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Full Length Research Paper

Glucosinolates within a collection of white head cabbages (*Brassica oleracea* var. *capitata* sub.var. *alba*) from Turkey

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Glucosinolates of a population of white head cabbages collected from different geographical regions of Turkey were determined at two different plant developmental stages (early and late development). The glucosinolates detected were glucoiberin, glucoraphanin, sinigrin of aliphatics and glucobrassicin, neoglucobrassicin, 4-methoxyglucobrassicin and 4-hydroxyglucobrassicin of indoles. Variation were observed in terms of glucosinolate profile and levels. However, the most abundant glucosinolate observed was glucobrassicin synthesized at significantly high levels. While total aliphatic glucosinolate content of genotypes ranged from 0.33 - 5.63 µmolg^{-1} dw and 0.46 - 16.14 µmolg^{-1} dw at early and late developmental stages, respectively, in the first year, the levels ranged from 0.05 - 7.72 µmolg^{-1} dw and 0.12 - 14.60 µmolg^{-1} dw at early and late developmental stages, respectively, in the second experimental year. In general, aliphatic glucosinolate content of cabbages were low compared to indoles; glucobrassicin in particular. The presence of glucoiberin and glucoraphanin in almost all cabbage genotypes is promising. Potential individuals synthesizing aliphatic glucosinolates together with glucobrassicin at relatively higher levels were selected for future breeding purposes aimed to develop novel lines with improved health benefits. Results suggested that both aliphatic and indole glucosinolate levels were higher at later stages of development.

Key words: Cabbage, brassica, glucosinolates, genetic resources.

INTRODUCTION

Brassica oleracea L. is an important vegetable crop species which includes fully cross-fertile cultivars and form groups with widely differing morphological characteristics (cabbage, broccoli, cauliflower, collards, Brussels sprouts, kohlrabi and kale) (Arın et al., 2008; Monteiro and Lunn, 1998). Cabbages are one of the most economically important member of the genus *Brassica.* In Turkey, there are local cultivars of cabbage (*B. oleracea* L. var*. capitata*) which are open-pollinated populations. Cabbage populations have been improved by farmers through mass selection for centuries (Balkaya et al., 2005). A comprehensive collecting program for the white head cabbage (*B. oleracea* var. *capitata* subvar. *alba*) populations of Turkey began in 1998 (Yanmaz et al., 2000). A collection of 95 local populations of white head cabbages was evaluated at the Black Sea Agricultural Institute in Samsun, Turkey.

Cabbages contain phytochemicals such as phenolics, vitamins, minerals and glucosinolates like all other crucifers. Glucosinolates are known to possess anticarcinogenic properties and regular consumption of crucifers is shown to be effective in the reduction of the risk of cancer (Fowke et al., 2003; Joseph et al., 2004; Moore et al., 2007; Munday et al., 2008; Seow et al., 2002; Traka et al., 2008; Traka et al., 2009; Wang et al., 2004; Zhao et al., 2001; Zickute et al., 2005). This activity is mainly associated with the presence of two isothiocyanates namely sulforaphane (1-isothiocyanato-4-methylsulphinylbutane) and iberin (1-isothiocyanato-3-methylsulphinylpropane) derived respectively from the precursor glucosinolates glucoraphanin and glucoiberin mostly abundant in broccoli, cabbage, kale, cauliflower, watercress and Brussels sprouts (Wang et al., 2005). Recently,

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erucin (4-methylthiobutyl isothiocyanate) an isothiocyanate from rocket salads structurally related to sulforaphane has also been shown to possess cancer chemopreventive properties (Melchini et al., 2009). Although rather complex, the activity of isothiocyanates mediating anticarcinogenic activity in general, is shown to be by inhibiting Phase 1 enzymes, inducing Phase 2 enzymes followed by the excretion of potential carcinogens from the metabolism, induction of apoptosis and cell cycle arrest and also as reported recently, by the inhibition of histone deacetylase activity (Juge et al., 2007). In addition to aliphatics, indolyl compounds derived from indole glucosinolates have also been associated with such activities (Nachshon-Kedmi et al., 2004).

The positive influence of glucosinolates on human health has led many researchers to explore the glucosinolate sources within *Brassica* species from different backgrounds as well as within different cultivars and potential ecotypes within species (Mithen et al., 1987; Sarıkamış et al., 2008). Cabbages are reported to synthesize mainly progoitrin, glucoiberin, glucoraphanin, sinigrin of the aliphatic group of glucosinolates and mostly glucobrassicin of the indole group depending on the genetic background of individuals (Cartea et al., 2008; Kushad et al., 1999). In this study, glucosinolate profile and content of a population of cabbages collected from different geographical regions of Turkey as a source of potential genetic material coding high levels of desired glucosinolates were determined.

Environmental factors during the growth period, date of planting, length of growing seasons and soil fertility have a major impact on glucosinolate content, nutritional quality as well as the yield of cabbage (Charron et al., 2005; Rosen et al., 2005; Shonhof et al., 2006; Velasco et al., 2007). Planting date is thought to affect concentrations of glucosinolates and their hydrolysis in cabbage (Radovich, 2004). Cartea et al. (2008) reported the variation in glucosinolate content of kale and cabbages at different growing seasons grown in part of Spain. In addition, the glucosinolate content of plants at different developmental stages varies. It is important to know the amount of glucosinolates produced at different developmental stages for determining optimum harvest time for improved benefits. In kales, it was determined that the amount of total aliphatics and indols were significantly higher when plants were fully mature compared to the early developmental stages (Sarıkamış et al., 2008).

In the present study, the glucosinolate production of cabbages were determined at two different developmental stages (early and late development) to identify the stages with the highest glucosinolate content.

MATERIALS AND METHODS

Plant material

A population of 70 cabbages taken from different geographical regions of Turkey was used for the analysis of glucosinolates. The present population was previously characterized morphologically (Balkaya et al., 2005). The seeds are currently being preserved at -20° C for long term storage in the Turkish seed gene bank (AARI) for use in future breeding programmes (Balkaya et al., 2005).

The experiment was conducted at the experimental plots in the Department of Horticulture, Faculty of Agriculture, Ankara University in 2006-2007. Seeds were sown into plug trays (5.5 cm width and 6 cm depth) on the first week of May (at both experimental years) seedlings were transplanted to the field when plants were at five - six leaf stage. Seedlings were planted in rows by leaving 60 cm within plants and 90 cm within rows. During field experiments, standard cultural practices were performed and plants were sprayed with an insecticide twice during the cultivation period. Leaf samples were taken at two different developmental stages in each experimental year. First sampling was from young plants (samples collected 1 month after transplantation to the field) and second sampling was when the plants were fully mature (samples collected 3 months after transplantation to the field), all samples were taken on the same day.

Analysis of glucosinolates

Analysis of glucosinolates was performed as the extraction of glucosinolates, conversion to desulfoglucosinolates and analysis by HPLC according to Sarıkamış et al. (2008). Leaf samples were freeze-dried prior to extraction. The extracted glucosinolates were purified on 0.5 ml column filled with 0.5 ml of DEAE Sephadex A-25 anion-exchange resin (Sigma Aldrich, Germany). Samples were analyzed and separated by HPLC-UV (Shimadzu®) detection in the HPLC laboratory at the Department of Horticulture, Faculty of Agriculture, Ankara University. A volume of 80 µl from the extract was injected onto a Waters Spherisorb® 5 µM ODS 2, 4.6 x 250 mm analytical cartridge. Analysis was carried out on a gradient of 99% water and 1% acetonitrile (Merck) at a flow rate of 1 ml/min for 24 min. The detection was carried out at a wavelength of 229 nm. The desulfoglucosinolates were identified using sinigrin and glucotropaeolin as standards.

Benzyl glucosinolate (glucotropaeolin) at a concentration of 16 mM was used as the internal standard for the quantification. The quantification of individual glucosinolates was carried out according to Heaney et al. (1986) and expressed as µmol.g^{-1} dry weight. Correction factors for glucoiberin, glucoraphanin, sinigrin, glucobrassicin, n-methoxyindolylmethyl and 4-hydroxyindolylmethyl glucosinolates were used for quantification.

Statistical analysis

Multifactorial variance analysis (ANOVA) was used to evaluate the data obtained, using MINITAB® version 14. Plant developmental stage and genotype were taken into consideration as variables. Significant differences were evaluated at $P < 0.001$ error level. Data were presented as mean values of genotypes ± standard error (SE) of the mean.

RESULTS AND DISCUSSION

Glucosinolate profile and content of cabbage genotypes

The results indicated that glucoiberin, glucoraphanin and sinigrin of aliphatics were synthesized together with glucobrassicin, neoglucobrassicin, 4-methoxyglucobrassicin and 4-hydroxyglucobrassicin of indoles. These find-

Table 1. Mean (µmol g^{-1} dw) glucosinolate content ± standard errors of the genotypes at two plant developmental stages*: (1) leaf samples taken 1 month after transplantation; and (2) leaf samples taken 3 months after transplantation to the field conditions (1st year).

Developmental Stage*	Glucoiberin	Glucoraphanin	Sinigrin	Total aliphatics	Glucobrassicin	neoglucobrassicin	4-methoxy glucobrassicin	4-hydroxy glucobrassicin	Total Indoles
1	1.261 ± 0.12	0.369 ± 0.05	0.565 ± 0.08	2.196 ± 0.15	21.37 ± 0.58	9.928 ± 0.46	1.605 ± 0.14	0.292 ± 0.07	33.195 ± 0.6
2	7.31 ± 0.21	0.547 ± 0.08	1.11 ± 0.12	8.967 ± 0.26	43.22 ± 1.78	9.42 ± 0.47	7.834 ± 0.32	1.119 ± 0.14	61.59 ± 2.03

Table 2. Mean (µmol g^{-1} dw) glucosinolate content ± standard errors of the genotypes at two plant developmental stages*: (1) leaf samples taken 1 month after transplantation; and (2) leaf samples taken 3 months after transnsplantation to the field conditions (2nd year).

Developmental Stage*	Glucoiberin	Glucoraphanin	Sinigrin	Total aliphatics	Glucobrassicin	neoglucobrassicin	4-methoxy glucobrassicin	4-hydroxy glucobrassicin	Total Indoles
1	0.570 ± 0.08	1.298 ± 0.16	1.46 ± 0.11	1.869 ± 0.17	28.56 ± 1.3	2.187 ± 0.18	2.966 ± 0.251	1.24 ± 0.12	34.95 ± 1.59
2	0.951 ± 0.12	1.75 ± 0.24	1.826 ± 0.12	2.701 ± 0.46	37.15 ± 1.65	2.952 ± 0.27	3.792 ± 0.29	1.799 ± 0.14	45.69 ± 1.82

ings are in agreement with other research groups reporting that glucoiberin, sinigrin, glucoraphanin, glucobrassicin, neoglucobrassicin and 4-hydroxy-glucobrassicin were detected in almost all cabbage varieties grown in Northwestern Spain (Cartea et al., 2008). We found that variation in terms of aliphatic glucosinolates was observed. While some individuals revealed a pattern such that glucoiberin, sinigrin and glucoraphanin existed together; some individuals had glucoiberin and glucoraphanin but not sinigrin. In some individuals glucoiberin was predominant whereas in some glucoraphanin was the predominant glucosinolate. Although glucoraphanin and glucoiberin were detected in almost all genotypes, the levels were low compared to indoles. When indoles are considered, glucobrassicin was the predominant glucosinolate present in all genotypes at very high levels which was followed by 4-methoxyglucobrassicin, neoglucobrassicin and 4-hydroxyglucobrassicin at much lower levels (Table 1 and 2). Glucobrassicin is the precursor of indole-3-carbinol which has been proven along with sulforaphane as a potent compound with anticancer activity present in cruciferous vegetables (Zhang and Talalay, 1994).

Separate studies reported the predominant glucosinolates in cabbages depending on the variety and different environmental conditions. Kushad et al. (1999) stated that glucobrassicin and sinigrin is present in cabbages followed by glucoiberin at much lower levels. Pocock et al. (1987) reported significant amounts of glucoiberin in three cabbage cultivars. Characterization of white cabbages from different regions of Europe in terms of glucosinolates revealed that glucobrassicin and sinigrin were the abundant glucosinolates accounting for about 30-70% of the total depending on the origin of plants (Kusznierewicz et al., 2008). The variation may be associated with the genetic background of individuals (Mithen et al., 2003; Sarıkamış et al., 2006) and the environmental factors such as soil properties and climatic conditions, date of planting and length of growing seasons having an influence on the glucosinolate content of plants (Chen et al., 2006; Li et al., 2007; Rosen et al., 2005; Zhao et al., 1994).

Considering the chemical classes, previous studies have suggested that indolyl glucosinolates are more susceptible to environmental effects than aliphatics (Kushad et al., 1999). Total glucosinolate content and the percentage of indole glucosinolates were reported to increase at higher temperatures during spring season (Cartea et al., 2008). It is believed that hot dry conditions may be related to the increased synthesis of amino acids and sugars, which are the precursors during the biosynthesis of glucosinolates. Summer temperatures rise up to 40°C in Ankara, where the field experiment was conducted, which may have probably enhanced the indole glucosinolate and total glucosinolate content of cabbage genotypes.

Developmental stage and glucosinolate content

Plant stage and plant parts should be considered when making breeding selections for

Table 3. Mean (µmol g^{-1} dw) total aliphatic and indole glucosinolate content ± standard errors of the genotypes at two plant developmental stages*: (1) leaf samples taken 1 month after transplantation; and (2) leaf samples taken 3 months after transplantation to the field conditions (1st year).

Genotype	Total Aliphatics		Total Indoles	
	1.	2.	1.	2.
B542 C	3.01 ± 1.05	10.98 ± 2.05	33.06 ± 4.63	53.57 ± 3.14
B144 C	2.73 ± 0.872	10.26 ± 1.15	35.23 ± 2.10	78.69 ± 7.62
B14	1.247 ± 0.196	9.377 ± 0.494	30.00 ± 0.722	57.6 ± 11.00
B228C	3.677 ± 0.495	10.897 ± 0.864	34.25 ± 2.18	51.4 ± 14.3

glucosinolate concentrations, because glucosinolate concentrations change with plant age and plant developmental stage. According to the results obtained from the study, it was suggested that the amount of aliphatic and indole glucosinolates at two different developmental stages (early development when plants were young and later developmental stage when mature) were significantly different from each other. First year results revealed that while total aliphatic glucosinolate content of genotypes ranged from 0.33 - 5.63 µmolg^{-1} dw with a mean value of 2.196 ± 0.151 µmolg^{-1} dw, and indolyls ranged from 23.57 - 45.53 µmolg^{-1} dw with a mean value of 33.195 ± 0.580 µmolg^{-1}dw. Glucobrassicin content varied from 9.40-37.07 µmolg^{-1} dw in younger plants. Similarly, the amount of total aliphatics varied from 0.46-16.14 µmolg^{-1} dw, with a mean of 8,967 ± 0,256 µmolg^{-1} dw and total indoles ranged from 22.76-94.09 µmolg^{-1} dw, with a mean value of 61.59 ± 2.03 µmolg^{-1} dw when plants were fully mature (Table 1). These results suggested that there was a significant increase in glucoiberin, sinigrin and total aliphatic glucosinolate content of cabbage plants when plants were mature ($P \leq 0.001$). Likewise, the increase in glucoraphanin content at maturity was statistically important ($P=0.05$). Among indoles, glucobrassicin, 4-methoxyglucobrassicin, 4-hydroxyglucobrassicin and total indole content increased at maturity ($P \leq 0.001$). Slight decrease in neoglucobrassicin content was not statistically important ($P > 0.05$). The experiment was conducted the following year as well in order to evaluate the effect of different developmental stages on the glucosinolate content of cabbages. The findings from the second year experiment also showed a similar profile. While total aliphatic glucosinolate content of individuals ranged from 0.05-7.27 µmolg^{-1} dw with a mean value of 1.869 ± 0.167 µmolg^{-1} dw, indolyls ranged from 10.69 - 60.60 µmolg^{-1} dw with a mean value of 34.95 ± 1.59 µmolg^{-1} dw. Glucobrassicin was the predominant indole when plants were young. Similarly, the amount of total aliphatics varied between 0.12-14.60 µmolg^{-1} dw, with a mean value of 2.701 ± 0.46 µmolg^{-1} dw and total indols ranged from 15.99 - 86.82 molg^{-1} dw, with a mean value 45.69 ± 1.82 µmolg^{-1} dw when plants were mature (Table 2). The increase in glucoiberin, sinigrin and total aliphatics were statistically important ($P < 0.05$); however, slight increase in glucoraphanin content was not statistically important ($P > 0.05$). In terms of indole glucosinolates, glucobrassicin and total indole glucosinolates increased significantly ($P \leq 0.001$). Likewise, neoglucobrassicin, 4-methoxyglucobrassicin and 4-hydroxyglucobrassicin contents also increased at maturity ($P < 0.05$). The changes in aliphatic and indolyl glucosinolate contents of genotypes at both experimental years are demonstrated in Figures 1a, b and 2a, b). These results are in agreement with other research teams (Charron et al., 2005; Rangkalidok et al., 2002; Velasco et al., 2007) demonstrating changes in glucosinolate content of plants during later stages of plant development.

The hydrolysis products of glucosinolates are isothiocyanates, nitriles and indoles. Isothiocyanates derived from the corresponding glucosinolates are associated with anticancer properties by providing a reduction in the risk of cancer (Fowke et al., 2003). In addition, sulforaphane has been reported to be effective in initiation and progression of a range of tumors in animal models (Gills et al., 2006; Hu et al., 2006). Therefore, the most promising varieties for future breeding purposes would be those with the highest total aliphatic glucosinolates. Within the collection of cabbages, B542C, B144C, B14 and B228C had the highest mean total aliphatic and total indole glucosinolate content at both experimental years (Tables 3 and 4). These genotypes have been identified as potential candidates with desired glucosinolate profiles for future breeding purposes for the development of new improved cultivars delivering potential health benefits.

Conclusions

The cabbage collection includes open-pollinated local varieties; so there is a high intravarietal diversity enabling selection of varieties with the highest glucosinolate content. The present study enabled characterization of head cabbage genetic resources in terms of glucosinolates. The results indicated that the genotypes were synthesizing more indoles than aliphatics, which were also reported to possess anticarcinogenic properties (Nachshon-Kedmi et al., 2004). The presence of

Table 4. Mean (µmol g^{-1} dw) total aliphatic and indole glucosinolate content ± standard errors of the genotypes at two plant developmental stages*: (1) leaf samples taken 1 month after transplantation; and (2) leaf samples taken 3 months after transplantation to the field conditions (2nd year).

Genotype	Total Aliphatics		Total Indoles	
	1.	2.	1.	2.
B542 C	1.590 ± 0.41	3.1 ± 0.88	33.49 ± 4.99	43.37 ± 6.81
B144 C	2.34 ± 0.26	3.1 ± 0.31	24.37 ± 7.18	33.68 ± 6.3
B14	1.17 ± 0.09	2.5 ± 0.4	35.27 ± 6.62	59.6 ± 15.2
B228C	1.8 ±0.98	2.36 ± 1.13	36.5 ± 12.5	55.1 ± 12.6

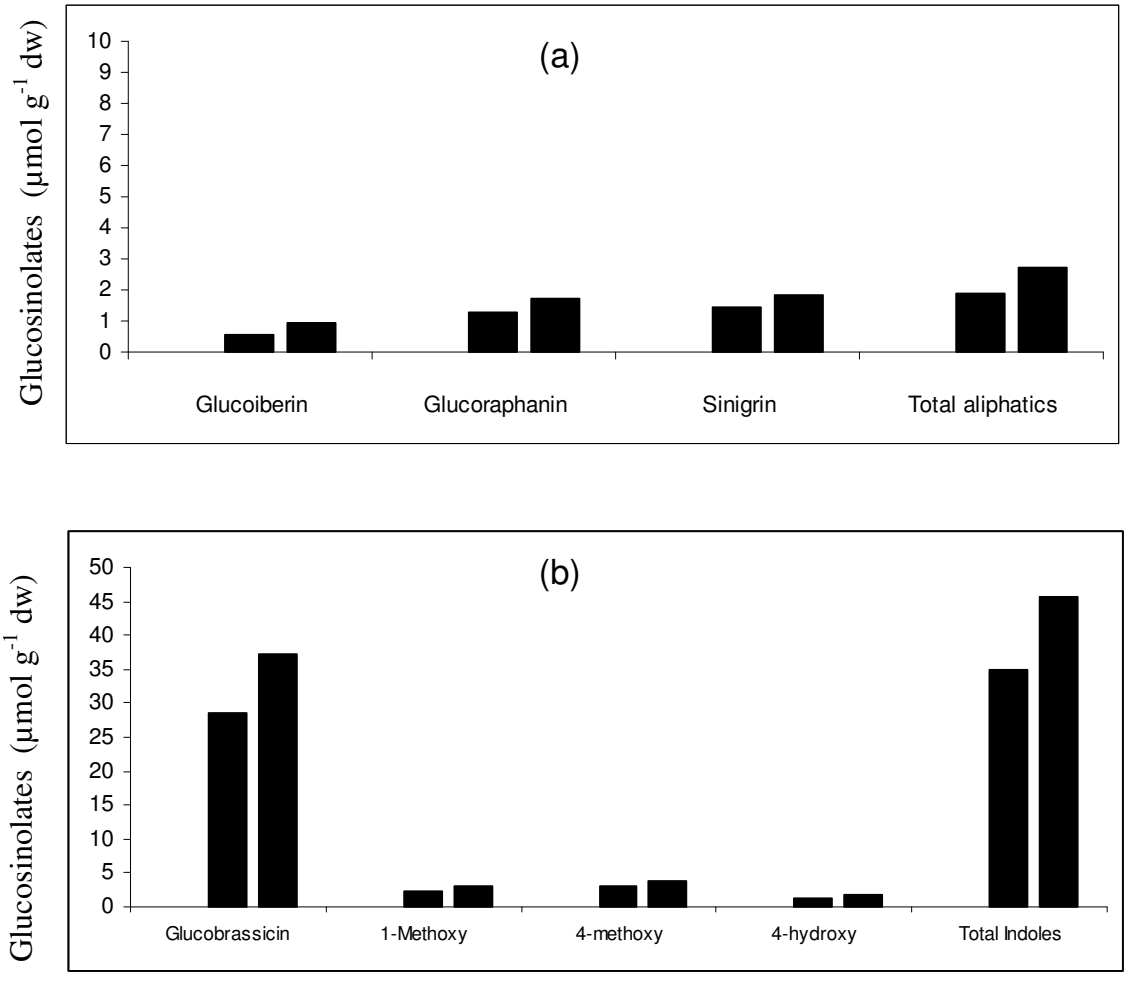

Figure 1 a and b: Graph demonstrating the changes in (a) aliphatic and (b) indolyl glucosinolate content of genotypes in Year 1.

glucoiberin and glucoraphanin in almost all genotypes is promising. Potential candidates synthesizing aliphatic glucosinolates together with glucobrassicin were selected among all. Together with their agronomic performance, these plants will be further utilized as a potential genetic source for breeding purposes aimed to generate novel cultivars with improved health promoting properties. In addition to glucosinolate contents, identification of the major glucosinolate hydrolysis products such as isothiocyanates, nitriles and indolyl compounds is also important in determining the anticarcinogenic activity of any crucifer.

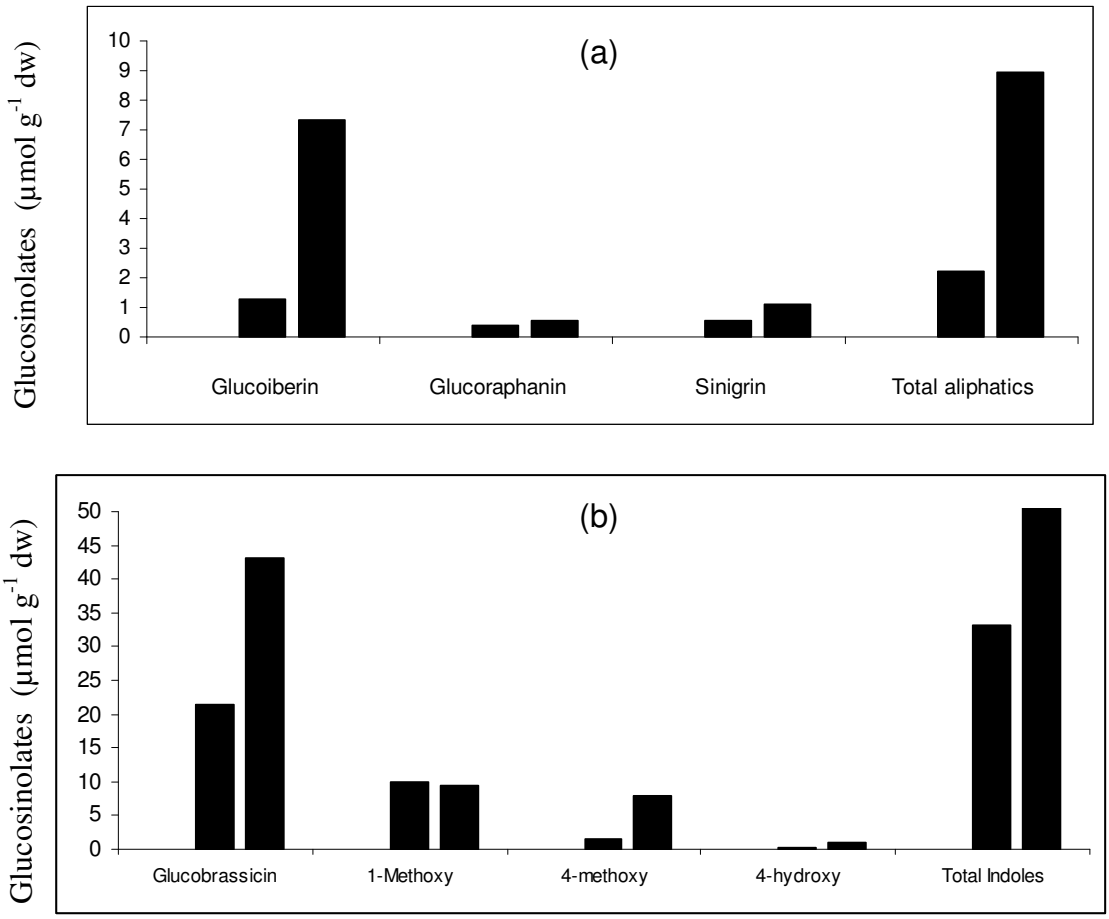

Figure 2 a and b. Graph demonstrating the changes in (a) aliphatic and (b) indolyl glucosinolate content of genotypes in Year 2.

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