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ORIGINAL PAPER

THE FLOWERING AND NUTRITIONAL STATUS OF *GLADIOLUS HYBRIDUS* CV. BLACK VELVET FOLLOWING A CYTOKININ TREATMENT*

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Abstract

The aim of this study was to assess the effect of benzyladenine used for soaking corms on the flowering of *Gladiolus hybridus* cv. Black Velvet, and on the content of micro- and macronutrients in its leaves. Before being planted, corms of 12-14 cm in diameter were soaked for 30 min in water solutions of benzyladenine (BA) at 100, 350 and 600 mg dm^{-3}. The control consisted of water-soaked corms. Following slight desiccation, corms were planted to openwork crates of 14 dm^3 in capacity, filled with peat substrate of pH 6.2 and supplemented with Osmocote Plus mixed fertilizer (3-4 M) at 3 g dm^{-3}. Five corms were planted into each crate. Plants grown in the greenhouse were watered regularly. After 4 weeks of culture, fertilization was started by applying 0.2% Peters Professional (20:20:20) mixed fertilizer containing micro- and macronutrients. Plants were fertilized once a week. Benzyladenine at concentrations within the 100-600 mg dm^{-3} range inhibited the elongation of inflorescence shoots in *Gladiolus hybridus* cv. Black Velvet; the substance only stimulated the elongation of inflorescences. Benzyladenine at 100 mg dm^{-3} in the first year of the study and at 350 and 600 mg dm^{-3} in the second year stimulated the development of flowers in inflorescences. Benzyladenine applied at 100-600 mg dm^{-3} stimulated the calcium uptake by cv. Black Velvet, while having no effect on the uptake of the other macronutrients. BA applied at 600 mg dm^{-3} stimulated the uptake of manganese and zinc, whereas at concentrations ranging from 100 to 600 mg dm^{-3} it stimulated boron uptake, while inhibiting copper uptake.

Keywords: *Gladiolus*, plant development, benzyladenine, micro-, macroelements.

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INTRODUCTION

Gladiolus hybridus is one of the most popular cormous plants grown practically throughout the world. It is planted in gardens and grown for cut flowers both outdoors and in plastic tunnels. Corms are its underground storage organs. The quality of its cultivars is determined by the colour of their flowers, the length and stiffness of their inflorescence shoots, the length of their inflorescences and the number of flowers developing within an inflorescence (HAVALE et al. 2008).

Growth regulators, such as cytokinins and gibberellins, are being applied increasingly often to enhance flowering and improve flower quality (POGROSZEWSKA, SADKOWSKA 2008, JANOWSKA, STANECKI 2012, 2013, JANOWSKA 2013). In ornamental plants, benzyladenine is applied primarily as a growth regulator responsible for the branching of plants propagated *in vitro*. The available references suggest that benzyladenine enhances the formation of flowers in *Zantedeschia aethiopica* (LURIA et al. 2005). In *Campanula persicifolia* cv. Alba, benzyladenine increases the number of 1st-order lateral shoots (POGROSZEWSKA, SADKOWSKA 2008). In turn, foliage-applied benzyladenine increased the yield of inflorescence shoots in Astilbe × arendsii Amethyst plants, (POGROSZEWSKA, SADKOWSKA 2007).

Cytokinins are a major class of plant growth regulators (PADHYE et al. 2008). They play a significant role in cell division and shoot branching, as they interact with auxins in apical dominance (FRANCIS, SORREL 2001). They also participate in chlorophyll biosynthesis (VREUGDENHIL 2004), enhance photosynthetic efficiency, and stimulate the uptake of nutrients, particularly nitrogen (SCHMULLING et al. 1997).

The aim of this study was to assess the effect of benzyladenine used for soaking corms on the flowering of *Gladiolus hybridus* cv. Black Velvet, and on the content of micro- and macronutrients in its leaves.

MATERIAL AND METHODS

The experiments were conducted in 2015 and 2016, at the Department of Ornamental Plants Poznań University of Life Sciences. The flowering and nutritional status of *Gladiolus hybridus* L. cv. Black Velvet plants were analysed following a cytokinin treatment.

Before being planted, corms of 12-14 cm in diameter were soaked for 30 min in water solutions of benzyladenine (BA) at 100, 350 and 600 mg dm^{-3}. The control consisted of water-soaked corms. One treatment (year × benzyladenine concentration) comprised 10 plants (2 replications, 5 plants each).

Following slight desiccation, corms were planted to openwork crates of 14 dm^3 in capacity, filled with peat substrate of pH 6.2 and supplemented with control release fertilizer Osmocote® Plus (3-4 M) at 3 g dm^{-3}. Five corms were planted into each crate. Plants grown in the greenhouse were watered regularly. After 4 weeks of culture, fertilization was started by applying 0.2% Peters Professional® (20 : 20 : 20) mixed fertilizer containing micro- and macronutrients. Plants were fertilized once a week.

Inflorescence shoots were cut above the second leaf after two bottom flowers in the spike had opened. The analyzed parameters included the inflorescence shoot length measured from the culture medium surface, inflorescence length as well as the number of flowers in an inflorescence. Chemical analyses of leaves were conducted to determine the content of macronutrients (nitrogen, phosphorus, potassium, calcium, magnesium) and micronutrients (iron, manganese, zinc, copper, boron).

Leaf tips of 10 cm in length were collected from each treatment as material for chemical analyses. Leaf tips were dried at 45-50°C and subsequently ground. The material was mineralized in concentrated sulphuric acid to determine the total content of nitrogen, phosphorus, potassium, calcium and magnesium. The nutrient content was analyzed using the following methods: total N – the distillation method according to Kjeldahl on a Parnas-Wagner apparatus, P – by colorimetry using ammonium molybdate (after Schillak), and K, Ca, Mg – by atomic absorption spectrometry (AAS). Leaves were mineralized in a mixture of nitric and perchloric acids (3:1, v:v) to assay the total iron, manganese, zinc, boron and copper content (Kamińska et al. 1972). After mineralization, Fe, Mn, Zn, B and Cu levels were determined by the AAS method (on a Carl Zeiss Jena apparatus). The content of K, Na, Ca, Mg, Zn, Mn, Fe and Cu was determined according to standard PN-EN 14084:2004. Samples of plant material were mineralized in 65% super pure HNO_3 using a CEM MARS-5 Xpress microwave oven, and the content of nutrients was determined by atomic absorption spectrometry using a Varian AA 280FS Atomic Absorption Spectrometer (Pasławski, Migaszewski 2006).

Results were analyzed statistically with the help of two-way ANOVA. Means were clustered according to the Duncan test at the significance level $\alpha = 0.05$.

RESULTS AND DISSCUSION

The comparison of inflorescence shoot length in *Gladiolus* cv. Black Velvet showed that both the year of the study and the benzyladenine concentration had a significant effect on this trait (Table 1). In both years, the longest inflorescence shoots were produced by the control plants. The application of benzyladenine at a concentration of 100-600 mg dm^{-3} resulted in significantly shorter inflorescence shoots growing from the treated corms in comparison to those of the control plants. Shortest shoots were reported in the combinations in which BA was applied at 600 mg dm^{-3}.

Table 1

Effect of benzyladenine on the quality of *Gladiolus hybridus* cv. Black Velvet

Concentration of BA (mg dm^{-3})	2015	2016
Length of inflorescence stems (cm)		
0	102.1 *c*	109.6 *e*
100	100.3 *b*	106.0 *d*
350	100.4 *b*	107.3 *d*
600	94.7 *a*	102.0 *c*
Length of inflorescence (cm)		
0	29.2 *b*	26.0 *a*
100	34.4 *c*	30.0 *b*
350	34.2 *c*	31.0 *b*
600	33.6 *c*	31.0 *b*
Number of flowers in inflorescence		
0	11.5 *c*	9.0 *a*
100	12.5 *d*	8.5 *a*
350	11.6 *c*	11.7 *c*
600	10.3 *b*	10.0 *b*

Means followed by the same letter do not differ significantly at $\alpha = 0.05$

The year of the study and benzyladenine concentration had an effect on the length of inflorescences in cv. Black Velvet (Table 1). In both years, significantly the shortest inflorescences were recorded in the control plants, being significantly longer in the first year of the study. The application of BA at a concentration of 100-600 mg dm^{-3} stimulated the elongation of inflorescences in both years, while no significant differences were recorded between the applied concentrations of solution.

Growth regulators may influence quality attributes of flowers, manifested in the length of pedicules and inflorescence shoots as well as the size and mass of flowers, while their effect may be positive or negative. These experiments indicate that benzyladenine in certain species has an inhibitory effect on the elongation of flowering shoots. It also influences flower quality. The formation of shorter pedicules following a benzyloadenine treatment was reported e.g. by Janowska et al. (2009) in *Anemone coronaria* cv. Sylphide, in which additionally the smallest flowers developed in the plants whose corms had been soaked in benzyladenine at 50 mg dm^{-3}. The development of shorter inflorescence shoots as a result of foliar benzyladenine treatment was also observed by Pogroszewska and Sadkowska (2008) in *Campanula persicifolia* cv. Alba grown in an unheated plastic tunnel in both years of this study. In plants grown in an open field, a similar phenomenon was observed only in older plants, i.e. in the second year of the study. In the case of *Hedera* *helix* cv. Brokamp, higher benzyladenine concentrations caused a very distinct shortening of both shoots and leaf blades. The shortest main shoots were produced by plants treated with benzyladenine applied at 5 mg dm^{-3} (Marcinek, Hetman 2006). However, benzyladenine had an adverse effect on the shoot length only in some species. In the study by Pogroszewska (2002), benzyladenine applied at 750 mg dm^{-3} effected the formation of longer peduncles and more showy inflorescence spathes in *Spatyphyllum* cv. Petite. Moreover, Sajjad et al. (2014) reported that *6*-benzylaminopurine has an advantageous effect on the length of inflorescence shoots in *Gladiolus* cv. White Prosperity, while Sajid et al. (2015) reported a similar phenomenon in *Gladiolus grandiflorus*.

The comparison of the number of flowers in an inflorescence in cv. Black Velvet showed that the year of the study and the applied BA concentration had a significant effect on this trait (Table 1). In the first year of this study, significantly the largest number of flowers in an inflorescence was recorded in the treatment in which BA was applied at 100 mg dm^{-3} to soak corms, while the lowest one was in the treatment with BA applied at 600 mg dm^{-3}. In the second year of the study, a stimulating effect of BA on flower development was observed in the treatments where BA was applied at 350 and 600 mg dm^{-3}. In ornamental plant production, benzyladenine is used primarily as a growth regulator responsible for the tillering of plants propagated *in vitro*. Benzyladenine is used much less frequently *in vivo*; however, research results indicate its high effectiveness in axillary shoot formation, which results in a more abundant development of inflorescences and flowers (Luria et al. 2005, Janowska, Stanecki 2012, 2013). Sajjad et al. (2014) reported that BAP stimulates flower development in *Gladiolus* cv. White Prosperity. At a 1 mM concentration, benzylaminopurine resulted in an increased numer of florets per spike (13.49) compared to non-treated plants (10.52 cm). In turn, Luria et al. (2005) showed that benzyladenine applied at 350 mg dm^{-3} had a positive effect on flower formation in *Zantedeschia aethiopica*. More abundant flowering following benzyladenine treatment was also reported by Gianfagna and Merritt (1998) in *Aguilegia vulgaris*. Much more abundant flowering after benzyladenine application was also found in *Doriotaenopsis* (Blanchard, Runkle 2008) and in *Zantedeschia* cultivars with coloured inflorescence spathes (Janowska, Stanecki 2012, 2013, Janowska 2013).

When comparing the macronutrient content in leaves of cv. Black Velvet following the benzyladenine treatment in both years of the study, it was found that this regulator had a significant effect only on the uptake of calcium by plants (Table 2). In both years of the study, a significantly higher content of Ca were recorded in leaves of plants grown from corms soaked in BA applied at 100-600 mg dm^{-3}. No differences were observed between the treatments in which various BA concentrations were applied. Different results were reported by Sajjad et al. (2014). These authors showed that the application of BAP at 100-1000 mg dm^{-3} stimulated the uptake of phosphorus

Table 2

Effect of benzyladenine on the content of macroelements in leaves of *Gladiolus hybridus* cv. Black Velvet (% D.W.)

Concentration of BA (mg dm^{-3})	2015	2016
Nitrogen		
0	2.67 *a*	2.89 *a*
100	2.66 *a*	2.76 *a*
350	2.45 *a*	2.80 *a*
600	2.49 *a*	2.82 *a*
Phosphorus		
0	0.47 *a*	0.49 *a*
100	0.41 *a*	0.50 *a*
350	0.40 *a*	0.47 *a*
600	0.42 *a*	0.43 *a*
Potassium		
0	2.53 *a*	2.66 *a*
100	2.77 *a*	2.76 *a*
350	2.60 *a*	2.65 *a*
600	2.86 *a*	2.53 *a*
Calcium		
0	0.60 *a*	0.67 *a*
100	0.78 *b*	0.80 *b*
350	0.88 *b*	0.80 *b*
600	0.77 *b*	0.82 *b*
Magnesium		
0	0.23 *a*	0.22 *a*
100	0.20 *a*	0.26 *a*
350	0.21 *a*	0.26 *a*
600	0.22 *a*	0.26 *a*

Means followed by the same letter do not differ significantly at $\alpha = 0.05$.

and potassium in *Gladiolus* cv. White Prosperity, while inhibiting the nitrogen uptake. According to Isaychev et al. (2013), growth regulators have a positive effect on the dynamics of nitrogen, phosphorus and potassium transport in plants. These authors showed that *Triticum* seed dressing with the use of growth regulators stimulates the uptake of these macronutrients by plants. The maximum content of nitrogen, phosphorus and potassium were reported by the cited authors in the initial phase of vegetative growth. The content of these macronutrients decreased with the progress in plant growth. The authors explained this phenomenon by the growth regulators stimulating numerous metabolic and physiological processes, which leads to increased yields owing to the improved nutrient uptake.

Both the year of the study and the benzyladenine concentration had an effect on the iron content in leaves of cv. Black Velvet (Table 3). In the study, variation in the content of this nutrient among plants treated with BA was examined. Following the application of BA at 100-600 mg dm^{-3}, the iron content decreased significantly in comparison to that in leaves of the control plants. The lowest iron content was recorded in the treatments in which BA

Table 3

Effect of benzyladenine on the content of microelements in leaves of *Gladiolus hybridus* cv. Black Velvet (mg kg^{-1} of D.W.)

Concentration of BA (mg dm^{-3})	2015	2016
Iron		
0	267.41 *f*	184.62 *d*
100	167.94 *c*	105.20 *a*
350	163.1 *c*	105.81 *a*
600	202.80 *e*	122.72 *b*
Manganese		
0	118.61 *a*	117.53 *a*
100	119.93 *a*	118.70 *a*
350	117.62 *a*	118.91 *a*
600	134.01 *b*	132.81 *b*
Zinc		
0	20.41 *b*	17.63 *a*
100	20.40 *b*	17.62 *a*
350	20.92 *b*	17.71 *a*
600	23.32 *c*	23.50 *c*
Copper		
0	12.66 *c*	11.70 *c*
100	6.61 *b*	4.95 *a*
350	7.61 *b*	4.62 *a*
600	7.00 *b*	4.81 *a*
Boron		
0	65.51 *a*	64.90 *a*
100	88.70 *b*	84.70 *b*
350	96.73 *c*	95.62 *c*
600	117.60 *e*	104.41 *d*

Means followed by the same letter do not differ significantly at $\alpha = 0.05$.

was applied at 100 and 350 mg dm^{-3}. According to Mukesh et al. (2001), foliar application of zinc, copper and iron at 250, 500 and 1000 mg dm^{-3} improves the quality of *Gladiolus* plants. Results of their experiments indicate the need for more intensive iron fertilization in *Gladiolus* when cytokinins are applied in order to improve plant quality. Iron is one of the major nutrients, as it participates in the processes of photosynthesis, respiration, reduction of nitrates and sulphates, as well as in the assimilation of N_2 (Grusak 2001).

The manganese content in leaves of the tested variety was significantly influenced only by the BA concentrations (Table 3). In both years of the study, the highest content of this element was recorded in leaves of plants grown from corms soaked in BA applied at 600 mg dm^{-3}. In the other treatments, the manganese content was comparable. The effeciency of growth regulators is largely dependent on their concentration, which was confirmed in this study. Manganese in plants activates enzymes and is involved in reactions of water splitting and release of oxygen in the process of photosynthesis. Moreover, it plays an important role in the metabolism of proteins, carbohydrates and lipids (Grusak 2001). The advantageous effect of growth regulators (auxin and cytokinin) on manganese uptake was reported by Sosnowski et al. (2014).

The zinc content in leaves of cv. Black Velvet depended significantly on both the year of the study and the BA concentration (Table 3). In the first year of the analyses, the zinc content was significantly greater than in the second year. However, in both years the highest content of this element was recorded in leaves of plants grown from corms soaked in BA applied at 600 mg dm^{-3}. In the other treatments, zinc concentrations were comparable. The results indicate that the uptake of both zinc and manganese was stimulated only in the treatment with benzyladenine applied at the highest concentration. The advantageous effect of growth regulators on the zinc uptake by *Medicago* was reported by Sosnowski et al. (2014). Zinc plays an important role in plants, as it is involved in the metabolism of carbohydrates and proteins (Grusak 2001)

The copper content in leaves of cv. Black Velvet depended significantly on the concentration of benzyladenine (Table 3). The application of benzyladenine inhibited the uptake of this element, while no differences were recorded between BA concentrations. Copper participates in photosynthesis. It is also involved in respiration, cell wall lignification, and in the metabolism of nitrogen compounds, proteins and carbohydrates (Grusak 2001). According to Sosnowski et al. (2014), growth regulators – cytokinin and auxin – have no effect on the copper uptake by *Medicago*.

The boron content in leaves of cv. Black Velvet depended significantly only on the concentration of benzyladenine (Table 3). The application of benzyladenine for corm soaking had an advantageous effect on the content of this element in leaves. The boron content in leaves of cv. Black Velvet in both years of the study increased in proportion to a BA concentration.

It could be reflected in the inflorescence length, as according to Naik et al. (2015), additional boron at 300 mg dm^{-3} in *Gladiolus* cv. American Beauty had a highly beneficial effect on the length of inflorescence shoots, spike length and the number of developing flowers. In plants, boron participates in cell division and tissue differentiation, while it is also an essential component forming cell wall structures (Brown et al. 2002). The positive effect of growth regulators (cytokinin and auxin) on the boron content in *Medicago* was reported by Sosnowski et al. (2014).

CONCLUSIONS

1. Benzyladenine at concentrations within the 100-600 mg dm^{-3} range inhibited the elongation of inflorescence shoots in *Gladiolus hybridus* cv. Black Velvet; it only stimulated the inflorescence elongation.

2. Benzyladenine at 100 mg dm^{-3} in the first year of the study and at 350 and 600 mg dm^{-3} in the second year stimulated the development of flowers in inflorescences.

3. Benzyladenine applied at 100-600 mg dm^{-3} in cv. Black Velvet stimulated calcium uptake, while having no effect on the uptake of the other macronutrients.

4. BA applied at 600 mg dm^{-3} stimulated the uptake of manganese and zinc, whereas at a concentration ranging from 100 to 600 mg dm^{-3} it stimulated the boron uptake and inhibited the copper uptake.

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