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The influence of particular biostimulators on some biochemical parameters in broccoli (*Brassica oleracea* L. Var. *Botrytis italica* Plenck)

Wpływ wybranych biostymulatorów na kształtowanie się parametrów biochemicznych u brokułu (*Brassica oleracea* L. Var. *Botrytis italica* Plenck)

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Słowa kluczowe: biostymulatory, brokuł, wzrost, prolina, dialdehyd malonowy

Abstract
The aim of this study was to determine the effect of biostimulators: Kelpak, Asahi SL and Goëmar Goteo on some biochemical parameters–proline and MDA concentration in leaves and florets of Monaco F1cultivars of broccoli grown under field conditions. Biostimulators applied in the experiment caused changes of some biochemical parameters in the plants. Kelpak and Goëmar Goteo significantly increased the oxidative stress parameters like the concentration of proline and malondialdehyde in leaves and florets of broccoli cultivar Monaco F1. Although, Asahi SL affected the content of chosen biochemical parameters although not seriously.

Streszczenie
Celem niniejszej pracy było określenie wpływu biostymulatorów: Kelpak, Asahi SL i Goëmar Goteo na kształtowanie się wybranych parametrów biochemicznych – stężenie proliny i dialdehydu malonowego, w liściach oraz różach brokułu odmiany Monaco F1, rosnących w warunkach polowych. Zastosowane w doświadczeniu biostymulatory wpłynęły na zmianę parametrów biochemicznych w badanych roślinach. Preparaty Kelpak i Goëmar Goteo istotnie podwyższały parametry stresu oksydacyjnego takie jak stężenie proliny i dialdehydu malonowego w liściach i różach brokułu odmiany Monaco F1. Natomiast preparat Asahi SL powodował nieznaczny wzrost stężenia MDA w liściach i różach brokułu oraz spadek zawartości proliny w różach brokułu.

1. INTRODUCTION

The European Union (EU) regulations impose restriction on the use and production of pesticides; thus, this demands searching for new and effective substances which are environment friendly. One of the solutions is to use biostimulators, which are safe both for human beings and for the environment and most importantly useful for reducing chemicals in agriculture [Poincelot 1993, Dhargalkar and Pereira 2005]. In recent years, biostimulators are being extensively used in agriculture and horticulture. Positive effects of its usage have yielded great results as confirmed by many experiments [Jelačić et. al. 2007, Smoleń et al. 2010, Smoleń and Sady 2010, Matysiak et al. 2011]. The mechanism of these substances has not been explained yet [Gawrońska and Przybysz 2011]. The active ingredients of biostimulators comprise many organic compounds (phenols, vitamins, polysaccharides, betaines, etc.), growth regulators, algae, humus, extract from grapefruit, garlic and also macro and micro elements [Gawrońska et al., Przybysz 2011, Truba et al. 2012].

It is known that biostimulators affect a number of physiological and biochemical changes in plant cell metabolism. Gawrońska et al. [2008] and Przybysz et. al. [2008] reported a positive effect of applied biostimulators on photosynthetic apparatus efficiency in plants (larger assimilation area, higher rate of photosynthesis, higher chlorophyll content). Wrochna et al. [2008] observed that biostimulators increased activity of antioxidant system during salt stress. Changes in some biochemical and physiological processes affect gene expression [Cambri et al. 2008].

Proline is a α-amino acid which protects plant enzymes like catalase, peroxidase or diphenol oxidase, looks for singlet oxygen and bounds redox active metal ions [Özturk i Demir 2002]. Many authors suggest that content of proline in plant tissues could be a good indicator of different environmental stresses [Chen i in. 2003, Zhu i in. 2008].

A widely used indicator of the level of stress in plants is also malonyldialdehyde (MDA), whose presence in a cell signifies lipid membrane peroxidation as an effect of oxidative stress [Woźny and Przybył 2004]. MDA is an end-product of fatty acids double bounds peroxidation [Głód et al. 2006].

Broccoli is a plant, whose popularity in the Polish market is increasing significantly every year. Consumers are interested in this vegetable not only due to its taste but also its high nutritional and medicinal properties [Anyszka et al. 2000].

2. MATERIAL AND METHODS

The experiment was conducted from 1st May 2012 to 3rd August/ 2012 at the Horticultural Experimental Station in Doluje (near Szczecin) and in the laboratory of the Department of Biochemistry at the West Pomeranian University of Technology in Szczecin. The research material in this experiment was broccoli cultivar Monaco F1. The field experiment was carried out in split-plot design in three replications. One of the factors of the experiment was the three types of biostimulators: Kelpak, Asahi SL and Goëmar Goteo. The control consisted of the plant not treated with biostimulators. Treatments (irrigating, herbicide and pesticides) were applied according to standard procedures.

During broccoli vegetation (from May to August), the average temperature was 16.2°C (similar to average multi-year air temperature; Table 1). The highest average temperature was observed in July (18.3°C); also in July, the sum of precipitation was the highest (114.8 mm, 21 days).

Table 1. Weather conditions during broccoli vegetation season in 2012 from Szczecin Dąbie Meteorological Station

Month	Average Air Temperature (°C)	Average Multi-year Air Temperature (°C) 1965–1994	Precipitation Sum (mm)	Average Multi-year Precipitation Sum (mm) 1965–1994	Number of Days of Precipitation
May	14.5	7.5	25.3	49.8	10
June	15.8	13.0	44.1	59.2	14
July	18.3	16.2	114.8	60.2	21
August	17.9	17.6	56.6	53.8	18

During the experiment, biostimulators were applied on plants three times, according to the instructions of producers. Goëmar Goteo was applied while watering, 0.1 per cent, solution, 75 ml for each plant. First application was done 7 days after seedling plantings, then at 14-day intervals. Biostimulators Kelpak (in concentration of 0.4 per cent) and Asahi SL (in concentration of 0.1 per cent) were applied in the form of spraying. Kelpak was applied 7 days after seedling plantings, then at 14-day intervals. Asahi SL was applied at 14-day intervals. During the field experiment, plant samples of leaves and florets were collected on 3rd August 2012. Proline and malondialdehyde concentration was measured colorimetrically with a UV 1800 Schimadzu spectrophotometer.

Free proline concentration was assayed according to the method of Batesa et al. [1973], while content of MDA in plant tissue was determined by the method described by Sudhakar et al. [2001]. The results were analyzed statistically using one-way ANOVA and Tukey's test at a significance level of $\alpha = 0.05$.

3. RESULT AND DISCUSSION

Changes in plant metabolism are often affected by various environmental conditions [Grzyś 2012]. Gawrońska and Przybysz [2011] said that in almost most instances, positive influence of applied biostimulators was observed. It is also possible that this kind of preparations might negatively affect and reduce growth, crop and condition of plants; moreover, it could also provoke some changes in cell metabolism. It is known that proline has significant role in plant response system protecting plants from different environmental stresses (water stress, salt stress, high amount of heavy metals in soil or temperature fluctuations), which is probably connected with some anti-oxidative and metal chelatic functions of proline [Özturk i Demir 2002, Hawrylak 2007]. In this study, biostimulators Kelpak, Asahi SL and Goëmar Goteo significantly influenced the proline concentration level in plants (Table 2). There was a statistically significant difference in the concentration of proline in leaves of broccoli [Monaco F1] between the control plants and plants with the addition of biostimulators. The highest concentration of proline was determined in leaves of plants treated with Kelpak (1.284 $\mu mol \cdot g^{-1}$ fm) and Goëmar Goteo (1.001 $\mu mol \cdot g^{-1}$ fm) as compared to control group, in which the concentration of proline was 0.436 $\mu mol \cdot g^{-1}$ fm. In plants treated with Kelpak, the concentration of proline increased to 194.1 per cent (compared to the control plants). After the application of Asahi SL, the concentration of proline increased slightly by 14 per cent (0.499 $\mu mol \cdot g^{-1}$ fm), and the difference was statistically significant. Applied biostimulators caused a change in the concentration of proline in florets of broccoli (Table 2).

Statistically significant increase of proline concentration was observed in plants treated with Goëmar Goteo (by 81 per cent) and Kelpak (by 31.5 per cent) in comparison with control plants. Decrease of proline content was noticed in florets of plant treated with Asahi (0.619 $\mu mol \cdot g^{-1}$ fm) but the difference was not statistically significant as compared to control plants. Borowski and Blamowski [2009] also observed that concentration of free proline in leaves of Ocimum basilicum L. increased after the application of biostimulators as compared to control plants.

Kelpak, Asahi SL, Goëmar Goteo caused an increase of MDA in leaves by 14–18 per cent compared to the reference group (Table 2). The highest concentration of MDA was noticed in plants treated with Kelpak (23.602 $nmol \cdot g^{-1}$ fm).

MDA concentration in florets was 21.183 $nmol \cdot g^{-1}$ fm (Table 3). Biostimulators affected statistically significant increase of this parameter from 1.7 to 38.5 per cent. The highest increase of MDA content, the same as in case of leaves was observed in plants treated with Kelpak (29.355 $nmol \cdot g^{-1}$ ś.m.). Above normal concentration of proline and MDA in plant tissue suggested that plant were affected by stress. The highest increase of Pro and MDA was observed in plants treated with Kelpak i Goëmar, both, in leaves and florets.

Gawrońska and Przybysz [2011] observed that the effectiveness of biostimulators is a sum of many factors, and some of them are out of control and difficult to predict by producers. Whereas biostimulator which caused small changes in the concentration of proline and MDA in leaves and florets was Asahi SL, it signified that under optimal conditions, this preparation changed the metabolism to a slight degree. Przybysz et al. [2010] reported that Asahi SL, applied under optimal conditions, influenced on measured physiological parameters in plants in a small extent. Obtained results proved that Asahi SL had a positively affected plants growing under both optimal and non-optimal conditions.

Table 2. Concentration of Pro [$\mu mol \cdot g^{-1}$ fm] in leaves and florets of broccoli depending on the applied biostimulator

Pro Concentration in Broccoli Leaves			
Control	**Kelpak**	**Asahi SL**	**Goëmar Goteo**
0.436 ± 0.149	1.284 ± 0.077	0.499 ± 0.043	1.001 ± 0.041
$NIR_{0.05} = 0.061$			
Pro Concentration in Broccoli Florets			
Control	**Kelpak**	**Asahi SL**	**Goëmar Goteo**
0.708 ± 0.029	0.932 ± 0.166	0.619 ± 0.281	1.282 ± 0.064
$LSD_{0.05} = 0.123$			

Note: $LSD_{0.05}$ – less significant difference $\alpha < 0.05$; ± SD – standard deviation

Table 3. Concentration of MDA [nmol • g^{-1} fm] in leaves and florets of broccoli depending on the applied biostimulator

MDA Concentration in Broccoli Leaves			
Control	**Kelpak**	**Asahi SL**	**Goëmar Goteo**
20.010 ± 0.322	23.602 ± 0.093	22.796 ± 0.094	23.226 ± 0.023
$LSD_{0.05}$ = 0,115			
MDA Concentration in Broccoli Florets			
Control	**Kelpak**	**Asahi SL**	**Goëmar Goteo**
21.183 ± 0.093	29.355 ± 0.161	21.559 ± 0.610	22.150 ± 0.093
$NIR_{0.05}$ = 0.214			

Note: $LSD_{0.05}$ – less significant difference α < 0.05; ± SD – standard deviation

4. CONCLUSIONS

1. Kelpak i Goëmar Goteo significantly increase proline and malonyldialdehyde concentration, both in leaves and florets of Monaco F1 cultivars.
2. Asahi SL insignificantly increases MDA concentration in leaves and florets of broccoli and decreases proline content in broccoli florets.

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