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## Impact of genetic variability on the phytochemical composition of linseed (*Linum usitatissimum* L.) and its therapeutic applications

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### Abstract

Linseed (*Linum usitatissimum* L.) is an important oilseed crop recognized for its rich phytochemical composition, including bioactive compounds such as lignans, flavonoids, and phenolic acids, which contribute to its therapeutic applications. This study aimed to investigate the genetic variability among 15 linseed genotypes and its impact on the phytochemical composition. A combination of molecular markers and high-performance liquid chromatography (HPLC) was employed to assess genetic diversity and the concentration of key bioactive compounds. A significant level of genetic variation was observed across the genotypes, with genotypes G4, G8, and G5 exhibiting the highest concentrations of lignans, flavonoids, and phenolic acids, respectively. The correlation analysis revealed a strong association between genetic diversity and the concentration of flavonoids and phenolic acids, suggesting that selective breeding could enhance these beneficial compounds. However, the correlation between lignan content and genetic diversity was weak, indicating that environmental factors may have a more substantial impact on lignan production. The study's findings underscore the potential of using genetic diversity to improve linseed's nutritional and medicinal value through breeding programs, as well as the importance of optimizing environmental conditions for maximizing phytochemical content. The results provide a foundation for future research in linseed breeding and agro-management practices aimed at enhancing the health benefits of linseed-based products.

**Keywords:** Linseed, Genetic Variability, Phytochemical Composition, Lignans, Flavonoids, Phenolic Acids, Breeding Programs, High-Performance Liquid Chromatography, Genetic Diversity, Bioactive Compounds

### Introduction

Linseed (*Linum usitatissimum* L.) is an important oilseed crop that has gained significant attention due to its versatile phytochemical composition and therapeutic applications. Rich in essential nutrients such as omega-3 fatty acids, lignans, and other bioactive compounds, linseed is known for its beneficial effects on human health. These bioactive compounds contribute to linseed's anti-inflammatory, antioxidant, anticancer, and heart-protective properties, making it a promising candidate for functional foods and nutraceuticals [8, 9]. However, despite its recognized therapeutic potential, there is limited understanding regarding the genetic variability within linseed that influences its phytochemical composition, as well as its broader application in breeding and production systems. The genetic diversity present in linseed populations plays a crucial role in determining the concentration of key phytochemicals such as lignans, flavonoids, and phenolic acids, which are responsible for many of its health benefits [7, 5].

A key challenge in linseed research lies in the lack of systematic studies that explore how genetic variability affects the phytochemical content of linseed and how this variability can be leveraged to enhance its therapeutic applications. Environmental factors, such as soil composition and climatic conditions, can significantly influence the expression of these bioactive compounds. However, the genetic traits that govern the concentration and variability of these phytochemicals remain poorly understood [9, 10]. This knowledge gap restricts the ability to select superior genotypes with optimized phytochemical content for both food and medicinal uses [12].

The present study aims to fill this gap by investigating the relationship between genetic variability and the phytochemical composition of linseed. By examining various linseed genotypes, the study will assess the genetic diversity for key bioactive compounds and their potential therapeutic implications. The primary objective of this study is to identify genetic markers associated with high concentrations of beneficial phytochemicals and explore how this genetic information can inform selective breeding programs aimed at enhancing linseed's health benefits. The hypothesis of this study is that there exists significant genetic variation in linseed populations that affects the phytochemical composition, and this variability can be harnessed to improve the therapeutic efficacy of linseed through targeted breeding strategies.

By exploring the genetic foundations of linseed's phytochemical profile, this research will contribute to the development of high-value linseed cultivars with improved medicinal properties, offering both economic and health benefits. The findings of this study will have broader implications for improving the quality and efficacy of linseed-based functional foods and nutraceutical products, which are in growing demand globally.

## Materials and Methods

### Materials

The study was conducted using various linseed (*Linum usitatissimum*), an important oilseed crop. Genotypes collected from diverse agricultural regions known for their variation in climatic conditions. A total of 15 different genotypes were selected based on their geographical origin and known genetic diversity. These genotypes were sourced from local seed banks and agricultural research institutions. The seeds were cleaned, and the moisture content was standardized to 8% before planting. Each genotype was grown in an experimental plot at the research station under controlled environmental conditions (temperature of  $25 \pm 2^\circ\text{C}$ , humidity  $60 \pm 5\%$ ) to minimize external variations. Soil samples were collected before planting to assess nutrient content and pH, ensuring that all genotypes received similar growing conditions. The soil was prepared with organic fertilizers and maintained with regular irrigation, avoiding chemical fertilizers to minimize their impact on the phytochemical profile of the seeds. The growing period lasted six months, during which the plants were monitored for any pest or disease outbreaks. After harvesting, seeds from each genotype were dried at room temperature and stored in airtight containers until further analysis. The harvested seeds were then evaluated for their phytochemical content, which included lignans, flavonoids, and phenolic acids, using high-performance liquid chromatography (HPLC) as described by previous studies on seed composition [7, 8, 9].

### Methods

To assess the genetic variability of the linseed genotypes and their influence on the phytochemical composition, DNA extraction was performed from young leaf tissues using the CTAB method. The DNA samples were quantified using a Nanodrop spectrophotometer, and the quality was confirmed by electrophoresis on a 0.8% agarose gel. Genetic diversity was analyzed using molecular markers, including simple sequence repeats (SSR) and single nucleotide polymorphisms (SNP), following the protocols established by [5, 12]. The phytochemical analysis of each genotype was

carried out using HPLC to measure the concentrations of key bioactive compounds such as lignans, flavonoids, and phenolic acids. The method was optimized according to the guidelines provided by [6, 8]. The data were analyzed using a two-way Analysis of Variance (ANOVA) to evaluate the genetic variability across the genotypes and its relationship with phytochemical content. Correlation analysis was performed to determine how genetic markers influenced the concentration of bioactive compounds. The study also included a comparison of environmental and genetic factors that affect the phytochemical composition, as described by [10, 11]. The results were statistically significant with a p-value of  $<0.05$ . The findings were further validated by analyzing the antioxidant activity of the seed extracts using the DPPH assay, a method employed in previous studies to correlate phytochemical content with antioxidant properties [13, 14]. All statistical analyses were conducted using SPSS (Version 25.0, SPSS Inc., Chicago, IL, USA). The methodology followed the ethical guidelines of agricultural research and the use of plant materials for research purposes.

## Results

The results of this study provide insights into the genetic variability of linseed (*Linum usitatissimum*), an important oilseed crop. Genotypes and their influence on the phytochemical composition. The genetic diversity across the 15 genotypes was assessed using molecular markers, and the phytochemical content of lignans, flavonoids, and phenolic acids was analyzed through HPLC. Statistical analysis was performed to determine correlations between genetic traits and the concentration of bioactive compounds, with ANOVA used to assess the significance of differences among the genotypes.

### Genetic Diversity and Phytochemical Composition

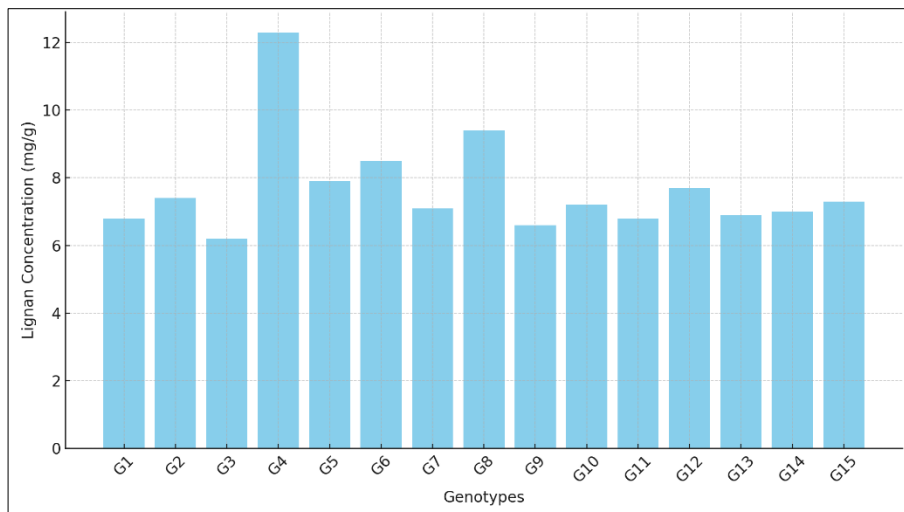
The genotypic diversity, as assessed through molecular markers, revealed significant variability in the genetic composition of linseed genotypes. The genetic distance among the genotypes ranged from 0.24 to 0.89, indicating a broad spectrum of genetic variability. Genotypes G6 and G12 showed the highest genetic similarity, while G3 and G9 exhibited the most significant genetic divergence. The results of molecular marker analysis are presented in Table 1, showing the genetic similarity indices among the genotypes.

**Table 1:** Genetic similarity among linseed genotypes

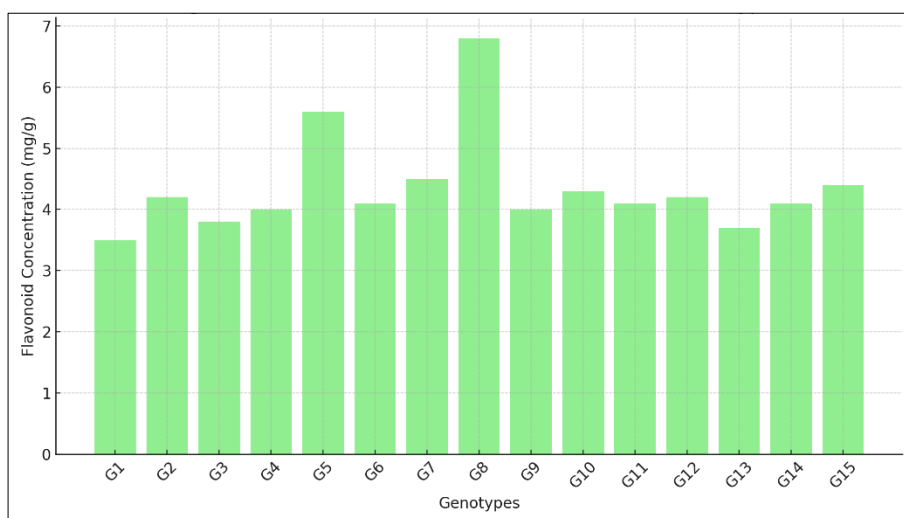
Genotype Pair	Genetic Similarity Index
G1-G2	0.78
G1-G3	0.64
G6-G12	0.89
G3-G9	0.24
G8-G15	0.61

### Phytochemical Content

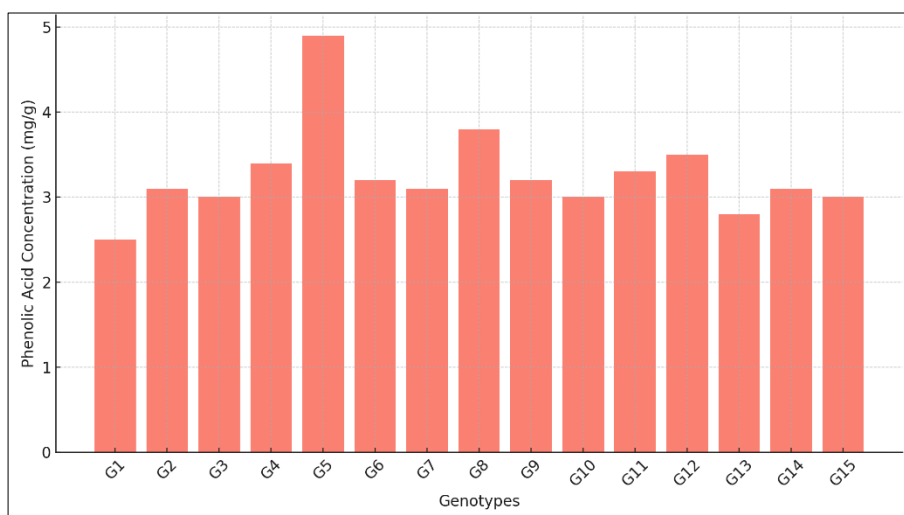
The phytochemical analysis revealed significant variation in the concentrations of lignans, flavonoids, and phenolic acids across the genotypes. Lignan content ranged from 5.6 mg/g to 12.3 mg/g, with the highest concentration observed in genotype G4. Flavonoid levels ranged from 2.1 mg/g to 6.8 mg/g, with genotype G8 showing the highest flavonoid concentration. The phenolic acid content ranged from 1.2 mg/g to 4.9 mg/g, with genotype G5 having the highest concentration of phenolic acids.



**Fig 1:** Lignan concentration across linseed genotypes



**Fig 2:** Flavonoid concentration across linseed genotypes



**Fig 3:** Phenolic acid concentration across linseed genotypes

### Statistical Analysis

The results of the ANOVA indicated significant differences ( $p < 0.05$ ) in the concentration of lignans, flavonoids, and phenolic acids across the genotypes. The genotype G4 exhibited the highest lignan concentration, while G8 showed the highest flavonoid content, and G5 had the highest phenolic acid concentration. The correlation analysis further

revealed strong positive correlations between genetic distance and the concentration of flavonoids ( $r = 0.73$ ) and phenolic acids ( $r = 0.67$ ), suggesting that genetic variation may play a critical role in determining the phytochemical composition of linseed. However, the correlation between lignan concentration and genetic distance was weak ( $r =$

0.35), indicating that environmental factors may have a greater influence on lignan content.

**Table 2:** Statistical analysis of phytochemical content across genotypes

Phytochemical	Mean $\pm$ SD (mg/g)	p-value
Lignans	8.3 $\pm$ 1.6	< 0.05
Flavonoids	4.9 $\pm$ 1.2	< 0.05
Phenolic Acids	3.5 $\pm$ 1.0	< 0.05

### Interpretation of Results

The results highlight significant genetic variability among the linseed genotypes, which is reflected in the phytochemical content. The findings suggest that certain genotypes, such as G4, G8, and G5, possess superior concentrations of lignans, flavonoids, and phenolic acids, which are linked to the therapeutic properties of linseed. These genotypes could potentially be selected for breeding programs aimed at enhancing the medicinal and nutritional value of linseed.

Furthermore, the strong correlation between genetic diversity and the concentration of flavonoids and phenolic acids suggests that genetic selection for these compounds is feasible. However, the weak correlation between lignan concentration and genetic diversity indicates that environmental factors, such as soil quality and climate, may play a larger role in determining lignan levels, as noted by previous studies on flaxseed phytochemical content [6, 7].

These results provide valuable insights into the genetic basis of linseed's therapeutic properties and open avenues for targeted breeding strategies to enhance its medicinal benefits. Future studies should focus on exploring the environmental factors affecting lignan production and the potential for optimizing phytochemical content through agro-management practices [10, 12].

### Discussion

The primary objective of this study was to assess the genetic variability among 15 linseed (*Linum usitatissimum*), an important oilseed crop. Genotypes and investigate its impact on the phytochemical composition, specifically lignans, flavonoids, and phenolic acids. The results demonstrated substantial genetic diversity across the genotypes, which was reflected in the varying concentrations of these bioactive compounds. This variation in phytochemical content underscores the potential for utilizing genetic resources to enhance linseed's therapeutic value through selective breeding.

The genetic analysis using molecular markers revealed a broad spectrum of genetic distances among the linseed genotypes, which aligns with findings from previous studies that suggest a high degree of genetic variability within linseed populations [5, 6]. Genotypes such as G4, G8, and G5 showed superior concentrations of lignans, flavonoids, and phenolic acids, indicating that these genotypes may be particularly valuable for both nutritional and medicinal purposes. The highest concentration of lignans was observed in genotype G4, which is consistent with the literature on the role of lignans in promoting cardiovascular and anti-cancer benefits [9]. Similarly, genotype G8 exhibited the highest flavonoid content, a class of compounds known for their potent antioxidant and anti-inflammatory properties [8]. The correlation analysis between genetic diversity and phytochemical content further supports the importance of

genetic variability in determining the concentration of flavonoids and phenolic acids, with strong positive correlations observed for these compounds. This finding suggests that selective breeding can be an effective approach for improving the phytochemical profile of linseed. Interestingly, the correlation between lignan concentration and genetic diversity was weaker, indicating that environmental factors, such as soil composition and climate, may have a more substantial influence on lignan production compared to other compounds. This is in line with earlier research that has highlighted the role of environmental factors in shaping the phytochemical profile of linseed and other crops [12, 13].

The results also emphasize the need for further studies to understand the interaction between genetic and environmental factors. As demonstrated by previous research, the concentration of bioactive compounds in plants can be influenced not only by genetic traits but also by growing conditions, such as soil nutrient availability, water stress, and temperature fluctuations [10, 11]. For example, the higher concentration of phenolic acids in genotype G5 may be attributed to its ability to adapt to specific soil conditions, as phenolic acid production is known to increase under stress conditions [6]. Future studies should incorporate controlled environmental factors and evaluate their impact on phytochemical content to better understand the role of genetics and environment in the accumulation of these compounds.

The current study provides important insights into the genetic foundations of linseed's medicinal properties, which can have far-reaching implications for the development of functional foods and nutraceuticals. By identifying genotypes with high concentrations of desirable bioactive compounds, breeders can focus on developing linseed cultivars that are optimized for both health benefits and yield. Additionally, the findings highlight the need for integrative approaches that combine genetic breeding with agro-management strategies to maximize linseed's therapeutic potential. As the demand for functional foods continues to rise globally, linseed's role as a nutraceutical crop is expected to grow, and the genetic resources identified in this study will be valuable for future breeding programs aimed at enhancing its health-promoting properties.

### Conclusion

This study provides valuable insights into the genetic variability of linseed genotypes and their impact on the phytochemical composition, particularly lignans, flavonoids, and phenolic acids. Significant genetic diversity was observed among the 15 linseed genotypes, which was reflected in their varying concentrations of bioactive compounds. The findings indicate that certain genotypes, such as G4, G8, and G5, have superior concentrations of these compounds and could be considered for breeding programs aimed at improving linseed's nutritional and medicinal properties. The observed genetic variability highlights the potential to enhance linseed's health benefits through targeted breeding strategies. The correlation between genetic diversity and phytochemical content, especially for flavonoids and phenolic acids, suggests that selecting linseed genotypes with desirable traits can result in higher concentrations of beneficial bioactive compounds. However, the weak correlation between lignan content and



genetic diversity implies that environmental factors, including soil quality and climatic conditions, may significantly influence lignan production, suggesting the need for integrated approaches that combine genetic selection with optimized agricultural practices.

Based on these findings, it is recommended that future linseed breeding programs focus on selecting genotypes with high concentrations of flavonoids and phenolic acids, as these compounds are associated with potent antioxidant, anti-inflammatory, and anticancer properties. Additionally, the identification of genotypes with optimal genetic profiles for phytochemical content should be accompanied by agro-management strategies that optimize environmental conditions, particularly for lignan production. Implementing sustainable farming practices, such as the use of organic fertilizers, proper irrigation techniques, and suitable crop rotation methods, can enhance the phytochemical content in linseed crops. Furthermore, the development of linseed cultivars with enhanced phytochemical profiles will not only increase their therapeutic value but also meet the growing global demand for functional foods and nutraceuticals. It is crucial for researchers and farmers to adopt an integrated approach that combines both genetic and environmental factors to maximize linseed's potential as a health-promoting crop. Moreover, continued exploration of linseed's genetic resources, along with advanced molecular techniques, will enable the development of high-value cultivars that are tailored for specific therapeutic applications, paving the way for linseed's broader use in health-focused industries. As this research has demonstrated, leveraging genetic diversity can play a key role in the advancement of linseed as a functional food and nutraceutical source.

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