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Comprehensive Characterization and Analysis of Key Agronomic Traits and Glucosinolate Profiles in Leaf Mustard

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ABSTRACT: Leaf mustard (*Brassica juncea*) is an important leafy vegetable highly valued for its diverse flavor and nutrient compounds, particularly glucosinolates. However, different varieties of leaf mustard exhibit substantial phenotypic variation and varying glucosinolate content. In this study, we systematically assessed the phenotypic variability and glucosinolate content among 86 genotypes of leaf mustard. The Shannon-Wiener index for qualitative traits ranged from 0.10 (leaf surface gloss) to 1.60 (leaf shape), while for quantitative traits, it ranged from 1.85 (petiole length) to 2.07 (leaf width). Cluster analysis grouped the accessions into three distinct clusters, with hierarchical clustering indicating that yield-related traits were the primary factors distinguishing these groups. Principal component analysis (PCA) revealed that eight components accounted for 87.44% of the total variance in yield and glucosinolate attributes. Based on comprehensive scoring, the top five genotypes (A645, A464, A512, A702, and A445), exhibiting diverse characteristics, were identified as promising candidates for breeding programs. Moreover, our results suggest that leaf mustard leaves with deeper or more numerous lobes contain higher concentrations of glucoraphanin. Collectively, these findings provide valuable genetic resources to advance leaf mustard breeding.

Keywords: *Brassica juncea*; Germplasm evaluation; Agronomic traits; Correlation analysis

1. Introduction

Mustard (*Brassica juncea*; AABB), an allopolyploid species in the Brassicaceae family, originated from the hybridization of *Brassica rapa* (AA) and *Brassica nigra* (BB) [1]. As an important vegetable crop, mustard species are classified into four categories based on the harvested organs: leaf-type (var. *multiceps*), stalk-type (var. *utilis*), stem-type (var. *tsatsai*), and root-type (var. *megarrhiza*) [2]. These distinct types reflect considerable genetic diversity and phenotypic variation [3,4]. The high diversity in mustard species

may be driven by widespread interspecific and intervarietal hybridizations [5]. Additionally, other morphological traits, such as leaf shape and plant height, also vary among species and varieties.

Leaf mustard, an important vegetable for both fresh consumption and processing, is particularly rich in glucosinolates—compounds responsible for its distinctive flavor and associated health benefits [6,7]. Glucosinolates are sulfur-containing secondary metabolites predominantly found in Brassicaceae species and play a crucial role in plant defense [8,9]. They are classified into three categories based on their amino acid precursors: aliphatic glucosinolates (AGS, derived from isoleucine, alanine, valine, methionine, and leucine), aromatic glucosinolates (RGS, derived from tyrosine and phenylalanine), and indolic glucosinolates (IGS, derived from tryptophan) [10]. Glucoraphanin is a known precursor of sulforaphane, a compound widely reported to inhibit several cancers, including lung, liver, and prostate cancers [11–14]. Given the moderate to high heritability of glucosinolate content and its demonstrated health benefits, selecting varieties with high glucoraphanin levels for breeding offers a promising approach to enhancing the nutritional profile of leaf mustard [15].

Germplasm resources form the foundation for cultivar improvement, genomic studies, and agricultural production. Genetic diversity within plant germplasm can be assessed through two primary approaches: phenotypic evaluation and molecular genetic analysis [16,17]. Phenotypic assessment, a traditional yet effective method, provides direct insights into the morphological and physiological characteristics of plants [18]. Phenotypic diversity, arising from the complex interaction between genotype and environment, plays a crucial role in biological research and crop improvement [5]. Recently, several studies have reported germplasm evaluations of vegetable crops, including purple flowering stalk [18], amaranth [5], and cabbage [19]. However, despite its commercial importance, research on the germplasm resources and nutritional traits of leaf mustard remains limited. Moreover, long-term selective breeding has narrowed the genetic background and reduced genetic diversity in leaf mustard [4]. Therefore, comprehensive investigation and evaluation of leaf mustard germplasm are essential for future breeding efforts. In this study, 86 accessions of leaf mustard were used to investigate genetic diversity based on key agronomic traits and glucosinolate content. Several statistical methods were employed to elucidate trait relationships. Additionally, we applied an integrated approach to identify promising germplasm for further breeding.

2. Materials and Methods

2.1. Plant Materials and Sample Preparation

In this study, a total of 86 leaf mustard varieties were selected from Hunan, Hubei, Fujian, and Beijing, China. The leaf mustard seeds were initially sown in a plant growth chamber, and seedlings at the four-leaf stage were subsequently transplanted into greenhouses at the Changde Academy of Agricultural and Forestry Sciences, Changde (29°02′ N, 111°41′ E), China. All plant samples were measured and harvested during the early flowering stage.

2.2. Morphological and Phenotypic Characterization

A total of 24 traits were identified based on previously reported methods for leaf mustard [3], including 17 qualitative and seven quantitative traits. The qualitative traits examined were plant type, leaf shape, serrated leaf margin, leaf lobe, leaf surface gloss, serrated leaf edge, leaf apex, leaf type, number of leaf lobes, leaf surface wrinkling, leaf surface prickles, petiole cross-section, tillering, leaf color, petiole color, mustard-like pungency, and heading ability. The quantitative traits assessed included plant width, plant height, petiole width, leaf petiole thickness, leaf width, leaf length, and petiole length.

2.3. Measurement of Glucosinolate Content

Glucosinolate extraction and quantification were performed using previously published methods [20,21]. Dried, powdered leaf samples (0.5 g) were initially extracted with 70% methanol in a water bath at 75 °C for 20 min. After adding 1 mL of barium acetate and subsequent centrifugation, the supernatant was collected in a polypropylene tube. The residue underwent a second extraction under identical conditions following the addition of sinigrin (Sigma, Kanagawa, Japan, internal standard). The combined supernatants were then loaded onto an ion-exchange column packed with DEAE-Sephadex A-25. Subsequently, the column was treated with 2 mL of 20 mM sodium acetate, after which 900 µL of sulfatase (Sigma) was added to facilitate desulfation. Following incubation overnight at 37 °C, the column was rinsed twice with deionized water. The eluate was collected into a sample vial for further processing after passing through a 0.45 µm membrane filter. Glucosinolate separation was performed using a high-performance liquid chromatography (HPLC) system. The mobile phase consisted of water and methanol at a flow rate of 1 mL/min. The gradient elution program was set as follows: 5% methanol (0–1 min), linear gradient to 100% methanol (1–9 min), 100% methanol (9–11 min), and re-equilibration to 5% methanol (11–13 min). Detection and quantification were conducted by UV absorbance at 229 nm, with desulfo-sinigrin serving as the reference standard.

2.4. Statistical Analysis

Principal component analysis (PCA) was performed using the prcomp function in R. Hierarchical cluster analysis (HCA) was conducted using Euclidean distance and Ward linkage in R. Correlation analysis was calculated using the cor.test function and visualized as heatmaps [22]. To generate a comprehensive ranking of varieties, a comprehensive evaluation value (D) was calculated for each accession as the weighted sum of PC1–PC8 scores, using their respective variance contribution rates as weights ($D = \Sigma\lambda_i \times PC_i$).

3. Results

3.1. Agronomic Traits of Leaf Mustard

The genetic diversity of 17 qualitative traits was evaluated, as summarized in Table 1 and illustrated in Figure 1. The Shannon-Wiener Index (H') averaged 0.98, ranging from 0.10 (leaf surface gloss) to 1.60 (leaf shape). Among these traits, 11—including leaf shape, leaf lobe, leaf color, leaf surface wrinkling, leaf surface prickles, petiole cross-section, number of leaf lobes, plant type, serrated leaf edge, leaf apex, and petiole color—exhibited H' values greater than 1, indicating substantial genetic diversity. In contrast, qualitative traits such as leaf surface gloss, heading ability, leaf type, and mustard-like pungency displayed H' values below 0.6, suggesting limited variability. For example, 98.04% of accessions exhibited glossy leaf surfaces, 86.27% had entire margins, 96.10% were non-heading types, and 80.00% showed mild mustard-like pungency.

Table 1. Qualitative traits of leaf mustard varieties.

Traits	Distribution								*H′*
	0	1	2	3	4	5	6	7	
Plant type		27.45	40.20	32.35					1.09
Leaf shape		2.94	13.73	1.96	20.59	41.18	11.76	7.84	1.60
Serrated leaf margin		9.80	22.55	67.65					0.83
Serrated leaf edge	14.71	59.80	18.63	6.86					1.09
Leaf apex		29.41	38.24	32.35					1.09
Leaf type		86.27	13.73						0.40
Leaf lobe	13.73	48.04	21.57	16.67					1.25

Number of leaf lobe	13.73	54.90	24.51	6.86			1.13
Leaf surface wrinkling		8.82	38.24	42.16	10.78		1.19
Leaf surface prickles	43.14	35.29	15.69	5.88			1.19
Leaf color		1.96	20.59	54.90	7.84	14.71	1.21
Petiole cross-section		42.16	22.55	33.33	1.96		1.14
Tillering		72.55	15.69	11.76			0.78
Leaf surface gloss	1.96	98.04					0.10
Petiole color		26.47	52.94	20.59			1.01
Mustard-like pungency		80.00	18.00	2.00			0.57
Heading ability	97.40	2.60					0.13

Qualitative traits were scored as follows: Plant type (1 = upright, 2 = half upright, 3 = spread), Leaf shape (1 = near circular, 2 = ovate, 3 = long ovate, 4 = obovate, 5 = elliptic, 6 = oblong-obovate, 7 = broadly obovate), Serrated leaf margin (1 = entire, 2 = gyrose, 3 = shallow sawtooth), Serrated leaf edge (0 = none, 1 = small, 2 = middle, 3 = large), Leaf apex (1 = wide circle, 2 = circle, 3 = pointed), Leaf type (1 = variegated leaf, 2 = flat leaf), Leaf lobe (0 = none, 1 = less, 2 = deeply, 3 = entirely), Number of leaf lobe (0 = no lobe, 1 = one lobe, 2 = two lobes, 3 = three lobes), Leaf surface wrinkling (1 = smoothness, 2 = slightly wrinkled, 3 = wrinkled, 4 = highly wrinkled), Leaf surface prickles (0 = none, 1 = less, 2 = middle, 3 = more), Leaf color (1 = light green, 2 = yellowish green, 3 = green, 4 = purplish-green, 5 = dark green), Petiole cross-section (1 = medium-round, 2 = broad-flattened, 3 = slender-rounded, 4 = broad-thick), Tillering (1 = weak, 2 = medium, 3 = strong), Leaf surface gloss (0 = non-glossy, 1 = glossy), Petiole color (1 = white-green, 2 = light green, 3 = green), Mustard-like pungency (1 = mild, 2 = medium, 3 = strong), Heading ability (0 = non-heading, 1 = heading).

Figure 1. Morphological characteristics of various leaf mustard varieties.

The *H′* values for quantitative traits ranged from 1.85 (petiole length) to 2.07 (leaf width), with an average of 1.98. Notably, leaf width, petiole width, plant height, and leaf petiole thickness also exhibited *H′* values equal to or greater than 2, indicating substantial genetic diversity across most quantitative traits (Table 2). Genetic diversity can also be assessed by the coefficient of variation (CV), where higher CV values indicate lower homogeneity [23]. In this study, CV values for quantitative traits ranged from 14.38% to 61.70%, with

an average of 32.27%. Traits such as petiole length and petiole width showed the highest CV values, while plant width and leaf length had the lowest (Table 2). This suggests that breeders have greater potential to select parental materials based on traits such as petiole length and width, whereas improving plant width and leaf length may be more challenging [24]. These findings underscore the potential for utilizing this genetic diversity in breeding programs aimed at developing superior leaf mustard varieties.

Table 2. Quantitative traits of leaf mustard varieties.

Traits	Min	Max	Mean	SD	Range	CV (%)	*H′*
Plant height	6.20	78.25	42.82	12.43	72.05	29.03	2.00
Plant width	34.60	107.80	84.95	12.21	73.20	14.38	1.95
Leaf length	25.15	103.58	61.90	12.90	78.43	20.84	1.99
Leaf width	9.08	48.10	28.84	8.55	39.02	29.65	2.07
Petiole length	0.30	19.15	4.89	3.02	18.85	61.70	1.85
Petiole width	0.45	6.10	3.00	1.34	5.65	44.64	2.03
Leaf petiole thickness	0.20	2.13	1.27	0.33	1.93	25.63	2.00

SD: standard deviation; CV: coefficient of variation.

3.2. Glucosinolate Profile of Leaf Mustard

Of the 86 varieties evaluated for agronomic traits, glucosinolates were detected at quantifiable levels in 45 varieties. Among these, a total of nine glucosinolates were identified, including five aliphatic glucosinolates (AGS: glucoiberin, progoitrin, sinigrin, glucoraphanin, glucoalyssin), three indolic glucosinolates (IGS: glucobrassicin, neoglucobrassicin, 4-methoxyglucobrassicin), and one aromatic glucosinolate (RGS: gluconasturtiin) (Figure 2). Significant variability in glucosinolate composition and content was observed among the varieties. Total glucosinolate content ranged from 0.09 μmol/g to 13.82 μmol/g. AGS constituted the predominant fraction, accounting for an average of 77% of total glucosinolates, while IGS and RGS represented averages of 16.39% and 6.61%, respectively. Notably, RGS was absent in 35 varieties.

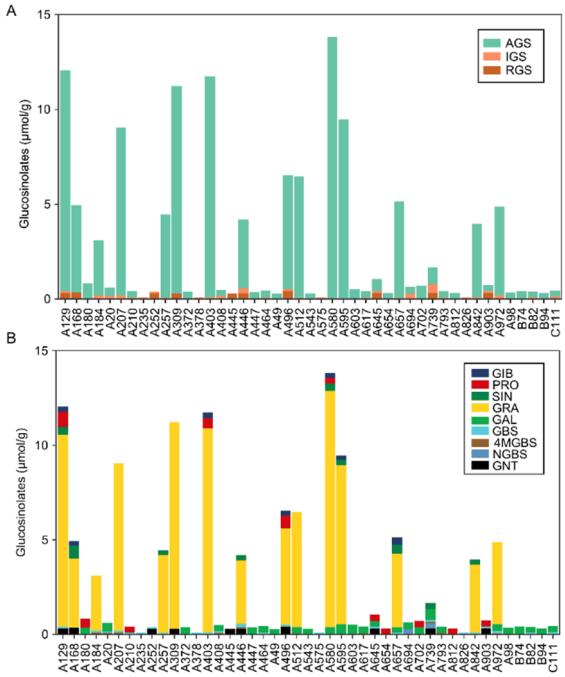

Figure 2. Total (**A**) and specific (**B**) glucosinolate content in different varieties of leaf mustard. AGS: aliphatic glucosinolates; IGS: indolic glucosinolates; RGS: aromatic glucosinolates; GIB: glucoiberin; PRO: progoitrin; SIN: sinigrin; GRA: glucoraphanin; GAL: glucoalyssin; GBS: glucobrassicin; 4MGBS: 4-methoxy glucobrassicin; NGBS: neoglucobrassicin; GNT: gluconasturtiin.

3.3. Principal Component Analysis (PCA) of Yield-Related Traits and Glucosinolate Content

Principal component analysis was conducted using yield-related traits (plant height, plant width, leaf length, leaf width, mustard-like pungency, tillering) and glucosinolate contents (Figure 3). Eight principal components (PCs) were extracted, collectively explaining 87.44% of the total variance. The first principal component (PC1), accounting for 24.68% of the variance, showed the highest loadings on variables including plant height, leaf length, leaf width, tillering, glucoalyssin (GAL), and glucobrassicin (GBS). The second principal component (PC2), explaining 16.83% of the variance, was predominantly associated with aliphatic glucosinolates: glucoiberin (GIB), sinigrin (SIN), 4-methoxy glucobrassicin (4MGBS), glucoraphanin (GRA), and progoitrin (PRO). Based on the PCA results, leaf mustard varieties were evaluated and ranked (Table 3). Among the top five varieties, 'A694', 'A512', 'A739', and 'A617' scored highly on PC1, indicating greater leaf length and width, taller plant height, reduced tillering, and lower levels of GBS. Varieties such as 'A617', 'A372', 'A464', and 'A694' exhibited higher scores on PC2, reflecting lower AGS content and higher IGS content.

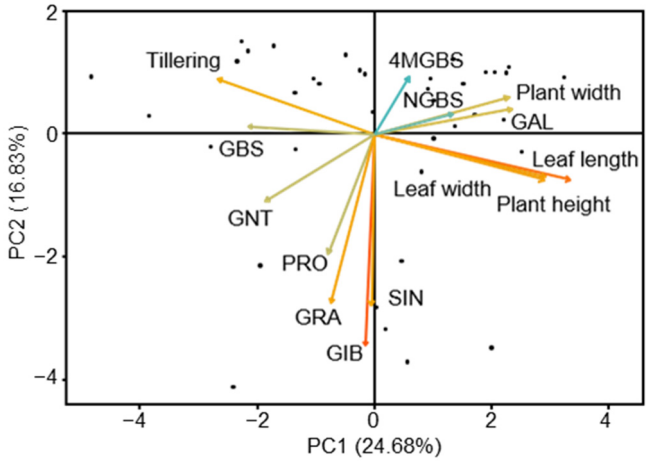

Figure 3. PCA plot of 45 leaf mustard varieties based on glucosinolate content and yield-related traits, including leaf width, leaf length, plant height, plant width, and tillering.

Table 3. Comprehensive evaluation of leaf mustard varieties by glucosinolate content and yield-related traits.

Accession	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	Comprehensive Evaluation Value (*D*)	Rank
A645	1.00	−0.06	−1.45	0.55	1.16	0.16	0.51	1.58	0.50	1
A464	−0.17	0.98	1.57	1.53	−0.46	−0.33	0.36	0.68	0.47	2
A512	2.49	−0.28	1.35	−2.99	−2.09	1.08	−0.49	1.97	0.46	3
A702	1.00	0.55	−0.84	1.47	−0.36	0.41	−0.70	0.36	0.45	4
A445	−0.95	0.82	−1.28	−0.40	0.27	1.71	0.36	−0.37	0.41	5

3.4. Hierarchical Cluster Analysis

Three distinct clusters were identified among all varieties through hierarchical cluster analysis (HCA) using the Ward method (Figure 4). The first cluster (red), comprising 15 accessions, was characterized by higher AGS content—particularly GRA, SIN, and GIB—as well as longer petioles, increased plant height, and larger leaves. The second cluster (blue), consisting of nine accessions, exhibited greater leaf and petiole widths but shorter petiole length. The third cluster (green), containing 14 accessions, was distinguished by a wider plant spread and fewer leaf surface prickles.

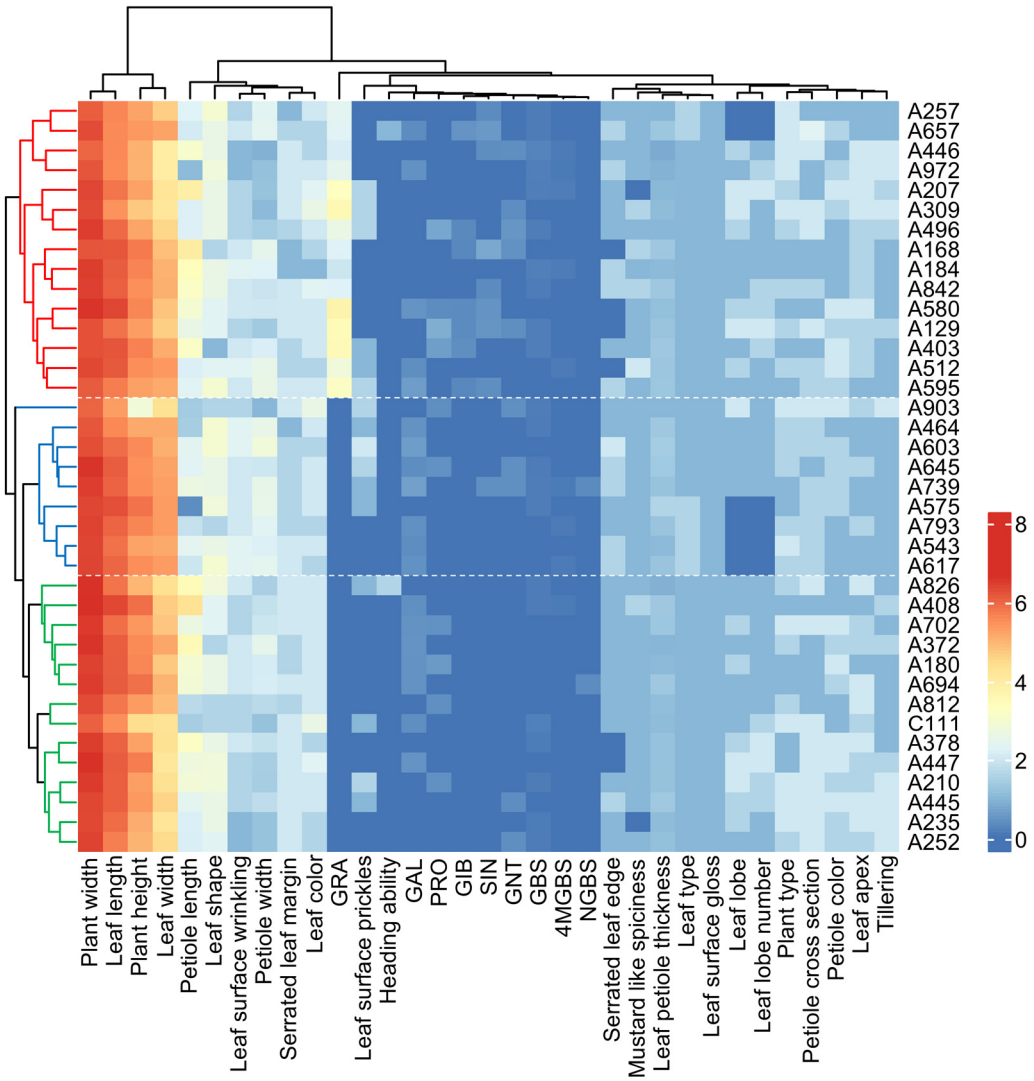

Figure 4. Hierarchical cluster analysis of leaf mustard.

3.5. Correlation Analysis

Correlation analysis revealed both positive and negative relationships between agronomic traits and glucosinolate contents. As shown in Figure 5, yield-related traits—including leaf length and plant width—were positively correlated with plant height, leaf surface wrinkling, and petiole width, but negatively correlated with plant type and leaf color. Glucosinolate content, especially GRA, was positively correlated with leaf lobe number and leaf lobe size. RGS compounds, such as GNA, were positively associated with tillering and negatively associated with leaf surface wrinkling. IGS, particularly GBS, showed negative correlations with leaf surface wrinkling, plant height, leaf width, and petiole width.

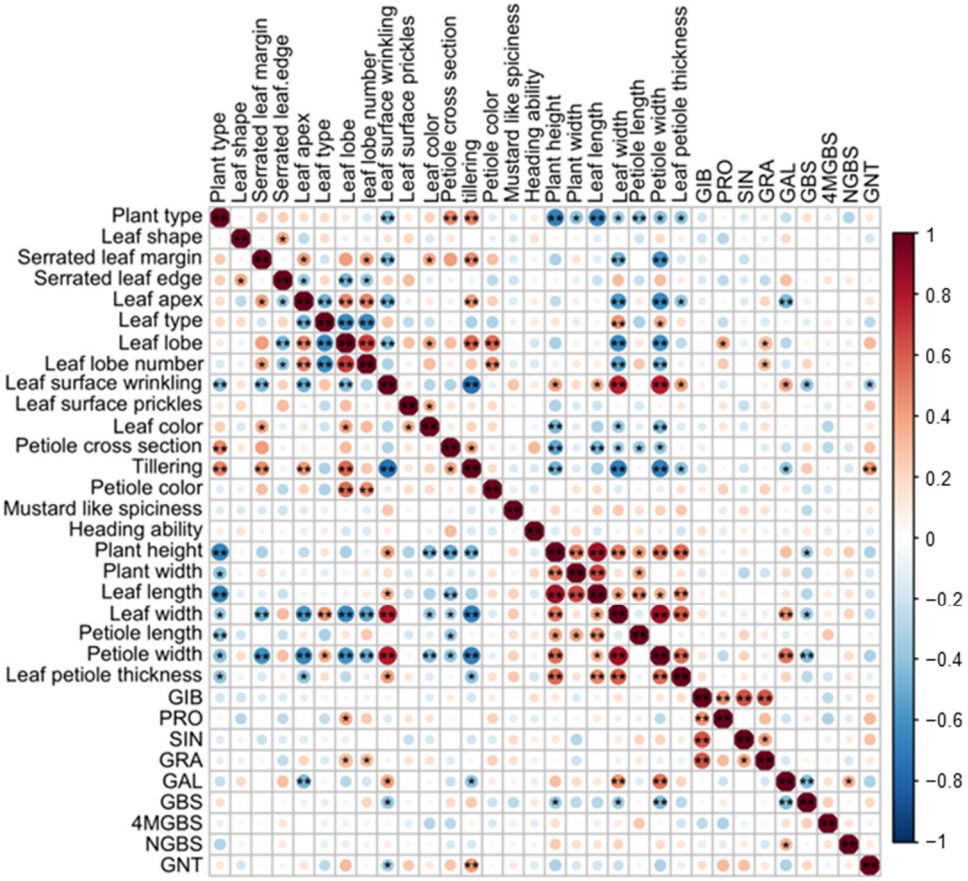

Figure 5. Correlation analysis between agricultural traits and glucosinolate content. * $p \leq 0.05$, ** $p \leq 0.01$.

4. Discussion

The leaf mustard materials in this study exhibit substantial genetic diversity. The Shannon-Wiener Index (H') exceeded 1 for all quantitative traits and 13 qualitative traits, including leaf shape, color, and yield-related characteristics. These traits are critical for breeding programs, as they provide a rich genetic reservoir for developing high-yield, high-quality varieties [25]. While leaf width and plant height showed high H' values, their coefficients of variation (CV) were notably low. In contrast, traits such as petiole width exhibited both high H' and high CV values. This discrepancy likely reflects differing market demands: stable preferences for optimal plant size contrast with evolving requirements for edible portion size and yield, necessitating the development of new varieties to meet these needs.

Leaf mustard exhibited a total glucosinolate content ranging from 0.09 to 13.82 μmol/g, which was lower than that of broccoli (0.467–57.156 μmol/g) but significantly higher than that of *B. napus* (0.238–7.384 μmol/g) [26,27]. These variations may be attributed to differences in tissue types, varieties, growing conditions, field management practices, and analytical methodologies. Aliphatic glucosinolates (AGS) were the predominant class, followed by indolic glucosinolates (IGS). This study identified nine glucosinolates, including five AGS, three IGS, and one aromatic glucosinolate (RGS). Although the number and composition of glucosinolates were similar to those reported in previous studies, some differences were observed. For example, Huang et al. detected nine glucosinolates, five of which overlapped with our findings [3]. Assefa et al. identified five glucosinolates, including glucoraphanin, progoitrin, and glucoalyssin [28].

PCA of yield-related and glucosinolate traits revealed that PC1, accounting for 24.68% of the total variance, was strongly associated with plant height, leaf width and length, GBS, and GAL. These findings align with HCA results, underscoring the importance of yield and glucosinolate traits in evaluating leaf

mustard diversity. The significant positive correlation between leaf lobe development and glucoraphanin (GRA) accumulation is highly relevant to both plant biology and agricultural practice. As the key precursor of sulforaphane, GRA content directly determines the nutraceutical potential of cruciferous vegetables [29]. Agronomically, this correlation suggests that leaf morphology, especially leaf lobation, could serve as a visual marker for selecting high-GRA cultivars [12,30]. Ecologically, this finding supports hypotheses that leaf lobation may represent a defense strategy, consistent with the role of glucosinolates in anti-herbivore defense [31–33].

Yield improvement and high quality remain key goals in leaf mustard breeding. Considering both yield-associated traits and glucosinolate content has facilitated the identification of the best-performing materials for breeding programs. Notably, the top five accessions exhibited complementary characteristics, enabling precise selection based on specific breeding objectives.

5. Conclusions

This study conducted a comprehensive analysis of genetic diversity in leaf mustard, focusing on agronomic traits and glucosinolate content. The evaluated accessions exhibited significant variability in leaf morphology and plant type. Based on a combined assessment of yield-related traits and glucosinolate profiles, the top five varieties were identified as superior accessions with both high yield potential and elevated glucosinolate levels. These varieties represent valuable genetic resources for improving leaf mustard cultivars. Consumers are encouraged to select leaf mustard cultivars with deeper or more numerous leaf lobes, as these may contain higher levels of glucoraphanin, which is beneficial for health.

Statement of the Use of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this manuscript, the authors used Grammarly for language polishing and grammar correction. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Author Contributions

Q.W. and K.H. designed the research. Z.H. and L.Y. performed the experiments. J.H., L.Y., X.S. and L.H. collected the data. Z.H., H.H. and J.W. analyzed the data. Z.H., L.Y., Q.W. and K.H. wrote the manuscript. All authors read and edited the manuscript before publication.

Ethics Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The authors declare that the data supporting the findings of this study are available within the paper.

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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.

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