

Full Length Research

Effects of some external treated plant growth regulators on stomatal aperture of cucumber (*Cucumis sativus* L.)

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In this study, cucumber (*Cucumis sativus* L.) seedlings grown singly in standard pots contain compost were exposed to two different concentrations (10^{-4} and 10^{-6} M) of BA (6-benzyladenine), NAA (α -naphthaleneacetic acid) and GA₃ (Gibberellic acid). Plant growth regulators were used as spraying (2 ml) on the lower surfaces of leaves for seven days. Beith alpha and Çengelköy, which are two elite and widely planting cucumber varieties in Turkey were used and the results were comparatively studied. Stomatal diameters from the epidermal sections of abaxial leaves in 1, 3, 5 and 7th days were examined by image processing and analysis software program. The data proved that, stomatal aperture were affected significantly with BA, NAA and GA₃ treatments, but responded variously in all treated plants compared to measurements of the control groups.

Key words: Cucumber, stomata, benzyl adenine, naphthalene acetic acid, gibberellic acid.

INTRODUCTION

Stomata are small pores found in epidermis of the leaves, which are open or close under the control of a pair of kidney-shaped cells called guard cells and stomata occupy a central position in the pathway for the transport of water vapor, CO₂ and O₂ (Pospíšilová, 2003a; Taiz and Zeiger, 2006). The regulation of stomatal conductance (g_s) is the main mechanism, by which plants control gas exchange and leaf temperature (Salleo et al., 2000). It is clearly known that, abscisic acid (ABA) synthesized in the roots under water stress and transported to the leaves together with ABA synthesized in the leaves, may act as a root-to-shoot chemical signal of water stress conditions to induce stomatal closure (Jia et al., 2001). However, other compounds may also play a role in the chemical signaling of stress conditions. Through the important regulatory role, which is played by cytokinins and auxins in modulating plant growth and development, it seems possible to expect their involvement in response of plants to adverse environmental conditions (Itai, 1999; Pospíšilová, 2003a).

Cytokinins (CKs) play roles in promoting cell division, acting both in synergy and in antagonism with other plant hormones; influence a wide range of events during plant growth and development (Pospíšilová, 2003a). They are

also responsible for accumulation of chlorophyll and conversion of etioplasts into chloroplasts and delay leaf senescence (Brault and Maldiney, 1999). In general, high concentrations of BA inhibit g_s and transpiration rate, while low concentrations stimulate them. CKs are often considered as ABA antagonists and they delay leaf senescence, reverse leaf and fruit abscission induced by ABA or water stress and release seed dormancy (Thimann, 1992; Pospíšilová, 2003a). CKs may affect stomatal behavior on a short-term basis, while ABA content reflects long-term water deficit (Fusseder et al., 1992). Although CKs induce stomatal opening, the effects are species specific and correlated with CK type, concentration and method of application (Pospíšilová, 2003b). In addition, different CKs could effect stoma and cause abnormal stomata development in tissue culture studies (Namli and Ayaz, 2007).

Auxins have wide range of effects on many processes such as cell division, cell enlargement, vascular tissue differentiation, root initiation, flowering, fruit setting and gravitropism (MacDonald, 1997). Before 80s it was believed that several of the synthetic auxins such as 2, 4-dichlorophenoxyacetic acid (2, 4-D) and naphthalene-1-acetic acid (NAA) inhibit stomatal opening, but the natural auxin indol-3-ylacetic acid (IAA) was believed to have no effects (Davies and Mansfield, 1987). Nowadays, it is believed that the type of auxin (IAA or NAA), which applied and its concentration have effects on stomatal conduc-

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tance (Assmann and Armstrong, 1999). In addition, it was indicated that synthetic auxins, which affect stomata, appear to function in an opposite manner to IAA (Davies and Mansfield, 1987). It could be suggested that the mode of action of IAA on guard cells is very specific to this molecule and is inhibited even by close analogues that can mimic the action of IAA elsewhere in the plant (Thomson et al., 1988).

Gibberellins (GAs) play roles on seed germination, endosperm mobilization, stem elongation, leaf expansion, flower and fruit set and their composition and contents are usually related with plant growth and development (Shah, 2007). In relation to environmental effects, such as irradiance and low temperature, Hedden (1999) reviewed induced changes in GAs metabolism, but little is known about changes induced by water stress. In addition, GA₃ also has reversal effects of applied ABA and water stress induced stomatal closure for a few plant species (Roper and Williams, 1989). Application of GAs to many different plant species shows different responses. While g_s of some plants were increased or decreased, depending on the type of the plant and the amount of GAs used, stomatal openings affected in three ways, opening, closing and non-responsive stopping (Pospíšilová, 2003a).

In this study, 2 months old cucumber (Beith alpha and Çengelköy genotypes) seedling's leaves were used as material and exposed to two different concentrations (10⁻⁴ and 10⁻⁶ M) of BA, NAA and GA₃ as spraying on the lower surfaces of the leaves (abaxial) every day. Stomatal diameters were measured in 2-day intervals and the data proved that, stomatal aperture affected significantly with BA, GA₃ and NAA treatments and showed different responses against the application number and accumulation in leaf tissues.

MATERIALS AND METHODS

Before germination, cucumber seeds were kept in running tap water for 1 h and then transferred into small petri dishes, which contained two sterilized filter papers in top and bottom sides. During one-week germination period, the seeds that were placed between filter papers were watered with Hoagland Solution (Hoagland and Arnon, 1950) and then they transferred into standard plastic pots containing sterilized compost (162.5 g) and maintained under growth room conditions. The plants were grown under fluorescent tubes giving an irradiance of 5000 lx and a (day/night - 16/8 respectively), temperature of 23 ± 2°C and relative humidity 45 - 50%. Each of the experimental groups of 8 replicates were watered by Hoagland's nutrient solution (40 ml) in two-day intervals for two months in which the hormone treatments were applied. 10⁻⁴ and 10⁻⁶ M of BA, NAA and GA₃ solutions were prepared and sprayed (2 ml) on the abaxial leaves everyday at 10 o'clock in the morning and at 12, cuttings were taken from epidermis tissues. The routine procedure was performed for 7 days and in days 1-3-5 and 7 cuttings were taken from abaxial leaves. In day 1 the first, in day 3 the second, in day 5 the third and in day 7 the forth leaves were used and 20 stomata's pore diameters were measured from 5 different areas. The preparations were photographed with an Evolution LC Color Camera and an Olympus BH-2 Microscope. The images were analyzed with

Image-Pro Express version 6.0 scientific image processing and analysis software. Eight different plants used for each treatment and the mean values and standard errors were calculated.

RESULTS AND DISCUSSION

In this study, Çengelköy and Beith alpha varieties, which are two elite and widely planting cucumbers in Turkey, were used and the measurements of stomatal diameters were comparatively studied. The diameters of stomata from the lower surfaces of leaves were measured by using Image-Pro Express version 6.0 scientific image processing and analysis software. The mean values were measured as 1.71 µm for Çengelköy and 1.64 µm for Beith alpha (Figures 1 - 2).

After the first BA (10⁻⁴ and 10⁻⁶ M) treatments in day one, the diameter values were rapidly increased and peaked. After second treatments, the values were decreased but they were still higher than control values. The reduction continued in day 3 and at the end of the last treatment (day 7), the levels were lower. By the way, while the stomata diameter values of 10⁻⁶ M BA treatments were closer to control groups' values, 10⁻⁴ treatment's values were a little bit less than stomata diameter values of controls at the end of the study (Figure 2, A-B). In a similar study, Rulcová and Pospíšilová (2001) applied 1 and 10 µM BA into the substrate (sand and nutrient solution) or sprayed (like this study) on leaves of control and water stressed *Phaseolus vulgaris* L. They observed an increase of stomatal conduction after 1 µM BA application while decrease after 10 µM BA application. They also observed slight positive effects (delayed leaf senescence or improvement of recovery during rehydration) of 1 µM BA on parameters of water relation and photosynthesis of primary bean leaves and negative effects of 10 µM BA and they related the effects of BA with concentration. In another study, Wachowicz et al. (2006) studied the effect of BA on stomatal aperture in senescing cut leaves of *Hosta* Tratt. 'Undulata Erromena' and they dipped the leaves in aqueous solutions contained 1 mmol dm⁻³ (= 225 mg dm⁻³) BA. They measured stomatal apertures of non-treated leaves as 4.55 µm while the treated were as follows: 5.12 (day 1), 4.03 (day 6) and 4.64 (day 19). Like our study, they observed an increase in stomatal aperture in the first treatment and then a decrease, and later an increase and a decrease respectively. The results, showed a quite similarity our study, a closer value of the non-treated (control) values. In a tissue culture study, Namlı and Ayaz (2007) added different concentrations of BA (0.25, 0.625, 1 and 2 mg/l) into the Murashige and Skog media and cultured *Pistacia vera* L. buds on them. They observed that the stoma cells were edematous and pores took the form chain in application of 0.25 mg/l BA and with the increasing of BA concentration, the abnormal stomatal morphologic characters were formed additionally as extended increase of distortion and reduction of extracellu-

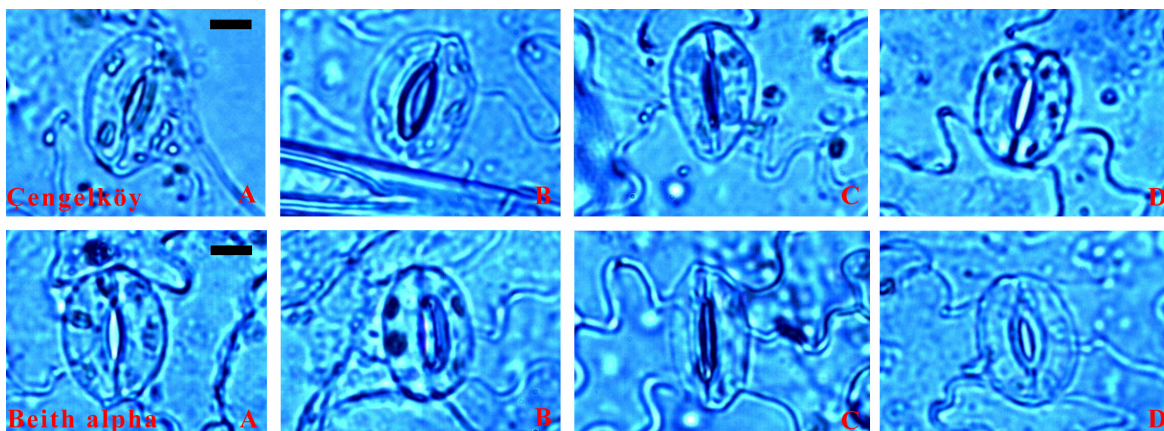


Figure 1. The stomatal response of first application onto abaxial leaves of 2 different cucumber genotypes (Çengelköy and Beith alpha) A- Control, B-BA, C-NAA and D-GA₃. The bars have indicated 10 micrometers.

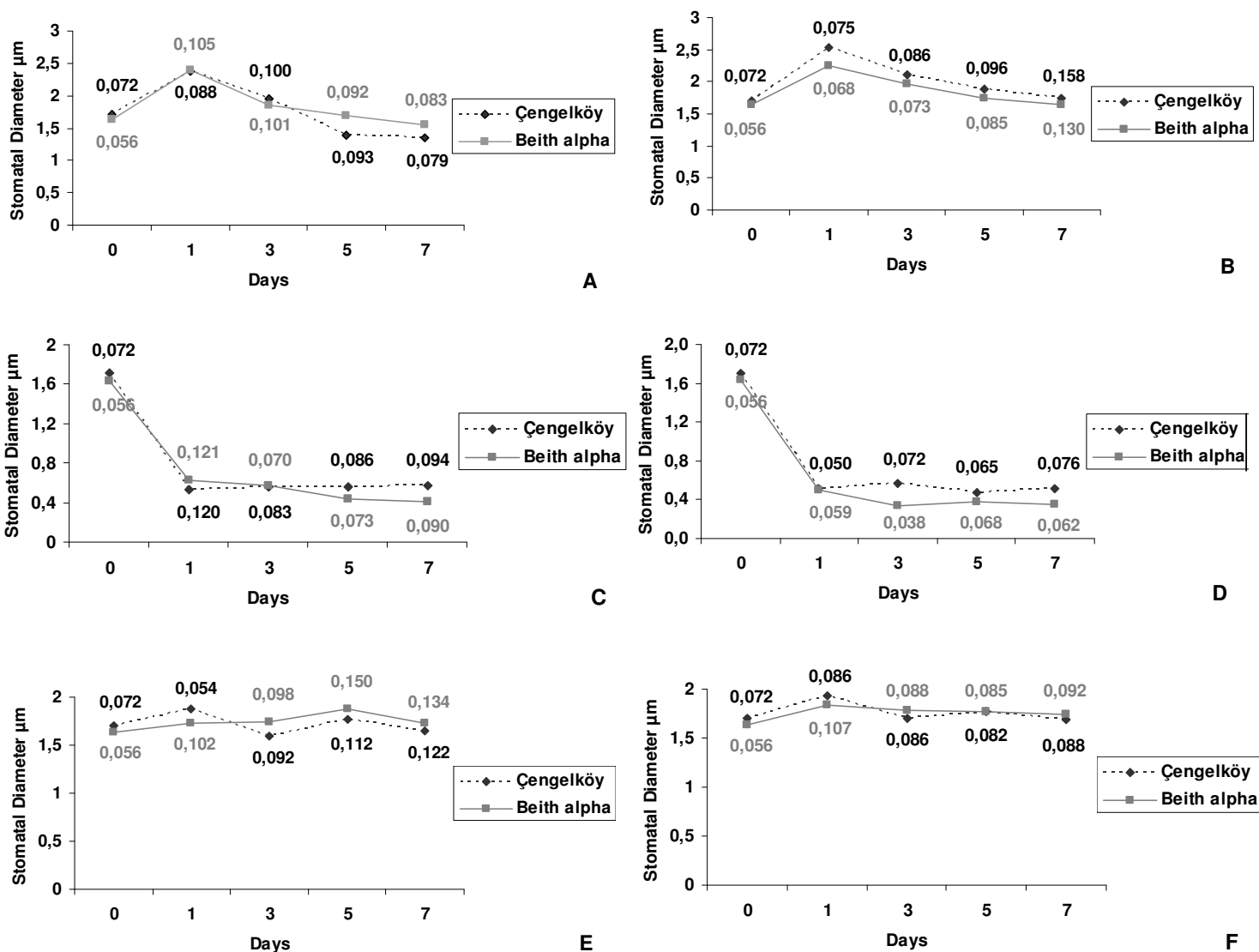


Figure 2. The stomatal diameter values of 7-day treated two elite cucumber (*Cucumis sativus* L.) genotype's (Çengelköy and Beith alpha) abaxial leaves. A-B) 10⁻⁴ and 10⁻⁶ M BA, C-D) 10⁻⁴ and 10⁻⁶ M NAA, E-F) 10⁻⁴ and 10⁻⁶ M GA₃. Standard errors were given numerically.

lar spaces. In addition, they obtained desirable results by using 1 mg/l BA compared to stomata of *Pistacia vera* L. developed *in vivo*. Furthermore, in *Nicotiana tabacum* L. and *Digitalis* plantlets, which have grown *in vitro*, addition of BA stimulated opening of stomata only in low concentrations (Diettrich et al., 1992; Pospíšilová et al., 1993).

Researchers observed that stomatal aperture in *Vicia faba* was stimulated by adenosine and kinetin riboside in darkness and in light, but in light kinetin itself had no effect or, at higher concentrations, inhibited stomatal aperture while stimulative effects in darkness (Morsucci et al., 1991; 1992). In epidermal strips or leaf fragments of *Commelina*, CKs had no effect on ABA-stimulated closure of *Commelina* stomata. When applied alone, at high concentration (10^{-1} mol m⁻³), to *Commelina* epidermis or leaf pieces both zeatin and kinetin restricted stomatal opening. In leaf fragments of maize, CKs alone did not promote stomatal opening either, but concentrations of both zeatin and kinetin from 10^{-3} to 10^{-1} mol m⁻³ caused some reversal of ABA-stimulated closure of maize stomata (Blackman and Davies, 1983).

Literature indicates that the responses of stomata to exogenous CKs are species-specific and depend on CKs concentration (Pospíšilová, 2003b) Nevertheless, our results and Wachowicz's results showed that, in addition to concentration, application time (continuous treatment) is also effective on stomatal aperture and long-time applications affect negatively the stomatal aperture while the short times positively.

In NAA treatments, the stomata were negatively affected by two different NAA concentrations and the diameter values were rapidly decreased. The reduction was between 65 - 80% and after the other treatments in day three, five and seven, diameter values were closer to these decreased levels (Figure 2, C-D). In previous studies, researchers tried to set apart IAA from other synthetic auxins, because of the effects of IAA are very different from the synthetic auxins. However, they observed concentration-dependent effects and mentioned that stomatal opening at low concentrations while closing in high concentrations (Assmann and Armstrong, 1999; Pospíšilová, 2003a). In a study, Snaith and Mansfield (1982) showed that there was no effect of IAA (0.1 µM-0.1 mM) on stomatal opening of *Commelina communis* in CO₂-free air, but the hormone eliminated the closing response attributed to CO₂. In contrast, Pemadasa (1982a) reported that IAA (10 µM - 0.1 mM) caused stomatal opening in *Commelina*, although Zelitch (1961) found that stomata in *Nicotiana tabacum* close in response to the synthetic auxins 2,4-D and NAA. In another study, Levitt et al. (1987) observed stomatal opening in *Vicia faba* L. by using IAA, at concentrations of 0.01 - 1.0 mM, and ethephon (0.3% v/v Ethrel) to epidermal peels in both light and dark. Lately, Wan and Li (2006) showed that NAA at high concentration increased ABA biosynthesis in peanut plants through up-regulation of the

AhNCED1 gene expression. The constitutive expression of the AhNCED1 gene in wild-type *Arabidopsis* resulted in an increase of ABA accumulation in transgenic plants in response to drought stress. This situation could be the cause of closing effect of NAA. In our study, the exogenous application of NAA rapidly decreased the stomatal aperture of abaxial leaves of 2 months old cucumbers.

Stomatal responses to endogenous auxins are depended not only on the auxin used and the concentration, but also plant species, age, environmental conditions and source of the epidermis (abaxial or adaxial) (Pessarakli, 2005). High concentrations of auxins can suppress such as PAA (Phenylacetic acid) and NAA stomatal opening (Permadasa, 1982b; Snaith and Mansfield, 1984). In addition, the effects of the auxin can be depended on atmospheric CO₂ concentration. In *Pisum sativum* and *Phaseolus vulgaris*, IAA increased g_s in the presence of CO₂ but not absence of CO₂. Furthermore there are some reports suggested that auxins have some antagonist effects ABA-induced stomatal closure in *Commelina communis* and *Vicia faba* (Snaith and Mansfield, 1982; Ricanek and Vicherkova, 1992; Dunleavy and Ladley, 1995). However, it is not known to what extend variation in endogenous auxin concentration influences stomatal sensitivity of ABA in plants (Pessarakli, 2005).

In this study, for GA₃ treatments, stomata were not directly affected negatively or positively from two different concentrations of GA₃. In the first treatment, a less increase and then less decrease were seen respectively. After the third and fourth treatments, the levels were closer the control values (Figure 2, E-F). It can be said that GA₃ affected slightly the stomatal aperture of 2 months old cucumbers. In a study, Thimann (1985) researched senescence of detached leaves of *Tropaeolum* and observed that GA₃ opened the stomata for the first 24 h, but later on the differences in aperture between leaves on GA₃ and the controls were insignificant. The increased opening was shown already after 2 h and in some experiments, it was still detectable after 48 h. In light, a small but consistent opening (confirmed in other experiments) was observed, but the effect of GA₃ on proteolysis in light was not significant. In another study, Roper and Williams (1989) sprayed GA₃ solution to *Vitis vinifera* L. leaves and they indicated that use of GA₃ had negated the effect of ABA on stomatal aperture as opened the stomata. Similar to our study, Wachowicz et al. (2006), studied the effect of GA₃ on stomatal aperture in senescing cut leaves of *Zantedeschia aethiopica* and they dipped the leaves in aqueous solutions contained (≈ 346 mg dm⁻³) GA₃. They measured stomatal apertures of non-treated (control) leaves as 9.38 µm while the treated were as follows: 11.37 (day 1), 9.02 (day 7) 12.25 (day 14) and 10.31 (day 22). Like our study, they observed an increase in the first treatment and then a decrease and later an increase and a decrease and the results, showed a quite similarity our study. Lately, Shah (2007) applied a pre-sowing treat-

ment d (5, 10 or 15 h in 10^{-6} , 10^{-5} , or 10^{-4} Maqueous solution of GA_3) to black cumin (*Nigella sativa* L.) and analyzed 50, 70 and 90 day old plants. They observed enhancement of g_s and carbonic anhydrase activity, brought about by the GA_3 treatment. In the literature, GA_3 is known to induce an influx of Ca^{2+} into the endoplasmic reticulum of guard cells, thereby initiating a process that leads to increase in stomatal activity (Assmann and Armstrong, 1999; Shah, 2007).

In summary, the observations presented in this study had a broad agreement with some of previous studies in many aspects. By the way, continuous application of those three hormones showed effects like high-concentration applications. The results obtained from *Cucumis sativus* should be supported by further experiments with other plant species.

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