

Article

Characterization of Radish Germplasm by Phenotypic Traits and Glucosinolate Composition

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Abstract

This study evaluated glucosinolate (GSL) composition and phenotypic traits in 72 radish (*Raphanus sativus* L. var. *sativus*) accessions, including 69 germplasm and 3 cultivars. Seven GSLs: glucoraphanin, glucoraphenin, glucoerucin, glucoraphasatin, glucobrassicin, glucobarbarin, and glucobetteroin were quantified using UPLC (mg/kg DW). Under the evaluated conditions, accession 10 (IT203308, Russia) showed high glucobarbarin (3976.9), while 38 (IT262009, Germany) and 39 (IT262067, North Korea) accumulated glucoraphasatin above 3500. Accession 40 (IT262091, North Korea) exhibited the highest glucoraphenin (612.9), and glucoerucin was enriched in 12 (IT204244, Israel), 19 (IT215036, Republic of Korea), and 35 (IT261962, Russia). Principal component analysis identified outliers: 10 (IT203308, Russia), 12 (IT204244, Israel), 19 (IT215036, Republic of Korea), 35 (IT261962, Russia), 38 (IT262009, Germany), 40 (IT262091, North Korea), 34 (IT261948, Kyrgyzstan), and 59 (IT306725, Malaysia), separated from central accessions. Multivariate analyses indicated that GSL accumulation and phenotypic variation were not consistently associated. These findings identify high-GSL and PCA-defined outlier accessions as potential donor resources for the breeding of radish with improved glucosinolate-related nutritional quality, while demonstrating that glucosinolate profiles cannot be reliably predicted from external phenotypes.

Keywords: glucosinolates; UPLC analysis; radish (*Raphanus sativus* var. *sativus*); germplasm; principal component analysis

1. Introduction

Radish (*Raphanus sativus* L. var. *sativus*) is a widely cultivated root vegetable in the Brassicaceae family, valued for its nutritional qualities and rich phytochemical composition. Among these, glucosinolates (GSLs), sulfur-containing secondary metabolites, are especially prominent for their roles in plant defense and the production of bioactive hydrolysis products such as isothiocyanates, which possess anti-cancer, anti-obesity, and anti-inflammatory activities [1–5].

While GSL profiles have been documented in radish sprouts and select cultivars, highlighting metabolites such as glucoraphasatin and glucoraphenin [6,7], comprehensive analyses across large germplasm collections remain limited. On the other hand, studies in *Brassica* crops have demonstrated extensive variation in GSL composition influenced by genetics and environment [8,9]. Radish also exhibits significant GSL-related genetic diversity [10], and GSL accumulation dynamics change with environmental factors like



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vernalization [11] or by tissue type [12]. A recent study even examined GSL stability under spaceflight conditions [13], underscoring the breadth of ongoing research.

Despite the growing interest, few studies have integrated GSL chemistry with morphological traits across broad radish germplasm panels. Addressing this gap, our study analyzed 72 diverse radish accessions, quantifying seven key GSLs (glucoraphanin, glucoraphenin, glucoerucin, glucoraphasatin, glucobrassicin, glucobarbarin, glucobetteroin) via UPLC and concurrently assessing root and shoot phenotypes. We applied Pearson correlation and principal component analysis (PCA) to examine interrelationships between chemical and morphological diversity. Our integrated approach aimed to characterize the phenotypic and glucosinolate diversity of the germplasm, identify chemotypically distinctive accessions, clarify the independence between GSL accumulation and external traits, and identify accessions with distinctive glucosinolate profiles as potential donor resources for future breeding.

2. Materials and Methods

2.1. Plant Materials and Experimental Design

A total of 72 radish (*R. sativus* var. *sativus*) accessions conserved at the National Agrobiodiversity Center, Rural Development Administration (RDA), Jeonju, Republic of Korea were used in this study. The experiment was conducted under open-field conditions at the National Agrobiodiversity Center (RDA-Genebank), Jeonju, Republic of Korea, in 2024. The experimental field measured approximately 50 m × 51 m. Each accession was grown in individual plots consisting of 10 plants. Plants were arranged with a spacing of 40 cm between plants and 50 cm between rows, resulting in a plot size of approximately 1.6 m × 0.5 m. A distance of approximately 1 m was maintained between adjacent plots. Seeds were sown on 29 August 2024, and standard cultivation practices were uniformly applied across all accessions. This study was conducted under a single growing season without spatial or temporal replication. Roots were harvested at approximately 60 to 90 days after sowing (DAS).

2.2. Morphological Traits

Morphological characterization was conducted according to the standard descriptors for radish germplasm provided by the National Agrobiodiversity Center and the guidelines of the International Union for the Protection of New Varieties of Plants (UPOV). A total of 22 morphological traits were evaluated, including root shape, root diameter, root color, root flesh color, root shoulder color, hypocotyl color, leaf length, leaf width, leaf number, leaf weight, leaf pubescence, plant vigor, and wind damage tolerance, among others [14–16]. Ten plants per accession were cultivated, and three representative plants showing normal growth were selected for evaluation. Plants exhibiting poor growth were excluded from the analysis.

2.3. Glucosinolate Extraction and Analysis

Whole radish roots were cut longitudinally into sections without removing the skin, freeze-dried, and ground into a fine powder. For each accession, 50 mg of the powdered sample (comprising both skin and flesh) was extracted twice with 70% methanol (MeOH) at 70 °C for 5 min. The extracts were centrifuged and the supernatants were collected. Desulfoglucosinolates were prepared using DEAE Sephadex A-25 anion-exchange chromatography (Merck KGaA, Darmstadt, Germany) [17,18]. A mini-column was prepared by packing approximately 300 µL of DEAE Sephadex A-25 resin, pre-equilibrated with 0.5 M sodium acetate, into a 1000 µL pipette tip plugged with cotton wool. The methanolic extract (2 mL) was loaded onto the column, which was then washed with 2 mL of distilled

water. Arylsulfatase solution (23 mg/mL, 60 μ L) was applied, and the column was sealed and incubated at room temperature for 16 h. Desulfoglucosinolates were subsequently eluted with distilled water (500 μ L $\times$ 3; total 1.5 mL) and confirmed by comparison of their retention times with those of an injected sinigrin standard and their characteristic UV absorption at 227 nm. Individual glucosinolates were identified based on retention times and previous reports, and quantified using response factors (RFs) relative to sinigrin as an external reference standard; no internal standard was used [7,19,20]. The retention times were as follows: glucoraphanin (7.1 min), glucoraphenin (7.6 min), glucobarbarin (10.7 min), glucoerucin (11.6 min), glucoraphasatin (12.0 min), glucobrassicin (12.3 min), and glucoberteroin (13.3 min) [Figure 1].

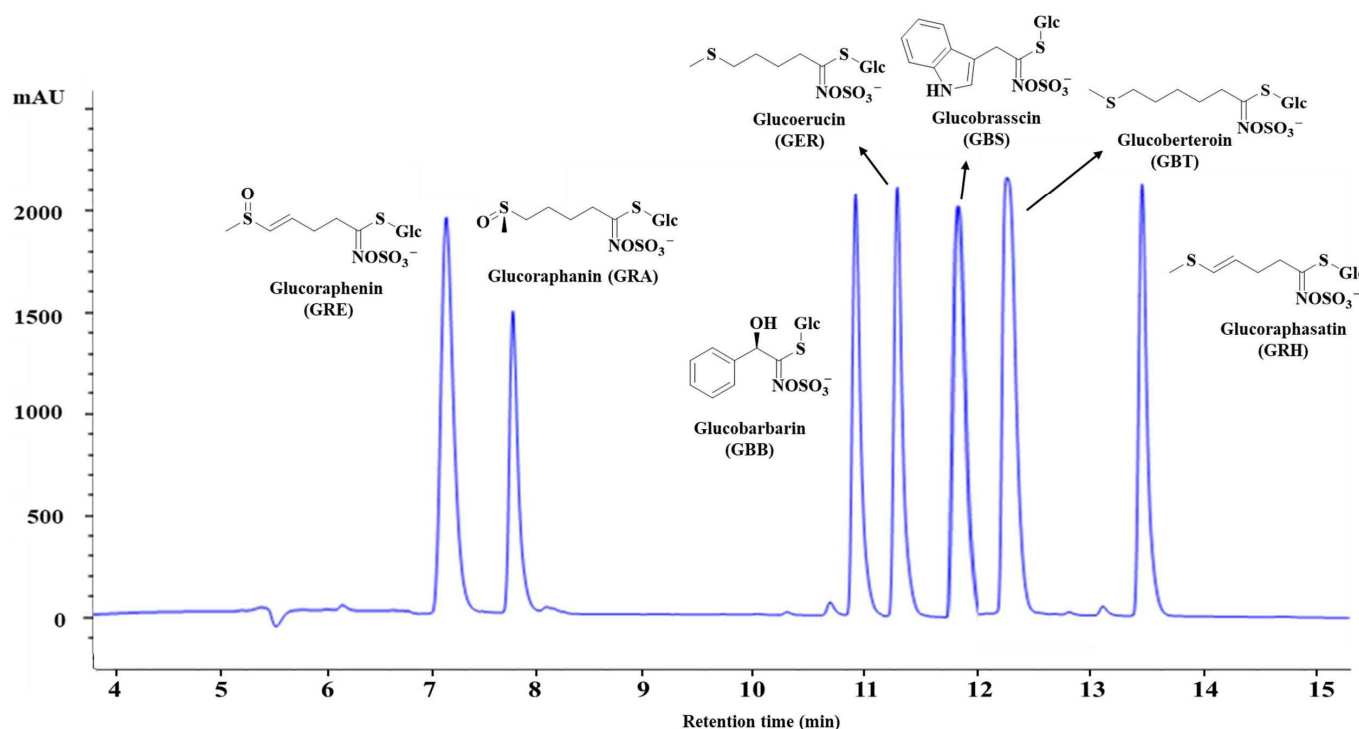


Figure 1. UPLC chromatographic profile of glucosinolate (GSL) standards detected at 227 nm. Identified compounds are glucoraphanin (GRA), glucoraphenin (GRE), glucoerucin (GER), glucoraphasatin (GPA), glucobrassicin (GBS), glucobarbarin (GBB), and glucoberteroin (GBT). Peaks correspond to individual GSLs, separated and quantified based on retention times and UV absorbance at 227 nm.

2.4. Method Validation

The analytical performance of the UPLC–DAD method for glucosinolate analysis was evaluated using sinigrin as an external calibration standard. Because authentic standards are not commercially available for many individual glucosinolates, quantification of individual glucosinolates was performed using response factors (RFs) relative to sinigrin, as previously described.

Calibration standards of sinigrin were prepared at six concentration levels (0.5, 1, 2.5, 5, 10, and 20 μ g/mL). Each concentration was analyzed in five replicate injections under the same chromatographic conditions as used for sample analysis. The calibration curve was constructed by plotting the mean peak area against sinigrin concentration using linear regression analysis.

The calibration curve showed excellent linearity over the tested concentration range, with the regression equation $y = 66.87x - 10.85$ and a coefficient of determination of $R^2 = 0.99$. The limit of detection (LOD) and limit of quantification (LOQ) were 0.133 μ g/mL and 0.402 μ g/mL, respectively. The retention time of sinigrin under the present chromatographic conditions was approximately 5.8 min. These results indicate that the developed

UPLC method provided reliable linearity and sensitivity for sinigrin-based quantification of glucosinolates. The use of sinigrin as a reference standard for glucosinolate quantification has been previously reported in *Raphanus sativus* and other Brassicaceae species [21]. The calibration curve and validation parameters are provided in Figure S2.

2.5. Statistical Analysis

Pearson correlation analysis was conducted using IBM SPSS Statistics version 27 (IBM Corp., Armonk, NY, USA) to evaluate the relationships among glucosinolate contents and morphological traits. Correlation coefficients (r) were calculated, and statistical significance was determined at $p < 0.05$ and $p < 0.01$ with a 95% confidence level. Principal component analysis (PCA) was performed using RStudio (version 2022.02.3 + 492 “Prairie Trillium”) with R version 4.3.2 (R Foundation for Statistical Computing, Vienna, Austria). The R packages FactoMineR, factoextra, ggplot2, and ggrepel were used for dimensionality reduction and biplot visualization. Two datasets were analyzed separately, comprising morphological traits and glucosinolate contents. All variables were mean-centered and scaled to unit variance prior to PCA. Pearson correlation used pairwise complete observations. For PCA, missing values were imputed by variable-wise medians before scaling. Biplots were generated to visualize accession distribution and variable contributions to the principal components.

3. Results

3.1. Identification of Glucosinolates in Radish

A comprehensive analysis was conducted on 72 radish (*R. sativus* var. *sativus*) accessions, consisting of 69 germplasm and 3 cultivars, representing diverse origins such as Türkiye, South Africa, Russia, Denmark, Afghanistan, Nepal, Kazakhstan, United States, Israel, Vietnam, both South and North Korea, Poland, Uzbekistan, Kyrgyzstan, Libya, Thailand, Germany, France, Yemen, Chile, Egypt, Bulgaria, Malaysia, Japan, and others, to evaluate the quantitative variation of seven glucosinolates (GSLs) (Table S1).

The identified compounds were glucoraphanin (GRA), glucoraphenin (GRE), glucorucin (GER), glucoraphasatin (GPA), glucobrassicin (GBS), glucobarbarin (GBB), and glucobetteroin (GBT), which exhibited remarkable differences in abundance across accessions (Table 1).

Table 1. Glucosinolate content (mg/kg dry weight (DW)) in 72 radish (*Raphanus sativus* L. var. *sativus*) accessions, comprising 69 germplasm and 3 cultivars ($n = 3$).

Samples	GRA	GRE	GBB	GER	GPA	GBS	GBT	SUM
1	101.2	207.9	ND ^a	119.9	1787.9	119.2	233.5	2569.5
2	59.2	181.7	ND	81.4	1653.4	ND	141.2	2116.9
3	38.2	169.2	ND	69.0	1323.0	158.5	753.2	2511.1
4	14.3	144.6	ND	40.2	893.0	19.6	231.2	1342.9
5	51.7	238.7	ND	62.9	1438.8	41.7	162.4	1996.1
6	44.3	190.8	ND	55.0	1204.7	194.5	247.0	1936.4
7	34.1	221.4	ND	62.3	1533.6	226.8	224.5	2302.6
8	ND	127.6	ND	92.2	793.5	73.0	78.2	1164.4
9	19.9	157.6	ND	ND	816.2	100.3	551.6	1645.5
10	10.5	205.1	3976.9	208.8	747.1	10.4	153.7	5312.4
11	11.9	226.2	1520.8	714.1	642.1	16.2	94.9	3226.1
12	ND	133.3	323.1	938.9	508.0	17.9	184.5	2105.7
13	56.1	237.1	ND	91.9	1556.0	205.2	313.1	2459.3
14	36.7	125.6	ND	47.5	1134.4	37.4	99.5	1481.1
15	79.1	201.8	ND	137.1	2082.2	56.7	232.3	2789.1

Table 1. Cont.

Samples	GRA	GRE	GBB	GER	GPA	GBS	GBT	SUM
16	97.6	114.3	ND	67.8	1321.9	27.4	113.6	1742.6
17	64.6	125.3	ND	97.4	1791.0	49.9	181.2	2309.5
18	57.1	153.6	ND	164.7	1654.8	24.1	221.3	2275.5
19	120.2	206.9	ND	928.6	977.2	ND	161.3	2394.2
20	49.6	112.5	ND	102.6	1883.9	306.4	169.7	2624.7
21	92.0	168.8	ND	107.4	1906.8	523.1	201.8	2999.8
22	45.7	157.1	ND	79.9	1239.5	269.3	156.3	1947.7
23	21.6	132.3	ND	85.2	1246.5	105.3	251.7	1842.6
24	27.7	155.5	ND	63.8	726.8	24.7	152.9	1151.4
25	15.4	130.9	ND	21.7	866.7	22.2	72.8	1129.7
26	63.7	158.5	ND	76.7	1414.0	26.7	226.5	1965.9
27	90.4	202.2	ND	64.1	1496.6	37.7	108.8	1999.8
28	ND	131.1	ND	335.5	1016.6	98.0	240.1	1821.3
29	22.3	99.4	ND	133.0	1757.6	171.3	243.8	2427.4
30	40.2	140.9	ND	211.9	1877.1	382.3	192.0	2844.4
31	35.1	172.5	ND	118.1	2039.5	226.4	279.0	2870.6
32	15.1	138.7	ND	71.0	917.2	40.1	68.5	1251.4
33	65.0	110.5	ND	91.2	1718.2	100.7	306.6	2392.2
34	17.2	84.4	ND	57.3	731.1	81.2	164.0	1135.3
35	ND	111.3	ND	860.2	421.2	24.3	52.4	1469.4
36	30.0	142.2	87.7	146.7	1650.1	53.0	335.3	2444.9
37	30.5	86.1	ND	79.0	1714.9	33.2	639.0	2582.6
38	56.8	323.4	ND	225.0	3557.3	171.5	659.2	4993.3
39	29.0	252.3	ND	155.0	3515.4	106.5	334.0	4392.3
40	50.0	612.9	ND	125.5	1992.3	279.8	165.5	3225.9
41	37.7	177.1	ND	201.5	1826.4	173.6	110.3	2526.6
42	35.6	55.4	ND	58.3	1448.2	20.9	210.1	1828.5
43	33.4	130.6	ND	217.7	2339.2	208.4	206.4	3135.5
44	13.8	119.3	ND	72.9	1871.2	312.9	218.8	2608.9
45	55.2	203.6	ND	40.5	401.0	38.5	120.5	859.3
46	38.0	121.9	ND	8.3	453.3	44.0	193.0	858.5
47	11.2	116.3	ND	62.8	1620.5	147.5	229.7	2188.0
48	17.8	173.8	ND	27.4	719.0	54.2	138.8	1131.0
49	23.0	32.9	ND	106.3	1927.3	136.1	264.1	2489.6
50	29.7	109.9	ND	107.8	2057.9	165.6	380.4	2851.2
51	47.5	136.3	ND	111.6	1929.3	141.8	249.0	2615.5
52	18.4	112.2	ND	269.4	759.7	84.7	465.1	1709.4
53	44.2	93.6	ND	623.6	763.7	200.6	59.9	1785.5
54	44.8	37.5	ND	82.9	1594.6	266.5	309.6	2335.9
55	31.4	201.2	ND	132.5	1968.8	252.4	365.3	2951.5
56	55.3	243.3	ND	167.1	2124.0	322.7	278.0	3190.5
57	47.5	153.5	ND	86.7	1734.8	391.0	347.8	2761.3
58	75.1	56.9	ND	77.9	1422.1	95.7	506.7	2234.4
59	39.7	42.1	ND	38.3	639.2	26.4	416.1	1201.8
60	40.8	56.5	ND	61.7	1343.0	91.0	345.6	1938.5
61	41.0	144.8	ND	122.6	1867.2	326.6	399.7	2901.9
62	50.5	52.3	ND	122.2	1620.8	156.6	299.8	2302.3
63	67.9	85.9	ND	158.6	2070.9	494.0	296.6	3173.9
64	68.9	56.2	ND	188.0	2178.8	415.8	384.6	3292.3
65	73.4	63.5	ND	81.7	1902.1	111.2	132.0	2364.0
66	58.3	207.8	ND	73.6	1671.7	144.4	109.4	2265.2
67	43.4	83.6	ND	70.6	1523.6	95.0	130.5	1946.7
68	54.9	66.9	ND	72.3	1595.1	187.3	114.0	2090.4
69	18.1	88.1	ND	91.2	2104.9	160.9	289.3	2752.5
C1 ^b	38.2	145.6	ND	70.1	1660.5	13.0	144.4	2071.8
C2	35.5	173.9	ND	69.5	1713.7	18.9	147.7	2159.2
C3	34.4	237.4	ND	76.4	1768.6	3.1	113.1	2233.0

Note. GRA: Glucoraphanin, GRE: Glucoraphenin, GBB: Glucobarbarin, GER: Glucoerucin, GPA: Glucoraphasatin, GBS: Glucobrassicin, GBT: Glucobertoin. ^a Not detected, ^b commercially available common cultivars.

Glucoraphasatin was the most abundant compound across the majority of accessions, with the highest concentrations detected in 38 (IT262009, Germany) and 39 (IT262067, North Korea), at 3557.3 and 3515.4 mg/kg DW, respectively. Glucoraphenin showed intermediate levels, reaching its maximum in 40 (IT262091, North Korea) at 612.9 mg/kg DW, while glucoraphanin was generally minor, though 19 (IT215036, Republic of Korea) accumulated up to 120.2 mg/kg DW. Glucoerucin was mostly low to moderate but peaked in 12 (IT204244, Israel) at 938.9 mg/kg DW, distinguishing this accession from the rest.

Indolic glucobrassicin was mostly absent or at low levels, yet 21 (IT 215044, Republic of Korea) contained 523.1 mg/kg DW, suggesting enrichment in specific accessions. Glucobacteroin was consistently detected in all accessions, with a median around 220.0 mg/kg DW, and the highest content observed in 3 (IT102573, Unknown) at 753.2 mg/kg DW. In contrast, glucobarbarin was absent in most accessions, but 10 (IT203308, Russia) reached an exceptionally high level of 3976.9 mg/kg DW, identifying it as an accession exhibiting higher glucobarbarin accumulation under the evaluated conditions.

Distinct accession-level profiles were evident. For example, 1 (IT102447, Türkiye) exhibited co-accumulation of glucoraphasatin (1787.9 mg/kg DW) and glucoraphenin (207.9 mg/kg DW). Accessions 12 (IT204244, Israel), 19 (IT215036, Republic of Korea), and 35 (IT261962, Russia) were characterized by relatively high glucoerucin contents, with 938.9, 928.6, and 860.2 mg/kg DW, respectively. In addition, 43 (IT302251, Russia) showed a relatively high total GSL content of 3135.5 mg/kg DW, mainly due to glucoraphasatin accumulation. The control cultivars (C1–C3, Republic of Korea) showed moderate total GSL contents ranging from 2071.8 to 2233.0 mg/kg DW, rather than representing the lowest-GSL group.

Total glucosinolate contents varied greatly, ranging from 858.5 mg/kg DW in accession 46 (IT305139, Yemen) to 5312.4 mg/kg DW in accession 10 (IT203308, Russia), representing more than a sixfold difference across the panel. High-GSL accessions such as 10 (IT203308, Russia), 38 (IT262009, Germany), and 39 (IT262067, North Korea) may represent potential donor resources for breeding functional radish varieties. However, as these values were obtained from root tissues grown in a single environment, further validation across multiple environments and growing seasons is required before these accessions can be reliably used in breeding programs. Conversely, low-GSL accessions, including 46 (IT305139, Yemen), 45 (IT305137, France), 25 (IT224118, China), 48 (IT305177, Chile), and 34 (IT261948, Kyrgyzstan), may represent germplasm with relatively low glucosinolate accumulation.

These results demonstrate that while aliphatic GSLs dominate the overall profile of radish germplasm, significant quantitative and qualitative variation exists among accessions. The identification of unique outliers, including 10 (IT203308, Russia, glucobarbarin-enriched) and 38 (IT262009, Germany) and 39 (IT262067, North Korea), both glucoraphasatin-enriched, reflects distinct glucosinolate accumulation patterns observed under the evaluated conditions within certain landraces and underscores the importance of conserving and utilizing diverse germplasm for breeding and functional applications.

3.2. Phenotypic Variation Among Accessions

The evaluated *R. sativus* var. *sativus* accessions displayed wide phenotypic variation based on the comprehensive characterization of traits summarized in Table 2, with the full dataset provided in Table S2. Root-related characteristics showed marked diversity, ranging from round to elongated/conical morphologies, with substantial differences in root length, diameter, and fresh weight. Such variation reflects contrasting storage-organ development strategies across accessions. Considerable heterogeneity was also observed in skin and flesh coloration, encompassing white, red, purple, pink, black, and green pigmentation patterns, although white predominated in both root skin (48.6%) and flesh (86.1%).

Table 2. Summary of phenotypic traits across 72 radish accessions.

Quantitative (Min–Max (Mean $\pm$ SD))	
Trait	Categories/Statistics
Root weight (g)	4.8–2783.3 (813.6 $\pm$ 660.5)
Root length (cm)	2.8–44.0 (15.3 $\pm$ 8.1)
Root diameter (cm)	1.4–15.0 (8.7 $\pm$ 3.5)
Leaf weight (g)	4.3–2438.3 (470.9 $\pm$ 407.1)
Leaf length (cm)	10.2–61.3 (37.9 $\pm$ 12.4)
Leaf width (cm)	4.6–19.3 (13.6 $\pm$ 3.8)
Leaf number	6–60 (24.1 $\pm$ 11.4)
Qualitative (Category: <i>n</i> (%))	
Trait	Categories/Statistics
Root skin color	White 35 (48.6), Red 13 (18.1), Purple 8 (11.1), Pink 7 (9.7), Black 5 (6.9), Green 4 (5.6)
Root flesh color	White 62 (86.1), Green 5 (6.9), Pink 4 (5.6), Red 1 (1.4)
Root base shape	Blunt 28 (38.9), Pointed 21 (29.2), Convex 10 (13.9), Flat 9 (12.5), Concave 4 (5.6)
Root shape	Elliptic 14 (19.4), Ovate 13 (18.1), Round 13 (18.1), Oblate ellipse 8 (11.1), Broad rectangular 8 (11.1), Rectangular 5 (6.9), Icicle-shaped 4 (5.6), Branched 2 (2.8), Narrow inverted triangular 2 (2.8), Narrow rectangular 1 (1.4), Others 1 (1.4)
Root texture	Hard 42 (58.3), Extremely hard 14 (19.4), Intermediate 9 (12.5), Soft 4 (5.6), Extremely soft 3 (4.2)
Root shoulder color	Green 20 (27.8), White 18 (25.0), Red 13 (18.1), Purple 9 (12.5), Pink 6 (8.3), Black 5 (6.9), Brown 1 (1.4)
Lateral rooting	Completely buried 21 (29.2), Half exposed 21 (29.2), Mostly buried 18 (25.0), Mostly exposed above soil 9 (12.5), Completely exposed above soil 3 (4.2)
Hypocotyl color	White 26 (36.1), Green 20 (27.8), Purple 13 (18.1), Red 13 (18.1)
Plant type (leaf angle) *	~20° 24 (33.3), ~45° 21 (29.2), 0° 15 (20.8), ~65° 6 (8.3), 90° 6 (8.3)
Plant vigor	Intermediate 31 (43.1), Strong 20 (27.8), Weak 17 (23.6), Very weak 4 (5.6)
Leaf color	Green 67 (93.1), Yellowish green 5 (6.9)
Leaf shape	Ovate 34 (47.2), Broad ovate 22 (30.6), Narrow ovate 16 (22.2)
Leaf lobing	Present 60 (83.3), Absent 12 (16.7)
Leaf pubescence	Few 30 (41.7), Intermediate 21 (29.2), Absent 15 (20.8), Many 6 (8.3)
Wind damage tolerance	Sound 44 (61.1), Very slight 9 (12.5), Slight 6 (8.3), Many cavities 6 (8.3), Intermediate 6 (8.3), Small cavity 1 (1.4)

Note. * Plant type was evaluated by measuring the angle of leaf insertion relative to the ground surface, expressed in degrees (°). Values for quantitative traits are presented as minimum–maximum (mean $\pm$ SD), based on three representative plants per accession. Values for qualitative traits are presented as the number of accessions (*n*) and their percentage (%) within each category; percentages may not total 100% due to rounding.

Shoot traits showed broad diversity, and leaf morphology, including leaf length, width, and shape, varied markedly among accessions, as did leaf number and overall plant vigor, indicating the presence of multiple canopy architectures and growth habits within the germplasm pool.

Trait associations were evident in several cases. Accessions with thicker root diameters generally produced heavier roots, while those with longer leaves also tended to exhibit broader leaf blades and greater leaf biomass. In contrast, pigmentation traits were largely independent from vegetative size, suggesting distinct genetic or regulatory controls.

Overall, the phenotypic dataset underscores the multidimensional variation present in radish germplasm, encompassing both belowground and aboveground traits. These results provide a valuable foundation for yield-focused breeding programs that target root size and morphology, as well as diversification strategies emphasizing pigmentation and canopy characteristics. Furthermore, this phenotypic baseline enables integrative analyses with glucosinolate composition in subsequent sections.

3.3. Pearson Correlation Between GSLs and Traits

Pearson correlation analysis was conducted to evaluate the relationships between glucosinolate (GSL) contents and morphological traits across 72 radish accessions. Overall, phenotypic traits were not consistently associated with GSL profiles, although several compound-specific correlations were observed. Glucoraphenin showed almost no asso-

ciation with root weight or root diameter, whereas glucoraphasatin exhibited moderate positive correlations with root weight ($r = 0.416, p < 0.01$), root length ($r = 0.381, p < 0.01$), root diameter ($r = 0.519, p < 0.01$), leaf weight ($r = 0.330, p < 0.01$), leaf length ($r = 0.513, p < 0.01$), and leaf width ($r = 0.565, p < 0.01$). Glucoraphanin also showed weak to moderate correlations with several traits, including root skin and leaf-related traits. However, these relationships were compound-specific and did not indicate a general predictive relationship between external morphology and overall GSL accumulation.

Taken together, these findings indicate that glucosinolate accumulation patterns are not consistently explained by external phenotypic variation in radish germplasm. Although some significant compound-specific correlations were detected, no single morphological trait reliably predicted total or individual GSL accumulation across the entire panel (Figure 2).

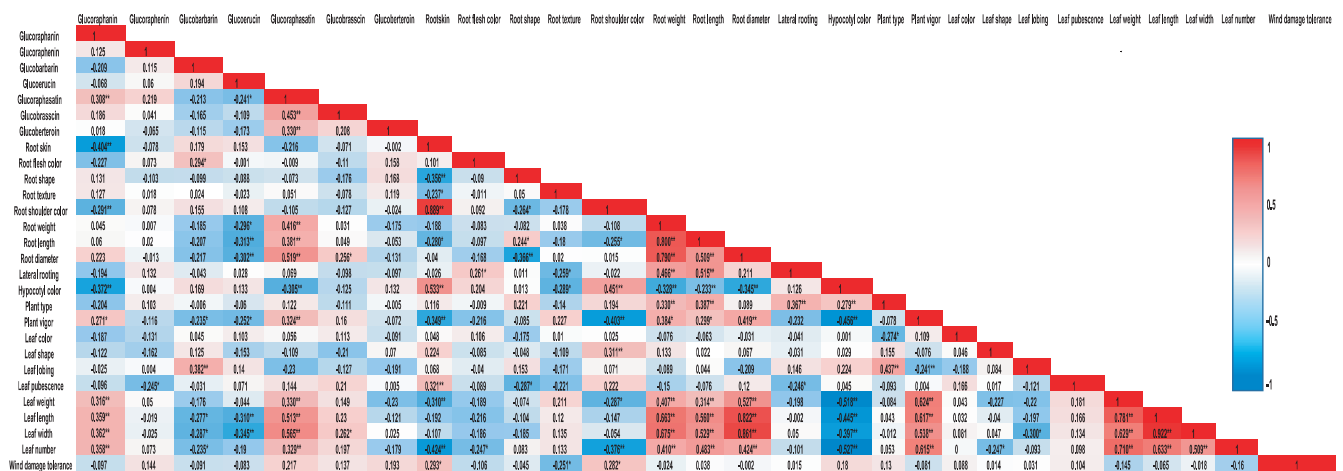


Figure 2. Pearson correlation coefficients between glucosinolate (GSL) contents and morphological traits in 72 radish accessions. Positive and negative values indicate positive and negative associations, respectively. Significance levels are indicated as follows: * $p < 0.05$ (2-tailed), ** $p < 0.01$ (2-tailed).

3.4. Principal Component Analysis of Glucosinolate Contents and Phenotypic Traits

Principal component analysis (PCA) of glucosinolate (GSL) profiles (Figure 3) revealed clear separation among accessions along the first two principal components, which together explained 46.9% of the total variance. Several accessions positioned at the margins of the PCA plot displayed distinctive chemotypic patterns. Accessions 10 (IT203308, Russia), 11 (IT204238, United States), and 12 (IT204244, Israel) were located on the positive side of PC1, mainly reflecting variation in glucobarbarin and glucoerucin levels; accession 35 (IT261962, Russia) showed enrichment primarily in glucoerucin. Accession 19 (IT215036, Republic of Korea) was placed in the upper quadrant and was mainly characterized by relatively high glucoerucin and glucoraphanin contents, rather than by glucoraphenin accumulation. On the opposite side, accession 38 (IT262009, Germany) was characterized by high glucoraphasatin and glucoberteroin contents, whereas accession 40 (IT262091, North Korea) was distinguished by the highest glucoraphenin content. In addition, accessions 34 (IT261948, Kyrgyzstan) and 59 (IT306725, Malaysia) were separated from the central cluster and appeared as outliers at the margins of the PCA plot without a clear dominance of specific GSLs. Accessions 37 (IT261985, Thailand) and 64 (IT321039, Republic of Korea) clustered on the lower side of PC2, showing stronger association with glucoberteroin compared with the central cluster. Together, these accessions represent chemotypic outliers clearly distinguished from the majority of accessions that were positioned near the center with intermediate or mixed glucosinolate compositions. Representative photographs of these distinctive accessions are presented in Figure S1.



Figure 3. Principal component analysis (PCA) of glucosinolate (GSL) contents in 72 radish accessions. Each accession is represented by a number. Red symbols indicate “20-day radish” accessions, while blue symbols represent all other radish accessions. Accessions labeled as “C” denote control cultivars. The labels positioned around the biplot correspond to the names of individual GSL compounds.

For phenotypic traits (Figure 4), PC1 (28.2%) and PC2 (13.9%) explained a total of 42.1% of the variance. In the phenotypic PCA, accessions 10 (IT203308, Russia), 11 (IT204238, United States), 12 (IT204244, Israel), and 35 (IT261962, Russia) were characterized by relatively small root and shoot traits, whereas accessions such as 41 (IT293115, Republic of Korea) and 68 (IT334026, Republic of Korea) exhibited larger root or shoot traits. These patterns indicate that the phenotypic PCA primarily separated accessions according to size-related traits, including root weight, root diameter, leaf weight, leaf length, and leaf width. PC2 captured pigmentation and morphological variation: accessions such as 26 (IT250741, Unknown), 42 (IT 293382, Russia), 55 (IT305391, North Korea), and 57 (IT305396, North Korea) appeared on the positive side with more pronounced pigmentation and distinct morphotypes, while accessions such as 32 (IT261945, Russia), 34 (IT261948, Kyrgyzstan), and 46 (IT305139, Yemen) were positioned on the negative side, representing less pigmented or less differentiated morphologies.

Together, these PCA results demonstrate that specific accessions occupy distinct metabolic or morphological spaces, while many remain grouped centrally with intermediate characteristics, underscoring both the diversity and continuity within the radish germplasm. It should be noted, however, that the first two principal components explained only 46.9% (GSL) and 42.1% (phenotypic) of the total variance, indicating that a substantial proportion of the variation was not captured by these axes. Accordingly, the separation patterns described here should be regarded as major descriptive trends rather than statisti-

cally defined clusters, and the positions of individual accessions in the biplots should be interpreted with caution.

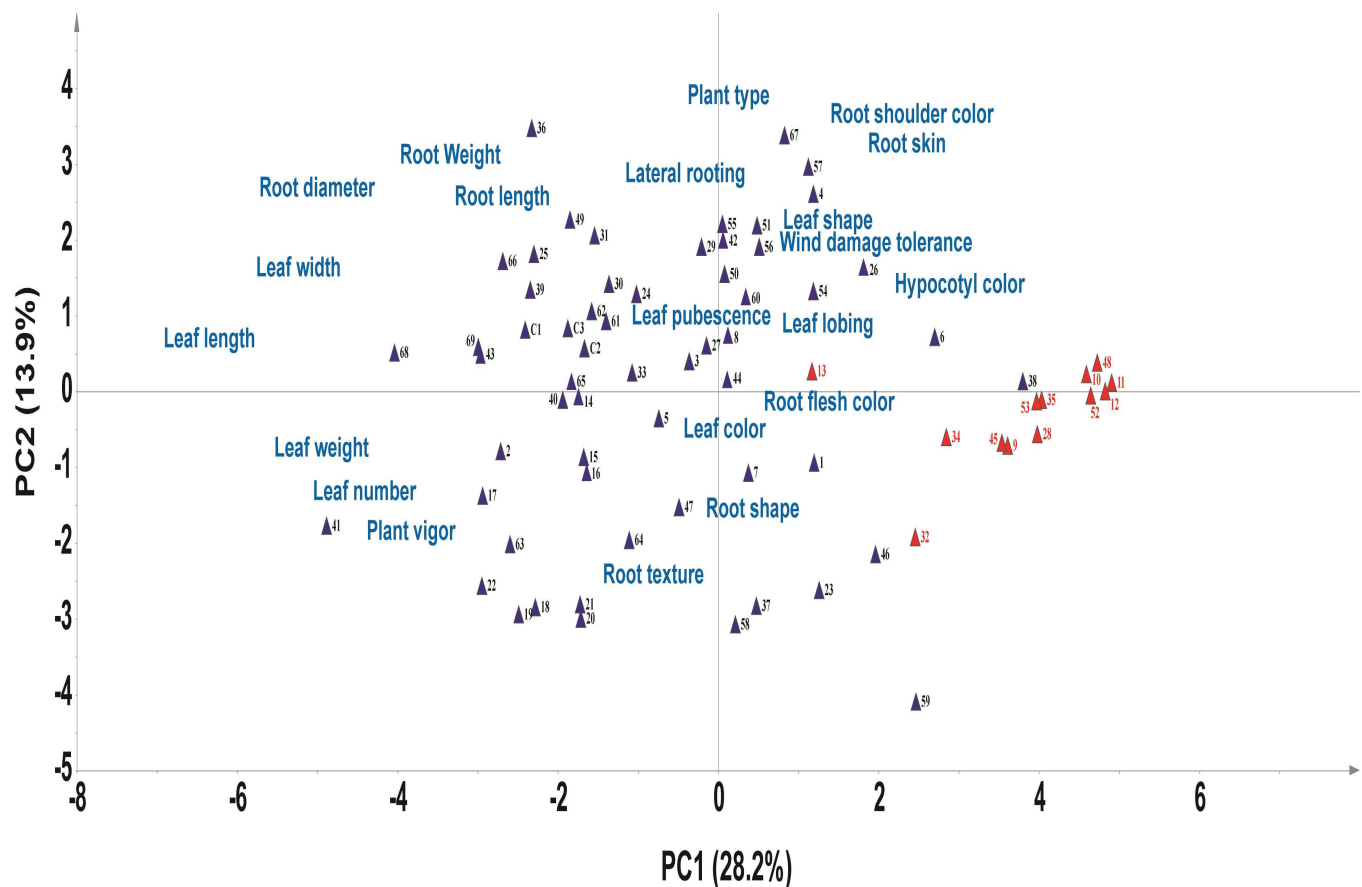


Figure 4. Principal component analysis (PCA) of phenotypic traits in 72 radish accessions. Each accession is represented by a number. Red symbols indicate “20-day radish” accessions, while blue symbols represent all other radish accessions. Accessions labeled as “C” denote control cultivars. Text labels correspond to the investigated phenotypic trait variables included in the PCA.

4. Discussion

This study revealed extensive variation in glucosinolate (GSL) composition and morphological traits among 72 radish accessions, consisting of 69 germplasm and 3 cultivars, thereby extending the scope of earlier investigations that examined smaller germplasm sets or focused on single traits. Importantly, correlation analyses (Figure 2) indicated that GSL accumulation and external phenotypes were largely independent, confirming that metabolic and agronomic traits should be considered separately rather than as proxies for each other [8,11].

Chemotypic outliers identified in the PCA plots highlight the diversity of radish GSL profiles. While glucobarbarin is generally absent or detected only in trace amounts in most radish accessions [10,22], accession IT 203308 (10, Russia) exhibited exceptionally high levels, clearly separating it from the majority. Similarly, glucoerucin was enriched in 11 (IT204238, United States) and 12 (IT204244, Israel), complementing previous reports that radish germplasm is usually dominated by aliphatic compounds [23,24]. Glucoraphenin was found at the highest level in accession 40 (IT262091, North Korea), whereas earlier studies emphasized its enrichment primarily in sprouts [24]. On the other hand, glucoraphasatin was particularly abundant in accessions 38 (IT262009, Germany) and 39 (IT262067, North Korea), whereas glucoberteroin was relatively high in accessions 3 (IT102573, Unknown), 38 (IT262009, Germany), and 37 (IT261985, Thailand). These results are consistent with the

recognition of these compounds as major aliphatic GSLs showing broad variability across radish landraces [22,23]. From an applied perspective, the choice of a source accession for commercial glucosinolate extraction depends not only on concentration but also on root biomass since the total recoverable yield is the product of concentration and root weight. Although accessions 38 (IT262009, Germany) and 39 (IT262067, North Korea) exhibited comparable glucoraphasatin concentrations (3557.3 and 3515.4 mg/kg DW, respectively), their root biomass differed markedly (27.2 g vs. 1813.3 g, respectively). Consequently, accession 39 would be the far more practical choice for commercial glucoraphasatin extraction, as it combines a high concentration with a much greater root biomass, whereas accession 38, despite its similarly high concentration, would yield only a small amount of total compound per plant. This example illustrates that agronomic performance and metabolite concentration must be considered jointly when selecting donor resources for practical applications.

Additional PCA-defined outliers included 34 (IT261948, Kyrgyzstan), 37 (IT261985, Thailand), 64 (IT321039, Republic of Korea), and 59 (IT306725, Malaysia); these accessions were separated from the central cluster in the biplot, but without a consistent dominance of specific GSLs across the dataset. This indicates that certain accessions occupy distinct chemotypic positions that cannot be fully explained by major compound enrichment alone.

Morphological variation was equally pronounced, encompassing pigmentation, canopy architecture, and root development, in line with earlier reports that documented wide morphological diversity in radish germplasm [15,16]. However, by combining these traits with metabolic data, the present study demonstrated that GSL accumulation could not be reliably predicted from external phenotypes, despite the presence of several weak to moderate compound-specific correlations (Figure 2). This independence corresponds with findings in Brassicaceae that secondary metabolite pathways segregate under separate genetic and environmental control [8]. For example, vernalization in radish has been reported to alter GSL metabolism without major morphological changes [11]. Although the present study was not designed to test these mechanisms directly, the observed correlations can be interpreted in light of several possibilities reported in related systems. The few significant correlations observed, such as the moderate positive associations between glucoraphasatin and root or leaf size (Figure 2), may reflect pleiotropic effects of shared regulatory genes or co-regulation during storage-organ development, rather than a direct functional link. Conversely, the general absence of strong correlations may indicate metabolic trade-offs, whereby carbon and sulfur resources are partitioned between growth-related processes and secondary metabolite biosynthesis. Because glucosinolate accumulation is additionally modulated by environmental conditions and developmental stage, the correlations reported here should be regarded as condition-specific rather than fixed relationships. Multi-environment and developmental-stage studies would be needed to disentangle these genetic, metabolic, environmental, and developmental contributions.

Previous studies often suggested morphological descriptors or geographic origin as practical criteria for identifying promising germplasm [15,16]. In contrast, our results demonstrate that rare or enriched GSL compositions cannot be reliably predicted from phenotype alone. This distinction underscores the importance of direct metabolic profiling when selecting germplasm for functional applications.

The glucosinolate profiles characterized here also carry potential nutritional and functional relevance. Both the total glucosinolate concentration and the compound-specific composition may determine the functional value of each accession, as aliphatic glucosinolates such as glucoraphasatin and glucoraphenin and the indole glucosinolate glucobrassicin are precursors of biologically active breakdown products previously linked to antioxidant and health-promoting properties [2,5,21]. This highlights that individual glucosinolate

profiles, rather than total content alone, should be considered when evaluating accessions. In this broader context, the accurate characterization of plant secondary metabolites is fundamental to linking chemical diversity with functional value [25], and the selection and optimization of extraction and analytical strategies critically influence the reliable recovery and quantification of bioactive compounds in complex plant matrices [26]; here, the desulfoglucosinolate-based UPLC approach provided consistent profiling across all 72 accessions.

Some limitations should be noted. The present work focused on root tissues under a single environment, while GSL profiles can differ among organs, developmental stages, and environments. Furthermore, the present study characterized biochemical (glucosinolate) diversity without accompanying molecular marker analysis; therefore, the observed chemotypic variation does not necessarily reflect underlying genetic divergence among accessions. Future integration of molecular diversity analyses, such as SSR or SNP genotyping, would help clarify the genetic basis of the glucosinolate variation reported here. The single-season, single-environment design is expected to affect the results in a specific manner: while the overall range of chemotypic and phenotypic diversity and the identification of distinct outlier accessions are likely to be robust, the absolute values and the relative ranking of individual accessions may shift under different environments or years, as glucosinolate accumulation is environmentally responsive. Replicated trials using a fixed plot size to measure root yield across multiple environments and years would allow the stability of these profiles to be quantified and would strengthen the identification of consistently promising genotypes. Accordingly, the present findings are best regarded as a robust first-season baseline rather than definitive selections, and confirmation of individual accessions for breeding should be considered preliminary until validated under multi-environment conditions. Despite this, by combining metabolic and morphological data across a diverse panel, this study provides a more comprehensive baseline than earlier radish evaluations and highlights the value of integrated approaches.

5. Conclusions

This study revealed substantial variation in glucosinolate composition and phenotypic traits among 72 radish accessions, with glucosinolate composition analyzed exclusively in root tissue. Under the evaluated conditions, several accessions exhibited distinctive chemotypes, including glucobarbarin-enriched (accession 10), glucoraphasatin-enriched (accessions 38 and 39), glucoraphenin-enriched (accession 40), and glucoerucin-enriched (accessions 12, 19, and 35) accessions. Correlation and PCA results indicated that glucosinolate profiles could not be reliably predicted from external phenotypic traits, highlighting the importance of direct biochemical profiling for germplasm evaluation. These accessions may serve as potential donor resources for breeding radish with improved glucosinolate-related quality. However, as glucosinolates were assessed only in root tissue under a single season, further validation across multiple environments, developmental stages, and tissues will be required to confirm the stability of these profiles and the associated agronomic traits of the root and foliage.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agronomy16151425/s1>. Figure S1: Calibration curve of sinigrin used as an external standard for glucosinolate quantification. Figure S2: Representative radish accessions identified as outliers from principal component analysis (PCA) of glucosinolate (GSL) contents. Table S1: Accession number and origin of 72 radish (*Raphanus sativus* L. var. *sativus*) accessions, comprising 69 germplasm and 3 cultivars. Table S2: Phenotypic characterization of 72 radish (*Raphanus sativus* L. var. *sativus*) accessions, comprising 69 germplasm and 3 cultivars, based on root and shoot traits.

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