Determination of glucosinolate contents in *Brassica* germplasm collections and inter- & intra-leaves distribution pattern using UPLC-MS/MS Multiple Reaction Monitoring scan mode

Awraris Derbie Assefa[1], Susanna Choi[1], Jae-Eun Lee[1], Jung-Sook Sung[1], On-Sook Hur[1], Na-Young Ro[1], Ho-Sun Lee[1], Jaejong Noh[1], Aejin Hwang[1], Ho-Cheol Ko[2], Yun-Jo Chung[3], Ju-Hee Rhee[1]*

[1]National Agrobiodiversity Center, National Institute of Agricultural Sciences, RDA, Jeonju 54874, Rep. of Korea

[2] Client Service Division, Planning and Coordination Bureau, RDA, Jeonju 54875, Rep. of Korea

[3] National Creative Research Laboratory for Ca^{2+} signaling Network, Chonbuk National University Medical School, Jeonju, 54896, Rep. of Korea

*Corresponding author: E-mail:- rheehk@korea.kr ; Tel. +82632384813

Abstract

Intact glucosinolate (GSL) profile (five aliphatic; three aromatic, and one indolic glucosinolate) in the leaves of 50 germplasm collections and commercial cultivars of *Brassica rapa*, *Brassica juncea*, and *Brassica oleracea* collected from six different countries and grown under uniform cultural conditions were compared by UPLC-MS/MS. Total GSLs content ranged from 36.80 to 2383.12 μmol/kg DW. Aliphatic GSLs predominated among the entire samples representing from 23.0 to 98.9% of the total GSLs content, where gluconapin and glucobrassicanapin contributed the greatest proportion. Other GSLs such as, progoitrin (PRO), glucotropaeolin (TRO), and glucobarbarin (BAR) were found in relatively low concentrations. Principal Component Analysis (PCA) yielded three principal components with eigenvalue ≥ 1, representing 70.33% of the total variation across the entire data set. Accessions IT260822 & IT32750, and commercial cultivar, "Hangamssam2", were well distinguished from other samples in the PCA plot due to their significantly high amount of BAR, glucobrassicin (GBC), and glucoerucin (ERU), respectively. The inter- and intra-leaf variations of GSLs were examined in three kimichi cabbage varieties. The GSLs content varied significantly among leaves in different positions (outer, middle, and inner) and sections within the leaves (top, middle, bottom, green/red, and white). Higher GSL contents were observed in the proximal half & white section of the leaves and inner positions (younger leaves) in most of the samples. GBC, gluconasturtiin (NAS), and glucoberteroin (BER) should be studied profusely in *Brassica* plants as some of their degradation products of GBC and NAS are useful in cancer chemopreventive functions, whereas BER takes part in the process of suppressing aging of the skin. GSLs are regarded as allelochemicals; hence, the data related to the patterns of GSLs within the leaf and between leaves at different position could be useful to understand the defense mechanism of *Brassica* plants. The observed variability could be useful for breeders to develop *Brassica* crops with high GSL content or specific profiles of GSLs as required.

Introduction

Glucosinolates (GSLs) also called β-thioglucoside-N-hydroxysulfates are class of sulfur-containing important plant secondary metabolites naturally occurring in almost all *Brassica* species [1]. GSLs could be classified as aliphatic, aromatic, and indolic glucosinolates based on their side chain structure (R group) which are derived from the amino acid precursors methionine (but also alanine, leucine, isoleucine, or valine in some cases), phenylalanine, and tryptophan, respectively [2]. Most glucosinolates share a basic chemical structure consisting of a β-D-glucopyranose residue linked via a sulfur atom to a (*Z*)-N-hydroximinosulfate ester and a variable R group [3]. Upon hydrolysis by myrosinases, glucosinolates produce several bioactive products including, isothiocyanates, thiocyanates, and nitriles. Glucosinolates and their biosynthetic products are implicated to reduce the risk of cancer in human [4, 5]. Reports also show that breakdown products of GSLs displayed antimicrobial activity [6, 7]. Although their contribution is complex to understand, GSLs are regarded as an important component of flavor in cooked vegetables [8]. GSLs and/or their degradation products serve as a feeding deterrent to wide range of herbivores such as birds, mammals, mollusks, aquatic invertebrates, nematodes, bacteria, and fungi [8, 9]. On the other hand, they also serve to attract and stimulate specialist herbivores such as the larvae of the lepidopteran species *Plutella xylostella* and *Pieris rapae* [9]. The biocidal activity of glucosinolate containing *Brassica* plants made them as a promising alternative to synthetic pesticides for pest and disease control [10]. *In planta* studies of various Brassicaceae seedlings have also showed a positive correlation between specific and/or total GLS contents and disease severity [11].

Glucosinolates are found in the vegetative and reproductive tissues of various dicotyledonous plant families, and are the major secondary metabolites in mustard-oil plants of *Brassicaceae* family [3, 12]. The content of glucosinolate accounts about 1% of the dry weight in *Brassica* vegetables and can go up to 10% in seeds of some plants [3]. The qualitative and quantitative profiles of total and individual GSLs in Brassica vegetables varies significantly due to several factors such as cultivar genotype [13, 14], developmental stage [15], environmental conditions (temperature, light, water, and soil) [16–19], growing seasons [20],

agricultural practices [21], level of insect damage [19, 22], and post-harvest conditions [23]. A wide geographic and evolutionary variation is recorded in broccoli [20], *Arabidopsis thaliana* [24], Chinese cabbage [14], and cabbage (*Brassica oleracea* L.) [25]. Apart from the aforementioned factors, glucosinolates tend to vary quantitatively and qualitatively based on plant part as observed in kale [19], in cabbage [17], and in Arabidopsis thaliana [15].

Analysis of glucosinolates in *Brassica* vegetables has been estimated using HPLC after extraction with boiling water/methanol followed by desulfation of the intact glucosinolates on sephadex-A25 columns [25]. However, desulfation process was seen to be time consuming [26] and some glucosinolates could be insufficiently desulphated at lower concentration of sulphatase [27]. GC-MS methods are often used for detailed analysis [28]. A simplified method of sample extraction and analysis of intact glucosinolates using UPLC-DAD-MS/MS in negative electron-spray ionization (ESI^-) mode and Multiple Reaction Monitoring (MRM) was employed [29]. In MRM, the precursor/parent ions are selected to make it through the first quadrupole and into the collision cell where they get fragmented. Certain fragment ions (also known as product or daughter ions) are selected to make it through the second quadrupole [30].

Leaves of kimichi cabbage, turnip, Brassica, mibuna, leaf mustard, and cabbage are commonly used for various dishes in many countries. Kimichi cabbage, is a major ingredient in “kimchi”-a widely consumed traditional fermented food in Korea [14]. A number of comparative studies of the profiles of glucosinolates in *Brassica* germplasm collections are available in the literature [1, 13, 20, 31–33]. However, GSLs profiles in large germplasm collections of *Brassica rapa*, *Brassica juncea,* and *Brassica oleracea* is limited except the work of Lee et al. (2014) [14] who identified and quantified ten GSLs of breed varieties of kimichi cabbage collected from the Republic of Korea. Many studies have determined the glucosinolates contents in the seeds [6, 13, 34], and less attention has been given to glucosinolates composition of the leaf of *Brassica,* of which the leaf tissue are usually consumed. Many plant natural products, including glucosinolates, serve as defenses against herbivores [22]. It is important to determine the glucosinolates content in different tissues of the plant to understand their actual defense role a potential herbivore would encounter. In this study, we

have identified and quantified the contents of nine GSLs namely, gluconapin, glucobrassicanapin, progoitrin, glucotropaeolin, glucoerucin, gluconasturtiin, glucoberteroin, glucobarbarin, and glucobrassicin in 50 germplasm of *Brassica rapa* subsp. *pekinensis* (kimichi cabbage), *Brassica rapa* subsp. *rapa* (turnip), *Brassica rapa* subsp. *nipposinica* (mibuna), *Brassica juncea* var. *integrifolia* (leaf mustard), and *Brassica oleracea* var. capitate (cabbage) collected form six countries and grown in a uniform agricultural conditions in an attempt to identify differences due to genetic factors. In addition, the accumulation patterns of glucosinolates within and between leaves of kimichi cabbage cultivars were also evaluated.

Materials and methods

Reagents and standards

All chemicals and solvents used in extraction and analysis were of analytical grade and purchased from Fisher Scientific Korea Ltd. (Seoul, South Korea) and Sigma-Aldrich (St. Louis, MO, USA). Glucosinolate standards (gluconapin, glucobrassicanapin, progoitrin, glucotropaeolin, glucoerucin, gluconasturtiin, glucoberteroin, glucobarbarin, and glucobrassicin) were purchased from Phytoplan Diehm &Neuberger GmbH (Heidelberg, Germany). All individual GSLs standards were with purity greater than or equal to 97%.

Plant materials

A total of 50 *Brassica* crops, belonging to *Brassica rapa*, *Brassica juncea*, and *Brassica oleracea* species, and originated from six different countries (China, Ethiopia, Japan, North Korea, South Korea, and Taiwan) was grown at the research farm of the National Agrobiodiversity Center (NAC), Jeonju (35°49′18″ N 127°08′56″ E), Republic of Korea. Seeds of *Brassica* species were sown in plug trays, and seedlings were grown in a greenhouse. Healthy looking seedlings were transplanted to an experimental field. Planting density was 60 x 40 cm. Plant cultural practices were followed in the field as per the recommendation of the Rural Development Administration (RDA), South Korea. Each accession was consisted of 25 plants. Plant

114 growth was maintained using nutrient solution throughout the growing season.

115 To study the glucosinolate spatial distribution within sections of the leaf of kimichi cabbage and
116 between leaves, two green pigmented ("Hangamssam" and "Alchandul") and one red pigmented ("Bbalgang
117 3-ho") commercial cultivars were selected. The inner, middle, and outer leaves were separated. Each leaf
118 was then dissected into top, middle, bottom, green/red, and white part as required. Sampling positions of
119 kimichi cabbage plant are done as shown in Fig. 1.

120 **Fig 1. Representative photos of sampling positions of kimichi cabbage based on:**

121 **a) Leaf sections: I, III, III refers to the upper, middle and bottom part of the leaf. The white**
122 **section is indicated in a triangular dashed line. Green/red part was sampled from the whole**
123 **leaf excluding the white section.**

124 **b) Location of the leaf: I, II, and III refers to the outer (two layers), middle (three layers), and**
125 **inner (the remaining part) part of the leaves.**

126 ## Sample pre-treatment, extraction and analysis of glucosinolates

127 Samples were harvested, placed in vinyl freezer bag and kept at -80°C until further processing. The
128 frozen samples were subsequently lyophilized for 48 h using LP500 vacuum freeze-drier (Ilshin biobase Co.,
129 Seoul, Korea). The freeze-dried samples were then ground to a fine powder using a mortar and pestle, and
130 held at -80ºC until analysis. 0.1 gram of lyophilized sample was mixed with 1 mL of 80% methanol in a 2
131 mL Eppendorf tube, and sonicated in ultra-sonication bath for 10 min at 30°C. The mixture was centrifuged
132 using VS-180CFi centrifuge (Vision Scientific Co., Daejeon, Korea) (centrifuge conditions set at: 14000 rpm,
133 4°C, and 10 min). The supernatant was transferred into a vial and glucosinolates were analyzed immediately
134 using UPLC-MS/MS.

135 The GSLs were analyzed using an Acquity UPLC System (Waters, Milford, MA, USA) coupled to
136 Xevo™ TQ-S system (Waters, MS Technologies, Manchester, UK). Chromatographic separation was carried
137 out using Acquity UPLC BEH C18 (1.7µm, 2.1 × 100mm) column (Waters Corp., Manchester, UK). The
138 flow rate was kept at 0.25 mL/min; the column temperature was maintained at 35°C; and the injection
139 volume was 5 µL. The mobile phase was composed of 0.1% trifluoroacetic acid in water as eluent A and

0.1% trifluoroacetic acid in methanol as eluent B. Elution conditions were as follows: Initial condition set at 100% of A; 0.0 – 1.0 mins, 100 to 95% of A; 1.0 – 4.0 mins, 95 to 0 % A; 4.0 – 4.5 mins, 0 % of A; 4.5 – 5.0 mins, 0 to 100% of A; 5.0 – 10.0 mins, 100% of A. The mass spectrometry instrument was operated in negative ion electrospray ionization (ESI^-) mode and Multiple reaction Monitoring (MRM). Data acquisition was performed using MassLynx 4.1 software. For MS/MS detection, the ionization source parameters were set as follows: capillary voltage was 3kV; the ion source and the desolvation temperatures were set as 150°C 350°C, respectively. The cone gas (nitrogen) and desolvation gas (also nitrogen) were set at flow rates of 150 and 650 L/h, respectively. Other MRM conditions are presented in Table 1 Glucosinolates were identified by comparing their retention time and MS and MS/MS fragmentation spectra with those of commercial standards. Individual glucosinolates were quantified by MRM, considering one MS/MS transition for each compound. Final concentration of individual glucosinolates was calculated using calibration equations derived from the calibration curves of the corresponding standards. Results are given as µmol kg^{-1} dry weight (DW) sample calculated from LC-peak areas.

Table 1 List of identified glucosinolates, retention times, calibration curves, and MRM conditions for quantitation of glucosinolates by negative ion MRM

Glucosinolates	RT (min)	MRM transition	CV. (V)	CID (ev)	Pol.	LOQ (ppb)	Calibration curve parameters
Gluconapin	2.26	371.89 > 96.0	54	18	ES^-	1.00	Y = 12.376X – 13.4682 (r^2 = 0.999)
Glucobrassicanapin	2.56	385.95 > 96.0	54	17	ES^-	1.00	Y = 32.466X – 18.5492 (r^2 = 0.994)
Progoitrin	1.11	387.91 > 96.0	54	18	ES^-	1.00	Y = 7.3288 X – 3.38502 (r^2 = 0.999)
Glucotropaeolin	2.58	407.97 > 96.0	54	18	ES^-	1.00	Y = 45.1909 X -47.6141 (r^2 = 0.993)
Glucoerucin	2.61	419.95 > 96.0	54	18	ES^-	1.00	Y = 13.135 X – 16.545 (r^2 = 0.998)
Gluconasturtiin	2.91	421.97 > 96.0	53	19	ES^-	1.00	Y = 22.8788 X – 21.2328 (r^2 = 0.999)
Glucoberteroin	2.94	433.95 > 96.0	54	18	ES^-	1.00	Y = 9.52375 X – 3.64797 (r^2 = 0.998)
Glucobarbarin	2.79	437.93 > 96.0	52	18	ES^-	1.00	Y = 9.84022 X – 13.2052 (r^2 = 0.996)
Glucobrassicin	2.68	446.95 > 96.0	54	18	ES^-	1.00	Y = 17.5328 X – 19.2757 (r^2 = 0.999)

CID= Collision Induced Dissociation; LOQ = Limit of Quantification, Pol. = Polarity, CV= Cone Voltage

Statistical analysis

Results were expressed as mean ± standard deviation (SD) of triplicates. The data were treated with Analysis of Variance (ANOVA) followed by Duncan's multiple range test ($p < 0.05$) using the SPSS V. 17.0 statistical program (SPSS Inc., Chicago, USA). The glucosinolate contents in 50 germplasm of *Brassica* were analysed using Principal Component Analysis (PCA). The PCA was performed using PAST (Palaeontological statistics, version 3.06) [35]. Data were visualized using principal components score and loading plots. Each point on the score plot represented an individual sample, and each line on the loading plot represented the contribution of an individual glucosinolate to the score.

Results

In this study, the GSLs profiles and their concentration were examined in leaves of five commercial varieties and 45 germplasm collections of *Brassica* plants belonging to *B. rapa*, *B. juncea*, and *B. oleracea*. The concentrations of GSLs were also evaluated in various leaf sections and positions of two green ("Hangamssam" and "Alchandul") and a red ("Bbalgang 3-ho") pigmented commercial *Brassica* varieties. Five aliphatic (GNA, GBN, PRO, ERU, and BER), three aromatic (TRO, NAS, and BAR) and an indolic (GBC) GSLs were identified. GSLs were examined by negative ionization electrospray LC-MS/MS using MRM mode by monitoring specific transitions originating the characteristic fragment ion at m/z 96 $[SO4]^-$. The detection and quantification conditions of the GSLs by LC-MS/MS are presented in Table 1. Information about the germplasm collections and commercial cultivars are presented as a supplementary file (S1 Table). The results of this study, which are to be presented and discussed in detail in the next sections, show the values varied widely among the entire germplasm collections and between different sections and positions of the *Brassica* leaves. Principal Component Analysis (PCA) helps to identify the glucosinolate exhibiting the greatest variance within a population and to determine closely related individual glucosinolates [36]. The data obtained were subjected to PCA to evaluate the glucosinolate difference among the germplasm collections.

Variation in GSL content between germplasm collections

As can be seen in Table 2, a significant difference in GSL content was observed among the germplasm collections and commercial varieties of *Brassica* plant. The total GSL content ranged from 36.80 ("Alchandul") to 2383.12 ("Shingatsuna", IT 135409) µmol kg^{-1} DW with an average value of 951.5 µmol kg^{-1} DW. Aliphatic GSLs were predominant throughout the entire collections which altogether represented from 22.9 to 98.9% (average 79.9%) of the total GSL content, followed by aromatic GSLs (0.2 to 60.4%; average 9.9%). Glucobrassicin, the only indolic GSL detected in our study represented as low as 0.97% and as high as 58.6% with an average of 10.2% of the total glucosinolates. GNA (ranging from 3.8 to 1,589.8 µmol kg^{-1} DW) and GBN (ranging from 0.06 to 800.0 µmol kg^{-1} DW) were the most dominant GSLs across the entire collections followed by NAS, GBC, and BER. GNA and GBN represented the greatest proportion (on average 49.8% and 32.3%, respectively) of the total glucosinolates in the leaves of the entire *Brassica* germplasm collection and commercial varieties. NAS, GBC, and BER had represented moderate proportion (7.2%, 5.0%, and 4.7%, respectively) of the total glucosinolates. The least dominant GSLs, TRO, BAR, and PRO contained an average values of 0.30, 0.84, and 1.69 µmol kg^{-1} DW in the entire collections, respectively. GNA and GBN were also documented as the most abundant GSLs in the leaves of kimichi cabbage in previous reports [14, 36]. However, GBN, 4-methoxyglucobrassicin, and progoitrin were the dominant GSLs of the leaves of kimichi cabbage in another study [37]. The identity and quantity of glucosinolates varies considerably between various crops of *Brassica*. For example, the predominant GSLs in broccoli were glucoraphanin, gluconapin, and glucobrassicin, while singrin was found to be the dominant GSL in green cabbage, Brussels sprouts, cabbage, cauliflower, and kale [13, 25].

201 Table 2 Glucosinolates contents (μmol/kg DW) in 50 germplasm accessions of *Brassica* (n=3)

S/No	GNA	GBN	PRO	TRO	ERU	NAS	BER	BAR	GBC
1	922.77±0.23uv	430.77±6.70pqr	2.65±0.22o	0.30±0.02lm	2.35±0.04bcdefg	83.68±1.40pq	27.90±0.93jkl	3.22±0.08qr	34.91±1.04m
2	858.01±21.89rs	539.65±8.11u	3.70±0.54qr	0.24±0.01hij	1.35±0.12abcd	154.89±1.49wx	33.61±2.41klmn	7.60±0.72s	28.05±0.41ijk
3	828.48±14.53q	595.11±4.17vw	4.31±0.50s	0.24±0.01hij	2.94±0.20defghi	117.28±1.94rst	63.82±4.27q	3.40±0.13r	72.11±2.67r
4	892.35±12.94t	557.32±7.80u	5.14±0.09t	0.13±0.01ef	4.86±0.15ij	163.10±4.28wx	77.69±2.75r	0.71±0.03ijk	34.80±0.27m
5	1368.50±14.26x	216.05±2.18fg	0.17±0.03abc	0.66±0.01u	1.34±0.12abcd	22.14±0.22defgh	1.68±0.06ab	2.90±0.08p	16.52±0.18de
6	757.82±27.74p	550.05±14.74u	0.72±0.01defgh	0.08±0.00bcd	1.60±0.03abcde	139.58±4.14v	40.74±2.11no	0.78±0.12ijk	36.19±1.12m
7	842.23±40.05qr	422.46±11.81pq	0.43±0.04abcdef	0.11±0.01de	1.77±0.11abcde	153.83±5.01wx	30.12±1.87ijklm	0.86±0.02jkl	19.84±0.71efg
8	775.31±24.07p	620.05±13.48w	0.12±0.01a	0.13±0.01ef	0.46±0.01ab	43.74±1.27jklm	8.02±0.39abcde	0.40±0.02cdefg	35.18±0.79m
9	1151.81±10.17w	270.26±2.79ijk	0.42±0.04abcdef	0.18±0.00g	0.66±0.04abc	14.81±0.13bcdef	2.59±0.16ab	0.88±0.01kl	22.22±0.20fgh
10	598.74±13.78m	492.80±7.73t	1.79±0.17mn	0.26±0.01jk	1.63±0.16abcde	119.51±2.19rst	31.69±3.26ijklm	1.39±0.03n	52.52±1.19p
11	915.47±20.91u	371.17±7.96m	1.04±0.04ghijk	0.24±0.00hij	14.72±0.15n	70.95±0.54o	60.99±0.74pq	0.44±0.01defgh	53.33±0.86p
12	866.94±8.93s	443.92±3.46qrs	0.21±0.03abc	0.45±0.02r	0.22±0.02a	25.03±0.34fgh	2.43±0.04ab	0.31±0.00abcdef	17.93±0.28ef
13	596.00±3.71m	403.66±2.10nop	1.14±0.17hijkl	0.06±0.00ab	0.74±0.02abc	112.63±0.57r	14.91±0.55ef	0.68±0.04hijk	33.07±0.37lm
14	767.16±8.30p	250.16±3.54hij	0.09±0.02a	0.18±0.00g	42.96±1.69p	90.55±1.71q	173.72±6.55v	0.57±0.02fghi	32.93±0.38lm
15	722.91±9.40o	475.35±2.70st	1.5±0.05lm	0.34±0.01no	0.49±0.02ab	18.31±0.22cdefg	12.00±0.51cdef	0.67±0.03hijk	24.58±0.08ghi
16	609.98±8.43m	552.97±4.18u	2.09±0.06n	0.18±0.01g	1.59±0.12abcde	87.95±0.99q	25.73±1.80ijk	0.54±0.03efghi	23.45±0.09ghi
17	686.80±8.77n	382.68±2.95mno	1.00±0.02ghijk	0.12±0.01e	7.05±0.33kl	152.39±0.89w	33.11±1.8jklmn	1.10±0.01lm	29.61±0.33jkl
18	820.76±9.14q	214.97±3.21fg	0.60±0.02bcdefg	0.24±0.00hij	18.18±0.51o	51.87±0.62lmn	36.13±1.26lmn	0.31±0.06abcdef	41.79±0.47n
19	874.87±15.09st	219.73±2.38fgh	0.50±0.02abcdef	0.81±0.02w	12.54±0.36m	25.87±0.30fgh	34.21±0.91lmn	0.29±0.03abcde	21.24±0.29efgh
20	384.29±6.14k	458.45±97.47rs	3.54±0.39q	0.41±0.05q	8.17±0.57l	164.25±33.26x	152.10±7.00u	1.01±0.1lm	101.13±12.02v
21	1589.80±2.92y	567.51±3.28uv	1.44±0.05klm	0.19±0.01g	7.29±0.17kl	131.75±1.23uv	25.17±0.76hij	0.44±0.05defgh	59.53±0.67q
22	210.67±2.36h	290.45±2.76kl	6.46±0.66v	0.72±0.01v	13.47±0.18mn	124.72±2.74stu	139.69±1.26t	1.01±0.11lm	285.68±6.23x
23	940.19±15.88v	65.22±0.50cd	0.04±0.01a	0.41±0.01q	0.12±0.01a	1.41±0.02a	0.07±0.01a	0.05±0.01a	9.43±0.06abc
24	462.58±15.94l	454.74±7.95qrs	0.28±0.00abcd	0.34±0.01no	1.09±0.04abcd	74.81±1.58op	17.98±1.16fgh	0.35±0.07bcdef	27.38±0.90ij
25	229.76±9.34h	408.72±12.94op	2.57±0.17o	0.60±0.02st	4.20±0.32ghi	152.26±4.98w	115.53±8.08s	1.14±0.07m	87.96±2.41t
26	292.95±1.79j	373.93±7.24mn	1.77±0.09mn	0.28±0.02kl	12.49±0.56m	84.72±2.71pq	216.00±11.01w	0.45±0.08defgh	47.94±1.22o
27	180.12±3.30g	429.44±4.61pqr	2.09±0.07n	0.82±0.00w	4.60±0.26hij	164.54±3.17x	84.08±5.69r	2.23±0.05o	69.80±1.40r
28	1132.99±9.19w	800.03±9.00x	1.23±0.06ijkl	0.10±0.02cde	0.53±0.05ab	126.91±1.79tu	30.28±0.99ijklm	0.62±0.03ghij	25.03±1.73hij

29	683.37±11.91n	193.19±6.33f	0.13±0.03ab	0.05±0.00ab	1.47±0.06abcd	9.60±0.35abc	3.97±0.25abc	0.12±0.00ab	9.52±0.14abc
30	258.39±13.36i	457.99±12.33rs	7.22±0.72w	0.35±0.01nop	3.63±0.28efghi	32.72±1.22hij	55.93±1.94p	0.29±0.02abcde	13.05±0.36cd
31	99.27±0.53e	314.34±4.23l	5.68±0.18u	0.25±0.00ijk	1.18±0.07abcd	72.95±0.62o	36.08±0.63lmn	0.21±0.03abcd	96.64±0.86uv
32	59.57±1.81cd	214.42±5.45fg	3.07±0.05p	0.30±0.00lm	3.63±0.19efghi	45.33±0.61klm	57.07±2.41pq	0.14±0.01abc	97.81±2.13uv
33	53.52±2.15cd	146.53±4.03e	2.51±0.11o	1.12±0.05x	2.73±0.21cdefgh	57.29±1.61n	47.13±4.41o	0.27±0.01abcd	61.98±2.85q
34	125.99±0.40f	243.15±1.94ghi	0.88±0.11fghij	0.38±0.01pq	0.31±0.00ab	37.75±0.25ijk	12.39±0.59def	0.17±0.01abc	78.37±0.39s
35	233.55±2.21h	280.20±0.85jk	1.02±0.06ghijk	0.21±0.01h	0.60±0.04abc	53.42±0.17mn	12.11±0.59cdef	0.29±0.00abcde	34.21±0.43lm
36	130.69±3.18f	212.84±2.55fg	1.00±0.02ghijk	0.28±0.01kl	6.23±0.09jk	43.08±0.55jklm	54.46±0.17p	0.18±0.03abcd	96.00±1.35u
37	15.24±0.70a	28.17±0.55ab	0.15±0.01ab	0.37±0.01op	0.55±0.05ab	41.62±0.55jkl	8.50±0.86bcde	0.21±0.03abcd	134.02±0.39w
38	40.88±0.65bc	78.44±0.92cd	0.50±0.15abcdef	0.15±0.00fg	1.92±0.06abcdef	15.06±0.15bcdef	17.09±0.38fg	0.10±0.02ab	54.15±0.16p
39	27.20±1.60ab	85.97±2.79d	0.80±0.07efghi	0.23±0.00hi	0.63±0.08abc	27.62±1.15ghi	15.81±2.47ef	0.09±0.00ab	43.91±1.20no
40	102.91±2.16e	353.98±5.67m	3.45±0.15pq	0.08±0.00abc	0.61±0.05abc	43.70±0.47jklm	24.17±2.06ghi	0.19±0.01abcd	6.46±0.05a
41	75.65±3.25d	146.77±4.62e	1.30±0.07jkl	0.08±0.01bcd	3.87±0.11fghi	42.88±0.99jklm	36.66±1.45mn	0.15±0.01abc	88.98±1.95t
42	396.05±6.22k	559.56±3.53u	3.43±0.07pq	0.09±0.00cd	1.24±0.01abcd	114.12±2.32rs	44.89±1.41o	3.13±0.03q	43.08±0.84n
43	20.59±0.82ab	90.50±2.29d	0.77±0.09efgh	0.17±0.01g	0.88±0.05abcd	13.52±0.26bcde	36.78±0.99mn	0.21±0.03abcd	6.24±0.10a
44	3.88±0.18a	14.43±0.63a	0.29±0.02abcd	0.21±0.01h	1.26±0.09abcd	5.62±0.21ab	5.97±0.51abcd	0.05±0.01a	5.09±0.19a
45	7.85±0.16a	13.62±0.27a	0.28±0.02abcd	0.07±0.00abc	0.67±0.09abc	5.44±0.06ab	8.89±1.80bcde	0.10±0.00ab	8.64±0.07abc
46	7.48±0.12a	17.78±0.17a	0.12±0.02a	0.05±0.00a	0.41±0.01ab	6.57±0.06ab	5.20±0.14abcd	0.08±0.01ab	11.38±0.04bc
47	27.45±1.52ab	0.06±0.01a	0.41±0.03abcde	0.44±0.02r	0.12±0.02a	7.97±0.38abc	0.01±0.00a	0.08±0.01ab	32.21±0.74klm
48	6.26±0.08a	5.68±0.01a	0.64±0.02cdefg	0.33±0.01mn	1.00±0.03abcd	12.23±0.36abcd	8.73±0.07bcde	0.15±0.01abc	18.55±0.44ef
49	9.73±0.33a	0.15±0.01a	0.05±0.00a	0.58±0.00s	0.01±0.00a	24.47±1.25efgh	0.01±0.00a	0.33±0.03abcdef	6.66±0.28ab
50	44.43±0.63bc	53.25±1.38bc	4.00±0.05rs	0.63±0.01t	83.06±5.87q	56.94±0.11n	247.65±14.4x	0.87±0.05jkl	100.97±1.31v

GNA = Gluconapin; GBN = Glucobrassicanapin; PRO= Progoitrin; TRO = Glucotropaeolin; ERU = Glucoerucin; NAS = Gluconasturtiin; BER = Glucoberteroin; BAR = Glucobarbarin; GBC = Glucobrassicin

The results of PCA are indicated by the principal components score and loading plots. The PCA of glucosinolates data yielded three principal components with eigenvalue ≥1, accounting 70.33% of the total variance across the entire data set. The first, second, and the third principal components (PCs) contributed 32.17, 25.6, and 12.18 % of the total variance, respectively. The loadings, eigenvalues, and percentage of variance for all principal components (PCs) yielded are attached in a supplementary file (S2 Table). Scores and loading plots of PC1 and PC2 of the PCA results obtained from glucosinolate content of 50 *Brassica* germplasm collections are presented in Fig 2. The loadings of glucosinolates (represented by green lines) show the extent of each glucosinolate concentration contributed to the principal components. All the glucosinolates were positively correlated with PC1 while only GNA, GBN, BAR, and NAS had a positive correlation with PC2. NAS was the predominant glucosinolate in PC1 while the aliphatic glucosinolates GNA and GBN had a major contribution to PC2. Three kimichi cabbage samples (2, 22 and 50) (see S1 Table for more), the first one located at the top right hand quadrant and others at the lower right hand quadrant of the PCA plot, were well distinguished from other samples. The location of these materials in the score plot could be described by their significantly higher content of BAR, GBC and ERU respectively.

Fig 2. Principal Component Analysis (PCA) plot of the scores (indicated by dotes) and loadings (indicated by lines) of the 50 *Brassica* plants based on the first and second principal components. The numbers 1-50 corresponds to the S/No in Table 2 and S1 Table. GNA = Gluconapin; GBN = Glucobrassicanapin; PRO= Progoitrin; TRO = Glucotropaeolin; ERU = Glucoerucin; NAS = Gluconasturtiin; BER = Glucoberteroin; BAR = Glucobarbarin; GBC = Glucobrassicin

Intra-and inter-leaf distribution of glucosinolates in kimichi cabbage

The leaves of three cultivars of green/ red pigmented kimichi cabbage namely, “Hangamssam” (green), “Alchandul” (green), and “Bbalgang 3-ho” (red) segregated based on their position in the whole leaf part as inner, middle, and outer parts and each leaf was further portioned into different sections (top, middle, bottom, green/red, and white). The GSLs content in kimichi cabbage significantly varied based on leaf section, position, and color. The GSLs content in different leaf sections/positions of three cultivars of

229 kimichi cabbage are presented in Table 3. The white sections of the leaf contained higher total sum of
230 glucosinolates (1.16 to 24.28-fold higher) than the green/red section except in the outer leaf of "Bbalgang 3-
231 ho" where the red section contained 2.8-fold greater total sum of GSLs concentration than the white section.
232 The trend in total GSLs content in different sections of the leaf (top, middle, and bottom) was not strictly
233 consistent. However, in most cases higher GSLs content were observed at the proximal half of the leaves. In
234 regard to the position of the leaf (outer, middle, and inner) in the whole plant, the average content of total
235 GSLs of the kimichi cabbage in the middle position were 1.8-, 2.2-, and 3.9-fold larger than in the outer
236 positions of "Hangamssam", "Alchandul", and "Bbalgang 3-ho", respectively. The content of total
237 glucosinolates evaluated in the inner position of "Alchandul" and "Bbalgang 3-ho" showed 2.8- and 1.2-fold
238 higher than the outer position, respectively. However, unlike "Alchandul", less total GSLs content was
239 recorded in the red pigmented "Bbalgang 3-ho" in the inner compared to the middle leaf. In general, the
240 younger (inner) leaves were found to contain higher concentrations of glucosinolates compared to older
241 (outer) leaves. In earlier study, a similar trend was observed in *Brassica oleracea* var. *capitate* where the
242 inner positions contained 1.1- to 1.8-fold greater GSLs concentrations than the outer positions [38]. In
243 another study form *Raphanus sativus*, younger leaves were found to contain higher glucosinolate
244 concentrations [39].

245

Table 3 Glucosinolate concentration in different leaf sections of three cultivars of kimichi cabbage (μmol/kg DW)

Cultivar's name	Plant part		GNA	GBN	PRO	TRO	ERU	NAS	BER	BAR	GBC	Sum of GSLs
	Outer leaf	top	821.9±15b	1013.4±37.4b	289±8.6b	1.5±0.2e	0.2±0a	62.8±8.3ab	2.5±0.5a	28.2±2g	44.8±1.1d	2264.3
		middle	1578.7±175.1f	1853.1±122.1e	534.8±42.8e	1.2±0c	6.4±0.2b	205±36.1de	92.8±6.4bc	26.6±1.6fg	46.4±4.7d	4345
		bottom	1425.8±214e	1464.4±164.7c	421±46.3c	0.5±0a	13.3±5.9c	352.5±79.1f	76±87.7bc	7.7±2.3c	38.6±6.9c	3799.8
"Hangamssam"		green	693.4±13.7b	1129.6±36.7b	320.6±9.6b	1.5±0d	0.2±0a	54.5±9.9a	0.9±0.2a	24.3±0.9f	40.8±2cd	2265.8
		white	1112.2±6.7c	1714.8±44.1d	484.7±15.8d	0.2±0a	14.7±0.5c	183±14.9cd	295.5±25.7e	5±1.6b	27.6±0.7b	3837.7
	Middle leaf	top	1227.1±9.6cd	1657.5±16.8d	466.3±2.4d	1.7±0f	0.5±0a	139.6±28.8c	8.5±2.1a	19.6±0.2e	91.2±3.8fg	3612
		middle	1576.3±12.6f	1999.2±44.7f	568.8±4.8e	2.4±0h	3.8±2.1ab	255.7±47.5e	48.9±34.9ab	16.2±0.7d	104.8±2.5h	4576.1
		bottom	1306.9±49.5de	1628.3±21.2d	462.8±5.9d	0.5±0b	16.6±0.9c	322.8±21.3f	174.3±20.4d	5.7±0.7bc	94.3±2g	4012.2
		green	1164.2±13.1cd	1874.5±33e	533.5±6.2e	2±0g	0.5±0.2a	123.5±15.3bc	9±0.7a	19.1±1.4e	87.8±0.2f	3814.1
		white	1644.3±24.9f	1636.8±39.5d	467.9±9.1d	0.5±0a	18±6.4c	434±48.6g	166±67.7d	3.6±0.7b	57.7±4.2e	4428.8
	Outer leaf	top	2.7±1.6a	24.8±0.8a	7.5±0.5a	3.4±0.2i	0±0a	4.3±0.7a	0.2±0a	1.8±0e	17.6±0.7e	62.3
		middle	30±2.1b	183.3±6.5c	51.2±1.9c	0.7±0de	0.2±0ab	41.8±2.4b	8±0.9a	0.2±0.2ab	29.2±1.1ab	344.6
		bottom	91.9±2.1cd	278±5.7d	80.1±2.1d	1.2±0g	1.4±0.2abc	71.1±6.6c	40±13.5b	0.9±0cd	12.5±0.4cd	577.1
		red	196.3±10.4fg	689.7±17.3g	195.5±7.2g	2.7±0.2h	2.4±0.2cd	135.5±2.4e	111.8±3.7d	3.2±0.5f	19±0.2f	1356.1
		white	95.6±3.7d	224.1±3.9c	62.4±1.6c	0.2±0a	0.5±0ab	57.6±0.9bc	34±1.8b	0±0a	4.9±0.2a	479.3
"Bbalgang 3-ho"	Middle leaf	top	207.8±35.1g	624.4±96.3f	178.6±28.1f	0.7±0e	6.6±2.6e	125.9±37.5ef	107.2±44.8d	1.1±0.2d	28.3±4.5d	1280.6
		middle	35.1±2.1b	99.1±2.1b	27.9±0.5b	0.2±0a	1.4±0bc	11.1±2.1a	33.8±1.6b	0.2±0a	2.9±0.2a	211.7
		bottom	154.3±2.7e	420.2±8.3e	120±3.7e	0.7±0cd	3.3±0.2d	94.5±5.2d	113.4±12.9d	0.5±0ab	18.3±0.2ab	925.2
		red	152.7±2.1e	595.8±13.4f	168.2±8f	6.6±0.2j	0.5±0.2ab	112.9±15.1de	34.4±16.1b	3.9±0.2g	19±0.9g	1094
		white	180.5±27.3f	617.4±55f	178.6±16.6f	0.7±0de	3.3±0.7d	142.1±22.2e	166.5±9.6e	0.2±0ab	29±1.6ab	1318.3
	Inner leaf	top	7.2±0.8a	33.8±1.3a	9.1±0.3a	0.2±0a	0.5±0ab	4±0.2a	3.9±0.2a	0±0a	3.8±0a	62.5
		middle	71.8±1.3c	318.3±5.7d	88.9±3.5d	0.5±0bc	2.6±0cd	54.3±2.1bc	71.4±2.8c	0.7±0.5bc	36.6±0.7bc	645.1
		bottom	42.6±0.3b	189.2±5.2c	54.1±1.3c	0.5±0b	0.9±0ab	41.6±0.9b	24.3±0.7ab	0.2±0a	15.6±0.2a	369
		red	6.2±0.3a	42.8±1a	12.1±0.5ab	0.2±0a	0.2±0ab	5.9±0.2a	3.9±0.5a	0±0a	8.7±0.2a	80
		white	292.2±19h	955.1±35.4h	274.8±12.9h	1±0f	14.7±0.9f	147.1±4.3e	212.9±9.6f	0.9±0.2cd	47.5±0.9cd	1946.2
	Outer leaf	top	1.1±0.8a	3.9±0.8a	1.3±0.5a	0.5±0a	0±0a	2.1±0.2a	0.5±0.2a	0.2±0a	3.1±0.7a	12.7
		middle	103.1±1.3f	343.3±0.3fg	95.9±1.1fg	6.8±0.2fg	3.1±0.9g	137.2±4.3a	28.5±7.6d	9.6±0.7c	31.2±0.9i	758.7
		bottom	88.4±12.1e	335.8±41.6fg	95.3±12.1fg	2.2±0.2fg	1.2±0d	195.5±20.8a	21.1±1.8g	7.1±1.4bc	34.6±3.3h	781.2
		green	9.4±0.5a	66.1±3.1b	18.2±0.8b	2.4±0.2b	0.7±0.2d	32.6±2.8a	5.7±0.9b	5.7±0.2ab	15.4±0.4g	156.2
		white	192.6±11.8h	573.6±29.9k	163.6±5.9k	1.2±0.2k	2.4±0.5c	266.1±10.4a	31±6.4i	9.1±0.9c	47.9±1.8i	1287.5
"Alchandul"	Middle leaf	top	21.7±2.7b	104.3±13.4c	29.5±3.7c	0.7±0.2c	25.4±4.5b	94.7±11.8f	94.8±17c	0.9±0f	14.5±2.2ab	386.5
		middle	144.3±4.8g	526.3±11.6j	148.4±5.9j	2±0.2j	62.4±0.5d	324.2±10.9h	316.6±6.9k	4.3±1.8i	39±1.3f	1567.5
		bottom	104.2±4.6f	424.4±19.1h	121.6±4.6h	2.2±0h	22.5±2.1d	247.9±4.5e	152.9±17.9hi	2.7±0.7g	42.6±1.3cde	1121
		green	28.4±3.5b	142.7±15d	41.2±3.7d	1±0.2d	15.9±1.4bc	162±13.7cd	52.1±6.4e	1.6±0d	13.8±1.8bc	458.7
		white	150.5±12.6g	471.6±30.2i	132.8±8.8i	2.2±0.2i	30.1±2.1d	296.3±21.7g	222±14.2j	2.3±0.5h	36.8±2.7cde	1344.6
	Inner leaf	top	56±0.5c	264.3±1.8e	74.2±1.3e	2.2±0e	18±0.2d	167.7±5d	71.4±1.4ef	3.2±0.5e	171.7±2def	828.7
		middle	83.3±12.1e	320.1±44.1f	88.6±13.1f	3.2±0.5f	10.4±1.9e	186.1±21b	53.5±14fg	1.8±0.7d	164.5±20.5bcd	911.5
		bottom	69.6±1.6d	363.7±4.6g	105±3.5g	6.1±0.2g	14.2±0.5f	237.3±14.2c	79.2±6.7h	3.6±0.7ef	125.5±3.1ef	1004.2

GNA = Gluconapin; GBN = Glucobrassicanapin; PRO= Progoitrin; TRO = Glucotropaeolin; ERU = Glucoerucin; NAS = Gluconasturtiin; BER = Glucoberteroin; BAR = Glucobarbarin; GBC = Glucobrassicin

Discussions

The data in this study, generated from evaluations conducted from *Brassica* germplasm collections and commercial cultivars originated from various countries and grown in the same location and season, revealed that a wide variety in the level of glucosinolates among genotypes, leaf position/section, and leaf color was observed. The difference observed in GSLs profile is both qualitative and quantitative. This could determine their level of nutritional and health promoting properties and supports the feasibility of developing germplasm with enhanced level of glucosinolates through genetic manipulation. Previous studies have shown that the temperature [19], amount of rainfall [40], radiation [41, 42], plant part examined [1], phenological stage of growth [15, 19], and level of insect damage [19, 43] have affected the level of glucosinolates. The degradation products (pent-4-enyl-isothiocyante and 5-(methylsulfinyl) pentyl isothiocyante) of the dominant GSLs found in this study, gluconapin and glucobrassicanapin, respectively, found to inhibit a wide range of bacteria and fungi indicating their promising antimicrobial potential [6]. The indolic glucobrassicin, the aliphatic glucoberteroin, and the aromatic gluconastrutiin were found in appreciable amount in our study, suggesting that the germplasm collections could be beneficial in terms of the health related activities of these compounds. The derivatives of GBC, indole-3-carbinol (I3C) and 3,3'-diindolylmethane (DIM), have been reported to induce apoptosis to suppress the growth of human prostate cancer cells *in vitro* [44], while glucoberteroin showed to strongly suppress protein glycation and carbonylation which in turn causes aging of the skin [45]. The hydrolysis product of NAS, phenethyl isothiocyanates (PEITC), shown to induce apoptosis in HeLa cells in a time- and dose-dependent manner [46] . In another study, PEITC was reported to create an oxidative cellular environment that induces DNA damage and *GADD153* gene activation, which in turn helps trigger apoptosis [47].

The enhancement of glucosinolate concentration upon plant damage [43], have long indicated that glucosinolates are plant defense chemicals where mostly their defensive properties are attributed to the toxicity and deterrence nature of their degradation products [9]. However, there are also cases that glucosinolates mediated by their volatile hydrolysis products could serve to attract adapted herbivores that

often use glucosinolates as cues for feeding or oviposition [9]. The spatial distribution of glucosinolates in different section of a single leaf and/or location of the leaf in the whole plant could be partly important to explain the patterns of herbivory. Studies devoted to glucosinolate spatial patterns within leaves of kimichi cabbage are elusive. The proximal half of leaves of *Raphanus sativus* contained higher mean concentration of glucosinolates compared to the distal halves of leaves [39]. Shroff et al. (2008) [48] studied the spatial distribution (midvein, inner lamina, and outer lamina) of glucosinolates in leaves of *Arabidopsis thaliana* and tried to relate the distribution to the pattern of herbivory caused by larvae of the lepidopteran, *Helicoverpa armigera.* These authors found out that the glucosinolate abundance in the inner vs. the peripheral part of the leaf affected insect feeding preference and anti-herbivore defenses. As stated in previous section and Table 3, the white part (midvein) of kimichi cabbage contained relatively higher glucosinolates compared to the green or red. This is consistent with *Arabidopsis thaliana* leaves where the midvein part exhibited the greatest concentration compared to the other sections of the leaf [48]. This could be due to certain biosynthetic enzymes are distributed exclusively to vascular bundles [49], resulting greater synthesis and storage of glucosinolates in the midvein (white part) of the leaf of kimichi cabbage. It could also be related to ecological significance [50], the midvein being critical to the function of the leaf as the transport of water and nutrients are carried through it. The greater concentration of glucosinolate in the white part of kimichi cabbage in our study corroborates the idea of storage of glucosinolates being associated with the vascular system. The higher content of glucosinolates in the inner (younger) leaves compared to the outer (older) leaves in this study is also in agreement with the predictions of optimal defense theory: young leaves are more valuable as they have a higher future photosynthetic potential needs higher degree of protection from damage [51]. In addition, glucosinolate concentration could tend to decrease in outer leaves due to dilution of glucosinolates as the leaf expands [51].

Conclusions

Nine glucosinolates were identified and quantified in *Brassica* germplasm collections and commercial varieties using UPLC-MS/MS method in Multiple Reaction Monitoring scan mode. Remarkable

differences in total and individual glucosinolates were observed among different samples. The variation in glucosinolate level suggests that the potential health benefits of *Brassica* plants could depend on the type of accession used. Glucosinolates and their degradation products are known for their chemopreventive properties, and the wide variability in glucosinolates among the germplasm collections in this study offer important information base for enhancing the level of glucosinolates in *Brassica* plants through breeding thereby enhancing their anti-cancer properties. In addition, the development of *Brassica* plants with specific GSL profiles of specific functions will allow for meaningful recommendations of dietary intake of *Brass*ica vegetables in respect to other biological activity. The PCA in this study allowed easy visualisation of the data; three kimichi cabbage samples (2, 22, and 50) were separated from the others in The PCA plot. The inter- and intra-leaf variations of GSLs were examined in three kimichi cabbage varieties. The GSLs content varied significantly among leaves in different positions of the plant (outer, middle, and inner) and sections within leaves (top, middle, bottom, green/red, and white). Higher GLS contents were observed the proximal half and white section of the leaves and inner positions (younger leaves) in most of the samples. Studying how the variability of GSLs content and composition is reflected within and between leaves would widen the present understanding of the accumulation pattern of GSLs in leaves of *Brassica* plants and provide information on the nature of plant defenses towards perceived danger. To better understand the extent and pattern of anti-herbivore glucosinolate defenses, further investigation of glucosinolate distribution in relation to the pattern of herbivory by insects is recommended.

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Conflict of interest

The authors declare that they have no competing interests

References

1. Bhandari SR, Jo JS, Lee JG. Comparison of glucosinolate profiles in different tissues of nine *Brassica* crops. Molecules. 2015; 20: 15827–15841. doi: 10.3390/molecules200915827

2. Ishida M, Hara M, Fukino N, Kakizaki T, Morimitsu Y. Glucosinolate metabolism, functionality and breeding for the improvement of Brassicaceae vegetables. Breed Sci. 2014; 64: 48–59 . doi: 10.1270/jsbbs.64.48

3. Fahey JW, Zalcmann AT, Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry. 2001; 56: 5–51. doi: 10.1016/S0031-9422(00)00316-2

4. Hong E, Kim GH. Anticancer and antimicrobial activities of β-phenylethyl isothiocyanate in *Brassica rapa* L. Food Sci Technol Res. 2008; 14: 377–382. doi: 10.3136/fstr.14.377

5. Kim H, Kim J, Kim H, Kim D, Jo H. Anticancer activity and quantitative analysis of glucosinolates from green and red leaf mustard. Korean J food Nutr. 2011; 24: 362–366. doi: 10.9799/ksfan.2011.24.3.362

6. Blažević I, Radonić A, Skočibušić M, Denicola GR, Montaut S, Iori R, et al. Glucosinolate profiling and antimicrobial screening of *Aurinia leucadea* (Brassicaceae). Chem Biodivers. 2011; 8: 2310–2321. doi: 10.1002/cbdv.201100169

7. Tierens KFMJ, Thomma BPHJ, Brouwer M, Schmidt J, Kistner K, Porzel A, et al. Study of the role of antimicrobial glucosinolate-derived isothiocyanates in resistance of *Arabidopsis* to microbial pathogens. Plant Physiol. 2001; 125: 1688–1699. doi: 10.1104/pp.125.4.1688

8. Mithen R. Glucosinolates - biochemistry, genetics and biological activity. Plant Growth Regul. 2001; 34: 91–103. doi: 10.1023/A:1013330819778

9. Halkier BA, Gershenzon J. Biology and biochemistry of glucosinolates. Annu Rev Plant Biol. 2006; 57: 303–333. doi: 10.1146/annurev.arplant.57.032905.105228

10. Lazzeri L, Baruzzi G, Malaguti L, Antoniacci L. Replacing methyl bromide in annual strawberry production with glucosinolate-containing green manure crops. Pest Manag Sci 2003; 59 :983–990. doi: 10.1002/ps.726

11. Aires A, Dias CSP, Carvalho R, Oliveira MH, Monteiro AA, Simões MV, et al. Correlations between disease severity, glucosinolate profiles and total phenolics and *Xanthomonas campestris* pv. *campestris* inoculation of different Brassicaceae. Sci Hortic (Amsterdam). 2011; 129: 503–510. doi: 10.1016/j.scienta.2011.04.009

12. Rodman JE, Soltis PS, Soltis DE, Sytsma KJ, Karol KG. Parallel evolution of glucosinolate biosynthesis inferred from congruent nuclear and plastid gene phylogenies. Am J Bot. 1998; 85: 997–1006. doi: 10.2307/2446366

13. Kushad MM, Brown AF, Kurilich AC, Juvik JA, Klein BP, Wallig MA, et al. Variation of glucosinolates in vegetable crops of *Brassica oleracea*. J Agric Food Chem. 1999; 47: 1541–1548. doi: 10.1021/jf980985s

14. Lee MK, Chun JH, Byeon DH, Chung SO, Park SU, Park S, et al. Variation of glucosinolates in 62 varieties of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) and their antioxidant activity. LWT - Food Sci Technol. 2014; 58: 93–101. doi: 10.1016/j.lwt.2014.03.001

15. Brown PD, Tokuhisa JG, Reichelt M, Gershenzon J. Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. Phytochemistry. 2003; 62: 471–481. doi: 10.1016/S0031-9422(02)00549-6

16. Khan MAM, Ulrichs C, Mewis I. Influence of water stress on the glucosinolate profile of Brassica oleracea var. italica and the performance of *Brevicoryne brassicae* and *Myzus persicae*. Entomol Exp Appl. 2010; 137: 229–236. doi: 10.1111/j.1570-7458.2010.01059.x

17. Rosa EAS, Rodrigues PMF. The effect of light and temperature on glucosinolate concentration in the leaves and roots of cabbage seedlings. J Sci Food Agric. 1998; 78: 208–212. doi: 10.1002/(SICI)1097-0010(199810)78:2<208::AID-JSFA104>3.0.CO;2-J

18. Volden J, Borge GIA, Bengtsson GB, Hansen M, Thygesen IE, Wicklund T. Effect of thermal treatment on glucosinolates and antioxidant-related parameters in red cabbage (*Brassica oleracea* L. ssp. *capitata f. rubra*). Food Chem.2008; 109 :595–605. doi: 10.1016/j.foodchem.2008.01.010

19. V Velasco P, Cartea ME, González C, Vilar M, Ordás A. Factors affecting the glucosinolate content of kale (*Brassica oleracea acephala* Group). J Agric Food Chem. 2007; 55: 955–962. doi: 10.1021/jf0624897

20. Brown AF, Yousef GG, Jeffery EH, Klein BP, Wallig MA, Kushad MM, et al. Glucosinolate profiles in broccoli: variation in levels and implications in breeding for cancer chemoprotection. J Amer Soc Hort Sci. 2002; 127: 807–813. doi: 10.21273/JASHS.127.5.807

21. Rosen CJ, Fritz VA, Gardner GM, Hecht SS, Carmella SG, Kenney PM. Cabbage yield and glucosinolate concentrations as affected by nitrogen and sulfur fertility. HortScience. 2005; 40: 1493–1498. doi: 10.21273/HORTSCI.40.5.1493

22. Ulmer BJ, Dosdall LM. Glucosinolate profile and oviposition behavior in relation to the susceptibilities of Brassicaceae to the cabbage seedpod weevil. Entomol Exp Appl. 2006; 121: 203–213. doi: 10.1111/j.1570-8703.2006.00480.x

23. Park MH, Arasu MV, Park NY, Choi YJ, Lee SW, Al-Dhabi NA, et al. Variation of glucoraphanin and glucobrassicin: anticancer components in *Brassica* during processing. Food Sci Technol,

Campinas. 2013; 33: 624–631. doi: 10.1590/S0101-20612013000400005

24. Windsor AJ, Reichelt M, Figuth A, Svatoš A, Kroymann J, Kliebenstein DJ, et al. Geographic and evolutionary diversification of glucosinolates among near relatives of *Arabidopsis thaliana* (Brassicaceae). Phytochemistry. 2006; 66: 1321–1333. doi: 10.1016/j.phytochem.2005.04.016

25. Park S, Valan Arasu M, Lee MK, Chun JH, Seo JM, Lee SW, et al. Quantification of glucosinolates, anthocyanins, free amino acids, and vitamin C in inbred lines of cabbage (*Brassica oleracea* L.). Food Chem. 2014; 145: 77–85. doi: 10.1016/j.foodchem.2013.08.010

26. Tian Q, Rosselot RA, Schwartz SJ. Quantitative determination of intact glucosinolates in broccoli, broccoli sprouts, Brussels sprouts, and cauliflower by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. Anal Biochem. 2005; 343: 93–99. doi: 10.1016/j.ab.2005.04.045

27. Hennig K, Verkerk R, Bonnema G, Dekker M. Pitfalls in the desulphation of glucosinolates in a high-throughput assay. Food Chem. 2012; 134: 2355–2361. doi: 10.1016/j.foodchem.2012.04.015

28. Mattha B, Fiebig H. Simultaneous determination of isothiocyanates, indoles, and oxazolidinethiones in myrosinase digests of rapeseeds and rapeseed meal by HPLC. J Agric Food Chem. 1996; 44: 3894–3899. doi: 10.1021/jf9603184

29. Gratacós-Cubarsí M, Ribas-Agustí A, García-Regueiro JA, Castellari M. Simultaneous evaluation of intact glucosinolates and phenolic compounds by UPLC-DAD-MS/MS in *Brassica oleracea* L. var. *botrytis*. Food Chem. 2010; 121: 257–263. doi: 10.1016/j.foodchem.2009.11.081

30. Cohen Freue GV, Borchers CH. Multiple Reaction Monitoring (MRM): principles and application to coronary artery disease. Circ Cardiovasc Genet. 2012; 5: 378. doi: 10.1161/CIRCGENETICS.111.959528

31. Velasco L, Becker HC. Variability for seed glucosinolates in a germplasm collection of the genus

Brassica. Genet Resour Crop Evol. 2000; 47: 231–238. doi: 10.1023/A:1008793623395

32. Griffiths DW, Deighton N, Birch AE, Patrian B, Baur R, Städler E. Identification of glucosinolates on the leaf surface of plants from the Cruciferae and other closely related species. Phytochemistry. 2001; 57: 693–700. doi: 10.1016/S0031-9422(01)00138-8

33. Bodnaryk RP, Palaniswamy P. Glucosinolate levels in cotyledons of mustard, *Brassica juncea* L. and rape, *B. Napus* L. do not determine feeding rates of flea beetle, *Phyllotreta cruciferae* (goeze). J Chem Ecol. 1990; 16: 2735–2746. doi: 10.1007/BF00988082

34. Song L, Morrison JJ, Botting NP, Thornalley PJ. Analysis of glucosinolates, isothiocyanates, and amine degradation products in vegetable extracts and blood plasma by LC-MS/MS. Anal Biochem. 2005; 347: 234–243. doi: 10.1016/j.ab.2005.09.040

35. Hammer Ø, Harper DAT, Ryan PD. PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron. 2001; 4: 1–9.

36. Kim JK, Chu SM, Kim SJ, Lee DJ, Lee SY, Lim SH, et al. Variation of glucosinolates in vegetable crops of *Brassica rapa* L. ssp. *pekinensis*. Food Chem. 2010; 119: 423–428. doi: 10.1016/j.foodchem.2009.08.051

37. Hong E, Kim SJ, Kim GH. Identification and quantitative determination of glucosinolates in seeds and edible parts of Korean Chinese cabbage. Food Chem. 2011; 128: 1115–1120. doi: 10.1016/j.foodchem.2010.11.102

38. Choi S, Park S, Lim YP, Kim S, Park J, An G. Metabolite profiles of glucosinolates in cabbage varieties (*Brassica oleracea* var. *capitata*) by season, color, and tissue position. Hort Environ Biotechnol. 2014; 55: 237–247. doi: 10.1007/s13580-014-0009-6

39. Shelton AL. Within-plant variation in glucosinolate concentrations of *Raphanus sativus* across multiple scales. J Chem Ecol. 2005; 31: 1711–1732. doi: 10.1007/s10886-005-5922-9

435 40. Ciska E, Martyniak-Przybyszewska B, Kozlowska H. Content of glucosinolates in cruciferous
436 vegetables grown at the same site for two years under different climatic conditions. J Agric Food
437 Chem. 2000; 48: 2862–2867. doi: 10.1016/S0550-3213(96)00633-5

438 41. Rybarczyk-plonska A, Fiskaa S, Iren G, Borge A, Bengtsson GB, Kåre M, et al. Postharvest biology
439 and technology glucosinolates in broccoli (*Brassica oleracea* L. var. *italica*) as affected by
440 postharvest temperature and radiation treatments. Postharvest Biol Technol. 2016; 116 :16–25. doi:
441 10.1016/j.postharvbio.2015.12.010

442 42. Banerjee A, Variyar PS, Chatterjee S, Sharma A. Effect of post harvest radiation processing and
443 storage on the volatile oil composition and glucosinolate profile of cabbage. Food Chem. 2014; 151:
444 22–30. doi: 10.1016/j.foodchem.2013.11.055

445 43. Birch ANE, Griffiths DW, Hopkins RJ, Smith WHM, Mckinlayc RG. Glucosinolate responses of
446 swede, kale, forage and oilseed rape to root damage by turnip root fly (*Delia floralis*) larvae. J Sci
447 Food Agric. 1992; 60: 1–9. doi: 10.1002/jsfa.2740600102

448 44. Nachshon-kedmi M, Yannai S, Haj A, Fares FA. Indole-3-carbinol and 3,3'-diindolylmethane induce
449 apoptosis in human prostate cancer cells. Food Chem Toxicol. 2003; 41: 745–752. doi:
450 10.1016/S0278-6915(03)00004-8

451 45. Hirano M, Takeda S, Hitoe S, Shimoda H. Glucosinolates and isothiocyanates from broccoli seed
452 extract suppress protein glycation and carbonylation. Funct Food Heath Dis. 2018; 8: 35–48 doi:
453 10.31989/ffhd.v8i1.391

454 46. Yu R, Mandlekar S, Harvey KJ, Ucker DS, Kong ANT. Chemopreventive isothiocyanates induce
455 apoptosis and caspase-3-like protease activity. Cancer Res. 1998; 58: 402–408

456 47. Powolny A, Takahashi K, Hopkins RG, Loo G. Induction of *GADD* gene expression by
457 phenethylisothiocyanate in human colon adenocarcinoma cells. J Cell Biochem. 2003; 90: 1128–1139.

458 doi: 10.1002/jcb.10733

459 48. Shroff R, Vergara F, Muck A, Ales Svatos A, Gershenzon J. Nonuniform distribution of
460 glucosinolates in *Arabidopsis thaliana* leaves has important consequences for plant defense. Proc
461 Natl Acad Sci USA. 2008; 105: 6196–6201. doi: 10.1073/pnas.0711730105

462 49. Reintanz B, Lehnen M, Reichelt M, Gershenzon J, Kowalczyk M, Sandberg G. bus , a bushy
463 Arabidopsis CYP79F1 knockout mutant with abolished synthesis of short-chain aliphatic
464 glucosinolates. Plant Cell. 2001; 13:351–367. doi: 10.1105/tpc.13.2.351

465 50. Hamilton JG, Arthur R, Delucia EH. The carbon-nutrient balance hypothesis : its rise and fall. Ecol
466 Lett. 2001; 4: 86–95. doi: 10.1046/j.1461-0248.2001.00192.x

467 51. Mccall AC, Fordyce JA. Can optimal defence theory be used to predict the distribution of plant
468 chemical defences? J Ecol. 2010; 98: 985–992 . doi: 10.1111/j.1365-2745.2010.01693.x

469 Supporting information

470 **S1 Fig. Representative MRM profiles of intact glucosinolates corresponding to *Brassica* germplasm**

471 **S1 Table. Accession number, scientific name, common name and origin of 50 germplasm accessions of**
472 ***Brassica* genus**

473 **S2 Table. Loadings, eigenvalues, and percentage of variance for the principal components (PCs) data**
474 **from germplasm collections**

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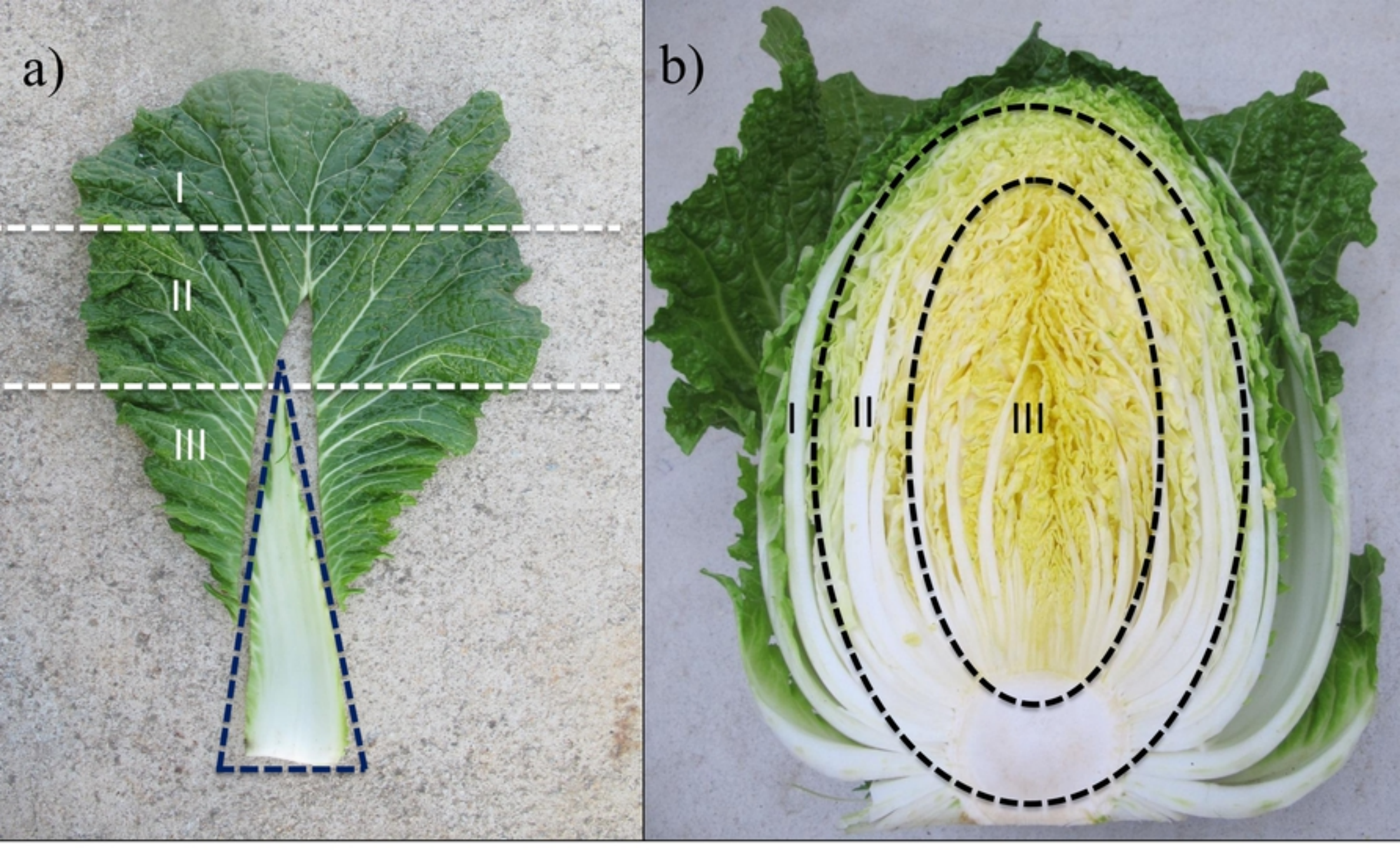

Figure 1

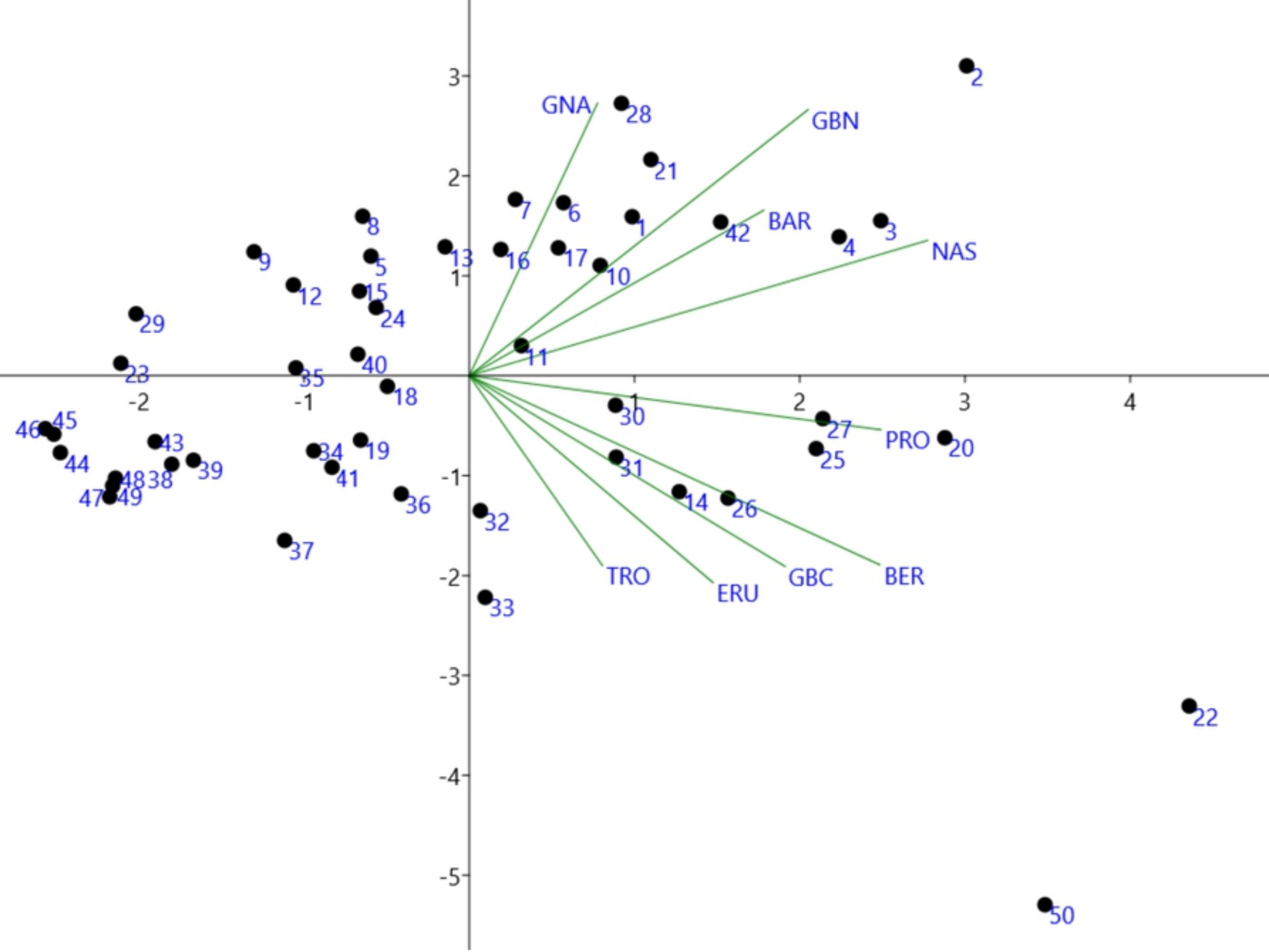
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Figure 2