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Diversity of leafy *Brassica oleracea* landraces from eastern Adriatic coast (Croatia): morphological characterization and glucosinolate content

Raznolikost lisnatih *Brassica oleracea* populacija s istočne obale Jadrana: morfološka karakterizacija i sadržaj glukozinolata

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ABSTRACT

Glucosinolates are compounds found in edible parts of *Brassica crops* and has been investigated comprenhensivley in last decades as have beneficial effect on human nutrition. Due to a lack of information, fifteen Croatian leafy *Brassica* landraces were evaluated for leaf glucosinolate content and morphological traits, and to find possible relation between them after field cultivation. Regarding morphological traits we found that most of the studied landraces belong to the collard morphotype with low curling density and leaf blistering and landraces showed high variability in leaf color. Regarding glucosinolates, we found a significant difference in these phytonutrients among landraces. Total glucosinolate content differed 10-fold between landraces with a mean value of 46,9 µmol/g leaf dry weight. Thirteen glucosinolates belonging to the three principal chemical groups were found, including seven aliphatic, four indolic and two aromatic glucosinolates. Seven compounds were found in all landraces. Correlation and multivariate analysis between morphological properties and glucosinolates content gave us limited conclusions, but obtained results provide a profile of kale and collard landraces phytonutrients useful for future research on nutritional value or breeding.

Keywords: kale, collard, correlation, multivariate analysis

SAŽETAK

Glukozinolati su spojevi koji se nalaze u jestivim dijelovima biljaka porodice *Brassicaceae*, a zadnjih desetljeća su provedena sveobuhvatna istraživanja koja su utvrdila njihov pozitivan učinak na ljudsku prehranu. Zbog nedostatka informacija u petnaest hrvatskih populacija lisnatih kupusnjača utvrđen je sadržaj i vrste glukozinolata u listu i morfološka svojstva, te se utvrdila moguća povezanost između njih nakon uzgoja u polju. Što se tiče morfoloških svojstava, utvrdili smo da većina proučavanih populacija pripada morfotipu raštike s malom gustoćom uvijenosti i mjehuravosti listova te populacije pokazuju visoku varijabilnost u boji lista. Kod glukozinolata utvrđena je značajna razliku u tim fitonutrijentima među populacijama. Ukupna koncentracija glukozinolata razlikovala se 10 puta između populacija sa srednjom vrijednošću od 46,9 µmol/g suhe mase lista. Pronađeno je trinaest glukozinolata koji pripadaju trima glavnim kemijskim skupinama, uključujući sedam alifatskih, četiri indolna i dva aromatska glukozinolata. U svim domaćim populacijama pronađeno je sedam spojeva. Korelacija i multivarijantna analiza između morfoloških svojstava i sadržaja glukozinolata dala nam je ograničene zaključke, ali dobiveni rezultati daju profil fitonutrijenata raštike i lisnatog kelja koji je koristan za buduća istraživanja nutritivne vrijednosti ili oplemenjivanja.

Ključne riječi: raštika, lisnati kelj, korelacije, multivarijantana analiza

INTRODUCTION

Kale (*Brassica oleracea* L. var. *acephala* DC.) is one of the oldest cultivated species from family *Brassicaceae* (Balkaya and Yanmaz, 2005). A long history and extensive horticultural use across Europe have resulted in a large number of kale genotypes showing huge genetic variability. Inter-population variability results from farmers' selection and adaptations to local ecological conditions. In addition, intra-population variability is generated by cross-pollination because of poor isolation of plants used for seed production (Cartea et al., 2008).

In everyday use and scientific literature, the word kale (kales) from acephala group includes different varieties, but the most common for the leafy, non-heading cabbages are kale and collards. Kale (*B. oleracea* L. var. *acephala* DC.) has dark green and curled leaves, while collard (*B. oleracea* L. var. *viridis* L.) has smooth, broad leaves without blistering (Šamec et al., 2019). The modernization of agriculture in the last decades along with the abandoning of rural areas has resulted in biodiversity loss, since farmers grow pure lines and hybrids and tend to abandon traditional landraces and cultivars (Negri et al., 2009). In the Croatian coastal region, kales are grown in traditional farming systems as a ruderal vegetable crop. In particular, kale is grown for leaves as an important vegetable during the winter, with leftovers being used as livestock feed.

The increased consumer interest in the consumption of safe and healthy food has resulted in growing demand for locally grown products such as kale. Due to known kale characteristic sensory and chemical properties, screening of kale landraces for agronomic and nutrient use efficiency traits was done (Batelja et al., 2009; Urlic et al., 2016) as a part of programmes to conserve local landraces within the framework of EU directives for agrobiodiversity.

Glucosinolates (GLS) are plant sulphur compounds predominant in the genus *Brassica*. GLS and their breakdown products (isothiocynates, thiocynates, nitriles) have been shown to have beneficial biological effects on human nutrition reducing the risk of several types of cancer (Traka et al., 2009; Orouji et al., 2023), reduce or stimulate insect attack, inhibit the growth of nematodes and fungi and the growth of neighbouring plants (Brennan et al., 2020), as also affect mostly specific flavor of *Brassica* vegetables.

GLSs variation in the amount and pattern has been attributed to genotype, tissue type, stage of development and environmental factors (temperature, drought, soil type, and nutrient availability (Velasco et al., 2007). Usually, a single *Brassica* specie has up to four different GLSs in significant amounts while, as many as 15 different GLSs can be found in the same plant (Verkerk et al., 2009). *B. oleracea* group has variation in GLS structure and the total GLS amount with all types containing glucobrassicin, glucoiberin and sinigrin. These three GLSs have been identified as major glucosinolates in kales and cabbages, where sinigrin was the most important GLS in kales (Cartea et al., 2008).

Trait selection in kale and nabicol varieties (*B. napus* var. *pabularia*) from Spain has shown that some morphological characteristics as glossy leaves are connected to resistance toward insects (Picoaga et al., 2003; Rodriguez et al., 2005). Despite leaf traits, it is well known that glucosinolates and their breakdown products can differently influence the feeding of monophagous and polyphagous insects (Renwick, 2002). Thus, the relationship between GLSs and plant resistance is often unclear and the possible influence of other secondary metabolites and nutrient levels can be involved in the resistance to different pest species (Cartea et al., 2010). The studies of the correlation between morphological traits and glucosinolates content in *Brassica* crops are elusive in the literature. Some information was done in experiments with *Brassica juncea* (Assefa et al., 2023) or Ethiopian mustard (*Brassica carinata* L.) (Teklehaymanot et al., 2019)

This study was an extension of work done in the evaluation of leaf morphological characteristics (Batelja et al., 2009) and has two new aims: (i) to determine the glucosinolate profile and content of this collection of leafy landraces that has not been evaluated before, and (ii) to identify any relationships that may exist between the leaf

morphological and chemical (GLSs) traits of Croatian leafy *Brassica* landraces. The results of this study will assist in the evaluation of *Brassica* biodiversity, allow us to detect genotypes with high-quality attributes and assist with potential future breeding efforts.

MATERIALS AND METHODS

Plant Material and Growth Conditions

The plant material used in this study consisted of 15 leafy landraces (*B. oleracea* var. *acephala*) collected along the Eastern Adriatic coast among local farmers (Table 1).

Table 1. Landrace number and geographic origin of the fifteen Croatian kale landraces included in the study

Landrace number*	Origin
1 - IJK 1	Blato n/C 43° 28′ 46″ N, 16° 50′ 28″ E
2 - IJK 6	Pridvorje (Konavle) 42° 36′ 3″ N, 18° 18′ 46″ E
3 - IJK 7	Opuzen 43° 0′ 51″ N, 17° 33′ 30″ E
4 - IJK 10	Tijarica 43° 35′ 2″ N, 16° 57′ 14″ E
5 - IJK 11	Vitaljina 1 42° 27′ 9″ N, 18° 28′ 24″ E
6 - IJK 12	Vitaljina 2 42° 26′ 25″ N, 18° 28′ 46″ E
7 - IJK 13	Vitaljina 3 42° 25′ 57″ N, 18° 29′ 10″ E
8 - IJK 14	Stravča 42° 36′ 34″ N, 18° 18′ 48″ E
9 - IJK 16	Mljet 42° 44′ 8″ N, 17° 33′ 3″ E
10 - IJK 17	Pula 44° 53′ 30″ N, 13° 55′ 20″ E
11 - IJK 18	Vižinada 45° 20′ N, 13° 46′ E
12 - IJK 19	Marčana 44° 57′ 35″ N, 13° 57′ 16″ E
13 - IJK 79	Svib 43° 33′ 35″ N, 16° 57′ 52″ E
14 - IJK 80	Zmijavci 43° 24′ 42″ N, 17° 12′ 45″ E
15 - IJK 81	Vrgorac (Banja) 43° 13′ 2″ N, 17° 22′ 46″ E

*Landrace number from the seed bank at the Institute of Adriatic Crops Split (Croatia)

At the end of July few kale seeds were sown in polystyrene trays with 30 ml cells in a commercial organic substrate (Brill Substrate, Georgsdorf, Germany). Seeds were allowed to germinate until the radicles were about 5 cm in length and then were thinned to one per cell. At the five-leaf stage, the seedlings were transplanted to ground beds 50 cm apart in rows and 70 cm between rows fertilized with slow-release fertilizer (15N:11P:13K). The wide spacing was applied to avoid competition between plants so that the phenotypic expression and differentiation were maximized. Plants were watered as required and once fertilized additionally with a 4% solution of liquid NPK (6:4:6) fertilizer. The trail was performed in Split, Mediterranean part of Croatia (43°30′15″N, 16°29′55″E), 50 m above sea level in calcareous alkaline silt loam soil. During the vegetation, weeds were removed mechanically to avoid the usage of herbicides and their potential impact on the morphological properties of the plant. The ecotypes were evaluated in a randomized complete block design with plots. Each plot included two rows with five plants per row.

The morphological characteristics were described by adapting the UPOV guidelines for Curly Kale (*Brassica oleracea* var. *sabellica* L.) (UPOV 2002) to the field observations and plant material and partially were already shown in study by Batelja et al., 2009.

For glucosinolates analysis, a sample of fresh and healthy fully developed leaves normally used for human consumption was used from four to five uniform plants per plot. Immediately after harvest leaves were frozen by liquid nitrogen and stored at -80 °C. Samples were lyophilized (Labconco, USA), ground into a fine powder and stored at -80 °C until glucosinolate analysis.

Extraction and desulphation of glucosinolates

Extraction, identification and quantification of glucosinolates were made in accordance with International Standard ISO 9167-1 (Rapeseed-Determination of glucosinolates content, Part 1: Method using HPLC).

The freeze-dried material (200 mg) was placed in centrifuge tubes and crude GSL was extracted with 2 ml of 70 % (v/v) boiling methanol maintained in a water bath at 700C for 15 min with 1 min vortex mixing. The mixture was centrifuged (5000 g, 5 min) and the supernatant was taken. The solid residue was re-extracted once again. The supernatants were collected and their volume was

brought to 5 ml with water. The ion-exchange column was, across bulbcut Pasteur pipet, with a glass wool plug in the neck. 0,5 ml of suspension of the exchange resin(DEAE-SEPHADEX A-25 in 2 M acetic acid, Sigma, USA) was quickly pipetted with a Pasteur pipette and poured into the prepared column. Liquid is drained by gravity. The resin was washed with 2 ml 6 M solution of imidazole formate and then washed with 2x1 ml of deionized water.

One ml of methanolic extract was applied to the ion exchange column. After dosing of extract to the column, it was flushed with 1 ml of 0,02 M Na-acetate buffer. 500 µl of sulfatase solution (25mg/2,5 ml of deionized water) from *Helix pomatia* (Sigma, USA) for desulfurization of GSL was put onto the column, capped and left overnight (16 h, RT). After the overnight reaction, desulfo GSLs were eluted into glass tubes with 1x1ml and 2x0,5 ml of deionized water. The eluates were analysed immediately or stored in the freezer (-20 °C) until HPLC analysis.

Desulphoglucosinolates analysis using HPLC

HPLC separation of desulfo-GSL was made on Zorbax Eclipse XDB-C18 column (4,6 x 250 mm, 5 µm) under the following conditions: injected volume 20 µm, flow rate 0,8 ml/min, column temperature 30 °C, wavelength detection 227 nm. The desulfo-GLS were separated using water (solvent A) and acetonitrile (solvent B) gradient, both with 0,1% trifluoroacetic acid.

Elution was carried out as follows: start with 96% A/4%B, linear gradient to 14% A/86%B at min 28, 14% A/86%B at 32 min, 96% A/4% B at 34 min using Perkin Elmer PE Series 200 HPLC system. Total analysis time was 42 min.

Identification of glucosinolates was done according to ISO 9167-1, using retention times of rapeseed glucosinolates and pure standards (singirin and tropaeolin) (Figure 1). Glucosinolate content was calculated using sinigrin as an external standard and the response factor of each compound relative to sinigrin (ISO 9167-1) and expressed in µmol/g of dry material. We used a calibration curve for siringin (Figure 2).

Statistical analysis

Analysis of variance (ANOVA) was performed to determine the difference among accessions for glucosinolate content. Differences among means were considered significant at $P \leq 0.05$ using Tukey's HSD test. Correlation coefficients (r) were determined by the Pearson correlation matrix method. A principal component analysis (PCA) was performed to evaluate interrelationships among accessions. Data obtained were analyzed using Statistica software version 11.0 (StatSoft, Inc. USA, 2012).

RESULTS AND DISCUSSION

The evaluation of kale leaves morphological traits is shown in Table 2. All genotypes included in this study belong to the same group (*B. oleracea* var. *acephala*) and they had a great range of morphological variation among them. Most of the landraces did not have curled leaves and without leaf blades blistered having a structure more like collards as was reviewed in Šamec et al. (2019).

The variation in color of the developed leaf was the most pronounced trait found in the germplasm evaluated.

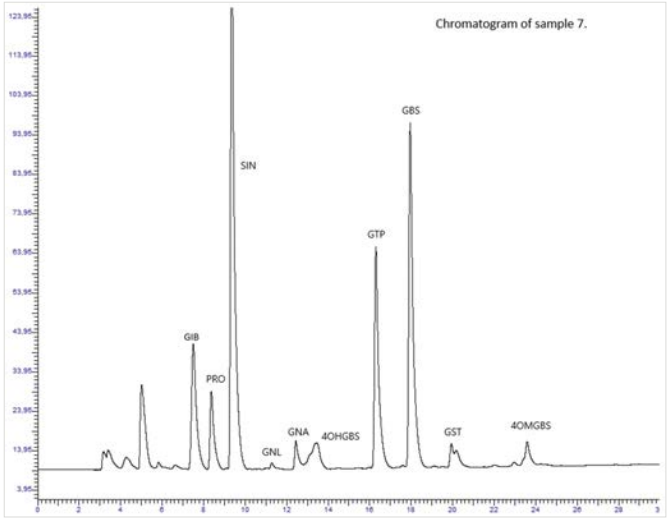

Figure 1. Glucosinolates determined in landrace IJK 13 (abbreviations for each compound are noted in Table 3)

Evaluation of glucosinolate levels showed significant differences between landraces (Table 2). Total glucosinolate concentration in studied landrace leaves ranged from 11.1 to 106.9 µmol/g DW with a mean value of 46,9 µmol/g DW. These high concentrations in some landraces are much higher than the ones found in other kale genotypes/landraces worldwide (Rosa et al., 1996; Kushad et al., 1999; Cartea et al., 2008). The chromatogram of identified glucosinolates in one landrace (IJK 13) is shown in Figure 1.

Table 2. Leaf morphological characteristics of studied landraces

Leaf trait	Landrace
Anthocyan coloration(Ant)	
1 - absent	1, 12, 17
2 - present	6, 7, 10, 11, 13, 14, 16, 18, 19, 79, 80, 81
Anthocyan coloration distribution (DAnt)	
1 - partial	1, 7, 10, 11, 12, 13, 16, 17, 18, 19, 79, 80, 81
2- entire leaf	6, 14
Color of developed leaf (Col)	
1- yellow green	
2 - green	18, 19, 79, 81
3 - grey green	1, 7, 12, 16, 80
4 - blue green	10, 11, 13, 17,
5 - purple (red)	6, 14
Curling density (Curl)	
1 - absent	1, 6, 10, 11, 12, 13, 14, 16, 19, 80
2 - low	7, 17, 18, 81
3 - medium	79
4 - high	
Leaf blade blistering (Blist)	
1 -none	6, 7, 10, 11, 12, 13, 14, 16, 18, 81
2 - low	1, 17, 19, 80
3 - intermediate	79
4 - high	
Leaf divison (Div)	
1 - entire	
2- sinuate	7, 17
3 - lyrate	1, 6, 10, 11, 12, 13, 14, 16, 18, 19, 79, 80, 81
4 - lacerate	

Thirteen glucosinolates belonging to the three principal chemical groups were found in the studied landraces from the eastern Adriatic coast, including seven aliphatic, four indolic, and two aromatic glucosinolates. Seven glucosinolates (glucoiberin, progoitrin, sinigrin, glucobrassicin, 4-hydroxiglucobrassicin, neoglucobrassicin, and gluconasturtin) were detected in all landraces, while epi-progoitrin was found in only 9 of them. Among aliphatics, sinigrin and glucoiberin were the predominant glucosinolates in all landraces, while among indoles glucobrassicin was at a much higher level than other compounds from the same group. Aromatic gluconasturtin had a higher content than glucotrapeolin. Studied landraces had significantly higher amounts of aliphatic glucosinolates than indolic, as reported for Polish varieties (Korus et al., 2014), while the opposite was found for Turkish ones (Sarıkamış et al., 2008). Although leaf morphological properties showed that most landraces belong to collard morphotypes, glucosinolate groups proportion did not indicate the same, as was found that collard had more indole glucosinolates with major one glucobrassicin (Delonga et al., 2007; Radošević et al., 2017; Kim et al., 2017). Also, in studied landraces, mostly collard types, glucoraphanin was not detected as was found in 25% of 81 US collard landraces evaluated (Stansell et al., 2015).

Results also showed significant differences in concentrations of individual glucosinolates among the kales tested. Sinigrin and glucoiberin are predominant GLS each one in 7 landraces, and only one (IJK 10) had highest gluconasturtiin concentration. As was differentially reported in kales main GLS was sinigrin or glucoiberin which was also most abundant in cabbage plants (Cartea and Velasco, 2008; Nilsson et al., 2006). These differences in the two main GLS could be the result of natural hybridization and selection that occur, as was shown that collard-cabbage hybrids have better agronomic performances and mostly maintain dominant non-heading (kale/collard) shape (Farnham et al., 2005). The glucosinolates gluconapin, sinigrin, progoitrin and indole glucobrassicin and neoglucobrassicin produce bitter and pungent isothiocyanates, so an excessive content might decrease consumer preference (Bell et al., 2018).

Landralpce IJK 10 had a very high concentration of gluconasturtiin. The phenethyl isothiocyanate, a breakdown of gluconasturtin, has been reported to have flavor attributes with a very low odor threshold when compared to the breakdown products of other GLS (Fenwick et al., 1983, Coolong et al., 2004). Some *Brassica* sp. showed higher resistance to the pest *Mamestra brassicae* larvae due to high gluconasturtiin concentration (Müller et al., 2018).

Studied kales contained on average higher progoitrin concentrations than previously reported levels in edible and ornamental kales (Kushad et al., 1999; Kushad et al., 2004; Cartea et al., 2008). High progoitrin content has been considered potentially goitrogenic and toxic in animals, which suggests a doubtful source of feed, at least for some accessions.

Cabbage genotypes with higher contents of glucobrassicin, glucoiberin and glucoiberverin were more resistant to diamondback moth larvae feeding preferences (Robin et al., 2017).

Multivariate analysis

The analysis of the correlation between morphological properties and glucosinolates content was done and gave us limited conclusions (data not shown). Glucoiberin and total glucosinolates were negatively correlated (r = - 0,68 and r = - 0,60, respectively) with leaf color having less of it in blue green, and red leaves. Kushad et al. (2004) found the opposite in cabbage with the highest total glucosinolates in the pink and red-leafed cultivars, which was also confirmed by Choi et al. (2014) who found that red cabbages had less sinigrin and more progoitrin and glucoraphanin than green ones.

The whole data set was subjected to principal component analysis (PCA) to obtain a broad view of accession dispersion (Figure 2(A)). Eigenvalues for the first two factors were 23,5% and 16,0% respectively, and cumulatively they capture 39,5% of the total variance in the data.

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Table 3. Total and individual glucosinolates concentrations (µmol/g DW) for 15 kale landraces from the eastern Adriatic coast

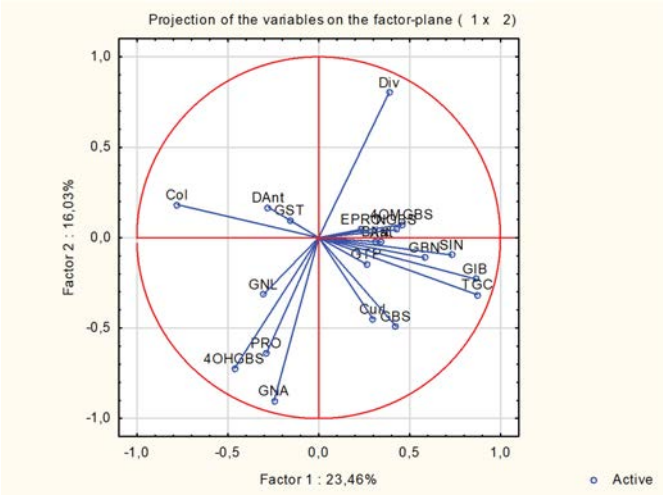
Genotype	Aliphatic							Indolic				Aromatic		Total
	GIB	PRO	EPRO	SIN	GNL	GNA	GBN	GBS	4OHGBS	4OMGBS	NGBS	GTP	GST	
IJK 1	7,72^{d}	0,08^{g}	nd^{d}	3,36^{de}	nd^{d}	0,03^{d}	0,02^{c}	3,89^{d}	0,03^{f}	0,03^{cd}	0,30^{de}	0,07^{e}	1,62^{d}	17,13^{ef}
IJK 6	3,13^{de}	2,58^{e}	nd^{d}	8,61^{de}	nd^{d}	0,04^{d}	0,01^{c}	4,40^{d}	0,07^{f}	0,09^{cd}	0,27^{de}	0,35^{a}	0,71^{ef}	20,26^{ef}
IJK 7	13,09^{c}	9,49^{a}	nd^{d}	8,49^{de}	0,03^{c}	0,98^{a}	0,02^{c}	9,73^{c}	0,95a	nd^{e}	0,41^{cd}	0,13^{cd}	1,66^{d}	44,98^{de}
IJK 10	1,77^{e}	0,27^{g}	0,02^{c}	1,79^{e}	nd	nd^{e}	0,13^{c}	2,35^{d}	0,01^{f}	nd^{e}	0,36^{cd}	<0,01^{f}	4,46^{a}	11,11^{f}
IJK 11	7,68^{d}	3,65^{d}	0,03^{c}	15,77^{c}	0,51^{bc}	0,04^{d}	0,03^{c}	6,61^{cd}	0,49^{b}	0,13^{bc}	0,34^{cd}	0,12^{d}	0,62^{ef}	36,01^{e}
IJK 12	5,65^{de}	4,60^{c}	0,04^{c}	7,66^{de}	0,43^{bc}	0,05^{d}	0,05^{c}	6,34^{d}	0,27^{de}	0,18^{bc}	1,49^{ab}	0,04^{ef}	1,47^{d}	28,26^{e}
IJK 13	3,13^{de}	3,59^{d}	0,04^{c}	25,02^{b}	0,38^{bc}	0,05^{d}	0,05^{c}	3,69^{d}	0,47^{b}	0,03^{cd}	0,22^{de}	0,05^{ef}	0,68^{ef}	37,40^{e}
IJK 14	11,04^{cd}	5,71^{b}	0,06^{b}	17,03^{c}	0,52^{bc}	0,03^{d}	nd^{d}	13,70^{b}	0,39^{c}	0,01^{d}	1,17^{b}	0,01^{f}	2,34^{c}	52,01^{d}
IJK 16	13,75^{c}	0,34^{g}	nd^{d}	6,08^{de}	0,01^{c}	0,03^{d}	nd^{d}	4,97^{d}	0,12^{ef}	<0,01^{e}	0,01^{e}	nd^{g}	0,37^{f}	25,67^{e}
IJK 17	9,23^{cd}	0,30^{g}	nd^{d}	8,82^{de}	1,26^{a}	0,42^{b}	0,12^{c}	7,62^{cd}	0,32^{cd}	0,03^{cd}	0,08^{e}	0,03^{ef}	1,03^{de}	29,23^{e}
IJK 18	55,75^{a}	1,46^{f}	nd^{d}	27,61^{b}	0,21^{c}	0,02^{d}	0,08^{c}	17,44^{a}	0,01^{f}	0,06^{cd}	0,68^{cd}	0,18^{cd}	3,48^{b}	107,0^{a}
IJK 19	52,44^{a}	1,41^{fg}	0,02^{c}	25,94^{b}	0,18^{c}	0,23^{c}	0,78^{a}	16,61^{ab}	0,01^{f}	0,10^{bc}	0,56^{cd}	0,30^{b}	0,40^{f}	98,97^{ab}
IJK 79	9,64^{cd}	0,56^{g}	0,50^{a}	17,22^{c}	0,08^{c}	0,02^{d}	0,11^{c}	7,08^{cd}	0,19^{ef}	0,01^{d}	0,57^{cd}	0,15^{cd}	0,92^{ef}	37,05^{e}
IJK 80	13,55^{c}	2,38^{e}	0,03^{c}	42,86^{a}	<0,01^{d}	0,02^{d}	0,31^{b}	4,18^{d}	0,30^{de}	0,14^{bc}	1,56^{ab}	0,02^{ef}	1,17^{de}	66,51^{c}
IJK 81	46,47^{b}	2,10^{ef}	0,04^{bc}	29,44^{b}	0,08^{c}	0,05^{d}	0,01^{c}	3,13^{d}	0,04^{f}	1,41^{a}	1,41^{ab}	8,67^{f}	0,05^{b}	

*Different letters within columns indicate significant differences between factors by Tukey HSD test at $P \leq 0.05$; GIB: glucoiberin; PRO: progoitrin; EPRO: epiprogoitrin; SIN: sinigrin; GNL: glukonapoleiferin; GNA: gluconapin; GBN: glucobrassicanapin; GBS: glucobrassicin; 4OHGBS: 4-hydroxyglucobrassicin; 4OMGBS: 4-methoxyglucobrasssicin; NGBS: neoglucobrassicin; GTP: glucotrapeolin; GST: gluconasturtin.

Factor 1 shows the strongest positive correlation with glucoiberin and total glucosinolate concentration, while the strongest negative correlation was leaf color. Factor 2 was strongly positively correlated with leaf division, while it was negatively correlated with gluconapin, 4-hidroxyiglucobrassicin and progoitrin concentrations (Figure 2(A)).

Five groups with different numbers of accessions can be separated on the plot of the principal component scores based on leaf morphological properties and glucosinolate content (Figure 2(B)).

(A)



(B)

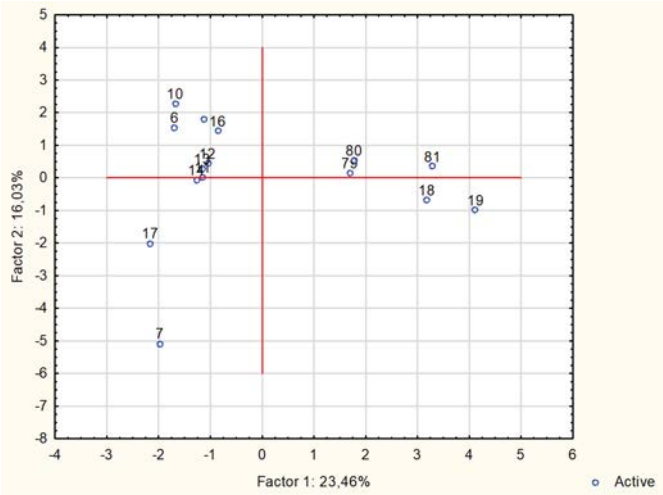


Figure 2. Principal component analysis of the first two factors showing dispersion of kale landraces based on the measured parameters; variable plot (A) and observation score (B)

IJK 7 appears quite different from all other accessions regarding progoitrin, gluconapin and 4-hidroxyiglucobrassicin concentration. IJK 17 is divided from others by the highest gluconapoleiferin concentration. All others, can be separated into groups different by leaf color (8 landraces). The group with IJK 18, 19, and 81 have high total glucosinolates and glucoiberin concentrations.

CONCLUSION

The present work is an important step forward in the knowledge of the variability in glucosinolates from leafy *Brassica olearacea* landraces collected along the Adriatic Coast. These data should be valuable tool for future direction for the conservation of interesting landraces and additional work on the evaluation of agronomic performance for breeding efforts aiming at the development of new kale cultivars with potential health benefits.

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