Journal of Agriculture and Food Research 25 (2026) 102555

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Comparative analysis of agronomic performance and bioactive compound profiles of the Sicilian landrace “ciurietto” (*Brassica oleracea* var. *botrytis* x *italica*)

Maria Concetta Di Bella [a,1], Hajer Ben Ammar [b,1], Sergio Argento [a,*], Sonia Bonacci [c], Maria Grazia Melilli [a,**], Ferdinando Branca [b]

[a] *National Research Council of Italy, Institute of Biomolecular Chemistry (CNR-ICB), Via P. Gaifami, 18, 95126, Catania, Italy*
[b] *Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Valdisavoia 5, 95123, Catania, Italy*
[c] *Department of Health Sciences, University Magna Græcia of Catanzaro, Germaneto, Catanzaro, Italy*

ARTICLE INFO

Keywords:
Brassica oleracea
Sicilian landraces
Cultivar comparison
Glucosinolates
TPC

ABSTRACT

The exploration of nutritional properties in landrace products, coupled with a thorough understanding of their cultivation environments, is paramount for developing effective and sustainable on-farm strategies for landrace conservation. *Brassica oleracea* L. comprises economically significant crops rich in bioactive compounds, including glucosinolates (GLSs), polyphenols, anthocyanins, and vitamins, contributing to their nutritional and health-promoting properties. This study, conducted within the BRESOV project (H2020), evaluated some landraces of the “Ciurietto” *Culton* in comparison with Marathon F1 of broccoli and the cultivar Snowball of cauliflower under organic cultivation in the southeastern of Sicily. Morpho-biometric traits and chemical composition were assessed, including GLSs (analyzed by HPLC), total phenolic content (TPC), determined using the Folin–Ciocalteu method), total soluble solids (TSS), and soluble solids (°Brix). Significant correlations were observed between morphological and biochemical traits. SPAD index were negatively correlated with harvest time ($r = -0.90$, $p < 0.001$), indicating a decline in chlorophyll content increasing the temperature for the late cultivars and landraces. Similarly, TPC was negatively correlated with plant height ($r = -0.82$, $p < 0.001$), showing the reduction of the phenolic by the increasing of the plant vegetative phase. Among the GLSs detected, neoglucobrassicin and 4-methoxyglucobrassicin exhibited a strong positive correlation between them ($r = 0.65$, $p = 0.0014$), while the total GLSs content was negatively correlated with 4-methoxyglucobrassicin ($r = -0.58$, $p = 0.0058$). The variation in GLSs content across accessions highlights differential metabolic regulation influenced by genetic and environmental factors. Notably, BN and BF accessions displayed high GLS levels, emphasizing their potential for health-oriented and agronomic applications. Ciurietto landraces exhibited high TSS levels, underscoring their value as genetic resources for broccoli breeding, particularly in organic and sustainable farming systems.

1. Introduction

The *Brassica oleracea* species encompasses a diverse group of economically and nutritionally significant crops, including cauliflower (*B. oleracea* var. *botrytis*) and broccoli (*B. oleracea* var. *italica*). These vegetables are widely cultivated for their edible inflorescences, rich in essential nutrients and bioactive compounds such as glucosinolates, polyphenols, and flavonoids [1]. These compounds are known for their antioxidants, anti-inflammatory, and potential anticancer properties, making them highly desirable in the growing market for functional foods [1,2]. Among these bioactive compounds, glucosinolates are sulfur-containing secondary metabolites that, upon hydrolysis, produce bioactive derivatives such as isothiocyanates and indoles, which have been extensively studied for their health-promoting effects [3,4]. Similarly, polyphenols, including flavonoids and phenolic acids, contribute to the antioxidant capacity of these vegetables and play a key role in

---

[*] Corresponding author. Via P. Gaifami, 18, 95126, Catania, Italy.
[**] Corresponding author.
E-mail addresses: sergio.argento@cnr.it (S. Argento), mariagrazia.melilli@cnr.it (M.G. Melilli).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jafr.2025.102555
Received 28 March 2025; Received in revised form 14 November 2025; Accepted 2 December 2025
Available online 3 December 2025
2666-1543/© 2025 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

reducing oxidative stress and inflammation [5]. As consumer demand for health-promoting foods continues to rise, there is an increasing need to identify cultivars with superior agronomic performance and enhanced nutritional profiles [6,7].

The relationship between cauliflower and broccoli has been extensively studied through bio-morphological [8,9], biochemical [10], and molecular analyses [11–13]. Cauliflower and broccoli are distinguished from other cultivars by the unique morphological transformation of their reproductive structures, which constitute the harvested produce [14]. Cauliflower is recognized for having a more diverse genetic background than broccoli, as indicated by its reproductive characteristics [15]. Genetic studies based on DNA polymorphism suggest that cauliflower likely originated from broccoli, with further genetic contributions from wild *Brassica species* via introgression [16]. This hypothesis is reinforced by morphological similarities between broccoli and various wild *Brassica species*, which are notably more pronounced than those observed between broccoli and cauliflower [17]. Both crops exhibit hypertrophic development of floral branches, though at different stages of growth. In cauliflower, this hypertrophy initiates early in development, prior to the elongation of flower stems, resulting in primordial reproductive buds becoming sterile [18]. These floral branches serve as primary sink organs for assimilates, leading to atypical proliferation and influencing the curvature of the curd. The transformation of floral meristems into inflorescence structures is regulated by a homeotic gene in a homozygous state, which disrupts normal reproductive development [19]. In contrast, broccoli undergoes hypertrophy at a more advanced floral development stage, allowing for the harvest of well-formed, fertile floral buds and enabling the plant to achieve a more advanced reproductive maturity [20].

Landraces are genetic resources that have been traditionally selected by farmers over generations, resulting in crops highly adapted to the specific environmental conditions where they have been cultivated for centuries. In southern Italy, the local varieties remain widespread and exhibit significant diversity [21]. The Sicilian landrace 'Ciurietto' exemplifies the rich genetic diversity within *Brassica oleracea*, underscoring the importance of landraces in this field of research. 'Ciurietto' (*Brassica oleracea* var. *Botrytis* x *italica*) has garnered attention for its unique combination of traits likely derived from its hybrid origins between broccoli and cauliflower. Traditionally cultivated in southeastern Sicily, 'Ciurietto' holds a significant place in local culinary culture, celebrated for its distinctive flavor and texture. Its hybrid nature allows 'Ciurietto' to exhibit desirable attributes from both parent varieties of *B. oleracea,* making it a compelling subject for evaluating agronomic performance and nutraceutical potential [22]. Exploring the nutritional properties of landrace products is crucial for developing effective and sustainable on-farm conservation strategies. A thorough understanding of the cultivation environments of these traditional varieties not only highlights their unique nutritional benefits but also emphasizes their vital role in enhancing dietary diversity and food security.

Despite the importance of landraces, there is a limited understanding of the agronomic performance and bioactive compound profiles of the 'Ciurietto' in comparison to more commonly cultivated varieties. Previous studies have explored the general agronomic traits of Brassica crops but have often overlooked the specific contributions of regional landraces [23,24]. This gap in knowledge highlights an opportunity to investigate the unique attributes of these traditional varieties, especially as consumer interest in health-promoting foods continues to rise.

Given this context, the cultivation of *Brassica oleracea* varieties, particularly traditional landraces like 'Ciurietto,' presents significant agronomic potential. These landraces will be compared with the hybrid F1 broccoli 'Marathon' and the cauliflower cultivar 'Snowball.' With their unique genetic diversity and biochemical profiles, these traditional varieties can be leveraged to improve crop resilience and enhance nutritional quality. This study aims to provide a comparative analysis of the agronomic performance and bioactive compound profiles of the Sicilian landrace 'Ciurietto' in relation to these commonly cultivated Brassica varieties. By focusing on key agronomic traits, including growth parameters yield, and plant morphology, alongside an in-depth analysis of bioactive compounds, this research seeks to contribute valuable insights into the integration of traditional landraces into modern agricultural systems, promoting both sustainability and public health.

2. Materials and methods

2.1. Plant material and experimental design

This study evaluated seven accessions of *Brassica oleracea* and its related complex species (n = 9) as outlined in Table 1.

The plant material of Ciurietti originates from seeds selections carried out by seed guardian in the province of Ragusa, (Sicily, South Italy), where the crop is widely cultivated and highly valued by local communities. The cvs Marathon and Snowball are F1 hybrids with stable agronomic and biochemical traits, making them suitable for comparative analysis of agronomic and technological responses to local agroecosystems, and used as Control in the study.

The seeds for each accession were sown in cellular trays filled with Brill soil (Geotec, Italy) and subsequently placed in a cold greenhouse located in Catania, Italy (37°31′01.00″N, 15°04′18.00″E), where they were exposed to natural light conditions. The temperature within the greenhouse was maintained at an average of 15.4 ± 5.8 °C throughout the growing period, providing optimal conditions for seed germination and early plant development (Fig. 1). Standard irrigation practices were applied consistently throughout the study, ensuring optimal moisture levels for plant growth.

At the four-leaf growth stage (October 2022), the plantlets were carefully transplanted into a certified organic greenhouse located in Santa Croce Camerina, Ragusa, Italy (36°51′13.300″N, 14°29′32.000″E). The plants were arranged in single rows, with a row spacing of 1.0 m and a plant spacing of 0.5 m along the rows, resulting in a crop density of 2 plants per square meter. The experimental design involved ten plants, replicated three times for each cultivar.

During the growth period, we implemented a fertilization regimen tailored to the needs of each cultivar. Regular observations of plant growth were conducted, noting key parameters such as height and leaf number at weekly intervals.

Harvest times varied for each accession based on the ripeness of the curds, and these were recorded meticulously. The common names of the plants were associated with the month of harvest. After harvesting, samples were collected, cleaned, frozen at −80 °C, and lyophilized at −50 °C for 72 h. The samples were then ground into a powder using a grinder for further analysis.

2.2. Morphometric trait

Morphological descriptors from the International Board for Plant Genetic Resources (IBPGR) and the International Union for the Protection of New Varieties of Plants (UPOV) were utilized to characterize the plants and their curds. The morphometric parameters assessed included plant height (cm), fresh and dry weight (g), stem diameter (cm), number of leaves (n), fresh biomass of leaves (g), and percentage of dry matter. These parameters provide a comprehensive evaluation of the plant's growth characteristics and overall productivity.

2.3. SPAD

The nutritional status of the plants was assessed using the Single Photon Avalanche Diode (SPAD) index. Chlorophyll measurements were taken from three fully developed leaves of each replicate weekly to ensure comprehensive monitoring throughout the growth period. Measurements were conducted using a portable chlorophyll meter (SPAD-502, Minolta Camera Co., Osaka, Japan) in the morning to minimize variability caused by environmental factors.

Table 1
List of *Brassica oleracea* accessions used in the study.

UNICT Code	Working Code	Name	Common Name		Harvest Period	Harvest Time (Julian Days)
UNICT 5082 B<superscript></superscript> R<superscript></superscript> 361	BN	*B. oleracea* var. *Botrytis* x *italica*	Natalino		December	356 ± 12
UNICT 5085 B<superscript></superscript> R<superscript></superscript> 363	BI	*B. oleracea* var. *Botrytis* x *italica*	Iannarino		January	23 ± 6
UNICT 5083 BR362	BF	*B. oleracea* var. *Botrytis* x *italica*	Friarolo		February	52 ± 18
UNICT 5080 B<superscript></superscript> R<superscript></superscript> 359	BM	*B. oleracea* var. *Botrytis* x *italica*	Mazzarolo		March	96 ± 14
UNICT 5088 BR365	BA	*B. oleracea* var. *Botrytis* x *italica*	Maiolino		May	143 ± 25
UNICT 4322 BR165	MA	*Brassica oleracea var. Italica*	Marathon F1		January	25 ± 4
UNICT 4937 CV213	SN	*Brassica oleracea var. Botrytis*	Snowball F1		February	60 ± 5

2.4. *Common quality parameters*

The dry matter (DM) content was quantified as a percentage based on the weight loss observed after lyophilization of a specified amount of fresh plant material. To measure the soluble solid residue (SSR), an aqueous extract was prepared by adding 2 g of lyophilized curd powder to 20 mL of distilled water. This procedure was performed in accordance with the method outlined by Lo Scalzo et al. [25]. The SSR was expressed in degrees Brix (°Brix) relative to the fresh weight (FW) of the plant material.

2.5. *TPC quantification*

The total phenolic content (TPC) was quantified using the Folin–Ciocalteu colorimetric method. In this procedure, an aliquot of the sample was combined with Folin–Ciocalteu reagent to initiate the reaction. Following this, sodium carbonate solution was added to the mixture, which was then allowed to incubate for a specified duration to ensure complete reaction. The absorbance of the reaction mixture was measured in triplicate at a wavelength of 760 nm using a spectrophotometer to enhance the accuracy of the results.

Results were expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g DE). A calibration curve was generated using gallic acid standards in the concentration range of 10–200 mg/mL, with a correlation coefficient (R^2) ranging from 0.989 to 0.998, demonstrating a strong linear relationship and confirming the reliability of the method.

2.6. *Glucosinolates extraction and analysis*

Extraction of GLS and enzymatic desulfation of GLS: the extraction method was based on the International Standard Method ISO 9167–1 (ISO 9167–1, 1992) [26] and European Commission (European Comission, 1990), with some modifications (Aires and Carvalho, 2017) [27].

Determination of GLS by HPLC: the extracts with desulphoglucosinolates were injected in an HPLC-DADUV/Vis equipped by a C18 (250 × 4.60 mm, 5 μm) column with a mobile phase of ultrapure water (solvent A) and 20 mL^{-1} acetonitrile: water (v/v) (solvent B), with a flow rate of 1.5 mL min^{-1} and an injection volume of 10 μL, with binary gradients according to Aires and Carvalho (2017). The GSs peak

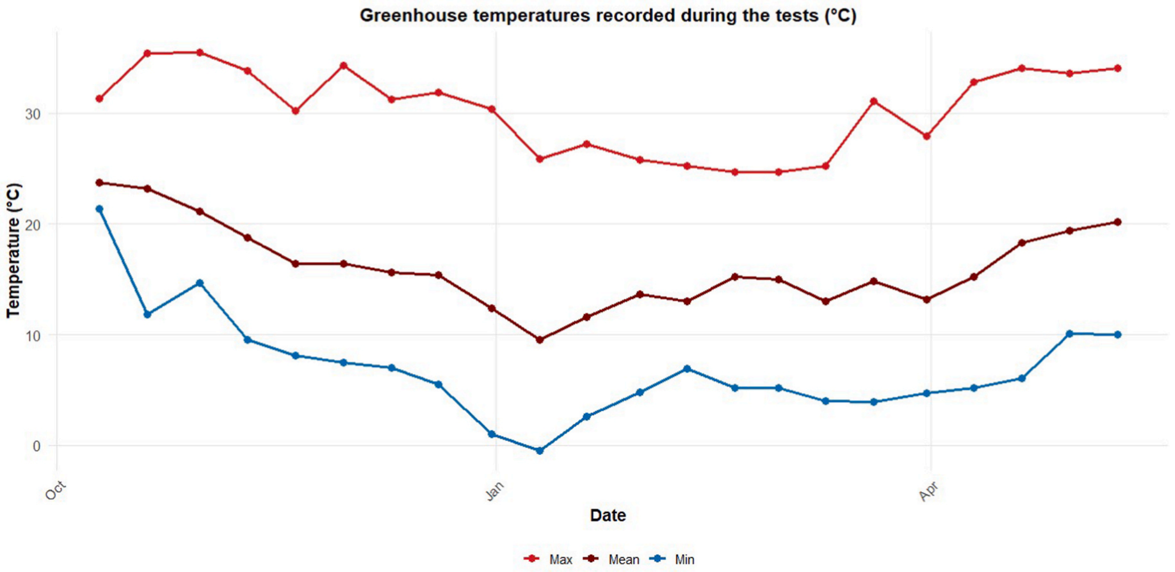

Fig. 1. Greenhouse temperature recorded during the experiment.

identification and quantitative estimations were made using pure standard GSs as internal standard (IS) (benzyl GL at 1 mg mL^{-1}). The chromatograms were recorded at 229 nm and used to identify GSs by retention time (RT) and UV spectra in comparison with commercial standards. All reagents used in analytical determinations were HPLC grade.

GLS Quantification. The quantification of GLS was based on the internal standard method, according to the fundamentals of HPLC and the guidelines of the International Conference on Harmonization (ICH) (IHC, 2005). Each GL content was quantified as μmoles·100 g^{-1} dry weight (dw):

$$C\text{GLS sample} = (C\text{is} \times \text{HPLC}_{\text{Area GLS sample}} \times \text{Rf} \times \text{Df})/\ (\text{HPLC}_{\text{area is}} \times \text{dw}) \times 100$$

Where *C*GLS sample is the concentration of each GL in the sample. *C*is is the concentration in μmoles·mL^{-1} of internal standards added to sample and injected in the HPLC, Rf is the response factor of each GLS, and Df is the dilution factor.

The Rf factors, for each GLS, detected at 229 nm, published in ISO 9617-1 standards were considered (ISO 9167–1, 1992).

2.7. Statistical analysis

The statistical analysis was performed using R software (version 4.4.2) (R Core Team, 2025). Data were initially checked for normality and homogeneity of variances using the Shapiro-Wilk test and Levene's test, respectively. Analysis of variance (ANOVA) was used to assess the differences between accessions for each trait, and Tukey's Honest Significant Difference (HSD) test was applied for multiple comparisons to identify which means were significantly different.

Principal Component Analysis (PCA) was conducted to explore the patterns of variation in the data and to examine the relationships between different accessions and traits. PCA was performed using the prcomp function from the stats package, and variable loadings were analyzed to evaluate each variable's contribution to the principal components Pearson's correlation analysis was performed to determine the relationships between various traits using the cor function in R. A correlation matrix was visualized using a heatmap generated with the ggplot2 package. Correlation coefficients were considered significant at $p < 0.05$.

For hierarchical clustering analysis, a distance matrix was first computed using the Euclidean method, followed by clustering using the hclust function in R. The dendrogram was plotted using the ggplot2 and ggdendro packages to visualize the hierarchical relationships between the samples.

Boxplots were created to compare the distribution of bioactive compounds, including total phenolic content (TPC), total glucosinolates (GLS), and other relevant traits, with the ggplot2 package. Outliers were identified using the standard method based on the interquartile range (IQR).

3. Results

3.1. Agronomic parameters analysis

3.1.1. Growth parameters

Table 2 presents the range of agronomic traits observed across the different accessions, revealing considerable variability in key growth and productivity parameters. Total weight exhibited a striking difference, ranging from 595.5 g in MA to 2976.74 g in SN, highlighting the substantial disparity in biomass accumulation. Total plant height varied between 19 cm in SNand 125 cm in BF, reflecting marked differences in plant stature and growth patterns. Similarly, stem weight varied from 20 g in U to 68 g in BM, and stem diameter ranged from 13.67 mm in BF to 46.71 mm in SN, illustrating the diversity in stem development across the accessions.

The number of leaves and leaf weight also displayed notable variability, with leaf count ranging from 12 leaves in MA to 35 leaves in SN, and leaf weight ranging from 397.3 g in BM to 1350.4 g in BI. These data

Table 2
Summary of minimum and maximum values for agronomic traits, with corresponding F-values and P-values from ANOVA analysis.

Variable (Units)	Min	Max	F_value	P_value
Total_weight (g)	595.50	2976.74	21.13	2.89e-06***
Total_height (cm)	19.00	125.00	42.7	3.29e-08***
Stem_weight (g)	20.00	68.00	18.26	7.01e-06***
Min_diameter (cm)	13.67	46.71	32.51	1.93e-07***
Max_diameter (cm)	21.00	56.87	4.642	8.46e-03**
Nbr_leaves (leaves)	12.00	35.00	10	2.17e-04***
Leaves_weight (g)	397.30	1350.40	3.523	2.45e-02*
Stem_weight2 (g)	78.00	368.10	11.26	1.14e-04***
Root_weight (g)	28.10	193.40	3.385	2.82e-02*
Fruit_weight (g)	215.19	718.80	20.41	3.57e-06***
Weight_dry_fruit (g)	17.82	34.76	6.672	1.69e-03**

suggest significant differences in leaf production and growth potential, which are essential factors in overall plant performance. Regarding fruit traits, fruit weight ranged from 215.19 g in BU to 718.8 g in BA, and dry fruit weight showed a similar range, from 17.82 g in BN to 34.76 g in SN, indicating variability in reproductive output across the cultivars.

Statistical analysis using ANOVA revealed significant differences for all agronomic traits, with F-values ranging from 3.38 for leaf number to 42.7 for total height. The corresponding *p*-values (all $p < 0.05$) strongly support the presence of meaningful differences among the accessions, underscoring the diverse performance of these cultivars. These findings provide valuable insights into the agronomic variability of the accessions, suggesting the potential for selective breeding and cultivation strategies aimed at enhancing desirable traits for specific agronomic goals.

3.1.2. *Chlorophyll content analysis by SPAD*

The analysis of SPAD values reveals significant variation in chlorophyll content across the different accessions (Fig. 2). BN (mean = 66.82, SE = 1.87) and BA (mean = 69.45, SE = 0.98) exhibit the highest SPAD values, suggesting superior chlorophyll content and potentially greater overall plant health compared to other accessions. Conversely, BF (mean = 45.05, SE = 1.83), BM (mean = 46.40, SE = 1.27), and SN (mean = 62.10, SE = 1.47) demonstrate lower SPAD values, indicating reduced chlorophyll content and potentially compromised plant health. The significant differences observed among the accessions emphasize the potential role of these traits in selecting plants with optimal chlorophyll content, which may contribute to improved photosynthetic efficiency and overall plant performance in various environmental conditions.

3.1.3. *Soluble solids analysis % SS*

The results reveal significant differences in the percentage of soluble solids (%SS) across plant parts, with roots and leaves showing the most pronounced variations (Table 3; Fig. 3). The roots have the highest %SS (mean = 40.71 %, p = 4.34e-06), likely due to their role in nutrient storage, while leaves also exhibit high %SS (mean = 22.98 %, p = 4.85e-06), reflecting their involvement in photosynthesis and nutrient transport. The steam and fruit show lower %SS (means = 17.89 % and 18.79 %, respectively), with statistically significant differences ($p < 0.01$) but smaller effect sizes. These findings highlight the strong link between plant part function and soluble solid content, emphasizing the importance of part-specific analysis in plant physiology studies.

Table 3
Variation in Soluble Solids Content Across Different Plant Organs in the studied cultivars.

Parameter	Min	Max	Mean	F Value	P Value
%SS Fruit	0.00	26.87	18.79	6.559	0.00183***
%SS Steam	6.198	27.671	17.89	8.296	0.000578***
%SS Leaves	16.77	29.75	22.98	19.4	4.85e-06***
%SS Roots	25.89	53.45	40.71	19.76	4.34e-06***

3.2. *Biochemical analysis*

3.2.1. *Total phenolic analysis*

The total phenolic content (TPC) of different accessions was determined and presented in Fig. 4. A significant variation in TPC was observed among the accessions ($p < 0.05$). The highest TPC was observed in the cauliflower SN (7.5 mg GAE/100 g DW), which was significantly different from all other accessions. This was followed by MA, which exhibited a mean TPC of 6.0 mg GAE/100 g DW. These two accessions displayed considerably higher phenolic contents, indicating their potential as rich sources of bioactive compounds.

In contrast, the lowest TPC was recorded in accession BA (2.0 mg GAE/100 g DW), significantly lower than all other accessions. The intermediate accessions (BN, BI, BF, and BM) exhibited moderate TPC values ranging between 3.0 and 4.5 mg GAE/100 g DW and suggesting no statistically significant differences among some of them.

3.2.2. *Glucosinolates analysis*

The analysis of glucosinolate content among various accessions demonstrates substantial variability in both mean levels and consistency as presented in Fig. 5. The BI accession exhibits a low mean glucosinolate content of 98.42 μmol/100g DW, accompanied by minimal variability (SD = 0.29), suggesting a high degree of consistency across replicates. In contrast, BF displays a significantly higher mean glucosinolate content of 471.47 μmol/100g DW, but with considerable variability (SD = 95.56), indicating pronounced differences among its replicates. The BM accession shows a moderate mean of 298.32 μmol/100g DW and a standard deviation of 22.13, reflecting some variability in glucosinolate levels. Conversely, BA has the lowest mean glucosinolate content at 50.42 μmol/100g DW and a standard deviation of 3.08, signifying relative consistency. Meanwhile, BN stands out with the highest mean glucosinolate content of 863.62 μmol/100g DW, alongside a standard deviation of 34.03, revealing some variability within its

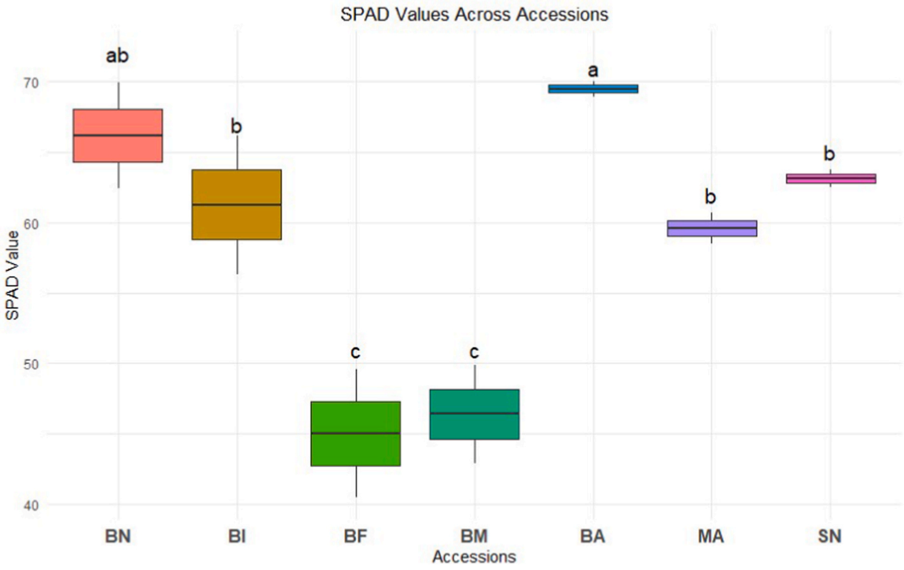

Fig. 2. Boxplot representing the variation in SPAD values across different accessions.

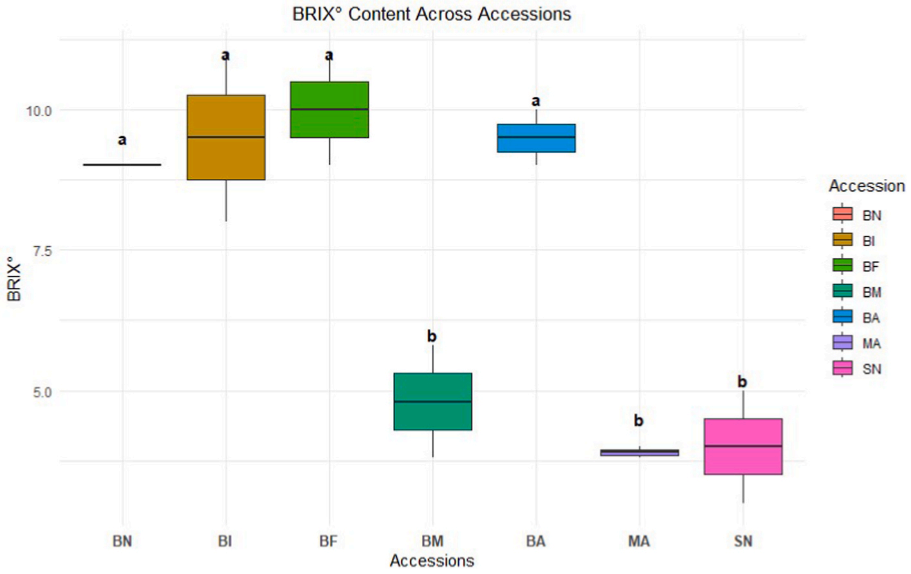

Fig. 3. *Boxplot showing the variation in BRIX levels across different accessions, the letters above the boxes indicate statistical groups, with accessions labeled the same being statistically similar.*

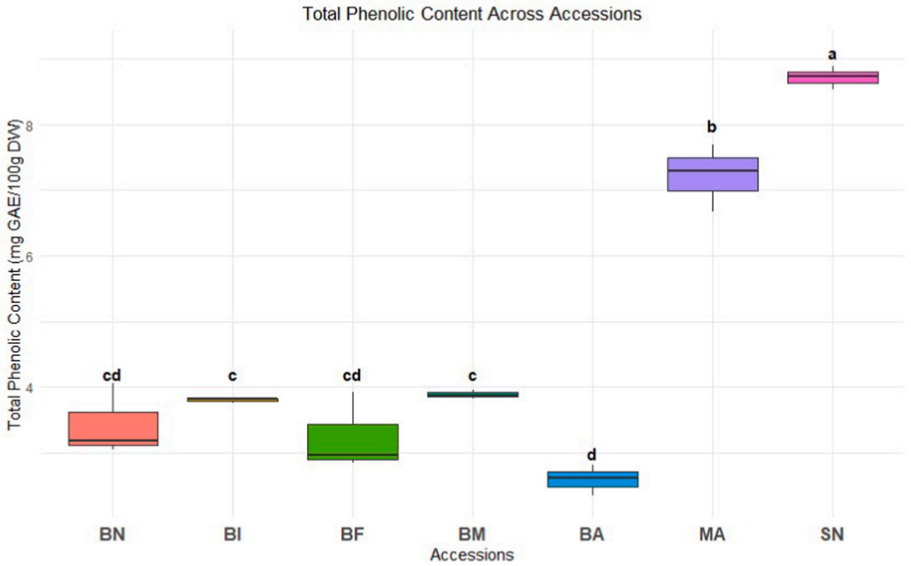

Fig. 4. Boxplot comparing the total phenolic content (TPC) (mg GAE/g DW) across different accessions, the letters above the boxes indicate statistical groups, with accessions labeled the same being statistically similar.

replicates. The MA accession presents a mean of 429.19 μmol/100g DW with a low standard deviation of 0.33, indicating stable glucosinolate levels. Lastly, Snowball SN exhibits a mean glucosinolate content of 289.64 μmol/100g DW and a standard deviation of 25.74, denoting moderate variability.

The glucosinolate profile among the studied accessions reveals considerable variability in both composition and total content. Among the aliphatic glucosinolates, glucoraphanin is the predominant compound, particularly in the BF and BN accessions, where it accounts for 69.55 % and 43.89 % of the total glucosinolate content, respectively. Similarly, progoitrin is notably abundant in the BI and BF accessions, whereas glucoiberin is present in significant amounts only in the BN (28.97 %) and MA (40.95 %) accessions. In contrast, the indolic glucosinolates—including glucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin—exhibit a more balanced distribution across accessions. However, BM (64.97 %) and SN (62.98 %) accessions show markedly higher total indolic glucosinolate levels. These variations highlight the distinct glucosinolate composition among accessions, as illustrated in Fig. 6.

3.2.3. Pearson s correlation between the studied traits

Significant correlations were observed between various morphological and biochemical traits, reflecting intricate relationships that shape plant development (Fig. 7). For example, SPAD readings exhibited a strong negative correlation with harvest time ($r = -0.90$, $p < 0.001$), suggesting that longer harvest times are associated with decreased chlorophyll content. Additionally, a negative correlation between stem weight and total weight ($r = -0.70$, $p < 0.001$) indicates that as stem weight increases, the overall plant weight decreases, possibly due to resource allocation favoring stem growth at the expense of other parts. The total height of the plant was negatively correlated with both root weight ($r = -0.59$, $p < 0.005$) and fruit weight ($r = -0.77$, $p < 0.001$),

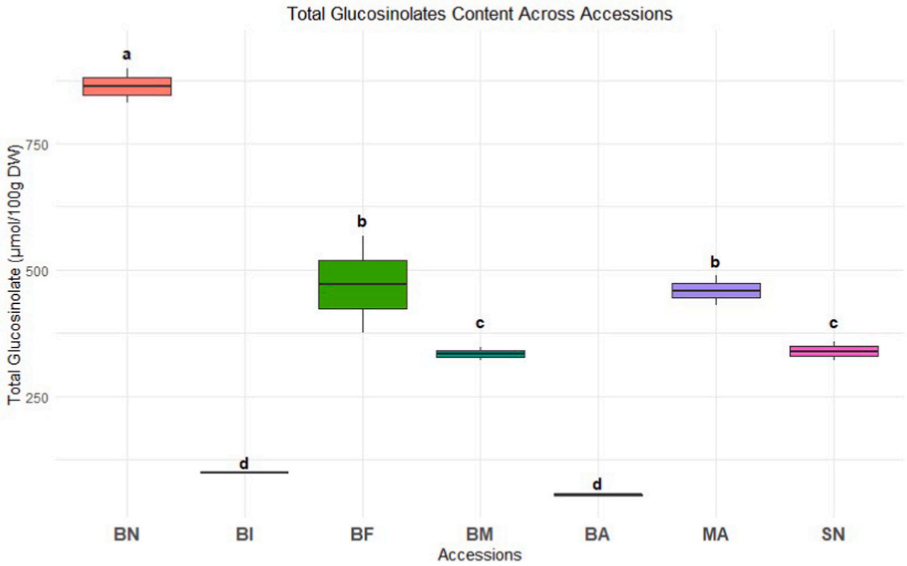

Fig. 5. Comparison of total glucosinolate content (µmol/100g DW) across different studied accessions reflecting genetic differences. The letters above the boxes indicate statistical groups, with accessions labeled the same being statistically similar.

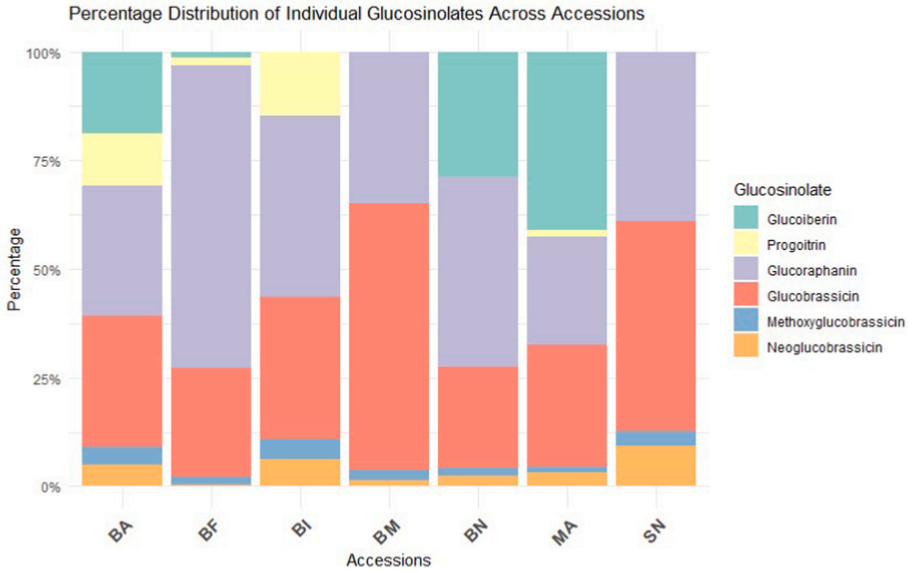

Fig. 6. Percentage distribution of individual glucosinolates in *Brassica oleracea* accessions. The chart illustrates the proportion of each glucosinolate in the total glucosinolate profile, expressed as a percentage.

implying that taller plants tend to have reduced root and fruit biomass. Furthermore, total phenolic content (TPC) showed a significant negative correlation with plant height ($r = -0.82$, $p < 0.001$), underscoring the complex interplay between growth parameters and biochemical composition. Other notable correlations included a positive relationship between fruit weight and dry weight of stems ($r = 0.78$, $p < 0.001$), indicating that larger fruit weight is associated with increased stem biomass.

In terms of glucosinolate compounds, several significant correlations were also identified. A moderate positive correlation ($r = 0.4725$, $p = 0.0305$) between progoitrin and 4-methoxyglucobrassicin suggests that higher progoitrin levels correspond to increased concentrations of 4-methoxyglucobrassicin. A strong positive correlation ($r = 0.6513$, $p = 0.0014$) was found between neogluco-brassicin and 4-methoxyglucobrassicin, indicating that elevated neogluco-brassicin levels are linked with higher 4-methoxyglucobrassicin concentrations. Conversely, a moderate negative correlation ($r = -0.5808$, $p = 0.0058$) between total glucosinolate content and 4-methoxyglucobrassicin suggests that as the total glucosinolate content increases, the concentration of 4-methoxyglucobrassicin decreases. These correlations emphasize the interconnected nature of glucosinolate compounds, with potential implications for the overall glucosinolate profile in the plants.

3.2.4. Principle component analysis PCA

This Principal Component Analysis (PCA) biplot illustrates the relationships between accessions and various phenotypic and biochemical traits. The two principal components, Dim1 (34.8 %) and Dim2 (22 %), together explain 56.8 % of the total variation (Fig. 8).

The cultivar MA is the most distinct accession, strongly associated with root weight, minimum diameter, %SS fruit, TPC, and glucoiberin, while SN also shows differentiation, aligning with root-related traits. In contrast, BM and BN cluster near the center, indicating phenotypic

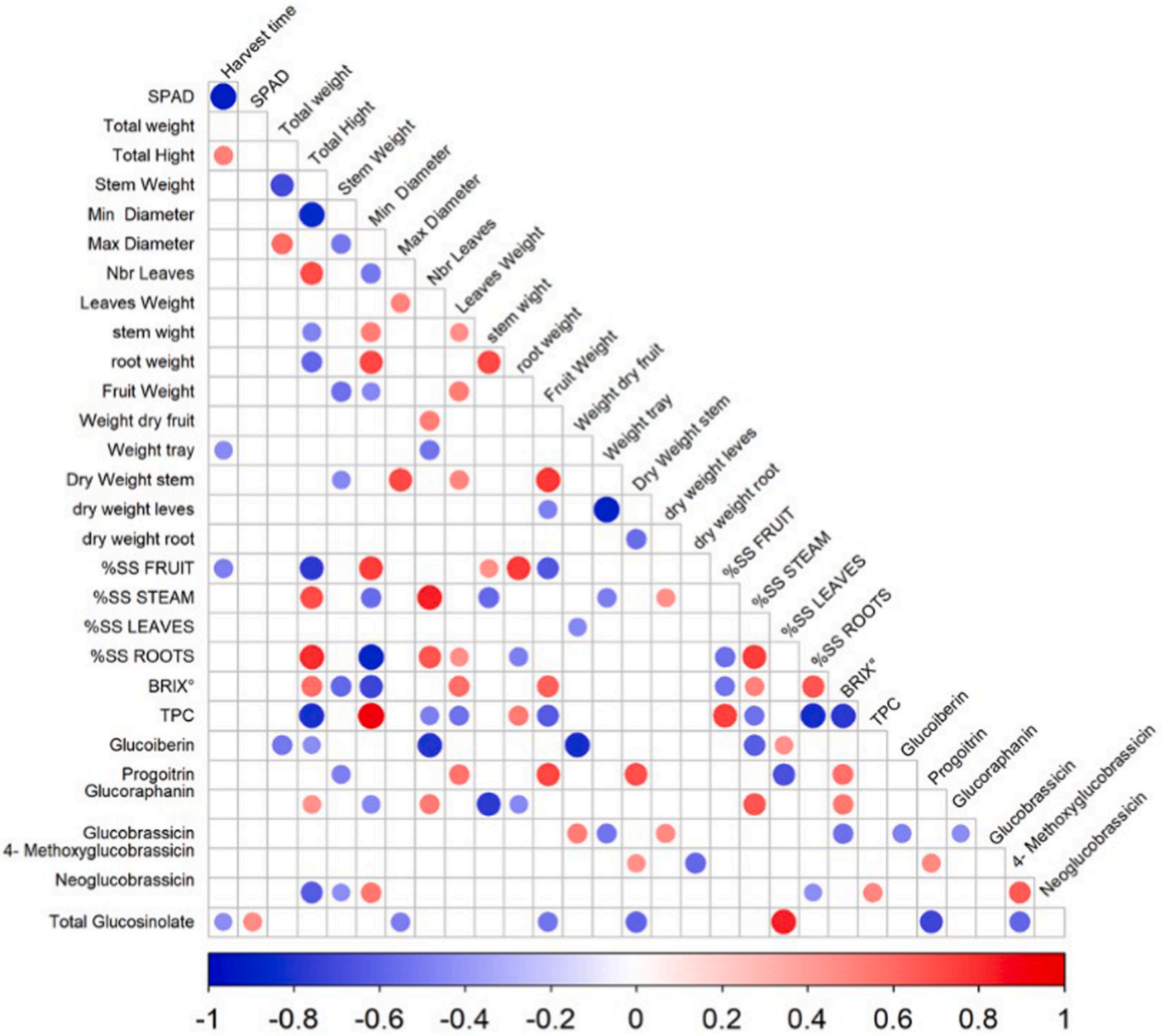

Fig. 7. Pearson's correlation matrix illustrating the relationships between the studied agronomic and bioactive traits across the *Brassica oleracea* cultivars. The correlation coefficients are represented by color gradients, with positive correlations shown in red, negative correlations in blue.

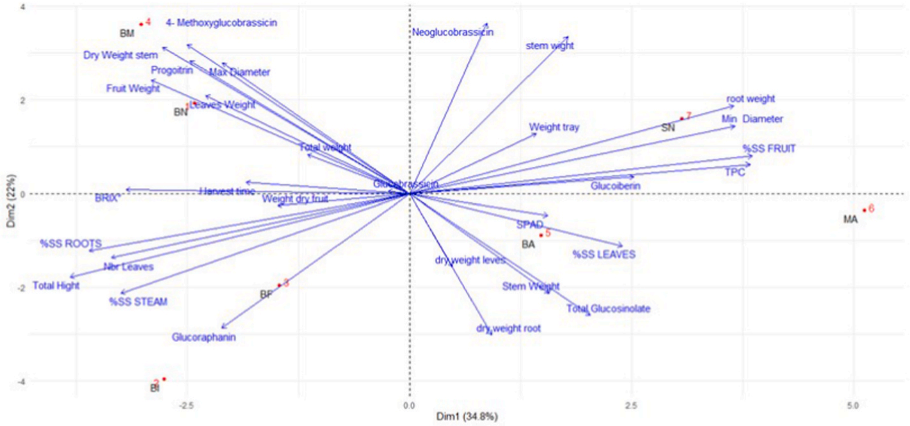

Fig. 8. Principal Component Analysis (PCA) of studied accessions based on agronomic and biochemical traits. The variance explained by each principal component is indicated, highlighting the relationships between accessions and their respective trait profiles.

similarity and moderate contributions from traits like dry weight of stem, fruit weight, and 4-Methoxyglucobrassicin. BI and BF, positioned negatively along Dim1 and Dim2, share characteristics related to glucoraphanin, %SS steam, and total height, whereas BA exhibits intermediate traits associated with SPAD, dry weight leaves, and total glucosinolate content. Traits such as stem weight and weight tray are positively correlated, while glucoraphanin and total height show an inverse relationship with root weight and %SS fruit. The longer vectors, particularly for root weight, %SS fruit, and total glucosinolate, indicate their strong influence in distinguishing accessions. This analysis highlights significant phenotypic and biochemical differences, particularly in MA and SN, while suggesting overall homogeneity among BN, BM, BI, and BF, which can be crucial for breeding strategies, conservation, or further study.

3.3. Hierarchical clustering of accessions based on Principal Component Analysis

The hierarchical clustering dendrogram presents the relationships among accessions based on dissimilarity distances, revealing two distinct clusters (Fig. 9). The first cluster groups SN, BA, and MA, with BA and MA being more closely related, while SN remains somewhat distinct yet still within the same grouping. The second cluster includes BN, BM, BI, and BF, where BN and BM form a subcluster, indicating strong similarity, and BI and BF form another closely related subcluster. The separation between the two major clusters, with a high dissimilarity value of approximately 9, suggests significant differentiation, potentially reflecting genetic, phenotypic variations among the accessions.

The significant separation of SN and MA from the rest implies that these accessions possess distinct morphological or genetic traits, which may be influenced by genetic divergence, environmental adaptation, or selective breeding. Meanwhile, the clustering of BN, BM, BI, and BF indicates high phenotypic uniformity, suggesting they either share a common genetic background or have been subjected to similar environmental pressures.

4. Discussion

The observed variability in agronomic traits across accessions highlights significant genetic diversity in *Brassica oleracea,* with implications for selective breeding and adaptation to different environments**.**

The evaluation of agronomic performance in *Brassica oleracea* accessions revealed substantial variability in key traits, reflecting differences in growth and productivity potential. These findings align with those of Rakshita et al. [28] in India, which also documented significant variation in cauliflower cultivars under distinct agro-climatic conditions. In our Sicilian trial, total plant weight exhibited a significant range, from 595.5 g to 2976.74 g, highlighting notable differences in biomass accumulation. Similarly, plant height varied between 19 cm and 125 cm, illustrating differences in vegetative growth patterns. These results further support the observations of Branca et al. [29], who identified considerable variation in plant height and biomass among different landraces of *Brassica oleracea*. In contrast, Rakshita et al. [28] reported total plant weights ranging from 475.0 g to 2883.3 g, with maximum plant heights reaching 16.1 cm. These discrepancies suggest that environmental factors such as temperature, photoperiod, and soil conditions play a crucial role in influencing genetic potential for plant height and biomass accumulation. Leaf traits, which are significant contributors to overall plant productivity, also exhibited notable differences. In our study, the number of leaves per plant ranged from 12 to 35, with corresponding leaf weights between 397.3 g and 1350.4 g. Notably, these values were higher compared to those observed in the cultivar grown under Indian conditions. This suggests that Sicilian accessions may have a higher leaf biomass allocation, potentially influenced by genetic factors or the effects of the Mediterranean climate on leaf expansion and retention [30]. The ANOVA conducted on the morphological traits revealed statistically significant differences among the studied accessions. This considerable variation highlights the significance of these traits in differentiating and further characterizing *Brassica oleracea* accessions, offering valuable insights into their phenotypic diversity and genetic variability, as previously demonstrated by El-Esawi et al. [31]. The observed differences in the percentage of soluble solids (%SS) across plant parts underscore the distinct physiological roles that each organ plays in plant development. The roots, exhibiting the highest %SS, serve a crucial function in nutrient storage, which is essential for supporting plant health and growth [32]. This elevated soluble solid content in roots not only indicates their role as energy reservoirs but also highlights their importance in ensuring plant resilience [33]. Leaves also showed a high %SS, with a mean of 22.98 % ($p = 4.85e\text{-}06$), reflecting their significant involvement in photosynthesis and nutrient transport. The high soluble solids content in leaves suggests a robust metabolic activity, as these organs are crucial for synthesizing and distributing carbohydrates produced during photosynthesis. This relationship between %SS and metabolic function reinforces the idea that leaves are not just passive structures but active participants in the plant's physiological processes. In contrast, the lower %SS in stems and fruits points to their different functional priorities. Stems primarily provide structural support and facilitate the transport of

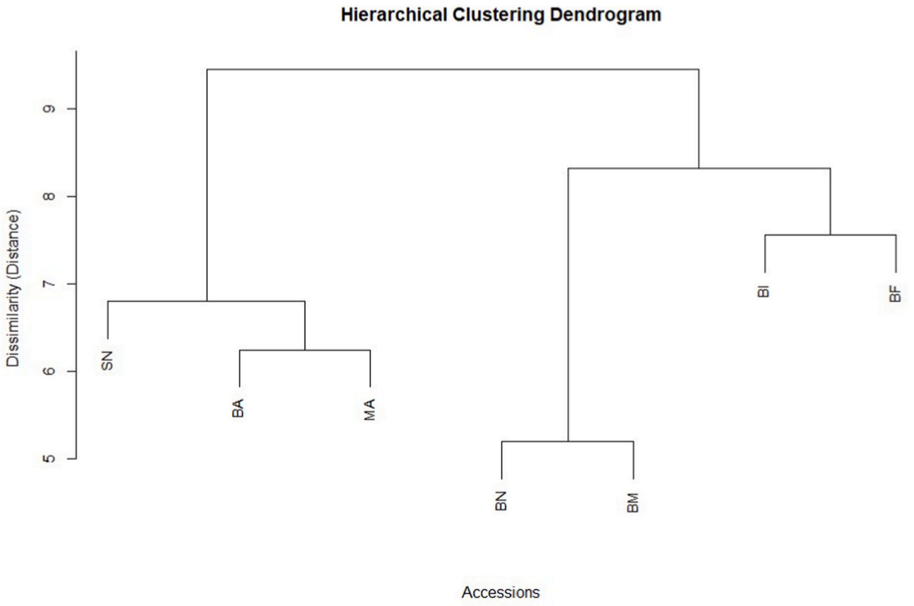

Fig. 9. Hierarchical clustering dendrogram of accessions based on phenotypic and biochemical data, showing the groupings and relationships between different accessions.

nutrients and water, which may limit their capacity for storing soluble solids. Similarly, the lower content in fruits indicates a focus on reproductive success rather than energy storage. This suggests a trade-off in energy allocation, where resources are directed more towards reproduction than accumulating reserves [34]. These findings highlight the significance of conducting part-specific analyses in plant physiological research, as the functions of different organs greatly influence their biochemical profiles. Understanding the interplay between %SS and organ function not only enhances our knowledge of plant biology but also has practical implications for agriculture.

The observed variation in glucosinolate content across different accessions reflects distinct metabolic regulation, likely influenced by genetic diversity and environmental factors [35,36]. This differentiation is particularly significant for breeding and agricultural applications, as glucosinolates play essential roles in plant defense mechanisms, bioactivity, and nutritional value. Glucobrassicin was the predominant glucosinolate in most accessions, particularly in BM, SN, and BI, where it accounted for more than 50 % of the total glucosinolate content. Glucoraphanin was also highly represented, especially in BF, BI, and BN, indicating a potential role in stress response mechanisms. Notably, MA and BN exhibited a markedly high proportion of glucoiberin, distinguishing them from the other accessions. Progoitrin was present in moderate amounts in BF, BI, and BN but was nearly absent in other accessions. Minor glucosinolates, including methoxyglucobrassicin and neoglucobrassicin, were consistently detected in all accessions, albeit at lower levels. The observed variations suggest genetic differences in glucosinolate biosynthesis pathways, which may influence both the adaptive potential of these accessions and their nutritional or phytochemical value. These findings highlight the importance of accession-specific glucosinolate profiling for future breeding programs and functional studies on plant defense mechanisms. In contrast, the significantly higher total glucosinolate content in BN and BF indicates their strong potential for health-related and agronomic uses. Overall, understanding these metabolic differences provides critical insights for optimizing glucosinolate profiles in breeding efforts, aiming for cultivars that enhance agricultural resilience and improve human health [37]. Future research should focus on developing targeted strategies to maximize the health benefits and agronomic potential of these diverse accessions. The significant correlations observed between various agronomic and biochemical traits further highlight the complex interplay between plant growth and biochemical composition. The strong negative correlation between SPAD values and harvest time suggests that accessions with longer growth periods tend to exhibit lower chlorophyll content, potentially indicating a trade-off between growth duration and photosynthetic efficiency. Additionally, the negative correlation between stem weight and total weight suggests that resource allocation may favor stem growth at the expense of other plant parts, such as leaves or fruit. The correlation between total phenolic content (TPC) and plant height further underscores the interconnectedness of morphological and biochemical traits, with taller plants tending to have lower phenolic content. These correlations suggest that plant growth strategies may influence the accumulation of bioactive compounds, which could have implications for the nutritional and medicinal value of the accessions.

The PCA performed on the studied accessions revealed key differences in the agronomic and biochemical traits that underlie their phenotypic diversity. By reducing the complex multivariate data into two principal components (Dim1 and Dim2), the PCA biplot helped identify the most significant trait variations and grouped accessions based on these trait profiles. MA and SN were positioned as the most distinct accessions compared to the rest of the studied accessions, both showing unique trait combinations. MA exhibited characteristics strongly associated with root weight, glucosinolate composition, and phenolic content (TPC). These traits suggest that MA might have evolved under specific environmental pressures, possibly favoring root development and secondary metabolite accumulation. Similarly, SN also showed significant differentiation, aligning with traits related to root development and biomass allocation, but with less emphasis on glucosinolate content. This separation in the PCA plot highlights the potential for MA and SN to be used in breeding programs focused on traits like root size or specific biochemical profiles, which could be important for agricultural systems emphasizing root-based productivity or specific chemical compositions for nutrition or medicinal purposes.

BN, BM, and BF clustered closely together, suggesting that these cultivars share a higher degree of phenotypic and biochemical similarity. This could indicate that they either share a common genetic origin or have adapted similarly to local growing conditions. These accessions displayed moderate levels of traits like glucosinolate concentration, soluble solid content in fruit, and total height. Such clustering suggests genetic uniformity or environmental convergence, which can be beneficial for breeding strategies focused on creating cultivars with consistent performance across various growing conditions [38].

The hierarchical clustering analysis provided deeper insights into the relationships among the accessions, revealing two major clusters that reflect both genetic and phenotypic differentiation. SN and MA, with their unique traits, should be considered in breeding programs targeting specialized traits, such as high glucosinolate levels, root size, or specific phenolic compounds. These cultivars can serve as sources of novel genetic material, enhancing the genetic diversity of breeding populations. For example, the introduction of these traits could lead to the development of crops with enhanced nutritional or medicinal properties, which are increasingly valued in contemporary agriculture. On the other hand, the closely clustered accessions (BN, BM, BI, and BF) should be prioritized in breeding efforts focused on consistency and reliability, especially for commercial cultivation. Their phenotypic uniformity makes them prime candidates for mass production, where predictable yield, uniform growth, and resistance to environmental stresses are essential.

Multivariate statistical analyses, particularly hierarchical clustering and Principal Component Analysis (PCA), serve as essential tools for uncovering cultivar-specific traits and the biochemical mechanisms influencing nutritional quality in *Brassica oleracea* [39]. These techniques, similar to those employed in the research by Granato et al. [40] and Sharma et al. [41], significantly enhance our understanding of both the biochemical diversity and the genetic relationships inherent among cultivars, including the Sicilian landrace 'Ciurietto'. By systematically categorizing and analyzing these traits, we can identify cultivars that not only demonstrate superior agronomic performance but also offer enhanced health benefits. This insight is crucial for developing informed selection strategies aimed at enriching the nutritional profiles of traditional landraces, ultimately supporting sustainable agricultural practices and promoting public health. While this study provides valuable insights into the agronomic and biochemical diversity of *Brassica oleracea,* several factors warrant further investigation. Environmental variability across growing seasons may influence trait expression [42, 43,], necessitating multi-year trials to assess trait stability under diverse conditions. Additionally, while biochemical assays offer useful indicators of antioxidant capacity, integrating metabolomic and transcriptomic approaches could provide a more comprehensive understanding of metabolic pathways governing trait variation [44]. Future research should also focus on elucidating the molecular mechanisms underlying observed trait correlations, thereby refining breeding strategies for improved crop performance and bioactive compound enrichment.

5. Conclusions

This study presents a comprehensive comparative analysis of agronomic and biochemical traits among diverse *Brassica oleracea* accessions, with the overarching goal of identifying superior candidates for cultivation and targeted breeding programs. Substantial variability was detected across key agronomic parameters,including plant height, biomass accumulation, and fruit weight—underscoring the considerable genetic diversity within the evaluated germplasm. Among the analyzed accessions, SN, the hybrid exhibited the highest total biomass, while BF attained the greatest plant height, identifying this population as promising genotypes for distinct agronomic objectives. Marked differences in SPAD values and soluble solids content further highlighted the pronounced physiological and biochemical heterogeneity across accessions. In particular, the elevated SPAD readings observed in BN and BA indicate enhanced chlorophyll content, potentially associated with greater photosynthetic efficiency and overall plant vigor. Similarly, the observed variation in soluble solids content among plant organs reflects the functional specialization of tissues in nutrient accumulation and translocation. Biochemical profiling revealed substantial differences in total phenolic content and glucosinolate composition, with the populations BF and BN showing significantly elevated levels of bioactive compounds. These findings point to their potential as valuable genetic resources for the development of *B. oleracea* varieties enriched in health-promoting phytochemicals. Correlation analysis elucidated key relationships between agronomic and biochemical traits, suggesting potential trade-offs between growth performance and secondary metabolite biosynthesis. Such insights provide a critical framework for integrating yield optimization with nutritional enhancement in future breeding strategies.

Overall, the results establish a robust foundation for breeding programs aimed at producing high-yielding, nutrient-dense *B. oleracea* cultivars adapted to a wide range of agro-environmental conditions. Further validation through multi-environment field trials will be essential to confirm the stability and heritability of these traits and to refine selection criteria for large-scale cultivation and commercial deployment.

CRediT authorship contribution statement

Maria Concetta Di Bella: Writing – original draft, Methodology, Formal analysis, Data curation. **Hajer Ben Ammar:** Writing – original draft, Formal analysis, Validation, Data curation. **Sergio Argento:** Writing – original draft, Methodology, Data curation. **Sonia Bonacci:** Data curation, Validation. **Maria Grazia Melilli:** Writing – review & editing, Writing – original draft, Supervision, Data curation. **Ferdinando Branca:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Funding

This research was supported by the project BRESOV (Breeding for Resilient. Efficient and Sustainable Organic Vegetable production) funded by EU H2020 Program SFS-07-2017. Grant Agreement n. 774244.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

[1] J. Quizhpe, P. Ayuso, M.D.L.Á. Rosell, R. Peñalver, G. Nieto, *Brassica oleracea* var *italica* and their By-Products as source of bioactive compounds and food applications in bakery products, Foods 13 (21) (2024) 3513, https://doi.org/10.3390/foods13213513.

[2] Di Bella, M. C., Melilli, M. G., Treccarichi, S., Tribulato, A., Arena, D., Ruffino, A., … & Branca, F. Influence of irrigation regime on productive and qualitative traits of kale (Brassica oleracea var. acephala DC) under the organic farming system. https://doi.org/10.17660/ActaHortic.2022.1354.39.

[3] K. Iwar, K.T. Desta, K. Ochar, S.H. Kim, Unveiling glucosinolate diversity in Brassica Germplasm and in Silico analysis for determining optimal antioxidant potential, Antioxidants 13 (3) (2024) 376, https://doi.org/10.3390/antiox13030376.

[4] R.M. Abdel-Massih, E. Debs, L. Othman, J. Attieh, F.M. Cabrerizo, Glucosinolates, a natural chemical arsenal: more to tell than the myrosinase story, Front. Microbiol. 14 (2023) 1130208, https://doi.org/10.3389/fmicb.2023.1130208.

[5] M.E. Cartea, M. Francisco, P. Soengas, P. Velasco, Phenolic compounds in Brassica vegetables, Molecules 16 (1) (2010) 251–280, https://doi.org/10.3390/molecules16010251.

[6] J.C. Lachance, S. Radhakrishnan, G. Madiwale, S. Guerrier, J.K. Vanamala, Targeting hallmarks of cancer with a food-system–based approach, Nutrition 69 (2020) 110563, https://doi.org/10.1016/j.nut.2019.110563.

[7] M. Herrero, M. Hugas, U. Lele, A. Wirakartakusumah, M. Torero, A shift to healthy and sustainable consumption patterns, Science and innovations for food systems transformation 59 (2023), https://doi.org/10.1007/978-3-031-15703-5_5, 2023.

[8] A. Tribulato, S. Toscano, M.C. Di Bella, D. Romano, F. Branca, *Brassica oleracea* complex species in Sicily: diversity, uses and conservation strategies, Acta Hortic. 1297 (2020) 61–68, https://doi.org/10.17660/ActaHortic.2020.1297.9.

[9] M.C. Di Bella, S. Treccarichi, D. Arena, R. Nicotra, A. Mazzaglia, M.G. Melilli, A. Bartoszek, B. Kusznierewicz, K. Parchem, F. Branca, Evaluation of Sicilian landraces of broccoli (*B. oleracea* var. *italica* Plenck) for quality traits, Acta Hortic. 1354 (2022) 343–350, https://doi.org/10.17660/ActaHortic.2022.1354.44.

[10] G. Bianchi, V. Picchi, R. Lo Scalzo, G. Campanelli, N. Ficcadenti, M.A. Dattoli, F. Branca, Agrobiodiversity in organic Brassica crops: relationship between pigment composition and antioxidant activity, Acta Hortic. 1354 (2022) 317–324.

[11] H. Ben Ammar, D. Sdouga, M.C. Di Bella, S. Treccarichi, R. Cali, E. Rosa, S. Marghali, Detection of glucosinolate metabolite pathway using SSR markers of *Brassica oleracea* complex species (n= 9) core collection, Acta Hortic. 1354 (2022) 105–110, https://doi.org/10.17660/ActaHortic.2022.1354.14.

[12] L.S. Shuang, H. Cuevas, C. Lemke, C. Kim, T. Shehzad, A.H. Paterson, Genetic dissection of morphological variation between cauliflower and a rapid cycling *Brassica oleracea* line, G3 (Bethesda) 13 (11) (2023), https://doi.org/10.1093/g3journal/jkad163 jkad163.

[13] F. Han, Y. Liu, Z. Fang, L. Yang, M. Zhuang, Y. Zhang, H. Lv, Y. Wang, J. Ji, Z. Li, Advances in genetics and molecular breeding of broccoli, Horticulturae 7 (9) (2021) 280, https://doi.org/10.3390/horticulturae7090280.

[14] L. Maggioni, R. von Bothmer, G. Poulsen, F. Branca, Origin and domestication of cole crops (*Brassica oleracea* L.): linguistic and literary considerations, Econ. Bot. 64 (2010) 109–123, https://doi.org/10.1007/s12231-010-9115-2.

[15] R. Chen, K. Chen, X. Yao, X. Zhang, Y. Yang, X. Su, D. Sun, Genomic analyses reveal the stepwise domestication and genetic mechanism of curd biogenesis in cauliflower, Nat. Genet. 56 (6) (2024) 1235–1244, https://doi.org/10.1038/s41588-024-01744-4.

[16] Z. Stansell, K. Hyma, J. Fresnedo-Ramírez, Q. Sun, S. Mitchell, T. Björkman, J. Hua, Genotyping-by-sequencing of *Brassica oleracea* vegetables reveals unique phylogenetic patterns, population structure and domestication footprints, Hortic. Res. 5 (2018), https://doi.org/10.1038/s41438-018-0040-3.

[17] M.E. Mabry, S.D. Turner-Hissong, E.Y. Gallagher, A.C. McAlvay, H. An, P.P. Edger, J.C. Pires, The evolutionary history of wild, domesticated, and feral *Brassica oleracea* (Brassicaceae), Mol. Biol. Evol. 38 (10) (2021) 4419–4434, https://doi.org/10.1093/molbev/msab183.

[18] E. Azpeitia, G. Tichtinsky, M. Le Masson, A. Serrano-Mislata, V. Gregis, C. Gimenez, F. Parcy, The making of cauliflowers: the story of unsuccessful flowers, bioRxiv 2021–02 (2021), https://doi.org/10.1101/2021.02.12.427428.

[19] D.V. Duclos, T. Björkman, Meristem identity gene expression during curd proliferation and flower initiation in *Brassica oleracea*, J. Exp. Bot. 59 (2) (2008) 421–433, https://doi.org/10.1093/jxb/erm327.

[20] S.M. Carr, V.F. Irish, Floral homeotic gene expression defines developmental arrest stages in Brassica oleracea L. vars. botrytis and italica, Planta 201 (1997) 179–188.

[21] D.L. Zuluaga, N. D'Agostino, E. Blanco, P.L. Curci, G. Sonnante, Genetic relationships and molecular signatures of divergence in traditional landraces and morphotypes of *Brassica oleracea*, Plants 14 (1) (2024) 20, https://doi.org/10.3390/plants14010020.

[22] F. Branca, G. Iapichino, Some wild and cultivated Brassicaceae exploited in Sicily as vegetables, Chron. Hortic. 48 (1) (1997) 20–25.

[23] M.W. Farnham, E.H. Davis, J.T. Morgan, J.P. Smith, Neglected landraces of collard (*Brassica oleracea* L. var. *viridis*) from the Carolinas (USA), Genet. Resour. Crop Evol. 55 (2008) 797–801, https://doi.org/10.1007/s10722-007-9284-8.

[24] G. Laghetti, F. Martignano, V. Falco, S. Cifarelli, T. Gladis, K. Hammer, "Mugnoli": a neglected race of *Brassica oleracea* L. from Salento (Italy), Genet. Resour. Crop Evol. 52 (2005) 635–639, https://doi.org/10.1007/s10722-005-8511-4.

[25] R. Lo Scalzo, G. Bianchi, A. Genna, C. Summa, Antioxidant properties and lipidic profile as quality indexes of cauliflower (*Brassica oleracea* L. var. *botrytis*) in relation to harvest time, Food Chem. 100 (3) (2007) 1019–1025, https://doi.org/10.1016/j.foodchem.2005.10.054.

[26] I.S.O. Rapeseed, Determination of Glucosinolates Content Part 1. Method Using high-performance Liquid Chromatography. Geneva, Sweitzerland, 1992, p. 9167, 1.

[27] A. Aires, R. Carvalho, Rapid separation of indole glucosinolates in roots of Chinese cabbage (*Brassica rapa* Subsp. *Pekinensis*) by High-Performance liquid chromatography with diode array detection, International Journal of Analytical Chemistry 2017 (1) (2017) 5125329, https://doi.org/10.1155/2017/5125329.

[28] K.N. Rakshita, S. Singh, V.K. Verma, B.B. Sharma, N. Saini, M.A. Iquebal, T. K. Behera, Agro-morphological and molecular diversity in different maturity

groups of Indian cauliflower (*Brassica oleracea* var. *botrytis* L.), PLoS One 16 (12) (2021) e0260246, https://doi.org/10.1371/journal.pone.0260246.

[29] F. Branca, G.L. Chiarenza, C. Cavallaro, H. Gu, Z. Zhao, A. Tribulato, Diversity of Sicilian broccoli (*Brassica oleracea* var. *italica*) and cauliflower (*Brassica oleracea* var. *botrytis*) landraces and their distinctive bio-morphological, antioxidant, and genetic traits, Genet. Resour. Crop Evol. 65 (2018) 485–502, https://doi.org/10.1007/s10722-017-0547-8.

[30] S. Matesanz, F. Valladares, Ecological and evolutionary responses of Mediterranean plants to global change, Environ. Exp. Bot. 103 (2014) 53–67, https://doi.org/10.1016/j.envexpbot.2013.09.004.

[31] M.A. El-Esawi, K. Germaine, P. Bourke, R. Malone, Genetic diversity and population structure of *Brassica oleracea* germplasm in Ireland using SSR markers, C. R. Biol. 339 (2016) 133–140, https://doi.org/10.1016/j.crvi.2016.02.002.

[32] H. Poorter, K.J. Niklas, P.B. Reich, J. Oleksyn, P. Poot, L. Mommer, Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control, New Phytol. 193 (1) (2012) 30–50, https://doi.org/10.1111/j.1469-8137.2011.03952.x.

[33] C. Agho, A. Avni, A. Bacu, A. Bakery, S. Balazadeh, F.S. Baloch, S. Fragkostefanakis, Integrative approaches to enhance reproductive resilience of crops for climate-proof agriculture, Plant Stress (2024) 100704, https://doi.org/10.1016/j.stress.2024.100704.

[34] S.E. Russo, G. Ledder, E.B. Muller, R.M. Nisbet, Dynamic Energy Budget models: fertile ground for understanding resource allocation in plants in a changing world, Conservation Physiology 10 (1) (2022), https://doi.org/10.1093/conphys/coac061 coac061.

[35] R. Ilahy, I. Tlili, Z. Pék, A. Montefusco, M.W. Siddiqui, F. Homa, M.S. Lenucci, Pre- and post-harvest factors affecting glucosinolate content in broccoli, Front. Nutr. 7 (2020) 147, https://doi.org/10.3389/fnut.2020.00147.

[36] D. Arena, H. Ben Ammar, V.M. Rodriguez, P. Velasco, G. Garcia, R. Calì, F. Branca, Exogenous melatonin affects the morphometric characteristics and glucosinolates during the initial growth stages of broccoli, Agronomy 14 (2) (2024) 286, https://doi.org/10.3390/agronomy14020286.

[37] E.L. Connolly, M. Sim, N. Travica, W. Marx, G. Beasy, G.S. Lynch, L. C. Blekkenhorst, Glucosinolates from cruciferous vegetables and their potential role in chronic disease: investigating the preclinical and clinical evidence, Front. Pharmacol. 12 (2021) 767975, https://doi.org/10.3389/fphar.2021.767975.

[38] Y. Xu, X. Zhang, H. Li, H. Zheng, J. Zhang, M.S. Olsen, Q. Qian, Smart breeding driven by big data, artificial intelligence, and integrated genomic-enviromic prediction, Mol. Plant 15 (11) (2022) 1664–1695, https://doi.org/10.1016/j.molp.2022.09.001.

[39] S. Aleem, M. Tahir, I. Sharif, M. Aleem, M. Najeebullah, A. Nawaz, A. Batool, M. I. Khan, W. Arshad, Principal component and cluster analyses as tools in the assessment of genetic diversity for late season cauliflower genotypes, Pakistan J. Agric. Res. 34 (1) (2021) 176–183, https://doi.org/10.17582/journal.pjar/2021/34.1.176.183.

[40] D. Granato, J.S. Santos, G.B. Escher, B.L. Ferreira, R.M. Maggio, Use of principal component analysis (PCA) and hierarchical cluster analysis (HCA) for multivariate association between bioactive compounds and functional properties in foods: a critical perspective, Trends Food Sci. Technol. 72 (2018) 83–90, https://doi.org/10.1016/j.tifs.2017.12.006.

[41] N. Sharma, R. Singh, R. Pandey, N. Kaushik, Genetic and biochemical stability assessment of plants regenerated from cryopreserved shoot tips of a commercially valuable medicinal herb *Bacopa monnieri* (L.) Wettst, Vitro Cell Dev. Biol. Plant 53 (4) (2017) 346–351, https://doi.org/10.1007/s11627-017-9826-5.

[42] R. Brooker, L.K. Brown, T.S. George, R.J. Pakeman, S. Palmer, L. Ramsay, M. J. Wilkinson, Active and adaptive plasticity in a changing climate, Trends Plant Sci. 27 (7) (2022) 717–728, https://doi.org/10.1016/j.tplants.2022.02.004.

[43] K. Kaur, P. Sharma, Effect of low light stress on photosynthetic pigments and antioxidative enzymes in field grown Indian mustard (Brassica juncea L.) genotypes, J. Agric. Sci. Technol. 11 (2021) 61–72, https://doi.org/10.17265/2161-6264/2021.02.002.

[44] A.S. Siomos, K. Koularmanis, P. Tsouvaltzis, The impacts of the emerging climate change on broccoli (*Brassica oleracea* L. var. *italica* Plenck.) crop, Horticulturae 8 (11) (2022) 1032, https://doi.org/10.3390/horticulturae8111032.