SUMMARY

of the doctoral thesis entitled:

STUDIES AND RESEARCH ON THE DISTRIBUTION RANGE, BIOLOGICAL CHARACTERISTICS, AND CONSERVATION AND PROPAGATION POSSIBILITIES OF CERTAIN ORCHID SPECIES FROM THE SPONTANEOUS FLORA OF ROMANIA

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The research included in this doctoral thesis was conducted at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, within the Faculty of Horticulture, as well as in the Plant Micropropagation Laboratory, an integral part of the Research Center for the Study of the Quality of Agri-food Products.

In this study, spontaneous orchid species were identified and characterized to provide an overview of the orchid flora in Romania. Additionally, various objectives were proposed to support the conservation and protection of these species, as well as to identify modern biotechnologies for the *in vitro* multiplication of certain species with significant horticultural value.

Chapter I offers a brief analysis of the history of orchid discovery, the origin and distribution of the Orchidaceae family and presents various estimates regarding the total number of species at the global level. In subchapter 1.1, the fascination that this family of plants has exerted on humanity, from its initial discoveries to the present day, is discussed, highlighting the fact that the history of orchids is closely linked to the evolution of human civilization.

Subchapter 1.2 presents, succinctly, the history of orchid seed germination, a process that has constituted a true mystery for botanists for centuries. This problem was elucidated only in 1899, by the French botanist Noël Bernard (1874–1911), who, through his pioneering observations, highlighted the essential role of mycorrhizal fungi in the germination process of orchid seeds. Subsequently, in 1921, the American

physiologist Lewis Knudson (1884–1958) developed the first asymbiotic method for germinating orchid seeds, nearly 400 years after they were first observed by the Swiss physician and naturalist Conrad Gessner (1516–1565).

Subchapters 1.3 and 1.4 provide information regarding the dynamic evolution and global distribution of the Orchidaceae family. With an existence of approximately 120 million years, orchids have managed to colonize all continents and climatic zones of the planet, adapting to a wide variety of habitats, which has allowed them to become, to date, the second most numerous family of plants. The taxonomic classification and estimates regarding the number of Orchidaceae species are detailed in subchapters 1.6 and 1.7.

Chapter II analyzes the general morphological and biological characteristics of this extremely diverse family of plants. The research details the structure of the vegetative organs, emphasizing the unparalleled complexity of the flowers, which distinguishes them from other plant families. The variability and extravagance of colors, forms, sizes and scents of orchid flowers, along with the complex structure of the gynostemium, give them a unique character among angiosperms. Additionally, a series of images obtained through modern macro photography techniques are included, illustrating, for the first time in Romania, the fascinating morpho-anatomical details of the structure of orchid flowers.

In the second part of the thesis, Chapters III and IV present the results of the author's research, including both taxonomic studies and those related to the conservation and protection of orchids through various methods, which include asymbiotic germination methods for taxons with significant horticultural value from the genus *Dactylorhiza*.

Chapter III states the purpose and objectives of the doctoral thesis, as well as the materials and methods used in the study of spontaneous orchid taxa.

To obtain a comprehensive view of the Orchidaceae family in Romania, the study carried out between 2019 and 2024 included all known wild orchid taxa to date. The research was conducted in most national and natural parks in Romania, in certain protected areas, as well as on private properties that host a significant number of wild orchid species. Additional details are presented in subchapters 3.2.2 and 3.2.3.

In the study of the distribution of orchid taxa, outlined in subchapter 3.2.4, the nine main types of habitats preferred by these taxa were analyzed, covering all altitude ranges present in our country. Subchapter 3.2.5 discusses the various mimicry strategies and pollination mechanisms observed for each taxon, based on field observations.

Measures and suggestions regarding the conservation of spontaneous orchids are included in subchapter 3.2.10. The study also contains assessments of the conservation status of all orchid taxa at the national level, conducted in accordance

with the guidelines and standards of the International Union for Conservation of Nature (IUCN).

Subchapter 3.2.11 describes the materials and methods of research used for the asymbiotic germination of immature seeds of the species *Dactylorhiza maculata*, intending to conserve this taxon and reintegrate the individuals into their natural habitats.

Chapter IV presents the results obtained over the five years research project (2019-2024), both from a taxonomic perspective through field studies and an *ex-situ* asymbiotic germination of orchid seeds, carried out in the Plant Micropropagation Laboratory within the Research Center for the Study of the Quality of Agrifood Products.

Subchapters 4.1.1 and 4.1.2 include taxonomic results, providing a general overview of the Orchidaceae family in Romania, which includes both previously described taxa and those recorded for the first time in the international flora (IPNI). Thus, the results indicate a total of 111 orchid taxa (72 species, 16 subspecies, 16 intrageneric hybrids, and 7 intergeneric hybrids) distributed among 24 genera that belong to the three major subfamilies of Orchidaceae. The new taxonomic descriptions made in this study are detailed in subchapters 4.1.3.1 (taxa described for the first time globally) and 4.1.3.2 (taxa described for the first time nationally).

In addition, a detailed study of the endemisms of the orchid flora at the national level is included in subchapter 4.1.4. The research results highlighted the existence of 11 endemic taxa of the indigenous orchid flora, described in subchapter 4.1.4.1, as well as 12 subendemic taxa, detailed in subchapter 4.1.4.2.

The results regarding the distribution of orchid taxa at the family and genus levels are presented in subchapters 4.1.5 and 4.1.6. The distribution of the ranges of the Orchidaceae in Romania and how the taxa are distributed according to habitat types are detailed in subchapter 4.2. In subchapter 4.2.2, the distribution of orchid taxa based on altitude range and root type is analyzed. The results demonstrated that the maximum density of taxa is found in the medium and upper areas, within the altitude range of 700 to 1700 meters. Additionally, taxa characterized by ovoid-spherical tubers predominately occur in low areas, while those with digitated tubers are more frequently found in higher subalpine and alpine regions.

Subchapter 4.2.3 presents the results concerning the general ecological correlations of orchid taxa.

In subchapter 4.3, the annual vegetaive life cycles and the durations of the antheses (flowering periods) characteristic of the orchid flora in Romania are discussed. Furthermore, studies of annual vegetaive life cycles are correlated with root types. The results of these studies demonstrate that taxa with green leaves during the winter (taxa that have migrated from their Mediterranean origin to northern Europe)

have largely maintained their specific phenological type, producing leaves at the beginning of the Mediterranean autumn, from September to November. The analysis revealed that they are exclusively characterized by hypogeal organs of the ovoidal-spherical tuber type.

Subchapter 4.4 analyzes the types of pollination and the mimetic strategies utilized by temperate/Romanian orchids. In this context, it includes and describes the seven types of deceptive mimetic strategies employed by non-rewarding taxa (nectarless) to attract pollinator insects through deception.

Additionally, subchapter 4.4 contains suggestions regarding conservation measures for orchids in Romania. Subchapter 4.5.1 presents a study on assessing the levels of threat and risk factors for orchids. In subchapter 4.5.2, conservation measures are proposed, both *in-situ* (actions undertaken to protect species and their habitats in their natural environment) and *ex-situ* (actions aimed at protecting species or ecosystems outside their natural environment, through the establishment of various asymbiotic or symbiotic germination programs on a large scale).

Subchapter 4.5.2.2 focuses on conservation measures based on legislative methods. The results of detailed studies regarding the conservation status of the taxa have led to proposals and suggestions for the first Red List of Romanian Orchidaceae (2024), presented in subchapter 4.5.2.2.2 and Appendix XI.

The inclusion and publication of this first version of the Red List exclusively for the orchids of Romania represents a crucial first step in raising awareness about the vulnerability of a significant number of orchid species, some of which are already threatened or at risk of extinction at the regional and national levels.

At the same time, this initiative would represent a first step towards integrating Romania into the group of Central European countries that implement national conservation measures, a group from which Romania is currently absent (see Appendix XIII).

Subchapter 4.6 presents the results and suggestions for the implementation of efficient asymbiotic germination procedures for the immature seeds of the species *Dactylorhiza maculata*. This species has significant horticultural value due to its spectacular appearance and unmatched chromatic variation of its flowers. *Dactylorhiza maculata*, whose wet habitats have suffered severe reductions in recent years, is the subject of *ex-situ* conservation measures aimed at developing effective large-scale propagation methods and acclimatization of individuals, cultivated in greenhouses and laboratories, back into their natural environments.

Subchapters 4.6.1-4.6.6 include the results of embryological analyses and detailed descriptions of the germination stages, emphasizing the specific stages of protocorm and young seedlings characteristic of Orchidaceae. Additionally, subchapter

4.6.10 is dedicated to the results concerning the efficiency of germination and the subsequent development of seedlings on various growth media.

Chapter V synthesizes the conclusions and recommendations formulated following the conducted research. The bibliography includes the sources consulted for the studies and research necessary for the development of this doctoral thesis.

The doctoral thesis is structured into 5 chapters, comprising a total of 140 pages, of which 40 pages are dedicated to the bibliographic study, while 98 pages contain original research, conclusions, and recommendations. Additionally, the thesis includes 29 tables and 74 original figures.

Additionally, the work includes 11 annexes dedicated to detailed studies on spontaneous orchids in Romania, conducted for the first time in this region.

These appendices encompass a complete taxonomy of orchids from Romania's flora, with newly discovered and described taxa during doctoral studies conducted between 2020 and 2024. The distribution of taxa is analyzed according to habitat types and altitudinal zones.

Moreover, the appendices include in-depth studies on annual vegetative cycles, flowering periods (anthesis), types of pollination (allogamous, autogamous), and the types of rewards offered by orchids (rewarding, non-rewarding).

The mimicry strategies used by non-rewarding (deceptive) species to attract pollinators are also discussed in detail.

Appendix XI presents an extensive study and proposes an initial version of the Red List of Romanian Orchidaceae, aiming to conserve this family of rare plants.

Furthermore, a list of publications is included, comprising 13 scientific articles (5 of which are indexed by ISI and the remaining 8 are included in the BDI database), two scientific books, and 43 scientific articles published within international orchid societies.

All articles contain original results that have contributed to the present study and are cited in the thesis.

These contributions showcase the efforts committed to orchid research and their influence on both national and international scientific communities.