



Evaluating polyphenol profile and antioxidant activity in relation to seed morphology in a collection of red lentils

Gaetano Giudice^{a,1} , Lorenzo Palombi^{b,1} , Stefano Pavan^a, Marzia Guerriero^a, Andrea Visioni^c , Barbara Laddomada^{d,*}

^a Department of Soil, Plant and Food Sciences (DISSPA), University of Bari Aldo Moro, Bari, Italy

^b Institute for Applied Physics "Nello Carrara" (IFAC), National Research Council (CNR), Sesto Fiorentino, 50019, Firenze, Italy

^c International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat, 10100, Morocco

^d Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Via Amendola 122/O, Bari, Italy

ARTICLE INFO

Keywords:

Lens culinaris

Whole grains

Pigmentation

Flavonoids

Multivariate analyses

ABSTRACT

Red lentils may have either a reddish-brown or grey seed coat with different pigmentation patterns, and cotyledons varying from yellow to orange-red depending on the genotype. This class of lentils is widely consumed for their nutritional and functional properties the latter being largely attributed to antioxidant polyphenols. However, the relationship between seed morphology and polyphenol composition remains poorly characterized. In this study, 59 red lentil genotypes were analyzed to investigate associations among seed color, polyphenol profiles, and antioxidant capacity. Total phenolic content and individual flavonoids were quantified, and antioxidant activity was assessed both in extracts and directly on whole-meal flour. ANOVA revealed significant variability among genotypes for all traits. Three-way ANOVA highlighted that different tegument pigmentation patterns were strongly associated with individual flavonoids and antioxidant capacity, while cotyledon color was influenced by total polyphenol content and gallic acid levels. Multivariate analyses (PCA, OPLS-DA) confirmed these relationships by achieving high classification accuracy for color classes based on combined biochemical and physical data. Overall, the results showed that morphological features of red lentils can be valuable tools for accelerating breeding programs for selecting and developing varieties rich in functional compounds.

1. Introduction

Lentil (*Lens culinaris* Medik.) is one of the oldest domesticated crops, with its origin dating back to 8500 BC in southwestern Asia, being an important source of protein and dietary staple in many cultures for thousands of years (Swamy, 2023). Nowadays, lentil is the fourth legume for production with 6.6 million tons produced in 2025 which confirmed the increasing growth trend over the last decade (Crop Production Statistics, 2024). A wide range of lentil landraces and cultivars are spread worldwide across the different growing regions which represent a valuable source for breeding programs aimed at improving nutritional quality and tolerance to climate constraints (Fiocchetti et al., 2009; Pavan et al., 2019).

Frequent consumption of lentils is associated with improved metabolic health due to the abundance of complex carbohydrates, dietary fiber and polyphenols (Adebamowo et al., 2005; Aslani et al., 2003;

Chamberlin et al., 2024; Clarke et al., 2022). Among these components, polyphenols help to prevent degenerative diseases due to their antioxidant capacity, anti-inflammatory properties, and anti-proliferative activities against cancer cells (Ganesan & Xu, 2017; Jung et al., 2022). The accumulation of specific phenolic compounds (e.g. flavan-3-ols, flavonols, flavones, anthocyanidins, proanthocyanidins, phenolic acids) in lentils may vary depending on the seed district, with flavonoids being more concentrated in the seed coat, and phenolic acids resulting more abundant in the cotyledons (Dhull et al., 2022; Manco et al., 2023; Shahidi & Ambigaipalan, 2015; Xu & Chang, 2010). Also, a significant variation occurs across lentil germplasm, with red lentils and highly pigmented genotypes being richer of cyanidin, delphinidin, myricetin, and proanthocyanidins (Irakli et al., 2021; Manco et al., 2023; Mirali et al., 2017).

Lentil seed color is the result of three main traits: i) seed coat background color; ii) seed coat pattern; and iii) cotyledon color (AGPG,

* Corresponding author.

E-mail address: barbara.laddomada@cnr.it (B. Laddomada).

¹ Equal contribution.

1985). In particular, red lentils are characterized by a reddish-brown or grey seed coat with different pigmentation patterns (e.g., absent, spotted, marbled, or complex), whereas the cotyledons may vary from yellow to orange-red depending on the genotype (Swamy, 2023). These lentil varieties are the most common in global markets, and the most consumed worldwide (Canadian Grain Commission, 2024, chap. 18). Usually, they are microsperma, with seeds ranging from 2 to 6 mm in diameter. In fact, seed color in lentils is associated with seed size, and both these traits contribute to determining lentil market value influencing taste and consumer acceptability (Del Coco et al., 2022; Erdoğan, 2015; Mishra et al., 2022; Sharma et al., 2014).

Red lentil genotypes have attracted the attention of geneticists and food companies due to their high protein and mineral content, along with anthocyanins occurrence (Giusti et al., 2017). Indeed, the specific composition of red lentils is considered crucial for improving the nutritional profile of derived gluten-free foods, and meat substitutes (Erdoğan, 2015; Giusti et al., 2017; Manco et al., 2023).

The variability in seed color, seed size, and polyphenol profiles of red lentils has been the subject of several works in the past ten years (Mirali et al., 2017; Mishra et al., 2022; Xia et al., 2023). However, comparisons of results are hampered by differences in extraction procedures and analytical methods (Mustafa et al., 2022; Purves et al., 2025). Moreover, previous studies focused on either morphological traits or polyphenol composition, but using limited number genotypes (Manco et al., 2023; Mirali et al., 2017; Mishra et al., 2022). Multivariate approaches were previously applied to metabolomic fingerprints, but to date an integrated framework combining seed morphology, targeted polyphenol profiling, and chemometric modeling has not yet been implemented (Longobardi et al., 2017; Palombi et al., 2025). Our study addresses this gap by analyzing a broad collection of 59 red lentil genotypes from diverse origins, differing in cotyledon and seed coat background color, and showing multiform seed coat pattern. The genotypes were characterized by applying a combined strategy that includes morphological characterization, total polyphenols quantification, HPLC-DAD determination of individual polyphenols, and advanced multivariate models (PCA, OPLS-DA). The quali-quantitative composition of individual flavonoids including anthocyanidins, flavonols, flavans and flavones, and phenolic acids was analyzed by HPLC-DAD. Total polyphenol content was measured by spectrophotometric assay and assessed for antioxidant capacity by ABTS assay. In parallel, a direct ABTS assay (ABTS_{dir}) was performed directly on the whole meal flour to estimate the antioxidant activity of the overall metabolites with antioxidant activity present in the lentil whole-meal flour without extraction. Using this approach, a robust discrimination of color classes was obtained through the identification of morphological predictors of lentil quality. As a result, the present work advances beyond previous studies by providing a scalable and reproducible framework for genotype characterization to support practical breeding for enhancing red lentil qualitative traits associated with market value and health-promoting properties. This approach may also promote the use of selected genotypes in high-value products, such as functional foods and beverages.

2. Materials and methods

2.1. Chemicals

Authentic reference standards of phenolic compounds, including (+)-catechin, (–)-epicatechin, kaempferol, luteolin, myricetin, delphinidin, cyanidin and gallic acid, ABTS (2,2'-Azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), potassium persulfate, AAPH [2,2'-azobis (2-methyl-propionamide)] were purchased from Sigma-Aldrich (Milan, Italy), while Folin-Ciocalteu reagent, sodium hydroxide, formic acid and HPLC (high performance liquid chromatography) grade solvents were obtained from Carlo Erba Reagent (Milan, Italy).

2.2. Plant materials

Fifty-nine red lentil varieties, with different pedigree, year of release, country of origin, and seed morphology were provided by ICARDA, Lebanon (Supplementary Table 1). The plants were grown at ICARDA experimental station, Terbol, Lebanon (33.81° N, 35.98° E), during the 2023 growing season.

2.3. Morphological analyses

Seed coat background color was measured using a Minolta CR-400 spectrophotometer (Minolta Camera Co, Osaka, Japan) according to the International Commission on Illumination CIELAB color space (CIE Lab, 1978). Whole seeds were transferred into the Minolta collector to measure lightness (L*) and color (a*, red index; b*: yellow, index). Seed coat background color and pattern, and cotyledons color were classified based on the descriptors reported by AGPG (1985). Seed size and hundred kernel weight (HKW) were also measured. These morphological traits were previously reported by our research group (Palombi et al., 2025) and the analytical data used in the present work are summarized in Supplementary Table 2.

2.4. Extraction of total polyphenols and Folin-Ciocalteu assay

Total polyphenols were extracted following Manco et al. (2023). Whole grain seeds were milled using a Mixer Mill (MM 400 Retsch, Verder Scientific S.r.l. Pedrengo, Italy) with a frequency of 30 Hz for 1 min. The whole-meal flour (0.1 g) was extracted by adding 1 mL of methanol/water/formic acid (80/15/5) at 20 °C and incubating overnight. Then, samples were centrifuged at 4000 × g for 10 min, and the slurry was re-extracted with an additional 1 mL of same solvent on a rotary shaker for 1 h, at 20 °C. The two supernatants were collected in one tube. The extractions were carried out in triplicate. Total polyphenol content of the extracts was evaluated using the method by Magalhães et al. (2010) in 96-well plates (Corning) using a microplate reader (Tecan, Infinite M200). Folin-Ciocalteu reagent (1:5, v/v) (50 µL) was placed in each well, and then 100 µL of sodium hydroxide solution (0.35 mol/L) was added. The absorbance value at 760 nm was recorded after 5 min of incubation. Gallic acid was used to obtain a calibration curve in the range from 2.5 to 40.0 mg/L ($R \geq 0.9997$), and total phenol content was expressed as gallic acid equivalents (GAE).

2.5. Flavonoids extraction and HPLC analyses

Flavonoids were extracted from the whole-meal samples according to Lee et al. (2017) by adding 1.2 mL of 50 % methanol containing 1.2 mol/L HCl to 0.1 g of whole meal flour. The samples were heated in a water bath at 80 °C for 2 h and then centrifuged at 10,000 × g at 4 °C for 5 min. The extractions were carried out in triplicates. Flavonoid extracts were analyzed by HPLC for quali-quantitative detection of individual anthocyanins and flavonoids associated with lentil seed pigmentation, antioxidant activity, and health-related functionality (Manco et al., 2023; Viscusi et al., 2022). An Agilent-1100 liquid chromatograph (Agilent Technologies, Italy) was used equipped with a DAD detector (Agilent 1260 Infinity), and a C18 column (5 µm UltraSphere 80 Å, 4.6 mm internal diameter x 250 mm length). For anthocyanins, the mobile phases were: (A) water/formic acid = 95/5 and (B) acetonitrile/formic acid = 95/5, using the following linear gradient: 1 min of isocratic elution with 6.7% B, 25 min of linear gradient from 6.7% to 16.7% B, 9 min of linear gradient from 16.7% to 55.6% B, 5 min of isocratic elution with 55.6% B, 3 min of linear gradient from 55.6% B to 80% B, and 8 min of isocratic elution with 80% B. An equilibration time of 10 min was set before the next injection while the flow rate was set at 0.7 mL/min. For flavonoid analysis, a linear gradient from 20 to 60 % acetonitrile was used in 40 min (solvent A: 1 % phosphoric acid, solvent B: 100 % acetonitrile) with a flow of 1 mL/min at 25 °C. The chromatographic

analysis was performed with UV detectors by comparisons with external standards. The metabolite concentrations were obtained on the base of calibration curves and were expressed in $\mu\text{g/g}$ of dry matter.

2.6. Determination of antioxidant capacity of TPC extracts

The antioxidant capacity of TPC methanol/water/formic acid (80/15/5) extracts was performed according to the original protocol by Re et al. (1999). Briefly, an ABTS $\bullet$ + stock solution was prepared by reacting to a 7 mmol/L aqueous solution of ABTS with 2.45 mM potassium persulfate overnight. The ABTS $\bullet$ + working solution was prepared by diluting the stock solution in ethanol 96 % to reach a final absorbance of 0.7 at 750 nm. A volume equal to 950 μL of ABTS $\bullet$ + were mixed with sample extracts (50 μL) and placed in a rotating disk for 20 min. The absorbance of samples was read at 750 nm, and the antioxidant capacity was expressed as mmol Trolox equivalent antioxidant capacity (TEAC) per g of dry matter.

2.7. Determination of total antioxidant capacity of whole meal flour

Total antioxidant capacities of whole-meal samples were assessed by a direct procedure according to the method described by Serpen et al. (2008). Briefly, the whole meal flour samples were made to react directly with the colored radical cation ABTS $\bullet$ +. An ABTS $\bullet$ + working solution was prepared by diluting the stock solution in a mixture of ethanol/water (50:50, v/v) to a final absorbance of about 0.7 at 740 nm. This ethanol/water mixture was chosen to overcome the solubility-dependent low reactivity of antioxidants toward ABTS $\bullet$ +. The lentil whole meal flour (1.5 mg) was dissolved into 15 mL of ABTS $\bullet$ + working solution to start the decolorization reaction (Romano et al. (2022)). The samples were placed on a rotating disk for 30 min to facilitate the surface reaction between the grounded samples and the ABTS $\bullet$ + reagent. After centrifugation at $9200 \times g$ for 2 min, the clear supernatant was separated, and the absorbance was read at 740 nm. The antioxidant capacity was expressed as mmol Trolox equivalent antioxidant capacity (TEAC) per Kg of whole meal flour.

2.8. Statistical analysis

Normal distribution of the data was verified using the Shapiro-Wilk normality test implemented in the stats R package. Non-normal data were transformed with the bestNormalize package for further analysis. Genotype effects on individual polyphenols, total polyphenol content, antioxidant activity and CIELab indices were tested by one-way ANOVA. Three-way ANOVAs, followed by the Tukey's HSD test, were conducted to assess the effects of cotyledon color (CC), tegument color (TC), and tegument pigmentation pattern (TPP) on individual polyphenols, total polyphenol content (TPC), and antioxidant capacity. The same approach was applied to evaluate the effects of CIELAB color indices. Statistical significance was determined at $P \leq 0.05$ using the Agricolae R package (de Mendiburu F. 2023). Principal Components Analysis (PCA) was performed with the *prcomp* function implemented in the basic stats R package and the results visualized using ggplot2 (Wickham, 2016).

Hierarchical clustering of genotypes and physicochemical variables based on collected data was performed using the complete method implemented in the *phclust* function of the basic stats R package and rendered with heatmap (Kolde, 2019).

Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) was employed to discriminate seed morphological traits, specifically tegument color and cotyledon color, based on polyphenol profiles, antioxidant activity (ABTS and ABTS dir), colorimetric parameters (CIELab indices), and seed size traits (diameter and hundred kernel weight). OPLS-DA was performed on genotype-level averaged data across replicates, and all variables were standardized prior to model fitting to ensure comparability and avoid scale-driven bias. The optimal number of PLS components was first determined through five-fold cross-

validation, repeated over 200 Monte Carlo randomizations of training and test datasets. The number of components that maximized both classification accuracy and the area under the ROC curve (AUC) was selected (Aslani et al., 2003; Szymanska et al., 2012). Using this configuration, a PLS-DA model was trained on the complete dataset. The OPLS-DA model was then derived by applying Singular Value Decomposition (SVD) to isolate orthogonal variation unrelated to class discrimination (Trygg & Wold, 2002). For each OPLS-DA application, correlation circle plots were generated to visualize the contribution of individual variables to the first and second orthogonal components. Additionally, confusion matrices were reported to assess classification performance, and Variable Importance in Projection (VIP) scores were summarized in histogram plots to highlight the most influential features. All OPLS-DA analyses and related visualizations, including correlation circle plots, confusion matrices, and VIP score histograms, were performed using MATLAB R2024a (MathWorks Inc.).

3. Results and discussion

3.1. Relation between physico-chemical composition, seed morphology and antioxidant capacity

This study addressed the same red lentil germplasm collection previously reported by Palombi et al. (2025), which included 59 red lentils from different countries worldwide (Supplementary Table 1). While Palombi et al. (2025) characterized the collection for seed morphological traits (Supplementary Table 2) and by untargeted ^1H NMR fingerprinting, here we focused on major individual phenolic compounds and total phenolic content (TPC) with the aim to investigate the biochemical basis underlying morphological variation in red lentils.

Red lentils are particularly rich in polyphenols, with TPC varying from 0.79 to 6.38 mg GAE/g (Irakli et al., 2021; Xia et al., 2023). In this study, we observed a narrower variation for TPC from a minimum of 3.012 mg GAE/g in IG784, and a maximum of 5.499 mg GAE/g in IG70076 (Table 1). These results could be ascribed both to the different genotypes comprised in the present red lentil collection along with the different environmental conditions as compared to previous works (Ahmed et al., 2020; Irakli et al., 2021). Moreover, it was shown that using different extraction procedures influence the extraction rate of polyphenols (Mango et al., 2023; Xu & Chang, 2007).

Polyphenols may act as reducing agents, hydrogen donating antioxidants, mineral chelators, and singlet oxygen quenchers (Rudrapal et al., 2022; Scarano et al., 2023). In this study, the antioxidant activity of TPC extracts was evaluated as radical scavenging capacity (ABTS) and the results are shown in Table 1. In addition, the radical scavenging capacity was estimated directly on whole-meal flour samples (ABTS dir) (Romano et al., 2022; Serpen et al., 2008). Indeed, literature evidence suggest that multiple methods of measurements are required to evaluate the antioxidant capacity of food's extracts or matrices (Zulueta et al., 2009). We found that ABTS of TPC extracts varied across the genotypes from 0.034 TE mmol/Kg (IG784) to 0.103 (IG5722). These results confirm previous works conducted on red lentils showing that different compositions in phenolic compounds result in different antioxidant activity of the extracts (Irakli et al., 2021; Manco et al., 2023; Xia et al., 2023). However, polyphenol antioxidant capacity can be significantly influenced also by extraction solvents rather than by genotypes (Alshikh et al., 2015; Dueñas et al., 2006; Manco et al., 2023). Thus, using the direct ABTS assay, we aimed to assess the antiradical scavenging capacity of antioxidant molecules excluding the effect of solvents. Also, we aimed to estimate possible differences in the synergistic effects among the antioxidants present in the raw materials compared to those present in the phenolic extracts (Serpen et al., 2008). Indeed, a similar range of variation was observed between ABTS and ABTS dir values among the genotypes, with ABTS dir values ranging from 0.052 TE mmol/Kg (IG129142) and 0.163 TE mmol/Kg (IG5748) (Table 1). However, the two assays were not correlated and TPC correlated only with ABTS,

Table 1
Total Phenolic Content (TPC, mg GAE/g), Trolox Equivalent Antioxidant Capacity of total phenolic extracts (ABTS, TE mmol/g) and of whole-meal flour samples (ABTS_{dir}, TE mmol/Kg) and individual phenolic compounds (μg/g d.m.) detected in the fifty-nine red lentil genotypes considered in this work. In bold: minimum and maximum values for each trait.

Nr.	Genotype	TPC	ABTS	ABTS _{dir}	Delphinidin	Cyanidin	Catechin	Epicatechin	Luteolin	Kaempferol	Myricetin	Gallic acid
1	IG358	4.904 ± 0.214	0.062 ± 0.004	0.127 ± 0.019	134.49 ± 17.53	77.70 ± 12.06	514.74 ± 30.48	323.91 ± 30.37	2.17 ± 0.02	117.34 ± 7.80	2.84 ± 0.04	92.82 ± 4.44
2	IG590	3.991 ± 0.179	0.045 ± 0.004	0.143 ± 0.016	70.20 ± 3.06	32.84 ± 2.43	514.44 ± 10.91	266.64 ± 5.07	2.03 ± 0.21	111.57 ± 0.01	2.48 ± 0.21	99.09 ± 9.37
3	IG481	4.265 ± 0.056	0.050 ± 0.001	0.143 ± 0.004	78.08 ± 4.78	45.45 ± 3.57	381.87 ± 1.14	379.25 ± 3.82	1.06 ± 0.08	156.04 ± 0.70	2.69 ± 0.01	100.18 ± 0.28
4	IG784	3.012 ± 0.242	0.034 ± 0.001	0.113 ± 0.015	56.83 ± 3.25	29.52 ± 2.48	212.85 ± 0.74	329.15 ± 6.76	4.68 ± 0.28	160.72 ± 0.80	3.22 ± 0.61	100.50 ± 0.36
5	IG854	4.490 ± 0.402	0.067 ± 0.001	0.127 ± 0.023	85.02 ± 1.80	53.10 ± 1.75	362.28 ± 0.16	377.56 ± 4.06	3.24 ± 0.10	132.84 ± 2.74	2.07 ± 0.05	89.65 ± 7.25
6	IG1005	3.000 ± 0.041	0.040 ± 0.001	0.097 ± 0.014	121.62 ± 8.88	79.40 ± 7.85	389.88 ± 2.08	359.85 ± 15.70	0.88 ± 0.03	156.62 ± 4.46	3.68 ± 0.09	97.26 ± 14.00
7	IG4399	3.858 ± 0.191	0.054 ± 0.002	0.097 ± 0.015	112.23 ± 1.42	71.13 ± 0.15	330.08 ± 5.97	338.14 ± 3.72	0.98 ± 0.04	139.13 ± 5.49	2.35 ± 0.17	98.85 ± 8.30
8	IG4400	4.427 ± 0.050	0.060 ± 0.003	0.093 ± 0.007	98.86 ± 6.53	56.82 ± 3.59	384.23 ± 8.77	435.83 ± 18.41	1.23 ± 0.12	168.79 ± 6.79	2.57 ± 0.15	90.53 ± 5.53
9	IG4605	4.023 ± 0.204	0.059 ± 0.005	0.107 ± 0.005	118.50 ± 0.40	47.81 ± 2.12	411.04 ± 72.41	543.75 ± 104.44	3.09 ± 0.42	204.12 ± 26.67	3.42 ± 0.47	100.57 ± 12.16
10	IG73661	4.767 ± 0.278	0.062 ± 0.004	0.077 ± 0.007	77.01 ± 6.16	39.74 ± 3.60	384.00 ± 2.84	336.29 ± 10.55	1.31 ± 0.09	131.29 ± 5.55	2.57 ± 0.05	98.86 ± 7.42
11	IG76027	3.340 ± 0.028	0.050 ± 0.002	0.092 ± 0.004	111.89 ± 8.41	69.60 ± 6.25	167.79 ± 6.15	251.63 ± 8.28	9.46 ± 0.28	145.62 ± 0.42	2.54 ± 0.01	92.82 ± 3.48
12	IG5562	4.699 ± 0.289	0.073 ± 0.006	0.103 ± 0.008	89.31 ± 4.58	45.66 ± 2.63	327.82 ± 2.05	375.96 ± 9.74	1.70 ± 0.06	160.33 ± 2.10	2.55 ± 0.21	106.63 ± 12.58
13	IG5582	5.159 ± 0.254	0.069 ± 0.004	0.084 ± 0.014	117.17 ± 3.12	74.49 ± 2.88	567.12 ± 95.90	661.87 ± 129.04	2.01 ± 0.29	231.62 ± 21.51	3.40 ± 0.33	113.93 ± 13.69
14	IG5588	4.078 ± 0.200	0.081 ± 0.003	0.119 ± 0.012	90.51 ± 0.05	56.71 ± 1.29	462.52 ± 43.31	395.30 ± 40.17	3.32 ± 0.25	173.60 ± 8.40	3.14 ± 0.11	96.62 ± 6.21
15	IG5722	4.851 ± 0.166	0.103 ± 0.007	0.129 ± 0.013	206.70 ± 7.60	102.95 ± 5.12	276.38 ± 6.05	2095.88 ± 66.89	1.07 ± 0.13	90.20 ± 6.33	2.29 ± 0.12	63.78 ± 0.06
16	IG5748	4.065 ± 0.232	0.057 ± 0.004	0.163 ± 0.022	198.69 ± 5.84	121.72 ± 3.12	341.97 ± 21.06	2672.44 ± 58.66	0.82 ± 0.00	107.77 ± 9.59	3.56 ± 0.16	54.96 ± 4.43
17	IG69492	5.004 ± 0.112	0.068 ± 0.001	0.107 ± 0.008	134.94 ± 3.75	89.07 ± 2.50	437.12 ± 10.57	1968.38 ± 23.01	2.28 ± 0.18	71.94 ± 1.83	2.05 ± 0.10	58.74 ± 9.49
18	IG70043	4.572 ± 0.112	0.061 ± 0.002	0.107 ± 0.009	134.29 ± 12.24	66.06 ± 8.49	180.59 ± 0.96	2957.83 ± 87.33	3.13 ± 0.09	115.88 ± 1.65	3.04 ± 0.05	53.58 ± 0.61
19	IG73979	4.495 ± 0.392	0.069 ± 0.002	0.092 ± 0.011	111.29 ± 7.29	68.80 ± 5.59	316.78 ± 16.75	458.43 ± 30.70	0.78 ± 0.03	132.01 ± 4.45	2.98 ± 0.11	83.97 ± 4.75
20	IG70076	5.499 ± 0.251	0.079 ± 0.004	0.098 ± 0.014	100.55 ± 2.00	41.43 ± 0.90	448.65 ± 51.39	375.34 ± 57.48	0.88 ± 0.03	174.56 ± 11.58	2.98 ± 0.02	84.37 ± 6.61
21	IG156736	5.058 ± 0.133	0.076 ± 0.001	0.115 ± 0.007	96.40 ± 6.10	64.20 ± 3.90	367.90 ± 6.12	222.12 ± 19.74	2.47 ± 0.16	154.03 ± 2.02	2.89 ± 0.03	72.28 ± 1.57
22	IG70092	5.692 ± 0.123	0.077 ± 0.005	0.127 ± 0.009	84.87 ± 4.32	43.94 ± 1.54	449.43 ± 1.50	644.90 ± 4.13	4.86 ± 0.02	203.80 ± 2.62	3.38 ± 0.05	73.05 ± 0.87
23	IG71144	5.848 ± 0.215	0.074 ± 0.004	0.093 ± 0.009	81.96 ± 0.88	43.36 ± 0.24	305.04 ± 8.91	345.37 ± 12.86	2.64 ± 0.01	155.59 ± 2.80	3.02 ± 0.15	77.96 ± 1.77
24	IG117646	4.848 ± 0.263	0.069 ± 0.004	0.101 ± 0.008	55.42 ± 2.59	29.77 ± 5.91	519.50 ± 101.72	349.78 ± 59.35	2.18 ± 0.22	159.42 ± 2.16	3.39 ± 0.17	96.44 ± 13.26
25	IG71178	5.196 ± 0.592	0.066 ± 0.004	0.080 ± 0.013	54.64 ± 4.18	30.66 ± 3.50	383.18 ± 7.37	548.04 ± 16.24	0.72 ± 0.04	133.38 ± 2.42	2.38 ± 0.10	88.44 ± 0.96
26	IG117679	4.343 ± 0.181	0.062 ± 0.005	0.106 ± 0.010	58.87 ± 6.14	32.46 ± 0.13	431.74 ± 6.37	380.15 ± 30.11	2.02 ± 0.13	140.82 ± 8.82	3.17 ± 0.21	94.61 ± 12.92
27	IG73685	3.562 ± 0.144	0.056 ± 0.008	0.093 ± 0.008	64.09 ± 0.18	29.05 ± 0.31	302.08 ± 5.78	447.70 ± 6.99	3.20 ± 0.20	166.55 ± 3.91	3.04 ± 0.21	79.67 ± 3.32
28	IG122918	3.972 ± 0.454	0.057 ± 0.010	0.086 ± 0.013	140.85 ± 6.72	78.25 ± 4.21	238.20 ± 0.17	339.26 ± 11.71	3.94 ± 1.67	164.21 ± 8.39	3.24 ± 0.61	141.12 ± 3.02
29	IG73858	3.877 ± 0.168	0.064 ± 0.004	0.107 ± 0.011	95.66 ± 10.53	50.21 ± 7.26	353.55 ± 5.26	427.89 ± 7.61	3.38 ± 0.03	172.27 ± 0.27	2.93 ± 0.05	158.13 ± 6.63
30	IG73874	4.274 ± 0.403	0.068 ± 0.008	0.108 ± 0.003	103.67 ± 6.63	67.57 ± 7.26	428.20 ± 49.76	495.15 ± 73.52	3.61 ± 0.47	171.19 ± 16.82	3.90 ± 0.42	167.58 ± 7.02
31	IG75896	3.779 ± 0.285	0.058 ± 0.004	0.086 ± 0.012	109.89 ± 4.72	63.61 ± 2.83	247.19 ± 4.60	504.74 ± 14.16	2.35 ± 0.09	118.39 ± 4.18	4.49 ± 0.03	130.79 ± 2.37
32	IG122934	5.156 ± 0.242	0.079 ± 0.002	0.110 ± 0.015	101.79 ± 18.50	68.19 ± 1.24	518.51 ± 22.48	825.08 ± 0.14	1.03 ± 0.05	150.67 ± 22.18	3.45 ± 0.14	122.54 ± 14.86
33	IG75920	4.317 ± 0.171	0.063 ± 0.002	0.102 ± 0.008	59.92 ± 12.81	55.61 ± 2.10	423.48 ± 41.43	469.32 ± 52.76	0.96 ± 0.15	190.13 ± 25.65	1.65 ± 0.15	99.60 ± 5.37
34	IG75932	4.128 ± 0.153	0.061 ± 0.001	0.099 ± 0.012	105.58 ± 5.46	53.52 ± 2.64	324.97 ± 22.09	254.52 ± 18.06	2.54 ± 0.10	157.97 ± 11.49	1.62 ± 0.09	167.28 ± 18.84
35	IG76232	4.469 ± 0.141	0.069 ± 0.003	0.100 ± 0.015	74.04 ± 2.62	39.05 ± 6.95	404.21 ± 36.86	588.62 ± 4.37	2.82 ± 0.29	172.95 ± 9.35	4.56 ± 0.61	119.31 ± 1.72
36	IG114687	5.227 ± 0.254	0.075 ± 0.006	0.109 ± 0.015	92.34 ± 3.48	47.66 ± 2.51	348.77 ± 2.31	304.12 ± 17.35	2.34 ± 0.06	131.86 ± 3.44	6.31 ± 0.26	107.24 ± 3.37
37	IG114688	4.489 ± 0.145	0.073 ± 0.003	0.110 ± 0.017	121.94 ± 12.86	68.07 ± 10.16	350.25 ± 9.05	327.97 ± 8.35	3.01 ± 0.02	176.35 ± 1.96	2.61 ± 0.09	86.71 ± 4.47
38	IG114713	3.987 ± 0.064	0.069 ± 0.003	0.103 ± 0.012	95.19 ± 4.15	58.70 ± 7.28	343.47 ± 1.99	321.31 ± 1.83	1.74 ± 0.10	155.01 ± 0.24	2.62 ± 0.01	97.05 ± 0.82
39	IG117647	4.330 ± 0.215	0.077 ± 0.004	0.064 ± 0.006	97.18 ± 0.36	46.51 ± 0.31	312.93 ± 13.62	497.55 ± 32.09	2.64 ± 0.13	224.24 ± 11.04	4.17 ± 0.15	88.80 ± 4.00
40	IG117680	3.642 ± 0.117	0.061 ± 0.003	0.067 ± 0.001	82.88 ± 11.36	34.78 ± 5.76	408.10 ± 0.62	393.67 ± 3.27	1.68 ± 0.10	209.69 ± 1.55	2.96 ± 0.02	77.22 ± 1.37
41	IG117682	3.451 ± 0.168	0.054 ± 0.005	0.071 ± 0.002	90.49 ± 0.37	54.31 ± 0.06	330.97 ± 31.00	383.47 ± 34.23	5.19 ± 0.52	163.71 ± 13.09	3.69 ± 0.29	96.78 ± 2.49
42	ILL8006	3.612 ± 0.087	0.047 ± 0.001	0.064 ± 0.007	85.63 ± 8.00	46.65 ± 6.17	297.97 ± 10.89	466.82 ± 14.25	6.70 ± 0.15	191.28 ± 5.93	3.88 ± 0.55	91.50 ± 2.50
43	IG129142	4.161 ± 0.369	0.057 ± 0.004	0.052 ± 0.005	75.26 ± 0.57	38.18 ± 1.19	439.95 ± 3.69	373.45 ± 2.54	2.30 ± 0.10	108.98 ± 1.09	3.61 ± 0.04	104.19 ± 1.39
44	ILL8009	4.576 ± 0.735	0.049 ± 0.003	0.074 ± 0.012	117.19 ± 12.98	65.68 ± 7.92	166.69 ± 22.94	331.93 ± 45.08	6.94 ± 0.91	157.15 ± 16.76	5.44 ± 0.21	94.98 ± 3.79

(continued on next page)

Table 1 (continued)

Nr.	Genotype	TPC	ABTS	ABTS _{dir}	Delphinidin	Cyanidin	Catechin	Epicatechin	Luteolin	Kaempferol	Myricetin	Gallic acid
45	IG122878	5.295 ± 0.098	0.075 ± 0.009	0.120 ± 0.005	100.31 ± 9.23	45.10 ± 8.83	289.21 ± 0.93	373.11 ± 8.69	1.67 ± 0.13	135.71 ± 19.21	3.39 ± 0.52	143.07 ± 16.20
46	IG122880	4.493 ± 0.168	0.064 ± 0.001	0.087 ± 0.013	134.73 ± 3.26	65.42 ± 1.31	332.27 ± 2.95	639.10 ± 2.96	11.52 ± 0.61	179.62 ± 2.75	5.66 ± 0.24	91.60 ± 5.99
47	IG142268	3.704 ± 0.155	0.056 ± 0.001	0.089 ± 0.006	74.47 ± 0.74	35.14 ± 1.42	394.71 ± 1.69	382.21 ± 2.50	1.52 ± 0.04	154.71 ± 2.70	2.76 ± 0.05	84.45 ± 0.28
48	IG156670	3.162 ± 0.169	0.047 ± 0.003	0.086 ± 0.008	149.54 ± 0.74	81.69 ± 1.42	192.56 ± 1.69	164.84 ± 2.50	8.95 ± 0.04	100.37 ± 2.70	4.50 ± 0.05	117.62 ± 0.28
49	IG156514	4.561 ± 0.063	0.067 ± 0.009	0.099 ± 0.011	92.04 ± 14.53	22.38 ± 2.15	328.95 ± 5.98	517.54 ± 0.58	2.86 ± 0.03	204.68 ± 2.90	3.91 ± 0.06	82.47 ± 1.05
50	IG156631	4.121 ± 0.135	0.067 ± 0.005	0.074 ± 0.012	58.75 ± 5.99	22.79 ± 3.97	278.85 ± 5.40	455.34 ± 12.69	2.22 ± 0.01	154.75 ± 4.71	2.82 ± 0.32	83.10 ± 2.77
51	IG156693	4.209 ± 0.143	0.059 ± 0.004	0.099 ± 0.007	86.80 ± 5.82	50.82 ± 8.77	520.83 ± 83.89	806.63 ± 116.84	3.62 ± 0.01	214.20 ± 21.16	4.11 ± 0.47	108.25 ± 7.17
52	IG156695	3.076 ± 0.127	0.036 ± 0.004	0.100 ± 0.011	93.64 ± 12.99	95.93 ± 7.21	311.20 ± 3.36	433.95 ± 18.70	1.82 ± 0.09	157.57 ± 4.39	5.04 ± 0.12	90.61 ± 0.11
53	10867/10174/6SPS	4.371 ± 0.191	0.061 ± 0.005	0.100 ± 0.004	93.48 ± 6.58	50.44 ± 5.43	379.14 ± 30.85	152.54 ± 15.45	2.54 ± 0.14	137.58 ± 9.13	2.80 ± 0.02	102.45 ± 13.50
54	FIX2011S-132/...	2.837 ± 0.116	0.036 ± 0.001	0.080 ± 0.005	69.41 ± 4.33	28.43 ± 3.19	291.91 ± 8.24	310.53 ± 11.40	1.74 ± 0.14	153.86 ± 1.49	2.76 ± 0.45	95.43 ± 3.68
55	8114/7663/2SPS	4.089 ± 0.163	0.059 ± 0.004	0.090 ± 0.011	99.02 ± 16.48	77.00 ± 13.11	358.04 ± 58.98	423.34 ± 73.19	8.26 ± 1.26	204.66 ± 19.60	4.72 ± 0.11	108.39 ± 10.58
56	FIX2011S-132/...	3.105 ± 0.307	0.041 ± 0.004	0.073 ± 0.006	124.83 ± 11.46	59.57 ± 7.72	263.09 ± 3.96	319.20 ± 8.95	2.49 ± 0.09	190.50 ± 8.43	2.17 ± 0.06	84.97 ± 5.91
57	7978/DPL 62/10-8	3.412 ± 0.158	0.056 ± 0.002	0.089 ± 0.008	122.08 ± 6.81	70.49 ± 4.63	250.80 ± 0.22	491.11 ± 16.65	9.98 ± 0.41	200.84 ± 0.06	3.97 ± 0.12	89.99 ± 0.71
58	4605/3596/2SPS	3.791 ± 0.194	0.060 ± 0.005	0.094 ± 0.016	132.43 ± 2.45	86.04 ± 5.56	167.73 ± 14.80	343.22 ± 37.98	10.12 ± 1.26	189.97 ± 17.63	12.45 ± 0.50	101.51 ± 7.67
59	10072/1712/10-3	3.460 ± 0.141	0.055 ± 0.002	0.084 ± 0.008	128.21 ± 7.82	59.81 ± 4.62	362.03 ± 28.63	260.04 ± 22.73	2.61 ± 0.17	277.76 ± 31.32	2.82 ± 0.27	98.71 ± 3.43

^a Full genotype name: FIX2011S-132/FIX2011S-110/23-10.

^b Full genotype name: FIX2011S-132/FIX2011S-110/6-2.

suggesting that the antioxidants reacting in the whole-meal flour could be different from those present in TPC extracts.

Then, we analyzed the lentil collection for specific flavonoids subclasses, such as flavanols, flavanones, flavones, isoflavones, flavonols, and anthocyanidins, as they were previously suggested to play a major role in determining lentil seed color variation (Erskine et al., 1991; Galgano et al., 2021; Mirali et al., 2017; Mishra et al., 2022). Within each of these subclasses, a large complexity and high number of molecules was previously detected, due to differences in hydroxylation or conjugation arrangements (Beecher, 2003; Harborne & Williams, 2000). Such a complexity could be resolved in a smaller number of compounds by focusing on the corresponding aglycone forms (Manco et al., 2023). Following this approach, we detected the most significant metabolites associated to red lentil color, namely delphinidin, cyanidin, catechin, epicatechin, luteolin, kaempferol, myricetin and gallic acid (Table 1). Flavonol monomers catechin and epicatechin resulted the most abundant compounds, varying from 166.69 to 567.12 µg/g d.m. and from 152.54 to 2672.44 µg/g d.m., respectively. Second for abundance we found the anthocyanidins delphinidin (varying from a minimum of 54.64 µg/g d.m. to a maximum of 206.70 µg/g d.m.), followed by kaempferol and cyanidin (Table 1). The least abundant were luteolin and myricetin with contents ranging from 0.72 to 11.52 µg/g d.m. (luteolin), and from 1.62 to 12.45 µg/g d.m. (myricetin). These results were in line with preliminary studies carried out on a low number of genotypes suggesting that the large variation for individual polyphenols in red lentils allow the selection of genotype with the best combination of polyphenols to improve the functional properties of lentil-based foods and beverages (Ganesan & Xu, 2017; Kushi et al., 1999; Lastras et al., 2021; Romano et al., 2023). In fact, while both catechins and anthocyanins contribute to the high antioxidant activity in lentils, studies suggest catechins (flavan-3-ols) and other flavonoids (like flavonols) are major contributors to overall antioxidant capacity, though anthocyanins were also associated with several benefits on human health (Giusti et al., 2017).

ANOVA indicated significant genotypic effects ($p < 0.01$) for all the traits under investigation, including individual polyphenols, total polyphenol content (TPC), antioxidant capacity (ABTS and ABTS_{dir}), and the three CIELab color space indexes L*, a* and b* (Supplementary Table 3).

In addition, three-way ANOVAs indicated significant association ($p < 0.05$) between individual polyphenol content (except for kaempferol), TPC and antioxidant capacity, with different cotyledon color (CC: orange, orange/red, orange/yellow, and yellow), and different classes of tegument background color (TC) and tegument pigmentation pattern (TPP) (Table 2). Significant differences were observed among the four cotyledon color classes for gallic acid, epicatechin, TPC and ABTS. These results are of great interest because they show that differences in cotyledon color based on visual observation do not result in significant differences in anthocyanins as one would expect based on the role of anthocyanins as plant pigments (Giusti et al., 2017). On the other hand, tegument background color classes (TC) significantly differed for a higher number of individual polyphenols (i.e. cyanidin, gallic acid, luteolin, kaempferol) and for TPC, ABTS, and ABTS_{dir} (Table 2). Finally, tegument pigmentation pattern (TPP) classes significantly differed for delphinidin, cyanidin, catechin, luteolin, myricetin, namely the compounds that are mostly associated to color, along with TPC, ABTS, and ABTS_{dir} (Table 2). Therefore, based on these results, tegument color and pigmentation are confirmed to be more linked with antioxidant polyphenols and health-promoting properties (Manco et al., 2023).

The use of linear regression models revealed significant associations between at least one of the three CIELab color space indexes and individual polyphenols (gallic acid, catechin, epicatechin, luteolin and myricetin), TPC and ABTS ($p < 0.05$, Supplementary Table 4). Indeed, the association between phenolic compounds and lentil seed color (measured by color space indexes) was previously shown by evidence on a limited number of genotypes (Manco et al., 2023; Mirali et al., 2017).

Table 2
Three-way ANOVA results of cotyledon color (CC), tegument pigmentation pattern (TPP) and tegument color (TC) effects on the concentration of individual polyphenols, total polyphenol content (TPC), and antioxidant capacity (ABTS, ABTSdir) in lentil genotypes. Different letters indicate significant differences ($p < 0.05$, Tukey HSD test).

Seed trait	Delphinidin	Cyanidin	Catechin	Epicatechin	Luteolin	Kaempferol	Myricetin	Gallic acid	TPC	ABTS	ABTSdir
CC Orange	99.53 ± 30.73	56.93 ± 20.44	348.3 ± 96.61	466.06 ± 381.85b	3.34 ± 2.69	158.89 ± 37.54	3.45 ± 1.75	98.5 ± 21.4b	4.08 ± 0.72b	0.06 ± 0.01 ab	0.1 ± 0.02
CC Orange/Red	83.83 ± 32.84	50.45 ± 24.12	424.79 ± 124.18	343.96 ± 34.98b	1.58 ± 0.7	149.28 ± 12.2	2.87 ± 0.62	97.64 ± 9.14 ab	4.35 ± 0.58 ab	0.06 ± 0.01 ab	0.1 ± 0.01
CC Orange/Yellow	96.49 ± 9.5	56.62 ± 13.42	378.01 ± 64.69	435.56 ± 81.05 ab	2.65 ± 1.14	165.76 ± 11.62	3.22 ± 0.82	137.1 ± 36.16a	4.49 ± 0.39 ab	0.07 ± 0.01a	0.11 ± 0.01
CC Yellow	108.03 ± 33.12	55.91 ± 23.23	353.14 ± 95.63	825.12 ± 836.37a	3.15 ± 2.56	165.99 ± 42.31	3.52 ± 0.87	91.83 ± 25.32b	4.65 ± 0.63a	0.07 ± 0.01a	0.1 ± 0.03
F-value	1244	0.286	1234	8127	1380	0.408	1476	4578	9547	5145	1847
p-value	0.297	0.835	0.301	6.21E-05	0.253	0.748	0.225	0.005	7.41E-06	0.002	0.141
TPP Absent	97.39 ± 33.7b	52.33 ± 21.6b	381.56 ± 92.36a	646.23 ± 643.69	2.19 ± 0.95b	156.61 ± 37.87	3.11 ± 0.89b	96.14 ± 25.94	4.46 ± 0.69a	0.07 ± 0.01a	0.1 ± 0.02a
TPP Complex	126.85 ± 19.11a	76.52 ± 9.25a	229 ± 71.72c	348.95 ± 108.82	8.03 ± 2.35a	169.53 ± 38.44	5.72 ± 3.23a	108.93 ± 18.24	3.83 ± 0.57b	0.05 ± 0.01b	0.09 ± 0.01 ab
TPP Dotted	102.88 ± 25.76 ab	62.58 ± 19.95 ab	300.62 ± 73.13bc	390.5 ± 122.14	5.36 ± 3.61a	179.11 ± 41.9	3.82 ± 1.06a	94.97 ± 5.59	3.43 ± 0.48b	0.05 ± 0.01b	0.09 ± 0.02 ab
TPP Spotted	95.55 ± 17.15b	54.7 ± 12.98b	364.49 ± 37.13 ab	349.25 ± 62.56	2.13 ± 0.89b	152.45 ± 21.95	2.8 ± 0.4b	103.85 ± 26.11	4.34 ± 0.45a	0.06 ± 0.01a	0.1 ± 0.03a
F-value	5287	4835	16,128	1907	19,100	2689	14,140	1736	15,437	15,812	3165
p-value	0.002	0.003	9.58E-09	0.133	5.02E-10	0.050	7.53E-08	0.164	6.47E-09	4.26E-09	0.026
TC Brown/Orange	104.17 ± 32.31	60.79 ± 19.85a	350.26 ± 102.13	465.44 ± 338.95	4.02 ± 3.07a	163.22 ± 33.68	3.68 ± 1.94	101.22 ± 22.83a	4.03 ± 0.65b	0.06 ± 0.01b	0.09 ± 0.02b
TC Pink/Red	98.8 ± 33.31	54.66 ± 22.83 ab	359.95 ± 101.77	684.78 ± 751.95	2.47 ± 1.91b	153.87 ± 42.62	3.02 ± 0.6	89.54 ± 18.84b	4.38 ± 0.81a	0.06 ± 0.01b	0.1 ± 0.02a
TC Yellow/Green	94.33 ± 11.13	44.43 ± 11.4b	341.9 ± 40.89	424.47 ± 116.44	2.3 ± 0.61 ab	170.88 ± 33.54	3.79 ± 1.46	114.1 ± 31.08a	4.63 ± 0.45a	0.07 ± 0.01a	0.1 ± 0.02 ab
F-value	1116	4584	0.116	1071	9099	1276	2903	9382	5688	4190	3353
p-value	0.331	0.012	0.890	0.346	2.22E-04	0.283	0.059	1.74E-04	0.004	0.017	0.037

Based on hierarchical clustering, TPC was highly correlated with antioxidant capacity expressed as ABTS, suggesting that ABTS is a reliable proxy to assess the antioxidant capacity of phenolic extracts (Fig. 1). Especially catechin was strongly associated with a^* , and both correlated with b^* , TPC and ABTS (Fig. 1). These results support previous findings indicating that while both catechins and anthocyanins contribute to the high antioxidant activity in lentils, catechins are major contributors to the overall antioxidant capacity of lentils (Mirali et al., 2017; Mishra et al., 2022; Xu & Chang, 2010).

Finally, our results showed that ABTSdir was specifically correlated with three individual flavonoids, namely epicatechin, delphinidin and cyanidin (Fig. 1), indicating that the ABTS assay conducted directly on the whole-meal flour is effective to estimate the antioxidant activity of specific individual compounds and their synergic effect (Rudrapal et al., 2022).

Overall, our results also suggest that selecting certain TPP and TC classes can result in higher content of bioactive polyphenols and antioxidant activity that could be used to improve the functional properties of lentil-based food products (Table 2).

3.2. Multivariate analysis of morphological and biochemical traits

Relationships among genotypes were further investigated by Principal Component Analysis (PCA). In PCA, the score plot displays the distribution of genotypes along principal components, highlighting similarities or differences among samples, while the loading plot indicates which variables most strongly influence these components. Variables positioned farther from the origin exert a greater effect on sample separation. The first two components explained 28.55% (PC1) and 18.62% (PC2) of the dataset variation (Fig. 2). Notably, the PCA biplot successfully separated accessions with complex or dotted TPP from those with spotted or no TPP. Examination of PCA loadings highlighted traits mainly driving this separation, including myricetin, luteolin, TPC, catechin content, ABTS, and CIELab color space indexes (Fig. 2).

Hierarchical clustering provided information on variables with similar variation trends and genotypes with similar physicochemical features (Fig. 1). Three main variable clusters were found. The first (V1) included luteolin, myricetin, kaempferol and gallic acid, with the first two variables exhibiting the most similar variation patterns (Fig. 1). The second cluster (V2) revealed very similar variation patterns for delphinidin and cyanidin, and relation of these two compounds with epicatechin and ABTSdir. The third cluster (V3) grouped the remaining variables, with L and b^* , TPC and ABTS, and a^* and catechin forming three sub-clusters with similar variation patterns.

Genotype clustering substantially confirmed PCA results, as two main clusters were identified, one mostly containing complex and dotted TPP accessions, and the other absent or spotted TPP accessions. The first cluster was characterized by below-average levels of V3 variables, while the second lacked a clear pattern of variation. In addition, a divergent genotypic cluster was found, including the accessions 15A, 16A, 17A and 18A, all characterized by no TPP. This was characterized by below-average levels of V1 variables, and above-average levels of V2 variables. The genotype 58C, displaying a complex TPP, showed a clearly distinct profile, with above-average levels of V1 variables (including the highest level of myricetin), and below-average levels of variables included in the V3 group (including the lowest value of the b^* color index).

Overall, our results are consistent with those of Palombi et al. (2025), showing that the biochemical composition of red lentils, evaluated by ^1H NMR, is correlated with seed coat and seed cotyledons colors, as well as with seed coat patterns. In turn, this indicates that higher abundance of specific compounds is associated with different colors and patterns. Our results are also in agreement with other works on lentil, whose conclusions were largely speculative due to the low number of accessions under study (Manco et al., 2023; Mirali et al.,

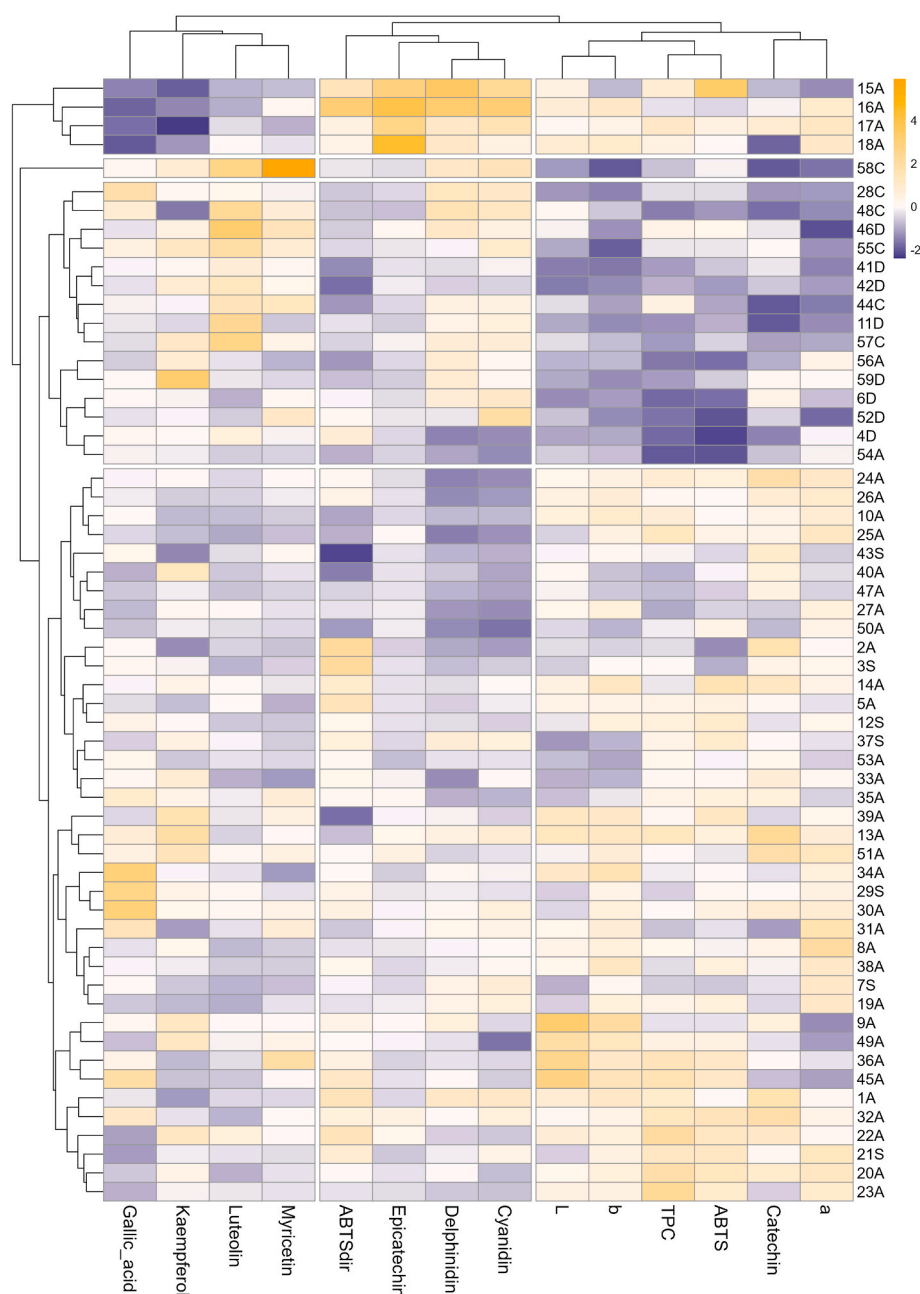


Fig. 1. Heatmap and hierarchical clustering based on the physicochemical features of 59 lentil genotypes. Each row refers to a single lentil genotype, labeled from 1 to 59 according to accession number, with letters denoting the tegument pigmentation pattern (TPP): A, absent (no pigmentation); C, complex; D, dotted; and S, spotted. The color scale represents scaled average values for each variable. L*, a*, b*: CIELAB color space indexes; TPC: Total phenolic content; ABTS: Antioxidant capacity by the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay on extracts; ABTSdir: Antioxidant capacity by direct ABTS assay. Lentil genotypes on the left are reported with the progressive order number as in Table 1 along with a letter indicating different TPP (A: Absent; C: Complex; D: Dotted; S: Spotted). Color intensity reflects standardized values for each variable, ranging from purple (lowest values) to yellow (highest values). Dendrograms group genotypes and variables with similar variation patterns, allowing identification of clusters associated with specific morphological or biochemical traits.

2017; Mishra et al., 2022). To our knowledge, this is the first in-depth study investigating polyphenol differences among red lentils. In addition, different polyphenolic profiles were associated with specific morphological features, thereby providing a basis for breeding programs aimed at the selection of biofortified red lentil genotypes (Ganesan & Xu, 2017; Kushi et al., 1999; Lastras et al., 2021; Romano et al., 2023).

Moreover, high heritability estimates for biochemical traits have been reported for several legume species (Mohammadi et al., 2025; Morris et al., 2023; Nadeem et al., 2019; Ujwal et al., 2025), suggesting that substantial genetic gains can be achieved.

3.3. Discriminant modeling of color classes using OPLS-DA

To investigate the relationship between biochemical and physical traits and seed color morphology, OPLS-DA was applied. OPLS-DA is a supervised multivariate method that separates predictive variation (related to class discrimination) from orthogonal variation (unrelated to classes). Score plots show how samples cluster according to predefined categories, whereas correlation circle plots indicate which variables contribute most to predictive or orthogonal components. In addition, VIP (Variable Importance in Projection) values summarize the relative influence of each variable, with values above 1 generally considered

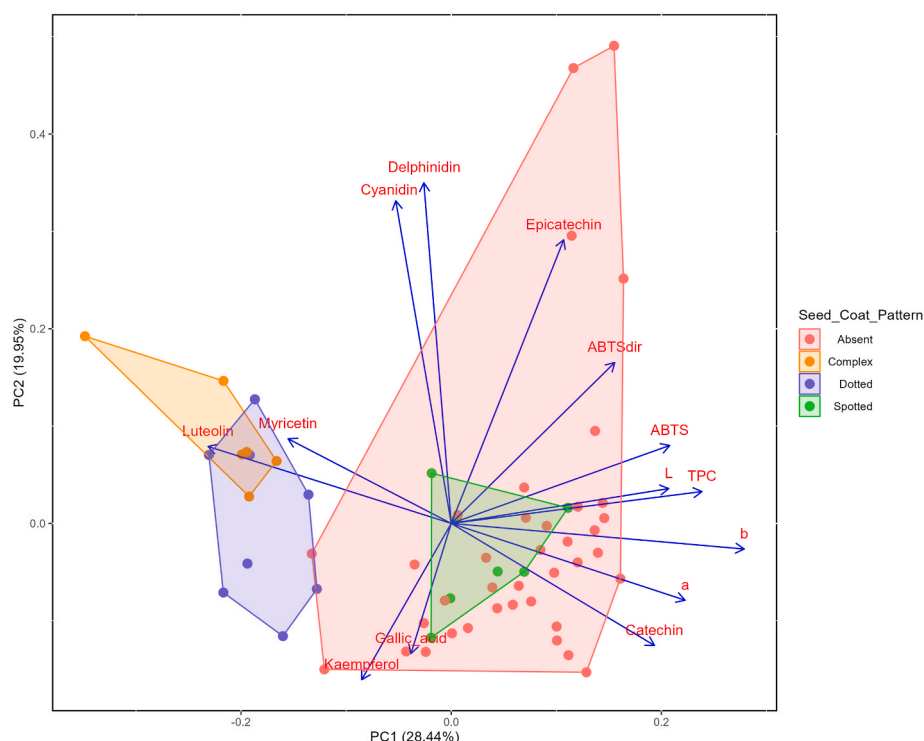


Fig. 2. Principal Component Analysis (PCA) of physicochemical features in 59 lentil genotypes. Each dot represents a genotype (1–59), where both color and letter correspond to the tegument pigmentation pattern (TPP): A, absent (red); C, complex (orange); D, dotted (purple); and S, spotted (green). The plot shows the first (PC1) and second (PC2) principal components. Arrows indicate variable loadings. L^* , a^* , b^* : CIELAB color space indexes; TPC: Total phenolic content; ABTS: Antioxidant capacity by the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay on extracts; ABTS_{dir}: Antioxidant capacity by direct ABTS assay. The axes indicate the first two principal components, which explain most of the variance in the dataset. Genotypes closer together share similar physicochemical profiles, while arrows represent variable loadings, with longer arrows indicating stronger influence on sample separation.

significant. The goal was to classify lentil genotypes according to cotyledon and tegument color categories using a multivariate dataset including polyphenol profiles, antioxidant activity (ABTS and ABTS_{dir}), seed size traits (diameter and hundred kernel weight), and CIELab colorimetric parameters (L^* , a^* , b^*). Although L^* , a^* , and b^* are instrumental measures of color, they were included in the model to assess how quantitative colorimetric variation interacts with biochemical composition and contributes to the discrimination of visually defined color classes. This approach allowed us to validate the consistency between visual classification and instrumental measurements, and to explore the extent to which biochemical traits reinforce or diverge from colorimetric data.

Given the strong imbalance in class distribution across the full dataset of 59 genotypes, only the most represented categories were considered for classification. Specifically, cotyledon color was restricted to the two dominant classes: Yellow (14 genotypes) and Orange (41 genotypes), while tegument background color was limited to Brown/Orange (29 genotypes) and Pink/Red (23 genotypes). This selection ensured sufficient statistical power and reduced the risk of overfitting due to underrepresented groups.

3.4. OPLS-DA analysis on cotyledon color

The optimal number of PLS components was determined through five-fold cross-validation with 200 Monte Carlo randomizations of training and test sets. The configuration that maximized classification performance included three predictive components, yielding a cross-validation accuracy of 86.4%, an AUC of 0.935, and an explained variance (R^2X) of 50.0%. Using these three components, the PLS-DA model was then applied to the complete dataset, achieving an accuracy of 94.5%, an AUC of 0.993, and an R^2X of 49.1%, confirming strong predictive ability and model stability. The confusion matrix (Fig. 3D)

indicated excellent classification performance for both cotyledon color classes, with only minor misclassifications.

The OPLS-DA decomposition separated predictive variation from orthogonal variation, improving interpretability. The score plot (Fig. 3A) revealed clear clustering of genotypes into the two cotyledon color classes (Yellow and Orange), with minimal overlap, indicating robust discrimination. The correlation circle plot (Fig. 3B) showed that the predictive component (explaining 16.6% of variance) was dominated by Hundred Kernel Weight, seed Size, and L^* , indicating that physical traits and brightness are the strongest drivers of cotyledon color classification. Antioxidant activity (ABTS), b^* , and TPC also contributed moderately, while polyphenols such as catechin and epicatechin were positioned closer to the center, suggesting a secondary role. The second component, orthogonal to the predictive axis, captured variation primarily associated with anthocyanidins (cyanidin and delphinidin) and some flavonoids (luteolin, myricetin), reflecting within-class biochemical diversity rather than predictive power.

The VIP score histogram (Fig. 3C) confirmed these patterns: Hundred Kernel Weight and Size had the highest VIP values (>1.8), followed by L^* , ABTS, and b^* , while TPC and selected flavonoids (luteolin, kaempferol) were moderately influential. Anthocyanidins and gallic acid had VIP values well below 1, indicating limited relevance for class discrimination. These findings underscore that cotyledon color is primarily associated with seed size and brightness, supported by antioxidant capacity and overall polyphenol content, while anthocyanin variation explains orthogonal differences unrelated to color class separation.

3.5. OPLS-DA analysis on tegument color

The OPLS-DA model was applied to classify genotypes according to tegument background color (Brown/Orange vs. Pink/Red) using the same multivariate dataset. The optimal number of PLS components was

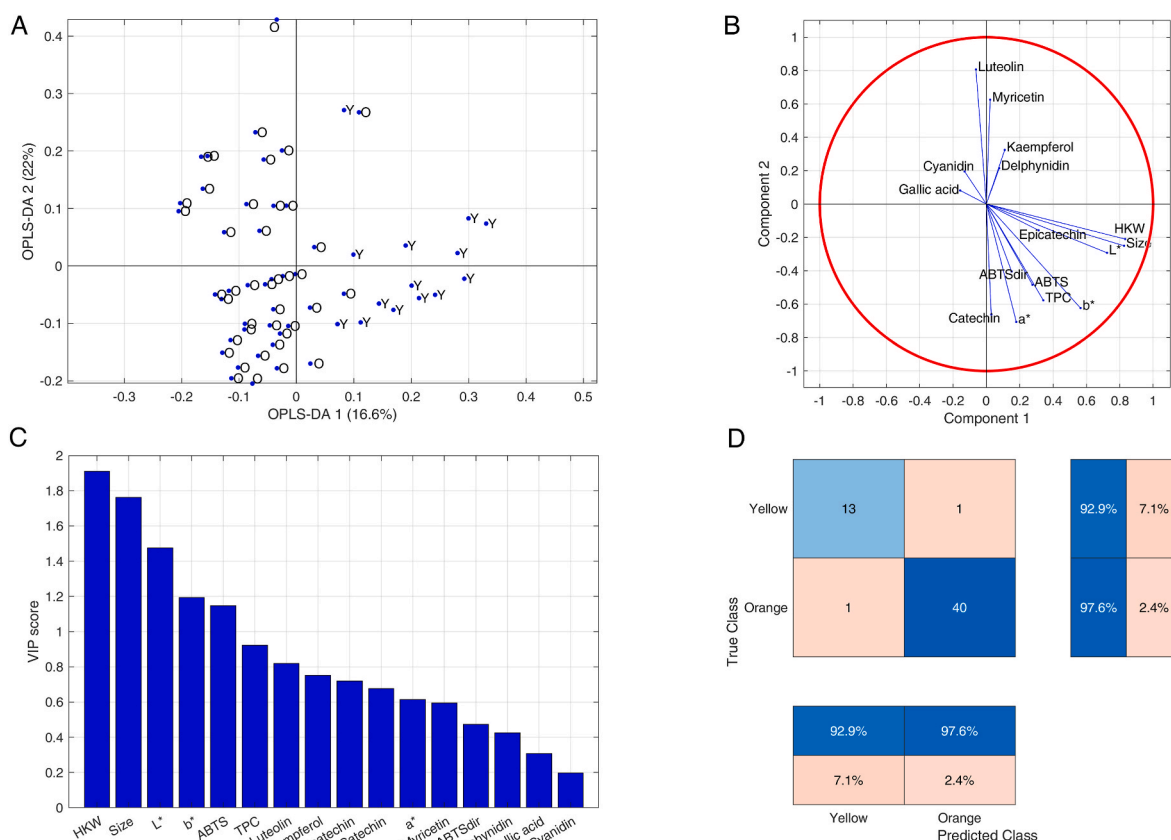


Fig. 3. OPLS-DA analysis for cotyledon color classification (Yellow vs. Orange). A): Score plot along the predictive component (OPLS-DA 1, 16.6%) and orthogonal component (OPLS-DA 2, 22.0%). Yellow and Orange genotypes are indicated by “Y” and “O” respectively. B): Correlation circle indicating variable contributions to the predictive and orthogonal component. C): VIP score histogram. D): Confusion matrix reporting both the number of genotypes correctly and incorrectly classified for each cotyledon color class and the corresponding percentages. Score plots show sample clustering by cotyledon color classes, while correlation circles highlight variables contributing most to predictive and orthogonal components. VIP histograms summarize variable importance for discrimination, with values above 1 generally considered influential. Confusion matrix reports the number and percentage of correctly and incorrectly classified genotypes for each cotyledon color class, providing an immediate measure of model accuracy.

determined through five-fold cross-validation with 200 Monte Carlo randomizations, resulting in four predictive components. This configuration achieved a cross-validation accuracy of 66.3%, an AUC of 0.679, and an explained variance (R^2X) of 61.4%. When applied to the complete dataset, the model performance improved substantially, with an accuracy of 84.6%, an AUC of 0.864, and an R^2X of 61.0%, indicating good predictive ability despite the complexity of tegument color variation.

The score plot (Fig. 4A) showed a clear separation between the two tegument color classes along the predictive component (OPLS-DA 1, explaining 12.9% of variance), with minimal overlap, while the orthogonal component (OPLS-DA 2, 21.9%) captured variation unrelated to class discrimination. The correlation circle plot (Fig. 4B) highlights the variables most associated with tegument color. The predictive axis was dominated by the a^* index, which directly reflects redness and is expected to discriminate Pink/Red from Brown/Orange seeds. Gallic acid, myricetin, and luteolin also showed moderate alignment with the predictive component, suggesting that these phenolic compounds contribute to tegument color differentiation. In contrast, catechin, ABTS antioxidant activity, seed size, hundred kernel weight, TPC, and L^* were positioned closer to the orthogonal axis, indicating that they explain variation unrelated to class separation and contribute mainly to within-class heterogeneity.

The confusion matrix (Fig. 4D) confirmed satisfactory classification performance, with correct predictions for 82.6% of Pink/Red and 86.2% of Brown/Orange genotypes. Misclassifications were limited. The VIP score histogram (Fig. 4C) corroborated these findings: a^* was the most

influential variable ($VIP \approx 2$), followed by gallic acid, ABTS, TPC, and b^* , while luteolin and myricetin were moderately important. Catechin, epicatechin, seed size, and L^* had lower VIP values, and anthocyanidins (cyanidin and delphinidin) were among the least relevant for tegument color discrimination. Anthocyanidins and seed size traits had VIP values below 1, confirming their minor contribution to predictive power.

Overall, these results demonstrate that tegument color classification is primarily driven by instrumental color parameters, particularly a^* , supported by antioxidant activity and selected polyphenols, while anthocyanin variation contributes to orthogonal differences unrelated to class separation.

4. Conclusions

This study provides a comprehensive evaluation of 59 red lentil genotypes for polyphenol profile and antioxidant capacity with the aim of assessing the correlation with seed morphological traits. The results show that genotypes with darker and more complex pigmentation patterns result in a higher accumulation of specific flavan-3-ols monomers (catechin, epicatechin), anthocyanidins (delphinidin, cyanidin), flavons (luteolin) and flavonols (myricetin), as well as higher TPC and antioxidant activity. In addition, cotyledon color was associated with seed size and brightness contributing to the overall antioxidant potential. Indeed, linear regressions supported the link between instrumental color parameters and phenolic content. Multivariate approaches, including PCA and hierarchical clustering, identified coherent genotype groups and variable clusters, reinforcing the role of pigmentation in shaping

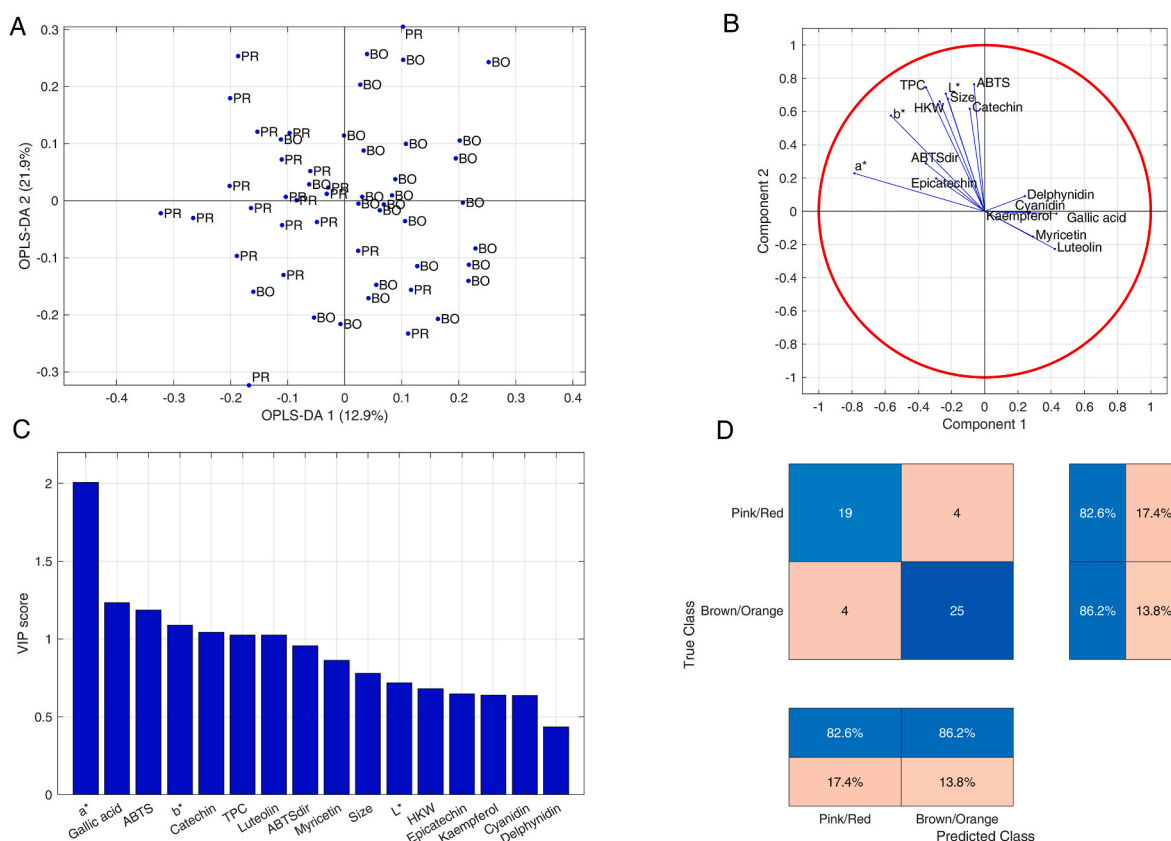


Fig. 4. OPLS-DA analysis for tegument color classification (Pink/Red vs. Brown/Orange). A): Score plot along the predictive component (OPLS-DA 1, 12.9%) and orthogonal component (OPLS-DA 2, 21.9%). Pink/Red and Brown/Orange genotypes are indicated by “PR” and “BO” respectively. B): Correlation circle indicating variable contributions to the predictive and orthogonal component. C): VIP score histogram. D): Confusion matrix reporting both the number of genotypes correctly and incorrectly classified for each tegument color class and the corresponding percentages. Score plots illustrate separation of tegument color classes, correlation circles indicate variable contributions, and VIP histograms rank variables by importance in class discrimination, with values above 1 generally considered influential. Confusion matrix summarizes classification performance for each tegument color class, showing correct predictions and misclassifications as percentages.

biochemical profiles. Discriminant modeling via OPLS-DA further demonstrated that cotyledon and tegument color classes could be accurately predicted based on combined physical and biochemical data, with instrumental color indices and antioxidant capacity emerging as key discriminants. Overall, this study highlights the potential of using seed morphological traits of red lentils, as practical indicators of potential functional quality offering valuable tools for breeding programs focused on enhancing the health-promoting properties of lentils.

CRediT authorship contribution statement

Gaetano Giudice: Writing – review & editing, Writing – original draft, Software, Formal analysis, Data curation. **Lorenzo Palombi:** Writing – review & editing, Writing – original draft, Software, Data curation. **Stefano Pavan:** Writing – review & editing, Funding acquisition, Data curation. **Marzia Guerriero:** Writing – review & editing, Data curation. **Andrea Visioni:** Resources, Data curation. **Barbara Laddomada:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The Authors declare that the work described in this article has not been published previously, that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including

electronically without the written consent of the copyright holder.

Acknowledgments

This research was partially funded by the following projects: PRIN2022 project acronyms GRANARIUS (Project Nr. 202282ZTPL) and LENTIGO (Project Nr. 2022SFMSW3); AgriTech National Research Center, receiving funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 June 17, 2022, CN00000022). The authors acknowledge Antonio Manco for skillful assistance in laboratory analyses. The authors also acknowledge the support from Dr. Simonetta Martena and Marinella Cavallo for GRANARIUS Project management.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fbio.2026.108452>.

Data availability

Data will be made available on request.

References

- Adebamowo, C. A., Cho, E., Sampson, L., Katan, M. B., Spiegelman, D., Willett, W. C., & Holmes, M. D. (2005). Dietary flavonols and flavonol-rich foods intake and the risk

- of breast cancer. *International Journal of Cancer*, 4, 628–633. <https://doi.org/10.1002/ijc.20741>
- AGPG. (1985). *AGPG. Lentil descriptor; international board for plant genetic resources*. Rome, Italy, 1985.
- Ahmed, T., Rahman, A., Salma, U., Akter, Z., Ansary, M. M. U., Khalil, M. I., Karim, N., & Bari, L. (2020). Nutritional, phytochemicals and antioxidant properties of some popular pulse varieties of Bangladesh. *Journal of Agricultural Chemistry and Environment*, 9, 343–368. <https://doi.org/10.4236/jacen.2020.94025>
- Alshikh, N., de Camargo, A. C., & Shahidi, F. (2015). Phenolics of selected lentil cultivars: Antioxidant activities and inhibition of low-density lipoprotein and DNA damage. *Journal of Functional Foods, Natural Antioxidants*, 18, 1022–1038. <https://doi.org/10.1016/j.jff.2015.05.018>
- Aslani, Z., Barker, M., & Rayens, W. (2003). Partial least squares for discrimination. *Journal of Chemometrics*, 17, 166–173. <https://doi.org/10.1002/cem.785>
- Canadian Grain Commission. (2024). *Official grain grading guide*. Ottawa, ON: Lentils (Chapter 18).
- Chamberlin, M. L., Wilson, S. M. G., Gaston, M. E., Kuo, W. Y., & Miles, M. P. (2024). Twelve weeks of daily lentil consumption improves fasting cholesterol and postprandial glucose and inflammatory responses - A randomized clinical trial. *Nutrients*, 31(16), 419. <https://doi.org/10.3390/nu16030419>, 3.
- CIELab. (1978). International Commission on Illumination/Commission Internationale de l'Éclairage/Internationale Beleuchtungskommission. <https://cie.co.at/>. (Accessed 16 May 2023).
- Clarke, S. T., Sarfaraz, S., Qi, X., Ramdath, D. G., Fougere, G. C., & Ramdath, D. D. (2022). A review of the relationship between lentil serving and acute postprandial blood glucose response: Effects of dietary fibre, protein and carbohydrates. *Nutrients*, 14(4), 849. <https://doi.org/10.3390/nu14040849>
- Crop production statistics. <https://data.tuik.gov.tr/Bulten/Index?p=Crop-Production-Statistics-2024-53447&dil=2>, (2024)–. (Accessed 5 March 2025).
- de Mendiburu, F. (2023). *Agricolae: Statistical procedures for agricultural research* (Version 1.3-7). <https://doi.org/10.32614/CRAN.package.agricolae>
- Del Coco, M., Laddomada, B., Romano, G., Carcagni, P., Kumar, S., & Leo, M. (2022). Characterization of a collection of colored lentil genetic resources using a novel computer vision approach. *Foods*, 11(24), 3964. <https://doi.org/10.3390/foods11243964>
- Dhull, S., Kinabo, J., & Uebersax, M. (2022). Nutrient profile and effect of processing methods on the composition and functional properties of lentils (*Lens culinaris* Medik.): A review. *Legume Science*, 5(4), e156. <https://doi.org/10.1002/leg3.156>
- Dueñas, M., Hernández, T., & Estrella, I. (2006). Assessment of in vitro antioxidant capacity of the seed coat and the cotyledon of legumes in relation to their phenolic contents. *Food Chemistry*, 98, 95–103. <https://doi.org/10.1016/j.foodchem.2005.05.052>
- Erdogan, C. (2015). Genetic characterization and cotyledon color in lentil. *Chilean Journal of Agricultural Research*, 75(3), 383–389. <https://doi.org/10.4067/S0718-58392015000500001>
- Erskine, W., Williams, P. C., & Nakkoul, H. (1991). Splitting and dehulling lentil (*Lens culinaris*): Effects of seed size and different pretreatments. *Journal of the Science of Food and Agriculture*, 57, 77–84. <https://doi.org/10.1002/jsfa.2740570109>
- Fiocchetti, F., Laddomada, B., Roselli, M., Crinò, P., & Lucretti, S. (2009). Fingerprinting of three typical macrospira Italian lentil (*Lens culinaris* Medik.) landraces using fluorescence-based AFLP markers. *Scientia Horticulturae*, 121, 383–387. <https://doi.org/10.1016/j.scienta.2009.02.006>
- Galgano, F., Tolve, R., Scarpa, T., Caruso, M. C., Lucini, L., Senizza, B., & Condelli, N. (2021). Extraction kinetics of total polyphenols, flavonoids, and condensed tannins of lentil seed coat: Comparison of solvent and extraction methods. *Foods*, 10, 1810. <https://doi.org/10.3390/foods10081810>
- Ganesan, K., & Xu, B. (2017). Polyphenol-rich lentils and their health promoting effects. *International Journal of Molecular Sciences*, 18(11), 2390. <https://doi.org/10.3390/ijms18112390>
- Giusti, F., Caprioli, G., Ricciuti, M., Vittori, S., & Sagratini, G. (2017). Determination of fourteen polyphenols in pulses by high performance liquid chromatography-diode array detection (HPLC-DAD) and correlation study with antioxidant activity and colour. *Food Chemistry*, 221, 689–697. <https://doi.org/10.1016/j.foodchem.2016.11.118>
- Harborne, J. B., & Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry*, 55, 481–504. [https://doi.org/10.1016/S0031-9422\(00\)00235-1](https://doi.org/10.1016/S0031-9422(00)00235-1)
- Irakli, M., Kargiotidou, A., Tigka, E., Beslemes, D., Fournomiti, M., Pankou, C., Stavroula, K., Tsvetelika, N., & Vlachostergios, D. N. (2021). Genotypic and environmental effect on the concentration of phytochemical contents of lentil (*Lens culinaris* L.). *Agronomy*, 11(6), 1154. <https://doi.org/10.3390/agronomy11061154>
- Jung, Y.-S., Lee, S.-H., Chun, S. Y., Kim, D. H., Jang, B. I., Han, M.-H., & Lee, S.-O. (2022). In vitro and in vivo protective effects of lentil (*Lens culinaris*) extract against oxidative stress-induced hepatotoxicity. *Molecules*, 27(1), 59. <https://doi.org/10.3390/molecules27010059>
- Kolde, R. (2019). *Pheatmap: Pretty heatmaps (version 1.0.12)*. CRAN [R package] <https://r-drr.io/cran/pheatmap/>.
- Kushi, L. H., Meyer, K. A., & Jacobs, D. R., Jr. (1999). Cereals, legumes, and chronic disease risk reduction: Evidence from epidemiologic studies. *American Journal of Clinical Nutrition*, 70(3), 451S–458S. <https://doi.org/10.1093/ajcn/70.3.451s>
- Lastras, C., Revilla, I., González-Martín, M. I., & Vivar-Quintana, A. M. (2021). Prediction of fatty acid and mineral composition of lentils using near infrared spectroscopy. *Journal of Food Composition and Analysis*, 102, Article 104023. <https://doi.org/10.1016/j.jfca.2021.104023>
- Lee, S.-Y., Yeo, Y.-S., Park, S.-Y., Lee, S.-G., Lee, S.-M., Cho, H., Chung, N.-J., & Oh, S.-W. (2017). Compositional analysis of lentil (*Lens culinaris*) cultivars related to colors and their antioxidative activity. *Plant Breeding and Biotechnology*, 5, 192–203. <https://doi.org/10.9787/PBB.2017.5.3.192>
- Longobardi, F., Innamorato, V., Di Gioia, A., Ventrella, A., Lippolis, V., Logrieco, A. F., Catucci, L., & Agostiano, A. (2017). Geographical origin discrimination of lentils (*Lens culinaris* Medik.) using ¹H NMR fingerprinting and multivariate statistical analyses. *Food Chemistry*, 237, 743–748. <https://doi.org/10.1016/j.foodchem.2017.05.159>
- Magalhães, L. M., Santos, F., Segundo, M. A., Reis, S., & Lima, J. L. F. C. (2010). Rapid microplate high-throughput methodology for assessment of Folin-Ciocalteu reducing capacity. *Talanta*, 83, 441–447. <https://doi.org/10.1016/j.talanta.2010.09.042>
- Manco, A., Gerardi, C., Romano, G., D'Amico, L., Blanco, A., Milano, F., Di Sansebastiano, G. P., Balech, R., & Laddomada, B. (2023). Phenolic profile of whole seeds and seed fractions of pigmented lentils and its impact on antioxidant activity. *Food Bioscience*, 54, Article 102887. <https://doi.org/10.1016/j.fbio.2023.102887>
- Mirali, M., Purves, R. W., & Vandenberg, A. (2017). Profiling the phenolic compounds of the four major seed coat types and their relation to color genes in lentil. *Journal of Natural Products*, 80, 1310–1317. <https://doi.org/10.1021/acs.jnatprod.6b00872>
- Mishra, G. P., Ankita, M., Aski, M. S., Tontang, M. T., Choudhary, P., Tripathi, K., Singh, A., Kumar, R. R., Thimmegowda, V., Stobdan, T., Kumar, A., Bhardwaj, R., Praveen, S., Yadava, D. K., Kumar, S., & Dikshit, H. K. (2022). Morphological, molecular, and biochemical characterization of a unique lentil (*Lens culinaris* Medik.) genotype showing seed-coat color anomalies due to altered anthocyanin pathway. *Plants*, 11, 1815. <https://doi.org/10.3390/plants11141815>
- Mohammadi, S., Purves, R. W., Paliocha, M., Uhlen, A. K., & Zanotto, S. (2025). Multi-environment field trials indicate strong genetic control of seed polyphenol accumulation in faba bean. *Euphytica*, 221, 47. <https://doi.org/10.1007/s10681-025-03486-8>
- Morris, J. B., Tonniss, B. D., Wang, M. L., & Bhattarai, U. (2023). Genetic diversity for quercetin, myricetin, cyanidin, and delphinidin concentrations in 38 blackeye pea (*Vigna unguiculata* L. Walp.) genotypes for potential use as a functional health vegetable. *Journal of Dietary Supplements*, 20(5), 673–688. <https://doi.org/10.1080/19390211.2022.2077881>
- Mustafa, A. M., Abouelenen, D., Acquaticci, L., Alessandrini, L., Angeloni, S., Borsetta, G., Caprioli, G., Nzekoue, F. K., Sagratini, G., & Vittori, S. (2022). Polyphenols, saponins and phytosterols in lentils and their health benefits: An overview. *Pharmaceuticals*, 15(10), 1225. <https://doi.org/10.3390/ph15101225>, 3.
- Nadeem, M. A., Gündogdu, M., Ercişli, S., Karaköy, T., Saracoğlu, T., Habyarimana, E., ... Baloch, F. S. (2019). Uncovering phenotypic diversity and DArTseq marker loci associated with antioxidant activity in common bean. *Genes*, 11(1), 36.
- Palombi, L., Romano, G., Tufariello, M., Mancarella, S., Visioni, A., Angilè, F., Laddomada, B., & Fanizzi, F. P. (2025). Correlating the ¹H NMR fingerprinting of a collection of red lentil varieties with seed colour and morphology using advanced statistical analyses. *Food Bioscience*, 72, Article 107458. <https://doi.org/10.1016/j.fbio.2025.107458>
- Pavan, S., Bardaro, N., Fanelli, V., Marcotrigiano, A. F., Mangini, G., Taranto, F., Catalano, D., Montemurro, C., De Giovanni, C., Lotti, C., & Ricciardi, L. (2019). Genotyping by sequencing of cultivated lentil (*Lens culinaris* Medik.) highlights population structure in the mediterranean gene pool associated with geographic patterns and phenotypic variables. *Frontiers in Genetics*, 10, 872. <https://doi.org/10.3389/fgene.2019.00872>
- Purves, R. W., Khazaei, H., Ellessawy, F. M., Munro, R., Shurmer, B. O., & Vandenberg, A. (2025). Investigation of polyphenol diversity among lentil species (*Lens* spp.) using mass spectrometry-based metabolomics guided by photodiode array detection. *Food Research International*, 209, Article 116154. <https://doi.org/10.1016/j.foodres.2025.116154>
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9–10), 1231–1237. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3)
- Romano, G., Del Coco, L., Milano, F., Durante, M., Palombieri, S., Sestili, F., Visioni, A., Jilal, A., Fanizzi, F. P., & Laddomada, B. (2022). Phytochemical profiling and untargeted metabolite fingerprinting of the MEDWHEALTH wheat, barley and lentil whole-meal flours. *Foods*, 11, 4070. <https://doi.org/10.3390/foods11244070>
- Romano, G., Tufariello, M., Calabriso, N., Del Coco, L., Fanizzi, F. P., Blanco, A., Carluccio, M. A., Grieco, F., & Laddomada, B. (2023). Pigmented cereals and legume grains as healthier alternatives for brewing beers. *Food Bioscience*, 52, Article 102463. <https://doi.org/10.1016/j.fbio.2023.102463>
- Rudrapal, M., Khaimar, S. J., Khan, J., Dukhyil, A. B., Ansari, M. A., Alomary, M. N., Alshabmi, F. M., Palai, S., Deb, P. K., & Devi, R. (2022). Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism(s) of action. *Frontiers in Pharmacology*, 13, Article 806470. <https://doi.org/10.3389/fphar.2022.806470>
- Scarano, A., Laddomada, B., Blando, F., De Santis, S., Verna, G., Chieppa, M., & Santino, A. (2023). The chelating ability of plant polyphenols can affect iron homeostasis and gut microbiota. *Antioxidants*, 12, 630. <https://doi.org/10.3390/antiox12030630>
- Serpen, A., Gökmen, V., Pellegrini, N., & Fogliano, V. (2008). Direct measurement of the total antioxidant capacity of cereal products. *Journal of Cereal Science*, 48, 816–820. <https://doi.org/10.1016/j.jcs.2008.06.002>
- Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects—A review. *Journal of Functional Foods*, 18, 820–897. <https://doi.org/10.1016/j.jff.2015.06.018>
- Sharma, S. R. V., Khandagale, S., & Shukla, V. (2014). Comprehensive study of lentil (*Lens culinaris* Medik.) seed and their nutritional profile – A review. In *Proceedings of national conference on harmony with nature in context of environmental issues and challenges of the 21st century* (pp. 8–13). Udaipur, Rajasthan, November 28–30, 2014.

- Swamy, K. R. M. (2023). Origin, domestication, taxonomy, botanical description, genetics and cy togenetics, genetc diversity and breeding of lentil (*Lens culinaris* Medik.). *International Journal of Current Research*, 15(6), 25122–25147. <https://doi.org/10.24941/ijcr.45571.06.202>
- Szymanska, E., Saccenti, E., Smilde, A. K., & Westerhuis, J. A. (2012). Double-check: Validation of diagnostic statistics for PLS-DA models in metabolomics studies. *Metabolomics*, 8, 3–16. <https://doi.org/10.1007/s11306-011-0330-3>
- Trygg, J., & Wold, S. (2002). Orthogonal projections to latent structures (O-PLS). *Journal of Chemometrics*, 16(3), 119–128. <https://doi.org/10.1002/cem.695>
- Ujainwal, M., Singh, N., Langyan, S., & Singh, N. K. (2025). Genetic dissection of total protein content, phenolic content and seed quality traits in pigeonpea (*Cajanus cajan*) using 62K pigeonpea genic SNP chip. *Molecular Genetics and Genomics*, 300, 44. <https://doi.org/10.1007/s00438-025-02235-9>
- Viscusi, G., Lamberti, E., Gerardi, C., Giovinazzo, G., & Gorrasi, G. (2022). Encapsulation of grape (*Vitis vinifera* L.) pomace polyphenols in soybean extract-based hydrogel beads as carriers of polyphenols and pH-monitoring devices. *Gels*, 8, 734. <https://doi.org/10.3390/gels8110734>
- Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. New York: Springer-Verlag. ISBN 978-3-319-24277-4 <https://ggplot2.tidyverse.org>.
- Xia, M., Li, M., Serrano Pinheiro de Souza, T., Barrow, C., Dunshea, F. R., & Suleria, H. A. R. (2023). LC-ESI-QTOF-MS2 characterization of phenolic compounds in different lentil (*Lens culinaris* M.) samples and their antioxidant capacity. *Frontiers in Bioscience (Landmark Ed)*, 28(3), 44. <https://doi.org/10.31083/j.fbl2803044>
- Xu, B., & Chang, K.-C. (2010). Phenolic substance characterization and chemical and cell-based antioxidant activities of 11 lentils grown in the Northern United States. *Journal of Agricultural and Food Chemistry*, 58, 1509–1517. <https://doi.org/10.1021/jf903532y>