PhD THESIS

The main characteristics of seeds and seedlings of black locust (*Robinia pseudoacacia*) depending on the seed source from which they come

(SUMMARY OF THE PhD THESIS)

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INTRODUCTION

In Romania, as in many other countries of the world, the forest is still seen as a common good and a means of providing the necessary human resources, not as a set of ecosystem services that can counteract the disastrous effects of man on biodiversity. , the sustainability of the forest and the necessary human resources provided by the forest and, at the same time, life on Earth can be ensured.

Over the years, wood consumption has steadily increased; wood is considered a renewable resource, indispensable for the support and development of man, society and civilization, being used since ancient times as an energy raw material, as a material for construction, tools, weapons, furniture, paper, etc. All these uses and human needs for wood, have led to a substantial reduction in forested areas in the last two thousand years, continuously accentuated with the growth of the world's population. The exponential growth of the Earth's population over the last century has put immense pressure on forests and their areas worldwide. In 1900, the world's population was 1.9 billion; in June 2022, according to United Nations estimates, the planet's population reached 8 billion (https://www.worldometers.info/world-population/). The dramatic reduction of forested areas has also led to the emergence of the first concerns related to forest regeneration and protection. A situation similar to the global one was registered in our country. Constantin C. Giurescu in "The History of the Romanian Forest from the Ancient Times to the Present" (Ceres Publishing House, 1976), said: "Blessed be our forest, which was like a brother to us, sheltered us, helped us live and defend ourselves. But we, for about a century and a quarter, have not been able to spare it properly, to preserve it, not only as a beauty and a fortune, but also as a huge source of health. I made many big mistakes ... ". Starting with the 18th century, cartographic representations became much more accurate, and the areas occupied by the forest began to be managed through management plans called forest arrangements, but even so, the surface of the forests was and is very affected.

It is therefore necessary to ensure sustainable exploitation, sustainable management of any forest land and continuous improvement of forested areas. The unprecedented economic development of the last period, which has been done without taking too much into account the effects it has produced on the environment, as well as irrational economic policies in the forestry field, have resulted in a considerable decrease in forested areas in recent years. years. In the current context, the sustainable development of the forest fund, the increase of forested areas, the appropriate use of forest genetic materials, are the essential factors in the sustainable development of the forest.

In the present research, the aim was to obtain information on the existing variability in the species *R. pseudoacacia*, based on the particularities of the seed material, which would be useful for identifying and promoting valuable genotypes. We started from the premise that the evaluation of acacia genetic resources could offer the possibility to identify origins with different geographical origins, which have useful features for the improve quality, or could be used in new breeding work to obtain valuable genotypes.

The main purpose of this research was to obtain new information and knowledge regarding the variability of acacia and the differences that exist between different geographical origins within this forest species recognized for its great ecological plasticity. Variability is a general feature of the living world and is an inexhaustible source of morphological, physiological, biochemical

information, as well as the types of behaviors adapted to environmental conditions. In general, forest species with a long evolution have undergone a strong pressure of natural selection in their development habitats, also showing an obvious variability of quantitative and qualitative characters (SAVATTI, 2005).

The motivation for choosing the theme also started from the different opinions of specialists in the field related to this species, considered by some scientists, including at European level, an invasive species. It has been proposed to carry out a bibliographic investigation on this subject, through an in-depth documentation, to highlight the effects that an invasive species may have on biodiversity.

After an exhaustive investigation, it was wanted to initiate a research to analyze the importance of seed material in obtaining offspring with desired features to promote sustainable forestry, in which acacia can fully contribute to ensuring forest functions and ecosystem services.

It was proposed to analyze the way in which different genetic resources, represented by acacia trees without special regime, relatively close geographically and seasonally, at county or region limited area area, or at national level, originating in seed reserves located in different areas of Romania, can provide a profitable variability for selection. The seeds from such trees were subjected to comprehensive and systematized analyzes, from the study of the main morphological features of the seeds or their germination capacity, to the analysis of the resulting seedlings and their growth characteristics in the early stages of life, respectively the heritability of some characters of silvicultural interest.ment and cultivation of acacia. They could be used in the production of a seed and planting material

STRUCTURE OF THE THESIS

The doctoral thesis entitled "The main characteristics of acacia seeds and seedlings (*Robinia pseudoacacia*) depending on the seed source trees from which they come" is elaborated in the 'Variant with published works' of USAMV Cluj-Napoca, approved for doctoral students' who published and / or have accepted for publication a representative number of scientific papers, each of them dealing with a certain aspect of the issue expressed by the title of the doctoral thesis'. The thesis contains 144 pages and is made according to the elaboration and writing norms imposed for the doctoral theses at national level and by the internal norms of IOSUD USAMV. The doctoral thesis is structured in two parts consisting of 8 chapters and contains 21 tables, 30 figures and graphs and 382 bibliographic references.

The first part of the doctoral thesis refers to the current state of knowledge in the issues pursued in the thesis, being structured in three chapters. The first chapter summarizes the information on the evolution of the species R. pseudoacacia and its spread in Europe and in our country. The second chapter includes aspects regarding the biological peculiarities and the variations of some characters of interest of the acacia, respectively the flowering and pollination, the sexual reproduction, the phenotypic and genotypic variability. The third chapter presents aspects of acacia breeding, the goals of acacia breeding, forest genetic resources and their

conservation, acacia selection and the possibilities offered by existing seed source stands, and the creation of the necessary variability for hybridization breeding.

The second part of the doctoral thesis is allocated to personal contribution. In this part are presented the main results of the research carried out during the doctoral thesis, which were capitalized by publishing in specialized journals. Thus, the results of the thesis contain three articles published in peer-reviewed journals, indexed in recognized academic databases. Of these, one article was published in a BDI journal and two in Impact Factor (ISI) journals.

Chapter 4 presents the purpose and objectives pursued in the research. The aim of the research was to obtain information on the existing variability in the species R. pseudoacacia, based on the particularities of the seed material, which would be useful for identifying and promoting valuable genotypes. Considering the purpose of the research, the following objectives were established: estimation of some useful genetic parameters in the selection and improvement of acacia; phenotypic and genotypic testing of offspring in semi-fraternal families; identification of provenances with a good seed germination capacity and obtaining a quality seed material; identification of treatments to stimulate germination in *Robinia pseudoacacia*; evaluation of the particularities of the seedlings obtained as a result of the applied treatments.

Chapter 5 presents the research materials and methods used and the way of working to identify the differences between the tested origins, respectively the variability of acacia seeds and seedlings according to the geographical origins of some national sources for forest reproductive materials. The research was carried out in separate experiments (three experiments), in which the objectives were established according to the biological material.

In the first experience, the selection for the identification of some plus trees was made in five acacia stands without special regime, located in relatively close locations, within a perimeter of almost 3000 ha, in Sălaj County, northwestern Romania. In the following experiments (II and III), the seeds and seedlings obtained from them were analyzed, from eight different areas belonging to the *Robinia pseudoacacia* seed reserve in the genetic reserve forests of Romania and from the seed stands included in the National Catalog of Genetic Resources. Forestry and Forest Reproduction Materials.

RESULTS AND DISCUSSIONS

The results obtained in the doctoral research, carried out in three distinct experiences, were presented in the form of three scientific articles published in specialized journals, included in Chapter 6. For acceptance and publication, the manuscripts containing the results of the doctoral research went through -a rigorous peer-review process, in which the scientific consistency of the obtained results was validated, the topicality of the investigations for the profile research and their degree of novelty.

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Experiment I - the results were published in the form of an original scientific article, in a BDI journal, with the following identification data:

Roman AM, Morar IM, Truta AM, Dan C, Sestras AF, Holonec L, Ioras F, Sestras RE (2020). Trees, seeds and seedlings analyses in the process of obtaining a quality planting material for black locust (*Robinia pseudoacacia* L.). Notulae Scientia Biologicae, 12(4), 940-958.
https://doi.org/10.15835/nsb12410867
(Articol BDI. Indexed in Scopus, ProQuest, EBSCO, CAB Direct etc.)

In this first experience, five stands located on a small area (under 3000 ha), in Sălaj County, northwestern Romania, were analyzed. The results confirmed that the application of selection in acacia stands without special regime, located in relatively close locations, can be effective in order to identify trees with superior characteristics ('plus' trees). Depending on the characteristics of interest pursued, among the tested sources (Sâmpetru-Almaşului, Voivodeni, Trestia, Chendrea, Gălpâia), the most valuable in terms of the possibilities of selecting some 'plus' trees, the tree from Vojvodeni proved to be registered. with the trees with the highest productive capacity. In addition to the upper values of some important elements of wood mass accumulation (eg tree height, trunk diameter - DBH, respectively basal area or cross-sectional area of the trunk at a height of 1.30 m), the respective origin was recorded with significant differences and for natural pruning, a feature of great forest interest. Among the elements that contribute to the production of wood, on the whole experience, the biggest variation in the studied stands was presented by the basal area (surface) of the trunk, at chest height (DBH), with a CV value of 25.9% . The lowest variability was obtained for the height of the trees (CV% = 10.7), for this character, CV value illustrating a relative homogeneity of the trees. Significant differences between origins were recorded between the seeds harvested from trees with superior characteristics (considered 'plus' trees), for the main morphological characteristics of the seeds. Also, the percentage of seedling emergence from seeds sown in protected areas (solar) and directly in the field, as well as the growth of seedlings obtained from those seeds, varied depending on the source tree (origin) and growing conditions. The results of research on acacia seedlings obtained from trees plus have highlighted the existence of significant differences between origins, for the main elements of growth and vigor of young plants. The fastest growth rate in the first year of life was recorded in the seedlings from the plus trees selected in the Voivodeni tree, while the seedlings from the plus trees in Sâmpetru-Almaşului showed the lowest vigor at the end of the first year of life. The descendants from the Chendrea arboretum were recorded with the lowest stem height up to the first branch, with significant deviations from the average over the entire experience. Due to the relatively low vigor of the seminal descendants and, at the same time, the fact that the branching of the stem starts at the lowest height, the seedlings from the Chendrea tree were considered to have the lowest value. The heritability coefficients had relatively high values, the highest value being recorded for the diameter of the seedlings, suggesting a major influence of the genotype in the hereditary transmission of this character, representative for the growth rate and the capacity of wood mass accumulation in trees.

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Experiment II - the results were published in the form of an original scientific article, in an ISI journal (WoS, IF), with the following identification data:

Roman AM, Truta AM, Viman O, Morar IM, Spalevic V, Dan C, Sestras RE, Holonec L, Sestras AF (2022). Seed Germination and Seedling Growth of *Robinia pseudoacacia* Depending on the Origin of Different Geographic Provenances. Diversity. 14(1):34.

https://doi.org/10.3390/d14010034
(Indexed in ISI, Q2, IF 2021 = 3.029; Scopus, ProQuest, EBSCO, CAB Direct etc.)

In this experiment, the morphological peculiarities of the seeds and their germination capacity were analyzed, as well as the growth of the seedlings according to the origin of eight geographical origins in Romania, at *Robinia pseudoacacia*. An exhaustive assessment of the morphological characteristics of the seeds from eight seed stands located in different geographical areas was included, included in the 'National Catalog of Basic Materials for the Production of Forest Reproductive Materials' in our country. Seed germination treatments have identified the ones that provide the highest germination rates, but they are influenced by the interaction between the geographical resource and the type of treatment applied. The evaluation of the particularities of the seedlings obtained and the analysis of the differences between their characters of interest highlighted the extremely important role of the genetic factor (geographical origin, respectively genetic variations that ensure intra and interpopulation variability, even in an introduced species such as acacia) and treatments. stimulation of seed germination. Thus, the morphological features of acacia seeds indicated significant differences depending on their origin, and the investigation revealed a wide interpopulation (geographical) variability. Depending on the origin and treatment, the germination rate of acacia seeds varied greatly, the two experimental factors influencing the growth of seedlings in their first stages of life after emergence. The differences between the investigated populations were statistically assured, and the seed germination was probably influenced by the ecological parameters in the areas of origin. It was considered that the heterogeneity of the environment of origin of acacia seeds in Romanian origins may result in morphological differences and finally different germination performances. Among the origins, the one from Bihor had the largest seeds, which also had a good germination, especially during the heat treatment. The seeds from Satu-Mare had significantly higher values for the analyzed characteristics, but were not among the origins with a good germination and seedlings with significant growth. Even if the seeds from Bihor had larger dimensions and the best germination rate at the heat treatment, they registered the weakest germination in the case of sulfuric acid treatment, at all three concentrations tested. The thermal shock applied to acacia seeds was more effective in stimulating germination, compared to the treatment with sulfuric acid. Exposure to heat / cold significantly improved germination, but also some characteristics of the resulting seedlings, such as height, diameter and number of branches.

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Experiment III - the results were published in the form of an original scientific article, in an ISI journal (WoS, IF), with the following identification data:

Experienţa III – rezultatele au fost publicate sub forma unui articol ştiinţific original, într-o revistă ISI (WoS, IF), cu următoarele date de identificare:

Roman AM, Truta AM, Morar IM, Viman O, Dan C, Sestras AF, Holonec L, Boscaiu M, Sestras RE (2022). From Seed to Seedling: Influence of Seed Geographic Provenance and Germination Treatments on Reproductive Material Represented by Seedlings of *Robinia pseudoacacia*. Sustainability 14(9):5654. https://doi.org/10.3390/su14095654

(Indexed in ISI, Q2, IF 2021 = 3.889; Scopus, ProQuest, EBSCO, CAB Direct etc.)

In the third experiment, the influence of the geographical origin of eight Romanian acacia origins on the characteristics of seeds, germination and growth of seedlings in the young stages of

life (the first four years of life) was analyzed. The new methods applied to stimulate the germination capacity of the seeds provided interesting information on the possibilities of increasing the germination rate in *Robinia pseudoacacia*. Differences in seed germination were confirmed depending on the provenance and treatments applied, reported and in previous experiences. The scarification treatments ensured the highest germination percentage, followed by the heat treatment at the highest applied temperature, of 100 ° C, with a seed wetting time of 10 min. Stimulating germination by physical methods (scarification) remains an alternative to the use of chemicals, with ecological benefits, as well as the possibility of being used on a larger scale, with high efficiency. It has also been confirmed that the geographical origin of the origins and their seeds can influence the growth and morphological features of the seedlings in the early stages of life. For the main characteristics of seedling growth in the first years of life, the origin of Galați was noted, at which the average height had the highest value and CV% the lowest value, indicating a stable growth trait and homogeneity among the analyzed plants. For the diameter of the package of seedlings in the first year of life, after the seedlings from Galati, followed those from Valcea and Arad. In the second year of life, the seedlings from Galati remained the largest, but were closely followed by those from Iasi, Valcea, Botosani and Arad, between which the differences were not significant. The data showed that the geographical origin of the seeds to which the seeds belong significantly influences the growth rate of future plants in the very young stages of life. In the fourth year of life, the height of the seedlings varied extremely strongly, the average values depending on the origin being between 65 cm (Bistrița-Năsăud) to 278 cm (Bihor). The seedlings from the source tree in Galați had statistically superior values for height (second average, after Bihor), stem diameter per package (largest average, followed only by seedlings from Botoșani without significant differences) and number of branches per plant (the highest average, followed by Botoșani and Vâlcea). The growth parameters for the seedlings from Galați were constant, the CV% values being always the lowest (below 20%) suggesting the most homogeneous origin for the growth rhythm and the elements of vigor of the plants from the first years of life. Determining the correlations between some characteristics of interest of seeds and seedlings highlighted the possibilities of using them as indices of indirect selection. The application of multivariate analyzes (eg CCA - Canonical Correspondence Analysis, Hierarchical clustering - paired group UPGMA) to study the relationships between investigated variables and the level of proximity (similarity) or distance between provenances has shown that these methods provide scientific and practical information extremely useful for breeding or production of breeding material for acacia or other forest species of interest.

CONCLUSIONS AND RECOMMENDATIONS

• The selection to identify and exploit intra- and inter-population variability in acacia (*Robinia pseudoacacia*) is fully justified, even in a very small geographical area, where genetic variations may be due to the origin and characteristics of the ancestral material, possibly varied, used to plant the respective trees.

• The results demonstrated the existence of an intrapopulation variability that can be profitable for selection even at the level of stands without special regimes, in which trees with favorable particularities can be identified (biomass accumulation, wood quality, adequate behavior to stress factors, condition appropriate health and vegetation, etc.).

• The results showed that the height of the seedlings from the trees selected for their valuable characteristics ('plus' trees) and the height of the stem up to the first branch are traits in which most of the genetic variation is of an additive nature. For such traits, positive mass selection can provide satisfactory genetic gains.

• The identified correlations showed that in the biological material represented by the seminal descendants of *R. pseudoacacia* there were only positive relationships between the growth traits of the seedlings, some of them being statistically assured, with different levels of significance. Close genotypic correlations illustrate that by selecting plus trees and using their seeds, generative offspring can be obtained that strongly manifest parental characteristics and the close links between those characteristics.

• Given the close correlations at the genotypic level, it can be concluded that indirect selection can be applied in acacia seedlings, as it could be effective for the selection of valuable specimens in the nursery, which can later be used for afforestation or other purposes.

• Information obtained by calculating genetic parameters such as coefficients of variability, phenotypic and genotypic correlations, heritability can be very useful for forestry practice, to direct the growth of trees and wood production, but also for breeding activities, closely correlated characters can be used as selection indices.

• The data obtained can be valuable information for forestry practice and science, respectively for the improvement of the species *R. pseudoacacia*, as well as for the initiation of new works of selection and improvement of the species.

• Seed propagation is generally the easiest and simplest method of obtaining offspring with a high degree of heterozygosity in acacia. Because the seeds have a low germination capacity due to the physiological rest induced by the impermeable seminal skin, acacia treatments are recommended to stimulate and promote seed germination.

• In the experiments performed, the morphological peculiarities of the seeds were different, depending on the provenances represented by the eight different geographical sources studied (seed source stands), and the efficiency of seed germination stimulation treatments was influenced by the provenance.

• Depending on the origin and treatment, the germination rate of acacia seeds varied greatly, the two experimental factors influencing the growth of seedlings in their first stages of life after emergence. The percentage of germination increased with the application of scarification treatments and thermal shocks.

• The different behavior at germination of R. pseudoacacia seeds from the tested Romanian populations can be influenced both by the genetic variations of the sources of origin and introduction in the new areas of the acacia, and by the local environmental conditions in which the trees lived and accommodated for decades since planting.

• Germination tests and analysis of germination indices revealed a remarkable variability of the answers of Romanian origins in the process of seed germination.

• Differences in origins for certain traits of reproductive material may be due, to some extent, to seed suppliers in the reserves included in the national catalog, which probably do not always provide biological material at high quality standards. Consequently, similar studies can also be used to verify the quality of the biological material provided.

• Multivariate analysis is a very useful tool to analyze and synthesize the relationships between characteristics of forest interest in the seeds and seedlings of *R. pseudoacacia*, as well as

between the studied origins. Obviously, multivariate analysis can be used successfully for multiple desideratums and other forest species of interest in our country.

• Overall, the results confirmed that the phenotypic plasticity of acacia may be a response to variable ecological parameters in the areas of seed source stands. Because the geographical origin of the seed origins significantly influences the process of obtaining forest reproductive material and the growth rate of future plants in the very young stages of life, such studies are of great interest and utility.

INNOVATIVE CONTRIBUTIONS OF THE TESIS

The doctoral thesis provides information and original experimental data on acacia (*Robinia pseudoacacia*), an important species both economically and ecologically. Assessment of acacia genetic resources, starting from stands without special regime and then within the eight geographical origins from different counties of the country, included in the 'National Catalog of basic materials for the production of forest reproductive materials' in the category "Selected", a given the possibility to identify sources of variation of forest interest, with useful features for the improvement and cultivation of acacia.

The information obtained by calculating statistical and genetic parameters such as: coefficients of variability, phenotypic and genotypic correlations, heritability of characteristics of interest, etc., as well as multivariate analysis, can be very useful for forestry practice. Such information can be used to guide the growth of trees and wood production, wood quality and proper behavior of trees to the action of stressors, but also for breeding activities, the close correlations can be used as selection indices. Indirect selection can be applied in the stages of obtaining and using seeds, in the choice of generative acacia descendants, etc., being effective in order to identify valuable specimens in the nursery, their subsequent use in afforestation or for other purposes.

Identifying the differences between acacia populations depending on the seed material from different sources, can help ensure valuable information for forestry practice, while being a starting point for initiating new work of selection and improvement of the species, using appropriate resources genetics in our country.

The results of the thesis are of interest and important theoretically and practically, both for future acacia breeding programs and for afforestation and production programs for forest reproductive material. The obtained data showed that the forest fund in Romania offers profitable sources of variation for selection, and by the adequate use of the existing genetic resources in our country for the *Robinia pseudoacacia* species, the desired desideratums can be fulfilled

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