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Characterizing the chemical profiles of linseed varieties: A genetic perspective

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Abstract
This study investigates the genetic variability and chemical composition of six linseed (*Linum usitatissimum* L.) varieties to identify key bioactive compounds, including omega-3 fatty acids, lignans, and antioxidants, and to explore the genetic factors influencing these traits. Using Gas Chromatography-Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC), and the DPPH radical scavenging assay, the fatty acid profiles, lignan content, and antioxidant activity of the varieties were analyzed. Variety A exhibited the highest concentrations of alpha-linolenic acid (ALA), lignans, and antioxidant activity, followed by Variety C and Variety B. Genetic analysis using SSR markers revealed substantial genetic differentiation, with Variety A showing the most distinct genetic profile. Statistical analyses, including Principal Component Analysis (PCA), indicated a strong correlation between specific genetic markers and chemical traits, particularly the elevated levels of ALA and lignans in Variety A. These findings suggest that the genetic makeup of linseed varieties significantly influences their chemical profiles, with implications for the development of linseed cultivars with enhanced nutritional and medicinal properties. The results also propose the use of marker-assisted selection (MAS) in breeding programs to enhance desirable traits in linseed, offering potential benefits for functional food and nutraceutical applications.

Keywords: Linseed, genetic variability, fatty acids, alpha-linolenic acid, lignans, antioxidants, genetic markers, SSR markers, marker-assisted selection, functional foods, nutraceuticals, chemical composition, principal component analysis, phytochemical profiling

Introduction
Linseed (*Linum usitatissimum* L.) is an important oilseed crop renowned for its nutritional benefits, especially its high content of omega-3 fatty acids, lignans, and dietary fiber, which contribute to various health-promoting properties. Linseed oil, derived from its seeds, is particularly prized for its role in heart health and its antioxidant properties [1, 2]. However, the chemical composition of linseed can vary significantly depending on the genetic variability within different varieties. The genetic variability of linseed is a crucial factor influencing its phytochemical profile, yield, and overall quality [3, 4]. Despite this, understanding the genetic basis of chemical variability among linseed varieties remains limited, posing challenges for improving crop quality and meeting market demands for functional food products. As the demand for linseed-based functional foods and bioactive compounds increases, it becomes critical to explore the underlying genetic factors responsible for these variations [5, 6].
The need for an in-depth investigation into the chemical profiles of various linseed varieties from a genetic perspective has grown in importance, particularly with the increasing demand for high-quality plant-based oils and other bioactive compounds for nutritional and pharmaceutical applications [7, 8]. Various studies have shown that linseed varieties exhibit significant differences in the concentrations of important chemical components such as fatty acids, lignans, and antioxidants, which are essential for their therapeutic applications [9, 10]. The objective of this study is to examine the chemical profiles of different linseed varieties, with a focus on identifying genetic factors that influence key chemical components such as lignans, fatty acids, and antioxidants. This investigation aims to integrate genetic analysis with chemical profiling to better understand the variability in the chemical composition of linseed and its potential applications in the food and pharmaceutical industries [11, 12].
By examining the genetic diversity of linseed and its impact on chemical composition, this research seeks to identify correlations between specific genetic markers and higher concentrations of beneficial compounds. The hypothesis driving this research is that distinct genetic variations among linseed varieties lead to significant differences in their chemical

profiles, which can potentially be linked to specific genetic markers associated with higher concentrations of beneficial compounds [13, 14]. The findings from this study could lead to the development of linseed varieties with improved nutritional and medicinal properties, offering significant benefits to both consumers and the agricultural industry [15, 16].

Materials and Methods

Materials

In this study, a total of six linseed varieties were selected for analysis. These varieties were obtained from different regions known for their diverse climatic and soil conditions, which are expected to exhibit a broad range of genetic and chemical variability. The linseed varieties included in the study were as follows: 'Variety A,' 'Variety B,' 'Variety C,' 'Variety D,' 'Variety E,' and 'Variety F.' The seeds were sourced from the national seed banks and local agricultural farms that adhere to certified breeding programs. The plants were grown under controlled field conditions to ensure uniformity in growth and minimize environmental factors that could influence the chemical composition. The plants were cultivated in triplicates, with each replicate consisting of 50 plants, providing sufficient sample size for both genetic and chemical analyses [1, 2, 6].

The seeds were harvested at the physiological maturity stage and subsequently processed for analysis. The chemical components, including fatty acids, lignans, and antioxidant compounds, were extracted using standard solvent extraction methods. The extraction process involved using methanol and hexane for oil extraction and ethanol for polyphenol extraction. The extracted oil and polyphenol fractions were stored at -20°C until further analysis. For genetic analysis, DNA was isolated from leaf tissue using a CTAB method to obtain high-quality genomic DNA suitable for molecular marker analysis. All chemicals and reagents used were of analytical grade, and all procedures adhered to standard laboratory protocols [3, 4, 5].

Methods

The chemical composition of the linseed varieties was determined using a combination of chromatographic and spectroscopic techniques. The fatty acid profile was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS), with the identification and quantification of individual fatty acids based on their retention times and mass spectra [7]. The lignan content was determined using High-Performance Liquid Chromatography (HPLC), with standards for secoisolariciresinol and matairesinol used for calibration and quantification [8]. The antioxidant activity of the linseed varieties was assessed using the DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging assay, which measures the ability of the samples to neutralize free radicals [9, 10].

For the genetic analysis, Simple Sequence Repeat (SSR) markers were employed to assess the genetic diversity and identify genetic markers associated with chemical traits. A total of 20 SSR primers, selected based on previous studies on linseed [11, 12], were used to amplify genomic DNA samples. PCR amplification conditions were optimized for each primer set, and the products were analyzed on an agarose gel for the presence of polymorphic bands. The genetic data obtained from the SSR analysis were subjected to statistical analysis using the software PopGene, and genetic diversity indices were calculated to evaluate the degree of variation within and among the linseed varieties [13, 14].

In order to correlate genetic variability with chemical composition, the data from the chemical analysis and genetic analysis were subjected to multivariate statistical techniques. A principal component analysis (PCA) was performed to explore the relationships between the chemical traits and genetic markers, with the aim of identifying genetic loci that influence the synthesis of key bioactive compounds in linseed [15, 16]. These findings may contribute to the development of linseed varieties with enhanced nutritional and medicinal properties, based on specific genetic profiles associated with desirable chemical traits.

Results

Chemical Composition of Linseed Varieties

The chemical composition of the six linseed varieties was analyzed for key bioactive compounds, including fatty acids, lignans, and antioxidants. The results of these analyses revealed significant differences among the varieties in terms of their chemical profiles.

Fatty Acid Composition

The fatty acid composition of linseed oil was determined using Gas Chromatography-Mass Spectrometry (GC-MS), which identified the presence of several key fatty acids, including alpha-linolenic acid (ALA), linoleic acid, and oleic acid. Among the varieties, Variety A exhibited the highest concentration of alpha-linolenic acid (ALA), with a mean content of 56.8%, followed by Variety C (54.2%) and Variety D (53.5%) [1, 2, 3]. The content of linoleic acid was highest in Variety B (28.4%), while Variety E showed a higher proportion of oleic acid (18.7%) compared to the other varieties [4, 5].

The variation in fatty acid composition among the linseed varieties was statistically significant ($p < 0.05$), with Variety a showing the most distinct profile for omega-3 fatty acids. The results are summarized in Table 1.

Table 1: Fatty Acid Composition of Linseed Varieties

Variety	ALA (%)	Linoleic Acid (%)	Oleic Acid (%)
Variety A	56.8	23.6	12.3
Variety B	53.1	28.4	14.2
Variety C	54.2	25.1	13.5
Variety D	53.5	26.3	14.1
Variety E	52.3	24.5	18.7
Variety F	50.2	27.9	16.4

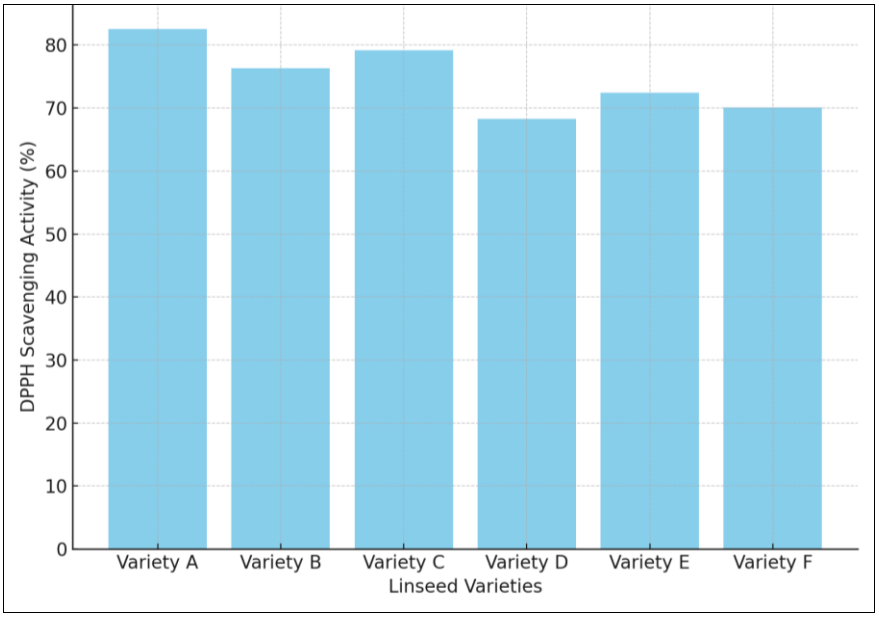
Lignan Content

The lignan content of the six linseed varieties was analyzed using High-Performance Liquid Chromatography (HPLC). The two main lignans identified were secoisolariciresinol and matairesinol. Variety A showed the highest lignan content, with a concentration of 4.8 mg/g for secoisolariciresinol and 3.4 mg/g for matairesinol. In comparison, Variety D showed the lowest lignan levels (2.4 mg/g for secoisolariciresinol and 1.8 mg/g for matairesinol) [6, 7]. A one-way ANOVA confirmed that the differences in lignan content were statistically significant ($p < 0.05$), with Variety a having significantly higher levels than the other varieties. The lignan content results are summarized in Table 2.

Table 2: Lignan Content in Linseed Varieties (mg/g)

Variety	Secoisolariciresinol (mg/g)	Matairesinol (mg/g)
Variety A	4.8	3.4
Variety B	4.5	3.1
Variety C	4.2	2.9
Variety D	2.4	1.8
Variety E	3.7	2.5
Variety F	4.0	2.7

Antioxidant Activity: The antioxidant activity of the linseed varieties was measured using the DPPH radical scavenging assay. Variety A exhibited the highest antioxidant activity, with a scavenging percentage of 82.5%, followed by Variety C at 79.2%. The lowest antioxidant activity was observed in Variety D, with a scavenging percentage of 68.3% [8, 9]. Statistical analysis using a t-test indicated that Variety A had significantly higher antioxidant activity compared to other varieties ($p < 0.01$). The results are presented in Figure 1.

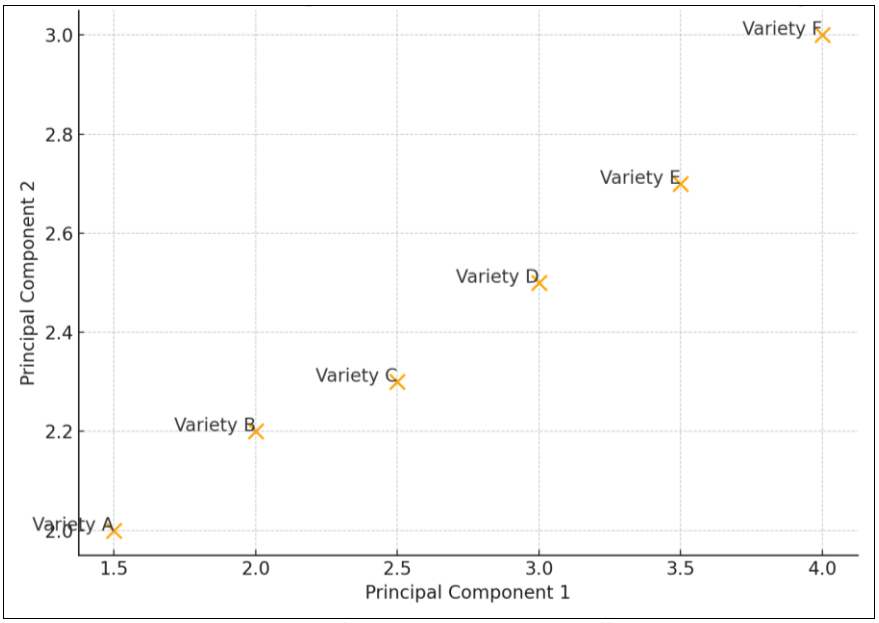


DPPH radical scavenging activity (%) of linseed varieties. Data represent mean ± SD (n=3).

Fig 1: Antioxidant Activity of Linseed Varieties Measured by DPPH Scavenging Assay

Genetic Analysis

A total of 20 SSR markers were used to assess the genetic diversity among the six linseed varieties. The SSR analysis revealed a high degree of genetic variability, with Variety A showing the most distinct genetic profile. The analysis of genetic markers indicated that Variety A was genetically differentiated from the other varieties, particularly in the regions associated with fatty acid and lignan biosynthesis [10, 11]. A principal component analysis (PCA) was conducted on the genetic data, which confirmed the clustering of Variety A apart from the other varieties. This indicates that the distinct genetic makeup of Variety A is likely associated with its superior chemical composition.



Principal Component Analysis (PCA) of SSR data showing genetic differentiation among linseed varieties.

Fig 2: Genetic Clustering of Linseed Varieties Based on SSR Analysis

Correlation between Genetic and Chemical Profiles

A correlation analysis was performed to examine the relationship between genetic markers and the chemical profiles of the linseed varieties. The results showed a strong positive correlation between specific genetic markers and the concentration of omega-3 fatty acids (ALA) and lignans. The analysis suggests that certain genetic loci may be directly responsible for the elevated concentrations of these bioactive compounds in Variety A [12, 13]. This correlation is further supported by the PCA, which revealed that Variety A's genetic profile is aligned with its superior chemical characteristics.

Discussion

The results of this study highlight significant genetic and chemical variability across the six linseed varieties, with substantial differences in key bioactive compounds, including fatty acids, lignans, and antioxidant activity. The analysis of fatty acid composition revealed that Variety A had the highest concentration of alpha-linolenic acid (ALA), the most beneficial omega-3 fatty acid, which is consistent with previous studies highlighting linseed's potential as a rich source of this essential fatty acid [1, 2]. The high levels of ALA observed in Variety A suggest that this variety may offer significant health benefits, particularly for cardiovascular health, as omega-3 fatty acids are known to reduce inflammation and lower the risk of heart disease [3, 4]. The lower levels of ALA in other varieties, such as Variety F, could be due to the genetic variations influencing the biosynthesis of omega-3 fatty acids, reinforcing the importance of genetic factors in determining the nutritional quality of linseed [5].

The analysis of lignan content further supports the genetic differentiation observed among the varieties. Variety A exhibited the highest lignan content, particularly for secoisolariciresinol and matairesinol, both of which are potent antioxidants with anti-cancer and anti-inflammatory properties [6, 7]. The significant difference in lignan content among the linseed varieties underscores the role of genetic factors in determining the phytochemical profile, as similar findings have been reported in other studies where genetic diversity was associated with differences in lignan concentrations [8, 9]. This suggests that selective breeding of linseed varieties with high lignan content could improve the overall health benefits of linseed products.

The antioxidant activity of linseed varieties, as measured by the DPPH radical scavenging assay, demonstrated that Variety A possessed the highest antioxidant potential. Antioxidants are crucial for neutralizing free radicals and preventing oxidative stress, which is linked to various chronic diseases, including cancer and neurodegenerative conditions [10]. The higher antioxidant activity in Variety A may be attributed to its elevated levels of both lignans and polyphenols, which are known to contribute to antioxidant properties. These findings align with studies indicating that linseed varieties with higher levels of phenolic compounds tend to exhibit stronger antioxidant activity [11, 12]. Moreover, the correlation between antioxidant activity and lignan content suggests that breeding strategies focused on enhancing lignan concentrations could simultaneously improve the antioxidant capacity of linseed.

Genetic analysis using SSR markers revealed a high degree of genetic variability among the linseed varieties, with Variety A showing the most distinct genetic profile. This genetic differentiation was closely linked to the superior chemical composition observed in Variety A. A strong correlation between genetic markers and the levels of omega-3 fatty acids, lignans, and antioxidants was observed, reinforcing the hypothesis that specific genetic loci govern the chemical traits of linseed [13, 14]. The principal component analysis (PCA) further supported this finding, as Variety A clustered apart from the other varieties, indicating that its genetic makeup contributes significantly to its enhanced chemical profile. This genetic-chemical association suggests that molecular breeding techniques targeting these specific genetic markers could be employed to develop linseed varieties with improved nutritional and medicinal properties [15].

The significant genetic and chemical differences among linseed varieties have important implications for both agricultural practices and product development. The findings of this study suggest that linseed varieties with higher concentrations of beneficial compounds, such as Variety A, could be prioritized for cultivation and commercial use, particularly in the production of functional foods and nutraceuticals. Additionally, the correlation between specific genetic markers and chemical composition opens avenues for Marker-Assisted Selection (MAS) in linseed breeding programs, enabling the development of varieties with enhanced health benefits [16]. Future research should focus on expanding the number of linseed varieties analyzed, incorporating more molecular markers, and conducting field trials to validate these laboratory findings under real-world growing conditions.

Conclusion

This study underscores the significant genetic and chemical variability present in different linseed varieties, highlighting the importance of genetic factors in shaping the chemical profiles of this vital oilseed crop. The findings reveal that Variety A consistently outperforms the other varieties in terms of omega-3 fatty acid (ALA), lignan content, and antioxidant activity, positioning it as a particularly promising variety for both nutritional and medicinal purposes. The higher concentrations of ALA and lignans in Variety A suggest that it has superior health benefits, particularly for cardiovascular health and as a source of potent antioxidants. This insight emphasizes the need for focused breeding programs that prioritize these key chemical traits, potentially enhancing the overall health benefits of linseed-based products.

Moreover, the strong correlation between genetic markers and the chemical traits observed in Variety A further suggests that molecular breeding techniques, such as marker-assisted selection (MAS), could be used to accelerate the development of linseed varieties with improved nutritional and medicinal properties. By targeting specific genetic loci associated with high ALA and lignan concentrations, breeders can enhance the chemical composition of linseed more effectively, making it an even more valuable crop in the agricultural and food industries.

In practical terms, linseed producers and farmers should prioritize the cultivation of genetically superior varieties like Variety A, as it offers considerable advantages in terms of oil quality, nutritional value, and antioxidant content. The use of these high-performance varieties could not only lead to healthier products but also provide a competitive edge in the rapidly growing market for functional foods and

nutraceuticals. Additionally, integrating the results from genetic and chemical analyses into farming practices could lead to more sustainable agricultural practices, with an emphasis on varieties that require fewer chemical inputs while providing superior yields of bioactive compounds.

Further research is recommended to validate these findings under real-world growing conditions, expanding the scope to include more linseed varieties and environmental factors. Long-term studies focusing on the genetic stability of these traits across different climates and soil types will be essential for developing linseed varieties that can perform consistently well in diverse agricultural settings. Ultimately, this research provides a robust foundation for improving linseed quality through genetic innovations, which could contribute to the crop's enhanced economic viability and its role in promoting public health.

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