Nauka Przyroda Technologie

2018
Tom 12
Zeszyt 1

ISSN 1897-7820 http://www.npt.up-poznan.net http://dx.doi.org/10.17306/J.NPT.00223

Dział: Ogrodnictwo i Architektura Krajobrazu

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THE EFFECT OF BIOSTIMULANTS ON THE YIELD AND QUALITY OF BROCCOLI HEADS DURING STORAGE

WPŁYW BIOSTYMULATORÓW NA PLONOWANIE I JAKOŚĆ RÓŻ BROKUŁU W CZASIE PRZECHOWYWANIA

Abstract

Background. Biostimulants are widely used in horticulture to increase yield and improve its quality as well as to increase plants' resistance to stress. There are publications providing information on the effects of different biostimulants on broccoli cultivation, but there is no information how the quality of broccoli heads changes during storage when plants are treated with biostimulants during the growth period. Therefore, the aim of this study was to evaluate the effect of amino acid biostimulants and amino acids applied in combination with *Ascophyllum nodosum* filtrate on the yield and quality of broccoli heads during cold storage for 1, 2 and 3 weeks.

Material and methods. The experiment was conducted between 2013 and 2014 and it comprised one broccoli cultivar 'Tiburon'. The following objects were used in the experiment: amino acid biostimulant, *Ascophyllum nodosum* filtrate + amino acid biostimulant, control plants (without biostimulants). The yield quantity and quality were determined. The harvested broccoli heads were cold-stored for 3 weeks at 1–2°C and 95% RH. The content of sugars, vitamin C and colour were measured in fresh heads and after each week of cold storage.

Results. There were no differences between the plants treated with biostimulants and the control plants in the total yield, mean weight and diameter of broccoli heads. In the first year of the study the biostimulants increased the sugar content after 3 weeks of storage, but in the second year of the study there was higher sugar content in the fresh heads. The content of vitamin C in the plants treated with the biostimulants was significantly higher after the first week of storage in the first year of the study. However, this trend did not continue in the second year.

Conclusions. The biostimulants did not increase the broccoli yield. The biostimulants and storage length affected the content of sugars and vitamin C, but there were considerable differences between the years of the research. The colour of broccoli heads did not change during storage.

Keywords: *Brassica oleracea* var. *italica*, amino acids, *Ascophyllum nodosum*

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Introduction

Plant biostimulants, or agricultural biostimulants, include various substances and microorganisms that enhance plant growth. Essential biostimulants comprise amino acids and marine algae-based biostimulants. Protein-based products can be divided into two main categories: protein hydrolysates consisting of a mixture of peptides and amino acids of animal and plant origin (Calvo et al., 2014). Biostimulants affect the uptake of nutrients from the soil and hence they affect the yield. They influence physiological processes and plant metabolism and they stimulate plants' resistance to both biotic and abiotic stress factors (Ertani et al., 2009).

Broccoli is a vegetable of high biological value due to the presence of such compounds as vitamin C, E, β-carotene, glucosinolates and polyphenols. The content of these ingredients in broccoli is higher than in other brassica vegetables such as cabbage, cauliflower or Brussels sprouts (Moreno et al., 2006; Singh et al., 2007). The main antioxidant found in brassica vegetables is vitamin C. It plays a very important role in plants' physiological response to stress as well as affects their growth and development. It takes part in detoxification of reactive oxygen species, plays an important role in photoprotection and in response to plant injuries and damage by insects (Podsędek, 2007).

The content of sugars and antioxidants in plants is affected by climatic (Gliszczyńska-Świgło et al., 2007), agrotechnical (Vallejo et al., 2003) and storage (Kałużewicz et al., 2012) conditions. According to Czeczko and Mikos-Bielak (2004) and Łyszkowska et al. (2008), the use of biostimulants increased the sugar content in lettuce leaves. Dobromilska and Gubarewicz (2008) observed a significant increase in the content of vitamin C in tomato fruits after the application of marine algae biostimulants. Many authors discuss the effect of storage conditions and length on the content of antioxidants in vegetables (Hasperúe et al., 2015; Starzyńska et al., 2003; Vale et al., 2015), but there are few studies presenting how the content of these compounds changes in plants treated with biostimulants.

The aim of this study was to present the influence of amino acids and amino acids combined with *Ascophyllum nodosum* filtrate on the yield and variation in the content of sugars and vitamin C as well as colour during cold storage for 1, 2 and 3 weeks.

Material and methods

Plant material

The experiment was conducted between 2013 and 2014. The study comprised one broccoli cultivar 'Tiburon'. Broccoli seedlings were produced in cell trays of 54 cm^3 capacity, filled with peat substrate for cruciferous vegetables (Kronen-Klasmann, Klasmann-Deilman Polska Sp. z o.o., Poland). The transplants were planted in a field in mid-July. 35 transplants were planted at the stage of four leaves and spaced at 0.5 × 0.5 m. The experiment was conducted on podzolic soil, the arable layer of which was loamy sand underlying sandy loam. The soil was tested before planting. The Yara-

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Mila Complex multi-nutrient fertiliser (600 $kg \cdot ha^{-1}$) was applied before soil preparation. Additionally, three doses of nitrogen (150 $kg \cdot ha^{-1}$) were applied in the growing season. The plants were cultivated without watering. The variants of the experiment were as follows: amino acid biostimulant – 1.5 $dm^3 \cdot ha^{-1}$ (the plants were sprayed 2, 4, and 6 weeks after planting), *Ascophyllum nodosum* filtrate – 1% (the seedlings were watered 4 and 5 weeks after sowing) + amino acid biostimulant – 1.5 $dm^3 \cdot ha^{-1}$ (the plants were sprayed 2, 4, and 6 weeks after planting), control plants (without biostimulants). Both biostimulants were produced by Arysta LifeScience Polska Sp. z o. o., Poland.

Broccoli heads were harvested as they grew, from September to October. After the harvest they were divided into marketable (compact, healthy, fully grown) and non--marketable ones (loose, unhealthy). Then the broccoli heads were weighed and measured in diameter.

The broccoli heads for storage in 2013 were collected on 22 October, whereas those to be stored in 2014 were collected on 23 September. 24 compact and even heads were collected from each variant. After harvesting six heads from each variant of the experiment, two 50 g samples were taken and frozen at –20°C. The remaining heads from each variant were placed in three boxes, six heads in each box. The heads were cold--stored at 1–2°C, 95% RH for 1, 2 and 3 weeks. After each week of storage samples were taken and frozen in the same way as the fresh heads.

Sugar and vitamin C analysis

The anthrone method was applied to determine the total content of soluble sugar in the broccoli (see Kałużewicz et al., 2015). The content of vitamin C in the broccoli heads was determined with the Tillmans method (PN-90/A-75101/11, 1990) at two replicates.

Colour

The colour of the broccoli was measured with a StellarNet Black-Comet UV-VIS spectrometer in each week of storage. The following parameters were measured: L – brightness, ranging from no reflection for black ($L = 0$) to perfect diffuse reflection for white ($L = 100$), a – redness, ranging from negative values for green to positive values for red, b – yellowness, ranging from negative values for blue to positive values for yellow. Four different positions on the surface of each broccoli head were measured.

Statistical analysis

The field experiment was conducted in a one-factor design, while the storage experiment was conducted in a two-factor design. There were four replicates in both experiments. The Stat program was used for statistical analyses. The influence of the biostimulants on the yield and quality of broccoli heads during storage was determined with ANOVA. The three-factor experiment was analysed statistically to determine the influence of the year of the study during storage. Differences between the means were estimated with the Duncan test at a significance level of $p < 0.05$.

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Results and discussion

The study did not show any increase in the yield after biostimulants had been used. In the second year of cultivation the marketable yield was even smaller than in the control variant (Table 1). This is consistent with the results noted by Łyszkowska et al. (2008) who researched the yield of lettuce after the application of both amino acids and *Ascophyllum nodosum* filtrate. The research by Kunicki et al. (2010) did not confirm the effect of the amino acid biostimulant on the yield of spinach. Arthur et al. (2003) obtained the opposite results and reported higher yield of peppers after treatment with the Kelpak biostimulant. Grabowska et al. (2012) observed a similar increase in the yield of carrots after using amino acid biostimulants. Mikiciuk and Dobromilska (2014) applied three biostimulants based on marine algae extracts and found that only two of them resulted in an increase in the tomato yield.

Table 1. The yield, mean weight of head and head diameter after treatment with biostimulants

Treatment	Yield ($t \cdot ha^{-1}$)		Mean weight of head (g)	Head diameter (cm)
	total	marketable		
	2013			
Control	18.2 a	18.2 a	462 a	15.5 a
AA	18.2 a	16.9 a	448 a	15.3 a
AA + AN	18.5 a	18.2 a	461 a	15.5 a
	2014			
Control	23.6 a	21.9 a	594 a	17.6 a
AA	21.4 a	18.8 b	577 a	17.5 a
AA + AN	22.6 a	19.6 ab	594 a	17.9 a

AA – amino acids, AN – *Ascophyllum nodosum* filtrate.
Values marked with the same letter in each column do not differ significantly at $p < 0.05$.

The analysis of variance showed that both the content of vitamin C and sugars depended on the length of storage, biostimulants, and the year of the study (Tables 2 and 3). In the second year of the study the sugar content in fresh broccoli heads treated with biostimulants was about 16% higher than in the control variant (Fig. 1). The results obtained in the first year did not confirm these findings. The increase in the sugar content following the use of biostimulants is consistent with the results of studies by Schiavon et al. (2008), Shehata et al. (2011) and Fan et al. (2014). Like in our study, there were considerable differences in the sugar content between the first and second year of the study conducted by Grabowska et al. (2012). According to the authors, the use of amino acids increased the sugar content in carrots only in the first year of the research, but this effect was not observed in the second year.

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Table 2. ANOVA F-values for the vitamin C and total sugar content in 2013 and 2014

Variant	2013		2014	
	vitamin C	total sugar	vitamin C	total sugar
Week	**	**	**	**
Treatment	*	**	ns	**
Week × treatment	ns	**	ns	**

* – significance level at $p < 0.1$, ** – significance level at $p < 0.05$, ns – insignificant at $p < 0.05$.

Table 3. ANOVA F-values for the vitamin C and total sugar content and colour parameters

Variant	Vitamin C	Total sugar	L	a	b
Week	**	**	**	ns	ns
Treatment	**	**	ns	ns	ns
Week × treatment	ns	**	ns	ns	ns
Year	**	**	ns	**	**
Week × year	**	**	ns	ns	ns
Treatment × year	ns	**	ns	ns	ns
Week × treatment × year	*	**	ns	ns	ns

* – significance level at $p < 0.1$, ** – significance level at $p < 0.05$, ns – insignificant at $p < 0.05$.

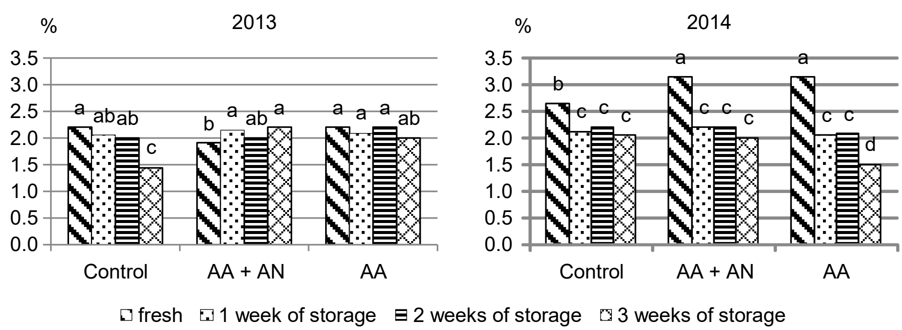

Fig. 1. The total sugar content in fresh broccoli heads and during cold storage in 2013 and 2014; AA – amino acids, AN – *Ascophyllum nodosum* filtrate; values marked with the same letter do not differ significantly at $p < 0.05$

In our study the sugar content increased or did not change with the length of storage only in the first year of the research. By contrast, in the second year the sugar content decreased with the length of storage in all the variants. In the second year of cultivation, after a week of storage the sugar content decreased from about 20% in the control variant to almost 35% after the application of the amino acid biostimulant. In the first year there was a significant drop in the control variant only after three weeks of storage. The decrease in the sugar content with the length of storage is consistent with the results obtained by other authors who did not use biostimulants in their studies (Hasperué et al., 2014, 2015; Pramanik et al., 2006). According to Hasperué et al. (2015), after three weeks of storage at 4°C the sugar content in broccoli heads was about 50%.

According to Lee and Kader (2000), during storage brassica vegetables lose less vitamin C than other vegetables due to the presence of sulphur and glutathione, which are involved in the mechanism responsible for the reduction of L-dehydroascorbic acid to L-ascorbic acid (Albrecht et al., 1990). In the first year of our study the vitamin C content decreased with the length of storage. However, in the second year the vitamin C content increased in all the variants in the first week, but it decreased in the subsequent weeks (Fig. 2). The reduction in the vitamin C content with the length of storage is consistent with the results obtained by Fernández-León et al. (2013). The authors stated that after 4 weeks of storage at 1–2°C the content of vitamin C in broccoli heads decreased more than three times. According to Serrano et al. (2006), when broccoli heads were stored at 1°C for 5 days, a 12% decrease in the vitamin C content was observed. Similarly, Galgano et al. (2007) observed that after 7 days of storing broccoli heads at 6°C there was an 18% decrease in the vitamin C content. Another trend was observed by Kałużewicz et al. (2012), who found that after a week of cold storage of broccoli heads the content of vitamin C did not differ significantly from the content in fresh heads.

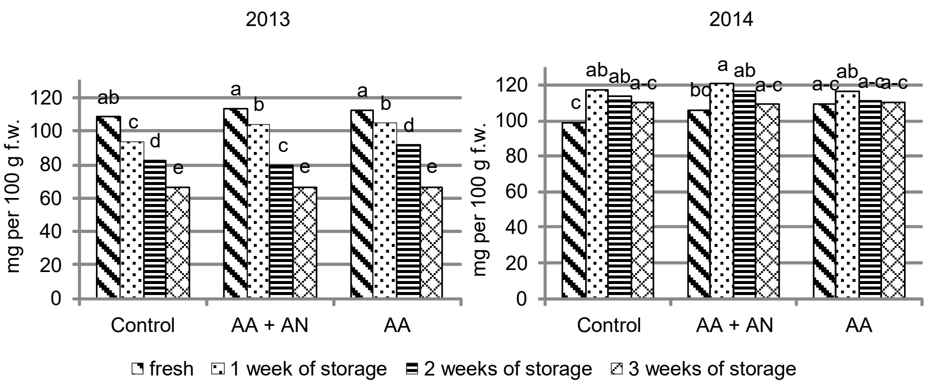

Fig. 2. The total vitamin C content in fresh broccoli heads and during cold storage in 2013 and 2014; AA – amino acids, AN – *Ascophyllum nodosum* filtrate; values marked with the same letter do not differ significantly at $p < 0.05$

The analysis of variance in our study showed that the biostimulants affected the vitamin C content only in the first year of the experiment (Table 2). Gajewski et al. (2008) reported that *Ascophyllum nodosum* significantly increased the vitamin C content in Chinese cabbage. Shehata et al. (2011) obtained the opposite results. They found that

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the use of amino acids and *Ascophyllum nodosum* did not increase the vitamin C content in broccoli heads.

Our study showed significant differences in the colour between the first and second year, as proved by parameters a and b (Table 3). However, neither the biostimulants nor the length of storage affected these parameters. This observation is consistent with the results of the study by Pramanik et al. (2006), who found that broccoli heads stored at 1°C for 14 days did not change their colour.

To sum up, in our study the year significantly affected the content of sugars and vitamin C. This could have been caused by the fact that between the first and second year there were large differences in the temperatures during 30 days preceding the harvest (Table 4). In the year when the total sugar content was higher, i.e. 2014, the average daily temperature of the air during that period was almost 4°C higher than in 2013. Moreover, in 2013 the plant growth period was more than one month longer than in 2014. In view of the fact that in both years biostimulants were used after the same number of days after planting, their effectiveness could have been influenced by the length of the plant growth period.

Table 4. Temperatures and total rainfall during broccoli cultivation

Year	Temperature (°C)			Total rainfall (mm)
	maximum	minimum	daily	
	From planting to harvest			
2013	20.7	10.6	15.7	143.8
2014	22.6	12.4	17.3	138.6
	30 days before harvest			
2013	14.4	5.3	9.9	21.0
2014	19.1	9.7	14.4	24.2

Conclusions

1. The biostimulants did not increase the yield of broccoli.
2. There were significant differences in the sugar and vitamin C content between the years of the research.
3. The biostimulants and storage length affected the content of sugars and vitamin C.
4. The biostimulants did not change the colour of broccoli heads during storage.

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WPŁYW BIOSTYMULATORÓW NA PLONOWANIE I JAKOŚĆ RÓŻ BROKUŁU W CZASIE PRZECHOWYWANIA

Abstrakt

Wstęp. Biostymulatory są szeroko stosowane w ogrodnictwie w celu zwiększenia plonu i poprawy jego jakości, jak również, aby zwiększyć odporność roślin na stres. W literaturze można znaleźć informacje dotyczące efektów stosowania różnych biostymulatorów w uprawie brokułu, brak jest jednak informacji dotyczących tego, jak zmienia się jakość róż brokułu w czasie przechowywania roślin traktowanych biostymulatorami w okresie wegetacji. Celem pracy była ocena wpływu biostymulatora zawierającego aminokwasy oraz połączenia biostymulatora zawierającego filtrat z *Ascophyllum nodosum* z biostymulatorem zawierającym aminokwasy na wielkość plonu i na jakość róż brokułu w czasie przechowywania w chłodni przez okres 1, 2 i 3 tygodni.
Materiał i metody. Doświadczenie, wykonane w latach 2013–2014, obejmowało odmianę brokułu 'Tiburon'. Zastosowano następujące warianty doświadczalne: biostymulator zawierający aminokwasy, biostymulator zawierający filtrat z *Ascophyllum nodosum* + biostymulator zawierający aminokwasy, rośliny kontrolne (nietraktowane biostymulatorami). Określono wielkość i jakość plonu róż. Zebrane róże były przechowywane przez 3 tygodnie w chłodni w temperaturze 1–2°C i przy 95% RH. W różach świeżych i po każdym tygodniu przechowywania określano zawartość cukrów, witaminy C i barwę.
Wyniki. Ani wielkość plonu ogólnego, ani średnia masa i średnica róż brokułów traktowanych biostymulatorami nie różniły się od roślin kontrolnych. W pierwszym roku badań zastosowanie biostymulatorów spowodowało wzrost zawartości cukrów po 3 tygodniach przechowywania, a w drugim roku istotnie większa zawartość cukrów była w różach świeżych. Zawartość witaminy C w roślinach traktowanych biostymulatorami była istotnie większa w pierwszym roku badań po pierwszym tygodniu przechowywania. Tendencja ta nie została potwierdzona w drugim roku.
Wnioski. Plon brokułu nie zwiększył się w wyniku zastosowaniu biostymulatorów. Zastosowane biostymulatory oraz czas przechowywania miały wpływ na zawartość cukrów i witaminy C, przy czym wystąpiły duże różnice między latami badań. Barwa róż brokułu nie zmieniła się w czasie przechowywania.

Słowa kluczowe: *Brassica oleracea* var. *italica*, aminokwasy, *Ascophyllum nodosum*

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Accepted for publication – Zaakceptowano do opublikowania:
5.02.2018

For citation – Do cytowania:
Kałużewicz, A., Spiżewski, T., Krzesiński, W., Zaworska, A. (2018). The effect of biostimulants on the yield and quality of broccoli heads during storage. Nauka Przyr. Technol., 12, 1, 45–54. http://dx.doi.org/10.17306/J.NPT.00223