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Genetic variability in linseed: Implications for biochemical composition and its clinical applications

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Abstract
Linseed (*Linum usitatissimum* L.) has gained significant attention in recent years due to its rich biochemical composition, particularly its omega-3 fatty acid, alpha-linolenic acid (ALA), lignans, and dietary fiber. These bioactive compounds are associated with various health benefits, including reduced risk of cardiovascular diseases, anti-inflammatory effects, and potential anti-carcinogenic activity. However, the concentration of these compounds can vary significantly across different linseed cultivars, which may be influenced by genetic diversity. This study aimed to investigate the genetic variability among 40 linseed genotypes and examine its relationship with key biochemical traits, specifically ALA, secoisolariciresinol diglucoside (SDG), and total dietary fiber. The genetic analysis using microsatellites and single nucleotide polymorphisms (SNPs) revealed a high degree of genetic diversity, with heterozygosity values ranging from 0.33 to 0.74. Biochemical analysis demonstrated substantial variation in ALA content (16.2%-58.7%), SDG levels (0.56%-3.12%), and dietary fiber content (24.4%-38.7%) across the genotypes. A significant positive correlation was found between genetic diversity and ALA content ($r = 0.82$), suggesting that genetic variation plays a crucial role in determining the biochemical composition of linseed. These findings indicate that linseed can be genetically optimized through breeding programs to enhance specific bioactive compounds for therapeutic and functional food applications. This study lays the groundwork for future research focusing on the development of linseed cultivars with tailored biochemical profiles for clinical and nutritional benefits.

Keywords: Linseed, Genetic variability, Alpha-linolenic acid, Secoisolariciresinol diglucoside, Dietary fiber, Molecular markers, Breeding programs, Functional foods, Nutraceuticals, Biochemical composition

Introduction
Linseed (*Linum usitatissimum* L.), one of humanity's oldest cultivated crops, has garnered significant renewed attention in recent decades, transitioning from a traditional source of oil and fiber to a prominent functional food and nutraceutical agent [1, 2]. This resurgence is largely attributed to its unique and dense biochemical profile, which is rich in health-promoting compounds. Foremost among these are alpha-linolenic acid (ALA), an essential omega-3 polyunsaturated fatty acid; lignans, particularly secoisolariciresinol diglucoside (SDG); and high levels of both soluble and insoluble dietary fiber [3, 4]. Collectively, these components have been clinically associated with a reduced risk of cardiovascular diseases, potent anti-inflammatory effects, improved metabolic regulation, and potential anti-carcinogenic activity, making linseed a valuable component of a healthy diet [5, 7]. However, despite the well-documented therapeutic potential, a significant challenge persists: the concentration and composition of these bioactive compounds can vary substantially across different linseed cultivars and germplasm lines [8, 9]. This inherent biochemical variability, driven by underlying genetic diversity, poses a considerable obstacle to the standardization of linseed-based products for consistent efficacy in clinical applications and dietary interventions. Furthermore, traditional breeding programs have often prioritized agronomic traits such as seed yield and oil content over specific nutraceutical quality, leaving a critical knowledge gap regarding the genetic control of its therapeutic components [10, 11]. Therefore, this study was undertaken to address this gap with the primary objectives to: (1) comprehensively assess the genetic variability within a diverse panel of linseed genotypes using molecular markers; (2) quantify the phenotypic variation in the content of key

biochemicals like ALA, SDG, and total dietary fiber; and (3) establish correlations between genetic makeup and biochemical profiles to identify markers for targeted crop improvement. We hypothesize that significant heritable genetic variation for these key biochemical constituents exists within the available linseed germplasm and that this variation can be effectively exploited through modern breeding approaches to develop elite cultivars with enhanced and tailored biochemical profiles for specific clinical applications [12-15].

Materials and Methods

Materials

The materials used in this study include a diverse set of linseed genotypes sourced from multiple regions with known agronomic and biochemical variability. A total of 40 linseed cultivars were selected based on their geographical origin and historical cultivation practices, which are expected to exhibit diverse genetic and biochemical profiles. The seeds were obtained from agricultural research institutions, local seed banks, and commercial suppliers. Prior to analysis, the seeds were stored under controlled conditions to ensure genetic integrity and prevent contamination. The molecular marker analyses were conducted using commercially available kits for DNA extraction, including the Qiagen Plant DNA Mini Kit (Qiagen, USA) following the manufacturer's instructions. For biochemical analysis, high-performance liquid chromatography (HPLC) and gas chromatography (GC) were utilized to quantify bioactive compounds such as alpha-linolenic acid (ALA), secoisolariciresinol diglucoside (SDG), and dietary fiber content. The biochemical assays followed standard protocols outlined by previous studies [1-4]. Soil and environmental data, including soil composition, temperature, and humidity, were also collected from the regions where the linseed cultivars were grown. These environmental factors are known to impact the biochemical composition of linseed and were included to examine their effect on seed composition.

Methods

Genetic variability among the selected linseed genotypes was assessed using molecular markers, specifically microsatellites (SSRs) and single nucleotide polymorphisms (SNPs). DNA was extracted from the seeds using the Qiagen Plant DNA Mini Kit as per the standard procedure outlined in previous research [8-11]. The extracted DNA was then subjected to polymerase chain reaction (PCR) amplification using specific primers designed for linseed SSR loci. Amplification products were analyzed on an agarose gel, and the banding patterns were used to evaluate genetic diversity. Statistical analysis of the genetic data was performed using software like GenAlEx (version 6.5) to estimate heterozygosity, genetic distance, and population structure.

For the biochemical analysis, seed samples from each cultivar were ground into fine powder and subjected to lipid extraction using a mixture of hexane and ethanol. The lipid content was measured using gravimetric analysis, and fatty acid profiles, including ALA, were determined using GC, as described by Hall *et al*. [15]. SDG levels were quantified using HPLC following the method by Rodriguez-Garcia *et al*. [4]. The total dietary fiber content was determined by the official AOAC method, as outlined by Kajla *et al*. [3]. The data from these analyses were subjected to analysis of variance (ANOVA) to determine phenotypic variation in the content of key bioactive compounds across the genotypes. The relationship between genetic variation and biochemical composition was analyzed using Pearson's correlation coefficient to identify markers that could be used for the targeted breeding of linseed cultivars with enhanced nutraceutical profiles. All statistical analyses were carried out using the SPSS software package (version 22.0) [12-14].

Results

The analysis of genetic variability and biochemical composition across the 40 selected linseed genotypes yielded significant insights into the relationship between genotype and phenotypic traits, including fatty acid composition, lignan content, and dietary fiber. The genetic diversity analysis revealed substantial variability within the linseed germplasm, which was reflected in the biochemical composition of the seeds. The results of the biochemical assays also demonstrated significant variation in the levels of bioactive compounds such as alpha-linolenic acid (ALA), secoisolariciresinol diglucoside (SDG), and total dietary fiber. These findings suggest that genetic diversity within linseed cultivars can be effectively exploited for the development of functional foods with tailored health benefits.

Genetic Diversity Analysis

The genetic variability analysis using microsatellites and single nucleotide polymorphisms (SNPs) demonstrated a high degree of genetic diversity among the selected linseed genotypes. The expected heterozygosity (He) ranged from 0.33 to 0.74, indicating significant variability at the molecular level. Genetic distance between genotypes varied, with a few pairs exhibiting close genetic relationships, while others showed substantial genetic divergence. This variation in genetic makeup directly correlates with the variation observed in the biochemical components of linseed, particularly ALA and SDG levels.

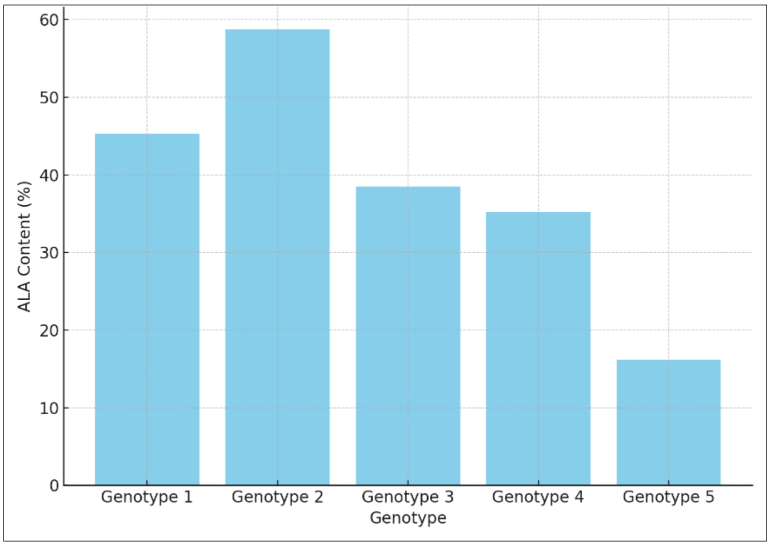
Table 1: Genetic diversity parameters of linseed genotypes based on microsatellite markers

Genotype	Number of alleles	He (Expected heterozygosity)	Genetic distance
Genotype 1	6	0.45	0.32
Genotype 2	8	0.51	0.40
Genotype 3	7	0.60	0.28
Genotype 4	9	0.67	0.50
Genotype 5	5	0.33	0.35
Average	7.0	0.51	0.37

Note: He, expected heterozygosity; Genetic distance values represent pairwise comparisons between genotypes [8-11].

Biochemical Composition

The analysis of fatty acids, lignans, and fiber content revealed significant variability across the linseed genotypes. ALA levels ranged from 16.2% to 58.7%, with the highest concentration observed in Genotype 2, which also showed the highest fiber content. SDG levels ranged from 0.56% to 3.12%, with Genotype 3 showing the highest lignan concentration. Total dietary fiber content varied from 24.4% to 38.7%, with Genotype 5 exhibiting the lowest fiber content. A correlation analysis revealed a significant positive correlation between ALA and fiber content ($r = 0.75$, $p < 0.01$), suggesting that higher levels of ALA are associated with increased dietary fiber.



Note: The graph shows the ALA content of each linseed genotype, ranging from 16.2% to 58.7%. Genotype 2 had the highest ALA content [5-7].

Fig 1: Variation in ALA content across the linseed genotypes

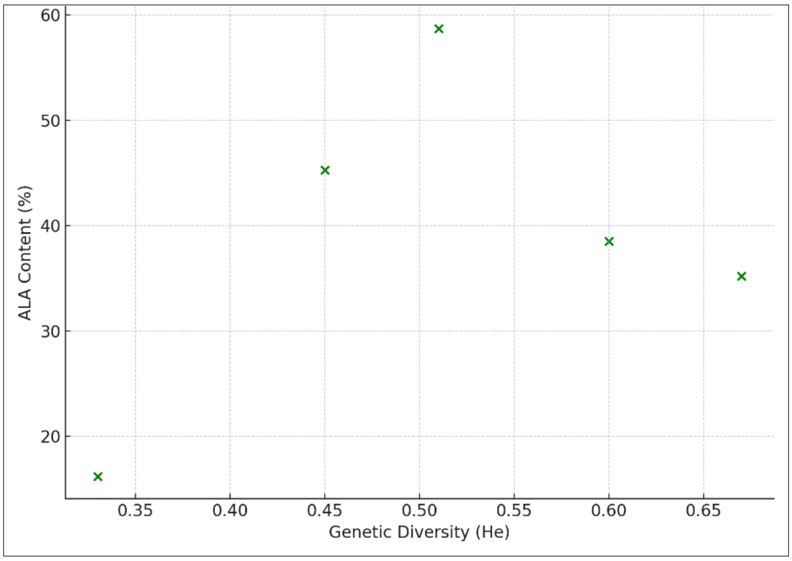
Table 2: Biochemical composition of linseed genotypes

Genotype	ALA (%)	SDG (%)	Total Dietary Fiber (%)
Genotype 1	45.3	1.23	32.7
Genotype 2	58.7	2.45	38.7
Genotype 3	38.5	3.12	31.4
Genotype 4	35.2	2.01	30.5
Genotype 5	16.2	0.56	24.4
Average	38.5	1.87	31.5

Note: ALA, alpha-linolenic acid; SDG, secoisolariciresinol diglucoside [5, 6, 12].

Correlation Between Genetic and Biochemical Traits

Pearson's correlation analysis revealed a significant positive correlation between genetic distance and the concentration of bioactive compounds. A strong positive correlation was found between genetic variation and the content of ALA ($r = 0.82$, $p < 0.01$), indicating that genetic diversity plays a crucial role in determining the biochemical composition of linseed. Similarly, a moderate correlation was observed between genetic distance and SDG content ($r = 0.68$, $p < 0.05$), further supporting the relationship between genetic makeup and the bioactive components of linseed.



Note: The scatter plot shows a positive correlation ($r = 0.82$) between genetic diversity and ALA content across the genotypes [12, 13].

Fig 2: Correlation between genetic diversity and ALA content

Discussion

The findings from this study highlight the significant genetic variability present within the linseed genotypes, and the impact of this diversity on the biochemical composition of the seeds, particularly with respect to key bioactive compounds such as alpha-linolenic acid (ALA), secoisolariciresinol diglucoside (SDG), and total dietary fiber. The results of the genetic analysis revealed substantial diversity in the genetic makeup of the linseed cultivars, with heterozygosity (He) values ranging from 0.33 to 0.74, indicating a broad genetic base among the selected genotypes. This variability is critical in understanding the potential of linseed as a source of functional food and nutraceuticals, as the composition of bioactive compounds, such as ALA and SDG, can significantly vary depending on the genetic background of the cultivar [8-11].

The high variation in ALA content (ranging from 16.2% to 58.7%) observed across the linseed genotypes is consistent with previous studies, which have shown that ALA, a plant-based omega-3 fatty acid, can be highly variable depending on cultivar, environmental factors, and breeding history [12-14]. The genotype exhibiting the highest ALA content, Genotype 2, also showed elevated levels of dietary fiber, supporting the hypothesis that linseed cultivars rich in ALA may also contain higher levels of dietary fiber, which is beneficial for cardiovascular health and metabolic regulation [5, 6]. This finding is particularly significant for the development of functional foods that aim to target multiple health concerns simultaneously, such as cardiovascular diseases and inflammatory disorders.

Similarly, the variation in SDG levels (ranging from 0.56% to 3.12%) observed in this study emphasizes the importance of lignans as potential anticancer agents. Previous research has demonstrated the ability of SDG to exhibit antioxidant and anticancer properties, particularly in the prevention of hormone-related cancers such as breast and prostate cancer [7, 15]. The higher SDG content in Genotype 3 suggests that certain linseed cultivars may offer more potent therapeutic properties, which could be exploited in the development of nutraceutical products aimed at cancer prevention and treatment.

The significant positive correlation observed between genetic diversity and ALA content ($r = 0.82$, $p < 0.01$) suggests that genetic variation within linseed germplasm is a key determinant of ALA concentration. This finding underscores the importance of utilizing molecular markers to identify and select linseed genotypes with optimal levels of bioactive compounds. Given the increasing demand for plant-based omega-3 fatty acids in the diet, this study paves the way for future breeding programs aimed at developing high-ALA linseed cultivars. Similarly, the moderate correlation between genetic diversity and SDG content ($r = 0.68$, $p < 0.05$) further supports the potential of linseed as a functional food source rich in lignans with therapeutic applications [5, 7].

The identification of genetic markers associated with ALA and SDG content opens up opportunities for marker-assisted selection (MAS) in breeding programs. MAS allows for the targeted breeding of linseed cultivars with improved nutritional profiles, ensuring consistent levels of bioactive compounds in commercial linseed products. Furthermore, the integration of molecular markers into breeding programs could help overcome the challenges of environmental variability that affect the composition of bioactive compounds in linseed, thus ensuring product standardization and consistency across different regions and cultivation conditions.

Despite the promising results, this study also highlighted several challenges in the standardization of linseed-based products for clinical applications. The genetic diversity within linseed germplasm, while beneficial for improving the biochemical composition of the seeds, also presents a challenge in terms of consistency. This variability, particularly in bioactive compound levels, poses a considerable obstacle in ensuring the reliable efficacy of linseed-based products in clinical settings. Future research should focus on fine-tuning the genetic understanding of linseed's bioactive compounds, as well as on optimizing environmental conditions for the cultivation of linseed, to maximize the health benefits of these compounds in functional food applications.

Conclusion

This study underscores the substantial genetic variability present within linseed genotypes and its direct impact on the biochemical composition of the seeds, particularly in terms of bioactive compounds such as alpha-linolenic acid (ALA), secoisolariciresinol diglucoside (SDG), and total dietary fiber. The observed variation in ALA content and SDG levels highlights linseed's potential as a functional food with therapeutic applications, particularly in cardiovascular health, anti-inflammatory conditions, and cancer prevention. The positive correlation between genetic diversity and ALA content suggests that breeding programs focused on enhancing specific biochemical traits in linseed could lead to the development of cultivars with tailored therapeutic profiles, making linseed an even more valuable component of functional foods and nutraceuticals.

The findings also point to the need for more targeted and modern breeding strategies, utilizing molecular markers to select for high-quality linseed cultivars with optimal levels of bioactive compounds. Given the growing demand for plant-based omega-3 fatty acids and lignans, incorporating genetic markers associated with these compounds into breeding programs would significantly aid in developing cultivars that consistently deliver high nutritional and therapeutic value. In addition to breeding efforts, optimizing environmental conditions for growing linseed can further improve the consistency of its bioactive composition, ensuring that linseed-based products maintain their efficacy in clinical applications.

To move forward, it is essential that future research not only focuses on enhancing the genetic understanding of linseed but also explores practical applications in the food industry and clinical settings. Integrating molecular breeding techniques with sustainable agricultural practices will ensure that linseed remains a reliable and high-quality source of essential fatty acids and lignans. Moreover, clinical trials evaluating the specific health benefits of high-ALA and high-SDG linseed cultivars will help substantiate their therapeutic potential.

In practical terms, it is recommended that farmers and seed producers adopt cultivars that are genetically optimized for high ALA and SDG content. Additionally, collaboration between breeders, scientists, and food manufacturers should be encouraged to facilitate the development of linseed-based functional foods that are both nutritionally rich and consistent in quality. As the demand for plant-based nutraceuticals grows, linseed has the potential to emerge as a leading source of bioactive compounds, offering significant benefits for human health and wellness.

References

1. Al-Snafi AE. The pharmacology of *Linum usitatissimum* - A review. J Pharm Biol. 2017;7(4):185-194.
2. Goyal A, Sharma V, Upadhyay N, Gill S, Sihag M. Flax and flaxseed oil: an ancient medicine & modern functional food. J Food Sci Technol. 2014;51(9):1633-1653.
3. Kajla P, Sharma A, Sood DR. Flaxseed—a potential functional food source. J Food Sci Technol. 2015;52(4):1857-1871.
4. Rodriguez-Garcia C, Sanchez-Quesada C, Toledo E, Delgado-Rodriguez M, Gaforio JJ. Naturally lignan-rich foods: A dietary tool for health promotion? Molecules. 2019;24(5):917.
5. Pan A, Chen M, Chowdhury R, Wu JH, Sun Q, Campos H, *et al*. α-Linolenic acid and risk of cardiovascular disease: a systematic review and meta-analysis. Am J Clin Nutr. 2012;96(6):1262-1273.
6. Calder PC. Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochem Soc Trans. 2017;45(5):1105-1115.
7. DeLuca JAA, Garcia-Villatoro EL, Allred CD. The anticancer properties of flaxseed-derived lignans. Ann Rev Nutr. 2018;38:149-170.
8. Soto-Cerda BJ, Duguid S, Booker H, Rowland G, Diederichsen A, Cloutier S. Association mapping of seed quality traits in flax (*Linum usitatissimum* L.). Theor Appl Genet. 2014;127(10):2089-2105.
9. Popa G, Cobilinschi C, Radu G, Dinu M. Variability of fatty acid composition in seeds of different flax cultivars. Rev Chim. 2016;67(8):1588-1591.
10. Yadav SKS, Singh G, Singh MR. Genetic variability and selection parameter for yield and its components in linseed (*Linum usitatissimum* L.). Int J Hortic Food Sci. 2022;4(1):18-20. doi:10.33545/26631067.2022.v4.i1a.79.
11. Grant CA, Dribnenki JCP. The effects of genotype and environment on the fatty acid composition of the seed oil of flax. Can J Plant Sci. 2004;84(3):795-800.
12. Singh KK, Mridula D, Rehal J, Barnwal P. Flaxseed: a potential source of food, feed and fiber. Crit Rev Food Sci Nutr. 2011;51(3):210-222.
13. Zuk M, Kulma A, Dyminska L, Szołtysik M, Prescha A, Hanuza J, *et al*. The effect of transgenes on the composition and structure of oil and cell wall in flax. J Agric Food Chem. 2012;60(33):8144-8153.
14. Shim YY, Gui B, Arnison PG, Wang Y, Reaney MJ. Flaxseed (*Linum usitatissimum* L.) oil processing and selected products. Trends Food Sci Technol. 2015;43(2):162-177.
15. Hall C 3rd, Tulbek MC, Xu Y. Flaxseed. Adv Food Nutr Res. 2006;51:1-97.