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Identification and Quantification of Novel Metabolites in (*Catharanthus roseus* (L) G. Don Parental types and their F1, F2 generation

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Abstract

This study investigates the variability of alkaloidal compounds in the aerial parts of Catharanthus roseus (periwinkle) parents, 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. Alkaloid concentrations were analyzed in the aerial parts (stems & leaves) of parental plants and F1 hybrids at the flowering stage, at the fruiting stage for F2 generation. Identification and Quantification of Novel Metabolites was conducted using appropriate biochemical methods to quantify and compare alkaloid levels across the different generations. The analysis revealed significant variability in the alkaloid profiles among the different generations. The analysis revealed significant variability in Quantification of Novel Metabolites 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 1.63%, and vindolinine at 1.81%. Alkaloids like coronardinine which was abundant in highest concentration in F1 hybrids Rr 10.1%, and showed decreased in F2 hybrids at 2.79%, and a slight recovery in F2 hybrids. Alkaloids like Pleiocarpamine (Pleiocarpamine) and Pericyvilinine (Pericyvilinine) were either absent or present in trace amounts in one of parental like RR or in F1 hybrids Rr or F2 hybrids Rr, indicating that this alkaloid might be regulated by complex genetic factors. Vindorosine alkaloid was a higher concentration in RR parent (4.46%) compared to R'R' (rr) parent (2.06%). It was increases in F1 hybrids (RR') (Rr), and continues to increase in F2 generation. Vindoline shows in a highest concentration in RR parent (82.33%), lower in rr parent (52.95%). In F1 Rr hybrid , concentrations are higher than both parents reaching (86.46%). In F2, concentrations are distributed among RR, Rr, and rr genotypes (around 80%). The study emphasizes that the genetic makeup of the Catharanthus roseus plant (also known as periwinkle) plays a crucial role in determining the quantity and types of alkaloids produces. Also crossing different strains of the Catharanthus roseus plant (also known as periwinkle) leads offspring with a wider rang of alkaloid productionin the aerial parts (stems & leaves) in this plant. This increased variability is valuable for developing varieties with enhanced medicinal properties."

Keywords: Metabolite; Indole alkaloids; *Catharanthus roseus*; Hybrid; Anticancer; Coronarridine; Vindorosine; Vindoline

1. Introduction

Catharanthus roseus is a medicinal plant belonging to the family Apocynaceae which produces terpenoid indole alkaloidsof high medicinal importance. Indeed, a number of activities like antidiabetic, bactericide and antihypertensive are linked to *C. roseus*. The most striking biological activity investigated has been the antitumour effect of dimeric alkaloids such as anhydrovinblastine, vinblastine and vincristine which are already in pre-, clinical or in use (Almagro *et al*., 2015).

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Catharanthus roseus G. Donhave a hypoglycemic as well as cytotoxic effects. They have been used to treat diabetes, high blood pressure and have been used as disinfectants. The vinca alkaloids are also important for being cancer fighters. There are four major vinca alkaloids in clinical use: Vinblastine, vinorelbine, vincristine and vindesine (Moudi *et al*., 2013).

*Catharanthus roseus*produces more than100 monoterpenoids indole alkaloids (TIA) in different organs Jordan *et al.,*(1991). The leaves and stems are the sources of dimeric alkaloids, vinacristine and vinblastine that are indispensable cancer drugs, while roots have antihypertensive, ajmalicine and serpentine Kulkarni, Baskaran, *et al.,*(1999) .

Catharanthus roseus has been found to contain as many as 130 constituents with an indole or dihydroindole structure. The principal component is vindoline (up to 0.5%), other compounds are serpentine, catharanthine, ajmalicine, akuammine, lochnerine, lochnercine and tetrahydroalstonine. Alkaloids present in various part of *Catharanthus roseus* are summarized, Leaf Catharanthine, Vindoline, Vindolidine, Vindolicine, Vindolinine, ibogaine, yohimbine, raubasine, Vinblastine, Vincristine, Leurosine, Lochnerine. Stem- Leurosine, Lochnerine, Catharanthine, Vindoline. Root- Ajmalacine, Serpentine, Catharanthine, Vindoline, Leurosine, Lochnerine, Reserpine, Alstonine, Tabersonine, Horhammericine, Lochnericine, echitovenine (Morgan *et al*.,1999). (Jossang *et al*., 1998) they reported the seedsof this plant hadvingramine, and methylvingramine. Svobada *et al.*, (1964) they conducted that the leaves *of Catharanthus roseus* (Apocynaceae) is one of the tropical vegetation Plants, includes, Lochnerine, Leurosine, Vindoline and Isovindoline to be used for treat antidiabetic activity. Renault *et al*., 1999, they reported that Vinblastine and vincristine are the dimers formed by the coupling of Monoindole alkaloids such as catharanthine and vindoline found abundantly in the aerial parts of the plants. Also Svoboda 1969, Marles and Farnsworth, 1995, and Almagro *et al*., 2015) they reported that the catharanthine and vindolinine exhibit hypoglycemic activity. Vindoline is one of the biologically active products derived from plants discovered to be important as possible starting materials for the synthesis of valuableanticancer antibiotics eg. vincristine and vinblastine, and other pharmaceuticals (Abbasi *et al*., 2010), and also by Imran *et al*., 2010). Henriques *et al*.,1996, Rates *et al.*,1993, Sharma and Cordell (1988) they reported that the coronardine is an iboga-type indole alkaloid found in many species of the plant kingdom , and it is Like others iboga alkaloids, coronardine was investigated for a wide variety of pharmacological effects, such as antitumor, anti-inflammatory, and bactericidal activities. we described the antiparasite effect of coronaridine (COR), isolated from the stem of the Peschieraaustral is shrub, against Leishmania amazonensis promastigotes and intracellular amastigotes) Bou-Habib *et al*., (1998); Delorenzi *et al*., (2001). Also Deecher*et al*., (1992), Glick *et al*., (1994), Glick *et al*., (1997),Glick *et al*., (1992). Svobada *et al*., (1964) they reported that the leaves of *Catharanthus roseus* (Apocynaceae) is one of the tropical vegetation Plants, includes, Lochnerine, Leurosine, Vindoline and Isovindoline to be used for treat antidiabetic activity.

Reasearsh in this plant led to isolation of over 100 alkaloids found in different parts of the plant, some are of pharmaceutical importance, for example, Cantharanthine, Vindolinc, Ajmalicine, Lochnerine, Serpentine, and Tetra-hydroalstonine (Dagnino *et al.,*1996). And of particular interest is a group of about 25 dimmeric alkaloid which contain those having antineoplastic activity, including leurocristine (vincristine) and vincaleukoblastine (vinblastine) which composed of indole alkaloid catharanthine and vindoline, both of which occur free in plant (Treas & Evans 1990). Alkaloid content varies considerably in various parts of the plant from different localities and the total alkaloids reported in the roots range from 0.15-1.34% by (Virmani *et al.,*1978). Masoud *et al.,* (1968) found that the total alkaloid in root vary from 0.53-0.77% while in the leaves it varies from 0.4-0.53% and stem it range from *C. roses* 0.17-0.4, in Tanzania the plant contain up to 0.93% total alkaloid. Masoud *et al*. (1968) found that the leaf content of Vinblastine range between 0.021-0.03% of the dry weight of leaves of intact plant. (Mandati *et al*., 1975) in Pakistan found that the whole plant yield about 0.3 alkalioid, while in Bengalore (Parkas-Roa *et al.,*1988) declared that the root contain about 1.87-2.02% and the leaves about 0.99-1.15% total alkaloids. Vinblastine and vincristine are found in small amounts Vinblastine being in the order 0.0045-0.007% in the leaf by (Paradasani *et al.,*1979).

Atta-Ur-Rahman *et al.*(1982) found that it is about 0.005% in the dry leaves and about 0.003%, in the dry whole plant. Atta-Ur-Rahman *et al.,*(1982)

Miura *et al*.,(1987) found that vinblastine content in tissus culture was about 0.015% of the dry weight of leaves. Morever, Miura *et al.,*(1988) found that Vinblastine content in the dry leaves of tissue culture was about 3% of that of the intact plant, or about 0.004%, while in the intact plant it is about 0.14% of the dry weight of leaves. While vincristine was obtained only in about 0.002% from the crude plant (Trease & Evans 1990). Ajmalicine, another alkaloid extracted from the roots, was found in a concentration of 0.034-0.1% in the dry roots by (Virmani *et al*.,1978). In France, Gleye*et al*., (1979) found that it occurred in the roots of C.roseus at a concentration about 0.1-0.8.

Periwinkle has become one of the very extensively investigated medicinal plants after the discovery of two powerful anti-cancer alkaloids, vinblastine and vincristine, in its leaves more than 50 years ago. These alkaloidal drugs are still in clinical use. Also, periwinkle is still the only source of these alkaloids and their precursors, catharanthine and vindoline Kulkarni *et al*., 2016. Due to the pharmaceutical importance and the low content in the plant of vinblastine and the related alkaloid vincristine, *Catharanthus roseus* became one of the best-studied medicinal plants. Consequently it developed as a model system for biotechnological studies on plant secondary metabolism (Heijden *et al*., 2004).

In plant breeding, the genetic inheritance of secondary metabolites like alkaloids is often complex, governed by multiple genes with both dominant and recessive alleles. Moreover, heterosis or hybrid vigor, a phenomenon where hybrids show superior traits compared to their parents, can also influence alkaloid content. This is important in the use of F_1 hybrid cultivars in many crops and vegetables. However, the molecular mechanism of heterosis is not clearly understood. Gene interactions between the two genomes such as dominance, over-dominance, and epistasis have been suggested to explain the increased biomass and yield (Fujimoto *et al*., 2018).

The diversity of secondary metabolites in hybrid plants, exploring the genetic factors that shape metabolite profiles. It emphasizes the evolutionary significance of hybridization in generating chemical diversity and the potential for hybrids to develop novel defense mechanisms against herbivores. The mechanisms responsible for the origin, maintenance and evolution of plant secondary metabolite diversity remain largely unknown. Decades of phenotypic studies suggest hybridization as a key player in generating chemical diversity in plants. Knowledge of the genetic architecture and selective constraints of phytochemical traits is key to understanding the effects of hybridization on plant chemical diversity and ecological interactions Caseys *et al*., 2015.

Hybridization plays a significant role in both speciation and species diversification, impacting traits like secondary metabolite production and morphological variation. Hybridization can result in significant changes in the production of secondary metabolites, which have implications for plant defense mechanisms and interactions with herbivores Chen *et al*., 2011. The objective of this study is to explore the variability in alkaloid profiles between the aerial parts (stems & leaves) of parental periwinkle plants and their hybrids (F1 and F2 generations).

2. Materials and Methods

This study was conducted at the Horticulture Administration of the Ministry of Agriculture ofKassala State, Sudan, during the period Jan 2016 and Sept 2019. Plant with pure red coloured and pure white coloured corolla were crossed and F1 plants were raised in the green house. F2 generation obtained by intercrossing F1 plants (Abdelmageed *et al*.2021), and Wesolowska *et al*., 2016). Crop management during the study period was carried out as the standard practice. Plants samples were collected during flowering and fruiting stages. Determination of corolla colour was determined. For laboratory analysis 100 g sample were taken from aerial parts (stems & leaves) for alkaloids content determination. Chemical speration of alkaloids was carried at Alawia Imam Pharmaceutical Research & Development Institute, University of Medical Sciences & Technology (UMST), Khartoum, Sudan. The indole alkaloids were extracted according to the methodology described by Misra *et al*., 2009, with small modification.

2.1. Extraction of Monoterpene Indole Alkaloids

Chemical analysis was performed at the Alawia Imam Pharmaceutical Research & Development Institute, University of Medical Sciences & Technology (UMST), Khartoum, Sudan. The indole alkaloids were extracted according to the methodology described by Misra *et al*.10 with a small modification.by Misra *et al*.,2009. (Misra et al ., 2009).

2.5 g of dried aerial parts (stems & leaves) 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 hours. 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.

2.2. 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-5ms, 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 minutes, then increased to 60°C over 30 minutes, and further increased to 230°C over 6 minutes, where it was held for 10 minutes. The final temperature of 230°C was maintained for 30 minutes. 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 minutes. 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.

3. Results

3.1. Production of F1hybrid and F2 generation

The result of this study shown that F1 plants produced from crossing of two parental of pink flower coloured, and white flowered, had Light pink corolla. The F2 progeny produced were atotal of 485 F2 plants They are classified according to the colours of the flowers. 125 plants had pink corollalike the parental stock. 227 plants had light pink corolla like the F1 plants and 133 plants had white corolla with the phenotypic ratio (1:2:1) Abdelmageed *et al*., 2021.

3.2. Production of Metabolites in two Parental F1 Hybrid and F2 generation

The results of the experiment shown Table 1 explores the variability of alkaloidal compounds in the aerial parts (Stem & Leaves) of Periwinkle (*Catharanthus roseus*) and their hybrids, focusing on the presence and concentration of specific alkaloids.

A total of 7 different alkaloids have been identified (Table 1). Among which Isovindolinine (0.70%), (2.01%), Vindolinine (0.94%), (1.23%). Pleiocarpamine (0.47%), (0%), Coronarridine (1.44%), (2.47%), Pericyvilivine (5.89%), (70.50%), Vindorosine (2.06%), (4.46%), and Vindoline (52.95%), (82.33%), respectively, were the most abundant in the parental plants (White, R'R' (rr)), and (Pink, RR). In contrast the Pleiocarpamine was absent in the pink-flowered parent (RR).

Table 1 Different levels of metabolites in the pure parents F1 hybrid and F2 generation in the aerial parts (Stem & Leaves)

Alkaloids (%)	W. P	P. P	F1	F2generation		
	Rr (rr)	RR	Rr	RR	Rr	Rr (rr)
Isovindolinine	0.70	2.01	0	2.19	1.63	3.31
Vindolinine	0.94	1.23	0	2.10	1.81	2.78
Pleiocarpamine	0.47	0	0	8.62	0	0
Coronarridine	1.44	2.47	10.01	1.90	2.79	1.46
Pericyvilivine	5.89	7.50	0	0	5.27	8.76
Vindorosine	2.06	4.46	3.53	3.71	3.60	4.11
Vindoline	52.95	82.33	86.46	81.48	84.90	79.58

W. P: White parent, P. P: Pink parent; F1: F1hybrid, F2: F2 generation; RR: Pink, Rr: light pink, rr: white

Experiment I: Parental lines :at onest flowering = after 140 days from seed crossing ; at flowering stages after 80 days from parental selfing at fruting stage = after 160 days from selfing of F1 individuals.

4. Discussion

The F1 plants produced from crossing of pink flower with white flowered, were all with light pink flower coloured . This indicate that the F1 plants had intermediate flower colour between the two parents which is probably due to incomplete dominance or there may be some minor modifier genes.

The experiment lists various alkaloids with their concentrations in the aerial parts (stems & leaves) of parental plants (both at the flowering stage), F1 (hybrids at the flowering stage), and F2 generation (at the fruiting stage). The alkaloids detected include:

Isovindolinine,Vindolinine, Pleiocarpamine, Coronarridine, Pericyvilivine, Vindorosine, and Vindoline, and Pleiocarpamine it is found only in white parent, rr), and absent in the pink-flowered parent (RR). This experiment focus on assessing the alkaloid profiles across different generations (parental and hybrid) at two different growth stages (flowering and fruiting). However, in the case of alkaloid compounds and chemical traits, there may be incomplete or lick of 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.

In the parental plants (White, rr) isovindolinine shows small amounts (0.70%), and in significant amounts in (RR) parent (2.01%). In the F1 hybrids, this alkaloids was absent (0%). In the F2 generation, the concentrations are significantly higher than in F1, and the (rr) parents, In F2 isovindolinine increases reaching (2.19%, 1.63% and 3.31%) in (RR), (Rr), and (rr), respectively. Vindolinine also shows in small amounts (0.94%%) in (White, R'R' rr) parent, and in significant a mounts in the (Pink, RR) parent (1.23%). In the F1 hybrids, this alkaloids was absent (0%), In the F2 generation concentrations of vindolinine were significantly higher than in F1, and the two parents reaching (2.10%, 1.81% and 2.78%) in (RR), (Rr), and (rr), respectively, suggesting that these compounds re-emerge in the F2 generation, possibly due to recombination and genetic segregation. Pleiocarpamine was found in parental plants (White, rr), in lower concentration 0.47% in the flowering stages and this alkaloids was absent in the parental plants (Pink, RR), and in the F1 hybrids with (0%). In the F2 generation alkaloid concentrations was found in significantly higher than in the (Pink, RR) parent, and F1 plants, it was abundant only in the parental plants (Pink, RR) hybrids with (8.62%), and it was absent (no deceted) in two hybrids (Rr), and (rr) respectively. suggesting that these compounds re-emerge in the F2 generation, possibly due to recombination and genetic segregation.

Coronarridine is found in significant concentrations with(1.44), and (2.47) in parental plants (White, rr), and (Pink, RR), respectively. At the flowering stages F1 (RR' Rr) hybrids, Coronarridine shows (in high amounts) with (10.01%). In the F2 generation the concentrations of this alkaloids was a round (1.90%), (2.79%), and (1.46) in the F2 generation (hybrids) of (RR), (Rr), and (rr), respectively. suggesting that these compounds re-emerge in the F2 generation, possibly due to recombination and genetic segregation. Pericyvilinine is a present small amounts in the parental plants (White, rr), (5.89%), and it was found (show) in high level (higher concentrations) in the parental plants (Pink, RR) with (7.50%). in the F1 hybrids F1 (Rr) hybrids this alkaloids it was absent (no deceted), and it was increasing in F2 (hybrids) in (Rr) with (5.27%) and (rr) with (8.76%), respectively. indicating that this alkaloid might be regulated by complex genetic factors.

Vindorosine is present in small amounts in the rr parent (2.06%), and it is found a higher in the RR parent at (4.46%). In the F1 hybrids F1 (Rr) hybrids this alkaloid concentrations are significantly in higher than in the white parent rr, and shows small level than the pink parent RR at (3.53%), and continues to decrease in F2 RR, and increase F2 hybrid Rr, and rr generation, suggesting that these compounds re-emerge in the F2 generation, possibly due to recombination and genetic segregation.

Vindoline is found in significant small of concentrations in the White parent rrwith (52.95%), and it was a highest level in the Pink parent RR with (82.33%), in the F1 hybrids F1 (RR') hybrids the alkaloid concentrations are significantly higher than in F1 the two parental plants (White, rr), and RR parent (parental plants (Pink, RR) vindoline reaching (35.70%), In the F2 generation the concentrations of this alkaloids was a round (81.84%), (84..90%), and (79.58%) in the F2 generation (RR), (Rr), and (rr), respectively. suggesting that these compounds re-emerge in the F2 generation, possibly due to recombination and genetic segregation. 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. 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. Our findings were consistent with those of previous studies in showing that hybridization can produce new alkaloid compounds and

alter production patterns across generations Abdelmageed *et al*., 2024. However, differences in the focus on color traits and the genetic ratios of alkaloid distribution suggest that our 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 Pais, and Jenny, 2018, and also in Liu *et al*., 2021.

Catharanthus roseus is one of the main source of terpenoid indole alkaloids used for preparation of diabetic drugs, cardiac drugs, in hypertension and in anticancer formation by the pharmaceutical industries[10]. Moreover, some of these therapeutic molecules are obtained by semi-synthesis using natural precursors extracted from *C. roseus* flowers and seeds.

5. Conclusion

This study investigates the genetic variability of alkaloid production in periwinkle plants and their hybrids. Alkaloids are organic compounds that have a wide range of pharmacological activities, making them important in the pharmaceutical industry. The study found that there were significant differences in alkaloid concentrations between the parental plants and their hybrid offspring. This variability highlights the complex inheritance patterns of alkaloid biosynthesis in *Catharanthus roseus*. The study provides insights into potential breeding strategies to optimize alkaloid production for pharmaceutical applications.

The study also examined the inheritance of flower color in periwinkle plants. Further research is needed to investigate the relationship between flower color and alkaloid production. Additionally, the study primarily examined alkaloid content in other parts of the plant (seeds and flower). Further research is needed to investigate assessing the of the alkaloid content in a pure line of the third hybrids of this plant (F3) .

This research has important implications for the pharmaceutical industry. By understanding the genetic basis of alkaloid production, breeders can develop new varieties of periwinkle plants that produce higher levels of these valuable compounds. This could lead to the development of new and more effective drugs.

Significance statement

This research investigated alkaloid profiles in white and pink flowered plants, and their hybrids, revealing distinct metabolite patterns. White flowered, and pink-flowered parents expressed multiple alkaloids, except Pleiocarpamine which was absent in pink-flowered parent. F1 hybrids showed there are four alkaloid in the two parents alkaloid (Isovindloine-Vindolinine- Pleiocarpamine- Pericyvilinine) showed no expression. In F2 generation, both parental expressed the all multiple alkaloids except Pleiocarpamine, which was absent in F2 rr. Also pericyvilininePericyvilinine which was absent in F2 RR. F2 hybrids Rr was expressed the all multiple alkaloids except Pleiocarpamine alkaloid. 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.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Authorship contribution statement

A H Abdelmageed : Writing – Original Draft, Methodology, Data Curation, Formal Analysis . H H. El-Kamali: Conceptualization, Supervision , Writing – Review and Editing. M E Abdelrahman : Supervision, Writing – Review and Editing.

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