Research Article

Total and Individual Glucosinolates of Newly Bred Open Pollinating Genotypes of Broccoli (*Brassica oleracea* convar. *botrytis* var. *italica*) Grown Organically: Effect of Genotype and Growing Season

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Abstract

Considering the demand for broccoli cultivars suitable for organic production and the prohibition of cultivating CMS-F1 hybrid cultivars under organic farming condition, current study evaluated glucosinolate content of eleven newly bred open pollinating genotypes of broccoli by comparison with F1 hybrid cultivars over two growing seasons. Effect of genotype, growing season and their interaction on glucosinolates was assessed as well. The results indicated the determination of six individual glucosinolates including glucoiberin, glucosinigrin, glucoraphanin, glucobrassicin, 4-methoxyglucobrassicin, neoglucobrassicin. Glucoraphanin was the major glucosinolate with the largest share in total-glucosinolates (more than 70%) and significantly higher concentration in fall. Total-glucosinolates and glucoraphanin ranged from 3.46 to 3.60 µmol g^{-1} DW and 1.44 to 1.69 µmol g^{-1} DW, respectively. We observed significant reduction in concentration of glucoraphanin, glucoiberin, 4-methoxyglucobrassicin and neoglucobrassicin in all genotypes in spring compared to fall growing season as the result of growing season significant effect. In contrast, glucobrassicin content of open pollinating genotypes was mostly stable across growing seasons. The genotype × growing season interaction did not affect the concentration of glucosinigrin and total-glucosinolates. Genetic factor affected the concentration of all glucosinolates significantly and resulted in differences in individual glucosinolates content of open pollinating genotypes and F1 hybrid cultivars. However, the level of total-glucosinolates of newly bred open pollinating genotypes was similar to F1 hybrid cultivars (3.46 - 3.60 µmol g^{-1} DW). A study on the agronomic performance of the open pollinating genotypes supplements the outcomes of this study and helps breeders and farmers to select the promising genotypes.

Introduction

In relation to the potential prevention of cancer and other diseases, species of the *Brassica* family are often in focus. Broccoli (*Brassica oleracea* L. var. *italic*) is considered as an important vegetable with health-promoting properties [1]. It is a cool-season crop, which is grown in temperatures ranging from 15 to 18°C [2]. Moreover, it is a favorite vegetable, consumed mostly cooked in Germany [3]. The composition of broccoli is 88.5% water, 3.8% protein, 0.2% fat, 2.7% available carbohydrates, 3.0% dietary fibers and 1.1% minerals [4]. On average, per 100 g of broccoli 58 mg of calcium, 15 µg of iodide, 459 µg of zinc and 700 ng of selenium are present. Additionally, broccoli contains vitamin C (94 mg 100g^{-1}), folic acid (114 µg 100g^{-1}) and many antioxidant compounds, such as carotenoids, tocopherols and Glucosinolates (GSLs) [4]. Including a high portion of *Brassica* species in diets showed a great reduction in the risk of some diseases like cancer [5]. High GSLs contents and their respective compounds,

which derived from an amino acid and glucose [6], act as cancer-chemoprevention agents [7]. Depending on the type of the amino acid; methionine, tryptophan and phenylalanine [8], the GSLs can be divided into three classes of aliphatic, indole and aromatic [9], respectively. Broccoli mainly contains indole and aliphatic GSLs [4]. The concentrations of aliphatic GSLs are mostly affected by genotype while the concentration of indoles is more affected by environment and genotype × environment interactions [10,11].

Due to current horticultural practices, broccoli cultivars that are on the market are almost exclusively F1 hybrid [12]. In organic production, due to the restrictions of the principles- according to the rules of International Federation of Organic Agriculture Movements (IFOAM)-, it is forbidden to develop F1 hybrid by using Cytoplasmic Male Sterility (CMS) [13]. Therefore, developing new Open Pollinating (OP) cultivars could be in favor of organic farming since it gives the farmers the possibility to produce their own seeds for reproduction [12]. OP cultivars are less homogeneous and differ from F1 hybrids in terms of composition [14]. Often, they are expected to contain higher amounts of health-benefitting secondary plant metabolites (such as glucosinolates, phenolics and flavonoids) compared to hybrid cultivars [13, 15].

Based on the information given above, we conducted a research study on the GSL composition of newly developed OP genotypes of broccoli, which were specifically bred for organic production (through on-farm breeding and single plant selection). Our current study is part of a larger project on the development of new OP cultivars of broccoli for organic farming in Germany. In this regard, we conducted two experiments during the 2015 fall growing season and during the 2016 spring growing season. We evaluated the results statistically over two different seasons to express the impact of growing season on the performance of the newly bred lines as well as the GSL pattern. Doing so, we were able to test the stability of the tested cultivars over the two growing seasons. We determined the GSL content of eleven OP genotypes and compared them with commercial control cultivars. In addition, we tested the effect of head weight, genotype and genotype × growing season interaction on GSLs content within and across growing seasons. Finally, we intended to select genotypes for the different growing seasons (fall and spring) based on their GSL content.

Materials and Methods

Plant Materials and Field Trials

Three commercial cultivars (F1 hybrids: "Batavia" and "Marathon", released OP: "Miranda") and eleven newly bred OP genotypes of broccoli (experimental lines) were our plant materials (listed in Table 2). The field trials were done under organic farming practices during fall growing season 2015 and spring growing season 2016 at the organic research station of the University of Hohenheim (Kleinhohenheim), Stuttgart, Germany (alt. 435 m, lat. 48.7, long. 9.2, long-term annual average precipitation and temperature 700 mm and 8.8°C). The soil type was sandy-loamy-clay. Broccoli seeds of fall and spring experiments were sown on July 10th, 2015 and March 21st, 2016, respectively. The seeds were pre-germinated in a greenhouse for two days at 18°C. Afterwards they were placed in another chamber of the greenhouse for further germination and grown at the same daily temperature matching that of the outdoors for 3-4 weeks. Seedlings were transplanted in the field at the stage of 3-4 true leaves and 10 cm stem length, 25 and 35 days after sowing for fall and spring experiments, respectively.

In order to ensure an adequate basic supply of Nitrogen (N) in the field, a preceding crop of one-year clover grass was used as green manure and incorporated into the soil. The nitrogen content of the soil was determined two weeks before planting. Soil samples were taken from two different depths (30 cm and 60 cm) and the nitrogen content was determined according to the $CaCl_2$ extraction method by the Association of German Agricultural Research and Research Institutes (VDLUFA). We applied necessary amount of slow-release Maltaflor fertilizer (5% N, 5% P and 5% K) to the field in order to keep the nitrogen content at 300 kg N ha^{-1}. The plants were covered by crop protection nets ((S48), with mesh sizes of 0.8×0.8 mm²), to protect against flea beetles (*Pyllotreta* ssp.) and swede midge (*Contarinia nasturtii*) until the first harvest. Irrigation was done directly after transplanting on 26.04.2016 (10 l m^{-2}) and on 20.05 2016 (15 l m^{-2}). In the 2015 fall growing season, the average daily temperature decreased from 22°C, at the transplanting time in August, to 7°C at the end of harvest in November. Throughout the 2016 spring season, the average daily temperature increased from 9°C to 20°C during April to July (from transplanting to the end of harvest). The average daily air temperature values were higher in fall season than spring season during the stages of growth and head formation up to the beginning of harvesting. At the time of harvesting broccoli heads in spring, the temperature was higher in comparison with fall growing season. The sum of precipitation was much higher in spring 2016 in comparison to fall 2015 over growing season with noticeable amount in the fifth and the seventh week after transplanting. Regarding the average relative humidity, the range was similar for both seasons from 60 % to 90 %, although the changing trend of the relative humidity during both seasons was different based on the amount of precipitation. Harvesting of the fall and spring experiment was done between 63-103 and 51-72 days after planting, respectively (Table 1).

Table 1: Harvesting period of broccoli heads in fall 2015 and spring 2016.

Growing season	Harvesting period	Harvesting window	Sampling interval
Fall 2015	07.10.-16.11.	6 weeks	7 times
Spring 2016	15.06.-06.07.	3 weeks	4 times

During harvest time, plots were visited regularly. On each assessment date three individual heads which were representative for the whole plot were picked for further analysis of GSL contents. All in all, each plot was assessed three to five times. To account for spatial trends in the field, the experimental design of the fall experiment was a randomized complete block design with three replicates, 14 plots per replicate. For the spring growing season 2016, planting direction and height gradient were orthogonal (due to slope of the field), therefore plants were arranged in a resolvable row-column design, which allowed to account for trends in both directions [16]. Again, plots were arranged in 14 rows and 3 columns, where a column corresponds to a complete replicate.

Sample Preparation

At each harvest, three broccoli heads were harvested fresh from each plot. The indicator of harvest was a head diameter of >10 cm. The stem was detached, and the heads were halved for sampling. The half heads were chopped into very small pieces and were placed into four bottles. Afterwards, they were frozen with liquid nitrogen, freeze-dried for one week, milled into 1 mm powder, stored at -20°C and finally mixed to one composite sample per plot per harvest. To analyze the GSL content, the samples were prepared similar to our previous study, Sahamishirazi et al. [17].

Glucosinolates Determination

The amount of total and individual GSLs was determined according to our former study by Near Infrared Spectroscopy (NIRS) [17]. NIRS was done using NIRS Model 5000 NIRS spectrometer (ISI Company, Germany). The amount of GSLs content was measured as previously described by Hernandez-Herrero et al. [6] and Sahamishirazi et al. [17]. The spectrums were obtained in the wavelength range of 400 to 2498 nm using the WIN ISI™ (Windows Infra Soft International, Germany).

Statistical Analysis

The data of both experiments was analyzed using a two-stage mixed model approach [19,20]. This approach accounts for all specifics of each experiment in stage one and calculates the means across growing seasons in stage two. The approach allows the handling of different designs in different trials while producing nearly identical results. For both experiments, the least square means of the genotype-by-harvest time from the first stage were estimated and subjected to an across-growing season analysis with the following second stage model:

$$\hat{\mu}_{ijn} = \mu + g_i + a_n + h_{in} + (ga)_{in} + (gh)_{jln} + f_{ijn} \quad (1),$$

where μ is the general intercept, g_i, a_n and h_{jn} are the fixed main effects of the ith genotype, n^{th} growing season and j^{th} harvest time within growing season n, respectively. Note that the effect of growing season is a confounded effect of experiment, year (2015 and 2016) and season (fall and spring). $(ga)_{in}$ and $(gh)_{jln}$ are assumed as random interaction effects between the ith genotype and the nth growing season or the ith genotype and the jth harvest time within growing season n, respectively. f_{ijn} are the error effects estimated in the first stages for genotype-by-harvest time means $\hat{\mu}_{ijn}$. To use error effects from the first stages, Smith weights [21] were calculated using a SAS macro [20]. We estimated both genotype main effects and genotype-by-growing season means from equation (1). Residuals were tested graphically for normality and homogeneity of variance. No means of across growing seasons for cultivar "Miranda" were calculated, as this cultivar did not produce any heads in spring 2016. After finding significant differences via F-test, a multiple t-test with α =0.05 was used to compare genotype means within or across growing seasons. Note that we also tried to extend the analysis of the first stage by adding a co-variable head weight, but it was non-significant for all traits. The rationale for adding this co-variable is that we want to avoid differences in head weight as reasons for differences in the content of glucosinolates. All statistical analysis was determined using SAS version 9.4.

Results and Discussions

Determination of GSL contents resulted in detection of six individual GSLs including three aliphatic (glucoiberin: GI, glucosinigrin: GS, glucoraphanin: GRA) and three indoles (glucobrassicin: GBS, 4-methoxyglucobrassicin: 4ME, neoglucobrassicin: NGB) similar to the study of Fachmann et al. [4]. The complete information on GSL contents of cultivars and genotypes of this study are provided in Table 2. Total glucosinolates (tGSLs) of each genotype, which is the sum of their individual GSLs, are also listed in the same table. In spring 2016, "Miranda" did not produce proper heads, which could have been the result susceptibility to high temperature at the time of head formation. Therefore, the concentrations of total and individual glucosinolates are not available for this cultivar. In line with the findings of Charron et al. [22] and Renaud et al. [15], in our study GRA, GBS and NGB were the dominating GSLs in all broccoli genotypes of both growing seasons. The proportions of the dominant individual GSLs in tGSLs were: GRA 36 to 41 %, GBS 16 to 19 % and NGB 16 to 18 %. In fall 2015 as well as spring 2016, the share of aliphatic GSLs in the samples were mostly higher than the indole ones. However, in spring the shares of the

dominant GSLs in tGSLs were lower (GRA 31 to 35 %, GBS 19 to 24 % and NGB 12 to 15 %).

Table 2: Comparison of individual and total glucosinolates content (µmol g^{-1} DW) and head weight (g) of broccoli samples in: a) fall growing season 2015, b) spring growing season 2016.

a) 2015	Genotypes	GI [1]	GS [2] *	GRA[3]	GBS [4]	4-ME [5]	NGB [6]	tGSLs [7] *	Head weight
Commercial control cultivars	Batavia F1	0.1956 [a A]	0.3683	1.69 [a A]	0.69 [bd A]	0.4557 [cd A]	0.67 [cd A]	4.06	358.67 ± 11.97
	Marathon F1	0.1949 [ab A]	0.367	1.64 [abc A]	0.68 [bd A]	0.4518 [d A]	0.64 [de A]	3.98	317.68 ± 13.74
	Miranda	0.1492 [e A]	0.366	1.44 [f A]	0.74 [ab A]	0.4615 [b A]	0.71 [ac A]	3.86	275.67 ± 13.42
Experimental open pollinating genotypes	CHE-BAL-A	0.1731 [cd A]	0.3658	1.56 [cde A]	0.70 [bd A]	0.4632 [b A]	0.7 [ac A]	3.96	312.51 ± 11.97
	TH-CAN-SPB	0.1633 [de A]	0.3685	1.51 [ef A]	0.70 [bd A]	0.4615 [b A]	0.7 [ac A]	3.9	273.63 ± 12.22
	Calinaro	0.1789 [ad A]	0.3659	1.57 [bde A]	0.70 [bd A]	0.4630 [b A]	0.69 [bc A]	3.96	274.63 ± 11.86
	TH-COA	0.1937 [ab A]	0.366	1.64 [abc A]	0.66 [cd A]	0.4705 [a A]	0.69 [bc A]	4.02	272.87 ± 12.8
	CHE-GRE-A	0.1738 [bcd A]	0.3677	1.55 [cde A]	0.73 [ab A]	0.4608 [b A]	0.70 [ac A]	4.01	250.22 ± 11.38
	CHE-GRE-G	0.1802 [ad A]	0.3658	1.56 [bde A]	0.68 [bd A]	0.4631 [b A]	0.70 [ac A]	3.95	305.5 ± 12.51
	TH-LIM-19-28	0.1978 [a A]	0.3668	1.66 [ab A]	0.65 [d A]	0.4600 [bc A]	0.64 [de A]	3.98	276.41 ± 12.26
	TH-LIM-20-68	0.1807 [ad A]	0.3658	1.60 [ad A]	0.65 [d A]	0.4642 [b A]	0.70 [bc A]	3.96	255.02 ± 11.53
	Linie 124	0.1860 [ac A]	0.367	1.53 [df A]	0.65 [d A]	0.4601 [bc A]	0.63 [e A]	3.83	253.3 ± 10.78
	Linie 701	0.1599 [de A]	0.3656	1.54 [cde A]	0.73 [abc A]	0.4642 [b A]	0.73 [ab A]	3.99	328.46 ± 14.75
	CHE-MIC	0.1487 [e A]	0.3638	1.46 [ef A]	0.77 [a A]	0.4634 [b A]	0.74 [a A]	3.94	294.95 ± 12.69

b) 2016	Genotypes	GI [1]	GS [2] *	GRA[3]	GBS [4]	4-ME [5]	NGB [6]	tGSLs [7] *	Head weight
Commercial control cultivars	Batavia F1	0.1298 [ab B]	0.3711	1.03 [ab B]	0.65 [bc A]	0.4307 [ef B]	0.41 [bc B]	3.04	274.61 ± 15.28
	Marathon F1	0.1306 [ab B]	0.371	1.01 [ab B]	0.64 [bc A]	0.4360 [ce B]	0.42 [bc B]	2.98	260.49 ± 18.88
	Miranda	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	No heads
Experimental open pollinating genotypes	CHE-BAL-A	0.1213 [bc B]	0.3704	1 [ab B]	0.69 [ab A]	0.4330 [def B]	0.44 [ab B]	3.09	287.55 ± 16.24
	TH-CAN-SPB	0.1324 [ab B]	0.3707	1.09 [a B]	0.64 [bc A]	0.4421 [ac B]	0.42 [ac B]	3.07	245.45 ± 17.65
	Calinaro	0.1307 [ab B]	0.3705	1.05 [ab B]	0.73 [a A]	0.4269 [f B]	0.42 [ac B]	3.12	247 ± 15.76
	TH-COA	0.1029 [c B]	0.3699	0.96 [ab B]	0.74 [a A]	0.4480 [a B]	0.48 [a B]	3.07	229.11 ± 16.95
	CHE-GRE-A	0.1338 [ab B]	0.371	1.05 [ab B]	0.63 [bc B]	0.4346 [ce B]	0.4 [bc B]	3.04	204.59 ± 15.26
	CHE-GRE-G	0.1496 [b B]	0.3716	1.07 [a B]	0.63 [bc A]	0.4324 [ef B]	0.38 [c B]	3.01	253.98 ± 15.25
	TH-LIM-19-28	0.1239 [bc B]	0.3713	1.01 [ab B]	0.63 [bc A]	0.4396 [bcd B]	0.4 [bc B]	2.96	223.04 ± 16.31
	TH-LIM-20-68	0.1361 [bc B]	0.3714	1.02 [ab B]	0.6 [c A]	0.4448 [ab B]	0.41 [bc B]	2.97	210.57 ± 15.75
	Linie 124	0.1283 [bc B]	0.3714	0.94 [b B]	0.68 [ab A]	0.4341 [cf B]	0.4 [bc B]	2.92	242.33 ± 15.74
	Linie 701	0.1150 [ac B]	0.3698	1.07 [ab B]	0.75 [a A]	0.4353 [ce B]	0.48 [a B]	3.2	257.29 ± 17.79
	CHE-MIC	0.1238 [bc B]	0.3704	1.05 [ab B]	0.7 [ab B]	0.4348 [ce B]	0.43 [ab B]	3.08	248.38 ± 16.4

[1]Glucoiberin, [2]Glucosinigrin, [3]Glucoraphanin, [4]Glucobrassicin, [5]4-methoxyglucobrassicin, [6]1-methoxyglucobrassicin, [7]total- glucosinolates. Means with the same letters were not significant (p < 0.05). Lowercase letters: comparison of genotypes within one year; Uppercase letters: comparison based on years within one genotype; n.a.: not available
* no letter display was created simple means for this variable, as the marginal means of genotypes across growing seasons should be compared (see Table 4).

To analyze the main effects (growing season, genotype, head weight) and their interactions (genotype × growing season), the output of the mixed model analysis for different GSLs are resented in Table 3. According to this table, the content of total and individual GSLs generally differed with growing season except for GBS. Variation due to genotype effect was significant for all individual GSLs and tGSLs, which is consistent with the results of Rosa and Rodrigues [23], Vallejo et al. [24], Schonhof et al. [25], Farnham et al. [11] and other former studies [10,15,32].

Table 3: Results of analysis of variance for the individual and total glucosinolates content.

Effects	GI [1]	GS [2]	GRA[3]	GBS [4]	4-ME [5]	NGB [6]	tGSLs [7]
Growing season	***	***	***	NS	***	***	***
Genotype	**	*	*	***	***	***	*
Genotype × Growing season	*	NS	*	*	**	*	NS

[1]Glucoiberin, [2]Glucosinigrin, [3]Glucoraphanin, [4]Glucobrassicin, [5]4-methoxyglucobrassicin, [6]1-methoxyglucobrassicin, [7]total-glucosinolates.
NS= non-significant; *, **, *** significant at a ≤ 0.05, 0.01, 0.001 by ANOVA

Since a lower level of GSLs content was observed in broccoli samples of spring season compared to fall season - similar to the results of Renaud et al. [15], the interaction of genotype × growing season was evaluated to check the possible effects. The effect was significant on all individual GSLs except GS and tGSLs. The interaction between the genotype and growing season illustrated the dependency of the relative performance of genotypes on the growing season or the dependency of difference between the growing seasons and the genotype. To test whether the weight of broccoli head has significant effects on GSLs content, the effect of head weight was evaluated on individual and total GSLs.

The results showed that none of the individual and total GSLs were influenced by head weight. In this regard, our findings were in line with the study of Farnham et al. [11] who reported no correlation between head weight and GSLs content of their broccoli samples. However, it was in contrast with the statement of Renaud [27] on the positive correlation between head weight and GRA. Similar to the study of Farnham et al. [11] and in contrast to the findings of Rosa and Rodriguez [23] our results indicated no dilution effect on GSL content of broccoli samples.

Glucoraphanin: Mainly, GRA represented the largest percentage of GSLs in broccoli, between 50 % and 80 % of total GSLs, therefore, it is considered as the key GSL [6,23,24,28,29,30,31,32]. Generally, concentrations of GRA in the broccoli samples of our study were similar to the amount of GRA of some experimental lines found by Vallejo et al. [23], some accessions tested by Kushad et al. [33] and in range with the GRA content of the study of Wang et al. [5]. GRA formed more than 70 % of the aliphatic glucosinolates in the samples of the fall and spring growing seasons. GRA content is greatly influenced by genotype [15,37] and less affected by environment and genotype × environment [37] since genetic factor is important in phenotypic expression of GRA

[10]. In this study, in addition to the effect of genotype, we found significant effects of growing season and genotype × growing season interaction on GRA content of our broccoli samples. In fall, among the OP genotypes, GRA ranged from 1.46 µmol g^{-1} DW (CHE-MIC) to 1.66 µmol g^{-1} DW ((TH-LIM-19-28). In this season, the GRA concentration of experimental genotypes was significantly lower when compared to the commercial cultivars, except for "TH-LIM-19-28", "TH-LIM-20-68" and "TH-COA". All commercial cultivars and experimental genotypes had significantly lower GRA content in spring 2016 compared to fall 2015 (Tables 2a and 2b). In the spring growing season (Table 2b), GRA ranged from 0.94 µmol g^{-1} DW (Linie 124) to 1.09 µmol g^{-1} DW (TH-CAN-SPB) among experimental genotypes. There were no significant differences between the commercial cultivars and the experimental genotypes except between "CHE-GRE-A", "TH-CAN-SPB" and "Linie 124".

Glucobrassicin: Up to 75 % (in fall) and 45 % (in spring) of indole glucosinolates belonged to the sum of GBS and NGB. In fall 2015 (Table 2a), "CHE-MIC" had significantly higher GBS contents than the tested commercial ones and all OP genotypes except "CHE-GRE-A" and "Linie 701". In spring 2016, only "CHE-GRE-A" and "CHE-MIC" had significantly lower GBS contents when compared to fall 2015 (Tables 2a and 2b). "Calinaro", "TH-COA" and "Linie 701" had significantly higher GBS contents than commercials. Similar to the outcomes of Renaud et al. [15], our findings showed that the level of GBS in OP genotypes tended to be higher than in hybrids. The comparison of the concentration of GBS of our samples with previous studies showed a lower level of GBS in samples of both growing seasons compared to the study of Vallejo et al. [24], Charron et al. [22] and Renaud et al. [15]. The lower concentration of GBS could be due to a higher level of GRA [15]. GBS was not significantly affected by growing season

Neoglucobrassicin: NGB ranged from 0.63 µmol g⁻¹ DW (Linie 124) to 0.74 µmol g⁻¹ DW (CHE-MIC) in fall 2015 (Table 2a). "Linie 124" had a significantly lower concentration of NGB compared to the most of the samples in fall 2015. All of the commercial cultivars and the experimental genotypes had significantly lower NGB contents in spring 2016 compared to fall 2015 (Table 2a and 2b). In spring 2016 (Table 2b), NGB ranged from 0.38 µmol g⁻¹ DW (CHE-GRE-G) to 0.48 µmol g⁻¹ DW (Linie 701 and TH-COA). "TH-COA" and "Linie 701" had significantly higher content of NGB than both commercial cultivars. The NGB contents of our samples were in range of the amount reported by Vallejo et al. [24]. Since, indole GSLs content is mostly influenced by environment rather and genotype × environment rather than genotype effects [37], differences in NGB content of the samples could be explained by different environmental conditions due to significant effect on regulating indole GSLs expression [10]. This could describe the higher NGB content of our samples compared to the work of Kushad et al. [33]. Moreover, different growing locations influence the content of GSL due to differences in nitrogen fertilizers, type of soil, spaces between plants and harvest date [33,34,35,36]. The rest of individual glucosinolates were available in smaller quantities in all genotypes and both growing seasons (Table 2a and 2b).

4-Methoxyglucobrassicin: The concentration of 4ME was in line with the amount and ranges previously reported in other studies [5, 7, 22, 24, 33]. All the commercial cultivars and the experimental genotypes had significantly lower 4ME content in spring 2016 compared to fall 2015 (Tables 2a and 2b). The concentration of 4ME was significantly higher in "TH-COA" within fall and spring growing season compared to the other experimental lines and commercial cultivars.

Glucoiberin: Effect of genotype is high on synthesis of aliphatic GSLs due to its significant effect on regulating aliphatic indole GSLs expression [10,37]. Therefore, in contrast to the study of Charron et al. [22], we could detect GI in broccoli samples of our study. GI levels of both seasons were in agreement with the outcomes of Wang et al. [5]. In fall 2015, GI ranged from 0.15 µmol g⁻¹ DW (CHE-MIC) to 0.20 µmol g⁻¹ DW (TH-LIM-19-28). In the same growing season, the concentration of GI was significantly lower in "CHE-MIC" compared to other OP genotypes except "Linie 701". The range of GI in spring decreased to 0.10 µmol g⁻¹ DW (TH-COA) and 0.14 µmol g⁻¹ DW (CHE-GRE-G). All the commercial cultivars and the experimental genotypes had significantly lower GI contents in spring 2016 compared to fall 2015 (Tables 2a and 2b). This could be due to higher temperature at the time of harvesting in the spring growing season. According to Rosa and Rodriguez [23], higher temperatures cause the increase of degradation of GSLs, hence reducing their concentrations in samples through stimulating myrosinase activity.

Glucosinigrin: Since the interaction of genotype and growing season did not affect the concentration of GS significantly, the level of this GSL across growing seasons is provided in Table 4 in which no differences in concentration of GS between the OP genotypes and F1 hybrid cultivars is observed. The levels of GS content of the current study were similar to the outcomes of Wang et al. [5].

Total Glucosinolates: Production of broccoli under organic farming affected the GSLs content of broccoli heads negatively. Studies showed lower GSLs level in organic broccoli compared to conventionally grown broccoli due to the optimum production conditions in conventional farming [38]. The comparison of the tGSLs content of the broccoli genotypes of the current study with former studies [7,24, 29,30] showed our findings were in line with the ranges achieved by the previous researchers. The outcomes of GSLs determination showed no significant differences between the tGSLs content of each genotype within both growing seasons (Tables 2a and 2b). However, since there was a significant genotype main effect, tGSLs of genotypes were significant across both seasons (Table 4). "Linie 124" had significantly lower tGSLs content value compared to both F1 hybrid cultivars and other OP genotypes except for "CHE-MIC" and "TH-CAN-SPB". In addition to the effect of genotype, climatic conditions could have affected the concentration of tGSLs by influencing the stimulation of myrosinase activity [23]. Other factors such as soil fertilization [39] also showed positive impacts on GSL content of *Brassica* vegetables.

Table 4: Comparison of broccoli genotype main effects across two consecutive seasons (fall 2015 and spring 2016) for the mean concentration of glucosinigrin and total glucosinolates (μmol g^{-1} DW).

	Genotypes	Glucosinigrin	Total glucosinolates
Commercial control cultivars	Batavia F1	0.3697 [b]	3.55 [a]
	Marathon F1	0.3690 [ab]	3.48 [ab]
	Miranda	n.a.	n.a.
Experimental open pollinating genotypes	CHE-BAL-A	0.3681 [bc]	3.53 [ab]
	TH-CAN-SPB	0.3695 [b]	3.49 [abc]
	Calinaro	0.3682 [bc]	3.54 [a]
	TH-COA	0.3679 [bc]	3.55 [a]
	CHE-GRE-A	0.3693 [ab]	3.52 [a]
	CHE-GRE-G	0.3686 [bc]	3.48 [ab]
	TH-LIM-1 9-28	0.3690 [ab]	3.47 [ab]
	TH-LIM-20-68	0.3686 [bc]	3.46 [ab]
	Linie 124	0.3692 [ab]	3.37 [c]
	Linie 701	0.3677 [abc]	3.60 [a]
	CHE-MIC	0.3670 [bc]	3.51 [ab]
Means with the same letters were not significant ($p < 0.05$). n.a.: not available			

Conclusion

Six individual GSLs were detected in the broccoli samples of this study. Among them, GRA, GBS and NGB were the main individual GSLs. There was a similar range of total and individual GSLs contents among the experimental genotypes and the commercial cultivars. We observed a significant effect of genotype on all individual GSLs and tGSLs contents of our broccoli samples. The interaction of genotype × growing season was significant on all indole GSLs, the main aliphatic GSL and GI. Generally, the GSLs content of the samples was higher when broccoli was cultivated in the fall growing season; however, the difference in the level of GSLs contents across seasons was significant only for GRA, NGB, 4Me and GI. Marketable head weight of broccoli genotypes showed no significant effect on GSL content of our samples. The OP genotypes performed similar to the F1 hybrid cultivars considering the content of tGSLs. Since the concentration of GSLs in the OP genotypes were mostly in the same ranges in each growing season, selection of specific genotypes was not noteworthy. A study on the agronomic performance of the genotypes supplements the outcomes of this study and helps breeders and farmers to pick genotypes, which perform well in both yield and quality.

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