ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume 62 59 Number 3, 2014
http://dx.doi.org/10.11118/actaun201462030561

THE EFFECT OF DIFFERENTIATED NUTRITION ON THE CONTENT OF ANTIOXIDANTS IN BROCCOLI

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Abstract

UHER ANTON, ŠLOSÁR MIROSLAV, LOŠÁK TOMÁŠ, HLUŠEK JAROSLAV. 2014. The Effect of Differentiated Nutrition on the Content of Antioxidants in Broccoli. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 62(3): 561–564.

The aim of this work is to determine the impact of differentiated nutrition, using different rates of nitrogen and sulphur, on the level of antioxidants, particularly vitamin E1 (α-tocopherol), vitamin C and β-carotene in the broccoli rosette. The experimental broccoli variety was Tiburon F1. It is a strong medium-late variety with a vegetation period of 82 days. In the 3-year field trial we observed the effects of different rates of nitrogen and sulphur on the amount of antioxidant compounds in broccoli. The experiment consisted of four fertilisation treatments: 1) unfertilised control, 2) fertilised with 200 kg $N.ha^{-1}$, 3) also fertilized with 200 kg $N.ha^{-1}$ and supplemented with 50 kg $S.ha^{-1}$, 4) 200 $kg.ha^{-1}$ and 60 kg $S.ha^{-1}$ was applied.

To determine the amount of β-carotene and vitamin E1, the slightly modified method of Olives Barb *et al.* (2006) was used. The vitamin C content was determined by titration.

Nitrogen nutrition has a significant impact not only on the amount of harvested broccoli, but also on the content of β-carotene, vitamin C and vitamin E1. The average content of β-carotene for the entire experimental period ranged from 24.84 $mg.kg^{-1}$ to 30.13 $mg.kg^{-1}$ of fresh mass. The content of β-carotene in broccoli rosettes increased as per the following order of treatments: control > N:S (200:50 $kg.ha^{-1}$) > N:S (200:60 $kg.ha^{-1}$) > N ($kg.ha^{-1}$). The β-carotene content increased significantly only in treatment 2 (30.13 $mg.kg^{-1}$) as compared to all the other treatments.

The level of vitamin C revealed the significant effect of fertilisation in all the treatments (567.9–614.2 $mg.kg^{-1}$) in contrast to the control variant (528.4 $mg.kg^{-1}$). What is more, in treatment 4 the content of vitamin C increased significantly in contrast to fertilised treatments 2 and 3.

The average content of vitamin E1 ranged from 4.33 $mg.kg^{-1}$ to 4.88 $mg.kg^{-1}$ of fresh mass. There were no significant differences among the untreated control and fertilised treatments 2 and 3. The only significant decrease in the content of vitamin E1 was detected in treatment 4 as against the unfertilised treatment 1.

Keywords: broccoli, fertilisation, antioxidants, β-carotene, vitamin C, E

INTRODUCTION

Given the current nutritional status of the Slovak Republic it is necessary to increase the production of products which are natural sources of bioactive substances, vitamins and antioxidants. Broccoli is one of the most valuable vegetables in our climatic zone. It contains high amounts of antioxidants which have a positive effect on the human body. Of all vegetables broccoli is also one of the richest sources of vitamin C. The aim of this work is to determine the impact of nutrition and fertilisation on the improvement of the quality of vegetables in terms of the content of substances such as vitamins (natural antioxidants). Vitamin C is an antioxidant which has an anticancer effect. It reduces the risk of cardiovascular diseases (Šlosár and Čeké, 2008). A sufficient amount of vitamin C increases the body's resistance against many diseases, it reduces the level of cholesterol in the blood and it is important in the process of bone formation (Šlosár and Ferusová, 2009). The content of vitamin C in broccoli depends on several factors, such as the variety (Singh *et al.*, 2007; Borowski *et al.*, 2008), climatic conditions (Vallejo *et al.*, 2003) and fertilisation (Kováčik and Takac, 2009) etc.

Byers and Perry (1992) found that carotenoids are important for the differentiation of epithelial cells. Olives Barb *et al.* (2006) reported a strong correlation between carotenoids and reduction of the risk of some diseases, such as cancer, bone calcification, eye degeneration and disorders of the nervous system.

The most active form of vitamin E is α-tocopherol (Vitamin E1). It affects the production of red blood cells, participates in recovery and muscle growth, fosters the activity of the gonads, slows the aging process and lowers blood pressure.

Broccoli can be fertilized with organic fertilizers. Manure at a rate of 40–60 $t.ha^{-1}$ is incorporated during autumn tillage. (Valšíková *et al.*, 1998). Broccoli has similar nutritional requirements as cauliflower. However, nitrogen rates are to be chosen cautiously as nitrogen cumulates nitrates (Small *et al.*, 1998).

MATERIALS AND METHODS

A small-plot field experiment with broccoli (*Brassica oleraacea* var. *Italica*) was established in 2008–2010 at the Slovak Agricultural University in Nitra, Department of Horticulture. The total area of the field trial was 67.5 m^2. Each variant took an area of 2.25 square meters in four repetitions. Nine plants were planted per each repetition at a spacing of 0.5×0.5m. Sowing was conducted at the Department of Horticulture on 26 May 2008, 20 May 2009 and 3 June 2010. Seedlings were planted on 24 June 2008, 21 June 2009 and 6 July 2010. The research project explored the influence of differentiated nutrition on the content of antioxidants in the edible parts of broccoli.

Fertilisation treatments:

1) Control – no fertilisers applied.
2) Treatment – nitrogen fertilization to the level of 200 kg $N.ha^{-1}$.
3) Treatment – nitrogen and sulphur fertilisation at rates of 200 kg $N.ha^{-1}$, 50 kg $S.ha^{-1}$.
4) Treatment – nitrogen and sulphur fertilisation at rates of 200 kg $N.ha^{-1}$, 60 kg $S.ha^{-1}$.

As a part of autumn soil preparation, 30 $t.ha^{-1}$ of manure was incorporated into soil. Phosphoric and potassium fertilizers were not applied because their supply in the soil was sufficient. Nitrogen and sulphur were applied in the spring before planting and during the growing season. Before planting, we applied the fertiliser DASA (26% N and 13% S, sulphate S), during the growing season we fertilised the soil with nitrogen LAD 27 (27% N). Fertilisation of the individual treatments was performed on the basis of soil nutrients analysis. The fertiliser DASA 26/13 was applied three weeks before planting. The LAD 27 fertiliser was applied in two stages – three and six weeks after planting.

Broccoli rosettes were harvested gradually based on the process of maturation. Harvested were whole rosettes with stem sections 0.1 m long. After the total yield identification (not published here) the crop samples were analysed for the content of antioxidants. The content of β-carotene and vitamin E1 in broccoli was determined in accordance with Olives Barb *et al.* (2006). The vitamin C content was determined by titration.

The results were evaluated by analysis of variance. Tukey tests were performed at a 95% probability level.

RESULTS AND DISCUSSION

The effect of fertilisation on the content of β-carotene in broccoli rosettes was manifested in all three experimental years in treatment 2, where nitrogen was applied at a rate of 200 $kg.ha^{-1}$ and where we observed an increase of 12.7% higher than the control. In other treatments where nitrogen and sulphur was applied (treatments 3 and 4) we discovered that the level of β-carotene was by 4.2% and 4.3%, respectively higher than the control but the differences were not significant.

The average content of β-carotene for the entire experimental period ranged from 24.84 $mg.kg^{-1}$ to 30.13 $mg.kg^{-1}$ of fresh mass. Analysis of variance confirmed a significant difference between the control variant and variant 2, i.e. by 5.29 $mg.kg^{-1}$ (Tab. I).

I: *The average content of β-carotene in broccoli rosettes ($mg.kg^{-1}$) in the years 2008–2010 (Test of Contrast, Tukey HSD, 95%)*

Variant	LS average	Homogenous groups	
1.	24.84	a	
3.	26.88	a	
4.	26.91	a	
2.	30.13		b

The effect of nitrogenous nutrition on the content of β-carotene has been the subject of research projects of several authors. Gajewski *et al.* (2010) investigated the effect of different rates of nitrogen on the accumulation of β-carotene in carrot roots. The content of β-carotene in carrot roots ranged from 30.5 $mg.kg^{-1}$ to 38.1 $mg.kg^{-1}$ of fresh mass, while the lowest content was recorded in the control variant not fertilized with nitrogen.

Evers *et al.* (1997) investigated the effect of decreasing doses of nitrogen (90, 60, 30 kg $N.ha^{-1}$) on β-carotene content in celery. The results indicated that decreasing the rate of nitrogen reduced the level of β-carotene in the edible part.

Čeké *et al.* (2009) found that nitrate nitrogen fertilisation rates of 250 kg.ha^{-1} significantly increased the amount of β-carotene in cauliflower rosettes. This represents an increase in the content of β-carotene by 24.7% versus the control variant.

Based on these results we can conclude that nitrogen nutrition has a positive effect on increasing the amount of β-carotene in broccoli rosettes.

Fertilisation was seen to have a significant effect on the content of vitamin C in broccoli rosettes in all treatments (567.9–614.2 mg.kg^{-1}) in contrast to the control (528.4 mg.kg^{-1}), Tab. II. On top of that in treatment 4 (200 kg N.ha^{-1}, 60 kg S.ha^{-1}) the level of vitamin C increased significantly in contrast to fertilised treatments 2 and 3 (200 kg N.ha^{-1} and 200 kg N.ha^{-1}, 50 kg S.ha^{-1}, respectively).

II: *The effect of fertilisation on the vitamin C content in broccoli rosettes in mg.kg^{-1} in the years 2008–2010 (Test of Contrast, Tukey HSD, 95%)*

Variant	LS average	Homogenous groups		
1.	528.4	a		
2.	567.9		b	
3.	578.2		b	
4.	614.2			c

Similar results were seen in the work of Ducsay and Varga (2004). They investigated the effect of nitrogenous nutrition on the content of vitamin C in Chinese cabbage. Vallejo *et al.* (2003) stated the positive effect of sulphur application on the content of vitamin C in selected varieties of broccoli; this is in accordance with our results.

In the respective years the average contents of vitamin E1 (Tab. III) ranged from 4.33 mg.kg^{-1} to 4.88 mg.kg^{-1} of fresh mass. There were no significant differences among the untreated control and treatments 2 and 3. The only significant decrease in the content of vitamin E1 was seen in treatment 4 in contrast to unfertilised treatment 1.

III: *The effect of fertilisation on the vitamin E1 content in broccoli rosettes in mg.kg^{-1} in the years 2008–2010 (Test of Contrast, Tukey HSD, 95%)*

Variant	LS average	Homogenous groups	
1.	4.88	a	
2.	4.67	a	b
3.	4.43	a	b
4.	4.33		b

CONCLUSIONS

From the results of 3-year experiments with broccoli applying nitrogen alone (200 kg N.ha^{-1}) or in combination with two rates of sulphur (200 kg N.ha^{-1} plus 50 or 60 kg S.ha^{-1}) we can conclude the following:

- the content of β-carotene in broccoli rosettes increased significantly only when nitrogen alone was applied,
- the content of vitamin C increased significantly in all the fertilised treatments in contrast to the unfertilised control,
- a combination of nitrogen and the highest rate of sulphur (200 kg N.ha^{-1} plus 60 kg S.ha^{-1}) significantly increased the content of vitamin C in contrast to the other fertilised treatments,
- there were no significant differences among the untreated control and fertilised treatments 2 and 3 in the contents of vitamin E1 (α-tocopherol),
- the content of vitamin E1 decreased significantly only in treatment 4 (200 kg N.ha^{-1} plus 60 kg S.ha^{-1}) as against unfertilised treatment 1.

SUMMARY

The aim of this work was to determine the impact of differentiated nutrition, applying different rates of nitrogen and sulphur, on the level of antioxidants, particularly vitamin E1 (α-tocopherol), vitamin C and β-carotene in the broccoli rosette. The experimental broccoli variety was Tiburon F1. The experiment consisted of four fertilisation treatments. The first, untreated control was not fertilised. In the second treatment 200 kg N.ha^{-1} was applied. Likewise in the third treatment 200 kg N.ha^{-1} was applied and supplemented with 50 kg S.ha^{-1}. Nitrogen with sulphur at 200 kg.ha^{-1} and 60 kg S.ha^{-1} was applied in the 4th treatment. In the 3-year field trial we observed different effects of applications of nitrogen and sulphur on the level of antioxidant compounds in broccoli.

The average content of β-carotene for the entire experimental period ranged from 24.84 mg.kg^{-1} to 30.13 mg.kg^{-1} of fresh mass. A significant increase in and the highest content of β-carotene were seen only in treatment 2 (nitrogen alone) in contrast to all the other treatments. The assessment of the level of vitamin C revealed the significant effect of fertilisation in all treatments (567.9–614.2 mg.kg^{-1}) as against the control (528.4 mg.kg^{-1}). Moreover in treatment 4 (nitrogen and sulphur) the increase in the content of vitamin C was significantly higher than in the fertilised treatments 2 and 3. The average content of vitamin E1 ranged from 4.33 mg.kg^{-1} to 4.88 mg.kg^{-1} of fresh mass. There were no significant differences among the untreated control and fertilised treatments 2 and 3. The only significant decrease in the content of vitamin E1 was seen in treatment 4 in contrast to unfertilised treatment 1.

If we increase the rates of nitrogen in the process of growing broccoli, it is necessary to take into account the accumulation of nitrate in the edible part. Any further increase in N rates above 200 kg.ha^{-1} has a positive effect on broccoli in terms of the sulphoraphane content, β-carotene. However it is also necessary to follow the permissible limit of an amount of nitrates in broccoli according to the Codex Alimentarius of the Slovak Republic.

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