RJB Journal of Botany

OPEN ACCESS

Alkaloid Profiling in Parental and Hybrid Generations of *Catharanthus roseus*: New Metabolite Accumulation

[1]*Awad Hamza Abdelmageed*, [2]*Hatil Hashim Alkamali and* [3]*Mohamed Elkheir Abdelrahman*

[1]*Department of Biology, University of Kassala P.O. Box 266, Fax 23501, Kassala, Sudan*

[2]*Department of Botany, Faculty of Science and Technology, Omdurman Islamic University, Omdurman, Sudan*

[3]*Department of Agriculture, University of Khartoum, Khartoum, Sudan*

ABSTRACT

Background and Objective: *Catharanthus roseus* is a valuable medicinal plant known for its alkaloid compounds with therapeutic properties. This study investigates the variability of alkaloidal compounds in the roots of *Catharanthus roseus* (periwinkle) and their hybrids at different growth stages. Alkaloids are of significant interest due to their pharmaceutical properties, and understanding their variation across generations can inform breeding strategies. **Materials and Methods:** Alkaloid concentrations were analyzed in the roots of parental plants at the flowering stage, F1 hybrids at the flowering stage, and F2 hybrids at the fruiting stage. Alkaloid profiling was conducted using appropriate biochemical methods to quantify and compare alkaloid levels across the different generations. **Results:** The analysis revealed significant variability in the alkaloid profiles among the different generations. Isovindolinine and vindolinine were prominent in the parental plants, but were absent in F1 hybrids and re-emerged in F2 hybrids, with isovindolinine at 38.28% and vindolinine at 35.70%. Alkaloids like vincoline and tetrahydroalstonine were either absent or present in trace amounts in the hybrids, indicating complex genetic regulation. Ajmaline, which was abundant in the parental plants (53.57%), decreased in F1 hybrids and showed a slight recovery in F2 hybrids. **Conclusion:** The study highlights the significant influence of genetic inheritance on alkaloid production in *Catharanthus roseus* and its hybrids. The findings underscore the potential of hybridization in increasing alkaloid variability, which could be valuable for optimizing alkaloid production for pharmaceutical applications. This research provides insights that could support breeding programs aimed at enhancing alkaloid yield in periwinkle.

KEYWORDS

Indole alkaloids, *Catharanthus roseus*, hybridization, genetic inheritance, alkaloid variability, pharmaceutical applications

INTRODUCTION

Catharanthus roseus, a member of the Apocynaceae family, is renowned for producing terpenoid indole alkaloids that have significant medicinal value. It is known for a variety of therapeutic properties, including antidiabetic, antibacterial, and antihypertensive effects. Among its notable compounds, the dimeric

ISSN: 2152-0461 (Online)
ISSN: 1816-4919 (Print)
https://doi.org/10.3923/rjb.2025.77.82

Received: 28 Sep. 2024
Accepted: 23 Dec. 2024
Published: 24 Jan. 2025

alkaloids such as vinblastine and vincristine have gained considerable attention for their anticancer activity, with some already being used in clinical settings[1].

This plant is also recognized for its hypoglycemic and cytotoxic effects, which have led to its use in treating conditions like diabetes and high blood pressure, in addition to its disinfectant properties. Vinca alkaloids, particularly vinblastine, vinorelbine, vincristine, and vindesine are vital for cancer treatment[2].

Catharanthus roseus has been extensively studied since the discovery of the potent anticancer alkaloids vinblastine and vincristine in its leaves over 50 years ago. These alkaloids remain in clinical use today and are still sourced exclusively from this plant, along with their precursors, catharanthine, and vindoline[3].

Given its pharmaceutical importance, *Catharanthus roseus* has been the subject of many studies, especially due to the low yield of vinblastine and vincristine in the plant. It has become a model for biotechnological research on plant secondary metabolism[4].

In plant breeding, the genetic inheritance of secondary metabolites, such as alkaloids, is often complex, controlled by multiple genes with both dominant and recessive alleles. Heterosis, or hybrid vigor, in which hybrid plants exhibit superior traits compared to their parents, can influence alkaloid content. However, the molecular mechanisms behind heterosis remain poorly understood, though gene interactions such as dominance, overdominance, and epistasis are believed to play a role in improving biomass and yield[5].

Hybridization is a key factor in the diversity of secondary metabolites in plants. It has been suggested that hybridization contributes to the generation of chemical diversity and could result in the development of novel defense mechanisms against herbivores. Despite decades of phenotypic studies, the exact mechanisms behind the origin, maintenance, and evolution of plant secondary metabolite diversity remain unclear. Understanding the genetic architecture of phytochemical traits and how hybridization impacts plant chemical diversity is critical for deciphering the ecological interactions and selective constraints of these traits[6,7].

The aim of this study was to investigate the variability in alkaloid profiles between the roots of parental periwinkle plants and their F1 and F2 hybrid generations.

MATERIALS AND METHODS

Study area: The experiments were conducted at the Horticulture Administration, Ministry of Agriculture, Kassala State, Sudan, from January, 2016 to September, 2019.

Plant material: The research material consisted of *Catharanthus roseus* (L.) G.Don (periwinkle) herb (leafy flowered stems).

The field experiment was carried out at the experimental station, Department of Horticulture of the Kassala State, Eastern Sudan. The method of plant propagation was a conventional method of cultivation from seeds. The seedlings of periwinkle were produced in the greenhouse. During the growing season, crop management was carried out. It included mainly irrigation, weeding, and pot cultivation. The plant harvest was done at the flowering and fruiting stages. For the laboratory analysis 100 g samples (periwinkle herb), for each experimental plot, were taken. The obtained plant material was dried in the shade.

Experimental design: Two genetically distinct parental pure lines of periwinkle (white and pink). The F1 hybrids (resulting from the cross between white×pink). The F2 hybrids (derived from the self-pollination or inter-cross of F1 hybrids). The investigation of the variability in alkaloidal compounds was carried out in the roots of periwinkle (*Catharanthus roseus*) and their hybrids[8,9].

Extraction of monoterpene indole alkaloids: Chemical analysis was performed at the Alawia Imam Pharmaceutical Research and Development Institute, University of Medical Sciences and Technology (UMST), Khartoum, Sudan. The indole alkaloids were extracted according to the methodology described by Misra *et al.*[10] with a small modification.

The 2.5 g of dried root powder from each genotype was weighed and placed into a 100 mL beaker. The sample was transferred to a 100 mL volumetric flask, and 50 mL of 96% ethanol was added. The sample was manually shaken for five minutes and left to stand for 24 hrs. The next day, the extract was filtered using filter paper. The residue was re-extracted with ethanol, and the pooled ethanolic extract was evaporated to dryness at room temperature.

The residue was dissolved in 25 mL of ethanol, diluted with 25 mL of distilled water, and adjusted to pH 1 using 3% hydrochloric acid. The sample was extracted three times with n-hexane, and the hexane layer was discarded. The remaining extract was cooled to 10°C and basified to pH 10 with 25% ammonia solution. It was then extracted three times with 25 mL of dichloromethane. The dichloromethane layer was collected, washed with 25 mL of brine, dried with anhydrous sodium sulfate, and evaporated to dryness. The alkaloid extract was dissolved in 2 mL of methanol and stored in a refrigerator for subsequent Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis: The qualitative analysis was performed using a GC/MS-QP2010 Ultra system (Shimadzu, Japan) equipped with a capillary column (Rtx-5 ms, 30 m×0.25 mm×0.25 µm). A 4 µL sample was injected in split mode, with helium as the carrier gas at a flow rate of 1 mL/min. The temperature program started at 40°C for 5 min, then increased to 60°C over 30 min and further increased to 230°C over 6 min, where it was held for 10 min. The final temperature of 230°C was maintained for 30 min. Mass spectra were recorded at 70 eV, with a mass range from 40 to 550 m/z. The total run time for each sample was 76 min. The injector temperature was set to 280°C, the ion source to 230°C, and the interface temperature to 250°C. The identification of the alkaloid was achieved by comparing retention indices and mass spectra with the National Institute of Standards and Technology (NIST) Library.

RESULTS AND DISCUSSION

The F1 plants were produced by crossing pink-flowered plants with strong violet-purple eye colour with white-flowered plants that had yellow and greenish eye colour. The F2 progeny consisted of 485 plants scored for flower colour. Among them, 125 plants exhibited pink corolla and strong violet-purple eye colour, resembling the parental stock. Another 227 plants displayed light pink corolla and pale red eye colour, similar to the F1 generation, while 133 plants had white corolla with yellow and greenish eye color, showing a phenotypic ratio of 1:2:1 observed in study of Abdelmageed *et al.*[8].

The F1 plants from the cross between pink-flowered, strong violet-purple-eyed plants and white-flowered, yellow-greenish-eyed plants all exhibited light pink flowers and pale red eyes. This suggests that the F1 plants showed an intermediate flower color, likely due to incomplete dominance or the influence of a minor modifier gene.

However, in the case of alkaloid compounds and chemical traits, there may be incomplete dominance, where neither parent fully dominates, and the hybrids exhibit intermediate or new chemical traits, such as the new compound that appeared in the first generation.

The new compound in the hybrid could result from the interaction between the genes responsible for producing alkaloids. This suggests that the interplay between the white and red genes influenced gene expression, leading to the production of a new compound.

Table 1: Variability in alkaloidal compounds in parental plant roots of periwinkle and their hybrids

	Parents (flowering stage)			F1 (flowering stage)	F2 (fruiting stage)	
Alkaloids (%)	R′R′	RR	R R′	RR	R R′	R′R′
Isovindolinine	10.88	0	0	38.28	28.75	31.28
Vindolinine	10.96	0	0	35.70	34.75	33.33
Vincoline	00.00	0	0	00.38	00.00	00.00
Tetrahydroalstonine	21.89	0	0	01.25	07.96	06.80
Ajmaline	53.57	0	0	07.98	12.59	16.63
Pleiocarpamine	02.70	0	0	05.44	09.61	00.00
19-Epivindolinine	00.00	0	0	01.10	03.56	01.63
20-Epivindolinine	00.00	0	0	00.22	00.00	00.00
16-Epivindolinine-N-oxide	00.00	0	0	00.51	00.00	01.09
Pericycline	00.00	0	0	01.20	02.78	00.00
Desipramine	00.00	0	0	07.08	00.00	00.00
Teberosnine	00.00	0	0	00.80	00.00	00.25
Coronaridine	00.00	0	0	00.00	00.00	08.99

Parental taxa at onset flowering = After 140 days from seed cultivation crossing, at flowering stages after 80 days from parental selfing crossing at fruiting stage = After 160 days from self-crossing of F1 individuals. Parental taxa roots were collected at the fruiting stage after 65 days from cultivated seeds, parent (white-flowered plant ŔŔ and pink-flowered plant RR) and their hybrids in F_2 fail to express any alkaloidal components

The results of the experiment shown in Table 1 explore the variability of alkaloidal compounds in the roots of Periwinkle (*Catharanthus roseus*) and their hybrids, focusing on the presence and concentration of specific alkaloids.

A total of 13 different alkaloids have been identified (Table 1), among which isovindolinine (10.88%), vindolinine (10.96%), pleiocarpamine (2.70%), tetrahydroalstonine (21.89%), and ajmaline (53.57% were the most abundant. In contrast, no alkaloids were detected in the pink-flowered parent (RR).

The experiment lists various alkaloids with their concentrations in the roots of parental plants (both at the flowering stage), F1 (hybrids at the flowering stage), and F2 (hybrids at the fruiting stage). The alkaloids detected include: Isovindolinine, Vindolinine, Vincoline, Tetrahydroalstonine, Ajmaline, Pleiocarpamine, 19-E, 20-E, 16-E, Peri, Desi, Teberos and Coronaridine.

In the parental plants (white, R′R′), isovindolinine and vindolinine are both found in significant amounts, with concentrations of 10.88 and 10.96%, respectively, at the flowering stage. In the F1 hybrids, however, both alkaloids are absent (0%). In the F2 generation, the alkaloid concentrations are significantly higher than in F1, with isovindolinine reaching 38.28%, and vindolinine at 35.70% in the (R′R′) hybrid, suggesting that these compounds re-emerge in the F2 generation, possibly due to recombination and genetic segregation.

This alkaloid is absent in all parental and hybrid samples except for a very small trace in the F2 (R′R′) hybrid at 0.38%. This suggests that vincoline is not a major component of the parental plants' alkaloid profiles, and its appearance in F2 may be a result of genetic variation or environmental influence.

The parent plants (R′R′) show a high level of tetrahydroalstonine (21.89%). However, the alkaloid is almost absent in F1 hybrids, and only small amounts are seen in F2 hybrids. This could indicate that the genetic makeup influencing the synthesis of this compound is not consistently inherited or expressed in F1 hybrids, but it reappears in the F2 generation due to genetic recombination.

Ajmaline is found in high amounts in the parental plants (R′R′) (53.57%). In F1 hybrids, the concentration drops significantly to around 7.98% R′R′ in, and slightly higher in the F2 generation, indicating that this alkaloid might be regulated by complex genetic factors, and its synthesis is likely influenced by both dominant and recessive alleles.

Pleiocarpamine is present in small amounts in the R'R' parent (2.70%), and it increases in the F2 hybrids (5.44 to 9.61%), indicating some genetic influence in the expression of this compound.

Alkaloids like 19-E, 20-E, 16-E, Peri, Desi, and Teberos appear in trace amounts in some of the hybrid generations, suggesting that their synthesis is influenced by specific hybrid combinations and genetic interactions.

The variability observed in the alkaloidal profiles between the parental plants and their F1 and F2 hybrids could be attributed to both genetic inheritance and environmental factors: The differences between the F1 and F2 generations in terms of alkaloid content likely result from genetic recombination. The F1 hybrids, being the first filial generation, often exhibit heterosis (hybrid vigor) but can also show altered or reduced expression of certain traits due to dominant and recessive alleles.

The significant variation in alkaloid concentrations between the generations and hybrids suggests that hybridization can lead to both increased variability and potentially enhanced or diminished levels of desired compounds. For example, the re-emergence of alkaloids like isovindolinine and vindolinine in the F2 generation could make certain hybrids more valuable for pharmacological uses[9,10].

Current findings were consistent with those of previous studies in showing that hybridization can produce new alkaloid compounds and alter production patterns across generations[10,11]. However, differences in the focus on color traits and the genetic ratios of alkaloid distribution suggest that the current study introduces new insights into these processes, warranting further research.

The chemical behavior of alkaloid compounds, especially in hybrid plants can be influenced by various factors including genetic, biochemical, and environmental interactions. It can be explained through a combination of genetic, enzymatic, epigenetic, environmental, and transport factors. Hybridization alters gene expression and metabolic pathways, leading to new alkaloids, changes in alkaloid levels, and modifications in their distribution. These changes are crucial for understanding how chemical traits evolve and are inherited in hybrid plants[11,12].

Catharanthus roseus is a key source of terpenoid indole alkaloids, which are utilized in the production of drugs for diabetes, cardiac conditions, hypertension, and cancer treatment by the pharmaceutical industry. Additionally, some of these therapeutic compounds are produced through semi-synthesis, using natural precursors extracted from the leaves of *C. roseus*.

CONCLUSION

The study demonstrates the genetic variability of alkaloid production in periwinkle plants and their hybrids, with notable changes in alkaloid concentrations from parental plants to hybrid generations. Thirteen alkaloids were identified, with ajmaline (53.57%), tetrahydroalstonine (21.89%), and others being the most abundant, while no alkaloids were detected in the pink-flowered parent (RR). This variability underscores the complex inheritance patterns of alkaloid biosynthesis in *Catharanthus roseus*, providing insight into potential breeding strategies to optimize alkaloid production for pharmaceutical applications. This study focused on the inheritance of two flower colors. Further research should investigate other flower colors and shades. Additionally, the study primarily examined alkaloid content in the roots; further research is needed on other plant morphological parts.

SIGNIFICANCE STATEMENT

This research investigated alkaloid profiles in white- and pink-flowered plants and their hybrids, revealing distinct metabolite patterns. While the white-flowered parent expressed multiple alkaloids, none were detected in the pink-flowered parent. The F1 hybrids showed no parental alkaloid expression, but in the

F2 generation, both parental and new alkaloids emerged. This suggests hybridization impacts alkaloid biosynthesis and the expression of secondary metabolites. The study highlights the complexity of genetic regulation in hybrids, with novel pathways and polymorphisms in loci influencing metabolite diversity and accumulation.

ACKNOWLEDGMENT

The authors thank Central Laboratory, Department of Chemistry, University of Science and Technology, and Ministry of Science and Technology, Khartoum, Sudan for the technical assistance.

REFERENCES

1. Almagro, L., F. Fernández-Pérez and M.A. Pedreño, 2015. Indole alkaloids from *Catharanthus roseus*: Bioproduction and their effect on human health. Molecules, 20: 2973-3000.
2. Moudi, M., R. Go, C.Y.S. Yien and M. Nazre, 2013. Vinca alkaloids. Int. J. Preventive Med., 4: 1231-1235.
3. Kulkarni, R.N., K. Baskaran and T. Jhang, 2016. Breeding medicinal plant, periwinkle [*Catharanthus roseus* (L) G. Don]: A review. Plant Genet. Resour., 14: 283-302.
4. Van der Heijden, R., D.I. Jacobs, W. Snoeijer, D. Hallard and R. Verpoorte, 2004. The *catharanthus* alkaloids: Pharmacognosy and biotechnology. Curr. Med. Chem., 11: 607-628.
5. Fujimoto, R., K. Uezono, S. Ishikura, K. Osabe, W.J. Peacock and E.S. Dennis, 2018. Recent research on the mechanism of heterosis is important for crop and vegetable breeding systems. Breed. Sci., 68: 145-158.
6. Caseys, C., C. Stritt, G. Glauser, T. Blanchard and C. Lexer, 2015. Effects of hybridization and evolutionary constraints on secondary metabolites: The genetic architecture of phenylpropanoids in European *populus* species. PLoS ONE, Vol. 10. 10.1371/journal.pone.0128200.
7. Cheng, D., K. Vrieling and P.G.L. Klinkhamer, 2011. The effect of hybridization on secondary metabolites and herbivore resistance: Implications for the evolution of chemical diversity in plants. Phytochem. Rev., 10: 107-117.
8. Abdelmageed, A.H., M.E. Abdelrahman and H.H. Alkamali, 2021. Genetics of flower colour in pink flowered "Rosea" and white flowers "Alba" in periwinkle *Catharanthus roseus* (L) G. Don. GSC Biol. Pharm. Sci., 14: 166-174.
9. Wesołowska, A., M. Grzeszczuk, J. Wilas and D. Kulpa, 2016. Gas chromatography-mass spectrometry (GC-MS) analysis of indole alkaloids isolated from *Catharanthus roseus* (L.) G. Don cultivated conventionally and derived from *in vitro* cultures. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 44: 100-106.
10. Misra, A., N.K. Srivastava, A.K. Srivastava and A. Khan, 2009. Influence of etherel and gibberellic acid on carbon metabolism, growth, and alkaloids accumulation in *Catharanthus roseus* L. Afr. J. Pharm. Pharmacol., 3: 515-520.
11. Pais, A.L., X. Li and Q.Y. (Jenny) Xiang, 2018. Discovering variation of secondary metabolite diversity and its relationship with disease resistance in *Cornus florida* L. Ecol. Evol., 8: 5619-5636.
12. Liu, Y., B. Patra, S.K. Singh, P. Paul and Y. Zhou *et al*., 2021. Terpenoid indole alkaloid biosynthesis in *Catharanthus roseus*: Effects and prospects of environmental factors in metabolic engineering. Biotechnol. Lett., 43: 2085-2103.