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CONTENT OF CHLOROPLAST PIGMENTS AND SACCHARIDES IN LEAVES OF POPPY ANEMONE (*ANEMONE CORONARIA* L.) 'SYLPHIDE' AFTER APPLICATION OF BENZYLADENINE AND GIBBERELLIC ACID

ZAWARTOŚĆ BARWNIKÓW CHLOROPLASTOWYCH I SACHARYDÓW W LIŚCIACH ZAWILCA WIEŃCOWATEGO (*ANEMONE CORONARIA* L.) 'SYLPHIDE' PO ZASTOSOWANIU BENZYLOADENINY I KWASU GIBERELINOWEGO

Summary. Tubers of the poppy anemone (*Anemone coronaria* L.) 'Sylphide' before planting were soaked for 24 h in water or gibberellic acid (GA_3) and benzyladenine (BA) at three concentrations: 50, 100 and 150 mg·dm^{-3}. From the 10th to 16th week of culture, leaves were sampled at weekly intervals to prepare weighed portions for determination of the content of saccharides and pigments: chlorophyll (total content) and carotenoids. Gibberellic acid and benzyladenine stimulated the production of chlorophyll and carotenoids as well as the accumulation of saccharides in leaves.

Key words: *Anemone coronaria*, growth regulators, saccharides, chlorophyll, carotenoids

Introduction

It is possible to obtain abundant flowering and high-quality cut flowers of many species of ornamental plants owing to the use of growth regulators from the group of gibberellins and cytokinins.

Gibberellins initiate the setting of flower buds and stimulate the development of flowers in many long-day plants, although the effects of their use differ depending on the species, application method and concentration. To improve flowering, gibberellic acid is used, e.g., in a number of species from the family *Araceae* (HENNY 1981, 1983, 1989, FUNNELL et AL. 1992, REISER and LANGHANS 1992, DENNIS et AL. 1994, HENNY and NORMAN 1999, BROOKING and COHEN 2002). Cytokinins, formerly employed in *in vitro* cultures, have recently started to be applied to ornamental plants growing *in vivo* in order to determine how they affect their growth and development. Both gibberellins and cytokinins are used to extend the postharvest longevity of flowers and 'cut greenery', because they retard ageing processes by preventing changes in the structure of cells and in their chemical composition. Leaves as photosynthesising organs are donors of saccharides indispensable for growth and the transition of plants into the generative stage (BUSH 1999, SHEEN et AL. 1999). Both gibberellins and cytokinins participate in the control of the formation and then growth of leaf blades and flowering.

The aim of the present research was to assess the photosynthetic activity of leaves of *Anemone coronaria* 'Sylphide' whose tubers were treated with gibberellin and cytokinin before planting on the basis of a quantitative analysis of saccharides and chloroplast pigments accumulated in the course of vegetation from the moment of formation of the first leaves.

Material and methods

Tubers of the poppy anemone (*Anemone coronaria* L.) 'Sylphide' were planted on 8 February 2008 to pots 13 cm in diameter into a medium composed of a peat substrate with a pH of 6.2 mixed with fresh crushed pine bark at 3:1 (v:v) and enriched with a slow-release fertiliser Osmocote (3-4M). Before planting the tubers were soaked for 24 h in water or gibberellic acid (GA_3) and benzyladenine (BA) at three concentrations: 50, 100 and 150 $mg \cdot dm^{-3}$. Use was made of Gibrescol 10 MG, a preparation in the form of microgranules, readily soluble in water and containing 10 mg of the pure component. In each combination four replications of 10 plants each were made. One treatment included 40 plants.

The plants were grown in the greenhouse of the Department of Ornamental Plants at a temperature of 18-20°C during the day and 16-18°C at night. On very warm and sunny days the greenhouse was aired vigorously and shaded to prevent the temperature from exceeding 24°C. The plants were fed starting the fifth week of culture. Every 10-14 days solutions of mixed fertilisers, Peters Professional and brown Superba, were applied at a concentration of 0.2%.

From the 10^{th} to 16^{th} week of culture, leaves were sampled at weekly intervals to prepare weighed portions which were then frozen in liquid nitrogen and stored at –20°C until the time of determination of the content of saccharides and pigments: chlorophyll (total content) and carotenoids.

The level of pigments was determined following HISCOX and ISRAELSTAM (1979), after their extraction with dimethyl sulphoxide (DMSO) without tissue maceration. Weighed portions (100 mg) were treated with 5 cm^3 DMSO and incubated in a water

bath at 65°C for 60 min. In the extract obtained, the levels of the pigments were determined spectrophotometrically at a suitable wavelength. For chlorophyll a, the absorbance of the extract was measured at a wavelength of 663 nm, for chlorophyll b, at 645 nm, and for carotenoids, at 470 nm. The content of the pigments was calculated with the help of ARNON'S (1949) formulae and given in milligrames per 1 g of fresh weight.

Total saccharides were determined using the antron reagent (BJÖRNESJÖ 1955). Under the effect of sulphuric acid, saccharides transform into derivatives of furfural, which, together with antron, yield blue-green products. The intensity of the colour is proportional to their content. Weighed portions (0.5 g) were crushed in a mortar with 5 cm^3 of distilled water and the homogenate was centrifuged at 10,000 g for 20 min. 1 cm^3 of the supernatant was added to 2 cm^3 of a cooled antron reagent (0.02% in concentrated H_2SO_4), and then the content of the test tubes were heated, while slowly mixed, on a water bath at 90°C for 14 min. After the tubes were cooled, the absorbance of the solutions was measured in a spectrophotometer at a wavelength of 620 nm. The content of saccharides was read from a standard curve prepared for glucose. The final results, which were means of four replications, were expressed in milligrames of glucose per 1 g of fresh weight.

Statistics. For the results obtained, arithmetic means were calculated for three replications, for which standard deviations were also computed. An analysis of variance was also performed to show statistically significant differences using Tukey's test. The test makes it possible to detect a difference at two levels of significance: $\alpha < 0.05$ (significant differences), and $\alpha < 0.01$ (highly significant differences).

Results

The levels of saccharides in control plants did not vary until the start of flowering. After the flowering ended in the leaves of plants treated with the growth regulators, the saccharides content was found slightly to increase. Their content was the highest after the use of gibberellic acid in treatments in which this growth regulator was applied at concentrations of 50 and 100 $mg \cdot dm^{-3}$. When benzyladenine was applied a similar dependence was observed in leaves collected in the 11^{th}, 14^{th} and 15^{th} week of culture (Fig. 1).

The content of chlorophyll in the leaves of control plants varied little in the course of the experiment. Gibberellic acid and benzyladenine caused growth of the total chlorophyll content in leaves, with the highest increase recorded directly before the start of flowering and towards its end (Fig. 2). Compared carotenoids content in leaves, in control of the changes was small. In treatment involving GA_3 increase was observed. At first carotenoids showed little change, but towards the end of flowering their level was very high after application of the GA_3. After the use of benzyladenine at the concentrations studied, the content of carotenoids was higher than in the control treatment throughout the entire growing season (Fig. 3).

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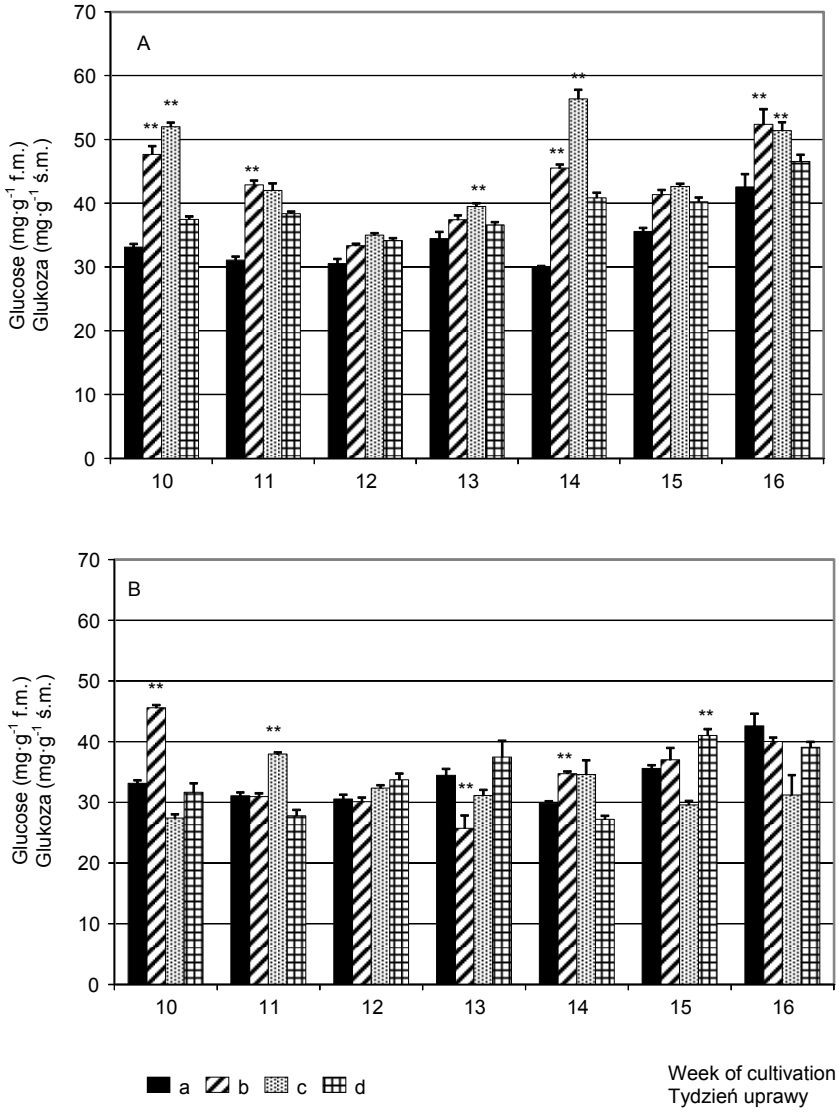

Fig. 1. Content of saccharides in leaves of the poppy anemone 'Sylphide' after the use of gibberellic acid (A) and benzyladenine (B): a – control, b – 50 mg·dm^{-3}, c – 100 mg·dm^{-3}, d – 150 mg·dm^{-3} (*α < 0.05, **α < 0.01)

Rys. 1. Zawartość sacharydów w liściach zawilca wieńcowatego 'Sylphide' po zastosowaniu kwasu giberelinowego (A) i benzyloadeniny (B): a – kontrola, b – 50 mg·dm^{-3}, c – 100 mg·dm^{-3}, d – 150 mg·dm^{-3} (*α < 0,05, **α < 0,01)

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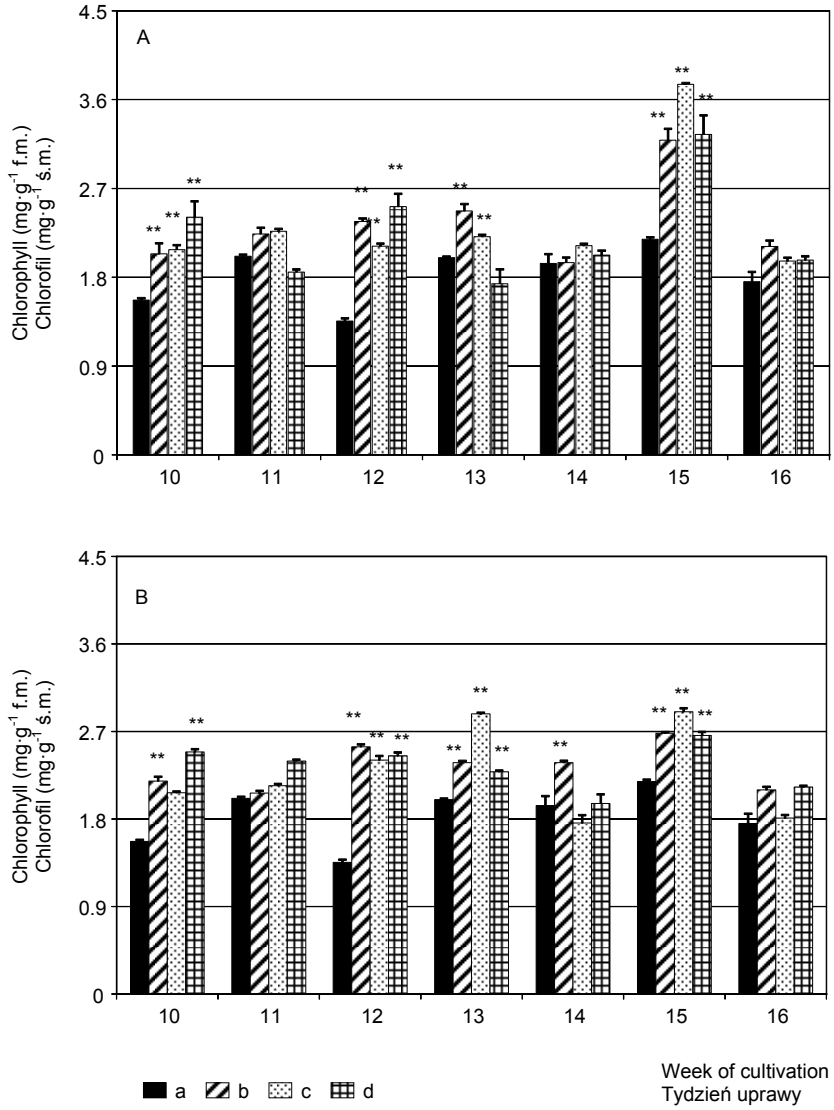

Fig. 2. Total content of chlorophyll in leaves of the poppy anemone 'Sylphide' after the use of gibberellic acid (A) and benzyladenine (B): a – control, b – 50 mg·dm^{-3}, c – 100 mg·dm^{-3}, d – 150 mg·dm^{-3} (*$\alpha < 0.05$, **$\alpha < 0.01$)

Rys. 2. Zawartość całkowita chlorofilu w liściach zawilca wieńcowatego 'Sylphide' po zastosowaniu kwasu giberelinowego (A) i benzyloadeniny (B): a – kontrola, b – 50 mg·dm^{-3}, c – 100 mg·dm^{-3}, d – 150 mg·dm^{-3} (*$\alpha < 0,05$, **$\alpha < 0,01$)

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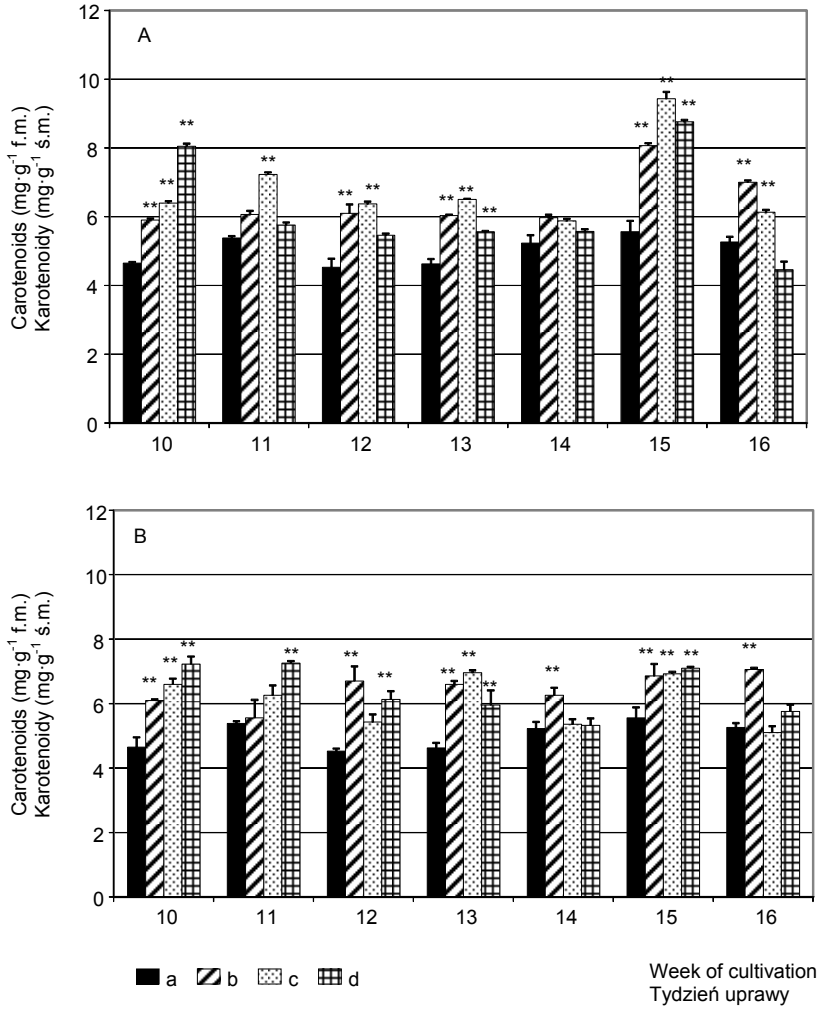

Fig. 3. Content of carotenoids in leaves of the poppy anemone 'Sylphide' after the use of gibberellic acid (A) and benzyladenine (B): a – control, b – 50 mg·dm^{-3}, c – 100 mg·dm^{-3}, d – 150 mg·dm^{-3} (*$\alpha < 0.05$, **$\alpha < 0.01$)

Rys. 3. Zawartość karotenoidów w liściach zawilca wieńcowatego 'Sylphide' po zastosowaniu kwasu giberelinowego (A) i benzyloadeniny (B): a – kontrola, b – 50 mg·dm^{-3}, c – 100 mg·dm^{-3}, d – 150 mg·dm^{-3} (*$\alpha < 0,05$, **$\alpha < 0,01$)

Discussion

In the experiment conducted, the use of gibberellic acid at concentrations of 50-150 mg·dm^{-3} for the soaking of tubers of *A. coronaria* brought about a heightened accumu-

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lation of saccharides in leaves over the entire growing season. Their highest content was noted in plants at full bloom. High saccharide levels were also observed just before flowering and after it had ended. Gibberellic acid in the applied concentrations had a similar effect on the content of saccharides in leaves during the growth of plants. When benzyladenine was applied it was found that, depending on its concentration and the growth stage of the plants, the level of saccharides was usually higher than in the control treatment, but the effect of this growth regulator was much weaker than that of gibberellic acid. There are few reports in the available literature on the effect of the growth regulators on the content of saccharides in leaves of ornamental plants. Changes in saccharide levels have been studied by SKUTNIK et AL. (2004) in cut leaves of *Zantedeschia aethiopica* and *Z. elliottiana*. With advancing senescence their content grew at first, to drop ultimately to 60-80% of the initial figure. Benzyladenine did not inhibit this process, but gibberellic acid turned out to be efficient means of retarding the degradation of saccharides in the leaves of *Z. aethiopica*, while in *Z. elliottiana* it brought about a rise in their content. In the research by KOZŁOWSKA et AL. (2007), gibberellic acid applied for the soaking of rhizomes of calla lilies with colourful spathes stimulated accumulation of saccharides in the leaves of this plant in the period before they started to function as donors of nutrients for flowers.

With the growth of plants, there occur many changes in the chemical composition of cells in their leaves, including chlorophyll degradation. This leads to the yellowing of leaves and the loss of ornamental values of the plants. The treatment of leaves with gibberellins and cytokinins may counteract the ageing of leaves, which finds practical use in efforts to extend the postharvest longevity of florist' green. Research shows that leaves and leafy shoots treated with cytokinin or gibberellin keep green for a longer time because they have more chlorophyll. For example, in *Alstroemeria* leaves have a tendency towards rapid yellowing, which greatly reduces the quality of its cut flowers. Gibberellic acid added to the solution inhibits the degradation of chlorophyll, thus enhancing the ornamental value of the flowers (HICKLENTON 1991). Also in *Lilium* gibberellic acid prevents a premature yellowing of leaves by inhibiting chlorophyll degradation (FUNNELL and HEINS 1998).

In the study reported here, with a rise in the content of chlorophyll in leaves after the use of the growth regulators was a heightened level of carotenoids also noted, which is an advantage because those compounds perform a supporting role in the process of photosynthesis: they absorb some ranges of visible radiation and then pass the excitation energy on to chlorophyll particles. The results obtained for *A. coronaria* seem to suggest that both gibberellins and cytokinins significantly enhance photosynthesis in the plants.

The stimulating effect of growth regulators from the groups of cytokinins and gibberellins on the content of saccharides and chloroplast pigments in the leaves of *A. coronaria* 'Sylphide' suggests a need for a more extensive research to gain a deeper insight into the dependence between the development stage of ornamental plants, the content of the above compounds, and the growth regulators.

Conclusions

1. Gibberellic acid and benzyladenine stimulated production of chlorophyll and carotenoids.
2. GA_3 and BA cause accumulation of saccharides in leaves.

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Streszczenie. Przed sadzeniem bulwy zawilca wieńcowatego moczono przez 24 h w wodzie lub kwasie giberelinowym (GA_3) i w benzyloadeninie (BA) w trzech stężeniach: 50, 100 i 150 $mg \cdot dm^{-3}$. Począwszy od 10. aż do 16. tygodnia uprawy, w odstępach tygodniowych, z roślin pobierano liście, służące do przygotowania naważek do oznaczeń zawartości sacharydów i barwników: chlorofilu (całkowita zawartość) i karotenoidów. Kwas giberelinowy i benzyloadenina stymulowały tworzenie się chlorofilu i karotenoidów oraz gromadzenie cukrów w liściach.

Słowa kluczowe: *Anemone coronaria*, regulatory wzrostu, cukry, chlorofil, karotenoidy

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