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The Effect of Nitrogen Fertilization on Nitrate Accumulation, and the Content of Minerals and Glucosinolates in Broccoli Cultivars

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Summary

Variable content of bioactive compounds and minerals is the main problem in the production of vegetables as functional food, due to genetic, abiotic and agronomic factors (especially fertilization), which may affect the nutritional and medicinal value of vegetables. In order to achieve the higher yield, producers often apply large amounts of nitrogen fertilizer, which can result in the accumulation of nitrate, reducing the quality of broccoli. There are no results about the content of bioactive compounds and minerals in broccoli inflorescence grown on the principles of Good Agricultural Practices in Croatia. Therefore, the research has been set up to determine the effect of nitrogen fertilization on the content of glucosinolates, some minerals (potassium, calcium and nitrogen) and nitrate levels in the broccoli top inflorescence during different growing seasons. The two factorial field trials were conducted in spring/summer and summer/autumn growing seasons in 2009 according to a randomized complete block design with four replications. Two broccoli cultivars (Marathon and Parthenon) and four levels of nitrogen fertilization (0, 60, 120 and 240 kg/ha) were tested. There was a significant effect of cultivar and rate of nitrogen fertilization on the nutritional quality of broccoli top inflorescence in both growing seasons. The average values of total glucosinolates per g of dry mass (12.82 μmol) and calcium (1.12 %) in broccoli top inflorescence were significantly higher during the spring/summer growing season. The prevailing glucosinolates in that growing season were glucobrassicin and neoglucobrassicin. In the summer/autumn growing season, higher amounts of nitrate per kg of fresh mass (553 mg), nitrogen (5.40 %) and glucoraphanin per g of dry mass (5.4 μmol) were achieved and the dominant individual glucosinolates were glucoraphanin and glucobrassicin. Combination of Marathon with N 120 or 240 kg/ha, which resulted in the highest values of determined glucosinolates and some minerals, with nitrate content in the allowed range, could be recommended for conventional broccoli production under climate conditions of northwestern Croatia.

Key words: *Brassica oleracea* L. var. *italica*, growing season, glucoraphanin, neoglucobrassicin, potassium, calcium, nitrogen

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Introduction

Numerous epidemiological studies indicate beneficial effects of *Brassica* vegetables (especially broccoli) on human health since they contain high concentrations of minerals, vitamins and a special group of phytochemicals called glucosinolates (*1,2*). Glucosinolates are a class of nitrogen- and sulphur-containing compounds with anticancer and chemoprotective properties (*3,4*). Glucosinolates are plant defence compounds and, consistent with this function, are accumulated preferentially in the organs that contribute most to the plant fitness at a particular moment in the growth cycle (*1*).

Variation of the amount and pattern of glucosinolates has been attributed to many different factors including genetics, plant development, plant part, environmental factors, cultural practices (soil type and fertilization), storage and processing (*1,5*). Variable content of bioactive compounds and minerals is the main problem in the production of vegetables as functional food. In order to acquire the complete benefits of nutritional and medicinal values of vegetables, it is necessary to know the natural variation in the content of health-promoting food components.

Broccoli requires relatively large amounts of nitrogen for optimum growth and production of high quality inflorescence (*6*). The amount of nitrogen found to give optimal yield varies greatly, due to soil properties, climatic conditions and product requirements (*7*). According to Lešić *et al.* (*8*), for broccoli yield of 12 t/ha, it is necessary to provide 200 kg of N, 80 kg of P_2O_5 and 215 kg of K_2O. Other authors (*9,10*) reported higher broccoli requirements for nitrogen (300 to 465 kg/ha).

Vegetable producers often apply large amounts of nitrogen fertilizer in order to achieve higher yield and economic benefit (*11*). However, excessive amounts of nitrogen may cause some physiological disorders like hollow stem and some pathological problems like head rot of broccoli (*6,12*).

Nitrogen oversupply can also lead to leaching of nitrate in the environment with negative effects on profitability, resource conservation, water pollution and human health due to the accumulation of detrimental nitrate (*6*). Nitrate is a naturally occurring compound that is part of nitrogen cycle and has an important role in the nutrition and function of a plant (*13*).

Nevertheless, human dietary nitrate and nitrite exposure should be controlled as they may be a health risk factor. Some nitrate metabolites and reaction products (nitrite and other N-nitroso compounds) have raised a concern because of the implications of adverse health effects such as methaemoglobinaemia and carcinogenesis (*2,13*). Modern fertilization recommendations must optimize crop yield and quality to minimize chances of negative environmental effects due to over-fertilization (*14*).

Previous works have shown that nutritional quality as well as the yield of cabbage crops can be affected by the date of planting, environmental conditions and length of growing seasons (*5*). Broccoli production in the environmental conditions of the continental Croatia is possible in the spring/summer and summer/autumn growing seasons since the optimum temperature for its growth and development is 15 to 18 °C (*15*).

With more knowledge on the effects of the complete vegetable production chain, a more effective choice can be made on how to enhance phytochemical levels in the final product (*16*). Therefore, it is necessary to strike a balance in order to produce broccoli heads that are rich in nutraceuticals, while maintaining high yields and protecting the environment (*14*).

There are no results about the content of bioactive compounds and minerals in broccoli inflorescence grown on the principles of Good Agricultural Practices in Croatia. Therefore, research has been set up to determine the effect of nitrogen fertilization on the content of glucosinolates, some minerals (potassium, calcium and nitrogen) and nitrate levels in the broccoli top inflorescence during spring/summer and summer/autumn growing seasons.

Materials and Methods

The two factorial field trials were conducted on a farm in Zagreb (45°49' N, 16°02' E) during 2009. Treatments were arranged in a randomized complete block design with four replications. Two broccoli cultivars (Marathon and Parthenon) and four levels of nitrogen (0, 60, 120 and 240 kg/ha) were tested. Each trial plot size was 12 m^2 with 40 plants. Seeds of broccoli cultivars were sown in polystyrene trays with 209 cells on March 25 and July 7. Broccoli seedlings were planted at 0.6×0.5 m spacing, with a plant density of 3.3 plants/m^2. Planting dates were May 6 for the spring/summer season and August 7 for the summer/autumn season, while harvest periods started on July 9 and October 23, *i.e.* 64 and 77 days after planting, respectively.

Basic mineral fertilization was conducted on the basis of major chemical soil parameters and planned broccoli yield (12 t/ha). Available P_2O_5 and K_2O mass fractions per 100 g of experimental plot soil in summer/autumn growing season were higher (12.04 and 19.5 mg), while organic matter was lower (2.40 %) compared to spring/summer season. Nitrogen level (0.15 %) and soil reaction (pH=7.3) were similar in both growing seasons. Before planting, the soil was fertilized with 80 kg of P_2O_5 and 215 kg of K_2O per ha, while nitrogen (34 %, Agrolinz Melamine International, Linz, Austria) was applied in the form of ammonium nitrate on three occasions (20, 30 and 40 days after planting). Weed and insect control, as well as overhead irrigation were based on standard agronomic recommendations.

Broccoli was harvested in the early morning and two subsamples of top inflorescence from each plot were cooled and transported to the laboratory for the analysis of minerals and glucosinolates. For the analysis of glucosinolate content, samples were frozen and lyophilizied. Freeze-dried samples were stored at –20 °C until analysis.

Determination of soil quality

Chemical properties of the experimental plot soil were analyzed in the Analytical Laboratory of the Department of Plant Nutrition (Faculty of Agriculture, Zagreb, Croatia) using the following methods: ISO method 10390:2004 for pH value (KCl) (*17*), gravimetric method for dry matter (*18*), dry combustion method for total nitrogen (*19*), Tjurin method for organic matter (*20*) and AL method (extraction with ammonium lactate/acetic acid) for the available phosphorus (P_2O_5) and potassium (K_2O).

Determination of minerals in broccoli top inflorescence

Water and dry matter content in plant samples were determined by gravimetric method (*18*). Mineral content of broccoli top inflorescence was determined in the Analytical Laboratory of the Department of Plant Nutrition using the following methods: dry combustion method for total nitrogen (*19*), digestion method with concentrated HNO_3 and flame photometer for potassium (*21*), digestion with concentrated HNO_3 and atomic absorption spectrophotometer for calcium (*21*), and in fresh plant samples by ISO method 10304-1:1998 for nitrate (*22*).

Extraction and determination of glucosinolates

The extraction, isolation and desulphation of glucosinolates were carried out according to the ISO method with minor modifications (*23*). Desulphoglucosinolate extracts were separated on Supelcosil C18 reversed-phase column (150×4.6 mm I.D., 5 µm, Supelco Park, Bellefonte, PA, USA) using a Varian LC Star System equipped with a Star Solvent Delivery System 9010, Injector Rheodine 7125, Polychrom 9065 (Palo Alto, CA, USA). A two-component solvent system consisting of water (A) and 20 % acetonitrile in water (B) was used. Detection was performed with UV-diode array detector at 229 nm. Results are expressed as µmol per g of tissue dry matter calculated from HPLC peak areas using response factors to correct the absorbance differences between the internal standard (sinigrin) and other identified glucosinolates according to the ISO method 10633-1:1995 (*23*).

Statistical analysis

Data analysis was performed using the Windows SAS® software v. 9.1 (*24*) and the samples were analyzed individually for each growing season. The effect of cultivar and nitrogen fertilization was determined using the analysis of variance (ANOVA) and average values were tested by the least significant difference (LSD) test at the significance level p≤0.05 or p≤0.01.

Results and Discussion

Meteorological conditions

The optimal temperatures for growing broccoli are between 18 and 27 °C, but preferably at the average of 20 °C (*25*). According to Lešić *et al.* (*8*), biological requirements of broccoli are mean daily air temperatures from 20 to 24 °C for the post-planting interval and 15 to 18 °C for generative development, also 400 L/m^2 of precipitation during growing period. Workflows of decade air temperature (minimum, maximum and mean) and precipitation sum throughout the spring/summer and summer/autumn growing season for broccoli in 2009 are presented in Fig. 1. The beginning and the end of top inflorescence harvest were on July 9 and July 27 in spring/summer growing season and on October 23 and November 26 in summer/autumn growing season. Mean air temperature of spring/summer and summer/autumn seasons (20.2 and 12.9 °C) indicated that temperature in spring/summer was on average 7.3 °C higher.

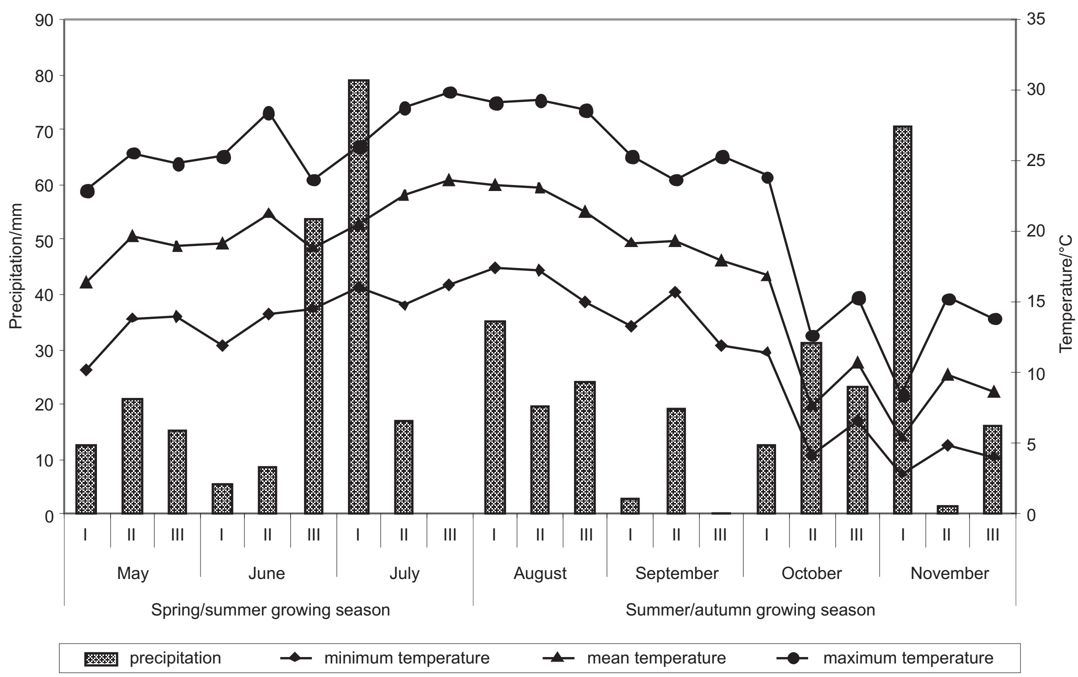

Fig. 1. Mean, minimum and maximum air temperature and precipitation sum per decade through spring/summer and summer/autumn 2009 growing seasons of broccoli

In the spring/summer growing season after planting, mean temperature values of the second and third decades of May (19.7 and 19.0 °C) were at a lower threshold range of the optimal temperature for broccoli post--planting period. In the first and second decade of June mean temperature (19.2 and 21.3 °C) was favourable for vegetative growth because it did not exceed 22 °C. That is very important because higher temperatures lead to prolongation of vegetative phase and to increasing leaf number until the inflorescence setting (*8*). Satisfactory temperature, slightly higher than recommended for generative development, continued in the third decade of June and first decade of July (18.9 and 20.5 °C). However, in the second and third decades of July mean decade temperature (22.6 and 23.7 °C) was significantly higher than optimal. In this period, maximum decade temperature was 27.5 and 29.0 °C. Such temperature conditions can cause appearance of a tiny and incoherent inflorescence, prone to premature blooming (*26,27*). During the spring/summer season precipitation was 213 mm, only 50 % of the broccoli total needs. The distribution was unfavourable due to long period with negligible amounts of rain followed by abundant amounts of precipitation, and then a subsequent dry period.

In the summer/autumn growing season temperature conditions for broccoli cultivation were very favourable during the first two months, from planting on August 7 to the second decade of October. Mean decade temperature (23.1, 21.4, 19.2, 19.3, 18.0 and 16.9 °C) corresponded to the optimal values for the post-planting period, vegetative growth and generative development of broccoli. Significantly lower than optimal were temperature values during the second and third decades of October (7.7 and 10.7 °C) and November (5.5, 9.9 and 8.6 °C). The decade minimum temperature in this period was bellow 10 °C, in a range from 2.9 to 6.6 °C. Velasco *et al.* (*28*) stated that low temperatures cause a reduction of glucosinolate content. Temperature was not the only environmental factor taken into account, since a lower average rainfall also increased glucosinolate content (*5*). During the summer/autumn growing season, 221 mm of precipitation fell, very well distributed, except during September (22.2 mm).

Mineral content

In the spring/summer growing season, the content of nitrate and minerals in fresh broccoli top inflorescence was significantly affected by cultivar×nitrogen fertilization interaction (Tables 1 and 2).

Marathon cultivar is one of the most commonly grown cultivars in southern Europe (*29*). In climate conditions of northwestern Croatia, Marathon had significantly lower nitrate but higher dry matter and nitrogen content in relation to Parthenon cultivar. Regarding the treatment, content of nitrate per kg of fresh mass was in the range from 240.0 (Parthenon×60 kg of N per ha) to 666.5 mg of NO_3 (Parthenon×240 kg of N per ha), which is within the recommended range for nitrate content in fresh broccoli (1000 mg of NO_3) according to EFSA (*13*). The highest nitrate accumulation was achieved by combinations Parthenon×240 kg of N per ha (666.5 mg of NO_3) and Marathon×120 kg of N per ha (445.3 mg of NO_3). The average amount of dry matter in the broccoli top inflorescence was 6.96 %, whereas the highest amount was found in plants treated with 0 kg of N per ha. In the research of Sørensen (*30*), dry matter content varied from 9.1 to 11.4 %, depending on the nitrogen dose per ha (20, 115, 210 or 400 kg).

The amount of nitrogen increased with the increase of nitrogen fertilization and the highest amount was determined in the Marathon fertilized with 120 and 240 kg/ha (5.46 and 5.05 %, respectively). All these values correspond to the amount of nitrogen from 4.2 to 5.0 % in broccoli top inflorescence, which was stated by Gutezeit (*31*) in a trial with nitrogen fertilization in the spring growing season. Higher values of nitrogen implicate higher crude proteins, which are important for nutritive quality of vegetables.

The highest potassium (4.90 %) and calcium (1.36 %) content (expressed on dry mass basis) was observed in Parthenon×60 kg of N per ha and Marathon×60 kg of N per ha, respectively. This indicates that in spring/summer season nitrogen fertilization had a negative impact on potassium and calcium accumulation. Sørensen (*30*) concluded that the concentrations of dry matter, potassium, calcium and phosphorus decreased due to increased nitrogen supply. However, under low nitrogen conditions,

Table 1. Analysis of variance for nitrate, mineral and glucosinolate content of broccoli top inflorescence

Source of variance	Nitrate and minerals					Individual and total glucosinolates			
	Nitrate	Dry matter	N	K	Ca	GRA	GBS	n-GBS	T-GSL
	Spring/summer growing season								
cultivar (C)	**	**	**	**	**	**	**	**	n.s.
fertilization (F)	**	**	**	**	**	**	**	**	**
C×F	**	**	**	**	**	**	**	**	**
	Summer/autumn growing season								
cultivar (C)	**	**	**	**	**	*	**	**	**
fertilization (F)	**	**	**	**	**	**	**	**	**
C×F	**	**	**	**	**	**	**	**	**

*significant at p≤0.05, **p≤0.01, n.s.=not significant

GRA=glucoraphanin, GBS=glucobrassicin, n-GBS=neoglucobrassicin, T-GSL=total glucosinolates

Table 2. Effect of cultivar and nitrogen fertilization on nitrate, dry matter and mineral content of broccoli top inflorescence

Cultivar	Treatment kg of N per ha	$w(NO_3)$/mg	w(dry matter)/%	w(N)/%	w(K)/%	w(Ca)/%
		Spring/summer growing season				
Marathon	0	302.5^F	8.76^A	4.15^G	3.78^D	1.25^C
	60	362.8^D	6.21^G	4.39^F	4.66^C	1.36^A
	120	445.3^B	7.99^B	5.46^A	3.65^E	0.86^F
	240	255.0^G	7.12^C	5.05^B	4.61^C	0.92^E
Parthenon	0	344.5^E	6.45^E	4.21^G	4.81^B	1.26^C
	60	240.0^H	6.57^D	4.59^E	4.90^A	1.30^B
	120	415.0^C	6.30^F	4.69^D	4.81^B	1.07^D
	240	666.5^A	6.28^{FG}	4.88^C	4.61^C	0.94^E
LSD value		4.283	0.063	0.089	0.063	0.02
		Summer/autumn growing season				
Marathon	0	650.5^B	9.74^C	5.56^B	4.56^C	0.48^{CD}
	60	530.0^D	9.90^{BC}	5.44^D	4.65^{AB}	1.00^A
	120	350.0^G	10.16^B	5.77^A	4.38^D	0.54^C
	240	337.5^H	10.80^A	5.49^{CD}	3.62^E	0.46^{DE}
Parthenon	0	522.5^E	10.12^{BC}	4.45^F	4.56^C	0.41^E
	60	570.0^C	9.26^D	5.17^E	4.60^{BC}	0.61^B
	120	437.5^F	10.20^{BC}	5.54^{BC}	4.60^{BC}	0.63^B
	240	1022.5^A	8.54^E	5.82^A	4.71^A	0.53^C
LSD value		3.856	0.400	0.063	0.063	0.063

*Mean values followed by the same letter within each column do not differ significantly at $p \leq 0.01$ according to the LSD test

heads often develop other undesirable characteristics such as enlarged flower buds, elongated branches and yellow or purple discolouration (*32*).

In the summer/autumn growing season, there was notable impact of cultivar×nitrogen fertilization interaction on the content of nitrate and minerals in broccoli top inflorescence (Table 2). Regarding the treatment, content of nitrate per kg of fresh mass in summer/autumn season was in the range from 337.5 (Marathon×240 kg of N per ha) to 1022.5 mg of NO_3 (Parthenon×240 kg of N per ha), which is also within the recommended range for nitrate content in fresh broccoli according to EFSA (*13*), but this is 1.5 times higher than in spring/summer season. High irradiance during spring/summer season reduces nitrate accumulation (*33*). Also, during the spring/summer growing season, precipitations were insufficient and adversely distributed (only about 50 % of broccoli total needs), which also resulted in lower accumulation of nitrate. According to Cárdenas-Navarro *et al.* (*33*), shoot nitrate and water content are always positively correlated due to the fact that nitrates are mostly transported towards the plant by mass flow.

An inverse relationship between nitrate and dry matter was determined, which is in agreement with a report by Siomos *et al.* (*34*). The reduction in dry matter content by increasing nitrogen fertilizer can be attributed to the replacement of organic acids and sugars by nitrate (*35*). Dry matter content was 1.4 times higher than in spring/summer season and varied from 8.54 to 10.80 %. Higher dry matter content (9.1 to 10.0 %) was also found in the autumn growing season by Gutezeit (*31*) and Tuncay *et al.* (*36*).

Content of nitrogen in summer/autumn growing season was 1.2 times higher, while the content of calcium was 1.9 times lower than in spring/summer season. The content of nitrogen, potassium and calcium was in a range from 4.45 to 5.82 %, 3.62 to 4.71 % and from 0.41 to 1.00 %, respectively. These values are mainly in accordance with the results of Mandelová and Totušek (*37*), and Jahangir *et al.* (*38*). In the research of Acikgoz (*39*) lower nitrogen content of Marathon inflorescence was determined and it varied from 2.91 to 4.01 %.

In summer/autumn growing season, Parthenon had a higher average content of nitrate per kg of fresh mass (638.1 mg) and potassium (4.62 %), while Marathon achieved higher average nitrogen and calcium content (5.56 and 0.62 % respectively). The highest rates of nitrogen per ha (240 and 120 kg) resulted in greater nitrate per kg of fresh mass (680.0 mg) and nitrogen (5.65 %) accumulation. Vågen (*7*) reported that nitrogen uptake in broccoli plants was increased with enhanced nitrogen fertilization. Increased nitrate value during summer/autumn season could be a result of mineralization due to favourable conditions for decomposition of organic matter. Also, accumulation of nitrate may be affected by the correlation of nitrogen and potassium, *i.e.* by a phenomenon called 'luxury consumption'. If nitrogen is taken up as nitrate (which carries one negative charge), the plant may take up potassium (which carries one positive charge) to ensure that the internal electrical status remains neutral (*8*).

Nitrogen is an important component of many structural, genetic and metabolic compounds such as amino and nucleic acids, proteins and chlorophyll (*36*). In order to achieve higher yield, producers often apply large amounts of nitrogen fertilizer, which can result in the accumulation of nitrate, reducing the quality of broccoli. The results presented in many reports concerning the optimization of the nitrogen dose to receive the maximum broccoli yield are very divergent. In research of Goodlass *et al.* (*9*), the most effective nitrogen rate in broccoli fertilization was 300 kg/ha, while Bakker *et al.* (*32*) recommended a rate of approx. 180 kg. Vågen (*7*) reported that the highest rate of N per ha (240 kg) fulfilled nitrogen requirement throughout the broccoli growing period. Split applications are often recommended as a way to reduce leaching of nitrogen or to improve nitrogen use efficiency (*7,40*).

Yoldas *et al.* (*41*) studied the effect of nitrogen doses per ha (0, 150, 300, 450 and 600 kg) on the quality and nutrient content in broccoli top inflorescence. The content of potassium, calcium, magnesium, iron and zinc increased with the increase in nitrogen. In summer/autumn growing season, the combination of Parthenon×240 kg of N per ha resulted in significantly higher nitrate per kg of fresh mass (1022.5 mg), nitrogen (5.82 %) and potassium (4.71 %) content. Similar values of potassium were determined in both growing seasons, which is in accordance with the results of Acikgoz (*39*), who stated that mineral levels in broccoli, excluding potassium, depended on the growing season. The highest content of calcium (1 %) was obtained with the combination of Marathon×60 kg of N per ha. Significantly higher values of calcium were determined in spring/summer season due to transpiration intensity. Transport of calcium towards the root is linked to the mass flow of water (*39*). A reduced water supply could lead to decreased contents of minerals in spring/summer season, *i.e.* without sufficient water to cool the soil, plant roots may be subjected to heat stress, which could affect the uptake of minerals. Insufficient or excessive amount of available water could affect translocations of minerals across root membranes so minerals may not be transported and deposited in the broccoli inflorescence.

Glucosinolate mass fraction

There were significant differences in the individual and total glucosinolate mass fractions among treatments in both growing seasons (Tables 1 and 3). The predominant group of glucosinolates found in broccoli inflorescences in spring/summer season was indole glucosinolates (glucobrassicin and neoglucobrassicin), followed by aliphatic glucosinolates (glucoraphanin), which is in accordance with the data reported by Rodrigues and Rosa (*42*), Vallejo *et al.* (*43*) and Hansen *et al.* (*44*). Glucoiberin, 4-hydroxyglucobrassicin and 4-methoxyglucobrassicin

Table 3. Effect of cultivar and nitrogen fertilization on individual and total glucosinolate content in broccoli top inflorescence

Cultivar	Treatment	Individual and total glucosinolates/(μmol/g)			
	kg of N per ha	Glucoraphanin	Glucobrassicin	Neoglucobrassicin	Total
		Spring/summer growing season			
Marathon	0	2.40^{BC}	2.51^{E}	5.64^{D}	11.10^{E}
	60	2.08^{D}	2.34^{E}	3.60^{E}	8.42^{F}
	120	2.13^{D}	4.43^{B}	11.11^{A}	18.67^{A}
	240	3.29^{A}	7.06^{A}	2.23^{F}	13.29^{CD}
Parthenon	0	2.14^{D}	4.66^{B}	8.79^{B}	16.35^{B}
	60	2.37^{C}	3.23^{D}	6.23^{CD}	12.34^{D}
	120	2.53^{B}	3.95^{C}	6.73^{C}	14.02^{C}
	240	1.62^{E}	2.59^{E}	3.64^{E}	8.39^{F}
LSD value		0.142	0.353	0.607	1.102
		Summer/autumn growing season			
Marathon	0	5.37^{C}	2.17^{E}	1.43^{C}	9.84^{D}
	60	6.94^{A}	2.94^{D}	1.30^{CD}	12.28^{B}
	120	4.04^{E}	3.30^{BCD}	1.83^{B}	10.14^{CD}
	240	5.01^{CD}	5.50^{A}	3.18^{A}	14.97^{A}
Parthenon	0	4.70^{D}	2.36^{E}	0.85^{E}	8.94^{E}
	60	6.30^{B}	2.99^{CD}	1.47^{C}	12.02^{B}
	120	5.09^{C}	3.31^{BC}	1.02^{DE}	10.57^{C}
	240	6.00^{B}	3.53^{B}	1.79^{B}	12.44^{B}
LSD value		0.390	0.375	0.283	0.587

*Mean values followed by the same letter within each column do not differ significantly at $p≤0.01$ according to the LSD test were also identified in the analyzed broccoli cultivars, although in significantly smaller amount. The glucosinolate profiles vary among species, cultivars of a single species and among tissues of a single plant (*44*). The proportion of each individual glucosinolate contributing to the total glucosinolate mass fraction may alter the biological effects of the crop at various growth stages (*45*).

In summer/autumn season, prevailing individual glucosinolate was glucoraphanin at mass fraction 2.3 times higher than in spring/summer season. This is in accordance with reports of Rosa and Rodriges (*46*), Borowski *et al.* (*47*), Cartea *et al.* (*48*) and Abercrombie *et al.* (*49*), who stated that aliphatic glucosinolates were the most common during the autumn season, whereas the percentage of indole glucosinolates increased during the spring season. Cartea *et al.* (*48*) also reported that the highest concentrations of total glucosinolates generally occurred when crops were harvested during high temperatures and long daylight periods. This is in agreement with findings by Rosa *et al.* (*45*) in Portuguese kale and by Cartea and Velasco (*5*) in cabbage, where the concentration of total glucosinolates in leaves harvested in spring season was higher than in autumn season.

Content of total glucosinolates in spring/summer season was 11.0 % higher than in summer/autumn season. Total glucosinolate values per g of dry mass in this paper are comparable with the values (3.0–28.3 and 2.4–35.0 μmol) stated by Vallejo *et al.* (*43*) and Kushad *et al.* (*50*). In spring/summer season, total glucosinolate content was higher due to higher levels of neoglucobrassicin (73 % higher than in summer/autumn season). A long period of slightly higher average air temperatures and decrease in the available water throughout 50 days after planting in spring/summer season resulted in higher content of neoglucobrassicin and total glucosinolates. A reduced water supply could lead to increased contents of phytochemicals. It has been shown that antioxidant compounds are sensitive to soil water content and that higher levels of phytochemicals were produced when plants experienced drought-like conditions caused by deficit irrigation practices, possibly due to related effects on nutrient uptake (*51*). A higher content of neoglucobrassicin during dry conditions in spring/summer season may be related to the increased synthesis of specific amino acids which are precursors in the biosynthesis of these individual glucosinolates. According to Kushad *et al.* (*50*), indole glucosinolates are more susceptible to environmental effects (especially high temperatures) than aliphatic glucosinolates. Glucoraphanin concentrations were significantly affected by average photosynthetic photon flux and daylight length (from transplanting to harvest), but not by temperature (*52*).

In regard to total glucosinolate mass fraction, there was no significant difference between cultivars in spring/summer growing season (Table 1), but the highest average mass fraction of glucoraphanin and glucobrassicin per g of dry mass was achieved in Marathon (2.47 and 4.09 μmol, respectively), while Parthenon had the highest average neoglucobrassicin mass fraction per g of dry mass (6.35 μmol). In summer/autumn growing season the highest average concentration of glucobrassicin, neoglucobrassicin and total glucosinolates per g of dry mass was achieved in Marathon (3.48, 1.94 and 11.8 μmol, respectively), while Parthenon had the highest average glucoraphanin mass fraction per g of dry mass (5.52 μmol). A variation in glucosinolate content among cultivars suggests differences in their health-promoting properties (*43*).

In *Brassica* species, strategies for nitrogen and sulphur fertilization are of primary importance for glucosinolate concentration since these nutrients are essential constituents of glucosinolate parent molecules (*53*). The glucosinolate content may be significantly affected by both sulphur and nitrogen fertilization (*54*). Aires *et al.* (*55*) observed detrimental effect of different nitrogen and sulphur fertilization combinations on the glucoraphanin concentration in broccoli sprouts. Nitrogen fertilization had a significant impact on the content of aliphatic and indole glucosinolates in rocket salad (*53*).

In the current research, the effect of nitrogen fertilization on glucosinolate mass fraction was positive. Therefore, in spring/summer growing season, the highest rates of nitrogen (240 and 120 kg/ha) resulted in the highest mass fraction of glucoraphanin and glucobrassicin, *i.e.* neoglucobrassicin and total glucosinolates. Combination of Marathon and the highest nitrogen rates (240 and 120 kg/ha) had the same effect on the mass fraction of determined glucosinolates. According to Omirou *et al.* (*53*), aliphatic glucosinolates in rocket leaves responded negatively to nitrogen fertilization, whereas indole glucosinolates showed a positive response. This difference is probably related to the effect of nitrogen on biomass production and biosynthesis.

In summer/autumn growing season the highest rate of nitrogen (240 kg/ha) resulted in the highest average mass fraction of glucobrassicin, neoglucobrassicin and total glucosinolates per g of dry mass (5.51, 2.49 and 13.71 μmol, respectively). It is clear that Marathon×240 kg of N per ha stands out with the highest values of determined indole glucosinolates, glucobrassicin and neoglucobrassicin per g of dry mass (5.50 and 3.18 μmol, respectively), which affected the content of total glucosinolates (14.97 μmol per g of dry mass). Contrary to this, Cartea and Velasco (*5*) reported that a high dose of nitrogen application tended to give lower glucosinolate levels.

Generally, in summer/autumn growing season, environmental conditions were more suitable for broccoli production but less favourable for the synthesis of indole glucosinolates. This is in agreement with the findings of Velasco *et al.* (*28*), Rosa *et al.* (*45*), and Rosa and Heaney (*56*), who found that short days, low temperatures and less radiation lead to lower glucosinolate levels. Glucosinolate biosynthesis is controlled by genes that regulate chain elongation of amino acids, the conversion of amino acids or derivatives to glucosinolates and secondary modifications to R groups of glucosinolates. Thus, there are a number of points in the biosynthetic process at which glucosinolate metabolism may be subject to environmental regulation by factors such as light or temperature (*52*). To conclude, it is possible that variations in glucosinolate mass fractions in this research resulted from variations in plant water potential induced by temperature and light.

Conclusions

Besides the yield, the main goal of nowadays' research in vegetable production is product with optimal level of health promoting compounds. Mineral and phytochemical content can be enhanced to some limits by Good Agricultural Practices. The simple tools for improving the levels of minerals and glucosinolates in the edible parts of vegetables are the management of fertilization, water supply, selection of appropriate cultivar and growing season.

The obtained results indicate a differential effect of cultivar and nitrogen fertilization on the glucosinolate and mineral content of broccoli top inflorescence during both growing seasons. The highest amount of nitrate, dry matter, nitrogen and glucoraphanin was recorded in summer/autumn growing season. In both growing seasons the highest content of nitrogen, individual and total glucosinolates was achieved by fertilization with the highest rates of nitrogen (120 and 240 kg/ha), except for glucoraphanin in the summer/autumn growing season. Nitrogen fertilization had a negative impact on potassium and calcium accumulation in the spring/summer growing season. Combination of Marathon×120 or 240 kg of N per ha, which resulted in the highest values of determined glucosinolates and some minerals, also with nitrate content in the allowed range could be recommended for conventional broccoli production under climate conditions of northwestern Croatia.

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