ACTA AGROBOTANICA
Vol. 43, z. 1, 2 – 1990
53-64

# The role of ethylene and 1-aminocyclopropane-1-carboxylic acid (ACC) in pollination and senescence of cut flowers of hippeastrum (*Hippeastrum* x *hybridum* hort.)

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(Received: December 28, 1988)

Abstract

Pollination of hippeastrum flowers did not affect the corolla life span. Only stigma and style from pollinated flowers wilted sooner than those from the unpollinated ones. Pollination or application of ACC to intact pistil accelerated synthesis of ethylene by the pistil only when stigma was mature. This was not observed when immature stigmas were treated. Various parts of the pistil showed different ability to synthesize ethylene after wounding. Production of this gas was the most intensive in the stigma, whereas decreased amounts of ethylene were emitted successively by the upper, middle and lower parts of the style. Increased amounts of ACC were found in pollinated or wounded pistils. Production of ACC was the highest in the stigma, and smaller in the style; amount of ACC decreased toward the basis of the style. The influence of ethylene and ACC on in vivo germination of pollen was also examined. Germintaion of pollen was accelerated by both ethylene and ACC.

## INTRODUCTION

Increased ethylene synthesis and accelerated senescence under the effect of pollination were observed in orchid by B u r g and D i j k m a n (1967). Similar phenomenon was observed in carnation (N i c h o l s, 1977), foxglove (S t e a d and M o o r e, 1979) and narcissus (P i s k o r n i k, 1986). B u r g and D i j k m a n (1967) and H a l l and F o r s y t h (1967) stated that ethylene synthesis by pistil was responsible for flower senescence after pollination. B u f l e r et al.

The project was partly financed by the Polish Academy of Sciences, project no. CPBP 05.02.4.14.

(1980) suggested that the factor responsible for senescence of intact carnation petals was not formed in the petals, and that ACC might have been this factor. Introduction of ACC on pistil stigma increased content of this compound in petals as well as ethylene synthesis by pistil and petals, resulting in accelerated flower senescence (Reid et al. 1984, Nichols and Frost, 1985). Pollen of a number of flowers contains ACC (Whitehead et al., 1983 a). Transformation of pollen ACC to ethylene takes place in stigma, this being reflected in rapid blowing away of increased amounts of this gas already before pollen germination (Whitehead et al., 1983 a, b). Reid et al. (1984) and Hoekstra and Weges (1985) expressed an opinion that ethylene produced by pollinated pistil style was not responsible for petal wilting. Ethylene appearing early after pollination may affect pollen germination in some flowers (Piskornik, 1986). This paper presents possible role of ethylene and ACC in pollination of hippeastrum flowers.

## MATERIAL AND METHODS

Studies were made on flowers of *Hippeastrum* x *hybridum* hort., cv. Red Lion. The plants were cultivated in a greenhouse, in autumn-winter period. Cut flowers were stored in 20°C ± 1°C. Shoot ands were immersed in distilled water to the depth of 10 cm.

Pistil treatment. The effect of pollination and stigma treatment with ACC production of ethylene by stigma and style was determined for intact pistils, eliminating the effect of traumatic stress on $C_2H_4$ synthesis. Pollen or 3 drops of 0,1 mM ACC were transferred onto pistils with mature stigma, covered with a characteristic excretion, or onto immature stigmas. Glass tubes sealed with Sirra wax (BDH) not excreting any traces of ethylene or other unsaturated hydrocarbons, were placed over pistil stiles. On the stigma side, the tube were sealed with septum stoppers. Four $cm^3$ the gas were collected throught this membrane with a syringe. The tubes were fixed to stands so as to avoid mechanical pressure on the pistils. Gas analyses were performed for 12 hours, at 1-hour intervals, and then after 24, 36, 48, 96 and 120 hours. Incubation lasted for 1 hour and then ethylene free air was blown through the tubes. Five repetitions were made for each experiment.

Determination of EFE activity. Stigma and style fragments (upper, middle and lower) were incubated in 11 $cm^3$ phials containing 0,5 $cm^3$ of 0,1 mM ACC. Phials were stopped with rubber stoppers and incubated in 25°C.

Ethylene content in gas samples was determined 1 and 2 hours after the segmentation. Incubation lasted for 1 hour each time. The experiment was repeated 4 times.

The effect of wounding. Cut off stigma and 3 style parts: upper, middle and lower, were placed on a moistened filter paper in 11 $cm^3$ phials at 25°C. Ethylene content was determined after 1 hour incubation. Gas analyses were performed 1, 2, 6, 12 and 24 hours after the segmentation of plant material. Six repetitions were made.

Ethylene determination. Gas chromatography Chrom-4 was used. Glass column 1,2 m long was filled with pelleted carbon Carbosive B 60/80 mesh (Supelco Inc.). Temperature of the injection chamber and the column was 160°C and 120°C respectively. Nitrogen was used as a carrier gas, the flow rate being 40 $cm^3$ x $min.^{-1}$. Ethylene was detected with flame ionization detector. Identification was performed using standard gas of known concentration (Supelco Inc.).

ACC determination. ACC content was determined in hippeastrum stigma and style fragments at pollination, and 12 and 24 hours later, as well as 24 hours after wounding. ACC was extracted from plant material with hot 80 % ethanol (Liu et al., 1984). ACC content was determined according to Lizada and Yang (1979) using standard ACC (Calbiochem AG). The experiment was repeated 3 times.

Flower longevity. After cutting, the flowers were placed in distilled water, at 20°C ± 1°C. When the pistils attained maturity, pollen was transferred onto the stigmas. The control consisted of unpollinated flowers. In order to determine flower longevity, observation were made of the time lapse between bud opening and:

- apperance of the first symptoms of wilting, such as loss of turgor and change of
- colour at petal edges,
- flower wilting,
- loss of turgor by stamens,
- loss of turgor by style.

The experiment was made in 5 repetitions, with 3 flowers in each. The results were verified statistically, calculating the standard error.

## RESULTS AND DISCUSSION

Pollination of hippeastrum flowers initiated ethylene evolution after several hours, the highest intensity of this process being served 36 hours after pollen

introduction onto the stigma (Fig. 1). Thereafter, $C_2H_4$ production decreased gradually. Similar effect observed after stigma treatment with 0,1 mM ACC solution. Fig. 2 shows changes in $C_2H_4$ production by the pistils with immature stigmas under the effect of pollination or ACC treatment of intact flowers. In both cases gas synthesis was not accelerated. Comparing the results presented in Figs. 1 and 2, it is readily seen that immature and mature stigmas differed as regards the response to ACC treatment and pollination. The two factors accelerated ethylene production in various flowers (Arditti and Knauft, 1969; Nichols, 1977; Gilissen, 1976; Halevy et al., 1984; Reid et al., 1984; Nichols and Frost, 1985) but did not stimulate ethylene synthesis by hippeastrum pistils when the stigmas were still immature. Similar results were obtained from immature tissues of pre-climacterical fruits (Yang, 1981; Sisler and Yang, 1984). Stigma cells in hippeastrum seem to acquire the ability to transform ACC to $C_2H_4$ only when they attain maturity. Reaction of immature tissues changed when an additional factor initiating ethylene synthesis (such as wounding) appeared. When immature hippeastrum pistils were cut and divided into 4 fragments, and placed in 0,1 mM solution of ACC, ethylene production was observed already 1 hour after the segmentation (Fig. 3). Hence, wounding induced the cells to transorm exogenous ACC into $C_2H_4$, this being so even in young tissues which in vivo had no such ability (Fig. 2). ACC transformation to $C_2H_4$ in plant cells is connected with active enzymatic system which transforms the gas precursor into a gaseous plant hormone. Enzymatic system able to transform ACC to ethylene was isolated from etiolated pea seedlings (Konza and Kende, 1979). Shimokava (1983) stated that *Citrus unshiu* fruits attained the ability to metabolize exogenous ACC into $C_2H_4$ already 1 hour after tissue wounding, and that this ability increased rapidly in the next 10 hours. Similar reaction was observed in wounded hippeastrum style and stigma.

Particular fragments of isolated from ovary hippeastrum pistil showed different the ability of ethylene biosynthesis from exogenous precursor. Stigma appeared to be the most active. Upper part of the style produced less ethylene, and this production decreased rapidly in the middle and lower part (Fig. 3). The same was observed for narcissus stigma and style fragments (Mareczek, unpubl. data). No anatomic and morphologic differences were observed between upper and lower style fragments (Rodkiewicz, 1973) that would expalin the differences in the ability to transorm egzogenous ACC to $C_2H_4$. This phenomenon was probably caused by different activity of amount of the enzyme responsible for ACC transformation into ethyelne (EFE). Sacalis et al. (1983) noted that fragments of isolated carnation petals also differed as to the ability of ACC

transformation. On the other hand, N i c h o l s et al. (1983) found high activity of the enzymatic system (EFE) responsible for $C_2H_4$ synthesis from ACC in carnation stigma.

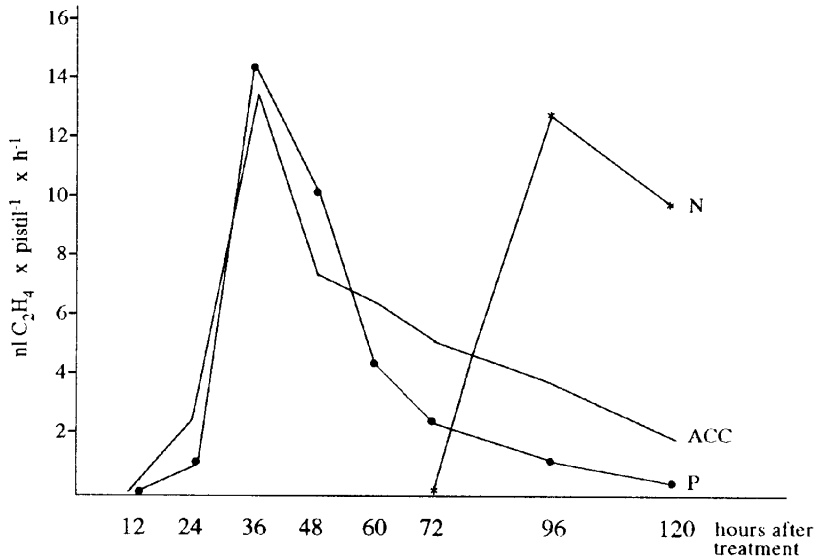


Fig. 1. Ethylene production by intact and mature pistils of hippeastrum after pollination and after ACC (0,1 mM) treatment of unpollinated stigmas
N – unpollinated, P – pollinated

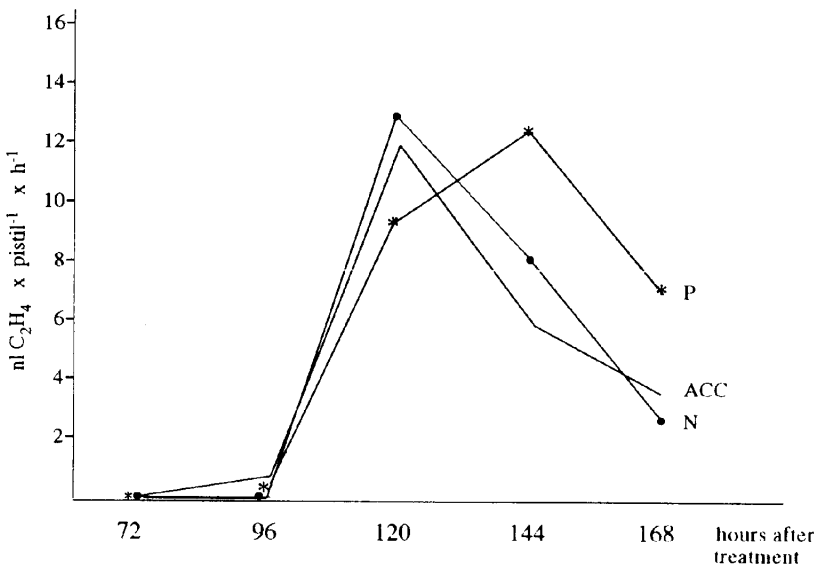


Fig. 2. Ethylene production by intact and immature pistils of hippeastrum after pollination and after ACC (0,1 mM) treatment of unpollinated stigmas
N – unpollinated, P – pollinated

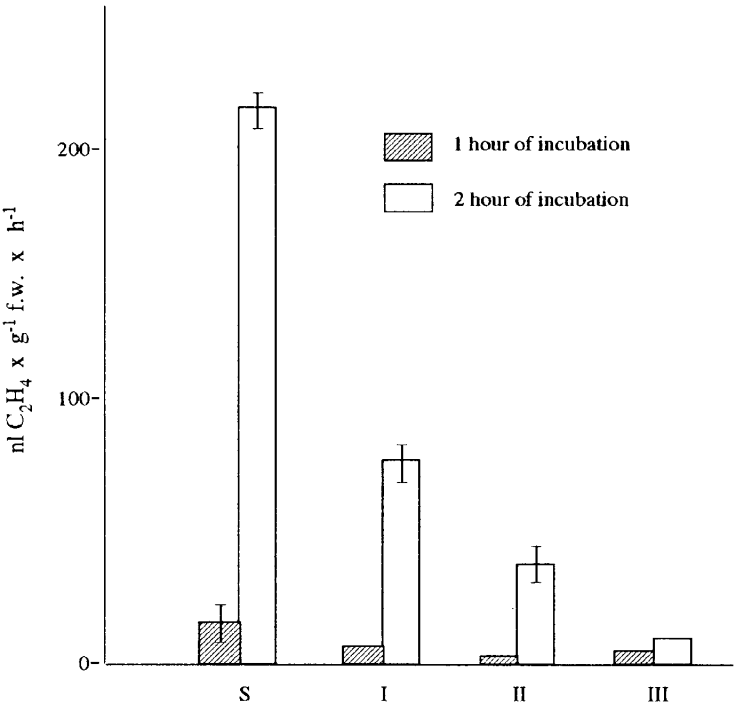


Fig. 3. EFE activity of immature stigma and style fragments isolated from fully opened flowers of hippeastrum. EFE activity of the portions was assayed adding exogenous ACC
S – stigma, I – upper, II – middle, III – lower part of style

Differences between stigma and style fragments were observed not only as regards EFE activity, but also the ability to synthesize ethylene from endogenic precursor. Wounding of the style tissues resulted in the highest ethylene production 24 hours later by stigma, but this production was lower in the upper style part, and very low in the middle parts (Fig. 4).

The same was observed in narcissus and gladiolus (P i s k o r n i k 1986), cherry and Japanese azalea (P i s k o r n i k, unpubl. data). S a l v e i t and D i l l e y (1978)showed that traumatic stress could result in ethylene synthesis within a few minutes to a few days after tissue wounding. These authors observed low ACC levels in tomato fruits just after wounding, but both ethylene production and ACC levels increased considerably already after 2 hours. B o l l e r and K e n d e (1980) stated that ACC and ethylene production in wounded tomato tissues took place as a result of rapid increase of ACC synthese activity.

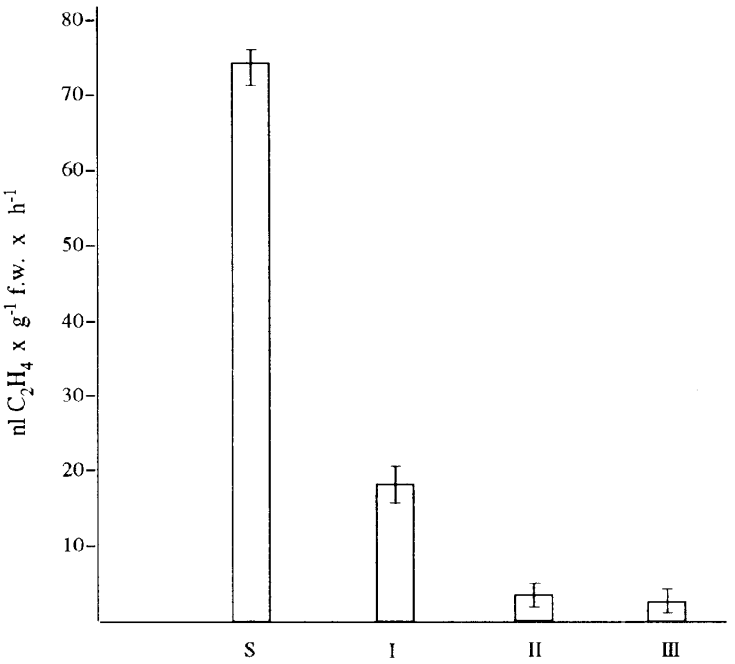


Fig. 4. Ethylene production by stigma and parts of a style of hippeastrum 24 hours after wounding
S – stigma, I – upper, II – middle, III – lower part of style

Gradient of ethylene production observed in the style may results from different precursor content of this gaseous hormone in plant tissues. No ACC was found in unpollinated hippeastrum pistils (Tab. 1). Pollination and wounding, which initiate synthesis of ethylene (Y a n g and H o f f m a n, 1984), resulted in the appearance of ethylene precursor in hippeastrum stigma as well as of its trace amounts in the style. The effect of wounding was observed also during growth of pollen-tubes in style tissue, this being reflected in intensive ethylene synthesis by pollinated pistils (G i l i s s e n, 1976, 1977).

Increased ethylene synthesis after pollination was observed in many flowers. This gas is produced mostly by the pistil (B u r g and D i j k m a n, 1962; H a l l and F o r s t y t h, 1967; N i c h o l s, 1977; S t e a d and M o o r e, 1983; P i s k o r n i k, 1986). The same was observed for pollinated hippeastrum flowers, in which mature uncut pistils reacted to pollen with ethylene synthesis in contrast to unpollinated flowers (Fig. 1). B u c h a n a n and B r i g g s (1969) stated that high ethylene concentration, which stimulated germination of peach pollen, inhibited growth of pollen-tubes. Possibly, decreasing ability of ethylene

synthesis in the style moving toward the ovary (Figs. 3 and 4), observed after wounding, represents a physiological necessity for proper growth of pollen-tubes in some plants.

Pollination accelerates flower wilting. This phenomenon was described for carnation (N i c h o l s, 1977), petunia (N i c h o l s et al., 1985), floxglove (S t e a d and M o o r e, 1979). N i c h o l s et al. (1983) stated that ACC moved from stigma toward pistil base, and then to flower petals, where its transformation to ethylene took place, and resulted in flower wilting. On the other hand G i l i s s e n and H o e k s t r a (1984) negated this role of ACC and advocated that some unidentified factor must be transported from stigma through style to petals, resulting in their wilting. My studies on cut hippeastrum flowers did not reveal accelerated flower wilting after pollination (Tab. 2), notwithstanding increased ACC levels in stigma already 24 hours after pollination (Tab. 1), and considerable acceleration of ethylene synthesis by pollinated pistil (Fig. 1).

T a b l e 1

Content of ACC (mM ACC x g -1 f.w.) in various pistil parts of hippeastrum

| Style | Unpollinated | Time after pollination (hours) | | | 24 hours after wounding |
|---|---|---|---|---|---|
| | | 2,5 | 10 | 24 | |
| Stigma | 0 | 0 | 0 | 1,70 ± 0,33 | 2,10 ± 0,28 |
| I | 0 | 0 | 0 | 0 | 0,89 ± 0,12 |
| II | 0 | 0 | 0 | 0 | 0,57 ± 0,25 |

I – upper and II – lower part of style

T a b l e 2

The effect of pollination on the time lapse to first morphological changes and wilting of cut hippeastrum flowers

| Flowers | Hours | | | |
|---|---|---|---|---|
| | First morphological changes | Wilting of flowers | Wilting of stamens | Wilting of pistil |
| Pollinated | 156,4 ± 4,2 | 198,3 ± 8,9 | 252,0 ± 6,3 | 228,1 ± 6,9 |
| Unpollinated | 159,7 ± 6,7 | 206,6 ± 8,2 | 248,0 ± 5,7 | 283,6 ± 9,2 |

Data presented in Tab. 2 suggest that pollination accelerated senescence processes only in pistils. Growth of stigma was also inhibited after pollination. Hence, a question arises whether ethylene produced in increased amounts after

pollination might destroy stigma and style tissues, the latter competing for nutritive substances with the developing ovary.

Figs. 5 and 6 present the effect of ethylene and ACC on hippeastrum pollen germination on agar. Both ethylene and ACC accelerated germination of pollen. Buchanan and Biggs (1969) also observed accelerated germination of pech pollen under the effect of ethylene. This gas stimulated narcissus pollen germination on agar (Piskornik, 1986). It is possible that in case of hippeastrum flowers, germination of pollen on stigma was accelerated rather by ethylene precursor present in the pollen (5 mM x $g^{-1}$ f.w.). Retarded ethylene synthesis by pistil after pollination (Fig. 1) suggests aslo that possibly it was not $C_2H_4$ that stimulated pollen germination on stigma, since this process took place within a few hours after pollen introduction. Unequivocal determination of the role of pollen ACC necessitates further extensive studies. Excluding ethylene of pistil origin as the factor responsible for flower wilting (as confirmed by Reid et al., 1984), one may still suggest that this gaseous plant hormone accelerated senescence processes in stigma and style tissues, as these had already fulfilled their biological function.

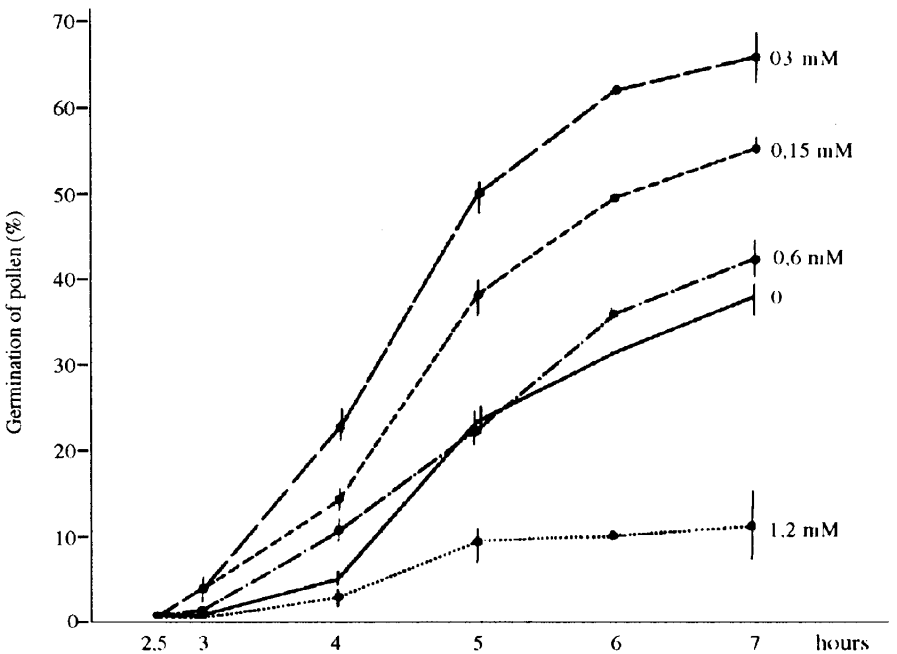


Fig. 5. Effect of ethephon on pollen germination of hippeastrum

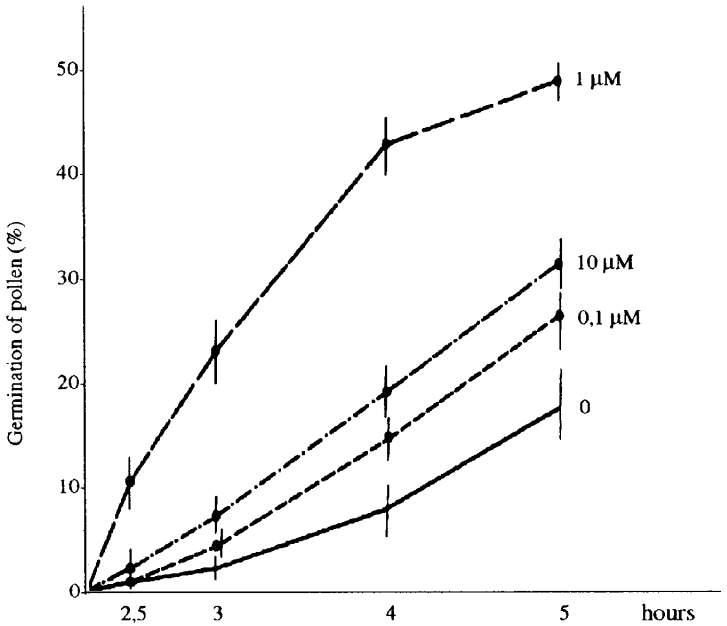


Fig 6. Effect of ACC on pollen germination of hippeastrum

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## Rola etylenu i kwasu 1-aminocyklopropano-1-karboksylowego (ACC) w zapyleniu i starzeniu ciętych kwiatów hipeastrum (*Hipeastrum* x *hybridum* hort.)

Streszczenie

Przeprowadzone doświadczenia wykazały, że zapylenie hipeastrum nie wpłynęło na trwałość ciętych kwiatów tego gatunku. Jedynie szyjka słupka i znamię więdły szybciej niż niezapylone. Zapylenie oraz traktowanie nienaruszonego słupka hipeastrum 0,1 mM roztworem ACC przyspieszało syntezę etylenu przez

słupek tylko wówczas, gdy znamię słupka było dojrzałe. Reakcji takiej nie obserwowano w przypadku traktowania słupków niedojrzałych. We fragmentach słupków hipeastrum zaobserwowano występowanie zróżnicowanej zdolności do syntezy etylenu po zranieniu. Najwięcej tego gazu produkowało znamię, natomiast malejące ilości etylenu wydzielały kolejno część podznamieniowa, środkowa i dolna szyjki słupka. Podobny gradient stwierdzono dla zdolności przekształcenia egzogennego ACC do etylenu przez znamię i poszczególne części szyjki słupka. Uzyskane wyniki wskazują także, że w słupku zapylonych lub zranionych kwiatów pojawia się kwas 1-aminocyklopropano-1-karboksylowy. Najwięcej tego prekursora etylenu występowało w znamieniu a znacznie mniej w szyjce słupka, przy czym ilość ACC malała ku podstawie szyjki. Przeprowadzono również doświadczenie nad wpływem etylenu i ACC na kiełkowanie pyłku hipeastrum w warunkach in vitro. Kiełkowanie pyłku hipeastrum przyspieszał zarówno etylen jak i ACC.