
PhD THESIS

Inducement and assessment of genetic variability in roses to obtain new varieties

(SUMMARY OF Ph.D. THESIS)

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INTRODUCTION

The breeding of roses, like the breeding of ornamentals and all cultivated species, is a biological discipline whose main objective is the creation of new cultivars with superior characteristics to the existing ones. Because roses enter the sphere of ornamental plants, obtaining new cultivars, with an increasing decorative and economic value, represents not only a science but also an art of enhancing and promoting beauty in a world in continuous scientific and technological evolution. Among the multiple valences of plants, so useful and welcome for meeting people's needs, some contribute to the beautification of the environment and ensure a clean, healthy environment, with major benefits both for life and physical health, respectively for the mental health of people. Among the ornamental plants, the rose is the true 'queen of flowers', thanks to both the special beauty of the flowers, as well as the impressive diversity and possibilities of use for different purposes and uses (SALCĂ ROMAN et al., 2024) .

Along with the evolution of modern man and the awareness of the importance of cultivating agricultural plants and raising animals, respectively the provision of food, man became more and more interested in 'beautiful' and how can ensure the ancestral aspirations for beauty in the immediate vicinity, under various forms, including through the lens of plants and flowers. Roses were among the first ornamental plants used to beautify people's lives. Gradually, with the evolution of society and sciences, as well as scientific and technical progress and lifestyle and living standards, the rose as a decorative species has seen a general and unanimous development, spread and recognition. The impressive number of varieties classified into different groups, with particularities, multiple destinations and uses in ecological conditions, and extremely varied locations, places, and purposes, offer just as many possibilities for the use and valorisation of roses.

Today, roses beautify the lives of people all over the world, being widely cultivated. Their multiple roles, economic, landscape, ecological, cultural, educational, etc. it is fully recognized and exploited, but new cultivars are necessary (ROMAN et al., 2021; SALCĂ ROMAN et al., 2024). In addition, the rose has symbolic or spiritual roles and meanings, often embodying love, beauty, harmony, or higher and uplifting feelings, aspiration for the better, as well as extreme feelings, from immaculate white, purity, peace and tranquility of soul and to fire, passion or intense love. As a full recognition of the 'queen' of flowers, the rose has become the most faithful witness of the most important events in human life.

STRUCTURE OF THE THESIS. MATERIAL AND METHOD

The doctoral thesis entitled "Induction and evaluation of genetic variability in the rose in order to create new varieties" is structured in five main parts, totalling a number of 160 pages, and contains 7 chapters, 37 tables, 41 figures, and 158 bibliographic references.

The first part of the thesis is represented by the introduction, which presents the current state of knowledge in rose breeding based on information from the specialized literature. Data on the spread, use, and socio-economic importance of roses, cultivation and distribution area, assortment diversity, as well as a brief history of culture and obtaining modern varieties are synthesized. The section on the origin and evolution of cultivated roses presents the origin and systematics of the genus *Rosa*, the multitude of species involved in the genetic background of cultivated roses, and the formation, diversification, and classification of cultivated roses. The basic characteristics of the main groups of roses are summarized and the way of appearance and evolution of garden roses is described. The information on the number of chromosomes and the genome of roses is supplemented in the genetics part with biological peculiarities and pollination in the rose, the importance of variability and artificial pollination as the main method of causing variability necessary in the creation of new varieties, as well as the heritability of some characters of interest in breeding. The last sections of this part are devoted to breeding objectives and methods of rose breeding. Within the breeding objectives, the emphasis is placed on the ornamental value of the slopes and the main desirables pursued in the breeding of modern roses (e.g. the architectural ideotype of the plants, the size and shape of the flowers, the recurrent flowering, the color and the fragrance of the flowers). Particular importance is given to the resistance to abiotic stress factors (especially to low temperatures, but also to drought and other abiotic stressors) and to biotic stress factors (respectively to the attack of the main pathogens and pests). Among the methods of rose breeding, according to the topic of the doctoral thesis, the emphasis is on artificial hybridization and selection, as a method of exploiting variability.

The second part of the thesis presents the personal contribution related to the purpose and objectives of the research, the particularities of the natural environment in which the research took place, respectively the description of the area and the environmental conditions. Information on biological material and experimental conditions is also presented, as well as how the most important characteristics for rose breeding and cultivation were analyzed.

In **the third part** of the thesis, the personal contribution related to the results obtained in the doctoral research is presented. This part has the largest weight in the whole thesis, the obtained results being presented in three distinct chapters. They were structured according to the experimental algorithm designed for conducting research, being focused on three major issues on which the rose breeding process is based:

- Investigating a germplasm pool represented by a rich genetic material.
- Analysis of parents used to create genetic variability through hybridization.
- Evaluation of F1 hybrids obtained from artificial pollination and genetic analysis.

In its turn, each issue was approached through an independent experience, so that, as a whole, the research included **three distinct experiences**, but closely related to each other from the point of view of the theme of the thesis, i.e. the improvement of roses. The germplasm fund represented by the rose collection and the biological material from the Horticultural Research Station of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca (USAMV Cluj-Napoca), as well as the existing technical and material base (laboratories, greenhouses, experimental fields, etc.) provided excellent premises for conducting research. The evaluation results were processed as average values of the characteristics analyzed over a period of at least five years for the genotypes in the collection and the field comparative trial (first and second experience), respectively as average values of the characteristics in young plants in the year of obtaining from the seed - F1 hybrids on their own roots, on the tables in the greenhouse, in the last decade of May (the third experience).

The fourth part of the thesis includes the sixth chapter elaborated to highlight the originality and innovative contributions of the thesis. The results obtained from the evaluation of the germplasm pool (rose collection), the selection of parents for artificial hybridizations and the analysis of F1 hybrids (obtained by diallel hybridizations) were extremely consistent. Since the results have been synthesized in the form of relevant and representative tables and graphs, the originality and novelty of the investigations carried out are revealed in the fourth part. In this sense, considerations were formulated regarding the actuality of rose breeding, increasing the efficiency of rose breeding through the optimal choice of parents and obtaining F1 hybrids with prospects for selection, the effectiveness of hybridization and artificial selection in obtaining new varieties of roses at SCH Cluj-Napoca and general perspectives in rose breeding.

In the last part of the thesis (**the fifth part**) the bibliographic references that constituted the scientific foundation of the subject addressed in the doctoral thesis (as well as the state of research in the field) are presented, as well as the basis of discussions and hypotheses formulated by comparing own results with results from similar research carried out internationally and as up-to-date as possible.

RESULTS AND DISCUSSIONS

The results obtained in **the first experience**, following the evaluation of 299 varieties of roses examined in the SCH Cluj-Napoca collection, highlighted the most representative and important particularities of the cultivars, which have a significant relevance for the culture and improvement of roses. The analysis of the genotypes according to the main classes or groups of roses accepted by the specialized literature, highlighted the fact that most varieties in the rosary belonged to the hybrid tea group (125), followed by floribundas (78) and shrubs (64). Hybrid tea varieties together with floribundas and shrubs represented 89.3% of the total varieties in the collection. Miniature and groundcover roses had low proportions (2.7 and 2.3%). Cultivars from the standard and weeping rose categories were not present, as grafting specific to these forms was not carried out (e.g. grafting on high-stem rootstocks), even though many cultivars from the previous categories lend themselves to these spectacular crown rose forms, such as tree, or 'the tree'. Accordingly, tree roses are not classified under the fundamental categories, since almost any rose that is grafted onto a tall trunk can be grown as a tree. Based on the habitus of the UPOV classification, most of the genotypes in the collection had vertical growth (38.5% of the total), 33.8% were classified as semi-spreading or semi-upright growth habit and 18.4% and moderately branched. The lowest percentages were obtained in cultivars with strong branching (4.0%) and intermediate habitus (5.4%). Most genotypes had double flowers (73.2%). The lowest proportion was recorded in the genotypes with single flowers and with wrapped or fully double flowers (2.7%). Most varieties had red flowers (26.8%), followed by those with pink (21.1%) and yellow flowers (12.4%). A significant proportion was represented by varieties with white or almost white flowers (8.4%), red mix (8.0%) and purple mix (5.0%). Not a single variety in the rosarium had purple or green flowers. Even if some winters are very cold in the study area, most varieties presented a good response to frost, being included in the "resistant" (46.8%) and "very resistant" (34.4%) class. Only 1.3% of the genotypes were considered very sensitive, and 9.0% sensitive. Regarding the response to the attack of the main diseases under natural infection conditions, 56.2% of the varieties were classified as "resistant", 31.1% as "very resistant" and 11.4% as "tolerant". Only 1.3% of the varieties were classified as "sensitive", while no variety was considered "very sensitive". According to the overall decorative value, on a scale of 1-10, 13.0% of the varieties were rated at maximum. The majority of varieties (56.9%) were registered with grade 9, 20.1% with grade 8, 9.7% with grade 7 and only 0.3% with grade 6. The genotypes tested showed a high decorative quality, and no varieties got low marks based on the evaluation scale (between 1 and 5).

In **the second experience**, the results obtained from the studies carried out for the selection of potential parents revealed the existing variability between 16 genotypes (Vigorosa, Lavender Dream, Angela, Nostalgica, Zburlici, Rosalinda, Pasiune mov, Rosagold, Romstar, Monica, Diorama, Diamond Jubilee, Rosarium Utersen, Ballerina, Perla Transilvaniei, Hermann Schmidt) for the main characters of interest in rose breeding. The differences between the genotypes tested in a comparative culture highlighted the possibility of identifying suitable parents for certain particularities or a set of valuable characteristics among those considered, e.g. the growth and vigor of the bushes, particularities of the foliage, the decorative value, the response to attack of main diseases (black leaf spot – *Diplocarpon rosae*, powdery mildew – *Sphaerotheca pannosa*, rust – *Phragmidium* sp.) response to cold (frost), etc. The height of the plants showed wide oscillations, between 55.5 cm (Zburlici) and 130.7 cm (Vigorosa). Tall plants also showed Rosalinda, Rosarium Utersen, Ballerina and Hermann Schmidt, and low bushes were also recorded in Lavender Dream, Purple Passion, Diorama and Diamond Jubilee. Long flower stalks were recorded at Hermann Schmidt (104.6 cm), at the opposite pole was Diamond Jubilee (35.8 cm). Lavender Dream, with the lowest value of the degree of attack, as well as the varieties Rosarium Utersen and Vigorosa, stood out for their adequate response to the black leaf spot attack. Hermann Schmidt, Angela, Perla Transilvaniei, Ballerina, Nostalgica and Diorama proved to be varieties with an adequate response to the powdery mildew attack, and the Zburlici variety presented the best response to the leaf rust attack. The Vigorosa variety stood out for the best response at negative temperatures during the winters, having the lowest degree of damage calculated based on the conditions caused by the frosts that occurred during the winters during the analyzed period. Passion Purple, Rosagold, Nostalgica, Romstar and Rosarium Utersen stood out for the fragrance of the flowers, and for overall value Monica, Diamond Jubilee, Passion Purple, Rosagold, Romstar, Diorama and Perla Transilvaniei.

In **the third experiment**, results with a high degree of originality were obtained, by analyzing the F1 hybrids resulting from four diallel hybridizations, in which the varieties studied in the second experiment participated as parents. The statistical analyzes were completed by genetic analyses, statistical variances being decomposed into genetic variances. The combinations Lavender Dream x Nostalgica, Vigorosa x Angela, Nostalgica x Lavender Dream, Ballerina x Perla Transilvaniei, Hermann Schmidt x Rosarium Utersen, Rosalinda x Rosagold, Passion purple x Zburlici, Passion purple x Rosalinda, Passion purple x Rosagold. Such hybrid combinations offer the best chances for the selection of elite plants leading to obtaining clonal selections with prospects for homologation as new varieties. In two of the four diallels, the genetic effects of general

combining ability (GCA) and those of specific combining ability (SCA), as well as reciprocal effects, significantly influenced the growth vigour of F1 hybrids. In the cultivar Vigorosa, the significant effects of GCA illustrated the fact that cultivar vigour (also suggested by its name) is highly transmissible in the seminal progeny, due to polygene additivity. Unlike Vigorosa, in Angela, the genetic effects of additivity act in the opposite direction. Non-additive genetic effects, dominance and epistasis were significant in the manifestation of growth vigour of F1 hybrids from the Vigorosa x Angela combination. Instead, the effects of GCA in Zburlici acted in the sense of obtaining F1 hybrids with a distinctly significantly lower height, compared to the other parents from the second diallel. In general, the additive effects of polygenes did not significantly influence the number of flowers per plant in diallel F1 hybrids, nor did the non-additive ones (dominance and epistatic interactions). In the first diallel, additivity acted in the direction of obtaining progeny with many petals in the flower. In the fourth diallel, the variety Rosarium Utersen stood out for its great ability to convey the character of flowers rich in petals. In contrast, in Hermann Schmidt and Vigorosa the GCA effects acted in the opposite direction. For the degree of powdery mildew attack in F1 hybrids, it was found that only in two diallels out of four were there real sources of variation that influence the response of the plants to the pathogen. In the second diallel, SCA effects and reciprocal effects were significant, and in the third diallel, SCA effects influenced the response of hybrids to mycosis. Dominance and epistasis effects, as well as reciprocal (maternal) effects contributed significantly to the manifestation of traits in the following direct and reciprocal hybridizations: ♀Zburlici x ♂Rosagold and ♀Rosagold x ♂Zburlici; ♀Rosalinda x ♂Purple Passion and ♀Purple Passion x ♂Rosalinda. In direct and reciprocal hybridizations between Vigorosa and Angela, genetic effects of a cytoplasmic nature were considered not to influence flower color, instead, extranuclear genetic effects greatly influenced a typical polygenic character, such as the height of F1 hybrids. The results of the multivariate analyzes provided extremely interesting and novel information for increasing the efficiency of rose breeding. The relatively close grouping in common quartiles of PCA (Principal Component Analysis) graphs of some direct and reciprocal hybridizations for the set of analyzed characteristics demonstrated that multivariate analyzes provide synthetic and relevant information for a multitude of data very useful for new variety creation programs.

CONCLUSIONS AND RECOMMENDATIONS

As a result of the studies carried out in the framework of this doctoral thesis, useful and original information was obtained regarding the existing variability in the

rose germplasm pool and the possibility of challenging genetic variation profitable for selection through an appropriate choice of parents. Knowing the ability to combine parental forms, and identifying optimal hybrid combinations, or parental formulas that can provide a wide variability in generative progeny, gives high chances for obtaining hybrid populations in which the selection is profitable and the efficiency of the breeding process and obtaining varieties us to be maximized.

In roses, the existence of a wide genetic variability and the heritability of the characters of interest (with a polygenic or quantitative determinism) is fundamental for ensuring success in any program aimed at increasing the varietal conveyor, respectively the assortment of varieties with multiple destinations and possibilities of use in the beautification of the landscape, of green spaces or interiors, or for use as cut roses in various arrangements and for various occasions. The hybridizations carried out and the results obtained have demonstrated that by combining some varieties from different groups or categories (tea hybrid, shrubs, etc.) the obstacle represented by the production limits of variability and restriction of the genetic base can be overcome, which constitutes a major blockage in breeding works and creation of new cultivars.

The research also looked at how different statistical analyzes and tests can be used appropriately for the processing and interpretation of results in roses, including when scoring or rating systems are used for the ornamental value and decorative features of plants and flowers. Apart from the Kruskal Wallis test, a non-parametric test commonly used for ranks to test the hypothesis that differences between categories or groups (in this case the studied genotypes) are due to random variation, chi-square (χ^2) tests and the F test were also performed. In all cases, the test statistics confirmed the hypothesis that the differences between cultivars are real and the mean scores for the characters of interest in roses were not due to random variation. The results of the ANOVA test (F-test) were also confirmed by the results of the non-parametric tests (KW and χ^2), which supported the hypothesis of using under certain conditions ANOVA analysis of variance for qualitative data, not only for quantitative data.

INNOVATIVE CONTRIBUTIONS OF THE THESIS

The studies carried out have shown that a good knowledge of genetic diversity is particularly useful in rose breeding to obtain hybrids with favourable particularities and perspectives in the selection and creation of new varieties. Such populations can ensure obtaining new hybrid varieties, produced with the greatest possible benefit through heterosis. The diallel hybridizations performed have demonstrated their great utility in

creating variability in F1 hybrid populations and obtaining information of great practical and scientific interest in rose breeding. Diallel hybridizations offered the possibility of calculating the genetic effects involved in the hereditary transmission of characters with a quantitative, polygenic determinism. The methods by which the general combining ability (GCA), due to the genetic effects of additivity, and the specific combining ability (SCC), due to the genetic effects of dominance and epistasis, were estimated based on diallel hybridizations, have a fundamental role in the identification of parental forms and genetic combinations (parental formulas) that can give rise to high-performance generative offspring, respectively in which an intense heterosis effect can be manifested for the desired characters.

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