

EVALUATION OF GLUCOSINOLATES IN NORDIC HORSERADISH (*ARMORACIA RUSTICANA*)
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Abstract

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Horseradish (*Armoracia rusticana* Gaertn., C.A. Mey & Scherb.) has a long history as food and medicinal plant. Glucosinolates (GLS) or their breakdown products are responsible for the pungent taste and claimed medicinal effects. The dominant GLS in horseradish is sinigrin (> 80%) followed by gluconasturtiin and glucobrassicin. A total of 168 Nordic accessions of horseradish were screened for the content of intact glucosinolates. Sinigrin levels varied between 10 and 45, gluconasturtiin between 1.3 and 7.4 and glucobrassicin between 0.1 and 2.6 μmol/g DM. Accessions with high levels of both sinigrin and gluconasturtiin were found. Horseradish accessions are kept as living plants in clonal archives in their respective countries. The task for plant gene banks is not only to conserve genetic resources for the future, but also to stimulate use of the collections for various products and breeding programmes. After further analyses to certify the screening results, selected accessions will form a base for breeding and increased use of horseradish as a condiment to food, natural preservative or in medical treatments.

Keywords: sinigrin, gluconasturtiin, glucobrassicin, genetic diversity, allyl isothiocyanate, mustard oil, 2-phenylethyl isothiocyanate.

INTRODUCTION

The *Brassicaceae* family contains about 3000 different species including many kinds of cabbages and root crops with a long history as food and medicinal plants (ANDERBERG & ANDERBERG, 2010). Horseradish (*Armoracia rusticana* Gaertn., C.A. Mey.&Scherb. was mentioned already by the Roman author and naturalist Pliny the elder (AD 23–79), who recommended it freshly grated after a heavy meal to promote digestion (BOSTOCK, 1855; COURTER & RHODES, 1969). It was introduced to the Nordic countries as a medi-

nal plant in the 13th century (LANGE, 1999). The root was used to cure cough and heart- and lung diseases, and was taken as stimulation for the digestion and against stomach troubles. Horseradish was also used as an agent to prevent or relieve scurvy (WEDELSBÄCK BLADH & OLSSON, 2011). In the 17th century, the use turned from medicine to a condiment to various food (GRIEVE, 1979). The strong flavour of horseradish is appreciated in both meat and fish dishes and is also used as a substitute to the more expensive wasabi (WEDELSBÄCK BLADH & OLSSON, 2011).

The chemical substances responsible for the pungent taste and claimed medicinal effects of horseradish were long unknown. Today, we know that plants of the *Brassicaceae* family are rich in sulphur containing glycosides, so called glucosinolates (GLS) (VAN DOORN et al., 1998; KUSHAD et al., 1999). More than 120 structures of GLS have been described and about 20 are present in *Brassica* vegetables (FAHEY et al., 2001). A number of studies have shown that the dominant GLS differ between plant species. In white cabbage, glucobrassicin is found in the highest concentrations (SARIKAMIŞ et al., 2009). Progoitrin is more prevalent in swedes, (SONES et al., 1984), glucoerucin and glucoraphenin are the major components in wild radish (*Raphanus raphanistrum* L.), whereas glucoraphasatin and glucoraphenin in commercial radish (*Raphanus sativus* L.) (MALIK et al., 2010). Glucoraphenin is also found in high concentrations in broccoli together with glucobrassicin (SARLI et al., 2012). Sinigrin is dominant in Brussels sprouts, cauliflower and horseradish (KUSHAD et al., 1999; FAHEY et al., 2001; LI & KUSHAD, 2004).

The concentrations of specific GLS differ between cultivars within species and between various parts of the plants in *Brassica* vegetables (JURGES & THIES, 1980; SONES et al., 1984; NILSSON et al., 2006; VERKERK et al., 2009). The variation is also shown in horseradish, where the relation between the GLS varies between the leaves and the roots (AGNETA et al., 2012). In the study made by LI & KUSHAD (2004), the sinigrin level accounted for about 83% in the roots and 91% in the leaves. Environmental factors such as climate, soil and fertilizer affect the GLS levels in the plants (ZHAO et al., 1993; CÍSKA et al., 2000; KOSSON & HORBOWICZ, 2008). Maturity and harvest time (ROSA et al., 1996; SARIKAMIŞ et al., 2009) also influence the GLS levels.

A recent study has identified 12 GLS in the roots and 16 GLS in the sprouts of horseradish (AGNETA et al., 2012). The strong and bitter taste derives from sinigrin and gluconasturtiin after degradation to allyl isothiocyanate (AITC, mustard oil) and 2-phenylethyl isothiocyanate (PEITC), respectively. The isothiocyanates are formed from the GLS with the help of the enzyme myrosinase when plant tissues are damaged. The study of different German horseradish types (NEBEL, 1987) also showed that the AITC level increases during growth season. AITC is known to in-

hibit prostate cancer (SRIVASTAVA et al., 2003), induction of lung cancer, the development of tumours in liver and fore stomach (KOSSON & HORBOWICZ, 2008) and is effective as a cancer chemo-preventive compound in the bladder (ZHANG, 2010). PEITC has been shown to inhibit development of tumours in lungs and oesophagus of laboratory rodents (MORSE et al., 1993; HECHT et al., 1996; MORSE et al., 1997; FAHEY et al., 2001; KOSSON & HORBOWICZ, 2008).

The aim of this study was to screen the variation of intact GLS in Nordic horseradish accessions. NordGen, the Nordic Genetic Resource Centre at Alnarp in Sweden, has collected a large number of horseradish clones from Denmark, Finland, Norway and Sweden. The accessions are kept as living plants in clonal archives in their respective countries. The task for plant gene banks is not only to conserve genetic resources for the future but also to stimulate the use of the collections for various products and breeding programmes. Gene bank materials, therefore, need to be described for morphological characters and to be evaluated for factors of value for production, e.g. resistance against pests, diseases and stress as well as nutritional quality.

Another purpose of this study was to analyse the concentration of GLS in different parts of the horseradish root. Knowledge about the distribution is very useful to achieve representational samples for chemical analysis. Also, horseradish is usually consumed in small amounts. With information about the levels in the clones and distribution in the root, it will be possible to maximize the consumption of valuable active biochemical compounds.

MATERIALS AND METHODS

Plant material

The material (Table 1) consisted of an older Danish collection with 23 accessions of horseradish established in the 1960–70s (Group I) representing A) European accessions together with one accession from Israel and one from Japan, B) accessions collected in Denmark and C) Danish breeding lines. In 2002 and 2003, another 145 horseradish accessions were collected mainly from old gardens in Sweden, Norway, Denmark and Finland (Groups II–V) (Table 1). Since 168 accessions were grown in different

countries and analysed in different years, the material will be referred to five groups.

Three plants per accession were grown in trial fields in their respective home country. Root cuttings (30 cm in length) from the mother plants were planted in the soil at a 45-degree angle in early May. Plant space was 0.5 m in all directions. Fertilizer, weed control and irrigation were carried out according to local practices. The roots were harvested in late autumn and stored at 90–95% relative humidity (RH) at 4° C until analysis.

The distribution of GLS within the root was analysed in six Danish accessions (D1, D9, D10, D22, D25 and D27). The selection of the material was based on the results from the first year, when two accessions had high levels, two accessions had medium levels and two accessions had low levels of GLS.

Sample preparation

From each horseradish accession in groups I–V, two roots with a diameter of ca 30 mm were chosen from two different plants. The roots were washed with distilled water, air-dried and a 30-mm-long piece was cut from the middle part of each root. After peeling 1 mm with a potato peeler, the two root samples were rapidly macerated together in a food-processor into smaller pieces and the GLS were extracted and determined as below.

For determination of the GLS distribution within the root, two roots of the same thickness (middle part 30 mm in diameter) from each accession were chosen. The roots were washed with distilled water

and air-dried, but not peeled. Samples 1 (outer 5 mm tissue) and 2 (inner tissue) were taken close to the shoulder, samples 3 (outer tissue) and 4 (inner tissue) from the middle part of the root and samples 5 (outer tissue) and 6 (inner tissue) from the root tip. Corresponding tissues from the two roots were pooled and rapidly macerated in a food processor and the GLS were extracted and determined as below.

Extraction and determination of glucosinolates

After maceration, 3 g of each sample were immediately boiled in 10 ml 99.5 % ethanol in a water bath for 10 minutes to hinder degradation of the GLS by myrosinase. Each sample was mixed with 100 µl glucotropaeolin (benzyl glucosinolate, internal standard, 120.8 mg in 50 ml water) and another 10 ml ethanol.

After homogenization for 30 seconds at 12000 rpm in Ultra Turrax (IKA T 18 basic), the extraction by boiling was continued for another 15 min. After cooling, the samples were centrifuged at 1481 × g (Rotixa/RP centrifuge, Hettich). Each supernatant was applied on a DEAE Sephadex A-25 column and treated according to the method described by HEANEY & FENWICK (1980). After washing with 6 ml water, 400 µl purified sulfatase solution (*Helix pomatia* type H1, EC 3.1.6.1) was added to the samples and left over-night. The desulphoglucosinolates were eluted with 2 ml water and analysed by high-performance liquid chromatography according to the method described in COMMISSION OF THE EUROPEAN COMMUNITIES (1990).

Table 1. The number, origin and cultivation place of the analysed horseradish accessions in five different groups

Group (number of accessions)	Origin of accessions	Cultivation site (Coordinates)
Group I: Denmark Old collection (23)	A) 10 accessions from Great Britain, Germany, Italy, Sweden, Denmark, Poland, former Czechoslovakia and Yugoslavia), Israel and Japan	The Danish Institute of Agricultural Sciences DIAS, Årslev (55° 30' N, 10° 48' E)
	B) 4 accessions Collected in old Danish gardens	The Danish Institute of Agricultural Sciences, DIAS, Årslev (55° 30' N, 10° 48' E)
	C) 9 accessions Danish breeding lines	The Danish Institute of Agricultural Sciences, DIAS, Årslev (55° 30' N, 10° 48' E)
Group II: Sweden (67)	Collected in old Swedish gardens	Svalöf Weibull AB, Landskrona (55° 52' N, 12° 49' E)
Group III: Norway (21)	Collected in old Norwegian gardens	Bioforsk Landvik (58° 20' N, 08° 31' E)
Group IV: Denmark New collection (31)	Collected in old Danish gardens	The Danish Institute of Agricultural Sciences, DIAS, Årslev (55° 30' N, 10° 48' E)
Group V: Finland (25)	Collected in old Finnish gardens	Agrifood Research Finland, MTT Pikkio (60° 23' N, 22° 30' E)

Statistical analysis

The statistical system “R” was used for the t-test, ANOVA and the bivariate boxplot methods. The home page for R, <http://www.r-project.org/>, provides access to panoply of resources and information, including link to the comprehensive R Archive Network (CRAN), from which R software can be downloaded.

RESULTS AND DISCUSSION

Variation of glucosinolates between accessions in the old Danish collection

Our study on GLS variation in horseradish started in 2003 with the Danish collection of mainly European market varieties and a few Danish breeding clones (group I). The following eight GLS were detected in all 23 accessions: glucoiberin, sinigrin, gluconapin, 4-hydroxyglucobrassicin, glucobrassicinapin, glucobrassicin, gluconasturtiin, and 4-methoxyglucobrassicin. In two accessions, progoitrin was also detected. These GLS have also been identified in horseradish by LI & KUSHAD (2004) and AGNETA et al. (2012).

Sinigrin was by far the most abundant GLS and constituted ca 87% of the total amount followed by gluconasturtiin with ca 8% and glucobrassicin with ca 3%. These three GLS covered 98–99 % of the total amount.

The European market varieties together with the accessions from Japan and Israel (group I A) showed a variation of sinigrin between 25 and 38 $\mu\text{mol/g DM}$ (mean value 32 $\mu\text{mol/g DM}$) (Table 2). Material

from one country may have low as well as high levels. The two accessions from Germany, thus, had mean sinigrin levels of 26 and 37 $\mu\text{mol/g DM}$, respectively, and the two Swedish accessions had 25 and 38 $\mu\text{mol/g DM}$, respectively.

Gluconasturtiin varied between 1.9 and 3.8 $\mu\text{mol/g DM}$ (mean value 3.0 $\mu\text{mol/g DM}$) and glucobrassicin between 0.7 and 1.9 $\mu\text{mol/g DM}$ (mean value 1.2 $\mu\text{mol/g DM}$). The non-European varieties did not exceed these ranges. The remaining GLS were found only in minor concentrations (1–2%) and will not be further discussed here. Several of these compounds are found in higher levels in other *Brassica* vegetables (SONES et al., 1984; SARIKAMIŞ et al., 2009). Analyses of the limited material collected from home gardens in different areas of Denmark (group I B) did not broaden the variation much. Only one out of four clones had a slightly higher sinigrin level (45 $\mu\text{mol/g DM}$) than material from group I A. Further analyses of nine Danish breeding clones (group I C) showed a range in sinigrin levels between 27 and 45 $\mu\text{mol/g DM}$.

LI & KUSHAD (2004) found much larger variation in their material of 27 accessions with origin from different parts of the world. They had a range in sinigrin levels from 2 to 258 $\mu\text{mol/g DM}$ in *unpeeled* horseradish roots. In their study, the variation between accessions from the same country could be up to 30-fold. This encouraged us to study a much larger horseradish material consisting of 145 accessions, which were collected in 2002–2003 from the Nordic countries (groups II–V). Before screening this material we decided to check the distribution of GLS within the root.

Table 2. Variation and mean values of sinigrin, gluconasturtiin and glucobrassicin and percentage of sinigrin of total glucosinolates (GLS) in Nordic horseradish (groups I–V)

Collection cultivated in	Group	Sinigrin ($\mu\text{mol/g DM}$) Min–Max (Mean \pm SD)	Gluconasturtiin ($\mu\text{mol/g DM}$) Min–Max (Mean \pm SD)	Glucobrassicin ($\mu\text{mol/g DM}$) Min–Max (Mean \pm SD)	Sinigrin of total GLS content (%) Min–Max (Mean \pm SD)
Denmark	I (A)	24.9–38.4 (32.2 \pm 5.1)	1.9–3.8 (3.0 \pm 0.6)	0.7–1.9 (1.2 \pm 0.3)	82.1–90.5 (87.0 \pm 0.02)
Denmark	I (B)	34.2–45.3 (39.2 \pm 4.6)	3.9–6.2 (5.1 \pm 1.0)	0.8–2.0 (1.2 \pm 0.6)	81.3–86.4 (84.0 \pm 0.03)
Denmark	I (C)	27.1–45.1 (33.9 \pm 5.7)	2.1–4.2 (2.6 \pm 0.6)	0.8–2.6 (1.2 \pm 0.5)	84.7–91.7 (89.0 \pm 0.02)
Sweden	II	13.6–38.6 (26.6 \pm 4.5)	1.3–7.4 (4.4 \pm 1.4)	0.07–1.2 (0.4 \pm 0.2)	75.2–91.8 (83.0 \pm 0.04)
Norway	III	10.0–36.9 (22.4 \pm 6.8)	2.2–7.2 (4.2 \pm 1.4)	0.04–0.5 (0.2 \pm 0.11)	73.7–87.2 (82.0 \pm 0.04)
Denmark	IV	17.2–36.2 (28.8 \pm 4.6)	1.3–4.8 (2.7 \pm 0.8)	0.07–0.6 (0.3 \pm 1.2)	84.6–93.9 (90.0 \pm 0.02)
Finland	V	15.7–33.3 (21.9 \pm 5.2)	1.6–5.6 (3.4 \pm 1.0)	0.04–0.2 (0.1 \pm 0.04)	78.0–91.1 (85.0 \pm 0.04)

Variation of glucosinolates within the root

Different studies show that the GLS are not evenly distributed in the plants. CARLSSON et al. (1981) found higher concentrations of gluconasturtiin and glucobrassicin in the peelings of turnip compared to the inner parts. Higher concentrations of progoitrin, 4-methoxy-3-indolylmethyl, neoglucobrassicin and gluconasturtiin were also found in the outer parts of swedes analysed by SONES et al. (1984). NILSSON et al. (2006) showed that the sinigrin levels were higher in the outer than in the inner, younger leaves in cabbage heads.

To learn more about the distribution of the three most frequent GLS within the horseradish root, different parts were analysed (Table 3). ANOVA analysis showed significant differences in sinigrin ($P < 0.001$) between the shoulder region and the middle or tip regions with higher levels in the shoulder.

Somewhat higher levels of both sinigrin and gluconasturtiin were found in the inner parts of the root compared to the outer parts from the same section. Glucobrassicin seemed, however, to be more evenly distributed within the root (Table 3). The differences between outer- and inner tissue were tested by the *t*-test. Only the difference in glucobrassicin for shoulder was significant at 5% level.

In our study of Nordic accessions, we decided to peel all roots before sampling and analysis of GLS. This handling did not cause loss in sinigrin and gluconasturtiin levels. The total amount of GLS as well as sinigrin was higher in the shoulder zone than in the middle and tip zones of horseradish roots. Sampling the

middle part of the root was, however, a more workable solution since this part usually does not have splits or cracks, which were more frequent in the shoulder region. Such damage to the tissues causes stress, which might influence the GLS levels and also makes it more difficult to clean the sample before analysis.

Variation of glucosinolates between accessions in the new Nordic collections

In addition to the GLS that were found in the European market varieties and Danish breeding clones (group I), a number of unidentified GLS were detected in small amounts in the Nordic collections (groups II–V). AGNETA et al. (2012) analysed only one variety, but identified 12 GLS in the root by using mass spectrometry. Seven of these GLS were the same as in our investigation, but they also found 2 methyl sulfonyl-oxo-ethyl-glucosinolat, glucosativin, glucoibarin, 5-hydroxy- glucobrassicin and glucoarabishirsutain. It is possible that one or more of our unidentified GLS are identical with the GLS found by AGNETA et al. (2012). As our material was not cultivated at the same location or in the same year, the results had to be treated separately. In Table 2, the variation of the levels of the three most frequent compounds in each group is shown. The difference in total GLS content was 3-fold among the Swedish accessions (group II) and 3.5-fold among the Norwegian accessions (group III). The latter material consisting of 21 accessions also showed the largest range of sinigrin (10–37 $\mu\text{mol/g DM}$). The smallest variation in sinigrin content (16–33 $\mu\text{mol/g DM}$) was found in

Table 3. Distribution of glucosinolates ($\mu\text{mol/g DM}$) in the outer (5 mm) and inner tissues of the shoulder, middle and tip zones in horseradish roots. Mean values of six accessions selected from group I included with standard deviation, *t*-test and *p*-value

GLS	Shoulder region			Middle region			Tip region		
	Outer tissues ($m \pm \text{SD}$)	Inner tissues ($m \pm \text{SD}$)	<i>t</i> -test <i>p</i> -value	Outer tissues ($m \pm \text{SD}$)	Inner tissues ($m \pm \text{SD}$)	<i>t</i> -test <i>p</i> -value	Outer tissues ($m \pm \text{SD}$)	Inner tissues ($m \pm \text{SD}$)	<i>t</i> -test <i>p</i> -value
Sinigrin	40.2 \pm 11.7	41.6 \pm 7.5	0.23 0.82	31.1 \pm 6.6	33.7 \pm 5.6	0.76 0.47	28.0 \pm 6.1	29.63 \pm 0.8	0.54 0.60
Gluconasturtiin	5.8 \pm 2.0	6.6 \pm 1.5	0.78 0.45	5.2 \pm 1.4	5.9 \pm 1.5	0.89 0.39	5.0 \pm 1.2	5.9 \pm 1.6	1.14 0.28
Glucobrassicin	1.0 \pm 0.2	0.8 \pm 0.1	2.57 0.03*	0.8 \pm 6.2	0.6 \pm 0.2	1.25 0.24	0.7 \pm 0.2	0.7 \pm 0.3	0.30 0.31
Total GLS	47.6 \pm 13.7	49.4 \pm 7.7		37.4 \pm 7.6	40.7 \pm 6.0		34.0 \pm 7.1	36.5 \pm 4.0	
Sinigrin % of total GLS	84 \pm 0.02	84 \pm 0.04		83 \pm 0.02	83 \pm 0.04		82 \pm 0.02	81 \pm 0.04	

* significant differences at $p < 0.05$

the Finnish material (group V) with 25 accessions. The highest mean value of sinigrin (29 $\mu\text{mol/g DM}$) was found in the Danish group (IV), followed by the Swedish (27 $\mu\text{mol/g DM}$). The Norwegian and Finnish accessions both had a mean value of 22 $\mu\text{mol/g DM}$. The sinigrin part of the total GLS content varied between 82 and 90% in groups II–V, with the highest percentage in the Danish material (Table 2). The much larger variation from 2 to 258 $\mu\text{mol sinigrin/g DM}$ in Li & KUSHAD's study (2004) of 27 accessions (mean value 81 $\mu\text{mol/g DM}$) might be due not only to a broader genetic background in his material compared to ours, but also to environmental conditions.

The mean value for gluconasturtiin varied between 2.7 (group IV) and 4.4 (group II) $\mu\text{mol/g DM}$. In Li & KUSHAD's study (2004) the mean value for this GLS was higher with 10.3 $\mu\text{mol/g DM}$. In our study, the mean values for glucobrassicin in groups II–V was 0.1–0.4 $\mu\text{mol/g DM}$ and in Li & KUSHAD's study (2004) – 0.5 $\mu\text{mol/g DM}$. The Swedish material showed the largest variation (0.1–1.2 $\mu\text{mol/g DM}$) among the groups.

The three major GLS in horseradish roots are sinigrin, gluconasturtiin and glucobrassicin, which all have

anti-cancerogenic effects (KUSHAD et al., 1999; VERKERK et al., 2009). The bivariate boxplot method was used to show the relationship between sinigrin and gluconasturtiin shown in Fig. 1 for groups II–V. This type of boxplot is useful for the indication of correlation as well as possible outliers. It consists of two concentric ellipses, one of which (the “hinge”) includes 50% of the data and the other (the “fence”), which delineates potential outliers. Furthermore, regression lines of both y on x and x on y are shown, with intersection showing the bivariate location of estimator. The size of angle between the regression lines indicates the correlation (GOLDBERG & IGLEWICZ, 1992; EVERITT & HOTHORN, 2011).

None of the accessions in any of the groups in Fig. 1 is found outside the “fence”, so no potential outliers are identified. However, several accessions are just on the “fence” as S13 in group II, N13 and N16 in group III and F6 in group V. It might be those accessions a breeder would look for. The results may also guide growers and consumers, who look for a specific taste or strength for culinary purposes. However, since the present study is only a first screening of GLS in the Nordic material, further analyses of the accessions are needed.

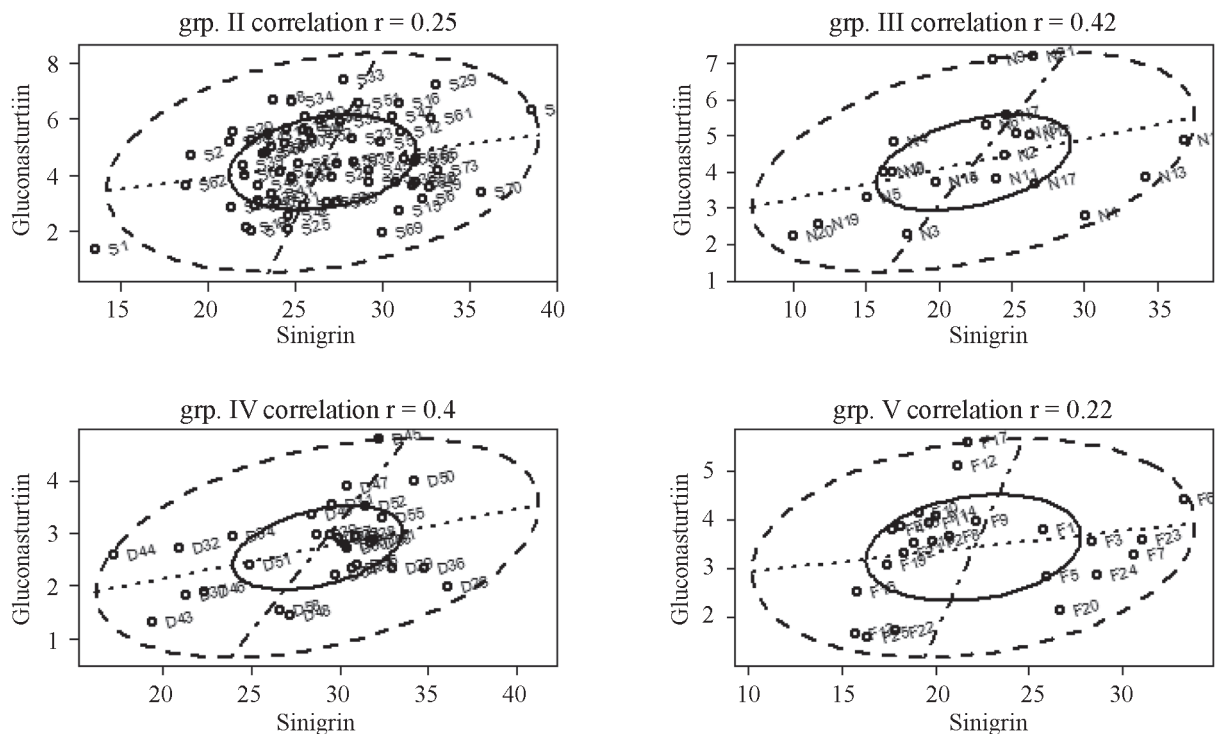


Fig. 1. The relationship and correlation between sinigrin and gluconasturtiin content ($\mu\text{mol/g DM}$) in horseradish (groups II–V) shown by the bivariate boxplot method

The bivariate boxplot method was not used to show the relationship between sinigrin and glucobrassicin. The glucobrassicin levels are very low in horseradish compared to the levels in other *Brassica* vegetables such as white head cabbage (21–43 µmol/g DW) (SARIKAMIŞ, 2009) and cauliflower (7–79 mg/g fresh weight) (SONES et al., 1984b). The intake of this GLS through horseradish is so low that it must be negligible.

Potential use

In the large collection of horseradish from the Nordic countries it should be possible to find interesting accessions for different purposes. Accessions with high sinigrin and gluconasturtiin levels could be interesting as functional food or used in medical treatment to inhibit different cancer forms or gastric lesions or used in spray against nasal and sinus dysfunction. As breakdown products from these compounds give a bitter taste, the large variation in the material should satisfy chefs and consumers, who look for specific flavour, when horseradish is used as a condiment in food. Allyl isothiocyanate (breakdown product from sinigrin) also strongly inhibits the growth of several bacteria, and horseradish rich in sinigrin could be used as a natural food preservative (WEDELSBÄCK BLADH & OLSSON, 2011).

It is not possible to conserve all horseradish accessions in the gene bank because the material must be kept as living plants, which costs too much. Based on our results of GLS levels together with finger print analysis (WEDELSBÄCK BLADH et al., submitted) and morphological studies to find material with good growth characters (not yet published), interesting accessions will be selected for conservation and use in the future breeding programmes. Our results also indicate that there are differences of GLS levels in different parts of the root. In the future it may be possible to find accessions or even parts of the plants with very high levels of the active chemical compounds.

In the present work, the myrosinase levels were not studied. This enzyme has numerous medical, industrial and agricultural application fields (WEDELSBÄCK BLADH & OLSSON, 2011). To assure the findings from our first screening of Nordic horseradish, the accessions need to be cultivated at the same site and tested for more than one year.

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REFERENCES

- AGNETA R., RIVELLI A.R., VENTRELLA E., LELARIO F., SARLI G., BUFO S.A., 2012: Investigation of Glucosinolate Profile and Qualitative Aspects in Sprouts and Roots of Horseradish (*Armoracia rusticana*) Using LC-ESI-Hybrid Linear Ion Trap with Fourier Transform Ion Cyclotron Resonance Mass Spectrometry and Infrared Multiphoton Dissociation. – *Journal of Agricultural and Food Chemistry*, 60(30): 7474–7482.
- ANDERBERG A., ANDERBERG, A.L., 2010: Den virtuella floran. [online] Available from: <http://linnaeus.nrm.se/flora/di/brassica/armor/armorus.html>. [Accessed 3rd March 2013].
- BOSTOCK J., 1855: Pliny the Elder. The Natural History. A Description of Plants, and of the Remedies Derived from them. – London: Taylor and Francis.
- CISKA E., MARTYNIAK-PRZYBYSZEWSKA B., KOZŁOWSKA H., 2000: Content of Glucosinolates in Cruciferous Vegetables Grown at the Same Site for Two Years under Different Climatic Conditions. – *Journal of Agricultural and Food Chemistry*, 48(7): 2862–2867.
- COMMISSION OF THE EUROPEAN COMMUNITIES, 1990: Commission regulation (EEC) 1864/90. Brussels Belgium. – *Official Journal of The European Communities* 170.
- COURTER J.W., RHODES A.M., 1969: Historical Notes on Horseradish. – *Economic Botany*, 23: 156–164.
- EVERITT B., HOTHORN, 2011: An Introduction to Applied Multivariate Analysis with R. – New York Dordrecht Heidelberg London: Springer.
- FAHEY J.W., ZALCMANN A.T., TALALAY P., 2001: The chemical diversity and distribution of glucosi-

- nolates and isothiocyanates among plants. – *Phytochemistry*, 56(1): 5–51.
- GRIEVE M., 1979: *A Modern Herbal*. (5th ed). – Thetford: Lowe and Brydone Printers LTD.
- GOLDBERG K., IGLEWICZ B., 1992: Bivariate Extensions of the Boxplot. – *Technometrics*, 34: 307–320.
- HECHT S.S., TRUSHIN N., RIGOTTY J., CARMELLA S.G., BORUKHOVA A., AKERKAR S., DESAI D., AMIN S., RIVENSON A., 1996: Inhibitory effects of 6-phenylhexyl isothiocyanate on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone metabolic activation and lung tumorigenesis in rats. – *Carcinogenesis*, 17(9): 2061–2067.
- JURGES K., THIES W., 1980: Quantitative analysis of the indole glucosinolate content of seeds and leaves of *Brassica napus* and *B. campestris*. – *Zeitschrift für Pflanzenzüchtung*, 84(2): 168–178.
- KOSSON R., HORBOWICZ M., 2008: Effect of Long Term Storage on Some Nutritive Components and Isothiocyanates Content in Roots of Two Horseradish Types. – *Vegetable Crops Research Bulletin*. 69: 155–164.
- KUSHAD M.M., BROWN A.F., KURILICH A.C., JUVIK J.A., KLEIN B.P., WALLIG M.A., JEFFERY E.H., 1999: Variation of Glucosinolates in Vegetable Crops of *Brassica oleracea*. – *Journal of Agricultural and Food Chemistry*, 47(4): 1541–1548.
- LANGE J., 1999: *Kulturplanternes indførelshistorie i Danmark* (Introduction History of Cultivated Plants in Denmark. (2 Ed), Fredriksberg: DSR Forlag.
- LI X., KUSHAD M.M., 2004: Correlation of glucosinolate content to myrosinase activity in horseradish (*Armoracia rusticana*). – *Journal of Agricultural and Food Chemistry*, 52(23): 6950–6955.
- MALIK M.S., RILEY M.B., NORSWORTHY J.K., BRIDGES W. JR., 2010: Variation of glucosinolates in wild radish (*Raphanus raphanistrum*) accessions. – *Journal of Agricultural and Food Chemistry*, 58(22): 11626–11632.
- MORSE M.A., ZU H., GALATI A.J., SCHMIDT C.J., STONER G.D., 1993: Dose-related inhibition by dietary phenethyl isothiocyanate of esophageal tumorigenesis and DNA methylation induced by N-nitrosomethylbenzylamine in rats. – *Cancer Letters*, 72(1–2): 103–110.
- MORSE M.A., LU J., GOPALAKRISHNAN R., PETERSON L.A., D'AMBROSIO S.M., WANI G., STONER G.D., 1997: Mechanism of enhancement of esophageal tumorigenesis by 6-phenylhexyl isothiocyanate. – *Cancer Letters*, 112(1): 119–125.
- NEBEL H., 1987: *Untersuchungen über einflüsse von herkunft, anbau und lagerung auf die qualität von Meerrettich (Armoracia rusticana P. Gaertn. et al.)*. Dissertation Technische Universität München.
- NILSSON J., OLSSON K., ENGVIST G., EKVAL J., OLSSON M., NYMAN M., ÅKESSON B., 2006: Variation in the content of glucosinolates hydroxycinnamic acids, carotenoids, total antioxidant capacity and low-molecular-weight carbohydrates in *Brassica* vegetables. – *Journal of the Science of Food and Agriculture*, 86: 528–538.
- ROSA E.A.S., HEANEY R.K., PORTAS C.A.M., FENWICK G.R., 1996: Changes in Glucosinolate Concentrations in *Brassica* Crops (*B. oleracea* and *B. napus*) Throughout Growing Seasons. – *Journal of the Science of Food and Agriculture*, 71(2): 237–244.
- SARIKAMIŞ G., BALKAYA A., YANMAZ R., 2009: Glucosinolates within a collection of white head cabbages (*Brassica oleracea* var. *capitata* subvar. *alba*) from Turkey. *African Journal of Biotechnology*, 8(19): 5046–5052.
- SARLI G., LISI A., AGNETA R., GRIECO S., IERARDI G., MONTEMURRO F., NEGRO D., MONTESANO V., 2012: Collecting horseradish (*Armoracia rusticana*, *Brassicaceae*): local uses and morphological characterization in *Basilicata* (Southern Italy). – *Genetic Resources and Crop Evolution*, 59(5): 889–899.
- SONES K., HEANEY R.K., FENWICK G.R., 1984: The glucosinolate content of UK vegetables – cabbage (*Brassicae oleracea*), swede (*B. napus*) and turnip (*B. campestris*). – *Food Additives and contaminants*, 1(3): 289–296.
- SONES K., HEANEY R.K., FENWICK G.R., 1984b: Glucosinolates in *Brassica* vegetables. Analysis of Twenty-seven Cauliflower Cultivars (*Brassica oleracea* L. var. *botrytis* subvar. *cauliflora* DC). – *Journal of the Science of Food and Agriculture*, 35: 762–766.
- SRIVASTAVA S.K., XIAO D., LEW K.L., HERSHBERGER P., KOKKINAKIS D.M., JOHNSON C.S., TRUMP D.L., SINGH S.V., 2003: Allyl isothiocyanate, a constituent of cruciferous vegetables, inhibits growth of

- PC-3 human prostate cancer xenografts in vivo. – *Carcinogenesis*, 24(10): 1665–70.
- VAN DOORN H.E., KRUK VAN DER G.C., HOLST VAN G.-J., RAAIJMAKERS-RUIJS N.C.M.E., POSTMA E., GROENEWEG B., JONGEN W.H.F., 1998: The glucosinolates sinigrin and progoitrin are important determinants for taste preference and bitterness of Brussels sprouts. – *Journal of the Science of Food and Agriculture*, 78(1): 30–38.
- VERKERK R., SCHREINER M., KRUMBEIN A., CISKAKA E., HOLST B., ROWLAND I., SCHRIJVER DE R., HANSEN M., GERHÄUSER C., MITHEN R., DEKKER M., 2009: Glucosinolates in *Brassica* vegetables: The influence of the food supply chain on intake, bioavailability and human health. – *Molecular Nutrition & Food Research*, 53(S2): S219–S219.
- WEDELSBÄCK BLADH K., OLSSON K.M., 2011: Introduction and Use of Horseradish (*Armoracia rusticana*) as Food and Medicine from Antiquity to the Present: Emphasis on the Nordic Countries. *Journal of Herbs, Spices & Medicinal Plants*, 17(3): 197–213.
- WEDELSBÄCK BLADH K., LILJEROTH E., POULSEN G., YNDGAARD F., KOLODINSKA BRANTESTAM A., 2013: Genetic diversity in Nordic horseradish, *Armoracia rusticana*, as revealed by AFLP markers (*submitted*)
- ZHANG Y., 2010: Allyl isothiocyanate as a cancer chemopreventive phytochemical. – *Molecular Nutrition & Food Research*, 54(1): 127–35.
- ZHAO F., EVANS E.J., BILSBORROW P.E., SYERS J.K., 1993: Influence of sulphur and nitrogen on seed yield and quality of low glucosinolate oilseed rape (*Brassica napus* L.). – *Journal of the Science of Food and Agriculture*, 63(1): 29–37.

GLIUKOZINOLATŲ ĮVERTINIMAS ŠIAURĖS ŠALIŲ (NORDIC) KRIENUOSE (*ARMORACIA RUSTICANA*)

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Santrauka

Valgomieji krienai (*Armoracia rusticana*) seniai naudojami augalai, pasižymintys maistinėmis ir vaistinėmis savybėmis. Aštrų krienų skonį ir gydomąsias savybes lemia gliukozinolatai (GLS), kurių tarpe dominuoja sinigrinas, sudarantis daugiau nei 80% viso GLS kiekio, toliau seka gliukonasturtinas ir gliukobracisinas. Gliukozinolatai buvo tirti 168 skandinaviškuose kolekcinuose pavyzdžiuose. Sinigrino kiekis skirtinguose augaluose įvairavo nuo 10 iki

45 μmol/g, gliukonasturtino – 1,3–7,4 μmol/g, gliukobracicino – 0,1–2,6 μmol/g orasausės jų masės. Buvo rasti pavyzdžiai, kaupiantys didelius sinigrino ir gliukonasturtino kiekius. Augalų genų banko tikslas yra ne tik išsaugoti genetinius šių augalų išteklius, bet kartu skatinti jų panaudojimą ir selekciją, todėl atrinkti kolekciniai pavyzdžiai pasitarnaus tolesnei krienų selekcijai bei skatins jų vartojimą maisto priedų ir natūralių konservantų gamybai bei gydymo tikslams.