

## Flowering and nutritional status of *Gladiolus hybridus* L. 'Black Velvet' following gibberellin treatment

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### Abstract

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Flowering and nutritional status in *Gladiolus hybridus* L. 'Black Velvet' were assessed following gibberellic acid treatment (GA<sub>3</sub>). Treatment were applied to corm (12–14 diameter) by soaking for 30 min in water solutions of GA<sub>3</sub> at 100, 350 and 600 mg/dm<sup>3</sup> with a control consisting of soaking-in water. All GA<sub>3</sub> treatments inhibited elongation of inflorescence shoots and stimulated spike elongation. None of the tested concentrations affected the number of developing flowers in the spike, except for the 100 mg/dm<sup>3</sup> in the 2nd year of the study. All GA<sub>3</sub> treatments stimulated calcium uptake, but had no effect on the uptake of other macronutrients. All the GA<sub>3</sub> treatments increased manganese content in leaves bud did not affect copper content. GA<sub>3</sub> at 600 mg/dm<sup>3</sup> stimulated uptake of iron and boron at 600 mg/dm<sup>3</sup> but inhibited both at lower concentrations. Zinc uptake was stimulated at 100 mg/dm<sup>3</sup> but inhibited at higher concentration.

**Keywords:** gladioli; quality; micro- and macro-elements; gibberellic acid

*Gladiolus* family Iridaceae (COHAT 1993) contains approximately 260 species, 10 found in Eurasia and 250 in Africa (MANNING, GOLDBLATT 2008) and over 10,000 cultivars (SINHA, ROY 2002).

*Gladiolus hybridus* is the most popular species with numerous cultivars grown worldwide for cut flowers in the ground and under cover. In 2008 it ranked 23 among cut flowers and 8<sup>th</sup> among geophytes sold at Dutch flower auctions. Sixty million inflorescences were sold in the United States in 2011, accounting for 4.5% all produced cut flowers. Pakistan produces annually 10–12 tons of cut *Gladiolus*, which is the second most important species after roses grown for cut flowers (SAJJAD et al. 2014).

An important aspect of flower production is to obtain cut flowers of the highest possible quality.

In *Gladiolus* the key attributes include the length and rigidity of the flowering shoot, flower size, the number of flowers in the inflorescence and spike length. Growth regulators such as gibberellins and cytokinins are applied increasingly often to improve flower yield and quality. Gibberellic acid is the most popular gibberellin, while among cytokinins benzyladenine is most commonly used growth regulator. For example, growth regulators from cytokinin and gibberellin groups have significant effect on flowering of *Zantedeschia* with colourful spathes increasing yield of cut flowers (KOZŁOWSKA et al. 2007; JANOWSKA 2013; JANOWSKA, STANECKI 2013; JANOWSKA et al. 2014). According to the research conducted by NGAMAU (2001), an increase in the flower yield is also possible to obtain in

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*Zantedeschia aethiopica* by application of a mixture of BA+GA<sub>3</sub>. POGROSZEWSKA and SADKOWSKA (2008b) report that benzyladenine in *Liatris spicata* 'Alba', foliage-applied at a concentration of 400 mg/dm<sup>3</sup> in plants grown in an unheated plastic tunnel and in the soil, increased the number and fresh weight of inflorescence shoots. Similarly, in *Campanula persicifolia* 'Alba', benzyladenine at the same concentration increases the number of 1<sup>st</sup>-order lateral shoots (POGROSZEWSKA, SADKOWSKA 2008a). In turn, in *Astilbe* × *arendsii* 'Amethyst' plants grown in the soil, foliage-applied benzyladenine at a concentration of 200 mg/dm<sup>3</sup> increased the yield of inflorescence shoots (POGROSZEWSKA and SADKOWSKA 2007).

The aim of this study was to assess flowering and nutritional status of *Gladiolus hybridus* 'Black Velvet' following the application of gibberellic acid in corm soaking - most effective method of using growth regulators in geophytes.

## MATERIAL AND METHODS

Analyses were conducted in 2015 and 2016 at the Department of Ornamental Plants, the Poznan University of Life Sciences. Flowering and the nutritional status in *Gladiolus hybridus* L. 'Black Velvet' were assessed following gibberellic acid treatment.

Cv. 'Black Velvet' used in the study has long shoots and compact inflorescences built out of violet flowers. Its tubers with very good health are reproduced in Poland.

Prior to planting corms of 12–14 cm in diameter were soaked for 30 min in water solutions of gibberellic acid (GA<sub>3</sub>) at 100, 350 and 600 mg/dm<sup>3</sup>. The control comprised corms soaked in water. One treatment (year × gibberellic acid concentration) consisted of 10 plants (2 replications, of 5 plants each).

After slight desiccation corms were planted to openwork crates of 14 dm<sup>3</sup> filled with peat substrate of pH 6.2 supplemented with Osmocote Plus mixed fertilizer (3–4M) at 3 g/dm<sup>3</sup>. Five corms were planted in each crate. Plants grown in the greenhouse were watered regularly. Fertilization was started after 4 weeks of cultivation. Peters Professional (20:20:20) mixed fertilizer containing micro- and macronutrients was applied at concentration 0.2%. Plants were fertilized once a week.

Inflorescence shoots were cut above the second leaf when two bottom flowers in the spike opened.

The recorded parameters included the length of the inflorescence shoot measured from the substrate surface, the length of the inflorescence and the number of flowers in the inflorescence. Contents of macronutrients (nitrogen, phosphorus, potassium, calcium, magnesium) and micronutrients (iron, manganese, zinc, copper, boron) were assayed in leaves.

In each treatment leaf tips of 10 cm in length were collected for chemical analyses. They were dried at a temperature of 45–50°C and then ground. To determine the total contents of nitrogen, phosphorus, potassium, calcium and magnesium, they were mineralised in concentrated sulphuric acid. The nutrient contents were determined using the following methods: total N – the distillation method after Kjeldahl on a Parnas-Wagner apparatus, P – the colorimetric method with ammonium molybdate (after Schillak), and K, Ca, Mg – atomic absorption spectrometry (AAS). To determine total iron, manganese, zinc, boron and copper, the leaves were mineralised in a mixture of nitric and perchloric acids (3:1, v:v) (KAMIŃSKA et al. 1972). After mineralization Fe, Mn, Zn, B and Cu contents were assayed by the AAS method (on a Carl Zeiss Jena apparatus).

Results were analysed statistically using the two-way analysis of variance. Means were clustered using the Duncan test at the significance level  $\alpha = 0.05$ .

## RESULTS

A comparison of inflorescence shoot length in *Gladiolus hybridus* 'Black Velvet' showed that corms soaked in gibberellic acid in both years of the study produced significantly shorter inflorescence shoots (Table 1). In the first year of the study the shortest inflorescence shoots grew from plants, which corms were soaked using GA<sub>3</sub> at 100 and 350 mg/dm<sup>3</sup>. Following the application of gibberellic acid at 600 mg/dm<sup>3</sup> inflorescence shoots were significantly longer, although they were still by 10.3 cm shorter than those of the control plants. In the second year of the study the shortest inflorescence shoots were recorded in the treatment, in which corms were soaked in gibberellic acid at 350 mg/dm<sup>3</sup>.

Gibberellic acid treatment had a significant effect on spike length in cv. 'Black Velvet' in both years of the study (Table 1). Significantly shortest inflorescence shoots in both years of the study were

Table 1. Effect of gibberellic acid on quality of *Gladiolus hybridus* 'Black Velvet'

Concentration of GA <sub>3</sub> (mg/dm <sup>3</sup> )	2015	2016
	Length of inflorescence stems (cm)	
0	108.1 <sup>d</sup>	109.6 <sup>d</sup>
100	92.9 <sup>a</sup>	100.3 <sup>c</sup>
350	91.5 <sup>a</sup>	98.0 <sup>b</sup>
600	97.8 <sup>b</sup>	101.0 <sup>c</sup>
	Length of inflorescence (cm)	
0	26.9 <sup>a</sup>	26.0 <sup>a</sup>
100	29.0 <sup>b</sup>	30.3 <sup>b</sup>
350	30.4 <sup>b</sup>	30.6 <sup>b</sup>
600	33.2 <sup>c</sup>	34.6 <sup>c</sup>
	Number of flower in inflorescence	
0	11.5 <sup>b</sup>	9.0 <sup>a</sup>
100	12.5 <sup>b</sup>	12.0 <sup>b</sup>
350	11.3 <sup>b</sup>	8.7 <sup>a</sup>
600	10.7 <sup>b</sup>	8.7 <sup>a</sup>

means followed by the same letter do not differ significantly at  $p = 0.05$

found in control plants. Gibberellic acid applied at the tested concentrations caused a significant elongation of inflorescences, with the longest inflorescences recorded in plants, which corms were soaked in GA<sub>3</sub> at a dose of 600 mg/dm<sup>3</sup>.

A comparison of the number of flowers developing in the spike showed that only in the second year of the study after gibberellic acid treatment at 100 mg/dm<sup>3</sup> their number was greater than in the other treatments. However, their numbers were comparable in all the treatments in the first year of analyses (Table 1).

When comparing macronutrient contents in leaves of cv. 'Black Velvet' following gibberellic acid treatment it was found that only in the case of calcium this growth regulator had a significant effect on the uptake of this nutrient by plants (Table 2). In both years of the study leaves of plants growing from corms soaked in GA<sub>3</sub> at 100–600 mg/dm<sup>3</sup> contained significantly greater amounts of Ca in comparison to its levels in leaves of control plants, while no differences were found between treatments differing in the applied gibberellic acid concentrations.

A significant effect on iron content in leaves of cultivar 'Black Velvet' was found both for the year of the study and the concentration of gibberellic acid (Table 3). Despite varying levels of iron in the

control leaves in both years of the study an analogous variation was observed in the content of this nutrient. Following the application of gibberellic acid at 100 and 350 mg/dm<sup>3</sup> iron content decreased in comparison to its levels in the control leaves. The lowest levels of iron were recorded in the treatment with GA<sub>3</sub> applied at 350 mg/dm<sup>3</sup>. In turn, corm soaking in a gibberellic acid solution at 600 mg/dm<sup>3</sup> resulted in a significant increase in iron content in leaves.

The concentration of gibberellic acid was the only factor having a significant effect on manganese content in leaves of the tested cultivar (Table 3). Significantly the lowest amounts of manganese were recorded in leaves of control plants. Manganese content in leaves increased significantly fol-

Table 2. Effect of gibberellic acid on content of macrolelements in the leaves of the *Gladiolus hybridus* 'Black Velvet' (% d.w.)

Concentration of GA <sub>3</sub> (mg/dm <sup>3</sup> )	2015	2016
	Nitrogen	
0	2.67 <sup>a</sup>	2.89 <sup>a</sup>
100	2.77 <sup>a</sup>	2.82 <sup>a</sup>
350	2.69 <sup>a</sup>	2.90 <sup>a</sup>
600	2.67 <sup>a</sup>	2.90 <sup>a</sup>
	Phosphorus	
0	0.47 <sup>a</sup>	0.50 <sup>a</sup>
100	0.46 <sup>a</sup>	0.58 <sup>a</sup>
350	0.44 <sup>a</sup>	0.55 <sup>a</sup>
600	0.44 <sup>a</sup>	0.51 <sup>a</sup>
	Potassium	
0	2.53 <sup>a</sup>	2.66 <sup>a</sup>
100	2.73 <sup>a</sup>	2.72 <sup>a</sup>
350	2.59 <sup>a</sup>	2.64 <sup>a</sup>
600	2.55 <sup>a</sup>	2.82 <sup>a</sup>
	Calcium	
0	0.69 <sup>a</sup>	0.67 <sup>a</sup>
100	0.82 <sup>b</sup>	0.81 <sup>b</sup>
350	0.87 <sup>b</sup>	0.80 <sup>b</sup>
600	0.83 <sup>b</sup>	0.85 <sup>b</sup>
	Magnesium	
0	0.23 <sup>a</sup>	0.22 <sup>a</sup>
100	0.24 <sup>a</sup>	0.24 <sup>a</sup>
350	0.22 <sup>a</sup>	0.21 <sup>a</sup>
600	0.25 <sup>a</sup>	0.23 <sup>a</sup>

means followed by the same letter do not differ significantly at  $p = 0.05$

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Table 3. Effect of gibberellic acid on content of microelements in the leaves of the *Gladiolus hybridus* 'Black Velvet' (mg/kg<sup>1</sup> in d.w.).

Concentration of GA <sub>3</sub> (mg/dm <sup>3</sup> )	2015	2016
	Iron	
0	267.40 <sup>f</sup>	184.61 <sup>d</sup>
100	173.20 <sup>c</sup>	142.43 <sup>b</sup>
350	111.41 <sup>a</sup>	110.70 <sup>a</sup>
600	275.33 <sup>g</sup>	219.51 <sup>e</sup>
Manganese		
0	88.61 <sup>a</sup>	87.12 <sup>a</sup>
100	93.80 <sup>b</sup>	93.41 <sup>b</sup>
350	96.32 <sup>c</sup>	94.63 <sup>b</sup>
600	102.01 <sup>d</sup>	95.50 <sup>b</sup>
Zinc		
0	20.43 <sup>e</sup>	17.62 <sup>c</sup>
100	20.41 <sup>e</sup>	17.31 <sup>c</sup>
350	18.72 <sup>d</sup>	12.90 <sup>a</sup>
600	18.62 <sup>d</sup>	14.10 <sup>b</sup>
Copper		
0	6.66 <sup>a</sup>	11.71 <sup>b</sup>
100	6.25 <sup>a</sup>	11.73 <sup>b</sup>
350	6.47 <sup>a</sup>	12.40 <sup>b</sup>
600	6.59 <sup>a</sup>	11.90 <sup>b</sup>
Boron		
0	65.50 <sup>f</sup>	60.90 <sup>e</sup>
100	50.40 <sup>d</sup>	29.60 <sup>b</sup>
350	37.00 <sup>c</sup>	20.70 <sup>a</sup>
600	74.50 <sup>g</sup>	72.20 <sup>g</sup>

means followed by the same letter do not differ significantly at  $p = 0.05$

lowing gibberellic acid treatment using the tested concentrations. In 2015 the higher the concentration of gibberellic acid, the greater the content of Mn detected in leaves. In 2016 no differences were recorded between the applied concentrations of gibberellic acid, with Mn content in leaves 6.3- to 8.4-fold greater than in the control plants.

Zinc content in leaves of cultivar 'Black Velvet' depended significantly on the year of the study and on the concentration of gibberellic acid (Table 3). In the first year of the study significantly highest zinc content was detected in leaves of the control plants and in those from plants which developed from corms soaked in gibberellic acid at a concentration of 100 mg/dm<sup>3</sup>. Significantly lower values of this nutrient in leaves were recorded following the ap-

plication of gibberellic acid at 350 and 600 mg/dm<sup>3</sup>. In the second year of the analyses, although the content of zinc in the control leaves was significantly lower than in the first year a similar dependence was found, since zinc content in leaves of the control and those of plants growing from corms soaked in a gibberellic acid solution at 100 mg/dm<sup>3</sup> was significantly highest. In turn, following the application of gibberellic acid at 350 and 100 mg/dm<sup>3</sup> a significant decrease was observed in the content of this nutrient, with the lowest value recorded in the treatment with gibberellic acid concentration of 350 mg/dm<sup>3</sup>.

Copper content in leaves depended significantly only on the year of the study (Table 3). Irrespective of the gibberellic acid concentration a significantly greater Cu content was recorded in the second year of the study.

Boron content in leaves of *Gladiolus* 'Black Velvet' depended significantly on the year of the study and on the concentration of gibberellic acid (Table 3). The application of gibberellic acid at 100 and 350 mg/dm<sup>3</sup> in both years of the study significantly reduced contents of this nutrient in leaves in comparison to the control treatment. Despite significant differences in boron content in leaves of the control plants in both years of the study, this dependence was similar: significantly lowest boron contents were recorded in the treatments with gibberellic acid treatment at 350 mg/dm<sup>3</sup>, while they were significantly greater, although lower than in the control, with the gibberellic acid concentration of 100 mg/dm<sup>3</sup>. Significantly greatest boron concentrations in both years of the study were found in the treatments, in which gibberellic acid was applied at 600 mg/dm<sup>3</sup>.

## DISCUSSION

In this study corm soaking in gibberellic acid applied at 100-600 mg/dm<sup>3</sup> in *Gladiolus hybridus* 'Black Velvet' inhibited the elongation of inflorescence shoots, while it stimulated inflorescence elongation. Although gibberellic acid is a growth regulator responsible first of all for elongation growth, its effect is to a considerable extent dependent on the species, cultivar, concentration and method of application, as it was shown in this study. In the case of ornamental plants grown *in vivo* the effect of GA<sub>3</sub> may be positive or negative. SAJID et

al. (2015) reported that spraying of *Gladiolus grandiflorus* leaves with gibberellic acid at a concentration of 25, 50 and 100 mg/dm<sup>3</sup> stimulates growth of inflorescence shoots. The longest inflorescence shoots were found in the treatment with gibberellic acid applied at 100 mg/dm<sup>3</sup>. Moreover, gibberellic acid at a concentration of 50 and 100 mg/dm<sup>3</sup> stimulated spike elongation and flower development. Shoot and inflorescence elongation following gibberellic acid treatment in *Gladiolus* 'H.B.Pitt' was observed by SABLE et al. (2015). These authors also reported that in that cultivar gibberellic acid applied at a concentration of 100–200 mg/dm<sup>3</sup> stimulated flower development, while its concentration of 200 mg/dm<sup>3</sup> caused considerable elongation of inflorescences. The advantageous effect of gibberellic acid on the length of shoots and inflorescences and on flower development in *Gladiolus* 'White Prosperity' was reported by SAJJAD et al. (2014).

In this study gibberellic acid in cultivar 'Black Velvet' stimulated calcium uptake, while it had no effect on the uptake of other macronutrients. Different results were obtained by SAJJAD et al. (2014). Those authors showed that in *Gladiolus* 'White Prosperity' gibberellic acid applied at a concentration of 100–1,000 mg/dm<sup>3</sup> stimulates uptake of phosphorus and potassium, while it inhibits uptake of nitrogen at the simultaneous highly advantageous effect on contents of chlorophyll, carotenoids and saccharides. In the opinion of many authors growth regulators stimulate nutrient uptake and participate in their distribution and accumulation (MEUWLY, PILET 1991; SVENSON 1992; ALI et al. 2008). This is partly confirmed by a study by NOWAK and WRÓBEL (2015). These authors showed an increase in the contents of calcium, potassium and magnesium and a decrease in nitrate nitrogen content in seeds of *Glycine max* following the application of indole-3-butyric acid, 6-benzylaminopurine as well as a mixture of these growth regulators. Moreover, BAP has an advantageous effect on contents of Ca and Mg. In the opinion of SOSNOWSKI et al. (2014), auxins and cytokinins contained in the commercial preparation Kelpak SL stimulate uptake of phosphorus and potassium by *Medicago* plants.

Micronutrients play a key role in metabolic and physiological processes of plants. It also needs to be stressed that they affect to a greater extent yield quality (KHOSA et al. 2011) rather than its volume (LAHIJE 2012). Micronutrients are contained in proteins and they serve as catalysts (LAHIJE 2012). This study

showed that micronutrient uptake was influenced by gibberellic acid, while its efficacy was dependent on the applied concentration. It was confirmed that gibberellic acid at the tested concentrations caused increased contents of manganese in leaves of *Gladiolus* 'Black Velvet', at the same time having no effect on copper content. Manganese in plants activates decarboxylases, dehydrogenases as well as other enzymes. It also participates in reactions of water decomposition and oxygen release in the process of photosynthesis, in the synthesis of chlorophyll as well as metabolism of proteins, carbohydrates and lipids (GRUSAK 2001). In turn, copper found in chlorophylls participates in photosynthesis, respiration, cell wall lignifications, as well as metabolism of nitrogen compounds, proteins and carbohydrates (GRUSAK 2001). Results of these studies are confirmed by a study of SOSNOWSKI et al. (2014). These authors observed a similar dependence in *Medicago*, except for the fact that in their experiments they applied auxins and cytokinins contained in Kelpak SL.

This study showed that gibberellic acid at a concentration of 600 mg/dm<sup>3</sup> stimulated uptake of iron and boron, while at a concentration of 100 and 350 mg/dm<sup>3</sup> it inhibited them and at 100 mg/dm<sup>3</sup> it stimulated zinc uptake, whereas at 350–600 mg/dm<sup>3</sup> it inhibited it. MUKESH et al. (2001) reported that foliar application of zinc, copper and iron at a concentration of 250, 500 and 1000 mg/dm<sup>3</sup> improves quality of *Gladiolus*. In turn, SOSNOWSKI et al. (2014) reported that in *Medicago* plants spraying of growth regulators has an advantageous effect on zinc content. According to KOCHIAN (1991), the capacity of plants to obtain optimal nutrient levels is dependent on nutrient contents in the substrate and the presence of a respective transport protein. GRUSAK (2001) indicated an important role of biotic factors in the intensity of nutrient uptake.

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