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ONE HUNDRED YEARS OF PTERIDOLOGY IN ROMANIA

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Abstract
In the last one hundred year the pteridology domain had a spectacular evolution. In the first half of the century was noted the floristic and embryological researches; also, the editing the Flora RPR-RSR (Pteridophyta, first volume, 1952) was done. In the last two decades new data have been added regarding: morphology and anatomy of the pteridophytes, somatic and zygotic embryogenesis, ex situ and in situ conservation of the pteridophytes biodiversity, evaluation of the fern extracts bioactivity, usage of the spores and gametophytes in the toxicity tests, in vitro bioaccumulation capacity of the heavy metal, phytosynthesis of the silver nanoparticles. Current research seeks to obtain extracts and phytosynthesized nano-architectures with practical applications in a field of great importance at national level: combating the main fungal diseases affecting the vineyard culture and apple culture.

Keywords: biodiversity, embryogenesis, morphoanatomy, phytosynthesized nanoarchitectures, toxicity tests.

1. INTRODUCTION

The current paper presents a review of the research conducted in the last 100 years on the embryogenesis, morphoanatomy, biodiversity conservation and toxicology of pteridophytes as well as on the bioactivity of pteridophyte extracts. In addition to these research directions, which have been less addressed at the national level, there are numerous studies on flora and vegetation that also comprise data on pteridophytes. However, these data will not be detailed in this paper.

2. RESEARCH CONDUCTED BETWEEN 1918 AND 1968

Among the first research studies carried out in the field of pteridology are those by Aurelian Vlădescu, focused on the embryogenesis and organogenesis of leptosporangiate ferns. The research conducted for his Ph.D. degree comprises aspects of zygotic and experimental embryogenesis of leptosporangiate ferns, as well as aspects regarding apogamy, apospory and polyembryony. His Ph.D. thesis, "Recherches morphologiques et expérimental es sur l'embriogénie et l'organogénie des fougères leptosporangiées", was published in Paris in 1934 (Vlădescu, 1934).

In 1952, Romanian botanists began to publish "the scientific botanical work of Romania" (Mititelu, 1980-1993), namely the first volume of the Flora of the Popular Republic of Romania - Socialist Republic of Romania (Flora RPR-RSR), which includes the division Pteridophyta. The taxa presented in this volume were described by Gh. Grințescu, E.I. Nyarady, E. Pop, L. Alexandrescu and E. Țopa. Each species is presented along with its Latin and popular denominations, morphological description, period of spore formation, station, spreading area in the country, variability of species (subspecies, varieties and forms), uses, general spreading, as well as drawings

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combined in seven drawing sheets. In total, there are 71 pteridophyte species described in the above-mentioned volume (Grințescu, 1952).

3. RESEARCH CONDUCTED BETWEEN 1969 AND 2018

In 1969 Todor and collab. identified the species *Botrychium virginianum* in the Argeş county, thus adding new information about its chorology. In the first volume of the Flora RPR-RSR, the spreading of the species in the country was limited to the following localities: Săcărâmb (Hunedoara county), Moldovița (Moldova Nouă region), Cârlibaba, Țibău Valley of the Golden Bistrița River (Vatra Dornei region) (Grințescu, 1952). Ther herbarium sheet with the collected material is in the Scientific Herbarium of the University of Piteşti.

In 1999, at the "Babeş-Bolyai" University, Rodica Bercu defended her Ph.D. thesis, entitled "The Structural Organization of the Corm in Some Polypodiaceae (Pteridophyta) with Special Referral on the Vascular System", under the supervision of L.Ş. Peterfi. The findings of her research helped to enrich the specialist literature by 30 articles, a book chapter, "The corm structure at fern *Athyrium filix-femina* (L.) Roth" (In I. Moustakas, M. Tsecos (Eds.) Progress în Botanical Research, Chapter 4: Structure and its Dynamics, Kluwer Academic Publisher, 1998), and the monograph "Structura histo-anatomică a cormului la unele ferigi autohtone cu referiri asupra sistemului vascular" (2001).

In 2000 another Ph.D. thesis brought data on the root, stem and leaf anatomy of 30 native fern species. The thesis, entitled "Morphological and histo-anatomical researches on the Filicatae species (Pteridophyta) from Romanian flora" was written by Daniela Dorin from the "Al. I. Cuza" University of Iaşi, under the supervision of C. Toma.

An identification guide dedicated solely to the identification of pteridophytes was edited in 2000 by M. Andrei; his work includes identification keys for 59 native pteridophyte species.

Aspects of the zygotic and somatic embryogenesis of pteridophytes were presented in the Ph.D. thesis "Research upon zygotic and somatic embryogenesis in some pteridophytes" by Liliana Cristina Soare. The thesis was written under the supervision of M. Andrei. The author made original contributions regarding the *in vitro* culture of some native and allochthonous fern species with not fully differentiated green sporangia (sori) as explants, including that of *Osmunda regalis*, considered to be extinct in the flora of Romania. Furthermore, the thesis contains descriptions of prothallial cells that differentiate and carry trichomes and rhizoids (trichomic-rhizoidal), trichomes and antheridia (trichomic-antheridial) or rhizoids and antheridia (rhizoidal-antheridial) as well as a particular type of prothallial collenchyma.

Research on the *in vitro* culture of the threatened species *Marsilea quadrifolia*, especially on its morphogenetic, biochemical and ultrastructural aspects, was conducted by Cristian Banciu, a researcher at the Institute of Biology Bucharest, during his doctoral degree, under the supervision of dr. Brezeanu Aurelia, a scientific researcher (qualification level I). The biochemical aspects revealed the electrophoretic spectra of estherase, peroxidase, phosphatase, glutamate-oxaloacetate transferase and malate dehydrogenase in plants originating from their natural habitats, in plants acclimatized to soil conditions and also in plants regenerated *in vitro* (Banciu et al., 2009). The ultrastructural aspects focused on the leaf mesophyll cells of *in vivo* and *in vitro* plants, respectively (Brezeanu and Banciu, 2009).

In the period 2008-2011, the University of Piteşti coordinated a research project on the conservation of pteridophyte biodiversity in the protected area of the Vâlsan Valley. The aim of the project was the *ex situ* conservation, which was accomplished through the creation of a plant collection, conservation of spores, *in vitro* culture and conservation of pteridophytes, and *in situ* conservation.

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Ex situ conservation was achieved through the creation of a pteridophyte collection at INCDBH Ştefăneşti Argeş. At that moment, in 2011, the collection was composed of the following species: *Asplenium scolopendrium*, *A. trichomanes*, *A. trichomanes-ramosum*, *Blechnum spicant*, *Athyrium filix-femina*, *Cystopteris fragilis*, *Dryopteris affinis*, *D. filix-mas*, *D. carthusiana*, *D. dilatata*, *Equisetum hyemale*, *Phegopteris connectilis*, *Polypodium vulgare*, *Polystichum aculeatum*, *P. setiferum*, and *P. braunii* (Soare et al., 2011).

Ex situ spore conservation consisted in dry and wet conservation of the species *Asplenium scolopendrium*, *Athyrium filix-femina*, *Dryopteris affinis*, *Phegopteris connectilis* and *Polypodium vulgare* at 5 and 20°C (Soare and Aldoiu, 2010). The conservation of pteridospores for a period of one year indicated that in the species *Asplenium scolopendrium* and *Athyrium filix-femina* the viability of spores is maintained mainly at 20°C, while in the species *Dryopteris affinis*, *Phegopteris connectilis* and *Polypodium vulgare* conservation is more efficient at 5°C.

The research on the *in vitro* culture and conservation of pteridophytes was performed at The National Institute for Research and Development for Biotechnology in Horticulture (INCDBH) Ştefăneşti, Argeş and at the Institute of Biology Bucharest. The researchers of INCDBH Ştefăneşti, Argeş developed the technology for the in vitro multiplication and conservation of pteridophyte gametophytes and sporophytes from sporangia (sori) in the species *Asplenium trichomanes*, *A. trichomanes-ramosum*, *Athyrium filix-femina*, *Cystopteris fragilis*, *Dryopteris affinis*, *Phegopteris connectillis*, and *Polypodium vulgare*, and the group of researchers from the Institute of Biology Bucharest developed the technology for the *in vitro* multiplication and conservation of pteridophyte gametophytes and sporophytes by gametophyte homogenization in the species *Asplenium trichomanes*, *Athyrium filix-femina*, and *Polypodium vulgare*. The two technologies encompassed all the stages, starting from the initiation of culture to the transfer of rooted plants into the soil in the external environment. A part of these species was included in the *ex situ* pteridophyte collection and the material thus obtained was also analyzed from a physiological and biochemical point of view (Soare et al., 2011).

As regards the *in situ* conservation, the research showed that the area under study is characterized by a high specific diversity; in the field, 30 pteridophyte species were identified, representing 42.85% of the total autochthonous species. Out of the 11 species considered to be sporadic in the flora of Romania, *Huperzia selago*, *Botrychium multifidum*, *Blechnum spicant*, *Polystichum braunii*, *Gymnocarpium robertianum*, *Dryopteris dilatata* and *Dryopteris expansa* are present with a small number of populations, each of them with a low number of individuals. *Botrychium multifidum*, a species mentioned in the documents of the Bern Convention, was identified in only one site, in which there were only a few specimens (Soare et al., 2011).

Other research studies regarding the *in vitro* micropropagation of some endangered species (*Asplenium adulterinum* and *Osmunda regalis*), the proteomic profile of the gametophyte and sporophyte of the species *Athyrium filix-femina* obtained by bidimensional electrophoresis (Aldea et al., 2013), etc. were conducted by Florentina Aldea during her Ph.D. studies, under the supervision of dr. Aurelia Brezeanu. The cryoconservation of the species *Athyrium filix-femina* was also tested by the same team of researchers from the Institute of Biology Bucharest (Banciu et al., 2013).

The *in vitro* morphogenesis of some ornamental alochthonous species such as *Nephrolepis exaltata* was also observed (Pop Maghiar, 2012).

The preoccupation with this research field led to the idea of establishing a professional association. Thus, in 2012, a group of teaching staff from the University of Piteşti succeeded in founding the Romanian Pteridological Society. Researchers from other institutions, such as the University of

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Bucharest, Institute of Biology Bucharest, The National Institute for Research and Development in Chemistry and Petrochemistry (ICECHIM) Bucharest, etc. have joined the association ever since.

Since 2012 new research themes have emerged in Romanian pteridology, specifically those concerning the biological activity of extracts obtained from native fern species. As a result, tests were performed to establish the antimicrobial activity of raw methanol extracts from leaves of *Athyrium filix-femina*, *Dryopteris affinis* and *D. filix-mas* against saprophytic and parasitic microorganisms (Soare et al., 2012a) and new therapeutical formulations were obtained by associating extracts and antibiotics (Soare et al., 2012b). The antimicrobial activity of the gametophytic tissues obtained *in vitro* for the species *Asplenium trichomanes*, *Asplenium trichomanes-ramosum*, *Cystopteris fragilis* and *Polypodium vulgare* (Soare et al., 2012c; Deliu et al., 2013) was also tested.

The use of pteridophytes in acute and chronic toxicity tests has represented another direction of research in Romania since 2013. Both pteridophyte spores and gametophytes have the necessary chracteristics to be used in toxicity tests (Catalá et al., 2009; Rodriguez-Gil et al, 2010; Marugán et al., 2012). The influence of some heavy metals and pesticides on spore germination and gametophyte differentiation was tested and modifications were recorded, such as the damage caused to the germination process, the delay in gametophyte differentiation, disruption of rhizoid elongation, formation of a tridimensional cellular mass, etc. (Soare et al., 2013 a, b, c; Soare et al., 2014) New data were gathered regarding the influence of heavy metals on the amount of chlorophyllous pigments and polyphenols in pteridophyte gametophytes (Drăghiceanu et al., 2015; Drăghiceanu and Soare, 2016), as well as on the accumulation of cadmium on the gametophyte surface, a property with an extremely important potential for bioremediation (Drăghiceanu et al., 2016a).

Bioaccumulation of lead was investigated *in vitro* using gametophytic and sporophytic tissues taken from the following species: *Asplenium scolopendrium, Asplenium trichomanes-ramosum, Cystopteris fragilis, Polypodium vulgare*. The highest bioaccumulation coefficient was recorded in the species *Asplenium scolopendrium* for a concentration of 573.64 $mg.g^{-1}$ Pb in its culture environment (Soare et al., 2015).

After 2016 the research has been oriented towards the phytosynthesis of nanoparticles in pteridophyte extracts, as well as towards their analytical and biological characterization (Șuțan et al., 2016, Soare and Șuțan, 2018). Researchers performed the phytosynthesis of silver nanoparticles in leaf extracts of *Asplenium scolopendrium* (Șuțan et al., 2016), in spore extracts of *Athyrium filix-femina* (Soare and Șuțan, 2018), in leaf extracts of *Polystichum setiferum*, etc.

The current research is directed at obtaining some extracts and phytosynthesized nanoarchitectures with applications in the control of fungal diseases that affect apple and vine crops.

4. CONCLUSIONS

At the national level, in the last 100 years, pteridological research has been centered around the following themes: embryogenesis, morphoanatomy, biodiversity conservation, toxicology, biological activity of extracts, and nanoparticle phytosynthesis. Besides these studies, there are many others dealing with the flora and vegetation which also include data on pteridophytes. Over the last one hundred years, Romanian pteridology has had a spectacular evolution: from plant identification to nanotechnologies.

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