gametophyte develops according to Polygnum type from chalazal megaspore (Przywara, 1974).

Totally, 1350 closed tubular disk flowers of three size classes (I – 0.8–1.5 mm with archespore, megaspore mother cells or meiocytes in meiotic stages, II – 1.5–2.0 mm with tetrads, and III – 2.0–3.0 mm with 1- to 4-nucleate embryo sacs) were inoculated on eight media based on MS (Murashige and Skoog, 1962) medium and supplemented with growth regulators (2,4-D, BAP, Kin, NAA) in different combinations and concentrations. Additionally hormone-free MS medium was used. Explants were cultured 11 days; flowers were fixed and embryologically analyzed after 3, 5, 7, 9 and 11 days of culture. In culture, the frequency of viable ovules or embryo sacs was rather low ranging from 10–26%, depending on the medium and stage at inoculation with the highest (26%) on MS+1mg/l 2,4-D+1mg/l Kin. Degeneration and necrosis affected 81,1 % of ovules, comprising necrosis of somatic and generative cells as well as degeneration of whole embryo sacs or even ovules. Only 5% of ovules continued development in vitro and the frequency depended on explant size: 0.5% in ovules of the I size class, 10.3% of the II size class and 4.7% in ovules of the III size class. The most sensitive were ovules at the early stages of development (from flowers of I size class). Mature megagametophytes were recorded only in flowers inoculated at the stage of one- to four-nucleate embryo sacs (III size class). Disturbances in meiosis and mitosis along with abnormalities in the female gametophyte developments were found in the material cultured in vitro. Although, in several ovules, mature, 7-celled female gametophyes developed in vitro were typically organized. In vitro conditions did not influence egg cell proliferation. Autonomous endosperm was noticed in one ovule after 11 days of culture on hormone-free MS medium.

It was concluded that flowers of *Leucanthemum ircutianum* are not a good material for experimental studies of embryo sac development under in vitro conditions.

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SEM studies on fruit and seed of some *Chenopodium* L. species (Chenopodiaceae)

Jagna Karcz[1], Bożena Kolano[2], Jolanta Małuszyńska[2]

[1]*Laboratory of Scanning Electron Microscopy, University of Silesia, 28 Jagiellońska str., 40–032 Katowice, Poland, e-mail: jmkarcz@us.edu.pl*
[2]*Department of Plant Anatomy and Cytology, University of Silesia, 28 Jagiellońska str., 40–032 Katowice, Poland*

It is well known that fruit and seed characters are useful in the identification and classification of plant taxa, and in establishing phylogenetic and evolutionary relationships among taxa. In *Chenopodium* genus some economically important species, especially *Chenopodium quinoa*, *Chenopodium berlandieri* and *Chenopodium album* are interested from a taxonomical and phylogenetical point of view. To date, no detailed study on fruit and seed surface structure have been carried out on these species except for some physiological and cytotaxonomical reports by Wilson (1980), Wood et al. (1993) and Prego et al. (1998). In our study mature fruits and seeds of wild and cultivated *Chenopodium* species: *C. quinoa*, *C. berlandieri* subsp. *berlandieri*, *C. berlandieri* subsp. *Nuttalliae* with two varieties: *Huauzontle* and *Quelite* (sect. *Chenopodium* subsect. *Cellulata*) and polymorphic species of *C. album* (sect. *Chenopodium* subsect. *Leiosperma*) were studied with scanning electron microscopy. *C. album* is often defined as a poliploid complex or a single plymorphic species with 3 cytotypes on the diploid (2n=2x=18), tetraploid (2n=4x=36) and hexaploid (2n=6x=54) level.

This work was initiated with the objective of using pericarp and seed coat surface patterns to identify taxa and to establish their interrelationships. The SEM analysis revealed that alveolate-fruited taxa (*C. quinoa*, *C. berlandieri*) are phenotypically distinct from the smooth-fruited *C. album* group. Similarly, seed surface characteristics of each species were reported in detailed description. Three general types of seed primary sculpture were observed: reticulate (*C. berlandieri*), flately tuberculate (*C. quinoa*) and smooth and finely lineated (*C. album*). Testa patterns of *C. berlandieri* and *C. album* were more variable than those of *C. quinoa*. In addition, seed coat topography in *C. berlandieri* taxa varied only in the extent of protrusion of the epidermal cell walls. These micromorphological seed surface features were also taxonomically informative Thus, our carpological analysis showed that *C. quinoa* is more closely related to *C. berlandieri* than to the *C. album*. Relationships among these taxa were also analysed using cytogenetic and molecular methods. Comparative study of repetitive sequence organization indicated that repetitive DNA fraction of *C. quinoa* genome is more similar to *C. berlandieri* than *C. album*. It was also showed that there is intraspecific polymorphism in repetitive sequences genome organization in *C. berlandieri*. Therefore, mature seed coat surface patterns may be used as parameters for species identification and indicate the different phylogenetic relationships among *Chenopodium* species.

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Floral modification and expression of floral homeotic genes in the smut-infected female plants of *Silene latifolia*

Shigeyuki Kawano[1], Yusuke Kazama[1], Ayako Koizumi[1], Amr Ageez[1], Wakana Uchida[2]

[1]*Department of Integrated Biosciences, Graduate School of Frontier Sciences, University of Tokyo, FSB–601, 5–1–5 Kashiwanoha, Kashiwa, Chiba 277–8562, Japan, e-mail: kawano@k.u-tokyo.ac.jp*
[2]*Present address: Mol Membrane Biol Lab, RIKEN, Wako, Saitama 351–0198, Japan, e-mail: w-uchida@riken.jp*

Silene latifolia is a dioecious plant that has X and Y chromosomes. Female plants initiate development of stamens by infection of the dimorphic smut fungus *Microbotryum violaceum*. Until stage 7, no floral modification is observed between healthy and infected females. At stage 8, the healthy female does not develop stamens, whereas the infected female initiates to develop stamens. They develop to the mature stage (stage 12), at which anthers are full of the fungus teliospores instead of pollen grains. We used electron microscopy to examine young floral buds and fungus-induced anthers that had been fixed by a high-pressure freezing method. The parasitic hyphae have a consistent appearance up to stage 8 and then they are observed adjacent to the dying sporogenous cell in the infected female anther. At stage 9, an increasing number of dead and dying sporogenous cells appear, among which the fungus sporogenous hyphae develop and form initial teliospores. The fungus accelerates cell death in the anther and utilizes constituents of the dead host cell to form the mature teliospore. Expression of the floral-B function gene, *SLM2*, an ortholog of *PISTILLATA* in *Arabidopsis*, was examined by in situ hybridization. Until stage 7, *SLM2* is expressed on whorl 2 and 3 of the healthy and infected females. At stage 8, *SLM2* transcript is supressed in the stamen primordia of the healthy females. However, developing stamen of the infected female expresses *SLM2* as intense as developing petals. The suppression of *SLM2* expression is removed by the smut-infection independent of the presence of Y chromosome. Smut-infected females should provide a useful system for clarifying the relationship between the B-function gene and the sex determination factor.

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Cis-regulatory regions responsible for suspensor-specific transcription

Tomokazu Kawashima[1], Xing-Jun Wang[1], Yuping Bi[2], Koen Weterings[3], Robert B. Goldberg[1]

[1]*Department of Molecular, Cell, and Developmental Biology, University of California, Los Angeles, California 90095, U.S.A., e-mail: tomokazu@ucla.edu*
[2]*Crop Institute, Shandong Academy of Agricultural Sciences, Shandong, China*
[3]*Bayer Crop Science N. V., Technologiepark 38, B–9052 Gent, Belgium*

We are interested in how the suspensor is differentiated from the basal cell and what gene regulatory network(s) activate genes in the suspensor during early stages of embryo development. Using the scarlet runner bean (*Phaseolus coccineus*) as a model system to study gene regulation during embryogenesis, we have identified several genes that encode mRNAs that are highly localized in the suspensor during early embryo development. One of the genes, designated as *G564*, encodes an unknown protein, and is transcriptionally regulated in the embryo basal region. We previously found several 80-bp repeat sequences in the *G564* promoter and showed that transcriptional activity in the suspensor is abolished when all 80-bp repeat sequences are deleted in transgenic tobacco plants. In addition, we used computational analysis to identify a conserved 10-bp motif within the region responsible for transcription in the suspensor. To determine what sequences are responsible for the suspensor-specific transcription, we carried out gain-of-function and fine scale 5'-deletion analyses. We determined that one 80-bp repeat sequence driven by the cauliflower mosaic virus 35S minimal promoter is sufficient to activate suspensor-specific transcription in transgenic tobacco embryos. In addition, we observed that the 10-bp conserved motif is sufficient to activate transcription within the suspensor when it is fused with a truncated *G564* promoter region that is not active in the suspensor. By Contrast, the 10-bp motif cannot activate transcription with the 35S minimal promoter in the suspensor, suggesting that additional elements are required for suspensor-specific transcription. Currently, we are carrying out experiments to determine what these additional sequences are and what transcription factors interact with suspensor *cis*-acting elements to activate transcription in the basal embryo region early in development.

Relationship between pre-anthesis development and seed formation in spring rape (*Brassica napus* L.)

Danguolè Kazlauskienè

Institute of Botany, 49 Žaliuju ežeru str., 08–406 Lithuania, e-mail: danguolekazlauskiene@takas.lt

Shoot apex development is determined by realization of a genetic program, nevertheless vegetative growth and generative development can be regulated through internal and external factors. The aim of this work was to study the process of embryogenesis in spring rape, beginning with apex development, flower primordia, flower formation – its anatomical and morphological structures up to seed formation, as well as the possiblity to modify these processes with the aid of the auxin type compound TA-14. Spring rape 'Muscat' plants were treated with compound TA-14 (1×10^{-3} M) in the 2nd true leaf development stage. Samples of rape vegetative cones for anatomical investigation were taken in 2nd, 3rd, 4th, 5th, 6th,7th true leaves stages. The excised apices – stable preparations wereprepared by a commonly used method (Kublickienè, 1978). In the 2nd true leaf stage (time of exposure to the compound) plants apices are in the vegetative growth phase: the apical meristem generates lateral meristems initially recognized as semicircular ridges along the main apex. At the stage of 3–4 leaves compound TA-14 induces rape shoot apical meristem cell division: in the test variant plants around the apical axis the flower cones and buds are already formed; from them, later on lateral branches will appear, whereas in control plants the cone apex is cone-shaped and contains the daveloping leaves, while flower primordia are not yet seen. Analyzing the developing rape inforescence at the 4–5 leafes stage, we see that both in the test and the control variants the apex is in the generative stage of development, however, plants exposed to the effect of TA-14 have already formed peduncles, petals and carpels, weareas only carpels are formed in the control variant. In the subsequent stage (6–7 leaves), under the effect of TA-14 a fecundated ovule is seen in the ovary, together with seedbuds and the initiated embryo development inside the endosperm, while in the control variant only flower seedbud sacks are observed.

The obtained data have shown that the auxin type compound TA-14 induces the rape embryogenetic process by enhancing apex development, the inflorescence, flower structure, embryo and seeds formation.

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Genetic analysis of gametophytic parental effect mutants in *Arabidopsis*

Nirma Skrbo[1], Moritz Nowack[2], Arp Schnittger[2], Gerd Jürgens[3], Martin Hülskamp[4], Reidunn B. Aalen[1], Paul E. Grini[1]

[1]*Molecular Biosciences Department, University of Oslo, Oslo, Norway*
[2]*University Group at the Max-Planck-Institute for Plant Breeding Research, Cologne, Germany*
[3]*ZMBP, Developmental Genetics Department, University of Tübingen, Tübingen, Germany*
[4]*Botanical Institute III, University of Cologne, Cologne, Germany*

Seed development requires a coordinated interplay of embryo, endosperm and the maternal seed coat. What roles gametophytic parental (maternal and paternal) factors play in this process is not clear. We have performed various screens to identify haplo-phase specific genes required in the gametophytic phase, or required in a gametophytic parental effect specific manner for embryo and endosperm development Here we present an outline of these screens. Our main focus is to identify and characterize the action of genes that have a parental effect on seed development. In the gametophytic maternal-effect *capulet* (*cap*) mutants, both embryo and endosperm development is arrested at early stages. The *cap* mutant phenotypes are not rescued by wild-type pollen and removal of silencing barriers from the paternal genome by *METHYL TRANSFERASE1* antisense transgene expression or by mutation in the DECREASE IN DNA METHYLATION1 (*DDM1)* gene also fails to restore seed development. The mutants display no autonomous seed development and were epistatic to *medea / fertilisation-independent-seed1 (fis1)* in both autonomous and sexual endosperm development. Embryo and endosperm specific *GUS* and *GFP* markers show ectopic expression in *cap* mutant endosperms. Molecular characterization of the *CAPULET* genes is in progress to verify whether the *CAPULETs* represent novel maternal functions supplied by the female gametophyte that are required for embryo and endosperm development. As an additional tool to dissect the involvement of maternal and paternal gene programmes in seed development we explore the use of a paternal effect cdka;1 mutant line, where mutant pollen induce embryo and endosperm developmental arrest after fertilization. In *cdka;1* a single generative-like cell preferentially fertilizes the egg cell, leading to partial development of both embryo and (unfertilized) endosperm, thus allowing a genetic dissection of parental contribution to the fertilization product.

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Is *Viola tricolor* L. from calamine waste heaps in the vicinity of Olkusz (Southern Poland) a metal-tolerant taxon?

Kristin Kellner[1], Elżbieta Kuta[2]

[1]*Institut für Systematische Botanik und Geobotanik, Freie Universität Berlin, Germany, e-mail: kristinkellner@o2online.de*
[2]*Department of Plant Cytology and Embryology, Jagiellonian University, 52 Grodzka str., 31–044 Cracow, Poland, e-mail: e.kuta@iphils.uj.edu.pl*

Metal-tolerant taxa are known in *Violaceae*. Two metalophytes named the blue (*V. lutea* ssp. *westfalica*) and the yellow (*Viola lutea* ssp. *calaminaria*) zinc violets belong to the *Melanium* Ging. section of the genus *Viola* L. (Ernst, 1968). In Southern Poland, on calamine waste heaps near Olkusz a large population of *V. tricolor*, other representative of the *Melanium* section, exists as an effect of natural succession.

We focused on reproductive processes of plants from calamine heaps. Control represented material from Botanic Garden in Cracow (Poland) and Hohenheim (Germany). Heavy metal (Pb, Zn) content in embryos was detected using SEM-EDS.

Neither irregularities in female and male gametophytes development, pollen, embryo and endosperm formation nor necrosis in somatic and generative tissues were observed in plants from calamine heaps. Embryos, isolated from seeds harvested on calamine heap although normally developed, showed reduced viability estimated in histochemical topographic tetrazolium test (3%), resulting probably from the low respiratory activity of embryos. In seed germination test the frequency of seedlings not exceeded 50%. Contrary, the embryos of control material from Botanical Garden in Hohenheim showed very high viability as assessed by staining using tetrazolium and indigocarmine 95% and 83%, respectively. Pollen viability, based on Alexander's staining, was very high reaching 89% in plants from waste heap and 98% in control material. In species with pollen heteromorphism, the ratio of pollen with lower and higher aperture numbers might be a response of the plant to extreme living conditions when tends toward lower aperture numbers (Nadot et al., 2000). In V. tricolor from the waste heaps 4-apertured pollen grains were in majority; the frequency of 5-apertured pollen grains was very low (0–1.4%) while in control material reached 9%.

SEM-EDS analysis of embryos isolated from seeds collected on calamine heaps clearly indicated that embryo cells not contain any remarkable amounts of the heavy metals (Pb, Zn). It can be concluded that *V. tricolor* is well adapted to the conditions on calamine waste heaps and that explains its frequent occurrence on this territory.

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Temporal and spatial distribution of pectin and arabinogalactan epitopes during androgenesis from wheat anther callus

Robert Konieczny[1], Joanna Świerczyńska[2], Andrzej Z. Czaplicki[3], Jerzy Bohdanowicz[2]

[1]*Department of Plant Cytology and Embryology, Jagiellonian University, ul. Grodzka 52, 31–044 Kraków, Poland, e-mail: r.konieczny@iphils.uj.edu.pl*
[2]*Department of Genetics and Cytology, University of Gdańsk, ul. Kładki 24, 80–822 Gdańsk, Poland*
[3]*Plant Breeding and Acclimatization Institute, 05–870 Błonie, Poland*

In anther culture of Polish winter wheat cv. Apollo the microspores at middle and late unicellular stage produce callus of embryogenic or organogenic potential (Konieczny et al., 2003). Microscopic observations at the onset of regeneration (6 days after culture on induction medium) revealed the presence of two distinct types of cells on the callus surface: large, loosely attached parenchymatous cells and small, tightly packed meristematic cells arranged in multicellular clusters. Cell walls of parenchyma cells at the callus periphery were labeled by several anti-AGPs antibodies i.e. JIM4, JIM14, JIM 16, but unlabeled by anti-pectin JIM5 and JIM7 ones. In contrast, strong signal of pectic epitope JIM7 on the surface of meristematic clusters was observed. None of anti-AGPs antibodies used (JIM4, JIM8, JIM13, JIM14, JIM15, JIM16, LM2, MAC 207) labeled the cell wall of meristematic cells. After subculture on regeneration medium (6–8 weeks after explantation) the peripheric regions of the callus became compact and nodular, whereas the inner parts remained parenchymatous. From the meristematic regions labeled strongly by anti-pectin JIM7 antibody numerous shoots and/or leaves were formed. Leaf formation often preceded shoot regeneration so that shoot apices differentiated from a broad meristematic zone at the base of the leaves. Very sporadically, distinct shoot buds were also found. Immunofluorescence microscopy indicated that anti-AGP LM2 antibody recognized the inner surface of the cell walls in the parenchyma regions of the callus and regenerated leaves. Additionally, the anti-pectin JIM5 was found to label the surface of the walls surrounding the intercellular spaces. Interestingly, the epitopes of AGPs present on the callus cells at the early stages of culture (JIM4, JIM14, JIM16) were not found after subculture on regeneration medium. Role of cell wall composition in relation to cell differentiation and cell proliferation in wheat androgenesis is discussed.

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SDS-PAGE protein profiles of developing seeds of *Abies concolor*

Andrej Kormuťák, Gabriela Libiaková, Ján Salaj, Jana Libantová, Jana Moravčíková, Božena Vooková

Institute of Plant Genetics and Biotechnology SAS, Akademick 2, P.O.Box 39A, SK–950 07 Nitra, Slovakia, e-mail: nrgrkorm@savba.sk

SDS-PAGE protein profiles were followed during embryogeny and seed germination of *Abies concolor*. The protein fraction of 42 kDa size dominated the soluble protein profiles of megagametophytes and embryos since the first stage analyzed. The proteins of 30–33 kDa molecular size range appeared in megagametophytes at the end of June, whereas those of 22 and 24 kDa in the midJuly. The synthesis of 36 kDa protein started on August 8 only when megagametophytes became rather compact. Soluble protein profiles of embryos consisted of 42, 32, 28, 22, 18 and 16 kDa proteins. Like in megagametophytes, their synthesis was regulated developmentally. Insoluble protein pattern of megagametophytes was essentially similar with that of soluble proteins. The only exception was a 40 kDa protein which was much more distinct in insoluble than in soluble protein fraction. Both 42 and 40 kDa proteins were detected in the first anylyzed stage already along with 32 and 18 kDa proteins. The synthesis of low molecular weight proteins of 22, 14 and 6.5 kDa started at the midJuly. A characteristic feature of the insoluble protein pattern of embryos was their 40 kDa protein exhibiting higher abundancy than 42 kDa protein. These proteins may be detected throughout the whole period investigated along with the proteins of 36, 32, 30, 18 and 14 kDa size. On the contrary, the 22 kDa protein appeared in embryos at the stage of their physiological maturation in August. Based on degradation dynamics of individual proteins during stratification and subsequent germination of seeds, the nutritive function was ascribed to nearly all soluble proteins of megagametophytes and embryos. Of the insoluble proteins, the megagametophyte proteins of 42, 22, 18, 14 and 6.5 kDa were depleted completely, whereas those of 32 and 30 kDa only partially. In embryos only 36 and 14 kDa insoluble proteins were degraded as compared with a partial digestion of 42, 40, 34, 32, 22 and 18 kDa proteins. The study has not confirmed the deviating nature of *Abies* storage proteins from other *Pinaceae* as quoted by Jensen and Lixue (1991).

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Application of fluorescent method to fast evaluation of interspecific crossing ability in *Rhododendron*

Dorota Korzonek, Maria Klein

Department of Genetics, Plant Breeding and Seed Science, Krakow Agricultural University, Al. 29 Listopada 54, 31–425 Krakow, Poland, e-mail: mklein@ogr.ar.krakow.pl

Interspecific crossing is one of ways for generation of initial materials for breeding new *Rhododendron* cultivars (Williams et al., 1990). However, pre- and postzygotic barriers exist, disabling hybrid production (Williams and Rouse, 1989; Zhang et al., 1998). On the basis of fluorescent method, crossing ability was tested for four cultivars from the *R. catawbiense* group, i.e. Album Novum, Boursault, Nova Zembla, and Old Port, in combination with wild Asian species *R. brachycarpum* and *R. purdomii*, carrying genes conferring resistance to low temperatures. Pollination was performed in controlled conditions on detached inflorescences collected from maternal plants. Pistils for cytological evaluation were collected after 3 hours, 7 hours, and during the next 10 days after pollination. Pollen tubes were stained with anilin blue following pistil maceration in 30% NaOH in 36˚C. Most often, pollen germination was initiated 3 hours after pollination. Typically one or two tubes germinated from the tetrad. Pistils of maternal plants differed in length, from 30,9 mm for cv. Old Port to 42,2 mm for cv. Album Novum. Pollen tubes needed 2 to 7 days to grow through the full length of the pistil. After 4 to 8 days pollen tubes started penetrating ovules. In most cases pollen germination was normal, some disturbances being observed mostly in Nova Zembla and Old Port after pollination with the Asian species. Obtained results indicate that there are no clear prezygotic barriers and the work leading to production of hybrids between the analyzed taxons should be continued.

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Structural and biochemical changes in the micropylar region of *Chondrilla juncea* apomictic ovule

Maria Kościńska-Pająk[1], Barbara Chudzik[2], B. Zarzyka[2], J. Pawelec[3]

[1]*Department of Plant Cytology and Embryology, Jagiellonian University, ul. Grodzka 52, 31–044 Cracow, Poland*
[2]*Department of Cell Biology, Institute of Biology, Maria Curie-Skłodowska University, ul. Akademicka 19, 20–033 Lublin, Poland*
[3]*Electron Microscope Laboratory, Institute of Biology, Maria Curie-Skłodowska University, ul. Akademicka 19, 20–033 Lublin, Poland*

During maturation of amphimictic plant ovules tissues in micropylar part of nucellus and integuments as well as synergids underwent structural and biochemical changes preparing a sufficient environment for pollen tube growth and fertilization.

In the present work we examined the anatomical structure and changes of the micropylar region during the development of obligatory apomictic *Chondrilla juncea* ovules in which the pollen tube does not enter the synergid and the embryo and the endosperm develop autonomously.

The observations in light and transmission electron microscopes showed that in young ovules at the stages from the megaspore mother cell to the one-nucleate embryo sac, the part of funiculus tissue protrude toward the ovule micropyle. This tissue had a meristematic character with isodiametric cells surrounded by thin cell walls. In ovules with two- and four-nucleate embryo sac the cells of this tissue elongated, started the secretory activity and formed on obturatore. At the stage of embryo sac maturation the space of the micropylar canal was filled with the extracellular matrix substance originated from the secretory activity and lysis of the obturatore cells. Immunolabelling with monoclonal antibodies recognizing the esterified (JIM 7) and deesterified (JIM 5) pectins showed that both substances were abundant in the micropylar canal matrix, but were not present in the micropylar part of the embryo sac. Ultrastuctural studies indicated that synergids did not form a filiform apparatus and had little endoplasmic reticulum and few dictiosomes. Our observations suggest that in the apomictic ovules of *Chondrilla juncea* the synergids do not secrete substances present inside the micropylar canal. This difference in comparison to amphimictic ovules coincides with lack of fertilization.

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Near isogenic lines for study spike morphology in bread wheat

S.F. Koval, V.S. Koval

Institute of Cytology and Genetics, 630090 Novosibirsk, Lavrent'eva 10, Russia, e-mail: kovalvs@bionet.nsc.ru

The success of investigations in plant special genetics considerably depends on the presence of gene collections. NIL sets produced on the base of single recurrent parent and marked on various genes are important among them. Such collections could be used not only as convenient testers in a genetic analysis and for studying phenotypic allelic manifestation but also in applied research – estimate of trait yield contribution and construction of a cultivar model etc. The analogues, isogenic and alloplasmic lines, are usually used as model objects in these or that experiments or as breeding stocks (donors, prospective would-be cvs). For this purpose isogenic and alloplasmic lines are to be convenient for working in a wide range of environments, both in a field experiment and artificial climate. Observation results should be well reproduced and have a free inambiguous consideration (Koval and Shamanin, 1999). Conclusions will be reasonable not only for the case under study but for a wide range of phenomena with good correspondence of an object to the aims of investigation.

The NIL series of the Institute of Cytology and Genetics, SB RAS has been developed from the onset considering the above-mentioned problems. Earlier published NIL catalogues of ANK series based on cv Novosibirskaya 67 (Watanabe et al., 2003) have become outdated as this series has been replenished with new lines for the recent years.The series has lines marked on plant morphological traits and habitus, terms of developmental phases, resistance to brown rust and powdery mildew . There are 94 lines in the set of NIL. Some of line marketed genes which controlled spike morphology characters. These characters are: spike color (white, red, black, brown glume), color of grain (white, red, purple) awned spike, haired glume, spike density (closely packed ear, compact and speltoid spike), form of grain. These lines can be used in research for genetic of spike morphology and flower development mechanism. Most part of the lines carry out certain marking genes. Another field of application near isogenic lines is study of pleiotropic effect marking genes on the plant productivity and duration of vegetation. At this moment monograph about these lines are published (Watanabe et al., 2003) and NILs can be used any researchers who are interesting in.

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The length of anther as an indicator of pollen developmental stage of triticale (× *Triticosecale* Wittmack)

Janusz Kozdój

Department Plant Biotechnology and Cytogenetics, Plant Breeding and Acclimatization Institute, 05–870 Błonie, Poland, e-mail: kozduj@ihar.edu.pl

The anther lengths were analysed during microsporogenesis and pollen development of seven Polish triticale cultivars (Bolero, Dagro, Grado, LAD 285, Largo, Lasko and Presto). Fresh anthers were harvested from three florets of mid-ear spikelet. The lengths were determined using binocular equipped with measuring ocular. For analysis of microsporogenesis and pollen developmental stages the squash preparations stained with 3% acetocarmine were made from fresh anthers. The microphotographs of meiocytes and developing pollen grains were taken using Nikon eclipse e400 microscope.

The analysis of anther length at consecutive stages of microsporogenesis since meiocyte to microspore and pollen development since microspore to three celled pollen grain allow us to use the anther length as a indicator of particular stage of pollen development, e.g. the anther length of 2.7 ± 0.19 mm corresponds to stage of microspore released from tetrad, the length of 3.4 ± 0.14 mm corresponds to stage of growing microspore with aperture visible, 4.2 ± 0.38 mm corresponds to stage of fully developed microspore with central vacuole, the length of 5.6 ± 0.20 mm corresponds to mature three celled pollen grain stage. Such correlations can serve as valuable tool for experiments on androgenic embryogenesis of cereals.

Microtubular cytoskeleton of embryo-suspensor in *Sedum acre* L.

Małgorzata Kozieradzka-Kiszkurno, Joanna Świerczyńska, Jerzy Bohdanowicz

Department of Genetics and Cytology, University of Gdańsk, 24 Kładki str., 80–822 Gdańsk, Poland, e-mail: stokrota@univ .gda.pl

Cytological processes of differentiation in the embryo-suspensor of *Sedum acre* L. were compared with the development of the embryo-proper. After the first division of the zygote, two cells of unequal size are formed: a large basal cell (BC) and smaller apical one. The BC undergoes no division, becomes much enlarged, and produces haustorial branches invading the micropyle and adjacent tissues, and protruding out of the ovule. The apical cell develops into the embryo-proper and chalazal suspensor. The mature suspensor consists of a large, pear-shaped BC and a few chalazal cells.

Ovules of *Sedum acre* were isolated from pistils in various developmental stages, immediately fixed and embedded in Steedman's wax. Immunohistochemical reactions were performed on sections using monoclonal antibody (Mab): mouse anti-tubulin. Antigen-Mab complexes were localized by a secondary, Alexa 488-conjugated anti-mouse, antibody. Fluorescence was studied with light microscopy.

During the early phase of development of suspensor BC, the microtubules were found to localize from micropylar to chalazal pole of the cell. These microtubules formed irregular bundles in cytoplasm. In the fully differentiated BC and the micropylar haustorium, the tubulin filaments formed a prominent network. At the micropylar end of the suspensor BC, a high concentration of longitudinally oriented tubulin filaments was present. The nucleus of the BC was located centrally. The microtubules were found distributed around the nucleus and radiating from the nuclear surface. A distinct tubulin cytoskeleton with numerous microtubules was observed in the cytoplasmic layer adjacent to the wall, separating the BC from the first layer of the chalazal suspensor cells.

These results reveal new details of the microtubular cytoskeleton in the developing suspensor.

Culture of carrot isolated microspores – induction of divisions

Dorota Krzyżanowska, Urszula Kowalska, Waldemar Kiszczak, Krystyna Górecka

Instytut Warzywnictwa, 96–100 Skierniewice, ul Konstytucji 3 Maja 1/3, e-mail: kgoreck@inwarz.skierniewice.pl

Carrot breeding has been currently dominated by F_1 hybrids, which form equal roots of very high quality. It is possible to obtain completly homozygotic lines by the use of biotechnological methods, first producing haploids and then doubled haploids. Microspore culture is one of the ways to obtain doubled haploids. The tests experiments were carried out on carrot genotype 1484. Microspore cultures of carrot were established on medium B5 (Gamborg et al. 1968) modified by Keller and Armstrong (1978) and used by Andersen et al.(1990) for carrot anther cultures. Microspores isolated from umbellets, after rinsing, were suspended in mediumin at such the ratio so 40 thousand microspores were contained in 1 ml and then were placed in the incubator at +30˚C. The following observations were made: developmentel stage of microspores, sterility of culture and divisions of microspores. Dishes with visible structures were exposed to continuous light at +27˚C.

First divisions of microspores were observed in the light microscope after about 2 weeks of culture. Further divisions took place successively and structures containing ten to hundred cells were formed. After 6 weeks of culturing, structures visible with naked eye were formed. Two weeks after the dishes were exposed to continuous light, some of the embryoids reached the size which allowed their passage onto the regeneration medium. The rest of the structures left on the dishes, to small to be transferred, after further two weeks were suitable for transferring too. Succesive formation of new embryoids was also observed. Six weeks after embryoids passage onto regeneration medium, they formed various structures: secondary embryos, deformed plants and callus-like rosettes. First complete plants were also obtained.

After elaboration of adaptation stage of obtained plants to external growing conditions, it should be possible to work out the optimum conditions of carrot microspore culture.

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Histological analysis of direct somatic embryogenesis of *Arabidopsis thaliana* (L.) Heynh.

Ewa U. Kurczyńska[1], Agnieszka Ujczak[2], Małgorzata D. Gaj[2]

[1]*Department of Biophysics and Cell Biology, University of Silesia, ul. Jagiellońska 28, 40–032 Katowice, Poland, e-mail: euk@us.edu.pl*
[2]*Department of Genetics, University of Silesia, ul. Jagiellońska 28, 40–032 Katowice, Poland*

In *A. thaliana* (L.) Heynh., a model plant in genetic research, in vitro culture of immature zygotic embryos (IZE) results in effective production of somatic embryos via direct embryogenesis, DSE (Gaj, 2001). Efficient somatic embryo production and advanced structural and functional genomics available in *Arabidopsis* provide unique opportunities for studying somatic embryogenesis in this plant. Different approaches should be applied including histological analysis of somatic embryo origin to gain insight into events taking place during acquisition of embryogenic competence.

The histological study of somatic embryos development in DSE system aimed at analysis of: (a) morphological and histological changes; (b) symplasmic communication between cells; (c) localization of auxin. Information about the symplasmic communication between cells was achieved by tracing the localization of fluorochromes under the fluorescence microscope. Transgenic plants carrying *Arabidopsis* plants expressing a GUS gene driven by a synthetic, auxin-responsive DR5 promoter were used to visualize auxin distribution pattern.

The obtained results have shown that somatic embryos develop from adaxial side of IZE cotyledones including their epidermis, parenchyma or parenchyma and epidermis. Somatic embryos developing from epidermis have had one- or multi-cell origin while somatic embryos originating from epidermis and parenchyma have shown a multi-cell origin. It was visible that somatic embryos have displayed no connection with vascular tissue of the explantat. Analysis of symplasmic communication has shown the presence of tracer in all cells of zygotic embryo in globular and early heart stage. In the late torpedo stage the tracer was observed only in hypocotyl and radicle. After several days of culture the tracer was detected in the basal part of the explant cotyledons, mostly in their adaxial side. The fluorochrome tracer did not enter the somatic embryo suggesting a lack of symplasmic communication between the embryo and the explant tissue. The activity of the DR5::GUS promoter monitoring auxin distribution was detected in different cells and tissues of the explant depending on the stage of somatic embryogenesis. The pattern of GUS expression has indicated rapid accumulation of auxin in explant cells induced in the presence of 2,4-D and asymmetric distribution of auxin in somatic embryos.

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Identification of seed-specific transcription factors from a global analysis of gene activity during the *Arabidopsis* life cycle

Brandon H. Le[1], Anhthu Q. Bui[1], Julie Pelletier[2], Linda Kwong[2], Zixing Fang[3], Steve Horvath[3], Gary N. Drews[4], Robert L. Fischer[5], Jack K. Okamuro[6], John J. Harada[2], Robert B. Goldberg[1]

[1]*Department of Molecular, Cell, and Developmental Biology, University of California, Los Angeles, CA 90095, U.S.A.*
[2]*Section of Plant Biology, Division of Biological Sciences, University of California, Davis, CA 95616, U.S.A.*
[3]*Department of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, CA 90095, U.S.A.*
[4]*Department of Biology, University of Utah, Salt Lake City, UT 84112, U.S.A.*
[5]*Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, U.S.A.*
[6]*Ceres, Inc., 1535 Rancho Conejo Blvd., Thousand Oaks, CA 91320, U.S.A.*

We identified seed-specific transcription factors by carrying out a whole-genome analysis of gene activity during the *Arabidopsis* life cycle. We used Affymetrix GeneChips to profile the gene sets active during seed development at 24 hr post-fertilization, 7–8 days after pollination (DAP), 13–14 DAP, and 18–19 DAP. These gene sets were compared with those active in pre-fertilization ovules, seedlings three days after imbibition (DAI), and leaves, roots, stems, and floral buds of the mature plant. Collectively, we detected approximately 15,000 transcripts during seed development and a total of 19,000 transcripts during all stages of the life cycle investigated. Between 9,000 and 13,000 diverse transcripts were detected in seeds depending upon the stage, with the number of transcripts decreasing prior to dormancy and then increasing following germination. Most mRNAs are present throughout seed development, although the prevalences of these mRNAs can fluctuate significantly. Hierarchical analysis of shared mRNAs identified several mRNA clusters with elevated levels at specific stages of seed development. Stage-prevalent mRNA clusters encode proteins enriched for specific functional categories including unique transcription factors. Each seed development stage also has a mRNA set that is not detected at the level of the GeneChip in other stages and that includes transcription factor mRNAs. Real-time quantitative RT-PCR (qRT-PCR) experiments indicated that seed-stage-specific transcription factor mRNAs are either absent from other stages or present at reduced levels. Comparison of genes active in seed development with those active at other periods of the life cycle uncovered a small set of seed-specific transcription factor mRNAs that were validated using qRT-PCR. Collectively, our data should facilitate the identification of regulatory networks and genes that are required to make a seed and an entire plant.

Tapetal raphides of *Tradescantia bracteata*: the strategy of their transport from anther to stigma

Joanna Leśniewska[1], Bartłomiej Och[1], Maria Charzyńska[2]

[1]*Department of Botany, Institute of Biology, University of Białystok, Świerkowa 20 B str, 15–950 Białystok, Poland, e-mail: joanles@uwb.edu.pl*
[2]*Department of Plant Anatomy and Cytology, Institute of Experimental Plant Biology, Warsaw University, 1 Miecznikowa str, 02–096 Warszawa, Poland, e-mail: marlig@biol.uw.edu.pl*

Tapetal raphides are present in many families of Commelinales (monocotyledons) (Prychid et al., 2003), but their physiological significance remains poorly understood.

The formation of calcium oxalate crystals in tapetum might be related to the regulation of free calcium level in the tapetal cytoplasm during anther development. It has been suggested that such crystals could adhere to the pollen grains during the anther dehiscence and could be transported together with the pollen to the surface of the stigma, and after being dissolved in liquid exudate could become the source of calcium ions for pollen germination. It was shown recently that in Petunia (Iwano et al., 2004) the calcium crystals, originating not from the tapetum but from the tissues underlying the stomium, move with pollen grains to the stigma, and are the source of Ca^{2+} for pollen germination and pollen tube growth.

We studied the calcium oxalate crystals in tapetum of *Tradescantia bracteata* L. (Commelinaceae) in order to explain how the raphides are attached to the surface of pollen. In this species, the tapetum protoplasts fuse after the PMC meiosis and form a multinucleate plasmodium, which enters the anther locule and surrounds the tetrads and developing microspores/pollen grains. Upon degradation of the tapetum, its numerous raphides are released amid the pollen grains. Pollen grains of *Tradescantia bracteata* are isopolar and monocolpate – with a single, large aperture in the form of elongate furrow. During maturation in highly watered stage they are spherical in shape. Before opening the anther, the pollen grains partly dehydrate, their aperture areas depress and become "closed" by pollen wall with exine. The convex surface of exine is poorly covered by sticky pollen coat and the pollen grains use special strategy for the containment of the crystals: the raphides are trapped during the pollen dehydration and lie along the concaved aperture. We suggest that the pollen grains with their own store of calcium have greater chances for germination.

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Calcium distribution in anthers of non-pollen type male-sterile rice (*Oryza sativa* L.) and its maintaining line during anther development

Cheng-Ye Liang[1], Kuai-Fei Xia[1,2], Xiu-Lin Ye[1]

[1]*South China Institute of Botany, The Chinese Academy of Sciences, Guangzhou 510650, China, e-mail: liangcy@scbg.ac.cn*
[2]*College of life Sciences, Sun Yat-sue University, Guangzhou 510275, China, e-mail: flyxia936@163.com*

Potassium antimonite technique was used to explore the changes of Ca^{2+} distribution in anthers of non-pollen type male-sterile rice and its maintaining lile during their development. Data shows few Ca^{2+} precipitate was detected in fertile anthers at the pollen mother cell stage and the meiosis pollen cell stage. At the uni-microspore and bi-cellular pollen stage, Ca^{2+} precipitates were dramatically increased and they are mainly located in the tapetal cells,and on the sexine of the pollen and the surface of the Ubish Bodies. Abundant Ca^{2+} precipitates on the surface of the Ubish Bodies might have close contact with the nutrient transportation from the anther wall to the pollen sac.The mature pollen was full of starch. No Ca^{2+} was found in the cytoplasm and intine.Vacuole membrane in the epidermis and cytomembrane of tapetum deposited a few Ca^{2+} precipitates.

There were many Ca^{2+} precipitates in sterile anthers at pollen mother cell and uni-microspore stage mainly depositing in the anther wall and microsporocyte,and especially in the middle layer and the tapetal cells. Abundant Ca^{2+} precipitates in anthers at the early development stage were probably related with the promotions of sterile genes and the regulation of the metabolism. Although the microsporocyte after Meiosis I became a dyad. The dyad then underwent Meiosis II to become tetrad. The tetrad microspores begin to round up, then the microspores gradually lose their cytoplasm as soon as. All microspores abort soon after.

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Identification of genes differentially expressed in tobacco egg and zygote suggests zygotic genome activation occurs soon after fertilization

Jue Ning, Mengxiang Sun

Key Laboratory of MOE for Plant Developmental Biology, College of Life Sciences, Wuhan University, Wuhan 430072, China, e-mail: mxsun@whu.edu.cn

The fertilization triggers plant egg cell stepping on the way to embryogenesis. To investigate molecular mechanisms on fertilization, we applied suppression subtractive hybridization (SSH) and mirror orientation selection (MOS) to compare gene expression profiles of isolated *Nicotiana tabacum* cv SR1 eggs and zygotes. SSH/MOS analysis resulted in the isolation of 155 expressed sequence tags (EST) that were differentially expressed, of which 146 have significant similarity to known genes and classed into 42 clusters. Semi-quantitative RT-PCR confirmed that 29 clusters were differentially expressed. The present study showed that transcription composition of zygote is distinct from that of egg cell, indicating that zygotic genome activation (ZGA) occurs soon after fertilization. According to the data, the maternal-to-zygotic transition probably happens before zygote first division in higher plants, which is much earlier than that in animals. The present ESTs provide a valuable resource for future research on the fertilization and early embryogenesis.

Characteristics of chosen species pollen of genus *Taraxacum* Wigg.

Jolanta Marciniuk

Department of Botany, University of Podlasie, ul. Prusa 12, 08–110 Siedlce, Poland, e-mail: jolam@ap.siedlce.pl

Taraxacum Wigg. is a genus which is represented by over 2.500 species in the whole world, grouped in 51 sections (Kirschner and Štepánek, 1997, 2004). The presence of 369 species from 13 sections has been observed so far in Poland (Głowacki, 2004). In terms of cytographics Poland is situated in the zone where apomictic polyploid forms are prevailing. Sexual diploids are represened in Poland just by three species known for their single occurrence, namely *Taraxacum bessarabicum* (section *Piesis), Taraxacum pieninicum* (section *Erythrocarpa)* and *Taraxacum erythrospermum (section Erythrosperma).* Although a decisive majority of apomictic species of the genus *Taraxacum* produces pollen, there are relatively few papers on the morphology and viability of pollen. In Poland this research was only conducted by Małecka (1964) on a low number of species, whereas the knowledge of the morphology and viability of pollen is very important both in taxonomic and evolutionary terms. It is also very interesting that in the case of *Taraxacum* there is also, as in the other polyploid complexes (Ehrendorfer, 1964; Dąbrowska, 1971), a clear correlation between the size of a pollen grain and the level of polyploidy of each individual species.

The following paper is aimed at describing the morphological diversity and viability of the pollen of the genus *Taraxacum* species occurring in Poland. The research was carried out upon five species of the section *Palustria* of the three polyploidy levels, namely *Taraxacum paucilobum* Hudziok (2n=24), *T. portentosum* Kirschner & Štepánek (2n=24), *T. trilobifolium* Hudziok (2n=32), *T. vindobonense* Soest (2n=32), *T. mendax* Kirschner & Štepánek (2n=40).

For each species the following research was conducted:

- karyological observations to check the number of chromosoms reported by Kirschnera and Štepánka (1998) under Polish conditions
- measurements of a pollen grain size through determining a maximum inner diameter of a grain (without an exine) in pole position; for each species a hundred grains, which were collected from closed buds straight before flourishing, were measured; prior to this, the buds were subject to maceration in 10% KOH; the measurements were conducted via the use of ocular micrometer, with a magnification of 16×40; an analysis of variation was done of the results obtained
- photos were taken with the use of SEM
- percentage of degenerated pollen was determined in the range of vision (in 10 replications)
- pollen viability was measured on the medium.

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Effect of abscissic acid on the morphology of tulip (*Tulipa* L.) somatic embryos

Małgorzata Maślanka, Anna Bach

Department of Ornamental Plants, Agricultural University, 54 29 Listopada Avenue 31–425 Cracow, Poland, e-mail: maslankam@ogr.ar.krakow.pl; robach@cyf-kr.edu.pl

An introduction of a new cultivar of tulip in production takes 25 years. The in vitro cultures make it possible to shorten this period by the processes of organogenesis and somatic embryogenesis (Gude and Dijkema, 1997). However, the currently developed methods in somatic embryogenesis frequently yield embryos of poor quality (Bach and Ptak, 2001).

Somatic embryos of tulip, cv. "Victor" at the torpedo stage, 5–10 mm in length, were used in the experiment. They were treated with abscisic acid at the concentration of 5 ppm for 4 or 2 weeks.

An enrichment of the medium with the growth regulator affected the weight and length of embryos depending on the length of the ABA treatment. The initial size of the observed embryos was not reduced. During the first weeks of the experiment the growth of ABA treated embryos was much slower in comparison with the control. After 30 weeks increases in weight were non-significant in the case of the 4-week ABA treatment while in embryos treated for 2 weeks increases were 3.5-times smaller. An increase in length measured after a 16-week in vitro culture was many times reduced by the ABA treatment and this was a significant difference in comparison with the control. After 30 weeks of in vitro culture the embryos treated with ABA for a longer period turned green in 65% while the embryos treated with ABA for a shorter period turned green in 0% since 29% of green embryos died-off after a 12-week culture.

Depending on the length of the ABA treatment the degree of malformation of the embryos was different. No malformations of ABA treated embryos were recorded during the first weeks of culturing. After the 30-week culture malformations were observed in 45% of embryos treated with ABA for 4 weeks and in 33% of these treated for 2 weeks. Control embryos showed a 95% malformation. After 6 months of in vitro culture malformed embryos began to form shoots while in the control the first shoots appeared already after 3 months.

After 30 weeks of growth the survival of embryos subjected to a 4-week ABA treatment was at the level of 32% and in the 2-week treatment at the level of 24% and it did not significantly differ from the survival of control embryos.

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Apomixis – preliminary lessons from wheat egg cells, *Poa pratensis*, *Hypericum perforatum* and *Arabidopsis thaliana*

F. Matzk[1], S. Prodanovic[1], A. Czihal[1], J. Tiedemann[1], D. Koszegi[1], F. Arzenton[3], F. Blattner[1], J. Kumlehn[1], L. Altschmied[1], I. Schubert[1], A. Johnston[2], U. Grossniklaus[2] and H. Bäumlein[1]

[1]*Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany*
[2]*University of Zurich, Switzerland*
[3]*University of Padua, Italy*

Poa pratensis has been used to study the inheritance of apomixis. The data support a model with five major genes required to control asexual seed formation: apospory initiator and preventer, parthenogenesis initiator and preventer and a megaspore development gene. These genes segregated independently. Differences in penetrance, expressivity and interactions of these genes are responsible for the highly variable capacity of apospory and parthenogenesis.

In *Hypericum* controlled crosses between an obligate sexual diploid and apomictic tetraploid plants allowed the isolation of a putative apospory-related AFLP-marker. A first characterisation of putative *Arabidopsis* homologs will be reported.

The Salmon system of wheat comprises three isogenic alloplasmic lines with either zygotic or autonomous embryo development. cDNA libraries from egg cells have been used for molecular subtraction and EST sequencing. Egg cell specific candidate genes have been isolated. Putative *Arabidopsis* homologs have been identified and will be discussed.

Sex chromatin and Y chromosomes in dioecious species, *Rumex acetosa* L.

Magdalena Mosiołek[1,3], Andrea Pedrosa-Harand[2], Dieter Schweizer[2,3], and Andrzej J. Joachimiak[1]

[1]*Department of Plant Cytology and Embriology, Institute of Botany, Jagiellonian University, Grodzka 52, 31–044 Kraków, Poland, e-mail: magda.mosiolek@op.pl*
[2]*Department of Chromosome Biology, University of Vienna, Dr. Bohr-Gasse 1/ Viehmarktgasse 2A, A–1030 Vienna, Austria*
[3]*GMI – Gregor Mendel Institute of Molecular Plant Biology, Dr. Ignaz Seipel-Platz 2, A–1010 Vienna, Austria*

The majority of plants have evolved various mechanisms to promote outcrossing, of which dioecy is the most effective, because in fully dioecious plants outbreeding is obligatory. The overall distribution of unisexuality thorough angiosperms suggests that dioecy has arisen independently and many times within different plant lineages. Only about 6% of angiosperm species are dioecious, and sorrel (*R. acetosa* L.) is one of the few dioecious species which have sex chromosomes. The sex chromosome system in sorrel is XX/XY_1Y_2.

Although deprived of the typical (C-banding positive) constitutive heterochromatin, sorrel Y chromosomes frequently form heteropycnotic bodies in some tissues. This interphase condensation (heterochromatinization) of Y chromosomes in *Rumex* is of special interests as it contrasts with the generally euchromatic nature of Y chromosomes in other plants possessing sex chromosomes. The mechanism of heterochromatinization of male sorrel sex chromosomes at interphase is totally unknown.

We analysed *Rumex* chromosomes and cell nuclei both in seedlings and in the roots cultured in vitro with a special reference to the male sex chromatin. We found that Y chromosomes formed large DAPI-positive chromatin bodies in root-meristem cells in vitro, but not in vivo. Using immunodetection (immunostaining with polyclonal antibodies against methylated H3 histone) we showed that Y-derived, DAPI-positive sex bodies in cultured roots were enriched with H3 histone, demethylated at lysine 4 and methylated at lysine 9. The observed histone-tail modifications in male sex bodies are generally the same as those recently discovered in the inactivated (facultatively heterochromatinized) human X chromosome (Boggs et al., 2002). This is the first report of H3 histone-tail methylation in plant sex chromatin. Additionally, we showed that mitotic (prophase and metaphase) Y chromosomes of *Rumex* are the mosaics of AT-rich (probably genetically degenerated, at least in some extent) and AT-poor (probably not degenerated) chromatin segments. Our results do not fully correspond to the most recent view on *Rumex* Y chromosomes as globally constitutively heterochromatic (Vyskot and Hobza, 2004).

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Embryo and endosperm in *Rudbeckia bicolor* Nutt. – resumption of study

Krystyna Musiał[1], Maria Kościńska-Pająk[1], Elwira Śliwińska[2], Tomasz Ilnicki[1]

[1]*Department of Plant Cytology and Embryology, Jagiellonian University, ul. Grodzka 52, 31–044 Cracow, Poland, e-mail: k.musial@iphils.uj.edu.pl*
[2]*Department of Genetics and Plant Breeding, University of Technology and Agriculture, 7 Kaliskiego str., 85–789 Bydgoszcz, Poland*

In *Rudbeckia bicolor* Nutt. the occurrence of hemigamy was described in meiotic embryo sac (ES) of *Fritillaria* type (Kościńska 1980; Kościńska-Pająk 1985).

The present embryological observations are beginning of more advanced cyto-embriological studies on this interesting species.

In total, 1070 ovules were examined. The mature seven-nucleate ESs were present in 669 ovules, in the remaining different developmental stages of embryo (from two-celled to heart-shaped embryos) and endosperm were observed. Structure of mature ES was in accordance with previously described scheme. Some modifications of an egg apparatus organization or antipodal apparatus typical for *Rudbeckia* were also stated (e.g. two egg cells, two egg cell-like antipodals). In many ovules a trace of pollen tube was visible in the micropyle, also inside an egg cell a sperm was often noticed, however either the fusion of those nuclei or the fusion of sperm nucleus and secondary nucleus were not observed. For the first time polyembryony in *R. bicolor* was detected; two globular embryos at micropylar pole of ES were observed.

Observations on the embryo and endosperm development showed that retarded or completely stopped development of endosperm was a frequent phenomenon. Undivided secondary nucleus was found in 33.7% among104 ESs containing proembryos, in the remaining ESs cellular endosperm occurred. At the stage of globular embryo in 19.6% out of 245 investigated ESs endosperm development was stopped. Some embryos developing without endosperm were irregular in shape and had abnormal suspensor. In such ESs proliferation of endothelium was often noticed. In the present material no distinct differences in size of nuclei of embryo cells were found, however, binucleate cells and cells with irregular nuclei were observed. The cytological analysis confirmed the karyological diversity in young seedlings; euploid and aneuploid chromosome numbers (2n = 38, 48, 64, 66, 68, 76) occurred in root tips. However, stated previously by Kościńska-Pająk (1985) metaphase plates with haploid chromosome number were not found.

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Hybrids of *Capsicum frutescens* L. × *Capsicum annuum* L. as a material for androgenic haploids production

Aleksandra Niklas-Nowak, Paweł Nowaczyk

Department of Genetics and Plant Breeding, University of Technology and Agriculture, 7 Kaliskiego str., 85–791 Bydgoszcz, Poland, e-mail: niklas@mail.atr.bydgoszcz.pl

This communication will focus on the presentation of successful protocol for the anthers culture of the five pepper lines selected from diploid interspecific *C. frutescens* L. × *C. annuum* L. hybrids. Tested lines reveal differentiation of usable features: fruit size, plant habit and yielding earliness. Anthers were isolated from young flower buds, then displayed on agar medium and cultured according to the method of Dumas de Vaulx et al. (1981) with slight modifications. Genotype and culture conditions were factors which had the most prominent effect on embryogenic potential of anther cultures. We observed embryogenesis and callus induction with a frequency ranging from 0 to 1,3%, depending on genotype. Callus formation was achieved for 4.100/I genotype. Regeneration into mature plants was gained for 34/2/33 genotype via direct embryogenesis.

The ploidy level of the obtained plants was carried out by a Partec Ploidy Analyzer (Partec CCA, Münster, Germany). Plant material to analysis was preparated according to Galbraith's et al. method (1983). Flow cytometric analysis of *in vitro* plantlets revealed, that regenerants were haploids, sometimes diploids and mixoploids. Interspecyfic hybrid of 34/2/33 seems to be the most valuable source of material for the future research on optimization conditions of androgenic plants production. Whereas doubled haploid (DH), as a result of its diploidisation, should be a good initial material for the genetic improvement of the *Capsicum annuum* L.

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In vitro anther culture of *Capsicum frutescens* L. red- and yellow-fruited forms

Paweł Nowaczyk, Anna Kisiała, Dorota Olszewska

Department of Genetics and Plant Breeding, University of Technology and Agriculture, 6 Bernardyńska str., 85–029 Bydgoszcz, Poland, e-mail: warz@atr.bydgoszcz.pl

The aim of this research was to make a preliminary determination of the effectiveness of androgenesis of *Capsicum frutescens* L. This species is a source of a lot of interesting features and therefore should be wider included in pepper breeding programmes, what gives the opportunity of greater genetic variability. Anther culture of red- and yellow-fruited forms of the species, each bred by the researchers on their own, was performed following the method described by Dumas et al. (1981) for *C. annuum* L. with some modifications. Anthers were laid onto CP induction medium (0.01 mg/l 2.4-D and 0.01 mg/l kinetin), with the addition of 0.5 g/l of activated carbon and 5 mg/l of silver nitrate (Nervo et al., 1994), solidified with 8 g/l of agar. Petri dishes were incubated in the darkness at +35˚C for 8 days. Next they were cultured at +25˚C in the conditions of a 12-hour photoperiod. On the 14th day of culture anthers were replaced onto R1 regeneration medium (0.1 mg/l kinetin). Obtained embryos were transferred onto V3 hormone-free medium and well growing plants were planted in greenhouses. A total number of 25 anther-derived mature plants were obtained from 610 in vitro cultured anthers of both genotypes. The ploidy of regenerated plants was determined by flow cytometry. Among androgenic regenerants 13 were haploid, 10 diploid and 2 were mixoploid. Although the effectiveness of androgenesis for both *C. frutescens* L. genotypes was relatively low and it did not exceed 5%, the results obtained in this test are a proof of the existing possibilities of the use of androgenesis in breeding work on *C. frutescens* L. After the method has been improved and greater effectiveness achieved, a wide application of androgenesis in breeding of this species may be expected.

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Polyembrionic seeds of tomato (*Lycopersicon esculentum* Mill.) as a source of homozygous diploids?

Paweł Nowaczyk, Lubosława Nowaczyk

Department of Genetics and Plant Breeding, University of Technology and Agriculture, 6 Bernardyńska str., 85–029 Bydgoszcz, Poland, e-mail: warz@atr.bydgoszcz.pl

The frequency of occurrence of additional embryos in many species of plants varies and these are most often diploid forms. Their main sources are dividing proembryos. However, the development of additional diploid embryos as a result of spontaneous diploidisation of haploid elements of an embryo sac and the subsequent embryogenesis cannot be excluded. The aim of the undertaken research was an attempt to verify such an assumption on the basis of evaluation of F_3 generation plants of tomato. The generations constituted offspring of two diploid twin pairs (F_2) representing *nor nor* genotype. As far as its phenotype is concerned, it means no ripening of fruits and the fruits maintaining their firmness for a few months. Mutants of *nor* type are used as primary elements of hybrids with prolonged life of fruits.

The seeds being the source of twin pairs originated from commercial variety of fruits – 'Celina F_1' from Israel. The frequency of occurrence of polyembryonic forms was 0.035% in 42,000 of sprouting seeds. The subject of research was two twin pairs marked with 4a, 4b and 8a, 8b symbols. The plants of the first of the above-mentioned pairs were characterised by similar yield of fruits (2.730 g and 2.4109 g), and the other two considerably varies in this respect (2.687 g and 780 g). Next year an evaluation of phenotype equalization in offspring of each of the four plants was carried out. The observations proved a considerable homogeneity of 4a and 4b offspring within each of them. The value of variation coefficients for the tested fruits (mean weight, pericarp wall thickness, weight of jelly and its share in fruits, the contents of substances soluble in water and the contents of dry matter) was much lower than that evaluated for 8a and 8b offspring.

With an attempt to explain the phenotype equalization of offspring of F_3 generation twin plants, one can point to the spontaneous diploidisation of an embryo sac haploid element with the subsequent division of a proembryo into two independent organisms. This seems to be a logical explanation of the homozygous state of the plants and their offspring in question. The reliability of the above suggestion is confirmed by the presence of androgenic diploids in *in vitro* anther cultures in some species of plants (Kamiński et al., 2005).

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Androgenesis in soft – flesh *Capsicum frutescens* L. genotypes

Dorota Olszewska, Iwona Jędrzejczyk, Pawel Nowaczyk

Department of Genetics and Plant Breeding, University of Technology and Agriculture, 7 Kaliskiego str., 85–791 Bydgoszcz, Poland, e-mail: dorota@atr.bydgoszcz.pl

The aim of the research was to determine capability for forming haploid embryos in anther cultures of *Capsicum frutescens* L. characterized by soft flesh fruits as well as analysis of morphological features and the level of ploidy of the obtained callus tissue. The culture of plants was conducted under unheated foil tent. Agrotechnical treatment typical for *C. annuum* L was applied. Flower buds with 1:1 ratio of corolla lobes to calyx sepals were collected in two periods: from 1st to 15th April and from 1st to 15th September. In the summer period 150 flower buds (about 700 anthers) and in the winter period 40 flower buds (about 200 anthers) were laid. Anther cultures were cultured according to the method devised by Dumas de Vaulx et al. (1981). A degree of ploidy was evaluated with the use of flow cytometry. Plant material for the flow cytometry was prepared according to the instructions given by Galbraith et al. (1983) with slight modifications. The analysis was carried out with the use of Partec CCA cytometer.

In the summer period no embryos of androgenic origin were obtained. The rizogenesis was occasionally observed on the exposed anthers. In the autumn period few of the exposed anthers initiated the process of embryo formation. It has been ascertained that capability of embryo formation in anther cultures was 3% for the evaluated genotype. The cytometric evaluation has shown the presence of 5 haploid plants and one diploid plant. The research has also shown embryos which died in the early stages of development.

The analysis of morphological characteristic of callus tissue was carried out 4 months after the beginning of the experiment. In the summer and autumn periods the capabilities of callus tissue formation were 10% and 8% respectively. A similar number of yellow, orange and brown calluses were obtained. Most of them did not exceed 5 mm in diameter. The cytometric measurement did not show any presence of haploid nuclei in any of the tested samples. Among yellow and orange calluses there were cells with ploidy ranging form 2x to 32x. Brown callus was characterized by a larges share of damaged cells, which is the proof of the tissue degradation.

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Different pathways of embryo sac development in *Galinsoga parviflora* Cav.

Jacek Pietrusiewicz*, Marcin Domaciuk, Józef Bednara

*Department of Plant Anatomy and Cytology, Maria Curie-Skłodowska University, Akademicka 19, 20–033 Lublin, Poland, *e-mail: jacekp@biotop.umcs.lublin.pl*

Galinsoga parviflora is an annual weed which produces a head inflorescence consisting of a dozen-or-so flowers that develop asynchronously. It blooms from early spring until late autumn and most often produces seeds in the sexual way, though sometimes reproductive deviations occur as a means of adapting to difficult conditions.

Megasporogenesis and the development of the embryo sac proceed according to the monosporic Polygonum type. Oblong megasporocytes develop in tenuinucellate ovules which, after meiosis, form a linear tetrad of megaspores. From one of them, the chalazal megaspore, there develops an eight-nucleate embryo sac capable of receiving the pollen tube, fertilization, and developing the embryo and endosperm. Exceptionally, other ways of development are observed: 1-with meiosis and development of two active megaspores, 2-with omission of meiosis and formation of a diplosporic embryo sac.

In monosporic types of embryo sac development, only one megaspore out of the four created after meiosis is active and it lies on the chalazal or micropylar side of the tetrad. The positioning of the active megaspore and degenerating megaspores depends on the pattern of arrangement of callose in cell walls of megasporocytes and tetrads. Callose is synthesised in prophase meiocytes as a continuous layer on their side walls. At the end of the prophase, callose disappears at the chalazal pole in the Polygonum type or at the micropylar pole in the Oenothera type. It also appears in the transverse walls of megaspores formed during meiosis. Three megaspores, completely surrounded with callose, degenerate and die, whereas one, free from the callose barrier, becomes an active megaspore and develops into the embryo sac. The development of two active megaspores in *Galinsoga* is a result of the atypical pattern of callose synthesis. Namely, there is a complete lack of it in side walls of meiocytes, which makes isolation and later elimination of three megaspores impossible. In these conditions, more than one megaspore has a chance to compete and assume the role of the active megaspore. The production of diplosporic embryo sacs is, in turn, a manifestation of economical development. After three mitotic divisions, an embryo sac develops from the megasporocyte and, additionally, it has a form which does not require fertilization at seed formation.

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Reproductive processes in the yellow zinc violet [*Viola lutea* Huds. ssp. *calaminaria* (Ging.) Nauenb.] – a metal-tolerant taxon

Maria Pilarska, Elżbieta Kuta

Department of Plant Cytology and Embryology, Jagiellonian University, 52 Grodzka str., 31–044 Cracow, Poland, e-mail: e.kuta@iphils.uj.edu.pl

The yellow zinc violet [*Viola lutea* Huds. ssp. *calaminaria* (Ging) Nauenb.= *V. calaminaria* (DC.) Lej.], a representative of the *Melanium* Ging. section (pansies) of the *Viola* L. genus, exclusively thrives on soils reach in heavy metals (Pb, Zn). The taxon has a very limited area of distribution, occurring on abandoned zinc mines in Belgium, Germany and Holland (Ernst 1968). The yellow zinc violet is closely related to *V. lutea* and to *V. lutea* ssp. *westfalica* (blue zinc violet). It was suggested that both zinc violets are descendants of *V. lutea* and evolved as ecotypes on soils with high concentration of heavy metals (Hildebrandt et al. in preparation). As metal tolerant taxon, yellow zinc violet is expected to be well adapted to harsh conditions opposite to non-metal tolerant taxa growing on sites contaminated with heavy metals. In non-metal tolerant species polluted environment negatively influences reproductive processes leading to reduced pollen and seed fertility (see Czapik et al., 2002).

Reproductive processes including micro- and megagametophyte development, embryo and endosperm formation were analyzed in specimens from two natural sites – Prayon (Belgium) and Breinigerberg (Germany). In majority of examined plants sexual reproduction was not disturbed. Highly viable pollen were formed after normal microsporogenesis analyzed in 259 cells. Pollen viability estimated in acetocarmine and Alexander's stains reached 95% in plants from Breinigerberg and 88% in specimens from Prayon. For the yellow zinc violet, as representative of pansies, pollen heteromorphism is typical with 3-, 4-, 5- and 6 apertured pollen grains formed in one anther. It was suggested that in case of pollen heteromorphism, lower frequency of pollen with high aperture numbers can be recognized a adaptation to unfavorable conditions. Pollen from both sites had in majority 4-apertured grains (over 80%). In the female gametophyte development some abnormalities concerned tetrad stage. In the yellow zinc violet, as in other *Viola* species, embryo sac develops according to the Polygonum type from chalazal megaspore (three other megaspores degenerate). In examined material the process of megaspores degeneration was delayed and in majority of tetrads more than one megaspore continued development that could be considered a adaptation feature.

Analysis of mature capsules and seeds showed that in plants from both sites seeds with normally developed embryo and endosperm represented ~40%. Embryos viability estimated in tetrazolium histochemical, topographic test was even lower reaching ~19% and corresponding to seed germination test (18%). Reduced viability might be the consequence of the growth in the deleterious heavy metal soil. Nonetheless, the frequency of fertile seeds might be sufficient for yellow zinc violet to survive. Contrary to blue zinc violet from Blankenrode, yellow zinc violet reproduce normally that indicates its metal-tolerant status.

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Special placenta structures of *Utricularia sandersonii* Oliver

Bartosz Płachno[1], Piotr Świątek[2], Andrzej Jankun[1]

[1]*Department of Plant Cytology and Embryology, Jagiellonian University, 52 Grodzka st., 31–044 Cracow, Poland, e-mail: bartek78pl@ poczta.onet.pl; jankun@iphils.uj.edu.pl*
[2]*Department of Animal Histology and Embryology, University of Silesia, 9 Bankowa st., 40–007 Katowice, Poland, e-mail: swiatek@us.edu.pl*

Utricularia sandersonii forms a stalked c. spherical placenta, which is covered with numerous, anatropous, unitegmic and tenuinucellate ovules. The placenta consists mainly with large, highly vacuolated parenchyma cells. Within the parenchyma nuclei characteristic crystal structures are visible. The thin cell walls and plastids with starch are a regular feature in the cytoplasm of these cells. During the development of the ovule, a group of cells differentiates near its base, which forms the placental nutritive tissue. In contrast to those of the placental parenchyma, these cells have prominent thick cell walls, their weak vacuolated cytoplasm is rich in organelles such as mitochondria, Golgi bodies, endoplasmic reticulum, free ribosomes and lipid droplets. The micropylar part of the mature embryo sac has direct contact with placental nutritive tissue. We suggest that placental nutritive tissue plays a key role in transfer nutrients to the megagametophyte and later in the embryo. We can not neglect an idea that the placental nutritive tissue also takes part in pollen tubes attraction.

In the open flower, epidermis of the placenta is composed of secretory papilla-shaped cells which form the transmitting tract (obturator) for pollen tubes. The RER and Golgi bodies are regular feature in the cytoplasm of these cells. Numerous vesicles of various sizes are found near the Golgi bodies and in the cytoplasm close to the plasmalemma. Multivesicular bodies and small vacuoles also occur in the cytoplasm. Microtubules are found near the lateral cell wall. The nucleus occupies the cell center and is surrounded by large mitochondria and plastids.

A technique to study polarity issues during early steps of zygote development in wheat (*Triticum aestivum* L.)

Zsolt Pònya, Wei Wang, Mauro Cresti

Università di Siena Dipartimento di Scienze Ambientali "G. Sarfatti", Via P. A. Mattioli 4, 53100 Siena, Italy, e-mail: ponyazs@unisi.it

The microenvironment of the maternal tissues in which the developing zygote is enclosed during its development may play a crucial role in the establishment and maintenance of cell polarity thought to be a prerequisite for normal zygotic development. Although the influence on early zygote development of maternal factors is implicated in the mechanisms that control morphogenesis in the course of early embryogenesis, how the early steps of normal zygotic morphogenesis unfolds in angiosperms has not been rigorously investigated. Capitalizing on the elaboration of a reproducible "re-implantation" technique through which *in vitro* produced zygotes could be "re-implanted" in the maternal environment, the development of *in vivo* versus *in vitro* produced and "re-implanted" zygotes could be monitored and compared.

Raising an antibody, recognizing a cyclin-dependent protein kinase, cdc–2 in wheat (*Triticum aestivum* L.), which had previously been shown to drive the cell cycle machinery in several well-studied systems, and the exploitation of our "re-implantation" technique made it possible to assay the commitment to a polar axis fixation of the fertilised wheat egg cell. This approach combined with inhibition studies aimed at shedding light on how cell cycle dependent protein kinases may govern morphogenesis allowed for a hypothesis concerning how the fixation of the developmental axis during zygotic morphogenesis may occur *in planta* with particular regard to cell cycle progression.

Furthermore, a system in which somatic (leaf) protoplasts isolated from wheat and "implanted" in ovules was used to assess the ability of the ovular tissues to influence the polarity of cells of "foreign origine". Using a micro chamber insuring auxin-gradient the potential role of auxin in cell elongation was addressed in the presented study.

First approaches to the characterization of one h-type thiorredoxin possibly involved in Olive (*Olea europaea* L.) tree pollen-pistil interaction

Amada Pulido, José Angel Traverso, Ana Chueca, María Isabel Rodríguez-Garíca, Adela Olmedilla

Department of Plant Biochemistry, Cellular and Molecular Biology, Estación Experimental del Zaidín, Profesor Albareda 1, 18008 Granada, Spain, e-mail: adela.olmedilla@eez.csic.es

Plant sexual reproduction depends on the highly specific interaction between pollen and pistil (Knox, 1984). Cell-cell interactions between pollen and the different femenine tissues occur with the interchange of molecules such as ligands, receptors and/or nutrients localized in the extracellular matrix or in the membrans of both tissues. These interactions determine the inter and intraspecific incompatibility. Currently the signal transduction is one of the most studied aspects of the incompatibility. h-type thiorredoxins have been described to be involved in the signal cascades of incompatibility in both mono and dicots. Briefly, the expression of some thiorredoxins h are strongly related to self-incompatibility both in plants which display gametophytic self-incompability (GSI) (*Phalaris coerulescens, Poaceae*) (Li et al., 1994) and in plants which display sporophytic self-incompability (SSI) (*Brassica rapa, Brassicaceae*) (Bower et al., 1996, Cabrillac et al. 2001).

Although olive (Olea europaea L.) tree has been traditionally considered as self-compatible (Fernández-BolaÑos and Frías, 1969) recent data show a strong controversia and some authors have even considered it to have a high level of self-incompatibility (Cuevas and Polito, 1997). In our study of the olive pollen-pistil interactions we have cloned a partial sequence of an h-type thiorredoxin by RT-PCR. This h-type thiorredoxin is expressed in different stages of the flower development in both the anthers and the pistils and in the pistils in the progamic phase of the pollination. However this h-type thiorredoxin is not expressed in later stages.

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Role of transcription factors in early male gametophyte development of *Arabidopsis thaliana*

David Reňák[1,2], Nikoleta Dupľáková[1,3], David Honys[1,3]

[1]*Laboratory of Pollen Biology, Institute of Experimental Botany ASCR, Rozvojová 135, 165 02, Prague 6, Czech Republic, e-mail: renak@ueb.cas.cz*
[2]*Department of Plant Physiology and Anatomy, Faculty of Biological Sciences, University of South Bohemia, Branišovská 31, 370 05 Česká Budéjovice, Czech Republic*
[3]*Department of Plant Physiology, Faculty of Sciences, Charles University, Viničná 5, 128 44, Prague 2, Czech Republic*

The haploid male gametophyte generation of flowering plants consists of two or three celled pollen grains and pollen tubes as an evolutionary result of structural and functional reductions but performs a vital role in the plant life cycle. Hence pollen ontogeny provides an attractive model for the study of fundamental developmental processes like cell growth and division, cellular differentiation and intercellular communication. Despite a long-term research on the field of plant sexual reproduction, the developmental changes and regulatory mechanisms during pollen grain formation have not been properly described yet.

Our research aims to characterize the role of transcription factors (TF) in male gametophyte development of *Arabidopsis thaliana* with special focus on early developmental stages such as uninucleate microspores and bicellular pollen grains. Exploiting microarray technologies (Honys and Twell, 2003; 2004) followed by careful bioinformatic analyses, we selected about 30 genes encoding putative TFs expressed specifically during the developmental stage considered. To prove the importance of selected TFs in development, we analysed respective Garlic and SAIL T-DNA insertion mutant lines. After PCR verification of all insertions, we performed the phenotype screening for aborted or structurally abnormal pollen grains by both light and UV microscopy. The second experimental approach comprises the search for segregation ratio distortion demonstrating the functional significance of examined gene without visible phenotype. Selected positive mutant lines with relevant impact on developmental changes will be genetically characterised to reveal the transmissibility as well as the recessive or dominant character of the mutation. Moreover, the influence of TF genes on downstream regulation will be examined by comparative transcriptome analysis of both TF mutant lines and wild type plants in order to determine their role in gametophytic gene regulatory networks.

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Embryo development in *Cardaminopsis arenosa* (L.) Scop. from polluted sites

Monika Siwek, Romana Izmaiłow

Department of Plant Cytology and Embryology, Jagiellonian University, Grodzka 52, 31–044 Kraków, Poland, e-mail: izmailow@iphils.uj.edu.pl

The studies were performed in order to determine the effect of pollutants and the environmental stress conditions on embryogenesis in *Cardaminopsis arenosa* (L.) Scop. The examined plants originated from two sites polluted with Zn and Pb: (1) old spoil heap in Bolesław, (2) vicinity of ZGH "Bolesław"; the control plants grew in Kostrze near Kraków on uncontaminated soil. A total 1300 ovules from both polluted sites and 550 ones from the control plants were studied. In comparison with the control material, detailed embryological analysis showed that the frequency of ovules with typical and viable embryo sac, typical embryo and endosperm development was reduced in specimens from both polluted sites. Necrotic processes were detected in embryo sacs, in zygotes and young proembryos. In some embryos at globular stage necrotic cells have been detected in hypophysis and in suspensor; in more advanced stage of embryogenesis necrotic regions were visible in radicle and cotyledons. Developmental disturbances and necrotic processes resulted in defects of embryonal organs or even they eliminated a part (~30%) of ovules from further development and reduced fertility of the plants colonizing the polluted sites.

Concentration of metals in soil and in plant organs (roots, leaves, flowers, seeds) were measured by AAS. The heavy metals location in seeds (in necrotic regions of embryos, in endosperm and testa) was determined by scanning microscopy. Analysis showed metals in generative structures but in smaller amounts in comparison with vegetative organs.

Interspecific hybridization between *Cucumis sativus* and wild *Cucumis* species through embryo culture

Dagmar Skálová, Nikol Gasmanová, Aleš Lebeda, Božena Navrátilová

Department of Botany, Fakulty of Science, Palackého University, Šlechtitelú 11, Olomouc – Holice, 783 71, Czech Republic, e-mail: skalova@prfholnt.upol.cz; lebeda@prfholnt.upol.cz

Resistance to several cucumber (*Cucumis sativus*) pathogens have been found in some wild *Cucumis* species (Leppik, 1966). For example *C. melo* (line MR 1) and some other accessions has resistance to cucumber downy mildew (*Pseudoperonospora cubensis*) (Lebeda, 1999). One possibility to overcome crossing barriers between *C. sativus* and wild *Cucumis* species is using embryo rescue cultures (Ondřej et al., 2001; Skálová et al., 2004).

Selected genotypes of *Cucumis* species were used for the experiments (*C. sativus* (09H390768), *C. anguria* (PI 24989), *C. metuliferus* (PI292190), *C. zeyheri* (09H410196), *C. melo* (09H401114 and 09H400600). The plant material originated from Plant Introduction Station, Iowa State University, Ames, Iowa (USA) and the vegetable germplasm collection of the Department of Gene Bank, Workplace Olomouc (Czech Republic).

The female flower of *C. sativus* were hand-cross pollinated by the other wild *Cucumis* species and the dissected seeds (or embroys) were cultivated on 5 types of media (OK-, ON-, CW-, GP- and GA-medium; MS- media supplemented with ascorbic acid, caseinhydrolysate, coconut water, giberelic acid). Recently there were regenerated only some calli of hybrid between *C. sativus* × *C. melo*, however some morpohological changes of the seeds of hybrid *C. sativus* × *C. metuliferus* (the both on GA-medium) were observed.

There are some polyploidization methods based on colchicin application. These methods are recently used for producing of potential tetraploids of *C. sativus*. They will be cross-pollinated with the wild *Cucumis* species.

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Genome imprinting, development of grains and seed-setting in apomictic maize × gamagrass hybrids

Victor Sokolov, Tatjana Tarakanova, Irina Belova, Elvira Abdyrahmanova

Plant Apomixis Laboratory, Institute of Cytology & Genetics, Novosibirsk 630090, Lavrentjeva 10, Russia, e-mail: sokolov@bionet.nsc.ru

Interploid cereal crosses lead to seed abortion. The research carried out in maize showed the thing that dosage effects between products of the imprinted and still unknown genes expressed during grain development are the reason for this phenomenon (Kermicle, 1970; 1978; Grimanelli et al., 2005). Maternal : paternal genome ratio in the endosperm must be due 2:1, respectively, for viable seed formation (Lin, 1982; 1984). In our investigations with pseudogamic apomictic maize × gamagrass hybrids, maize maternal : paternal genome ratio (in the presence of 1–18 gamagrass chromosomes in maize) varied from 2:1 to 12:1 and, under these, viable seeds were obtained. Based on the experiments, it was hypothesized as for suppression of imprinting in the presence of even single gamagrass chromosomes. Dependence of male signal intensity on the ploidy of the used pollinator and its genotype, also their role in viable grain formation are discussed.

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More about the sources of polyembryony

M.P. Solntseva

Organism and environment, Görlitzer Str. 20, 90473, Nürnberg, Germany, e-mail: solntseva@freenet.de

Polyembryony means a formation of two or more embryos in the same seed. Polyembryony can be found in many families of Angiospermae but its origin may be different. In some cases two (rarely three) nucelli are observed in one ovule. Each nucellus develops its own embryo sac (ES). In other cases two or more ESs develop in one nucellus, and each of them produces an embryo. These cases are examples of false polyembryony. In cases of true polyembryony, the embryos develop from various cells of the ES or from the division of the embryo proper or its suspensor (cleavage embryony). Apart from the egg cell, the cells that give an embryo are commonly synergids, seldom are they antipodals. The phenomenon is named apogamety. Some embryos develop from vegetative cells of nucellus or integument, and while growing they always penetrate the embryo sac. The phenomenon is known as adventive embryony.

To make a distinction between adventive embryos and embryos formed in multiple embryo sacs of the same nucellus it is necessary to trace the stages of ES development up to maturity. Usually multiple ESs develop asynchronously and the earlier developed ones are bigger and occupy the central part of the nucellus. The others are placed laterally, their cells are smaller and it is possible (using proper staining) to notice the cell walls of central cells of each ES. All ESs may be fertilized and develop embryos. In such cases the embryos are situated in various parts of ES. Cell walls of ESs disintegrate at a later phase and their endosperms are shared by all embryos. Initials of nucellar adventive embryos appear within the nucellus tissue. They are usually positioned in apical parts of nucellus. In some plants a new meristematic tissue develops close to the micropylar part of ES. Some of its cells degenerate, the adjacent ones become rounded; they divide and form the embryos.

The presentation gives the original data for all types of polyembryony and the list of species with synergid apogamety. The latter phenomenon has been noted till now in 71 families of Angiosperms (Monocotyledons – 13 and Dicotyledons – 58) and in 71 and 140 species of Mono- and Dicotyledons correspondingly.

Ole e 10, a 1,3-β-glucanase from olive pollen, co-localizes to callose during pollen tube growth

Cynthia G. Suárez[1], Patricia Barral[2], Juan de Dios Alché[1], Rosalía Rodríguez[2], María Isabel Rodríguez-García[1*]

[1]*Department of Biochemistry, Cell and Molecular Biology of Plants, Estación Experimental del Zaidn. CSIC, Profesor Albareda 1, Granada, 18008, Spain*
[2]*Department of Biochemistry and Molecular Biology I, Complutense University, Madrid, Spain*
[*]*e-mail: mariaisabel.rodriguez@eez.csic.es*

Callose is one of the major polysaccharide components present in the walls of pollen tubes (Nakamura et al., 1984; Ferguson et al., 1998). In the olive pollen tube, this β-glucane is present in the wall as well as in the callose plugs. These structures are formed at intervals behind the cytoplasm of the tube as it grows.

Ole e 10 is a novel plant protein displaying homology with non-catalytic domains from plant 1,3-β-glucanases (Barral et al., 2004). In order to determine putative relationships between this protein and its better-known substrate (callose) during pollen tube growth, we have studied the cellular location of the protein using immunofluorescence techniques. The localization of callose materials was simultaneously accomplished by using sirofluor as a fluorofore in a Confocal Laser Scanning Microscope (CLSM). Immunolocation of Ole e 10 in germinating pollen indicated that both components (Ole e 10 and callose) co-localize in the growing pollen tube, suggesting a role for the protein in the metabolism of callose and in pollen tube wall reformation during pollen germination.

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Behaviour of stigma before and after pollination in olive tree (*Olea europaea* L.)

Cynthia Suárez[1], Irene Serrano[1], Hava F. Rapoport[2], Adela Olmedilla[1], Maria Isabel Rodríguez-García[1*]

[1]*Department of Plant Biochemistry, Cellular and Molecular Biology, Estación Experimental del Zaidín, Profesor Albareda 1, 18008 Granada, Spain*
[2]*Instituto de Agricultura Sostenible. Finca Alameda del Obispo, Apdo 4084, 14080 Córdoba, Espana*
[*]*e-mail: mariaisabel.rodriguez@eez.csic.es*

Pollen – pistil recognition and interaction is a major step in the complex series of events leading to fertilization. The olive tree, *Olea europaea* L., is generally wind pollinated and has a wide range of self-compatibility, partial incompatibility and incompatibility, according to cultivar. The specific reports of compatibility relationships in this species, however, are highly variable and even contradictory, with differences between locations, types of tests and authors (Cuevas and Polito, 1997; Moutier, 2002; Wu et al., 2002)

In addition to the genetic factors implied in pollen compatibility, the pistil plays a determinant role during pollen-pistil recognition. Nevertheless, in the olive little is known with regards to pistilar structures and their behaviour during the progamic phase. In the context of a project about pollen-pistil interaction in the olive tree we have started to study the morphology and changes observed in the olive stigma before and after the arrival of pollen, with special attention to the starch and lipid content.

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Immunolocalization of lipoxygenase in the anther of *Gagea lutea* (L.) Ker.-Gaw.

Ewa Szczuka[1], Ewa Skórzyńska-Polit[2], Bożena Pawlikowska-Pawlęga[3], Jolanta Sobieska[4], Antoni Gawron[3]

[1]*Department of Plant Anatomy and Cytology, Maria Curie-Skłodowska University, Akademicka 19, 20–033 Lublin, Poland, e-mail: eszczuka@biotop.umcs.lublin.pl*
[2]*Department of Plant Physiology, Maria Curie-Skłodowska University, Akademicka 19, 20–033 Lublin, Poland*
[3]*Department of Comparative Anatomy and Anthropology, Maria Curie-Skłodowska University, Akademicka 19, 20–033 Lublin, Poland*
[4]*Scientific Department of Biology and Horticulture, University College of Arts and Natural Science in Sandomierz, Krakowska 26, 27–600 Sandomierz, Poland*

Lipoxygenase (LOX) occurrence was revealed in different parts and types of the anther cells of *Gagea lutea* using the immunogold labelling method. LOX was found in the cytoplasm and close to ER elements in epidermal and endothecial cells of the anther; moreover, the enzyme was observed close to the cell walls of the latter. The positive immunoreaction to LOX was less intensive in the middle layers and in the loculus of the anther, where single immunogold particles were concentrated at the cell wall edges of these layers and in the area of protoplast masses, in vacuoles, close to mitochondria, inside plastids and in the liquid of the anther cavity. The enzyme occurred in the cytoplasm and around ER elements of the vegetative cell of pollen grains as well as in the exine layer, particularly in the places of connections between both the outer and inner exine layers.

LOX (EC 1.13.11.12) catalyses the oxygenation of the long chain of fatty acids containing *cis, cis*-pentadiene structure to hydroperoxides. The enzyme is involved in a number of important processes in plant cells. Some compounds of the LOX pathway can induce the programmed cell death or show antifungal activity. Some of the products of hydrperoxide lyase pathway (branch of LOX pathway) such as C6-volatiles (aldehydes and alcohols) are attractors, odours, show antimicrobial activity, or play role in plant signaling. Many pollen grains produce volatile derivates in their pollen coats, which act as either attractant or deterrents to insects and may also play a role in defence against microbiological attack. Some volatiles, like xylene, limonene and hectadecanone are to be released by various types of pollen (Porta and Rocha-Sosa, 2002). Due to these different functions, the presence and localization of LOX in the different anther cells is correlated with functioning of particular anther layers.

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Tendency towards facultative apomixis in breeding lines of sugar beet (*Beta vulgaris* L., *Chenopodiaceae*)

Teresa Szkutnik

Kutnowska Hodowla Buraka Cukrowego Sp. z o. o., Straszków, 62–650 Kłodawa, e-mail: t.szkutnik@khbc.pl

Studies on occurrence of apomictic processes in sugar beet was carried on breeding materials of Kutno Sugar Beet Breeding Station. Male sterile and fertile plants were investigated in controlled conditions of isolation and pollination. Ploidy degree of adult plants and of embryos taken from rape seeds were counted with flow cytometer of the firm Partec. Allelic composition of maternal plants and their progeny in five izozyme loci (PGI, PGM, MDH, IDH, GDH) was determined by means of electrophoresis. The developmental cycle of seeds was examined under light microscope in paraffin slides. The results of ploidy investigations and genetic segregation pointed to the occurrence of facultative apomixis in some plants. Living plants were obtained from seeds in which embryos developed in the process of reduced parthenogenesis with participation of pseudogamous endosperm. Seeds with pathenogenetic embryos in lines showing a tendency towards apomixis comported 0.7–19% according to the individual variability. Cytoembryological examination revealed the occurrence of parthenogenetic and adventitious embryos, autonomous endosperm in addition to the pseudogamous one and of aposporous embryo sacs. The plants in which adventitious embryos were observed did not develope viable seeds. The functionality of aposporous embryo sacs in examined plants has been not proved till now.

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The sporogenous tissue and tapetum of transgenic tobacco plants (*Nicotiana tabacum* L.) with different levels of the histone H1 variants

Joanna Ślusarczyk[1], Andrzej Wierzbicki[3], Teresa Tykarska[2], Andrzej Jerzmanowski[3], Mieczysław Kuraś[2]

[1]*Department of Ecology and Environmental Protection, Świętokrzyska Academy, 15 Świętokrzyska str., 25–406 Kielce, Poland, e-mail: J.Slusarczyk@pu.kielce.pl*
[2]*Department of Plant Morphogenesis, Warsaw University, 1 Miecznikowa str., 02–096 Warsaw, Poland*
[3]*Department of Plant Molecular Biology, Warsaw University, 5A Pawińskiego str., 02–106 Warsaw, Poland*

The phenomenon of male sterility has often been observed in investigations on the role of histone H1 in regulation of morphogenetic and cytological processes in transgenic tobacco plants (Ślusarczyk et al., 2001). These changes were accompanied by disturbances in flower development, consisting in lengthening of the pistil style in relation to anthers. This prevented pollination and production of seeds. As similar abnormalities occurred also in the present investigations, we carried out observations of ultrastructure of anther tapetum cells in view of abnormalities which occurred during microsporogenesis and development of male gametophyte. The H1 histone occurs in plant and animal cells in the form of sequentially different variants. There are six such variants in tobacco: two major ones (H1A and H1B), which constitute ca. 90% of all H1, and four minor ones (H1C, H1D, H1E, H1F), occurring in very small amounts. In the investigations, we analyzed four groups of transgenic tobacco plants with different level of histone H1 variants: K – control with the full set of histone variants, – AB – with inactivated A and B variants, – ABCD – with inactivated A, B, C and D variants, and – CD – with inactivated C and D variants.

The analysis of microsporogenesis in those plants, based on preparations squeezed in acetoorcein, revealed the asynchronous course of meiosis in – AB and – ABCD plants, occurrence of chromosomal aberration, and, consequently, the formation of sterile pollen grains (accordingly: 84,4% and 81,4%). In – CD plants, the percentage of aberration and sterile pollen grains was similar to the control material. Electron microscope observations of microsporogenesis showed ultrastructural changes. In – AB and – ABCD plants, a major portion of the pollen grains were degraded. The smallest number of degraded pollen grains, in comparison with the control, was found in the – CD group. It was found that tapetal development was normal in all investigated groups of plants, and the sequence of changes was similar as in the control. However, certain ultrastructural differences appeared when tapetum functioned as secretory tissue, and in the degeneration phase. In tapetal cell cytoplasm, with participation of rER, lipid bodies were formed, which, took part in formation of pollen coat. Both in the control and in the other combinations, excluding – ABCD, these bodies looked similar: they were gray, homogenous and surrounded with black jagged deposits. In – ABCD plants, these bodies were more translucent, slightly rarefied, and not surrounded with the deposits. Moreover, in – CD plants, large lipid deposits were frequently observed between remainders of degraded tapetal cells. They did not occur in the control and the remaining combinations.

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Changes in isoenzyme pattern of chosen enzymes during flax zygotic embryogenesis

Tomáš Takáč, Anna Pretová

Institute of Plant Genetics and Biotechnology, Slovak Academy of Sciences, Akademická 2, P.O. BOX 39 A, Sk–95007, Nitra, Slovak Republic, e-mail: tomas.takac@savba.sk

In the recent years increased effort is given to biochemical characterization of the embryo development in situ and also for embryo development in vitro (embryo cultures, somatic and gametic embryogenesis). It was shown, that isozymes of several enzymes, which are active during embryogenesis, can be useful for this purpose (Pretová et al., 2001). The aim of our study was to find a association between izoenzyme pattern of chosen antioxidant enzymes and the developmental stages of flax zygotic embryo formation. Antioxidant enzymes (such as superoxide dismutase, catalase, ascorbate peroxidase, etc.) are responsible for maintenance of concentration of reactive oxygen species (ROS) in the cell. It is known, that ROS have important role during various developmental processes including embryogenesis.

Embryos 8, 10, 14, 16 and 20 days old were harvested from flax fruits, grounded using liquid nitrogen in 0.1 M sodium phosphate buffer (pH 7.5) containing 1 mM EDTA, and centrifuged at 15,000 g for 20 min. Supernatant was used for enzymes analyses. Izozymes of superoxide dismutase, peroxidases and catalase were separated on 10% denaturating polyacrylamide gels. Specific activities of studied enzymes were examined spectrophotometrically.

We found significant changes in superoxide dismutase, peroxidases and catalase specific activities during embryo formation. Similarly, electrophoretic analysis revealed remarkable differences in the isoenzyme patterns through the zygotic embryo development. For peroxidases, nine multiple molecular forms or isozymes, were detected during flax zygotic embryo development. Four superoxide dismutase izozymes (three Cu/Zn SOD and one MnSOD) were expressed in developing flax embryos. From those only MnSOD was developmentally regulated in flax embryo. MnSOD izozyme showed growing expression throughout the embryo development. Our results showed, that izoenzymes of studied enzymes have specific pattern throughout the flax zygotic embryo development.

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Pollination biology of *Terminalia chebula*

Seema Talwar, A.K. Bhatnagar

Environmental Biology Laboratory, Department of Botany, University of Delhi, Delhi – 110 007, India, e-mail: seematalwar2003@yahoo.com

Terminalia chebula Retz., a member of Combretaceae, is widely distributed in the tropical and subtropical regions of the world. The fruits are credited with laxative, tonic and stomachic properties. It is used under the name of 'triphala' in combination with *Terminalia bellerica* and *Emblica officinalis*. Studies on floral biology of *Terminalia chebula* were conducted in the medicinal garden of College of Agriculture, Dapoli, Maharashtra (India) during 2003–2005. Flowering starts from the higher branches and extends to the lower ones. In 2004, the first flowering was observed on 22nd March, and in 2005 on 1st April. The pale yellow flowers, arranged in spikes, were bisexual, with 5 petals, 10 stamens, dry and non-papillate stigma, elongated style and an inferior ovary. Anthesis extended from 00.00 h to 03.00 h. During anthesis, the style emerged out first. On the second day of anthesis, style increased in size (3 mm) and stigma became receptive. Anthers emerged out on the third day of anthesis. First the five anthers of outer whorl emerged out, while five anthers of inner whorl remained appressed. Anthers dehisced asynchronously and dehiscence took place by longitudinal slits. After dehiscence, anthers moved towards the peripheral region. Pollen grains were pentacolporate. Pollen was shed at 2 nucleate stage. At the time of anther dehiscence, pollen viability was 91.2%. Pollen remained viable for 5 days. Ovary was unilocular with two ovules in each compartment. Ovules were anatropous, bitegmic and the micropyle was formed by both the integuments. Experiments conducted by bagging the flowers in mosquito nets to prevent insects from visiting the flowers did not show any fruit formation. The mode of pollination was entomophillic. The frequent floral visitors were *Apis dorsata, Apis cerena indica, Polistes hebraeus, Tirumala limnace* and *Spindosis vulcanus.* The bees proved important pollinators by the frequency of their visits, foraging speed and pollen pick up. Anthers moved towards the periphery after dehiscence, creating a small gap between anthers and pistil to allow the passage of insects to the nectaries at the base of stigma. Foraging activity started around 08.00 h in the morning and reached its maximum between 09.30 h and 11.00 h and then there was a sudden decline in the number of insect visitors from 12.00 h to 16.30 h because of high temperature. The possibility of wind pollination was also studied by hanging slides smeared with glycerine jelly at various heights and observed after 48 hours. No pollen grains of the species were found on any of the slides. The field pollination showed fruit set only in cross-pollinated flowers showing self-incompatibility. The natural fruit set rate was only 8%.

Searching for *Arabidopsis thaliana* (L.) Heynh. hormone-response mutants impaired in somatic embryogenesis

Aneta Trojanowska, Agnieszka Ujczak, Małgorzata D. Gaj*, Marzena Mędrek

Department of Genetics, University of Silesia, 40–032 Katowice, Poland,
**e-mail: mmdgaj@us.edu.pl*

Somatic plant cells cultured under appropriate *in vitro* conditions can regain their totipotency and produce somatic embryos via developmental process called somatic embryogenesis (SE). While later stages of somatic embryo development resemble zygotic embryogenesis in many aspects, the initial phase is obviously different. Especially little is known on the most intriguing phase of SE i.e. acquisition by a somatic cell of embryogenic competence. Among some factors considered to be essential for SE induction, a proper endogenous hormone regime and appropriate exogenous hormone treatment are believed to be crucial. Hence, the mutants presenting altered hormonal response could be very useful for genetic research on SE. Exceptionally numerous collection of different hormonal mutants is available in *Arabidopsis thaliana* (L.) Heynh. This model plant species with its advanced structural and functional genomics and efficient *in vitro* system for induction of somatic embryogenesis via direct morphogenic pathway (Gaj, 2001), provide a unique opportunity for dissection of the genes essential in SE.

To select mutants impaired in SE induction capacity for SE was studied in *in vitro* cultures of the mutants presenting defects in response to different plant hormones such as: auxin, ABA and gibberellins. SE was induced in culture of immature zygotic embryos on solid induction medium with 2,4-D (Gaj, 2001). Capacity for SE was evaluated in terms of efficiency of SE induction (frequency of SE-responding explants) and productivity of SE (an average number of somatic embryos produced per explant) in 2-week old mutants' and wild-type cultures.

Among the forms found to be impaired in their ability for SE were mutants resistant to auxin (*axr*) and ABA (*abi).* The selected mutants have shown significant decrease in embryogenic response manifested by a low efficiency and/or productivity of SE. Especially valuable material for further studies on genetic determination of SE have represented mutants which lost the ability for SE induction.

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Expression of *LEAFY COTYLEDONS* genes during somatic embryogenesis induced in *Arabidopsis thaliana* (L.) Heynh.

Agnieszka Ujczak, Mirosław Kwaśniewski, Małgorzata D. Gaj*

*Department of Genetics, University of Silesia, 40–032 Katowice, Poland, *e-mail: mmdgaj@us.edu.pl*

Somatic plant cells cultured under appropriate *in vitro* conditions are able to produce embryos via developmental process called somatic embryogenesis (SE). Understanding the key factors promoting vegetative-to-embryogenic transition and identification of genes involved in this process presents a challenge for modern molecular biology. Recently, *Arabidopsis thaliana* has met requirements of a model system for studying SE in plants due to establishment of *in vitro* methods enabling efficient production of somatic embryos and advanced structural and functional genomics.

The *Arabidopsis LEAFY COTYLEDON* (*LEC*) genes, *LEC1, LEC2,* and *FUSCA3* (*FUS3*) encode transcription factors and play a central role in controlling many aspects of plant zygotic embryogenesis (ZE). Recently, it was shown that besides ZE the *LEC* genes play also a crucial role in the induction of SE and thus in the acquisition of embryonic competence by cultured somatic cells (Gaj et al., 2005). The *lec* mutants were found strongly impaired in ability for SE induction and displayed mostly callus development instead of direct somatic embryos formation observed in wild-type cultures (Gaj et al., 2005).

To further elucidate the involvement of *LEC* genes in genetic determination of SE induction and development of somatic embryos, the quantitative analysis of *LEC* genes expression level has been carried out. Direct SE was induced in culture of immature zygotic embryos of Columbia ecotype on solid induction medium with 2,4-D (Gaj, 2001). Total RNA was isolated from explants induced and maintained on SE induction medium for 0 to 35 days. qRT-PCR analysis was performed with the use of LightCycler 2.0 System, ROCHE. The analysed *LEC* genes have shown changes in expression level during SE induction and somatic embryos development. The expression pattern of the *LEC* genes presented by cultured tissue undergoing SE has confirmed the essential role of these genes in acquisition of embryogenic competence by cultured somatic cells.

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Callus induction on leaves of *Salix viminalis* clones

M.K. Wojciechowicz, J. Krysiuk, M. Zenkteler, E. Zenkteler, A. Bagniewska-Zadworna

Department of General Botany, Institute of Experimental Biology, A. Mickiewicz University, ul. Umultowska 89, 61–614 Poznań, Poland

Willow is often use in bio-energy plantations for its potential as a renewable energy source, but knowledge about its possibility of in vitro regeneration is still limited. Therefore, we investigated effect of medium composition and stage of leaf development on callus induction on leaves of *Salix viminalis* clones.

Leaves of five clones (2, 38, 72, 75, 46) of basket willow in different stages of development were used as an initial explants. Shoots of *S. viminalis* of length of 60–80 cm with closed vegetative buds were cut off at one week intervals from the beginning of February until end of March. Shoots were kept in water and left standing on the window in laboratory rooms at a temperature of approximately 20˚C. The developing juvenile leaves, which were 10 mm to 50 mm long, were harvested and transferred to MS medium supplemented with auxins (2,4-D, NAA, dicamba, IAA or picloram) and cytokinins (BAP, kinetin or thidiazuron). Explants as leaves and leaves segments were placed with the adaxial side down onto the media. Cultures were maintained in darkness or continuous light (40 $\mu Em^{-2}s^{-1}$) at temperature of 24˚C.

The whole leaves of size from 10 mm to 15 mm and segments of them occurred to be the most suitable initial explants for induction of callus tissue. The MS media supplemented with 2,4-D 2.0 mgl^{-1} and BAP 0.2 mgl^{-1} were the most effective to induced callus tissue on leaves of *S. viminalis*. Under light condition leaves of all studied clones showed capacity to induce callus tissue. Callus was formed after one to four weeks of leaf cultures from the base of leaves and cut ends of lamina near the main vein. Callus was white, friable with green spots and grow fast. Additionally, red callus line appeared in the 38 clone. In darkness only leaves of clone 2 formed yellowish and friable callus but its growth was very slow. The obtained callus yields varied with the stage of leaf development, growth regulator compositions and light conditions.

In vitro embryo sac development *via* ovule cultures in *Doritis pulcherrima*

Xiu-Lin Ye[1], Cheng-Hou Wu[1,2], Cheng-Ye Liang[1]

[1]*South China Institute of Botany, The Chinese Academy of Sciences, Guangzhou 510650, China, e-mail: xlye@scbg.ac.cn*
[2]*Guangzhou Instiute of Garden, Guangzhou 510405, China, e-mail: wuchenghou2000@yahoo.com.cn*

The megasporgenesis in vitro by ovule culture can be induced on all media whether with exogenous hormones or not 3 d after inoculation, but the later magagametogenesis will stop on the medium without hormone or only with auxin or cytokinin. Mature embryo sac can form from megaspore mother cell on the modified VW medium supplemented with 0.5 mg/l BA and 0.1 mg/l NAA. The megasporocyte continued to enlarge along the cell axis and contained a prominent nucleus and dense cytoplasm when cultured *in vitro*. The polarity of the megasporocyte *in vitro* was as that *in vivo*. After the first meiotic division, a relatively equal dyad formed. The chalazal dyad cell enlarged and differentiated into the functional megaspore at the expense of the micropylar one. The functional megaspore after the second meiotic division so that a 2-nucleate enbryo sac came to expand. A large vacuole separated the two nuclei. Both nuclei divided and a 4-nucleate embryo sac formed, two nuclei displayed front and behind at the micropylar end and two nucleus stained more deeply due to the presence of more heterochromatin within the nucleoplasm. One further division took place and the embryo sac became 8-nucleate. At maturity, therefore, the embryo sac was seven-celled. The embryo sac developmental pattern was the same type as *in vivo*.

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Embryo sac development on facultative apomixis in *Woonyoungia septentrionalis* (Dandy) Law

Xiu-Lin Ye[1], Qing-Wen Zeng[1], Ya-Qin Wang[1,2], Cheng-Ye Liang[1]

[1]*South China Institute of Botany, The Chinese Academy of Sciences, Guangzhou 510650, P. R. China, e-mail: xlye@scbg.ac.cn*
[2]*College of Bioscience and Engineering, South China University of Technology, Guangzhou 510641, China, e-mail: wangyq@scbg.ac.cn*

Woonyoungia septentrionalis belongs to the family Magnoliaceae. This species can be found in a rare species and little information is available concerning its reproductive biology. A cytological study on *Woonyoungia septentrionalis* (Dandy) Law revealed that this is a facultative apomictic species with 30% of its embryo sac formed via apospory. The normal pattern of embryo sac formation conforms to the polygonum type. During the archesporial or the megasporocyte stage of megasporogenesis, some nucellar cells adjacent to the degenerated archesporial or the megasporocyte became an aposporous initial, which later developed into a panicum-type embryo sac. The aposporous embryo sac is composed of an egg, a single synergid and a central cell with 2 polar nuclei. Chromosome reduction did not occur.

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Towards embryology of Campanulaceae in connection with family systematics

Nadezhda A. Zhinkina

Department of Embryology and Reproductive Biology, V. L. Komarov Botanical Institute, Prof. Popov str., 2, 197376, Saint-Petersburg, Russia

Campanulaceae is one of the families with unclear systematic position. The typical genus Campanula is of special interest; it is heterogeneous, distributes in almost all Holarctic zones and also in mountain regions where the evolution of Campanulaceae mainly occurred. Embryology of different genera members was researched on light level. Taking into consideration Linneaus statement on significance of frutification and seed structure as important features for definition of systematic position of species and genera within the family, we paid attention to the study of seed structure in fruit (scanning microscopy). Ultrastructure of seed surface is closely connected with the structure of seed coat and is a reprint of outer epidermal and inner spermoderm layers. The most heterogeneity in seed surface ultrastructure is observed in the members of Campanulaceae. Great differences in shape and seed sizes were noted that could be also used as the important morphological feature in species description. Resemblance and differences in some embryological features and data on seed surface structure contribute much in systematics and phylogeny of Campanulaceae.

Distribution of poly(A)RNA and splicing machinery elements in mature pollen grains and *in vitro* growing pollen tubes of *Hyacinthus orientalis* L.

Krzysztof Zienkiewicz, Dariusz J. Smoliński, Elżbieta Bednarska

Department of Cell Biology, Institute of General and Molecular Biology, Nicolaus Copernicus University, 9 Gagarina str., 87-100 Toruń, Poland, e-mail: kzienk@biol.uni.torun.pl

In eukaryotic cells the newly formed transcript undergoes specific modifications among which are addition of a poly(A) tail at the 3' end and splicing, i.e. intron excision and exon joining. The splicing is performed by protein-ribonucleoprotein complexes called spliceosomes. Each splicing RNP is composed of a short snRNA with TMG cap at the 5' end and a complex of seven Sm proteins. Besides in forming a catalytically active spliceosome a SC35 protein participate.

The localization of poly(A)mRNA and molecules linked to pre-mRNA splicing: TMG snRNA and SC35 protein, in mature 2-cell pollen grains and *in vitro* germinating pollen tubes of *Hyacinthus orientalis* L. were investigated. In order to analyze this problem fluorescent *in situ* hybridization and immunocytochemistry in fluorescent and confocal microscope were used.

The performed investigations indicated a different pattern of poly(A)mRNA distribution in mature pollen grains before anthesis than in germinating pollen grains. Directly before anthesis poly(A)mRNA was present in the both pollen cells. In the vegetative cell it was homogenously distributed in the whole cytoplasm, in the generative cell poly(A)mRNA was localized in the cytoplasm present at the two oppositing poles of the cell. This poly(A)RNA could be long-life RNA, which is synthesized during differentiation of pollen grain and stored until anthesis and then used during pollen tube growth. After rehydration poly(A)mRNA is accumulated at one of the pollen poles. In the pollen tube it was present in the cytoplasm, mainly in the apical zone and under the plasmalemma.

Both in the mature pollen grains and in the growing pollen tubes molecules associated with pre-mRNA maturation have been found. In the mature pollen grains, which are considered as transcriptionally inactive, both TMG snRNA and SC35 protein were localized in form of small clusters within the nucleus of the vegetative and generative cell. Just after rehydration of the pollen grains the pattern of distribution and the level of the investigated antigens in the vegetative and generative nucleus were similar as before anthesis. During the growth of the pollen tube changes were observed in the distribution and in the level of TMG snRNA and SC35 protein in the vegetative nucleus. A significant increase in the level both analyzed splicing factors has been observed there. TMG snRNA was localized in a dispersed form in the whole nucleus and SC35 protein occurred in large clusters, which occupied a large part of the nucleus. The observed changes suggest that synthesis of splicing factors takes place there and does not exclude resumption of transcription and maturation of pre-mRNA in growing pollen tube.

Influence of growth regulators level on the initial phases of androgenic induction in isolated microspore culture of triticale (× *Triticosecale* Wittm.)

Iwona Żur, Ewa Dubas, Maria Filek, Jolanta Biesaga-Kościelniak, Elżbieta Golemiec, Maria Wędzony

Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, 30–239 Cracow, Poland, e-mail: zur@ifr-pan.krakow.pl

Androgenic induction was examined in the isolated microspore cultures of two triticale (× *Triticosecale* Wittmack) spring cultivars: Wanad and Mieszko. Tillers were harvested when the majority of microspores were at the mid- to late-uninucleate stage, and cold-treated for 3 weeks, at 4˚C, in the darkness. Anthers were aseptically isolated to low nutrient mannitol medium and kept in 32˚C for 4 days. Microspores were isolated and suspended at equal concentrations in liquid 190–2 medium containing 2,4-D and kinetin in proportion of 0.5/0.125; 1/0.25 and 2/0.5 mg/dm^3 respectively. Control medium was devoid of growth regulators (GR). Samples were taken for microscopic and biochemical analysis on the day of isolation before culturing and on the 1st, 3rd, 5th and 7th day of culture.

The tested genotypes differed in their reactions to the pretreatment: on the day of isolation microspores of Wanad were viable while on average 45% of isolated Mieszko microspores were dead. Up to 30% of Wanad microspores died during the first day of culture and the proportion of vital microspores decreased rapidly up to 5th day of culture in control and in the medium with the lowest concentration of GR. The two highest concentrations maintained microspore viability till the 5th day of culture, but this positive effect was lost before the 7th day of culture. In Mieszko, the rate of cell death was similar, however the effect of growth regulators treatment was less visible. Lower concentration of GR (0.5 mg/dm^3 2,4-D/0,125 mg/dm^3 kinetin) accelerated androgenic microspore development and its first symmetrical divisions till the 3rd day of culture. Starting from the 5th day of culture the higher concentration of GR inhibited previously initiated mitotic divisions and enhanced accumulation of starch granules. The analysis of 7 days old cultures revealed that the presence of GR in the medium had finally no significant effect on multicellular structures development. For both genotypes, at the optimal for androgenic development concentration of hormones, microspores accumulated significantly higher amounts of carbohydrates, calcium and potassium ions.

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Regeneration of haploid plants via zygote rescue after maize pollination of wheat

Ferenc Bakos, Katalin Jäger and Beáta Barnabás

Agricultural Research Institute of the Hungarian Academy of Sciences, 2 Brunszvik str. H-2462, Martonvásár, Hungary, e-mail: bakosf@mail.mgki.hu

The production of (doubled) haploid plants is advantageous for plant breeders because it offers a way of rapidly advancing chosen lines to complete homozygousity and of increasing the efficiency of subsequent selection. Although various methods (delayed pollination, pollination with unviable pollen grains, chemical induction of parthenogenesis) have been attempted in cases where microspore embryogenesis cannot be applied efficiently (e.g. recalcitrant *T. durum*, T. *triticale* and bread wheat genotypes), pollination with a distantly related species appears to be a real alternative technique for generating haploids by inducing embryogenic development (Laurie and Bennett, 1988).

Following the wheat × maize pollination, the embryos, which are not excised within 3 weeks, will perish because of the lack of a normal endosperm. In the most cases the embryos were rescued at a late stage, 2 weeks after the distant pollination. However, since the development of the embryos is hidered even at a very early stage (Wedzony and van Lammeren, 1996), the rescue of two-day-old zygotes may be advantageous, providing an efficient, well-optimized single cell culture method.

In the present study, early rescue was applied in an attampt to culture zygotes or proembryos which had not been exposed to the negative influence of endosperm abnormality or absence for a long period. The dissected, activated egg cells were cultured with young wheat pistils, as nurse tissue (Bakos et al., 2003).

Although the efficiency of this method seemed to be already comparable to that of embryo rescue, further optimisation of in vitro culture following early zygote rescue could make the efficiency of this new method even better, since the embryos are rescued earlier from the severely suboptimal environment, allowing their faster development and resulting in higher efficiency of the wheat × maize pollination system.

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Morphological and cytological characterisation of a microspore-derived structure, yielding the highest amount of spontaneous doubled haploid regenerants in maize anther cultures

Katalin Jäger, D. Kőszegi and Beáta Barnabás

Agricultural Research Institute of the Hungarian Academy of Sciences, Brunszvik u. 2, H-2462 Martonvásár, Hungary, e-mail: jkata@mail.mgki.hu, dkoszegi@mail.mgki.hu, bea@mail.mgki.hu

In anther culture, plants can become differentiated via two pathways: direct androgenesis, during which embryoids develop from the microspores, or indirectly through callogenesis followed by organogenesis. The first type of division in maize microspores is generally asymmetric, and calli are formed from most of the microspores developing androgenetically (Barnabs et al. 1987, 1999). Correlations between the morphological traits, ploidy level and regeneration ability of microspore-derived structures formed during the early induction phase in maize have not yet been investigated.

The applicability of the method and thus the efficiency with which doubled haploid maize lines of microspore origin can be produced is limited not only by the strong genotype dependence, but also by the fact that the regeneration ratio of the microspore-derived structures is low, despite the relatively high induction frequency. This means that there is little return in terms of regenerants for the labour invested in transferring large numbers of microspore-derived structures from one medium to the other.

The regeneration capacity of microspore-derived structures, with various morphological characteristics produced in anther cultures of maize (*Zea mays* L.) were studied in order to identify the morphotype resulting in the highest yield of spontaneous doubled haploid regenerants. Parallel to the morphological studies the ploidy level of microspore-derived structures and regenerants was analysed by flow cytometry. It was found that changes in the chromosome number and ploidy level had already taken place by the 3rd week of induction. In agreement with observations on embryogenic callus lines of wheat, published earlier, the present studies suggest that the growing conditions of the donor plants and differences in the composition of the induction medium did not have a decisive effect on the distribution of various morphotypes among the microspore-derived structures, or on their regeneration ability. The highest number of spontaneous doubled haploid plants was regenerated from white compact structures 2–3 mm in size, derived from the anthers of phytotron-grown donor plants.

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