PREFACE

Pollen is a fine to coarse powder containing the microgametophytes of seed plants, which produce the male gametes (sperm cells). Pollen grains have a hard coat that protects the sperm cells during the process of their movement between the stamens to the pistil of flowering plants or from the male cone to the female cone of coniferous plants. When pollen lands on a compatible pistil of flowering plants, it germinates and produces a pollen tube that transfers the sperm to the ovule of a receptive ovary. Among other topics, this book reviews research on pollen biology and the hybridization process in walnuts, modern conception on the structure and development of the sunflower anther; the role of anion channels in pollen germination and tube growth.

Chapter 1 - This chapter is an illustrated catalogue of meiotic division abnormalities, preferably cytoskeleton aberrations in karyo- and cytokinesis, leading to 2n gametes formation in plants. It includes 40 meiotic restitution mechanisms in pollen mother cells.

Chapter 2 - This review focuses on the pollen biology of *Juglans*, and in particular *Juglans nigra* (Eastern Black walnut) and *Juglans regia* (Persian or English walnut), which are economically important species in Europe, Asia and North America. Both species are monoecious, heterodichogamous and wind –pollinated. Their mating system is predominantly outcrossing, although under particular environmental conditions self-pollination is possible. Hybrids between the two species, *Juglans* × *intermedia* (Carr) can occur naturally, although they often have reduced fecundity. Compared to the parental species, most *J.* × *intermedia* (*J. nigra* × *J. regia*) hybrids show increased vegetative vigor, distinct disease resistance, high wood quality, and greater winter-hardiness. For these reasons here is great demand for *J.* × *intermedia* for forestry, especially in Northern Europe. The authors review several aspects of *Juglans* pollen biology that frustrate the production of *J.* × *intermedia* and limit the progress of researchers and plant breeders who work with this genus. The authors also discuss the ways in which scientists and breeders are working to overcome problems related to pollen storage and viability testing, pistillate flower abscission (PFA), fertilization and embryogenesis in *Juglans*, and the use of microsatellites to monitor gene flow, ploidy, parentage, and hybridogenesis all with an eye toward practical solutions to the current shortage of *J.* × *intermedia* for research and applied forestry.

Chapter 3 - The cold deserts in Western Himalayas spread over approx. 74,809 Km^2, cover Leh and Kargil districts of Ladakh in Jammu & Kashmir, Uttarkashi in Uttarakhand and Lahaul- Spiti along with some parts of Chamba and Kinnaur districts of Himachal Pradesh. These cold deserts consist of rugged mountains, snow clad peaks, bare rocks, steep

sandy slopes with rock gravels, low capacity of soil to retain moisture, and have oppressive and inhospitable conditions which include freezing temperature, dry arid weather, high velocity winds and low precipitation. As a consequence of such harsh climatic conditions prevailing in these regions, plants tend to become prostrate, thick, bushy, hardy, mat forming and spiny with long roots and small succulent or woolly leaves. Majority of these plants are perennials and survive through underground parts. Further, the plants of the area are exposed to high incidence of UV rays and are under considerable pressure of human intervention and natural disasters which include agriculture, heavy grazing, snow avalanches, windstorms, landslides and increasing entry of tourists and transport vehicles. As a consequence of such stresses, the plants of the area are expected to show considerable amount of irregularities during male meiosis and in pollen grains. Keeping above assumptions in mind the work on cytomorphological diversity in the plants of Lahaul-Spiti, a part of the Western Himalayan cold deserts, was undertaken. During the course of study spanning 5 years (2005-2009) the authors have come across several species which depict various irregularities during male meiosis resulting into the formation of variable sized apparently fertile/ stained pollen grains and considerable amount of pollen sterility. These heterogeneous sized pollen grains and pollen malformation are reported mainly in species depicting the phenomenon of cytomixis involving chromatin transfer among proximate pollen mother cells (PMCs) viz., (*Anemone rivularis*, 2n=16; *Aquilegia fragrans*, 2n=16; *Astragalus bicuspis*, 2n=16; *A. frigidus*, 2n=16; *A. himalayanus*, 2n=16; *A. rhizanthus*, 2n=16; *Caltha palustris*, 2n=32; *Clematis grata*, 2n=16; *C. orientalis* var. *acutifolia*, 2n=32; *Geranium pratense*, 2n=56; *Hedysarum astragaloides*, 2n=14; *Meconopsis aculeata*, 2n=56; *Medicago falcata*, 2n=16; *M. sativa*, 2n=32; *Melilotus officinalis*; 2n=16, *Parnassia laxmanii*, 2n=18; *Pleurospermum candollii*, 2n=22; *P. govanianum*, 2n=18; *Potentilla atrisanguinea* var. *atrisanguinea*, 2n=84; *P. atrisanguinea* var. *argyrophylla*, 2n=84; *P. cuneifolia*, 2n=28; *P. fruticosa* var. *rigida*, 2n=14; *Ranunculus hirtellus*, 2n=32; *R. laetus*, 2n=28; *Rosularia alpestris*, 2n=28; *Silene vulgaris*, 2n=24; *Thalictrum foetidum*, 2n=42; *Trifolium pratense*, 2n=14; *T. repens*, 2n=16 and *Trigonella emodii*, 2n=16). The resultant PMCs after partial or complete chromatin transfer in these species depict hyper-, hypoploid and anucleated nature which give rise to apparently fertile diploid (2n), haploid (n), and aneuploid, and sterile or micro pollen grains. Such variable sized fertile and sterile pollen grains are also resulted in species depicting irregular synapsis (*Dianthus angulatus*, 2n=30; *Ranunculus laetus*, 2n=28; *Rosularia alpestris*, 2n=28), spindle irregularities (*Potentilla atrisanguinea* var. *argyrophylla*, 2n=84; *Ranunculus hirtellus*, 2n=32; *R. laetus*, 2n=28; *Rosularia alpestris*, 2n=28), triploids (*Chrysanthemum pyrethroides*, 2n=27; *Heracleum brunonis*, 2n=33), pentaploids (*Agrimonia eupatoria*, 2n=70) and polyploids with irregular meiotic course (*Chrysanthemum pyrethroides*, 4x; *Clematis orientalis* var. *acutifolia*, 4x; *Potentilla atrisanguinea* var. *atrisanguinea* 12x; *P. atrisanguinea* var. *argyrophylla*, 12x; *Ranunculus hirtellus*, 4x; *R. laetus*, 4x). Double sized pollen grains referred as '2n' pollen grains resulted as a result of either direct fusion of two pollen grains (*Caltha palustris*, *Potentilla atrisanguinea* var. *argyrophylla*) or are the end products of fused PMCs (syncytes) as is the case in *Meconopsis aculeata* and *Clematis orientalis* var. *acutifolia* are also recorded. Though cytological status of such variable sized fertile pollen grains could not be ascertained presently, they can play an important role in the origin of intraspecific polyploids, aneuploids and taxa with B-chromosomes.

Chapter 4 - The process of double fertilization involves 2 sperm cells that are delivered into female gametes via pollen tubes. To analyze this process, the authors utilized the

bicellular pollen species *Alstroemeria* and *Cyrtanthus*, which produce 2 sperm cells in the pollen tubes. Novel procedures have been developed for the *in vitro* observation of pollen tubes under precise control conditions, for flow cytometric analysis with single cell manipulation, and for immunocytochemical analysis to detect cytoskeletons. Using these unique methods, male gamete dynamics have been elucidated during pollen tube development. In particular, the authors have focused on male germ unit (MGU) defining that the generative cells or the pair of sperm cells in pollen tubes or pollen grains are closely associated with the vegetative nucleus. This MGU is proposed to function as a vehicle for the transmission of male gametes to the female gametes and to participate in the fusion with the target female cell during fertilization. In this chapter, recent insights into male gamete dynamics using novel procedures are discussed.

Chapter 5 - Modern conception about the structure and development of sunflower anther in the normal state and under the different types of sterility are presented in the investigation. In the review different types of male sterility: nuclear male sterility, cytoplasmic male sterility and sterility which induces different factors is considered. This research is based on the scientific literature summarizing and experimental work results of the authors as for meiosis studying of culture sunflower and wild species and their interspecific hybrids. Spore formation capacity of androceum concerning practical breeding and seed production problems are also stated. The results of research in field of inheritance of nuclear mail sterility and cytoplasmic mail sterility, mapping of genes which control male sterility with use different molecular markers. The questions of relationship plasmone and genome in manifestation of cytoplasmic male sterility were shown. The importance of gametocytes for the induce of sunflower sterility was considered. A great attention is paid to meiosis violation in the process of chromosome formation rearrangement during the interspecific hybridization. The reasons of cytological instability of interspecific hybrids which have been obtained by crossing of annual wild species with culture sunflower and the ways of overcoming this cytological instability are discussed.

Chapter 6 - Restoration of pollen fertility in plants with cytoplasmic male sterility (CMS) is known to be controlled by specific nuclear fertility-restoring genes that suppress functioning of aberrant mitochondrial genes, expression of which destroys pollen development. Usually, these genes are dominant and manifest in the F_1 generation. In sorghum, pollen fertility restoration in some CMS-inducing cytoplasms (A4, '9E', 'M35-1A') has aberrant mode of inheritance: it is stably expressed in self-pollinated progenies of F1 hybrids with restored fertility but unstably manifested in new hybrid genome (F1 or test-cross hybrids to CMS lines with the same cytoplasm type). The authors found that in the '9E' cytoplasm this phenomenon is caused, evidently, by environmental conditions during test-cross hybrid plant development, namely water-availability and photoperiod. Experimental data testify to strong sensitivity of fertility-restoring genes to plant water-availability conditions at microspore- and gametogenesis: at high level of water availability these genes are dominant and can express in heterozygous state (in test-cross hybrids), while in drought conditions fertility-restoring genes are recessive and can function only in homozygous state. In addition, reduced photoperiod at photoperiodically-sensitive stage of sorghum plant ontogenesis also increased percentage of male-fertile F_1 plants, perhaps, by 'activation' of fertility-restoring genes, and these genes expressed in the next generation. Changes of environmental conditions during male-sterile hybrid plant ontogenesis can 'activate' expression of fertility-restoring genes. Cytological analysis revealed significant

polymorphism of pollen grain (PG) types in CMS-lines with the '9E' cytoplasm and in the F_1 hybrids with restored male fertility. Both sterile and fertile plants had "fertile" (normally colored) PGs, PGs with anomalous shape, with delay of development at one- or two-nucleate gametophyte, with disturbed starch accumulation (incompletely filled with starch and PGs of *waxy*-type), with entirely degenerated content. Additional watering increased percentage of fertile PGs but no clear correlation between their frequency and seed set has been found. The data obtained demonstrate that in the '9E' cytoplasm pollen fertility, obviously, is epigenetically-regulated trait depending both from genotype and plant environmental conditions.

Chapter 7 - The UPS (Ubiquitin Proteasome System) is a major post-translational regulation pathway in eucaryotes, allowing rapid and selective degradation of proteins. These proteins are either misfolded or damaged polypeptides (quality control), or key developmental/metabolic regulators. The latter are selectively recognized and labelled with a poly-ubiquitin chain by E3-ubiquitin ligases, prior to degradation in a large multi-subunit complex called the 26S proteasome. In plants, the UPS controls many processes, in particular most hormone signalling pathways, and several other important steps including cell cycle, shoot branching, biotic stress response and self-incompatibility. Recent data highlight that the UPS is also crucial for male gametophyte (i.e. pollen) development. Plant male gametophyte development requires several tightly regulated processes of nuclei movement, cell divisions and differentiation that are well described, but which molecular control still needs to be deciphered. Different transcriptomic data indicate that transcripts for most UPS actors are found in microspore and/or pollen cells. In the last few years, the role of several components of UPS, including proteasome subunits and proteins involved in targets recognition/ubiquitylation, has been demonstrated during male gametogenesis. In particular, the role of an F-box protein that is transiently transcribed in male germ cell and specifically required for mitosis entry, has allowed significant progress in building a model of the control of male gametophyte development. In this review, I try to state all published evidence for an implication of UPS components in male gametophyte development, and to highlight important ways that still need to be explored.

Chapter 8 - Orchids (Orchidaceae) are known to frequently hybridize. Especially in deceptive taxa, pollen flow between species is caused by lack of fidelity in pollinator behaviour, as pollinators tend to move to different-looking plants after an ineffectual visit. Because many of these deceptive species have no post-pollination reproduction barrier between them, production of hybrid seed in sympatric populations is not rare. As a result of interspecific fertilization, either intermediate or non-typical, usually tall plants with large inflorescences appear. These hybrid plants may be polyploids with varying number of chromosomes and they suffer from very low fertility. Many of these hybrids also totally fail to produce pollen, but production of normally structured and totally or partially in-viable pollinia is also possible. In this chapter, the quality of pollen in hybrid plants and its effects on reproductive success of original species are discussed using the hybrids between *Dactylorhiza incarnata* and *D. fuchsii* in Irish dune population as an example. Quality of pollen in hybrid plants was examined using fluorescent staining method, and was found to vary from totally in-viable to partially viable. The effects of pollen source on seed production were examined with hand-pollination experiments in parental species and hybrids. Production of F_1 seed between *D. incarnata* and *D. fuchsii* was comparative to intra-specific fertilization, but only a few seeds were produced following pollination of parental species with hybrid

pollen. Seed production in hybrid plants was very low compared to the parental species, but some seed was produced. Asexual reproduction via apomictic seed production in *D. incarnata* was examined experimentally stimulating stigma chemically and mechanically. Mechanical stimulation did not result in seed production but liquid containing chemicals from orchid pollen did trigger ovule maturation and production of some seed, as did non-related *Ranunculus acris* pollen. Hybrid pollen in the stigma did not essentially cause interference in seed production of *D. incarnata* either before or after pollination with intra-specific pollen. Pollen produced by hybrids thus seems not to play an important negative role in the reproduction of the parental species. Some hybrids, however, produce at least partially viable pollen which may lead to back-crossing and lower production of genetically normal progeny. This production of hybrids, possibility of apomictic seed production, and introgression at different levels may be important factors behind the high number of taxa and large intra-specific variation in deceptive terrestrial orchids.

Chapter 9 - Nowadays, the use of isolated and *in vitro* cultured microspores is not limited for the production of doubled haploids which are important part of modern plant breeding programmes. Microspores became also a powerful tool to investigate important biological processes such as cell totipotency, differentiation, embryogenesis, cell cycle, plant reproduction and development. In the normal pathway microspores develop into mature pollen *in vivo* or when cultured *in vitro* in a rich medium with sugars, however they can be reprogrammed into the totipotent state and further induced to become embryogenic and produce embryos when subjected to various stresses, such as nutrient starvation, heat or cold shock. Microspore cultures are the most efficient method to produce doubled haploids, excellent system for *in vitro* mutagenesis and selection, attractive target for genetic transformation and for gene targeting in plants. This review paper describes the potential applications of plant microspores in plant breeding, genetics, cellular and molecular biology and biotechnology.

Chapter 10 - The basic feature of polar growing cells is uneven distribution of organelles, forming distinct cytoplasmic zones. It is closely related to the cytosolic free ion gradients and transmembrane ion fluxes, which may cause uneven membrane potential distribution along the cell surface. Participation of Ca^{2+}, H^{+} and K^{+} in pollen tube growth has been proved in numerous studies. Data on inorganic anions contribution to the growth process are scarce and controversial. In somatic plant cells anion channels have vital functions, including membrane voltage and turgor pressure regulation. In this chapter the authors give an overview of recent findings in ionic regulation of pollen germination and give evidence for the important role of anion channels in this process. The use of inhibitory analysis combined with fluorescent methods has allowed them to observe both temporal and spatial changes of membrane potential and reveal the involvement of anion channels in the regulation of this value. The authors' data on the key role of anion channels in structural and functional compartmentalization of the polarized pollen tube cytoplasm are considered. A contribution of mitochondrial anion channels to the pollen tube growth regulation is also discussed.

Chapter 11 - Phenotypic plasticity in floral traits associated with mating systems has been demonstrated for many species. However, few studies have explored the existence of plasticity in pollen and ovule production. This is important because plant mating systems are often estimated as pollen:ovule ratio. In this chapter the authors determine the existence of phenotypic plasticity in gamete production and in mating system as estimated through pollen:ovule ratio in *Nicotiana longiflora* and *N. plumbaginifolia*. The authors measured

several morphological traits of flowers and then counted pollen and ovules directly on plants from greenhouse and natural environments. Then the authors developed regression models for both environments, using morphological traits to predict pollen and ovule number. The authors then applied both regression models to eight and six natural populations of *N. longiflora* and *N. plumbaginifolia*, respectively. The authors found that pollen production is plastic in *N. longiflora* but not in *N. plumbaginifolia*. There was a general trend towards a decrease in number of pollen grains in natural populations of *N. longiflora*; but it was significant in only four out of the eight populations sampled. In *N. plumbaginifolia* that trend was inverted, with natural populations producing more pollen grains; however this pattern was not significant for any of the six populations studied. Ovule production was not significantly different between environments in *N. longiflora* and data did not show any particular trend. In *N. plumbaginifolia*, ovule production had a general trend towards a decrease in natural populations, but this trend was significant in only three out of the six populations studied. Pollen:ovule ratio had a general trend towards a decrease in natural populations of *N. longiflora*, but this trend was significant in only one population, suggesting the existence of a trend towards selfing for that population. On the contrary, pollen:ovule ratio in *N. plumbaginifolia* tended to be higher in natural populations compared to greenhouse, however, it was not significant in any of the populations studied. The authors' results showed that plastic responses are population- and species- specific, possibly as a consequence of local adaptations to dominant selective pressures in each population.

Chapter 12 - Pollen grains are one of the major causes of respiratory allergies. The authors briefly review the role of aerobiological monitoring centers in providing information about airborne pollen concentration for helping allergic patients to reduce exposition to allergens and to start appropriate drug treatments. Spatial and temporal resolution of this information should be increased. However, the effort required by the technique currently used to identify and count the airborne pollen grains hinders this improvement. Therefore, innovative classification approaches were investigated. In particular, the authors studied the feasibility of methodologies developed in the spectroscopic and biomolecular field, with the aim at providing rapid, accurate and possibly automated airborne pollen concentration measurements. In this chapter the state of the art in this field is outlined as well as the authors' obtained results; the authors discuss both the proof of principle of the applicability of such techniques for pollen quantification and, from a more practical point of view, the feasibility of implementing them in aerobiological centers as routine identification tools. Possible future improvements of developed techniques to solve current weaknesses are also examined.

Chapter 13 - Orbicules or Ubisch bodies are corpuscles of sporopollenin that appear in the anther locule during pollen grain development. Their size ranges from 0.14 μm to 20 μm. They present different shapes with a smooth or ornamented surface. Orbicules often form aggregates and sometimes have a plaque-like appearance. Ultrastructurally, they may present a central core with different degree of transparency to electrons. Those that do not have a central core are observed completely solid. Orbicules are resistant to acetolisis, autofluorescent when irradiated with ultraviolet light and have the same reaction to colorants that the exine of pollen grains. Their presence is generally associated with a tapetal membrane in species with secretor type tapetum and with a peritapetal membrane in species with intermediate or plasmodial type tapetum.

Although the shed of orbicules out of the anther along with the pollen grains is cited, they are usually attached to the inner surface of the locule when the anther opens.

Investigations suggest that orbicules appear in approximately 80 families of Angiosperms and Gimnosperms. It is not certain whether orbicules are not developed in the rests of the families or are just not informed. Researches on ontogeny and ultrastructure of orbicules are rare. However, their tapetal origin and their simultaneous formation with the pollen grain wall are well established. The systematic value of orbicules is known and considered in a few families, such as Loganiaceae, Gentianaceae, Apocynaceae, Rubiaceae and Oxalidaceae. Evolutionary studies on these bodies or on its relationship with the different modes of pollination are lacking. Even though orbicules are so common among angiosperms, their function is unknown and only speculations are made. On this report a review on orbicules is made and an analysis of their presence, ontogeny and morphology is presented. The authors' aim is to supply information that will help understand orbicules function. Therefore, the orbicules morphology in relation with the pollination mode is studied.

Chapter 14 - Cotton breeders have long recognized the importance of alien germplasm from the Malvaceae family as sources of genes for crop improvement. An understanding of the biological nature of the incompatibility systems that prevent hybridization and/or seed development is necessary for the successful hybridization and introgression between cotton and other Malvaceae species. In this review, the authors survey the reasons for reproductive isolation between cotton and its wild or cultivated Malvaceae relatives.

Chapter 15 - The microspore and anther cultures have been used in plant breeding since the first reports on embryogenesis from pollen of *Datura innoxia* in 1960's. Large numbers of studies have been focused on formation of homozygous double haploid plants in many important crops by both methods. Microspore embryogenesis is an elegant system for a selection for dominant and recessive traits in haploid plants and after artificial diploidization of them, the plants homozygous for all genes can be obtained.

Reports on employment of the anther cultures are more frequent in contrast to isolated microspore cultures due to a more simple technical handling, despite the fact that there is generally a lower efficiency of this method for doubled haploids production than in pure microspore cultures. Moreover, the anther cultures lead to somatic tissue–derived plants and thus are used as the method for the somatic embryogenesis.

I used anther and microspore cultures methods prospecting their potential also in apomixis research. Dandelions (*Taraxacum* sect. *Ruderalia*) as plants representing an model for studying a diplosporous type of apomixis and producing three ploidy levels (haploid, diploid and triploid) of pollen grains were subjects to establishing new protocols of such artificial embryogenesis. A production of microspore-derived plants could have the potential for observation of recombinations and segregations of apomictic genes within microsporogenesis. Different culture media were tested and callus production, differentiated shoots, roots, and finally whole viable and fertile plants were obtained using anther cultures. Obtained regenerants were of somatic origin. The first attempts of establishing microspore embryogenesis were not successful also in isolated microspore cultures. This is the only one report on obtaining somatic embryogenesis based plants via anther cultures of dandelions with potential use in pharmacy (production of drugs) and particularly for further research purposes. These methods have not been published for this genus yet.

Chapter 16 - The aim of the present chapter was to describe the Chenopodiaceae-Amaranthaceae pollen dynamics in the atmosphere of two cities of the Middle-West of Spain

(Salamanca and Valladolid). Samples were collected by the volumetric method with the aid of two Burkard spore-traps located in the centre of both urban cities during years 2005 and 2006. This pollen type was mainly detected in the atmosphere between late Spring and late Summer, with a Main Pollen Season registered between late May and early October and maximum concentrations detected in August. The intra-diurnal pattern, calculated by means of three different methods, was very similar for both towns reaching a higher hourly concentration percentage in the second half of the day. The correlations obtained between daily pollen counts and different meteorological parameters showed that the airborne presence of this pollen type is positively associated with temperature and negatively with rainfall during MPS. According to known threshold (10-15 pollen/m^3), Chenopodiaceae-Amaranthaceae pollen concentrations exceeded this threshold during 1 day in 2005 and during 12 days in 2006.

Chapter 17 - Pollen viability in three hybrid swarm populations of *Pinus mugo* × *P. sylvestris* and in control populations of *P. mugo* in northern Slovakia was studied throughout two successive years. Germination percentage and pollen tube length were used as the principal parameters of pollen viability. In 2007, a slight reduction of pollen germination was recorded in hybrid swarms on the localities Habovka and Sucha Hora in comparison with the adjoining population of *P. mugo* from Rohače. Statistically significant differences between individual populations were revealed in pollen tube length only. In 2008, a pollen of improved quality was produced in all the populations tested. Pollen germination of hybrid swarms in Habovka, Sucha Hora and Tisovnica was comparable in the respective year with control population of *P. mugo* from Vratna valley. At the pollen tube level the hybrid swarms in Habovka and Sucha Hora deviated considerably from control population by their shorter pollen tubes. Obtained results are taken as an evidence supporting partial reduction of pollen viability in the respective hybrid swarms. Annual variation in pollen viability is supposed to be conditioned climatically.

Chapter 18 - *Grevillea rosmarinifolia* presents three -aperturate pollen grain organized according to Garside's rule, a pattern that is common in Proteaceae but otherwise rarely observed in the eudicot clade where this family belongs. In this chapter the authors describe into details features of early pollen development (microsporogenesis) in order to explore the impact of callose deposition on aperture pattern determination in this species. The authors show that the partitioning of the microspore mother cell during microsporogenesis occurs through centripetal cell-plate formation, followed by additional deposition of callose onto the cell plates. The authors' observations strongly suggest that these additional callose deposits are implicated in the determination of aperture localisation in *Grevillea*.