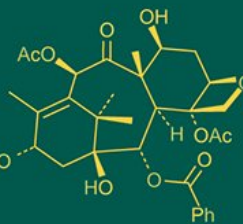


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Saket S Bhoyar
PG Scholar, Department of
Molecular Biology and
Biotechnology, Dr PDKV,
Akola, Maharashtra, India

AG Deshmukh
Professor, Department of
Molecular Biology and
Biotechnology, Dr PDKV,
Akola, Maharashtra, India

PV Jadhav
Professor, Department of
Molecular Biology and
Biotechnology, Dr PDKV,
Akola, Maharashtra, India

Dipika A Padole
Assistant Professor,
Department of Molecular
Biology and Biotechnology, Dr
PDKV, Akola, Maharashtra,
India

MM Wakode
Assistant Professor,
Department of Agricultural
Botany, Dr PDKV, Akola,
Maharashtra, India

Kalyani More
Ph.D. Scholar, Department of
Molecular Biology and
Biotechnology, Dr PDKV,
Akola, Maharashtra, India

Rupali Patle
PG Scholar, Dr PDKV, Akola,
Maharashtra, India
Corresponding Author: Saket S

Bhoyar
PG Scholar, Department of
Molecular Biology and
Biotechnology, Dr PDKV,
Akola, Maharashtra, India

Corresponding Author:
Saket S Bhoyar
PG Scholar, Department of
Molecular Biology and
Biotechnology, Dr PDKV,
Akola, Maharashtra, India

A Comparative Biochemical Study of a Black-Seeded and a White-Seeded Germplasm in Chia (*Salvia hispanica* L)

Saket S Bhoyar, AG Deshmukh, PV Jadhav, Dipika A Padole, MM Wakode, Kalyani More and Rupali Patle

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Abstract

Seed coat color is one of the most conspicuous and field-selectable qualitative descriptors in chia (*Salvia hispanica* L.), yet whether it is associated with the seed's nutritional and fatty-acid quality remains largely unexamined. In the present study, two contrasting chia germplasm accessions maintained at Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola a Black Seed Chia (IC-647150) and a White Seed Chia (IC-647154) were compared for fourteen nutritional and fatty-acid attributes. White seed chia recorded consistently higher macro-mineral and antioxidant values than black seed chia: calcium (7173.45 vs 5882.32 mg/kg, +22.0% in the white-seeded germplasm), magnesium (4105.91 vs 3321.23 mg/kg, +23.6%), phosphorus (8279.85 vs 6741.23 mg/kg, +22.8%), total phenolic content (4.61 vs 3.78 mg GAE/g, +22.0%) and total flavonoid content (1.36 vs 0.95 mg QE/g, +43.2%). Conversely, black seed chia markedly outperformed White Seed Chia in monounsaturated fatty acid quality, recording nearly four-fold higher oleic acid (2.12% vs 0.55%) and more than three-fold higher total MUFA (2.34% vs 0.68%), while white seed chia recorded a 63.7% higher total SFA content (5.11% vs 3.12%). Omega-3 (ALA), omega-6 (LA), protein and total PUFA content differed only marginally between the two germplasm (−0.8% to −4.9%). These results indicate that seed coat pigmentation in chia is associated with a genuine, trait-specific nutritional trade-off: white seed color for mineral and phenolic/flavonoid density, and black seed color for a more favourable, oleic-acid-rich unsaturated fatty acid profile rather than a uniform advantage of one seed color over the other, with clear implications for trait-targeted parental selection in chia breeding.

Keywords: *Salvia hispanica*; chia; seed color; black seed; white seed; nutritional profiling; fatty acid composition; germplasm characterization

Introduction

Chia (*Salvia hispanica* L.), a member of the family Lamiaceae native to Mesoamerica, has emerged over the past two decades as one of the most widely marketed 'superfood' seed crops, owing chiefly to its exceptionally high content of omega-3 (α -linolenic acid, ALA) polyunsaturated fatty acid, complete essential amino acid protein, dietary fibre, and phenolic antioxidants. Chia seed coat color is a highly visible and readily field-scored qualitative trait, commonly classified into black, brown, mottled and white phenotypes, with the white-seeded phenotype in particular commanding a premium in certain specialty food markets. Despite the commercial and varietal-identification importance attached to seed color, systematic biochemical comparison of black- and white-seeded chia germplasms evaluated side by side under identical agro-climatic conditions remains limited in the published literature, with most existing reports either pooling seed-color classes together or examining pigmentation and nutritional quality as separate, unconnected lines of enquiry.

The present investigation compares two chia germplasm accessions, procured from the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, and evaluated during Rabi 2024–25 at Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, for seed coat color and for fourteen nutritional and fatty-acid attributes. Accession IC-647150 was recorded as pure black-seeded and is hereafter designated Black Seed Chia, while accession IC-647154 was recorded as pure white-seeded and is hereafter designated white seed chia. The present paper compares these two contrasting germplasm accessions in detail.

with the specific objective of determining whether, and in which specific nutritional attributes, black-seeded and white-seeded chia differ in nutritional performance, so as to inform trait-targeted parental selection in future chia improvement programmes.

2. Materials and methods

2.1 Plant material and experimental site

The two chia germplasm accessions compared in this study, black seed chia (IC-647150) and white seed chia (IC-647154), were procured from the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, and evaluated in a Randomized Block Design with three replications during Rabi 2024–25 at the Nagarjun Medicinal Plants Garden and Biotechnology Centre, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth (Dr. PDKV), Akola, Maharashtra.

2.2 Seed Color Classification

Black seed chia was recorded as pure black and white seed chia as pure white in seed coat color, the basis on which the two germplasm accessions were selected for the present comparative study.

2.3 Estimation of Nutritional and Fatty Acid Attributes

Total protein content was estimated by the micro-Kjeldahl method (AOAC, 2000), based on digestion of the seed sample, distillation of liberated ammonia into boric acid, and titration against standard HCl, with nitrogen content converted to crude protein using a factor of 6.25. Calcium, magnesium and phosphorus content were estimated following microwave-assisted acid digestion and quantified by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) against a multi-element calibration curve, and expressed on a dry-weight basis (mg/kg). Crude seed oil was extracted by the Soxhlet method (AOAC, 2000) using

petroleum ether, converted to fatty acid methyl esters (FAME) by base-catalyzed transesterification, and the FAME profile including omega-3 (ALA, C18:3n-3), omega-6 (LA, C18:2n-6), oleic acid (C18:1), palmitic acid (C16:0), stearic acid (C18:0), and the summed total saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid fractions was quantified by gas chromatography with flame ionization detection (GC-FID) against authentic FAME reference standards, and expressed as per cent of total fatty acid methyl esters. Total phenolic content (TPC) of the methanolic seed extract was estimated by the Folin-Ciocalteu method (Singleton and Rossi, 1965) ^[14] and expressed as mg gallic acid equivalent (GAE) per g dry weight, and total flavonoid content (TFC) was estimated by the aluminium chloride colorimetric method (Chang *et al.*, 2002) ^[11] and expressed as mg quercetin equivalent (QE) per g dry weight.

2.4 Data Presentation

As the present comparison concerns two individual germplasm accessions rather than replicated group means, the germplasm-mean value recorded for each of the fourteen nutritional and fatty-acid attributes is presented directly for black seed chia and white seed chia, together with the percentage difference of the black-seeded germplasm relative to the white-seeded germplasm. Values for both germplasm were additionally scaled to a 0–100 range for each attribute using the lower and higher of the two recorded values, to allow direct visual comparison of their relative profile.

3. Results

3.1 Germplasms Compared

Table 1 presents the identity and seed-color classification of the two germplasms compared in the present study.

Table 1: Germplasm details of the black-seeded and white-seeded chia accessions compared in the present study

Sr. No.	Germplasm	IC No.	Seed Color	Source
1	Black Seed Chia	IC-647150	Black	NBPGR, New Delhi
2	White Seed Chia	IC-647154	White	NBPGR, New Delhi

3.2 Comparative Nutritional and Fatty Acid Profile

The germplasm-mean values recorded for Black Seed Chia and White Seed Chia across all fourteen nutritional and

fatty-acid attributes examined, together with the percentage difference between them, are presented in Table 2.

Table 2: Comparative nutritional and fatty acid profile of Black Seed Chia and White Seed Chia germplasm

Nutritional / Fatty Acid Attribute	Unit	Black Seed Chia	White Seed Chia	% Difference (Black vs White)
Omega-3 fatty acid (ALA)	%	14.23	14.34	−0.8
Omega-6 fatty acid (LA)	%	5.32	6.87	−22.6
Protein	%	21.40	22.36	−4.3
Calcium	mg/kg	5882.32	7173.45	−18.0
Magnesium	mg/kg	3321.23	4105.91	−19.1
Phosphorus	mg/kg	6741.23	8279.85	−18.6
Total phenolic content (TPC)	mg GAE/g	3.78	4.61	−18.0
Total flavonoid content (TFC)	mg QE/g	0.95	1.36	−30.1
Oleic acid	%	2.12	0.55	+285.5
Palmitic acid	%	2.05	2.02	+1.5
Stearic acid	%	1.48	1.30	+13.8
Total SFA	%	3.12	5.11	−38.9
Total MUFA	%	2.34	0.68	+243.0
Total PUFA	%	20.23	21.28	−4.9

White Seed Chia recorded higher values than Black Seed Chia for nine of the fourteen attributes examined, most

prominently the three macro-minerals calcium (+22.0% in the white-seeded germplasm), magnesium (+23.6%) and

phosphorus (+22.8%) and the two antioxidant attributes, total phenolic content (+22.0%) and total flavonoid content (+43.2%), the largest relative difference recorded for any attribute in the favour of the white-seeded germplasm. White Seed Chia also recorded higher omega-6 (+29.1% relative to Black Seed Chia) and total SFA content (+63.7%). Conversely, Black Seed Chia recorded strikingly higher values for the monounsaturated fatty acid attributes: oleic acid content in Black Seed Chia (2.12%) was almost four times that recorded in White Seed Chia (0.55%), and total MUFA content in Black Seed Chia (2.34%) was more

than three times that of White Seed Chia (0.68%), the two largest relative differences recorded for any attribute in the entire comparison. Black Seed Chia also recorded a modestly higher stearic acid content (+13.8%). Omega-3 (ALA) content was nearly identical between the two germplasm (14.23% in Black Seed Chia versus 14.34% in White Seed Chia, a difference of under 1%), as was protein content (21.40% versus 22.36%) and total PUFA content (20.23% versus 21.28%), indicating that these three attributes are effectively decoupled from seed color in this germplasm pair.

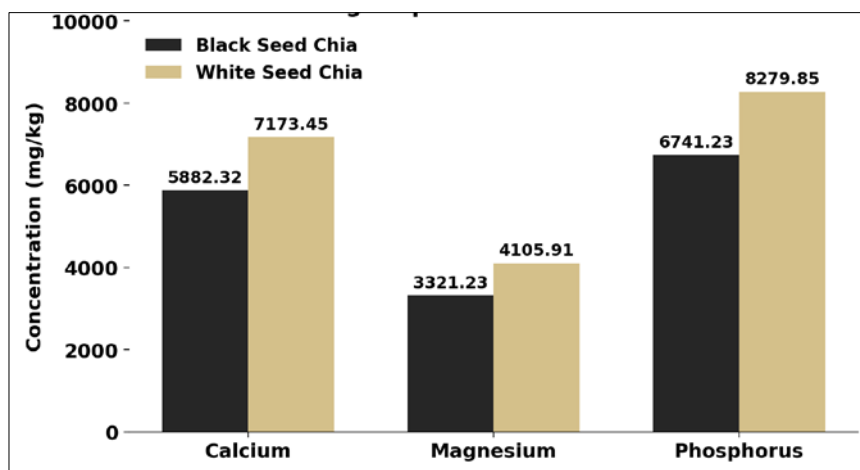


Fig 1: Macro-mineral content of Black Seed Chia and White Seed Chia germplasm

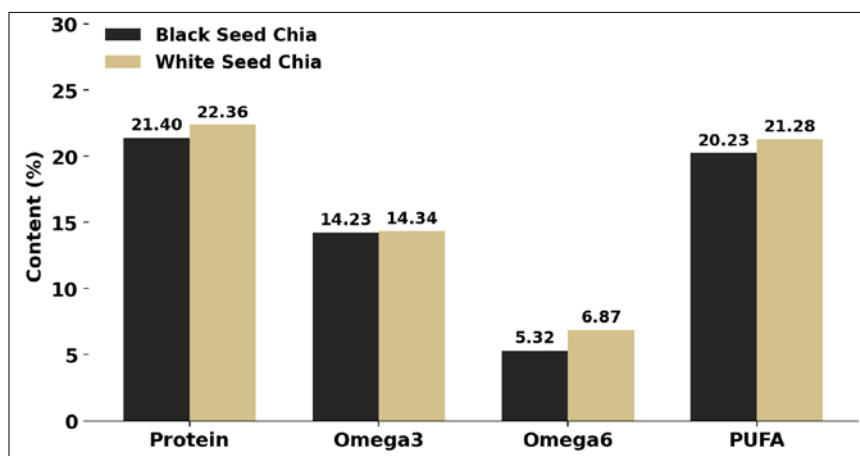


Fig 2: Protein and polyunsaturated fatty acid content of Black Seed Chia and White Seed Chia germplasm

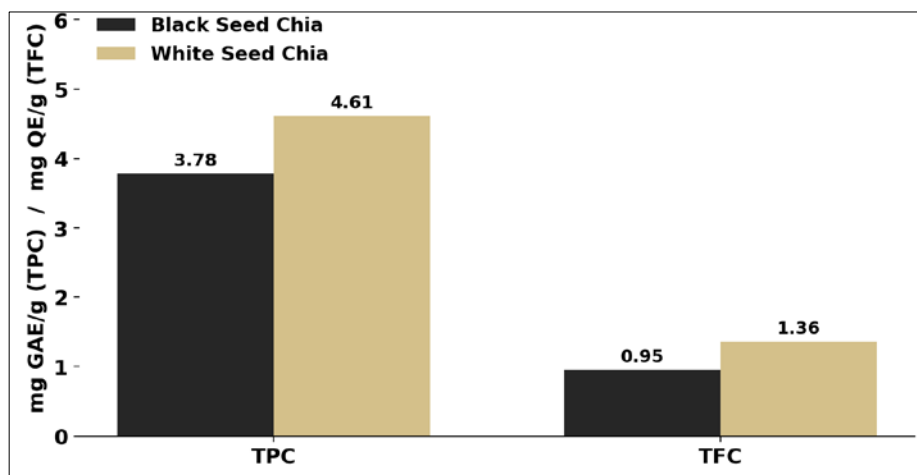


Fig 3: Antioxidant (phenolic and flavonoid) content of Black Seed Chia and White Seed Chia germplasm

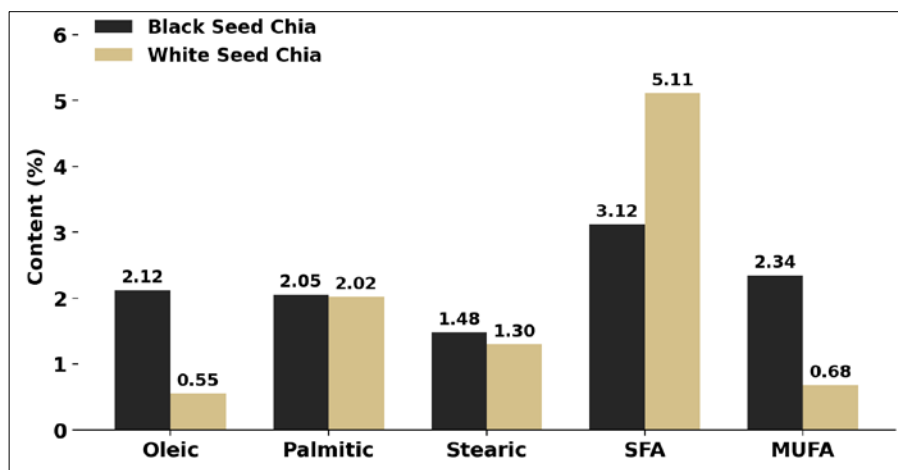


Fig 4: Individual fatty acid and saturation-class composition of Black Seed Chia and White Seed Chia germplasm

Figures 1 through 4 present the comparative bar-chart profile of Black Seed Chia and White Seed Chia across the mineral, proximate/PUFA, antioxidant, and individual fatty acid/saturation-class attribute groups, respectively. The macro-mineral profile (Fig. 1) and the antioxidant profile (Fig. 3) both show a consistent, one-directional advantage

for White Seed Chia, whereas the individual fatty acid profile (Fig. 4) shows a sharp reversal, with Black Seed Chia far exceeding White Seed Chia in oleic acid and MUFA content despite White Seed Chia's higher SFA content.

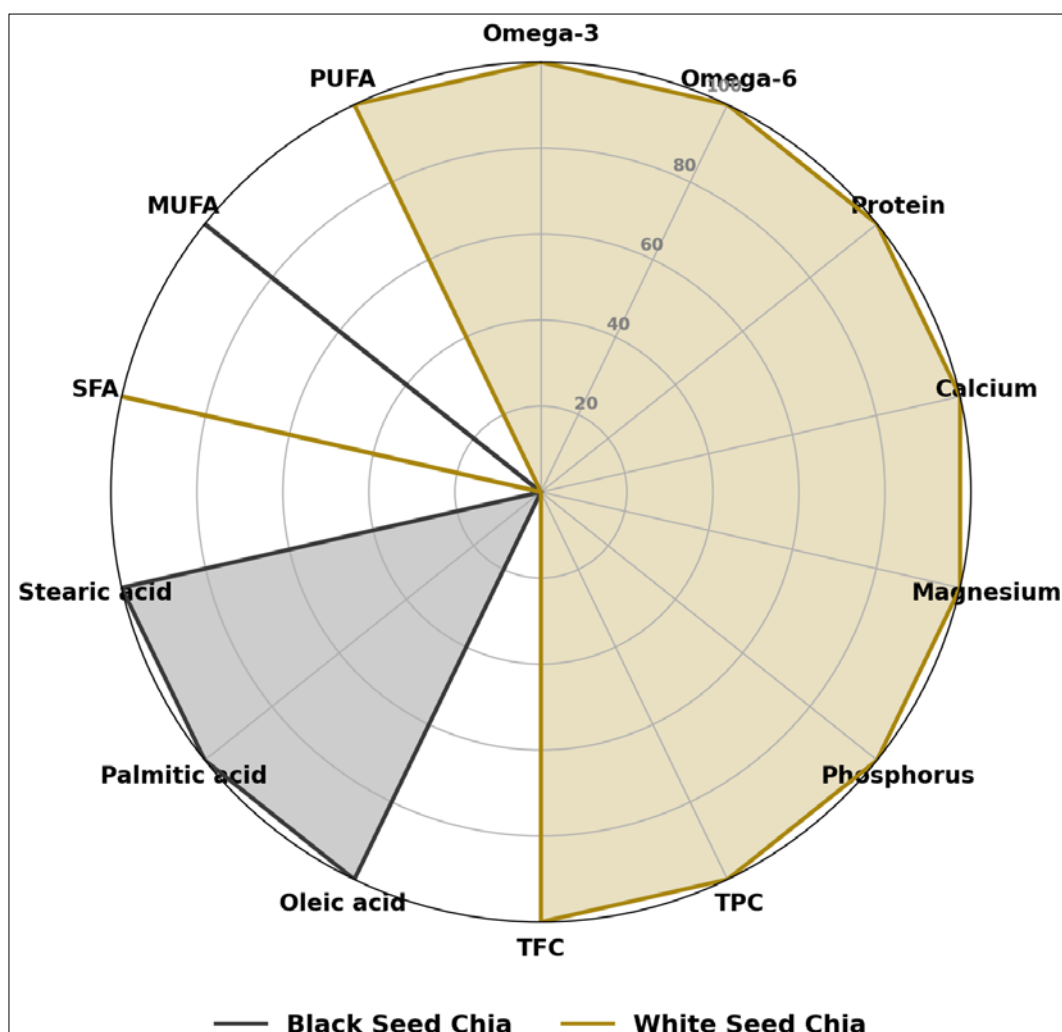


Fig 5: Normalized (min-max scaled, 0–100) nutritional and fatty acid profile of Black Seed Chia vs White Seed Chia

Figure 5 presents the normalized (0–100) profile of both germplasm across all fourteen attributes simultaneously, each attribute scaled between the two recorded values. The radar plot visually confirms that the two germplasm occupy

largely complementary, rather than overlapping, regions of the trait space: White Seed Chia dominates along the mineral and antioxidant axes (Calcium, Magnesium, Phosphorus, TPC, TFC, Omega-6, SFA), while Black Seed

Chia dominates along the monounsaturated fatty acid axes (Oleic acid, MUFA), with the two germplasm closely overlapping along the Omega-3, Protein and PUFA axes.

4. Discussion

The present comparison of a black-seeded and a white-seeded chia germplasm drawn from NBPGR and evaluated under identical agro-climatic conditions indicates that seed coat color in chia is associated with a genuine, but trait-specific, nutritional differentiation rather than a uniform advantage of either seed color. The markedly higher calcium, magnesium and phosphorus content recorded in White Seed Chia is consistent with the substantial origin- and germplasm-dependent variability in mineral concentration reported for commercial chia seed sources by Vera-Cespedes *et al.*, (2023) ^[15], and suggests that white-seeded material may be disproportionately represented among mineral-dense chia germplasm more generally. The higher total phenolic and flavonoid content recorded in White Seed Chia is similarly consistent with the substantial variability in phenolic and flavonoid content documented among chia seed sources by Reyes-Caudillo *et al.*, (2008) ^[13]; notably, this finding runs counter to the general expectation, common in other pigmented seed crops, that darker seed coats accumulate more phenolic compounds via anthocyanin and related flavonoid deposition in the testa, suggesting that the phenolic-flavonoid pool quantified here in chia is not primarily testa-anthocyanin-derived, or that pigment type rather than pigment presence per se governs the association.

The pronounced reversal observed for oleic acid and total MUFA content, in which Black Seed Chia recorded nearly four times the oleic acid and over three times the MUFA content of White Seed Chia, is in line with the significant germplasm-dependent differences in individual fatty acid proportions among chia selections reported by Ayerza and Coates (2009) ^[2], and indicates that fatty acid saturation profile in chia segregates largely independently of the mineral-antioxidant axis associated with seed color. Coates and Ayerza (1996) ^[12] similarly noted that white-seeded chia can carry a distinct fatty acid profile relative to pigmented seed, and the present result, in which White Seed Chia additionally recorded the highest total SFA content of the pair, is consistent with a genuine seed-color-linked divergence in oil fatty acid composition rather than incidental germplasm-to-germplasm noise. Omega-3 (ALA) content, by contrast, was almost identical between the two germplasm, reaffirming that the crop's principal nutraceutical attribute, its exceptionally high omega-3 fatty acid content, is not itself linked to seed coat pigmentation in this comparison.

These findings carry direct practical relevance for chia breeding programmes. Where mineral density or phenolic/flavonoid antioxidant enrichment is the primary breeding objective, white-seeded material such as White Seed Chia offers seed color as a low-cost, non-destructive visual proxy for probable nutritional performance. Where, instead, a favourable, oleic-acid-rich unsaturated fatty acid profile is the objective, dark-seeded material such as Black Seed Chia would be the more appropriate starting point, and seed color would again serve as a useful preliminary screening cue. For omega-3 enrichment specifically, however, the present results indicate that seed color offers little to no discriminatory value, and direct biochemical or

gas-chromatographic screening remains necessary irrespective of seed coat phenotype. It should be noted that the present comparison is based on a single black-seeded and a single white-seeded germplasm evaluated at one location and one growing season; broader validation across additional black- and white-seeded chia accessions, and across multiple environments, would be required before these trait associations can be generalized as fixed, seed-color-linked characteristics of the species as a whole.

5. Conclusion

White Seed Chia was consistently superior in macro-mineral density (calcium, magnesium, phosphorus) and phenolic-flavonoid antioxidant content, while Black Seed Chia was markedly superior in oleic acid and total monounsaturated fatty acid content, with omega-3, protein and total PUFA content essentially unaffected by seed color. These results position Black Seed Chia and White Seed Chia as complementary, rather than competing, parental resources for chia improvement: White Seed Chia for mineral- and antioxidant-enriched functional food breeding objectives, and Black Seed Chia for oxidative-stability-oriented, high-oleic breeding objectives, with combination breeding between the two representing a potential route toward a nutritionally comprehensive chia cultivar.

References

1. Abad A, Shahidi F. Compositional characteristics and oxidative stability of chia seed oil (*Salvia hispanica* L.). *Food Prod Process Nutr.* 2020;2:9.
2. Adamczyk G, Ivanišová E, Kaszuba J, Bobel I, Khvostenko K, Chmiel M, *et al.* Quality assessment of wheat bread incorporating chia seeds. *Foods.* 2021;10(10):2376.
3. Ali A, Lim XY, Choy CT, Chong MH, Yusoff AH. Beneficial fatty acid ratio of *Salvia hispanica* L. (chia seed) potentially inhibits adipocyte hypertrophy, and decreases adipokines expression and inflammation in macrophage. *PLoS One.* 2020;15(4).
4. Ali NM, Yeap SK, Ho WY, Beh BK, Tan SW, Tan SG. The promising future of chia, *Salvia hispanica* L. *J Biomed Biotechnol.* 2012;2012:171956.
5. AOAC. Official methods of analysis. 17th ed. Washington, DC: Association of Official Analytical Chemists; 2000.
6. Ayerza R, Coates W. Some quality components of four chia (*Salvia hispanica* L.) germplasms grown under tropical coastal desert ecosystem conditions. *Asian J Plant Sci.* 2009;8(7):301-307.
7. Cahill JP. Ethnobotany of chia, *Salvia hispanica* L. (Lamiaceae). *Econ Bot.* 2003;57(4):604-618.
8. Cahill JP. Genetic diversity among varieties of chia (*Salvia hispanica* L.). *Genet Resour Crop Evol.* 2004;51(7):773-781.
9. Cahill JP. Domesticated and wild chia (*Salvia hispanica* L. and *S. carduacea* Benth., Lamiaceae) in their aboriginal range. *Econ Bot.* 2005;59(3):236-241.
10. Cahill JP, Ehdaie B. Variation and heritability of seed mass in chia (*Salvia hispanica* L.). *Genet Resour Crop Evol.* 2005;52(2):201-207.
11. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal.* 2002;10(3):178-182.

12. Coates W, Ayerza R. Production potential of chia in northwestern Argentina. *Ind Crops Prod.* 1996;5(3):229-233.
13. Reyes-Caudillo E, Tecante A, Valdivia-López MA. Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds. *Food Chem.* 2008;107(2):656-663.
14. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic.* 1965;16(3):144-158.
15. Vera-Cespedes N, Muñoz LA, Poblete D. Compositional and mineral variability of commercial chia (*Salvia hispanica* L.) seed sources. 2023.