PhD THESIS

Assessing pear (*Pyrus* spp.) response against major diseases and pests and identifying useful genotypes for new breeding programs

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INTRODUCTION

Pear species (*Pyrus* spp.) represent important natural resources for biodiversity, as well as for the conservation and genetic improvement of cultivated species. Pear is an important fruit tree species, both worldwide and in Romania, which provides fruits that are particularly appreciated in current consumption, as fresh fruits, but which are also widely used in the processing industry (KORBAN, 2019; SIMIONCA MĂRCĂȘAN et al., 2022, 2023). Although there are thousands of varieties of pear worldwide, many of them present certain deficiencies, including: sensitivity to the attack of diseases and pests, sensitivity to the action of some abiotic stress factors, alternation in fruiting, poor fruit quality, including by the low content in useful substances, poor fruit preservation, high perishability, etc. (SESTRAȘ, 2004; KORBAN, 2019). In addition, relatively few cultivars are widely distributed and cultivated, so only a small fraction provides significant fruit production globally. An extremely limited number of varieties (around ten) have been found to contribute more than 90% to the world's pear production (QUINET and WESEL, 2019). The long selection carried out to obtain varieties with high production, large fruits and good quality, as well as the small number of varieties widespread in the world, determines a high degree of genetic vulnerability of the cultivated species (SIMIONCA MĂRCĂȘAN et al., 2022, 2023).

Pyrus spp. is also considered to be at increased genetic risk because wild species populations are rapidly declining due to the sixth mass extinction event. So, species of the genus *Pyrus* are vulnerable to different stress factors. The biodiversity of species and varieties of pear provide multiple sources of genes of interest for the new cultivars and for obtaining fruit productions that contribute to ensuring the food resources of mankind (given that the Earth's population has exceeded eight billion). Also, pear genetic resources have an immeasurable value from an ecological point of view, contributing to the diversity and sustaining of the living world, and being used by humans for various food, forestry, landscaping, pharmaceutical, etc. purposes. Widening the genetic diversity within the cultivated species and obtaining varieties resistant to various stress factors are highly topical desires, for the achievement of which it is necessary to preserve and study the germplasm fund, in order to identify and use the existing gene sources appropriately.

STRUCTURE OF THE DOCTORAL THESIS

The doctoral thesis entitled "Assessing pear (*Pyrus* spp.) response against major diseases and pests and identifying useful genotypes for new breeding programs" is structured in two main parts, totalling 150 pages and contains 8 chapters, 12 tables, 30 figures and 338 bibliographic references.

The first part of the thesis consists of a bibliographic investigation of research related to the issues pursued in the thesis and is structured in 3 important chapters. **Chapter 1** summarizes the information on the importance, origin and history of the genus *Pyrus* L**. Chapter 2** includes aspects related to the genus *Pyrus* L., presenting the cytogenetic and biological peculiarities of the pear and aspects regarding the culture and breeding of the species. **Chapter 3** presents aspects regarding the main objectives of pear breeding, namely fruit quality (shape, colour, texture, taste, and aroma), as well as resistance to attack by the main pests (*Psylla* spp.) and diseases (fire blight, pear scab, monilia, septoria and pear rust).

The second part of the doctoral thesis is represented by the personal contribution, through the prism of the results obtained from the research, being structured into five chapters. In this part, the main results of the research carried out during the doctoral studies, which were capitalized by publication in specialized journals, are presented. Three articles were published in peer-reviewed journals indexed in recognized academic databases, all with impact factor (IF), two in ISI journals located in Q1, and one article in a Q4 journal. **Chapter 4** presents the purpose and objectives of the research. The aim of the research was to investigate the existing phenotypic and genotypic diversity of Pyrus species and varieties, as well as their response to the main biotic stress factors, represented by the diseases and pests with the highest frequency in Romania. The research undertaken also sought to identify genetic resources that can later be used as parental forms in artificial hybridizations, thus increasing the efficiency of future pear breeding programs.

In accordance with the proposed purpose, the main objectives of the research were:

• Evaluation of the genetic resources within the genus *Pyrus*, represented by species, native varieties (Romanian ones), and foreign varieties.

• Analysis of the main phenotypic characteristics of pear genotypes and their response to the attack of the most important pear diseases and pest attack.

• Identification of rapid and efficient methodologies for determining the frequency, intensity, and degree of attack of the main pear diseases, allowing the evaluation of many genotypes (e.g., species, varieties, hybrids) in a pragmatic and objective way.

• Collection of new data and useful information on the population structure and biology of the main pests of pear (*Psylla* sp.), with a view to improving pest control and orchard management.

• Analysis of the genetic diversity of pear genotypes using SSR and RAPD molecular markers, correlation of molecular results with phenotypic ones, especially regarding the attack response to the main diseases and pests, and multivariate analysis for the use of complex data and obtaining information with scientific impact and application for pear breeding and crop.

Chapter 5 presents the materials and methods used to identify and evaluate the response of *Pyrus* genotypes to pest attack and the main diseases, the study of some interesting phenotypic characteristics of pear genotypes and their response to the most important diseases and pest attack, monitoring the emergence and the evolution of pests with the help of specific traps, as well as the methodology used to analyse the genetic diversity of pear genotypes with the help of SSR and RAPD molecular markers.

RESULTS AND DISSCUTIONS

In **Chapter 6**, the results obtained in the research related to the doctoral studies are presented, in the form of scientific articles published in specialized journals. The chapter includes three subchapters, each of which is represented by a research problem carried out according to the proposed objectives. The results obtained within the three research directions were capitalized in three articles published in ISI journals (WoS, with IF – impact factor).

The first theme addressed was the importance of assessing the population structure and biology of *psylla* species for pest monitoring and management in pear orchards. The monitoring and evaluation studies of the evolution of insects that attack pear, carried out in the north of Romania, in an area of tradition for the culture of fruit trees (Cireșoaia, Bistrița-Năsăud County), showed that the main pests of pear orchards were psyllids, called the "melliferous fleas" of the pear. They are Homoptera of the species *Cacopsylla* spp. (or *Psylla* spp.) and are considered the most dangerous pests of pear varieties belonging to the species *Pyrus communis* L., both in Europe and North America.

The investigations carried out provided in-depth information on the biology and

evolution of the two identified species, *Cacopsylla pyri* L. and *C. pyricola* Förster. The relative abundance of *C. pyri* was found to be significantly higher than that of *C. pyrisuga*. No specimens of the species *C. pyricola* and *C. bidens* were identified, and based on the results and bibliographic investigations, it was hypothesized that *C. bidens* is synonymous with *C. pyricola* Förster. In the most common species, *C. pyri* L., the adult insects of the hibernating generation started their activity in the first half of March, and the insects developed three generations: generation I (March-June), generation II (June-July) and the 3rd generation (August-March), with the possibility of some generations overlapping. The experimental results confirmed that psyllids are strongly attracted to the yellow colour of sticky traps, which are effective in monitoring the biological cycle of pests. The number of adults captured on the yellow traps was very high, indicating a greater preference of psyllids for sun-exposed areas of tree crowns. The Mann-Whitney U test demonstrated that the mean number of psyllids/traps was significantly higher ($p < 0.05$) on the southern canopy exposure compared to the northern exposure.

In the study carried out, slightly varied climatic circumstances between the two years had an insignificant effect on the development of phenology and generations of *Psylla*. The extension of such studies over a longer period is necessary and could better highlight the influence of climatic conditions on the response of psyllids to environmental factors, especially temperature. There is a clear trend of temperature increase in the study years compared to the multi-year average of the last 30 years. In these conditions, a large infestation of the 'Williams' variety was highlighted, the psyllids causing the appearance of 'honeydew' on the organs of the trees and later sooty, as well as the premature aging of the trees. In the monitored area, insecticide treatments were applied after the emergence of the larvae of each generation, but the droppings of the pests and the formed 'honeydew' reduced their effectiveness. Instead, the applied insecticides probably affected the populations of zoophagous species observed in the area, including *Anthocoris nemoralis* F. (Hemiptera: Anthocoridae), *Forficula auricularia* L. (Dermaptera: Forficulidae) and *Chrysopa* spp. (Neuroptera: Chrysopidae). The observations confirmed that variations in the density of psyllid populations can be determined by the interaction between the dynamics of climatic factors and the activity of zoophages, and *Psylla* sp. it can develop gradations at certain time intervals, a phenomenon also reported in other pest species.

The results obtained in this research were published in an ISI journal (WoS): **SIMIONCA MĂRCĂȘAN, L.I.**, HULUJAN, I.B., FLORIAN, T., SOMSAI, P.A., MILITARU, M., SESTRAS, A.F., OLTEAN, I., SESTRAS, R.E., 2022, The importance of assessing the population structure and biology of psylla species for pest monitoring and management in pear orchards. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*

50(4):13022 [IF 1.249, Q4] https://doi.org/10.15835/nbha50313022

The second research theme focused on the response of pear genotypes to the attack of the main diseases and pests and the comparative analysis of phenotypic and molecular data. The study was carried out in a germplasm collection of pear species and cultivars, and the research results were published in a peer-reviewed journal. The bibliographic investigation that preceded this original research revealed that within the genus *Pyrus* there is a wide genetic variability, represented by at least 22 recognized species of pear and more than 6,000 European and Oriental varieties. Although the diversity of genetic resources is relatively large, cultivated pear is vulnerable to the action of abiotic and biotic stress factors. Trees are often susceptible to the action of pathogens and pests, which limit both yield and fruit quality. Of the many pear diseases, the most dangerous is fire blight (bacterial disease of rosaceous plants) caused by *Erwinia amylovora*, which can even compromise plantations. Large damages can also cause some fungal diseases, e.g. pear scab (*Venturia pyrina*) and septoria (*Septoria pyricola*), frequently found in orchards in Romania. Among the pests, psylla species (*Psylla* sp. or *Cacopsylla*) can cause high production losses and affect the sustainability of orchards.

The study of the phenotypic and genotypic diversity of the response to diseases and pests of the different genotypes from the germplasm collection at SCH Cluj-Napoca, represented by 13 species of *Pyrus*, 17 Romanian varieties and 50 foreign varieties, allowed the identification of some potential parents for future programs of pear breeding. Thus, the genotypes with the lowest degree of attack on pear scab (Venturia pyrina) were: *Pyrus persica*, *P. lindlezi* and *P. longipes* (among species); 'Napoca', 'Ina Estival' and 'Haydeea' (from the Romanian varieties); 'Er Shi Shinge', 'Kristalli', 'Okusankichi', 'Olivier de Serres' and 'Précoce Trottier' (among foreign varieties). The best response against septoria was recorded in *P. persica* and *P. nivalis* (species); 'Primadona' and 'Republica' (Romanian varieties); 'Williams', 'Pierre Corneille' and 'Williams Bovey' (foreign varieties). No symptoms of *E. amylovora* attack were recorded in six species, six Romanian varieties, and 15 foreign varieties. Tolerance to *Psylla* attack was recorded in *P. lindlezi*, *P. persica* and *Sorbopyrus* (species); 'Haydeea' and 'Adria' (Romanian varieties, new creations obtained at SCH Cluj-Napoca); 'Olivier de Serres', 'Curé', 'Laxton Superb', 'Okusankichi' and 'Précoce Trottier' (foreign varieties). The identified genotypes could provide sources of genes of interest for increasing resistance to the analysed biotic stress factors.

The molecular investigations carried out with the SSR markers recommended within the European Cooperation Program for Plant Genetic Resources (ECPGR) revealed the genetic diversity of the studied accessions, among which were also well-

known varieties, e.g., 'Abbé Fetel', 'Conference' and 'Williams'. The phenotypic dendrogram for the response of the genotypes to the attack of pathogens and psyllids, as well as the molecular one, revealed the closeness or distance between the genotypes and their phylogenetic relationships, indicating possible ways to induce heterosis in the desired directions through pragmatic hybridization programs and genetic recombination. In addition, SSR markers can also be useful in identifying situations of homonymy and synonymy between genotypes, contributing to the reduction of management costs in germplasm collections by avoiding redundant accessions.

The results obtained in this research were published in an ISI journal (WoS) as follows: **SIMIONCA MĂRCĂȘAN, L.I**., OLTEAN, I., POPA, S., PLAZAS, M., VILANOVA, S., GRAMAZIO, P., SESTRAS, A.F., PROHENS, J., SESTRAS, R.E., 2023, Comparative analysis of phenotypic and molecular data on response to main pear diseases and pest attack in a germplasm collection. *International Journal of Molecular Sciences* 24(7):6239 [IF 6,208, Q1] https://doi.org/10.3390/ijms24076239

The third theme addressed was dedicated to *Pyrus* species and the complex potential they have as gene resources. The evaluation of the morphological and genetic peculiarities of the *Pyrus* species from the germplasm collection at SCH Cluj-Napoca and the bibliographic investigations related to the subject have highlighted the fact that the pear is a polyvalent species, which offers multiple possibilities of use, in various fields such as horticulture, agriculture, forestry, landscape, pharmaceutical, etc. Based on the results regarding tree growth and vigour, *P. lindlezi*, *P. betulaefolia* and *P. canescens* could represent valuable sources for wood production and improve the ability of trees to accumulate woody mass. It has been hypothesized that the plurivalence of some pear species, including in terms of tree growth and tree architecture (habitus), is the result of their biological versatility, adaptability (ecological plasticity) and favorable response to the ecological conditions in which the testing was carried out. Among the analysed species, the largest flowers were recorded in *P. lindlezi*, and *P. caucasica, P. variolosa* and *P. persica* had the largest fruits. Other species, such as *P. betulaefolia*, *P. drovara and P. caucasica* have been recorded as having flowers with large petals and corollas, showing decorative potential and possibilities for use in landscaping. Because *P. communis* cultivars are self-incompatible, wild species can also be used as pollinators in commercial pear orchards.

Similar to the cultivated pear, wild species of *Pyrus* are attacked by a large number of pathogens and pests. The main diseases that were identified under natural circumstances of occurrence and spread were *E. amylovora*, *V. pyrina*, *S. pyricola*, *Monilinia fructigena* and *Gymnosporangium sabinae*. The most frequent pests were *Psylla* spp., *Phyllonorycter corylifoliella*, *P. blancardella*, *Stigmella malella*, *Leucoptera*

scitella, *Neurotoma flaviventris*, *Aphis pomi* and *Eriophyes pyri*. A higher overall susceptibility to disease was observed in *P. caucasica* and *P. drovara*, and to pest attack in ×*Sorbopyrus*. On the other hand, *P. ×malifolia*, *P. korshinskyi* and ×*Pyronia veitkii* were recorded as having the lowest cumulative disease susceptibility scores, and *P. drovara*, *P. ussuriensis* and *P. betulaefolia* had the lowest scores to pests. The morphological diversity was also confirmed by the molecular one, and the dendrograms revealed the correspondence of the phenotypic and molecular relationships (using RAPD markers), but also confirmed the difficulties in evaluating the phylogenetic relationships and in the classification of *Pyrus* genotypes. The study revealed a high level of morphological and genetic variability between species, different geographical origins, or even accessions from the same geographical areas.

The results of the study highlighted the diversity of *Pyrus* species, and the great variability of response to diseases and pests specific to the study area offered the possibility of identifying and recommending possible sources of genes for new research programs. Among these can be found new, original directions for our country, among which the exploitation of the ornamental potential of pears by creating ornamental cultivars, used in landscaping and which, apart from beautifying green spaces, also have a significant ecological impact, or some cultivars to contribute to increasing the forestry and silvocultural value of pear trees.

The results obtained in this research were published in an ISI journal (WoS), as follows: **SIMIONCA MĂRCĂȘAN, L.I.**, POP, R., SOMSAI, P.A., OLTEAN, I., POPA, S., SESTRAS, A.F., MILITARU, M., BOTU, M., SESTRAS, R.E., 2023, Comparative evaluation of *Pyrus* species to identify possible resources of interest in pear breeding. *Agronomy 13(5):1264.* [IF 3,949, Q1]

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CONCLUSIONS

Chapter 7 is designated to the conclusions based on the obtained results.

Conclusions on the monitoring and analysis of the evolution of pest (*Psylla*) populations in pear orchards

Cacopsylla pyri L. was the most frequent psylla species found in pear plantations in the Cireșoaia region, located in the northern part of Romania, with three generations produced each year, which may or may not overlap. No specimens of the third species which appears in pear orchards in different areas, *C. pyricola*, nor of the *C. bidens* species were identified. The mean number of psyllids/traps was significantly higher ($p < 0.05$) on the southern tree crown exposure compared to the northern exposure. The

slightly varied climatic circumstances between the two years had an insignificant effect on the evolution of pest phenology and generations during the year. The extension of such studies over a longer period is necessary and could better highlight the influence of climatic conditions on the response of psyllids to environmental factors, especially temperature.

Conclusions on the diversity of *Pyrus* genotypes in response to the attack of the main diseases and pests

Among the many diseases of pear, the most dangerous is fire blight caused by *Erwinia amylovora*, but great damage can also be caused by pear scab (*Venturia pyrina*) and septoria (*Septoria pyricola*), which are also frequently found in orchards in Romania. Among the pests, psylla species (*Psylla* sp. or *Cacopsylla*) can cause high production losses and affect the sustainability of orchards. The investigation of the phenotypic and genotypic diversity of the response to diseases and pests of the different genotypes from the germplasm collection at SCH Cluj-Napoca, represented by 13 species of *Pyrus*, 17 Romanian varieties and 50 foreign varieties, allowed the identification of potential parents for future programs of pear breeding. The phenotypic dendrogram for the response of the genotypes to pathogen and psyllid attack, as well as the molecular dendrogram, revealed the closeness or distance between the genotypes and their phylogenetic relationships, indicating possible ways to induce heterosis in the desired directions through pragmatic hybridization programs and genetic recombination.

Conclusions regarding the study of some particularities of interest of rustic species of pear and their response to the attack of the main diseases and pests.

Like cultivated pear, wild species of *Pyrus* are affected by many pathogens and pests. No major problems occurred during the years of research, but previous fire blight and psyllid attacks resulted in significant losses, including the disappearance of some genotypes from the germplasm collection. The results of this study highlighted the differences between *Pyrus* genotypes and the great variability of their response to diseases and pests specific to the area where the research was carried out. This large diversity offered the possibility of identifying and recommending possible sources of genes, respectively parental forms useful for different breeding objectives and future research programs. Among these, we can also find some new, original directions for our country, among which the exploitation of the ornamental potential of pear by creating cultivars to be used in landscaping and which, in addition to beautifying green spaces, also have a significant ecological impact, such as an increasing the forestry importance and silvocultural value of pear trees.

RECOMMENDATIONS

As a result of the research related to this PhD thesis, the genotypes identified and recommended as potential sources of genes can be effectively used in the future to improve the resistance of trees to biotic stressors, as well as for other objectives related to the current and future situation and interests. Among these can be included the creation of pear rootstocks, the creation of ornamental varieties - research direction that in Romania has been relatively limited until now, the creation of pear genotypes suitable for various forestry and ecological requirements, as well as for the promotion of some genotypes as potential pollinators for edible cultivars from pear orchards.

The conservation of *Pyrus* species both inside and outside their natural habitat (in situ, respectively ex situ), as well as pear varieties in germplasm collections (gene banks – 'gene pool'), are vital for the role on which will have a part in the Earth's biodiversity and as a source of healthy food and the provision of other goods and means necessary for man. It is worth mentioning that in an academic city like Cluj-Napoca, the existence of germplasm collections, e.g., that of *Pyrus*, is also of great importance from a cultural and educational point of view, being an excellent source of learning and knowledge for pupils and students.

INNOVATIVE CONTRIBUTIONS OF THE THESIS

In the current context in which the world's population is constantly growing, exceeding eight billion people, and food needs are immense and overlapped with the reduction of natural resources, the loss of biodiversity, global warming and climate change, the results of this thesis are of interest, original and important both theoretically and practically. The research carried out provided results and information that contribute to science and knowledge in the field of plant breeding, respectively to an economically and socially significant horticultural species, with an old history and tradition in the culture of fruit trees in Romania. These results and the hypotheses formulated based on them can be a starting point in new breeding programs, with a view to exploring agricultural, horticultural, landscape and forestry innovations, contributing to securing food resources and improving food and nutritional security, supporting livelihoods, and protecting biodiversity.

The methods and working procedures designed and applied, the descriptors and evaluation scales, as well as the results obtained, contribute significantly to the originality of the doctoral thesis, and the contributions to knowledge are innovative, highlighting the importance of standardizing the assessment of pear diseases and identifying resistance genes that would be used effectively in improving the response of trees to biotic stressors. The phenotypic descriptors and the proposed systems are advantageous, simple, fast, and cheap. In addition, they can be used both in commercial orchards with a small number of cultivars and many trees per cultivar, and in germplasm collections with many genotypes, or in hybrid fields with thousands or tens of thousands of seed progeny, each being a distinct genetic entity. The proposed methods can be useful to both plant protection specialists and plant breeders, the common interest being to ensure the health of orchards in the context of sustainable agriculture, protecting the environment and maintaining an ecological environment, obtaining rich harvests and quality, healthy fruits for consumers. The collaboration between specialists in genetics and plant breeding and those in plant protection is the key to achieving such desired goals, given that ensuring integrated crop protection and control programs is becoming a priority in agriculture.

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