”Ion Ionescu de la Brad” Iasi University of Life Sciences.

**DOMAIN: AGRONOMY**

# HABILITATION THESIS

## CONTRIBUTIONS TO THE STUDY OF POLLEN AND THE CYTOGENOTOXIC POTENTIAL OF SOME PHYSICOCHEMICAL FACTORS

**Assoc. prof. Silvica PĂDUREANU, PhD**

**IAŞI, 2023**

## ABSTRACT

The habilitation thesis entitled "**Contributions to the study of pollen and the cytotoxic potential of some physicochemical factors**" represents a selection of the results published after obtaining the PhD degree, results considered relevant for one's own academic, scientific and professional career. The paper also mentions the main directions projected for the continuation of one's own career, as well as the possibilities of their fulfilment.

The thesis presented is structured and written in accordance with the legal provisions in force and the specific rules of USV Iasi, in this regard including: A. Abstract; B.I. Professional and academic scientific achievements; B.II. Professional, scientific and academic career development plan; B.III. Bibliographical references.

The thesis contains 24 tables and 95 figures, of which 47 are graphs and 48 are photographs taken under the light and electron microscope, all original, from scientific articles published as sole author or first author in ISI journals with impact factor and other journals indexed BDI.

**I**. The results selected to highlight the evolution and development of their own career, after obtaining the PhD degree, have been presented in section **B.I**., differentiated into two research directions, in which their own achievements are documented by references to articles published (as sole author or first author) between 1999-2023, in the context of the current state of knowledge in the field, highlighting the relevance and originality of personal contributions. For each of the two strands we presented: a short introduction, the general working method, a selection of results and discussions, conclusions (in Romanian and English).

**1. Research on pollen characterization of plant species:**

Research on pollen morphology has made important contributions to the development of new guidelines in plant taxonomy and phylogeny. The morphological characteristics of pollen - true diagnostic characters of plants - vary between species, genus, and family. Pollen germination and pollen tube growth can be influenced by a number of internal and environmental factors, some of which have been highlighted and demonstrated in our own research.

Pollen morphological characteristics are also indirect indicators of the degree of polyploidy as well as pollen fertility. Germination capacity directly reflects pollen fertility. Knowing the germination capacity of pollen makes it possible to identify valuable genotypes as pollinators in interfertile

combinations, but also for artificial hybridizations, in order to use them as pollinators in breeding programs.

In all the cases studied, there is a close and direct correlation between pollen germination capacity and pollen tube length, as well as between pollen tube length and floral style length.

The morphology of pollen from different genotypes but from the same botanical family showed marked similarities, which was demonstrated in five genotypes of the *Vitaceae* family: *Vitis vinifera – Fetească neagră* variety, two varieties of direct producer hybrids (HDP) - *Noah* and *Othello*, two liane of the genus *Ampelopsis* - *A. aconitifolia* and *A. brevipedunculata*.

Pollen morphological features are not influenced by environmental conditions, which proves the involvement of major genes in pollen character enhancement. This was demonstrated by *Lotus corniculatus* pollen from an unpolluted stationary and a stationary polluted with fine cement dust from the cement factory in Taşca, in the area bordering the Ceahlău National Park.

*Rhododendron racemosum* pollen remains in pollen tetrads provided with viscin threads indicating a strict adaptation to certain pollinators. My personal conclusion from this case is that the plant consumes minimum energy with maximum efficiency of pollination and therefore fruiting.

The pollen of the hybrid lily *Lilium Star Gazer* is morphologically distinguished by the presence of a single germinal pore - an essential diagnostic feature of monocots.

In our own research we have shown that for optimal germination pollen prefers a certain concentration of carbohydrates in the germination medium depending on species. Thus, optimal germination is achieved on medium enriched with: 15-20% sucrose in grapevine varietie - *Fetească neagră*, *Ampelopsis aconitifolia* and *A. brevipedunculata*; 15-25% sucrose for direct producer hybrid (HDP) varieties *Othello* and *Noah* (genus *Vitis*).

The level of pollen germination capacity differs between species, even within the same botanical family. Thus, germination is: more than 80% in *Fetească neagră*, 35% in HDP *Noah*, 58% in HDP *Othello*, 79% in *Ampelopsis aconitifolia*, 80% in *A. brevipedunculata*. The higher germinability of *Othello* pollen can be explained by the presence of *Vitis vinifera* genes in its genotype.

Pollen germination in the five *Vitaceae* was significant in the first 24 hours after inoculation, with an insignificant increase in the following hours. In *Ampelopsis aconitifolia* and *A. brevipedunculata*, pollen tube degeneration occurred after 48 hours.

In all five *Vitaceae*, pollen tube formation was preceded by the formation of a vesicle, confirming the hypothesis that *Vitaceae* pollen germinates “in vesicle”.

*Lotus corniculatus* pollen surprises by its high level of germinability (over 90%) after the first 24 hours after inoculation on nutrient mediums and the viability of pollen tubes for over 168 hours under varying conditions of carbohydrate concentration in nutritive mediums, from 5% - 200% sucrose. This could explain the eco-physiological plasticity and wide range of this species. Furthermore, our own research has shown that pollen germination of *L. corniculatus* is not affected by the polluted environment, which may be evidence that pollen germination is controlled by major genes and is not influenced by environment.

*Rhododendron racemosum* pollen germinates optimally but slowly on 25%-40% carbohydrate mediums, reaching the upper limit of 95% after 192 hours when pollen tubes are still viable.

*Star Gazer* lily pollen impresses with its optimal germination capacity (93%) in the first 24 hours on carbohydrate-deficient substrate and the longest pollen tubes grown on the same very nutrient-poor substrate. This reflects the sugar-rich composition of lily pollen, which ensures its ability to germinate on poor and very low carbohydrate mediums.

Pollen germination and pollen tube growth were also investigated as a function of environmental temperature, with research carried out on *Galanthus nivalis* pollen at two temperature regimes: 18 °C and 4 °C. Germination of snowdrop pollen was optimal (over 90%) on nutrient mediums with 15%-20% sucrose at both temperatures during the first 24 hours.

The longest pollen tubes were formed on media with 10-25% sucrose at 18 °C and on media with 10-15% sucrose at 4 °C, which maintained their viability for 120 hours.

At the same carbohydrate concentrations in the nutritive mediums, pollen tube lengths were similar at both temperatures.

In most plant species, low temperatures not only inhibit pollen tube growth but also induce flower abortion. The results of this research provide new evidence that the male gametophyte of the snowdrop fowl is genetically programmed to thrive at low temperatures. The optimal germination rate and pollen tube growth at 4 °C highlights the vernal character of *G. nivalis* pollen.



**2. Research on the cytotoxic potential of some physicochemical factors:**

Extensive industrialisation in all areas of human activity is responsible for current environmental pollution of air, water, soil, food, etc. All pollutant noxious gases affect the ecological balance and impact the Earth's living creatures. The effects of pollution on organisms are: cytotoxicity, genotoxicity and therefore mutagenicity, teratogenicity, oncogenesis, phenotypic changes.

A physicochemical factor can be cytotoxic if it affects the mitotic index. In this case, the mitotic index may increase or decrease under the influence of different environmental factors. These situations lead either to disordered proliferation of cells that can form tumours or to a mitodepressant effect that slows down the body's growth. In both cases, the cytotoxic effect is induced by various physico-chemical factors that affect cell functions.

A physicochemical factor can be genotoxic if it has the potential to induce DNA or chromosome damage. Such changes to genetic material may be heritable if they occur in germ cells; or they may be non-heritable if they occur in somatic cells, in which case they may cause a somatic mutation leading to cancer. Genotoxins alter the molecular structure of DNA strands, causing gene, chromosomal, genomic mutations.

Among the physicochemical factors whose cytotoxic potential we have determined, we have selected a few, which will be presented below.

Sodium hexanitrogen cobaltiate III, sodium pentacyano-mononitrozo-ferrate III, lead nitrate, lead acetate and sodium nitrite were administered as aqueous solution treatments at concentrations of 5%, 1% and 0.1% with exposure times between 2 h and 48 h on *Allium cepa* seeds.

The effects induced by each of the five chemical compounds were a significant reduction in mitotic index, induction of chromosomal aberrations and micronuclei. The reduction in mitotic index and the frequency of genetic abnormalities were directly proportional to the concentration and time of action of the chemical compound. The type of genetic abnormalities induced and their frequency demonstrate the mode of action of the chemical compounds tested. Thus, chromosomal bridges, fragments, associations between bridges and fragments indicate the clastogenic effect of the chemical compounds evaluated; lagging chromosomes and multipolar ana-telophases demonstrate their aneugenic effect; micronuclei are evidence of the clastogenic and aneugenic action of the five chemical compounds tested.

Sodium nitrite is a food additive. Regarding its use in the food industry I recommend that the cytotoxic potential of this chemical compound be taken into account.



Lead nitrate also has the potential to induce autopolyploid cells by inhibiting the division spindle.

Grape marc considered as waste from the winemaking industry is commonly used in the food, pharmaceutical and medical industries. However, studies have reported that certain concentrations of grape marc extracts can induce negative effects in animals. Our own research on grape marc aimed to assess whether grape marc induces abiotic stress with serious negative implications on plants. For this purpose, wheat caryopses were treated for 48 hours with aqueous extracts of *Merlot* and *Sauvignon blanc* grape marc in four concentrations: 0.025%, 0.05%, 0.1% and 0.2%. The germination rate of treated caryopses and cytogenetic parameters were investigated. The germination rate decreased moderately compared to the control for all treatment variants. The cytogenetic parameters investigated were mitotic index and genetic abnormalities.

As grape marc concentration increases, germination rate and mitotic index decrease moderately, while the frequency of cells with chromosomal aberrations (bridges, fragments, associations between bridges and fragments, multipolar ana-telophases) and micronuclei increases.

Treatments with *Merlot* grape marc extracts induced a higher percentage of genetic abnormalities.

The results indicate the cytogenotoxic potential of grape marc extracts, through a moderate reduction in mitotic index (reflected in a moderate decrease in caryopsis germination rate) and the induction of genetic abnormalities, both effects being directly proportional to grape marc extract concentrations. Therefore, we have shown that grape marc induce abiotic stress on wheat genetically, which is why we recommend that grape marc be depleted in polyphenols before being stored in the field. We estimate that a possible use of unprocessed grape marc could be as a bio-herbicide.

The physicochemical factor tested was non-thermal plasma-activated water (PAW) which is used in agriculture mainly for surface decontamination of seeds/cariops with possible positive effects on physiological processes. In our own research we used PAW generated in ambient air at atmospheric pressure in eight variants with different pH and reactive species doses ($H_2O_2$, $NO_3^-$). Through this research I investigated the indirect effect of PAW on wheat caryopses, focusing on the effects on genetic material through cytogenetic monitoring.

All PAW variants caused clastogenic and aneugenic events of the genetic material, with different intensities, in a dose-dependent manner of reactive species in the plasma composition.

PAW variants with the highest doses of $H_2O_2$ (13-22 mg/L) and $NO_3^-$ (49-68 mg/L) at pH 3.8-4.1 reduced the mitotic index the most and induced the highest frequencies of genetic abnormalities, of which the chromosomal bridges and the micronuclei dominated.

In correlation with this damage to the nucleus, germination rate and root and shoot (hypocotyl) length of wheat were significantly decreased in the variants with the highest doses of reactive species.

The PAW variants with the lowest doses of $H_2O_2$ (1-5 mg/L) and $NO_3^-$ (8-15 mg/L) at pH 5.5-5.1 induced a much lower cytotoxic potential, maintained a germination rate comparable to the control and even stimulated root and shoot length growth. In conclusion, the effects of PAW depend essentially on the dose of reactive species and pH.

Demonstrating the cytotoxic potential of non-thermal plasma, I recommend that cytotoxicity tests be included in the PAW generation protocol in order to establish by screening the most appropriate concentrations of reactive species and pH compatible with plant genome stability.

**II. In the second section of this thesis** the proposed objectives for the further development of teaching and research activity (appropriate to the strategic research objectives of the faculty and the university), and implicitly the possibilities for their realisation, have been outlined.