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THE EFFECT OF NITROGEN AND SULPHUR NUTRITION ON THE YIELD AND CONTENT OF ANTIOXIDANTS IN BROCCOLI

VPLYV DUSÍKATEJ A SÍRNATEJ VÝŽIVY NA ÚRODU A OBSAH ANTIOXIDANTOV V BROKOLICI

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The small field experiment with broccoli (variety ‚Tiburon F1') was established in The Botanical Garden of Slovak University of Agriculture in Nitra. In this experiment, we investigated the effect of four variants of nutrition on the broccoli yield, content of sulforaphane and vitamin C in broccoli florets. The first variant of nutrition was a control one – without application of fertilizers. The second one was fertilized at nitrogen level 200 kg.ha^{-1}. The third one had level of nutriments N : S = 200 : 50 kg.ha^{-1} and fourth one N : S = 200 : 60 kg.ha^{-1}. In each variant of nutrition, the yield of broccoli was significantly increased in comparison with the control variant. The highest yield of broccoli florets was reached in variant 4 (27.87 t.ha^{-1}). The highest amount of sulforaphane was also determined in variant 4 – 50.93 mg.kg^{-1} of fresh matter. However, this value did not represent significant increase of sulforaphane content compared to the control variant. The highest content of vitamin C was determined in variant 2 (585.78 mg.kg^{-1} of fresh matter). In this variant, we achieved significant increase of vitamin C content in comparison with the control variant. Thus, by application of nitrogen and sulphur nutrition, we reached significant increase of broccoli yield compared to the control variant. At the same time, the applied nutrition assisted to enhanced accumulation of sulforaphane and vitamin C in broccoli florets.

Keywords: broccoli, nutrition, nitrogen, sulforaphane, vitamin C

The recent years bring new knowledge concerning the effect of nutrition on health. Researches and studies of scholars open new views on origination of many diseases, in particular the civilization ones. Vegetables play a very important role in human nutrition, being the basis of a food chain with an indisputable health importance. Nevertheless, the annual consumption of vegetables is still insufficient (Šlosár a Čekey, 2008).

Compared to other grown crops, vegetables have relatively high nutrient requirements. That is why nutritions and fertilization are very important processes in growing vegetables (Hlušek, Richter a Ryant, 2002). Nitrogen nutrition considerably influences quantity and quality of the yield, however, other macroelements like phosphorus, potassium, calcium, sulphur and magnesium should not be forgotten, too (Fecenko a Ložek, 2000).

Broccoli (*Brassica oleracea* var. *italica*) contains large amount of phytochemicals with demonstrated anticarcinogenic activities. It is a rich source of vitamins A, C and E, folic acid, selenium, carotenoids, flavonoids, glucosinolates, and phenols. These substances are able to act protectively against cancers (Hollósy, 2004). Consumption of broccoli is linked to reduced risk of prostate (Joseph et al., 2004) and lung cancer (Spitz et al., 2000).

Cole crops have high contents of glucosinolates. Their amount ranges in dependance on types and cultivars of individual vegetables (Sivakumar, Aliboni and Bacchetta, 2007) as well as on individual parts of the plants (Kushad et al., 1999). Concerning glucosinates, broccoli contains higher amounts of sulforaphane, which was proved to restrain tumour extension. Sulforaphane also kills helicobacter pylori, the bacteria responsible for stomach ulcers (Fahey et al., 2002; Hollósy, 2004).

Thanks to its characteristics, vitamin C (L-ascorbic acid) belongs to very efficient antioxidants. It acts as an anticarcinogen and lowers the risk of cardiovascular diseases. When an organism is properly supplied with the vitamin, brain activity gets increased and neuromuscular reactions get faster (Pokluda, 2006). The antioxidant effects of vitamin C have been subject to many scientific studies. Byers a Perry (1992) imply that vitamin C prevents cancer, inhibiting creation of nitroso compounds in stomach and stimulating immunity system.

Material and methods

The small field experiment with broccoli was established in the territory of the Botanical garden of the Slovak University of Agriculture in Nitra in 2008. Medium late broccoli variety TIBURON F1 was included in the experiment. It forms dense, dark green florets and it is suitable for summer and autumn growing (vegetation period of 82 days).

The broccoli seeds were sown on May 26, 2008, in the outdoor area of the Department of Vegetable Production. The nursery plants were planted on June 24, 2008, having 2–4 first leaves at that time. An experiment field had an area of 2.25 m^2. As there was fourfold replication, the area of one experiment variant was 9 m^2. In a replication, 9 plants were planted into 0.5 × 0.5 m spacing.

In the field experiment, effect of four nutriotion variants on vitamin C content, sulforaphane content and yield of broccoli florets was investigated. The nutrition variants are characterized in the Table 2. The control variant (1) was free of any industrial fertilizers. In the nutrition variants 2, 3 and

Table 1 Agrochemical soil characteristics before the establishment of the experiment

pH/KCl	Nutrients content in mg.kg^{-1} of soil (1)						% of humus (2)
	N_{an}	P	K	S	Ca	Mg	
7.13	16.8	130.0	570	67.5	6300	695	3.42

Tabuľka 1 Agrochemické pôdne vlastnosti pred založením pokusu
(1) obsah živín v mg.kg^{-1} pôdy, (2) % humusu

Table 2 Scheme of nutrition variants

Nutrition variant (1)	Nutrient doses in kg.ha^{-1} (2)			
	N	P	K	S
1	–	–	–	–
2	200	–	–	–
3	200	–	–	50
4	200	–	–	60

Tabuľka 2 Schéma variantov výživy
(1) variant výživy, (2) dávky výživy v kg.ha^{-1}

4, fertilizers LAD27 and DASA 26/13 were used to replenish supplies of N and S, based on the agrochemical soil analysis (Table 1). The DASA 26/13 fertilizer was applied on June 6, 2008, i.e. three weeks before the planting. The LAD27 fertilizer was applied twice – July 17, 2008 (50% of the LAD27 dose) and August 5, 2008 (50 % of the LAD27 dose), i.e. 3 and 6 weeks after the planting. As the content of P and K was adequate to the broccoli growing requirements (40 kg P.ha^{-1}; 160 kg K.ha^{-1}), these elements were not applied. Within the basic soil preparation, 30 t.ha^{-1} of stable manure were ploughed into the soil in autumn 2007.

Protection from weeds was mechanical – manual hoeing. Protection from plant diseases was not necessary as the health condition of broccoli was good. In order to prevent pests intrusion, the following chemicals were used: Actellic 50 EC, Actara 25 WG, Pirimor 50 WG, Decis EW 50 and Karate Zeon 5 SC.

The broccoli florets were harvested gradually, since August 15, 2008 to September 9, 2008, the whole yield consisting of four partial harvests. The content of sulforaphane was evaluated with help of modified HPLC method according to Sivakumara et al. (2007) at the Department of Sustainable Agriculture and Herbology, Slovak University Agriculture in Nitra. The vitamin C content was evaluated by the titration method at the Centre of Plant Biology and Ecology in Malanta, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra. The analyses of monitored content substances were performed based on the samples, harvested on September 3, 2008. The evaluation of the obtained results was conducted by analysis of variance with help of Tukey HSD test, 95 % probability (Tables 3, 4, 5 and 6).

Table 3 Analysis of variance for yield of broccoli florets in t.ha^{-1}

Sources of variability (1)	Sum of squares (2)	Degree of freedom (3)	Mean squares (4)	F-ratio (5)	P-value (6)
A: variants (7)	97.95	3	32.65	8.15	0.0062
B: replications (8)	17.58	3	5.86	1.46	0.2890
Rezidual (9)	36.07	9	4.01		
Total (10)	151.60	15			

Tabuľka 3 Analýza variancií pre úrody ružíc brokolice v t.ha^{-1}
(1) zdroje variability, (2) súčet štvorcov, (3) stupne voľnosti, (4) priemer štvorcov, (5) F hodnota, (6) pravdepodobnosť, (7) varianty, (8) opakovania, (9) reziduál, (10) spolu

Table 4 Analysis of variance for content of sulforaphane and vitamin C in broccoli florets in t.ha^{-1}

Sources of variability (1)	Sum of squares (2)	Degrees of freedom (3)	Mean squares (4)	F-value (5)	P-value (6)
Content of sulforaphane (7)					
A: variants (9)	15.01	3	5.00	0.26	0.8495
B: replications (10)	59.37	3	19.79	1.05	0.4186
Rezidual (11)	170.38	9	18.93		
Total (12)	244.77	15			
Content of vitamin C (8)					
A: variants	17 885.90	3	5 961.97	12.84	0.0013
B: replications	965.88	3	321.96	0.69	0.5790
Rezidual	4 180.13	9	464.46		
Total	23 031.91	15			

Tabuľka 4 Analýza variancií pre obsah sulforafanu a vitamínu C v ružiciach brokolice v t.ha^{-1}
(1) zdroje variability, (2) súčet štvorcov, (3) stupne voľnosti, (4) priemer štvorcov, (5) F hodnota, (6) pravdepodobnosť, (7) obsah sulforafanu, (8) obsah vitamínu C, (9) varianty, (10) opakovania, (11) reziduál, (12) spolu

Results and discussion

The objective of the research was to determine the influence of nitrogen and sulphur nutrition on yield, sulforaphane and vitamin C content in broccoli.

The yield of broccoli florets significantly increased under the influence of applied nutrition in comparison with the unfertilized control variant; the rate of broccoli yield increase varied from 24.7% (variant 2) to 27.5% (variant 4). The highest broccoli yield was reached in variant 4 – 27.87 t.ha^{-1}, which means that compared to variant 1, the yield was 6.01 t.ha^{-1} higher. All monitored variants with applied nutrition marked significant differences in the yield parameter, compared to the control variant.

The outcomes of the research confirm the widely known fact that application of nitrogen fertilizers can significantly increase yields of crops (Varga, Ložek a Ducsay, 2004; Babik a Elkner, 2002).

The sulforaphane content in broccoli florets varied from 48.43 to 50.93 mg.kg^{-1} of fresh mater, its content in the variants increasing as follows: 1 < 2 < 3 < 4. The highest sulforaphane content was reached in variant 4 (50.93 mg.kg^{-1} of fresh matter). The increase of sulforaphane content compared to the control variant reached 2.5 mg.kg^{-1} of fresh matter, which stands for increase of 5.2 %. Though, it is not a statistically significant increase (Tabuľka 5). Thus, applied nutrition tend to cause higher accumulation of sulforaphane in broccoli florets, while the variant with applied sulphur marked higher sulforaphane content than the variant with applied nitrogen and the control variant.

The applied nitrogen and sulphur nutrition caused increase of vitamin C content; its content in the variants increasing as follows: 1 < 3 < 4 < 2. The variant 2 with applied nitrogen marked significant increase of vitamin C by 19 % (Table 6) compared to variant 1. The variants 3 and 4 also recorded increased vitamin C content compared to variant 1 – by 7.1 % and 8 %, respectively. This result does not confirm the argument that the application of nitrogen fertilizers cause decrease of vitamin C content in broccoli florets (Lisiewska and Kmiecik, 1996; Karitonas, 2002). On the other hand, the hypothesis saying that the optimization of nutrients doses can contribute to increased production of vitamin C (Ducsay a Varga, 2001).

Conclusion

The small field experiment was established in the Botanical garden of the Slovak University of Agriculture in Nitra in 2008. The effects of differentiated nutrition on broccoli yield and contents of sulforaphane and vitamin C in broccoli florets were evaluated in the experiment. All monitored nutrition variants marked significantly higher yields of broccoli florets compared to the control variant. The highest broccoli yield was recorded in variant 4 – applied nitrogen combined with sulphur in ratio N : S = 200 : 60 kg.ha^{-1}. In this variant, the yield reached 27.87 t.ha^{-1}, which means the increase of the yield by 27.5 % compared to the unfertilized control variant. The highest sulforaphane content was also reached in variant 4 – 50.93 mg.kg^{-1} of fresh mater; compared to the control variant, the increase of sulforaphane reaches 5.2 %. The highest vitamin C content was recorded in variant 2 (585.78 mg.kg^{-1} of fresh matter), which means the significant

Table 5 The yield of broccoli roses and content of sulforaphane in broccoli
Tests of Contrasts in t.ha^{-1} (Tukey HSD, 95%)

Sources of variability (1)	Yield of broccoli florets in t.ha^{-1} (2)			Content of sulforaphane in mg.kg^{-1} of fresh matter (3)	
Variant (4)	LS mean (5)	Homogenous groups (6)		LS mean	Homogenous groups
1	21.86	A		48.43	A
2	27.26		B	48.72	A
3	27.57		B	49.49	A
4	27.87		B	50.93	A

Tabuľka 5 Úroda ružíc a obsah sulforafanu v brokolici
Test kontrastov v t.ha^{-1} (Tukey HSD, 95%)
(1) zdroje variability, (2) úroda ružíc brokolice v t.ha^{-1}, (3) obsah sulforafanu v mg.kg^{-1} čerstvej hmoty, (4) variant, (5) LS priemer, (6) homogénne skupiny

Table 6 The content of vitamin C in broccoli in mg.kg^{-1} of fresh matter
Tests of Contrasts in t.ha^{-1} (Tukey HSD, 95%)

Sources of variability (1)	Content of vitamin C in mg.kg^{-1} (2)		
Variant (3)	LS mean (4)	Homogenous groups (5)	
1	492.33	A	
2	585.78		B
3	527.03	A	
4	531.65	A	

Tabuľka 6 Obsah vitamínu C v brokolici v mg.kg^{-1} čerstvej hmoty
Test kontrastov v t.ha^{-1} (Tukey HSD, 95%)
(1) zdroje variability, (2) obsah vitamínu C v mg.kg^{-1}, (3) variant, (4) LS priemer, (5) homogénne skupiny

increase of vitamin C content by 19 % compraed to the control variant.

Based on the outcomes it can be stated that application of nitrogen and sulphur nutrition substantially increased broccoli yield and also contributed to increased accumulation of sulforaphane and vitamin C in broccoli florets.

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Súhrn

Maloparcelkový poľný pokus s brokolicou (odroda Tiburon F1) bol založený v roku 2008 v areáli Demonštračnej záhrady SPU v Nitre. V pokuse sme skúmali vplyv štyroch variantov výživy na úrodu, obsah sulforafanu a vitamínu C v ružiciach brokolice. Prvý variant (1) bol kontrolný – neboli na ňom aplikované priemyselné hnojivá. Variant 2 bol vyhnojený na úroveň N 200 kg.ha^{-1}. Pri 3. a 4 variante boli živiny doplnené na úroveň N : S = 200 : 50 kg.ha^{-1}, resp. 200 : 60 kg.ha^{-1}. Na všetkých variantoch výživy sa úroda ružíc brokolice preukazne zvýšila v porovnaní s kontrolou. Najvyššia úroda ružíc brokolice bola dosiahnutá na variante 4 – 27,87 t.ha^{-1}. Najväčšie množstvo sulforafanu bolo taktiež zistené vo variante 4 – 50,93 mg.kg^{-1} čerstvej hmoty. Táto hodnota však nepredstavovala preukazné zvýšenie obsahu sulforafanu oproti kontrolnému variantu. Najväčší obsah vitamínu C bol dosiahnutý na variante 2, pričom sme zaznamenali preukazné zvýšenie obsahu vitamínu v porovnaní s kontrolou. A teda, aplikáciou dusíkatej a sírnatej výživy sme dosiahli preukazné zvýšenie úrody oproti kontrolnému variantu, ale zároveň aplikovaná výživa prispela k zvýšenej kumulácii sulforafanu a vitamínu C v ružiciach brokolice.

Kľúčové slová: brokolica, výživa, úroda, sulforafan, vitamín C, dusík, síra

References

BABIK, I. – ELKNER, K. 2002. The effect of nitrogen fertlization and irrigation on yield and quality of broccoli. In: Acta Horticulturae (ISHS), 2002, no. 571, p. 33–43. ISSN 0567-7572.

BYERS, T. – PERRY, G. 1992. Dietary carotenes, vitamin C and vitamin E as protective antioxidants in human cancers. In: Annual review of Nutrition, vol. 12, 1992, p. 139–159. ISSN 0199-9885.

DUCSAY, L. – VARGA, L. 2001. Vplyv dusíkatého hnojenia na tvorbu úrody, vitamínu C a akumuláciu dusičnanov pri pekinskej kapuste. In: Acta horticulturae et regiotecturae, roč. 7, 2001, č. 1, s. 20–23. ISSN 1335-2563.

FAHEY, J. W. –HARISTOY, X. – DOLAN, P. M. – KENSLER, T. W. – SCHOLTUS, I. – STEPHENSON, K. K. – TALALAY, P. – LOZNIEWSKI, A. 2002. Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of Helicobacter pylori and prevents benzo[a]pyrene--induced stomach tumors. In: Proceedings of the National Academy of Sciences of The United States of America, vol. 99, 2002, no. 11, p. 7610–7615. ISSN 0027-8424.

FECENKO, J. – LOŽEK, O. 2000. Výživa a hnojenie poľných plodín. Nitra : SPU, 2000. 452 s. ISBN 80-7137-777-5.

HLUŠEK, J. – RICHTER, R. – RYANT, P. – KOLÁŘ, L. Výživa a hnojení zahradních plodin. Praha : Zemědělec, 2002, 94 s. ISBN 80-902413-5-2.

HOLLÓSY, M. 2004. Dajme zelenú brokolici! In: APOTHÉKA – odborno-informačný štvrťročník pre zákazníkov lekárne, roč. 4, 2004, č. 2, s. 48–49. ISSN 1336-0930.

JOSEPH, M. A. – MOYSICH, K. B. – FREUDENHEIM, J. L. – SHIELDS, P. G. – BOWMAN, E. D. –ZHANG, Y. – MARSHALL, J. R. – AMBROSONE, C. B. 2004. Cruciferous vegetables, genetic polymorphisms in glutathione S-transferases M1 and T1, and prostate cancer risk. In: Nutrition and cancer, vol. 50, 2004, no. 2, p. 206–213. ISSN 0163-5581.

KARITONAS, R. 2002 Effect of nitrogen supply on yield and quality of broccoli. In: Plant nutrition, vol. 92, 2002, p. 298–299. ISSN 978-0-7923-7105-2.

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. In: Journal of agricultural and food chemistry, vol. 47, 1999, no. 4, p. 1541–1548. ISSN 0021-8561.

LISIEWSKA, Z. – KMIECIK, W. 1996. Effects of level of nitrogen fertilizer, processing conditions and period of storage of frozen broccoli and cauliflower on vitamin C retention. In: Food Chemistry, vol. 57, 1996, no. 2, p. 267–270. ISSN 0308-8146.

POKLUDA, R. 2006. Význam a nutriční hodnota zeleniny. In: Zahradnictví, roč. 10, 2006, č. 6, s. 18–19. ISSN 1213-7596

SIVAKUMAR, G. − ALIBONI, A. − BACCHETTA, L. 2007. HPLC screening of anti-cancer sulforaphane from important European Brassica species. In: Food Chemistry, vol. 104, 2007, no. 4, p. 1761–1764. ISSN 0308-8146.

SPITZ, M. R. − DUPHORNE, C. M. − DETRY, M A. − PILLOW, P. C. − AMOS, C. I. − LEI LEI − DE ANDRADE, M. − XIANGJUN GU − HONG, W. K − XIFENG WU. 2000. Dietary intake of isothiocyanates: evidence of a joint effect with glutathione S-transferase polymorphisms in lung cancer risk. In: Cancer Epidemiology Biomarkers and Prevention, vol. 9, 2000, no. 10, p. 1017–1020. ISSN 1055-9965.

ŠLOSÁR, M. – ČEKEY, N. 2008. Hlúbová zelenina – významná súčasť výživy človeka. In: Zahradnictví, roč. 12, 2008, č. 8, s. 16–17. ISSN 1213-7596

VARGA, L. – LOŽEK, O. – DUCSAY, L. 2004. Vplyv diferencovanej výživy na úrodu brokolice. In: 2. medzinárodná záhradnícka vedecká konferencia, 16.–18. september 2004, Nitra : SPU, 2004, s. 96–98. ISSN 1335-2563

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