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Ethylene is Involved in the Control of Male Gametophyte Development and Germination in Petunia

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Abstract The time courses of 1-aminocyclopropane-1-carboxylic acid (ACC) content and ethylene production in developing anthers of petunia fertile and sterile lines and the effects of exogenously applied ethylene and an inhibitor of ethylene action, 2,5-norbornadiene (NBD), on male gametophyte development and germination were investigated. Fertile male gametophyte development was accompanied by two peaks of ethylene production by anther tissues. The first peak occurred during microspore development simultaneously with degeneration of both tapetal tissues and middle layers of the anther wall. The second peak coincided with maturation and dispersal of pollen grains. The mature pollen is characterized by a high ACC content (up to 300 nmol/g). Exogenously applied ethylene (1–100 ppm) induced degradation of gametophytic generation at the meiosis stage. NBD completely inhibited anther development at the early stages of its development and delayed anther dehiscence. In anther tissues of the petunia sterile line, tenfold higher ethylene production was observed at the meiosis stage compared to that in fertile male gametophytes and this correlated with degeneration of both microsporocytes and tapetal tissues. In vitro male gametophyte germination was accompanied by an increase of ethylene production, whereas NBD completely blocked male gametophyte germination. These results suggest that ethylene is an important factor in male gametophyte development and germination.

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Introduction

In flowering plants, a diploid, spore-producing generation (sporophyte) alternates with a haploid, gamete-producing generation (gametophyte). The male gametophyte ends its early development within the anther. Male gametogenesis begins with a division of the diploid sporophytic cell, giving rise to the tapetal and the sporogenous (pollen mother cells) initials. The sporogenous cells undergo meiosis (Me), giving rise to a tetrad (T) of haploid cells. Individual cells of T are released as free microspores under the action of a callase produced by the tapetum layer of the anther. The tapetum contributes to pollen development by secreting a number of different compounds into the loculus. Cytoplasmic male sterility (CMS), a maternally inherited condition under which the plant is not capable of producing functional pollen, results from disturbed coordination between the processes of formation of tapetum cells and microsporocytes (Goldberg and others 1993; McCormick 1993).

Male sterile mutants are of agricultural importance for production of hybrids to improve crop yield. Different strategies have been developed in the past to engineer male sterility. These are based on interfering with both development and metabolism of the tapetum or pollen in transgenic plants. Thus, in transgenic tobacco, an introduced gene for endo-β-1,3-endoglucanase under the control of an anther tapetum-specific promoter (Osg6B promoter) caused digestion of the callose wall at the beginning of the T stage, preceding an appearance of

normal endogenous glucanase activity (Tsuchiya and others 1995). Transgenic tobacco plants transformed with an antisense construct of extracellular invertase *Nin 88* under the control of its own promoter exhibit a block in early pollen development leading to male sterility (Goetz and others 2001).

Recently, some reports indicating the involvement of the plant growth regulator ethylene in the control of formation of male fertility and sterility have appeared. Overexpression of mutated melon ethylene receptor genes *Cm-ETR1/H69A* and *Cm-ERS1/H70A* induced pollen abortion and altered flower architecture thereby causing sterility or reduced fertility in transgenic tobacco plants (Ishimaru and others 2006).

To develop a new system for inducible male sterility in tobacco plants, a mutated ethylene receptor gene *Cm-ERS1/H70A* was fused with the tobacco *Nin 88* promoter, known to function mainly in the tapetum and microspore, and the *CaMV 35S* promoter, known to be a constitutive promoter (Takada and others 2006). The fusion genes *pNin88*::*Cm-ERS1/H70A* and *p35S*::*Cm-ERS1/H70a* were introduced into tobacco plants, which generated two independent transformants. Transformants with *35S*::*Cm-ERS1/H70A* produced less normal pollen and had modified floral architecture, whereas those with *Nin88*::*Cm-ERS1/H70A* produced less normal pollen without modification of floral architecture. Histological observations of anthers showed that tapetum degeneration in transformants occurred later than in wild types, indicating that expression of the mutated gene was involved in this delay.

The purpose of the present work was to elucidate how ethylene participates in the development of the male gametophyte in fertile and sterile clones of petunia. In the fertile clone, microsporogenesis is known to end with formation of mature pollen grains, which germinate after landing on a perceivable stigma surface. Resulting pollen tubes grow through conducting pistil tissue up to the ovary. In contrast, in the sterile clone, microsporogenesis is stopped at the stage of sterile male gametophyte formation.

To reveal a role for ethylene in male gametophyte development, we performed the following analyses: (1) compared the structure of developing anthers and male gametophytes in fertile and sterile lines and checked how these were related to anther wall modifications; (2) evaluated changes in the ethylene production by anthers in the course of development of fertile and sterile male gametophytes; (3) followed the effects of exogenously applied ethylene and an inhibitor of ethylene action, 2,5-norbornadiene (NBD), on in vivo development and in vitro germination of fertile male gametophytes.

Materials and Methods

Plant Material and Treatments

Plants of petunia (*Petunia hybrida* L.) fertile and sterile clones from our laboratory collection (found in nature) were propagated vegetatively in tubes on Murashige and Skoog (1962) nutrient medium in a climatic chamber (25°C, 16-h photoperiod). The 45 60-day-old plants were transferred into 5-l plastic vessels with soil and grown in the greenhouse under ambient illumination during summer months.

To treat developing anthers with ethylene, detached flowering stalks were inserted into vessels containing 100 ml water and placed in 20-l glass chambers equipped with an inlet for ethylene influx. Ethylene was injected into chambers to produce ethylene concentrations of 0.1, 1.0, 10, or 100 μl l^{-1}. Chambers were opened daily for air exchange and ethylene concentration reestablishment. The treatment with ethylene was continued for 3 days. The anthers from flower buds of the stalks kept in the chambers at ambient ethylene concentration in the air (0.005 μl l^{-1}) were used as a control.

For treatment with NBD, flowering petunia stalks (3–6 and 10–15 mm) were placed for 1 week in an atmosphere of 500, 2000, or 6000 μl l^{-1} NBD in enclosed 6-l chambers opened daily for air exchange and NBD concentration reestablishment. Pollen collected from these plants germinated on the cultivation medium (0.4 M sucrose and 1.6 mM H_3BO_3). Pollen collected from the plants grown in the same chambers in the absence of NBD and germinating on the same cultivation medium was used as a control. Five stalks were used in each experiment.

For ethylene treatment of in vitro germinating pollen, immediately after pollen planting on the cultivation medium (0.4 M sucrose and 1.6 mM H_3BO_3, pH 6.83), ethylene was injected into the flasks to produce ethylene concentrations of 0.1, 1.0, 10, or 100 μl l^{-1}. The pollen grains were cultivated for 2–6 h at 26°C. A percent of pollen germination was determined by calculating the number of germinated pollen grains from 200 randomly selected grains.

For treatment with NBD, pollen germinating on the cultivation medium (0.4 M sucrose and 1.6 mM H_3BO_3) was incubated in an atmosphere containing 500, 2000, or 6000 μl l^{-1} NBD in closed 6-l chambers that were opened daily for air exchange and NBD concentration reestablishment. Pollen germinating in the chambers in the absence of NBD was used as a control.

Cytological Analysis

Anthers were excised from flower buds (2.5–50 mm). To isolate developing gametophytic and sporophytic cells, the

anthers were fixed in a mixture of acetone with 96% ethanol (1:1 v/v) and homogenized with a pestle, and the resulting homogenate was filtered through a capronic filter (40-μm pores) (Matveeva and others 1998). Thereafter, the cells obtained were centrifuged at 1,000 *g* for 3 min, placed into 70% ethanol, centrifuged under the same conditions, and stained with the luminescent dye Hoechst 33258 (Serva, Germany). For each developmental stage, anthers from 15 flower buds were used. The preparations were examined under an MBS-15 microscope (Lomo, Russia).

Ethylene Measurement

The rate of ethylene production was monitored by gas chromatography by measuring the changes in the ethylene concentration in incubation vials. Flowering stalks were excised 30 min prior to each experiment. Anthers from five to seven flower buds were placed into a 15-ml glass vial and hermetically sealed with a Red Rubber Septa (Aldrich, USA). The empty vials (without anthers) were sealed for measuring the initial level of ethylene in the air. Before the ethylene measurements, vials were incubated for 0.5 h in darkness at 26°C in a TCH-100 thermostat (Laboratorni Pristroje, Praha, Czech Republic). Ethylene concentration was measured in the headspace of vials using a Zvet 106 gas chromatograph (Russia) equipped with a flame ionization detector and a system for concentrating hydrocarbons. These accessories allowed us to analyze the whole air volume of the vial, thereby significantly increasing the sensitivity of the method.

Determination of ACC Content

The ACC content was determined by the method of Lizada and Yang (1979). Plant material (developing anthers and mature pollen) collected just before the experiments was frozen in liquid nitrogen and further stored at −70°C until use. Weighted samples were homogenized in 0.2 M trichloroacetic acid (TAA) (1.2 ml). ACC was extracted for 2 h and then centrifuged for 5 min at 200 *g*. Then 1 ml of the supernatant obtained, 0.6 ml of distilled water, and 0.2 ml of 0.01 M $HgCl_2$ were mixed in a 15-ml flask. The flask was hermetically closed with a resin plug through which 0.2 ml of a cold mixture containing 5% NaOCl and saturated NaOH (2:1 v/v) was added. All the operations were performed at 4°C. The ethylene formed from ACC was measured as described above (Rakitin and Rakitin 1986).

Statistical Analysis

Figures 2 and 3 show the means ± SD for data obtained in three to five independent experiments with two replicates in each one ($n = 6$–10). Figures 6 and 7 show means ± SD for data obtained in three independent experiments with ten replicates in each one.

Results

Dynamics of ACC Content and Ethylene Production in Developing Anthers

Taking into account the common criteria for identification of the developmental stages of anther (Koltunow and others 1990) and also the correlations revealed by us between flower bud length and developmental stages of petunia fertile male gametophytes (Dobrovol'skaya and others 2009), we followed the dynamics of the content of ACC (ethylene precursor) and ethylene production over the whole period of petunia anther development in both fertile and sterile lines (Fig. 1).

A proper anther analysis was conducted at time points that correspond to the following developmental stages in fertile male gametophytes: mother pollen cells (Ar), meiosis (Me), tetrads (T), early microspores (EM), late microspore (LM), mitosis (Mi), early pollen grains (EPG), middle pollen grains (MPG), late pollen grains (LPG). As established earlier (Dobrovol'skaya and others 2009), during the first stage, Ar, cells of three middle layers of the anther wall were fully elongated and thinned. By the time of T formation, the middle layers were already significantly destroyed. Tapetal cells became binuclear at the end of the Me stage and multinuclear at the T stage. At the EM stage, the tapetum became disorganized and two middle layers of the anther wall were destroyed as well. At the stage of LM,

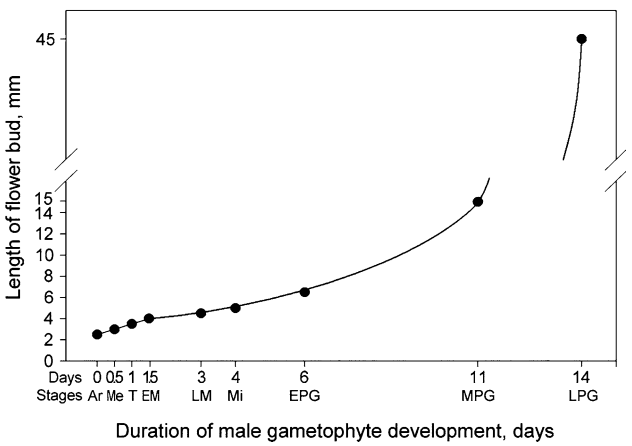

Fig. 1 Relationships between flower bud length and stages of male gametophyte development in the petunia fertile line. Stages of male gametophyte development: *Ar* stage of mother pollen cells, *Me* meiosis, *T* tetrads, *EM* early microspores, *LM* late microspores, *Mi* mitosis, *EPG* early pollen grains, *MPG* middle pollen grains, *LPG* late pollen grains

the tapetum exhibited partial destruction and its full disintegration took place before EPG formation.

As early as in the prophase of Me in the petunia sterile clone, we observed disorganization of tapetal tissue simultaneously with abnormal development of sporogenous tissue (Dobrovol'skaya and others 2009). Death of microsporocytes was accompanied by a premature degeneration and plasmolysis of tapetal cells. Although tapetum and microsporocytes became degenerated, the anther wall was still preserved. Simultaneously with complete destruction of the tapetum and microsporocyte cell contents, the middle layers of the anther wall underwent enlargement. The sizes of developing sterile anthers corresponded to those of fertile anthers and anther dehiscence in both lines occurred at the same time. Thus, a program of anther maturation in the sterile line appears to operate in the same manner as in the fertile one despite the fact that the pollen has perished.

We found that the dynamics of ethylene production in the anther–gametophyte system are characterized by specific features in both the petunia fertile and sterile clones (Figs. 2, 3).

The development of the fertile male gametophyte was accompanied by a gradual increase in the content of ACC (Fig. 2). The first increment took place at the stage of microspore development, in particular, at the stages T and Mi, whereas the last increment occurred at the late phase of pollen grain maturation. During these processes, fertile anther tissues displayed only a slight increase in ethylene production initiated by microspore release from T and peaked at the LPG stage (Fig. 2). The observed first maximum in the content of ACC and ethylene production appeared to coincide with degeneration of the middle layers of the anther wall and tapetum. The second enhancement of the above processes related to a 100-fold increase in the ACC content, and a 10-fold increase in ethylene production was correlated with maturation of pollen grains at the late stage and their dehydration (Fig. 2).

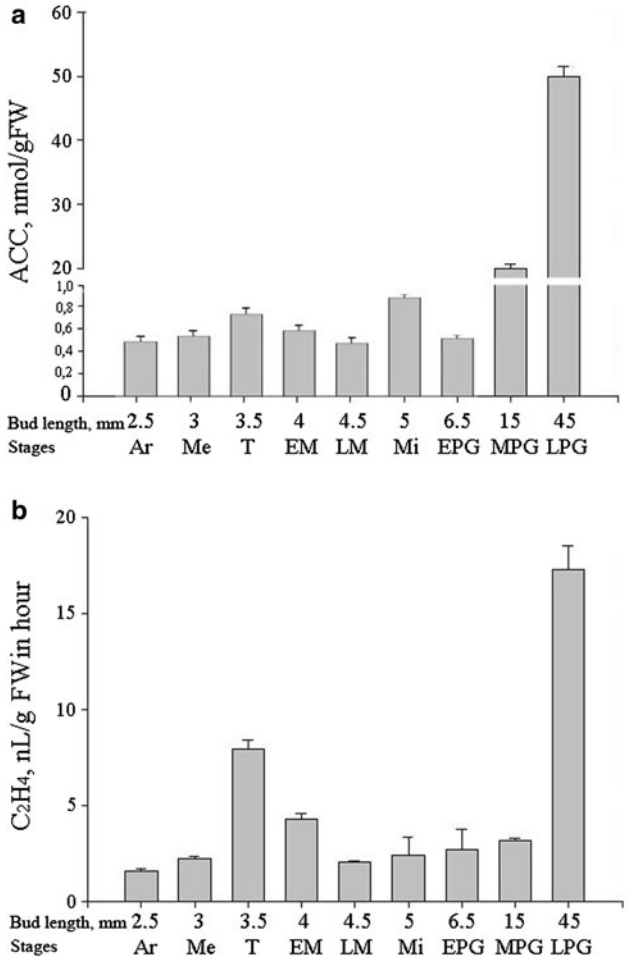

Fig. 2 Dynamics of ACC content (**a**) and ethylene production (**b**) in the developing anthers of the petunia fertile line. The means ± SD obtained from three to five independent experiments with two replicates in each one (n = 6–10) are shown

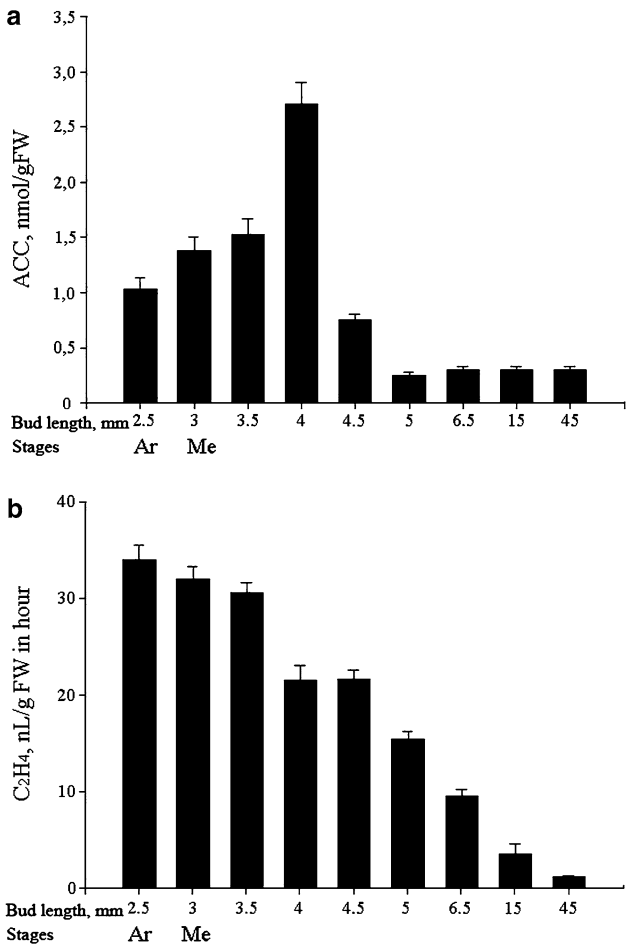

Fig. 3 Dynamics of ACC content (**a**) and ethylene production (**b**) in the developing anthers of the petunia sterile line. The means ± SD obtained from three to five independent experiments with two replicates in each one (n = 6–10) are shown

In the sterile line, ACC accumulation and high ethylene production were initiated at the Ar stage and were accompanied by degeneration of microsporocytes and the tapetum (Fig. 3). Dramatic ethylene production by developing anthers of the sterile line (accompanied by the degeneration of the tapetum and death of microsporocytes) appeared to be tenfold higher than that by developing anthers in the fertile line (accompanied by the degeneration of the tapetum and middle layers of the anther wall) (Fig. 3).

Thus, fertile male gametophyte development was accompanied by two peaks in ethylene production by anther tissues. The first increase in ethylene production occurred during microspore development with a peak at the T stage, simultaneously with degeneration of both tapetal tissues and the middle layers of anther wall. The second peak coincided with maturation and dispersal of pollen grains. In sterile lines, overproduction of ethylene by anther tissues at the meiosis stage accompanied male sterility formation.

Effects of Exogenously Applied Ethylene on Male Gametophyte Development

The data related to the effects of exogenous ethylene on in vivo development of fertile male gametophytes are schematically presented in Table 1 and Fig. 4. All generative cells at the Me stage experiencing action of this hormone died after 48 h of treatment with ethylene at a concentration of 1 μl l^{-1} or after 24 h of treatment at a concentration of 10 and 100 μl l^{-1}. Tetrads were injured after all ethylene treatments and died after 72 h of treatment with the hormone at concentrations of 10 and 100 μl l^{-1}. EM were injured after 72 h of ethylene treatment at a concentration of 10 μl l^{-1} or 48 h of treatment at a concentration 100 μl l^{-1}. Since microspores are in subsequent stages of development, that is, LM and Mi, they continued their normal development after the ethylene treatment. Thus, exogenous ethylene induced degradation of the generative cells during their development from Ar to microspores released from T, whereas vacuolated microspores and binucleate pollen grains under the same conditions did not exhibit any signs of degradation in the course of maturation.

Effects of Exogenously Applied Ethylene on Male Gametophyte Germination and Growth

In vitro germination of freshly collected petunia pollen was accompanied by ethylene evolution (Fig. 5). Maximal ethylene production was observed after 1-h cultivation of pollen tubes. Exogenously applied ethylene had no effect on pollen germination, whereas at concentrations of 1, 10, and 100 μl l^{-1} it inhibited pollen tube growth by 50% (Fig. 6). Thus, exogenously applied ethylene inhibited pollen tube growth.

Table 1 Effects of exogenous ethylene on male gametophyte development in a petunia fertile clone

Stage of male gametophyte development	Ethylene concentrations											
	0 (Control)			1 μl l^{-1}			10 μl l^{-1}			100 μl l^{-1}		
	1-st d	2-nd d	3-d d	1-st d	2-nd d	3-d d	1-st d	2-nd d	3-d d	1-st d	2-nd d	3-d d
Meiosis												
Tetrades												
Early microspores												
Late microspores												
Mitosis												
Pollen grains												

- uninjured cells
- injured cells
- death of cells

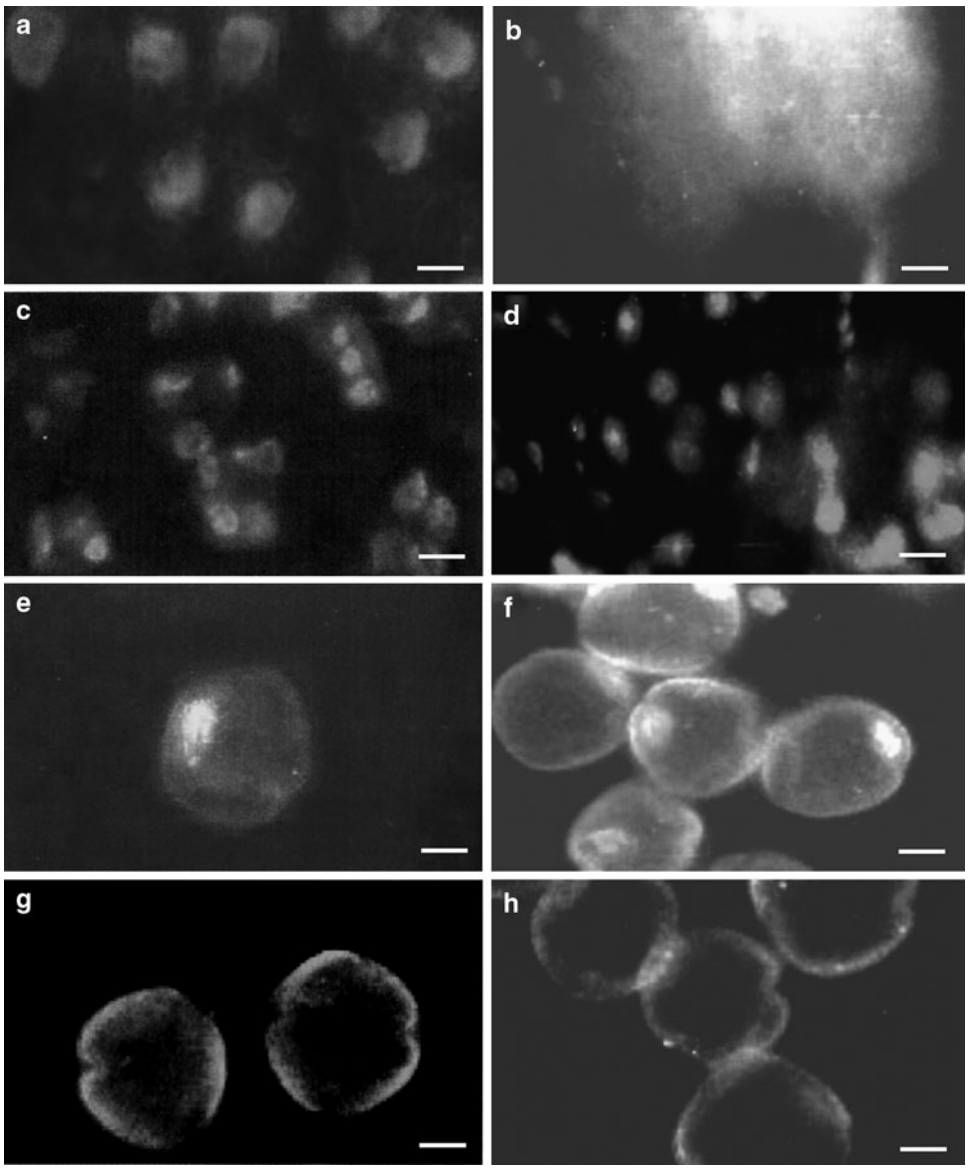

Fig. 4 Development of petunia male gametophyte in control: **a** Ar (stage of mother pollen cells); **c** T (stage of tetrads); **e** microspore; **g** pollen grains and after growing the flower buds in ethylene: Injuries of generative cells at the stages of Me (**b**) and T (**d**) and uninjured microspores (**f**) and pollen grains (**h**). Scale bar = 10 μm

Effects of NBD on Male Gametophyte Development and Germination

The inhibitor of ethylene action, NBD, suppressed germination of freshly collected pollen in a concentration-dependent manner (Fig. 7a). At concentrations of 500 and 2000 μl l^{-1}, it blocked male gametophyte germination by 30–40% and a nearly complete inhibition of this process (by 95%) was observed at a concentration of 6000 μl l^{-1}.

Similar results were also obtained when stalks with flower buds (3–6 and 10–15 mm) grew for a week in an atmosphere containing different concentrations of NBD. Buds of the first group and at different microspore development stages immediately stopped their development. In contrast, buds of the second group in which microspores were growing into pollen grains (EPG and MPG stages) continued their development after the NBD treatment but anther dehiscence appeared to be delayed. Pollen collected from closed anthers of buds of the second group germinated on the cultivation medium for 4 h (Fig. 7b). Germination of pollen from the plants was affected by NBD at concentrations of 500 and 2000 μl l^{-1} and it appeared to be 50% of that of controls (pollen collected on plants grown in atmosphere without NBD). At the same time, pollen from the plants treated with 6000 μl l^{-1} NBD did not germinate (percent of germination was only 3%). Thus, NBD completely inhibited the early stages of anther development and suppressed anther dehiscence and pollen germination in a concentration-dependent manner.

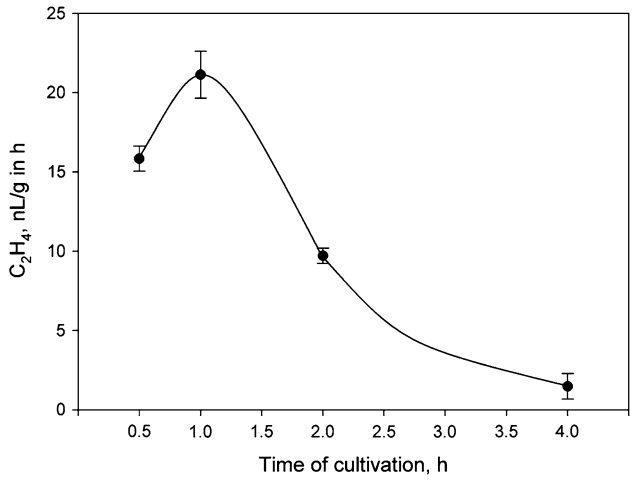

Fig. 5 Ethylene production by in vitro germinating petunia pollen on cultivation medium (0.4 M sucrose and 1.6 mM H_3BO_3). The means ± SD obtained from three independent experiments with ten replicates in each one are shown

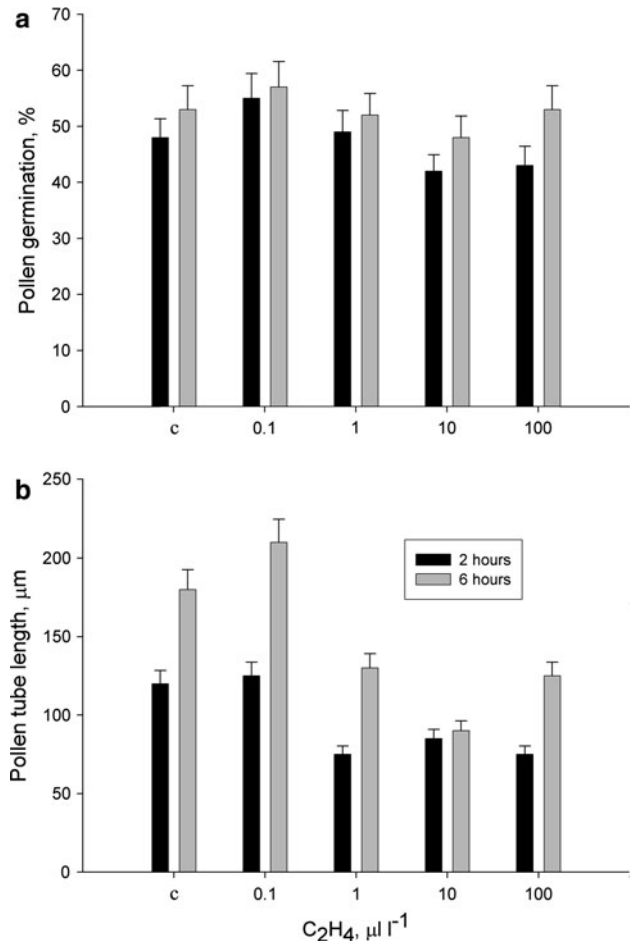

Fig. 6 Effects of exogenously applied ethylene on germination (**a**) and growth (**b**) of petunia male gametophytes

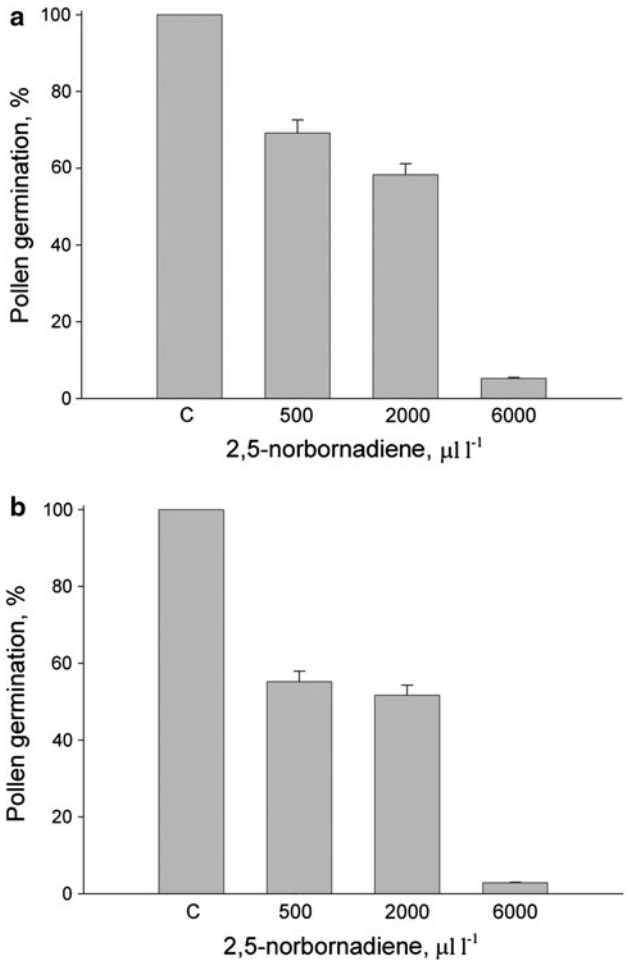

Fig. 7 Petunia pollen germination in vitro in the presence of the inhibitor of ethylene action, NBD (**a**), and after growing the flower buds in NBD (**b**). The means ± SD obtained from three independent experiments with ten replicates in each one are shown

Discussion

Ethylene regulates various plant processes, from seed germination to organ senescence (van Doorn and Woltering 2008). Such a multiple role of ethylene is supported by many reports dealing with floral induction (Achard and others 2007; Wilmowicz and others 2008), flower opening (Xue and others 2008), and abscission and fruit ripening (Bleecker and Kende 2000; Alexander and Grierson 2002; Butenko and others 2006). Transgenic petunia plants with reduced PheIN2 mRNA expression displayed reduced ethylene sensitivity in a wide range of physiological processes, including flower senescence and fruit ripening, suggesting an important role of PheIN2 in ethylene signal transduction during plant development (Shibuya and others 2004). In this article we showed a putative involvement of ethylene in male gametophyte development and germination.

Ethylene as a Factor in Sporophytic Control of Male Gametophyte Development

It is now known that ethylene is produced by all cells during plant development but the rate of this process may be different, with the highest rates associated with meristematic, stressed, or ripening tissues (Abeles and others 1992). There is now increasing evidence that, at least in some species, ethylene is involved in this genetic network in shoot meristems that specifies floral identity (Lin and others 2009). It has been proposed that flowering is triggered by a small burst of ethylene production in the meristem in response to environmental cues and, indeed, for example, in the pineapple (*Ananas comosus*) AcACS2 was found to be induced in the meristem during induction of flowering (Trusov and Botella 2006). In petunia ACO3 and ACO4 were found to be specifically expressed in developing pistil tissue (Tang and others 1994).

Currently, there is only little evidence for the role of ethylene in anther development. The results of the present work indicate that ethylene is required for the early stages of microsporogenesis. In the petunia fertile line, structural changes in the wall of developing anthers related to degeneration of the middle layers of the anther wall and tapetum at the stage of microspore formation were found to be accompanied by an increase in ethylene production by the anther (Fig. 2). Treatment with NBD of buds from the petunia fertile line at the earliest stages of their development (before initiation of M) led to a complete stop of anther and male gametophyte development.

For two floral cell types, the tapetum and pollen tubes, there is strong evidence for apoptotic-type cell death similar to that in animal cells (Rogers 2006). Onset of the programmed cell death (PCD) in tapetum at the proper time point is a required condition for development of fertile pollen (Goldberg and others 1993). At present, ethylene is suggested to be a mediator of PCD in plants (Woltering and others 1999). Introduction of a constitutively active form of the melon ethylene receptor into tobacco under the control of an anther-specific promoter was shown to cause a reduced number of pollen grains and delay in the PCD of tapetal cells (Takada and others 2006). Because ethylene is believed to be a key regulator of PCD (Gunawardena 2008), these observations suggest that ethylene is involved in the PCD of tapetal cells. LM-array analysis indicated that ethylene synthesis and signaling occur in the tapetum throughout anther development (Hirano and others 2008). The results obtained in the present work suggest that ethylene is involved in the coordination of the sequence of events in the anther that are required for male gametophyte development participating in PCD, first of all, in tapetal cells that determine completely the development of male generative cells. It may be proposed that ethylene initiates the mechanisms in the anther that underlie PCD, analogous to its action in other systems (Rogers 2006).

Indirect Evidence for Participation of Ethylene in Male Sterility Formation

As noted above, CMS results from a disturbed coordination of the processes related to development of the tapetum and microsporocytes. The critical stages of microsporogenesis most typical of emergence of male sterility include early Me stages and the T stage or binuclear pollen grains. In petunia, this abnormality was manifested in premature degeneration and lysis of the tapetum as well as in its prolonged maintenance and sometimes even in its active outgrowth after microspore formation. At the early Me stage, formation of CMS phenotypes can be due to insufficient metabolic activity or disorganization of the tapetum. At the T stage, this may be caused by too early or, in contrast, delayed tapetum destruction and may result from tapetum structural disturbance at the stage of microspore maturation.

We showed that in the petunia sterile clone, an abortion of male gametophyte development took place due to premature degeneration of the tapetum. Disorganization of tapetal tissue together with abnormal development of sporogenous tissue was observed in as early as the prophase of Me. Premature degeneration of the tapetum and death of sporogenous cells were found to be correlated with abundant production of ethylene (Fig. 3). In the petunia fertile line, high concentrations of exogenously applied ethylene caused degradation and death of male generative cells during developmental stages, from the beginning of Me to the release of microspores from T (Table 1). Therefore, the results obtained can be considered as indirect evidence of participation of ethylene in male sterility formation and are supported by the available literature data as well. Inflorescences of ethylene-treated plants of wheat exhibited 100% sterility (Campbell and others 2001).

Ethylene Controls Timing of Anther Dehiscence

Our findings indicate that a role for ethylene in male gametophyte development is not restricted to coordination of events in developing anthers during pollen grain formation. In particular, it appeared that maturation of pollen grains was coupled to ACC accumulation and ethylene production in them before anther dehiscence. This result is in a good accordance with the available reports.

PCD is known to be involved in flower development, in particular in later stages of anther development leading to its dehiscence and release of pollen (Rogers 2006). Tobacco flowers treated with the ethylene perception inhibitor 1-methylcyclopropane (1-MCP) displayed

delayed anther dehiscence that was accelerated by ethylene treatment (Rieu and others 2003). Antisense suppression of the ethylene receptor PhETR2 in petunia was shown to lead to stomium degeneration and anther dehiscence before anthesis, indicating that PhETR2 regulates synchronization of anther dehiscence with flower opening (Wang and Kumar 2007).

In our experiments, a similar effect (delay of anther dehiscence) was observed after treatment of buds of the petunia fertile clone with NBD. The percent of germination of pollen obtained from these anthers appeared to be considerably decreased (Fig. 7), indicating participation of ethylene in development of male gametophytes at the late stages of maturation as well. LM-array analysis revealed that during microspore and pollen development, ACS6 and ACO2 and ACO3 were predominantly expressed at the late stages of pollen grain development, indicating that these stages are characterized by active ethylene synthesis (Hirano and others 2008).

Ethylene Regulates Male Gametophyte Germination

Pollination leads to a rapid increase in ethylene production by pistil tissues and a subsequent wave of increased ethylene by other floral organs in several plant species, including petunia (*Petunia hybrida*) (Singh and others 1992; Tang and others 1994; Holden and others 2003; Kovaleva and Zakharova 2003). The development of petunia styles is associated with an increase in the activity of ACC oxidase in the stigma (Tang and others 1994). The petunia stigma is capable of converting applied ACC to ethylene but does not produce significant ethylene until pollinated (Pech and others 1987). This indicates that the increase in ethylene following pollination is likely due to an increased synthesis of ACC or the delivery of ACC to the stigma by the pollen (Tang and Woodson 1996).

To date, the role for ACC pollen in pollination-induced ethylene remains unclear. Thus, there are reports indicating that pollen contains significant levels of ACC, putatively providing evidence for its role in pollination-induced ethylene production (Whitehead and others 1983; Singh and others 1992). On the other hand, according to the data obtained by Hoekstra and Weges (1986), pollen ACC is not capable of participating in ethylene production on the stigma because pretreatment of the stigma with exogenously applied AVG completely prevented pollination-induced ethylene synthesis.

As follows from the results of the present work, in vitro petunia pollen germination was accompanied by ethylene evolution and NBD inhibited this process (Figs. 5, 7). According to the data obtained, ethylene synthesis is provided by ACC accumulated in pollen grains. As follows from Fig. 2, a 100-fold increase in ACC content in the anthers at the last stages of maturation of pollen grains is related to its accumulation in them. Possibly, in the course of pollen germination on the stigma, ethylene formed from pollen ACC, under the action of ACC oxidase, triggers autocatalytic ethylene synthesis in the stigma.

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