

Effect of BA and GA₃ on the Morphological Features of Stomata in the Leaf Epidermis of the *Zantedeschia albomaculata* cv. 'Albomaculata'

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Abstract

The study focused on the effect of benzyladenine at concentrations of 100, 350 and 600 mg·dm⁻³ combined with gibberellic acid at the same concentrations on the morphological features (length and width) and number of stomata in the lower and upper leaf epidermis in *Zantedeschia albomaculata* cv. 'Albomaculata'. To determine changes in the structure of stomata, rhizomes were soaked before planting in mixtures of the growth regulators at the above concentrations for 30 minutes. After the application of BA and GA₃ at concentrations of 100+100 mg·dm⁻³ and 350+350 mg·dm⁻³ the stomata in the upper leaf epidermis were larger than in the control treatment and fewer in number. In the lower epidermis a mixture of BA and GA₃ at concentrations of 100+100, 350+350 and 600+600 mg·dm⁻³ caused the formation of bigger stomata, and their number declined when the concentration employed was 350+350 mg·dm⁻³.

Keywords: *Zantedeschia albomaculata*; BA; GA₃; stomata; leaves

Introduction

Species of the genus *Zantedeschia* belong to the order Arales and the family Araceae (Erhardt *et al.*, 2000) embracing 2,823 species (Govaerts *et al.*, 2002). They have a characteristic inflorescence - a spadix, composed of a thick, fleshy main axis on which flowers are borne. They are usually dioecious, with the perianth reduced or none, two whorls of stamens (2+2 or 3+3), and an upper pistil, usually built of three carpels. The spadix sits in a leaf sheath corresponding to the inflorescence spathes of palms. With advancing specialisation, the sheath has taken the form of a protective casing of the spadix, often brightly coloured, and with time in many taxa has become part of a characteristic inflorescence which traps insects until the flowers have been pollinated. In calla lilies the spathes are 12 to 25 cm long and white, yellow and pink to dark purple in colour; they surround spadices coloured yellow, golden-yellow and creamy-yellow. In morphological terms, all species are similar. Petiole bases closely adhere to one another, forming 'an apparent stem'. Successive leaves and peduncles grow from their midst. Within the genus, leaf blades are lanceolate in shape, narrowly to broadly sagittate or cordate. Medium- to dark-green in colour, they are covered or not in transparent, white spots. The stomatal apparatus types of Araceae vary from anomocytic, actinocytic to paracytic, cyclocytic and transitional type between paracytic and cyclocytic; the numbers of subsidiary cells from zero to twelve. Epidermal cells

are nearly isodiametric in outline with straight, arched and undulate anticlinal walls. Striate ornamentation occurs on periclinal walls of epidermal cells in some species. Although the stomatal apparatus types in Araceae are of little taxonomical significance at infra-family level of Araceae, the combined characters of stomatal apparatuses, the shape of anticlinal wall and ornamentation of cuticles in guard cells may be useful for species identification (Wang and Zhao, 2002).

The calla lily is a native of the Congo River delta and seasonally inundated or swampy areas in South Africa. Funnell (1993) mentions six species and two subspecies of the genus *Zantedeschia*, Kuehny (2000) describes seven species and two subspecies. However, only four have been found useful in floricultural production: *Zantedeschia aethiopica* (L.) Spreng., *Z. elliottiana* Engl. W. Wats., *Z. rehmanii* Engl., and *Z. albomaculata* (Kuehny, 2000).

Initially, in cultivation the dominant species was the common florist's calla (*Z. aethiopica* L. Spreng.). Today, however, its significance has declined, while cultivars with colourful spathes have grown in importance. Flowering in the calla lily depends on the cultivar, the size of rhizomes, and the duration of their storage (Corr and Widmer, 1991, Funnell *et al.*, 1992). The research conducted in the world focuses on how to improve flowering with the use of growth regulators. For the calla lily, those shown to be effective are gibberellins, in particular gibberellic acid. The application of mixtures of growth regulators in ornamental plants is only rarely

employed as a method to improve their flowering. The study of the mixture of benzyladenine and gibberellic acid in the calla lily is based on the assumption that the effects of the growth regulators could add up or reinforce each other. The aim of the research was to determine changes occurring in the structure and number of stomata in the leaf epidermis of the cultivar 'Albomaculata' under the influence of benzyladenine and gibberellic acid.

Material and methods

The research was conducted in the years 2009-2010. The cultivar employed was 'Albomaculata' deriving from the spotted calla (*Zantedeschia albomaculata* Hook. Baill.).

The study focused on the effect of benzyladenine at concentrations of 100, 350 and 600 mg·dm⁻³ combined with gibberellic acid at the same concentrations on the morphological features (length and width) and number of stomata in the lower and upper leaf epidermis. To determine changes in the structure of stomata, rhizomes were soaked before planting in mixtures of the growth regulators at the above concentrations for 30 minutes. They were then planted into 20-cm pots in a medium consisting of peat with a pH of 6.2, enriched with a slow-release fertiliser Osmocote Plus (3-4M) in the amount of 3 g·dm⁻³ and mixed with fresh, shredded pine bark at a rate of 3:1 (v:v). Grown in a plastic tunnel, the plants were fed starting with the fifth week of cultivation. Every 10-14 days, solutions of mixed fertilisers, Peters Professional and brown Superba, were applied at a concentration of 0.2%. At the start of vegetation, when the leaves were fully developed, lime saltpetre was foliar applied once at a concentration of 0.2%.

In both years, ten leaves were selected from each rhizome treated with solutions of the growth regulator mixture at the various concentrations. Tested characteristics determined on 10 samples of each treatment. Leaf segment of an area of 0.5 cm square from each specimen was cut. The upper and lower surface were separated with dissecting needle and rinsed with clean water. The number of stomata per 1 mm² of the upper and lower leaf epidermis was determined as well as their length and width. Those observations and measurements were carried out in a light microscope under 400x

magnification. The measurements were conducted using the micrometric method with the help of an eyepiece scale. The density of stomata was calculated using a calibrated eyepiece graticule under 100x magnification (LM). The results were processed with the help of analysis of variance. The means were grouped using Duncan's test at the $\alpha=0.05$ significance level.

Results

The application of BA+GA₃ at concentrations of 100+100 mg·dm⁻³ and 350+350 mg·dm⁻³ changed both the length and the width of stomata in the upper leaf epidermis of the cultivar 'Albomaculata'. They were significantly bigger than in the control treatment (Tab. 1, Fig. 1 A-D). It was found that the bigger the stomata grew, the smaller their number was. At a concentration of 600+600 mg·dm⁻³, BA+GA₃ did not affect the size of the stomata, but they influenced their density: there were more of them per 1 mm² than in the control treatment (Tab. 1, Fig. 2 E-H).

BA+GA₃ in the concentration variants examined had a significant effect on the length of stomata in the lower leaf epidermis of the cultivar 'Albomaculata'. The longest stomata were observed to appear in the treatments with BA+GA₃ at concentrations of 100+100 mg·dm⁻³ and 350+350 mg·dm⁻³. When the growth regulators were used at a concentration of 600+600 mg·dm⁻³, the stomata were a bit shorter, but still longer than in the control. Their width also changed. BA+GA₃ at those concentrations had a similar effect on the formation of wider stomata (Tab. 1, Fig. 3 I-K). As to the number of stomata in the lower leaf epidermis, the results show that the growth regulators had no significant effect here, with the exception of the variant of BA+GA₃ at a concentration of 350+350 mg·dm⁻³, which caused a decline in their number (Tab. 1, Fig. 4 M-P).

Discussion

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 and Stanecki, 2013; Ranwala and Miller, 1998; Rodriguez-Perez et al., 2009). Regulators have also effect on post-harvest longevity

Tab. 1. Characteristic of stomata in upper and lower epidermis in leaves of *Zantedeschia* 'Albomaculata' after soaking in mixture of benzyladenine and gibberellic acid

Concentration of BA+GA ₃ (mg·dm ⁻³)	Length (μm)		Width (μm)		Number of stomata on 1 mm ²	
	2009	2010	2009	2010	2009	2010
Upper epidermis						
0	42.3 a	41.9 a	27.5 b	27.8 b	277.5 b	270.0 b
100+100	44.1 c	43.7 b	28.5 c	28.7 c	222.0 a	218.5 a
350+350	45.7 d	44.3 b	28.9 c	28.3 c	213.0 a	220.0 a
600+600	43.4 b	42.0 a	27.8 b	27.4 b	290.5 c	287.0 c
Lower epidermis						
0	42.1 a	41.9 a	26.7 a	27.0 a	305.0 b	300.5 b
100+100	44.6 c	45.0 c	29.0 b	27.9 b	310.5 b	299.0 b
350+350	45.2 c	49.9 c	29.3 b	28.5 b	210.0 a	215.0a
600+600	43.2 b	42.9 b	28.7 b	28.1 b	314.0 b	303.0 b

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

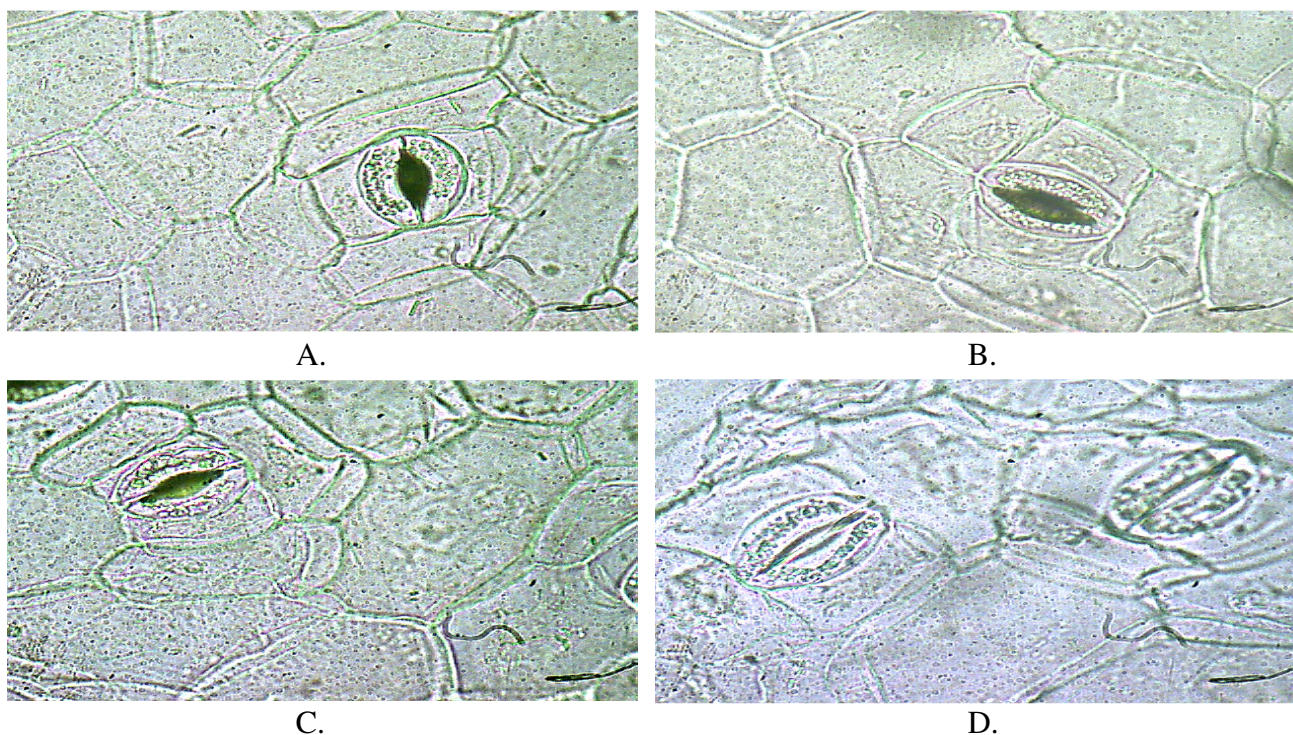


Fig. 1. Stomata in upper epidermis in leaves of *Zantedeschia*: A. Control plants
 B. Plants treated with mixture of BA+GA₃ at concentration of 100+100 mg·dm⁻³
 C. Plants treated with mixture of BA+GA₃ at concentration of 350+350 mg·dm⁻³
 D. Plants treated with mixture of BA+GA₃ at concentration of 600+600 mg·dm⁻³

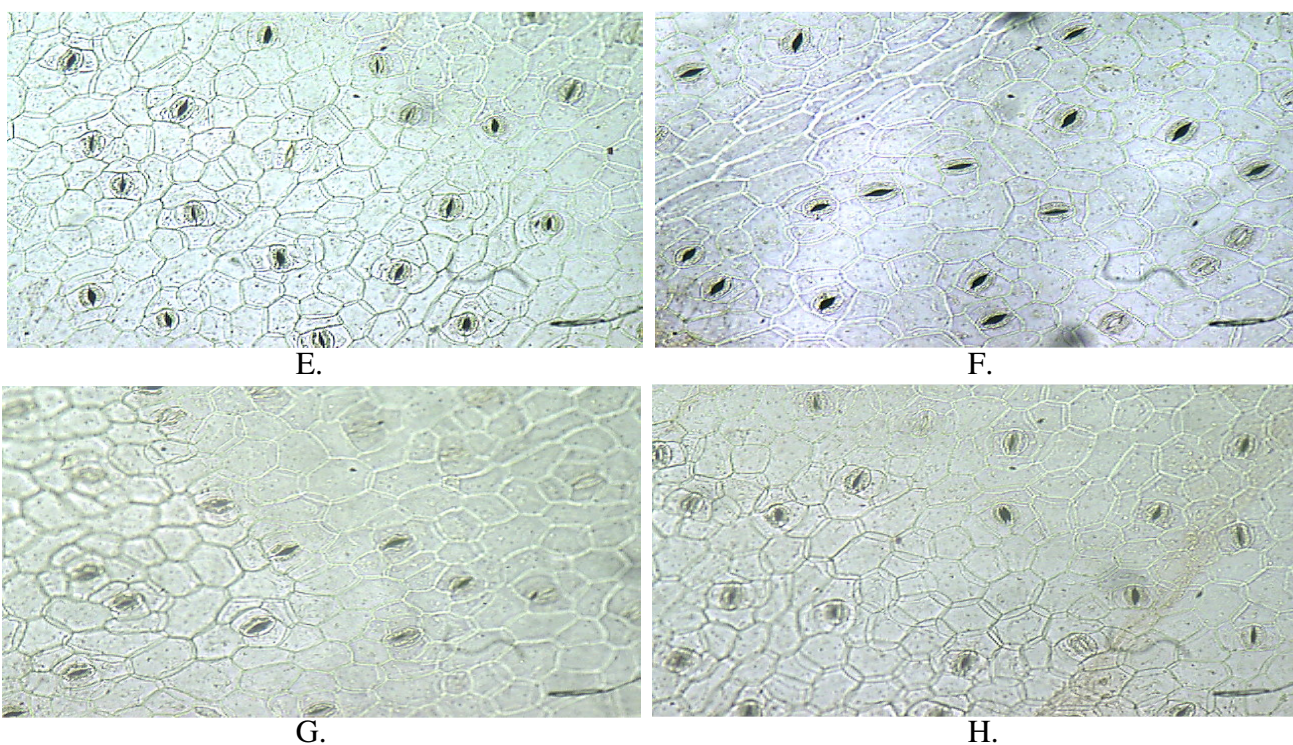


Fig. 2. Number of stomata in upper epidermis in leaves of *Zantedeschia*: E. Control plants
 F. Plants treated with mixture of BA+GA₃ at concentration of 100+100 mg·dm⁻³
 G. Plants treated with mixture of BA+GA₃ at concentration of 350+350 mg·dm⁻³
 H. Plants treated with mixture of BA+GA₃ at concentration of 600+600 mg·dm⁻³

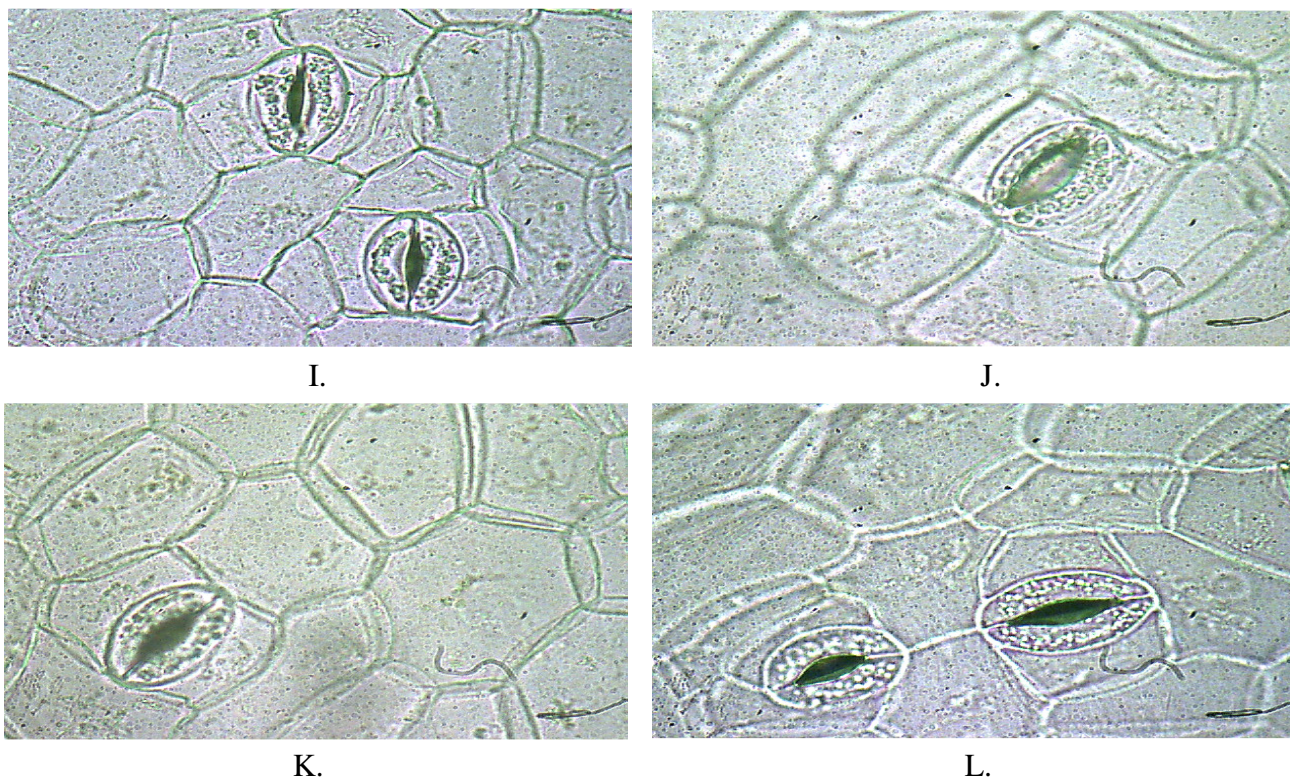


Fig. 3. Stomata in lower epidermis in leaves of *Zantedeschia*: I. Control plants
 J. Plants treated with mixture of BA+GA₃ at concentration of 100+100 mg·dm⁻³
 K. Plants treated with mixture of BA+GA₃ at concentration of 350+350 mg·dm⁻³
 L. Plants treated with mixture of BA+GA₃ at concentration of 600+600 mg·dm⁻³

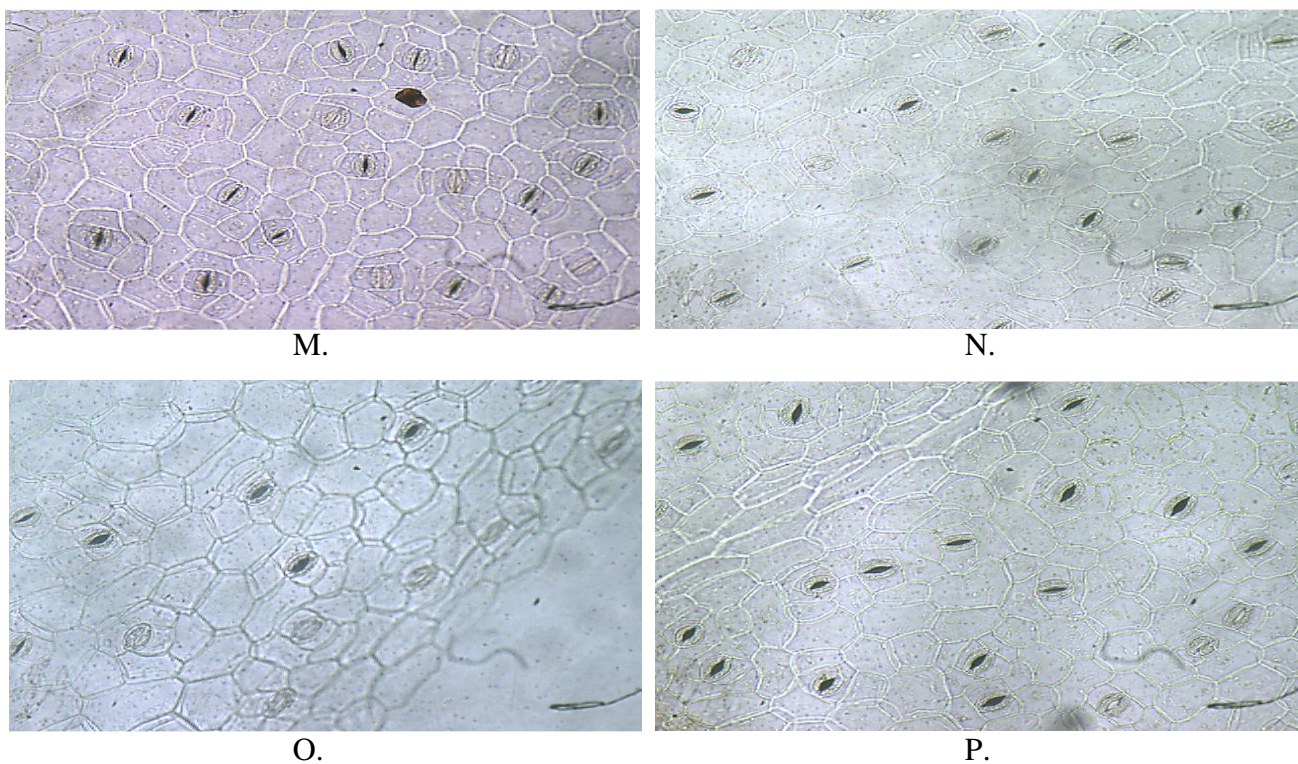


Fig. 4. Number of stoma in lower epidermis in leaves of *Zantedeschia*: M. Control plants
 N. Plants treated with mixture of BA+GA₃ at concentration of 100+100 mg·dm⁻³
 O. Plants treated with mixture of BA+GA₃ at concentration of 350+350 mg·dm⁻³
 P. Plants treated with mixture of BA+GA₃ at concentration of 600+600 mg·dm⁻³

of flowers and leaves of *Zantedeschia* cultivars (Janowska and Stanecka, 2011; Wachowicz et al., 2006).

This experiment determined the effect of gibberellic acid and benzyladenine on size and number of stomata in epidermis of 'Albomaculata' leaves.

As the results show, in the cultivar 'Albomaculata' after the application of a mixture of BA+GA₃ at concentrations of 100+100 and 350+350 mg·dm⁻³, stomata in the upper leaf epidermis were bigger than in the control treatment, while their number declined. In the lower epidermis a mixture of benzyladenine and gibberellic acid at those concentrations also led to the formation of bigger stomata, but their number dropped only when the concentration employed was 350+350 mg·dm⁻³. Gupta (1992) found less stomata in epidermis of *Vicia faba* grown from seeds soaked in solution of gibberellic acid but the stomata were bigger than stomata in control plants. The effect of gibberellic acid on the morphology of stomata was studied by Pogroszewska (2002). The author did not show GA₃ to have an effect on the length of stomata in the organs examined or on their width in the lower epidermis of the topmost leaf. In turn, in the lower epidermis of the spathe GA₃ made stomatal pores significantly narrower than in the control. The application of the acid did not bring about any significant change in the density of stomata in the lower leaf epidermis, but it did affect their number per 1 mm² in the lower bract epidermis. Gibberellic acid produced a decline in the number of stomata in the spathe epidermis. The author also assessed the aperture in stomata of the lower spathe epidermis. She observed them to be opened more widely in plants treated with GA₃ at a concentration of 1000 mg·dm⁻³ than in the control. Besides, in the bract of those plants there developed morphologically transformed stomata with four or three guard cells. She also noted asymmetrically built stomata and a narrowing of guard cells in the upper bract epidermis. In the present research no anomalies were observed in the structure of stomata. Carey (2008) suggests that the stomatal aperture is stimulated by cytokinins. These growth regulators induce stomata opening, the effects are species specific and correlated with cytokinin concentration and method of application (Pospíšilová, 2003). Wachowicz et al. (2006) reported that GA₃ in *Zantedeschia* and BA in *Hosta* enhanced stomata opening by 21% and 44%, respectively. In addition, different cytokinins could effect stoma and cause abnormal stomata development in tissue culture studies (Namli and Ayaz, 2007).

Conclusion

After the application of BA and GA₃ at concentrations of 100+100 ppm and 350+350 mg·dm⁻³ the stomata in the upper leaf epidermis were larger than in the control treatment and fewer in number. In the lower epidermis a mixture of BA and GA₃ at concentrations of 100+100, 350+350 and 600+600 mg·dm⁻³ caused the formation of bigger stomata, and their number declined when the concentration employed was 350+350 mg·dm⁻³.

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