Potential Involvement of Cell Cycle-Related Genes in the Arrest of Stamen Development of Female Flowers During Sex Expression in Cucumber (*Cucumis sativus* L.)

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Monoecious cucumber (*Cucumis sativus* L.) produces male and female flowers on the same plant, but application of ethylene promotes expression of female flowers. In the early bisexual stage, all flower buds contain primordia for both stamens and pistils and sexual differences are established by the selective arrest of sexual organ primordia. The expression of six cell cycle-related genes [*Cs Cyclin A* (*CsCycA*), *CsCycB*, *CsCycD3; 1*, *CsCycD3; 2*, *Cs Cyclin-dependent kinase A* (*CsCDKA*), and *CsCDKB*] was analyzed in male and female flower buds to investigate cell cycle activity in sexual organs. *CsCycA* was expressed specifically in the area containing arrested stamen primordia (ACASP) in female flower buds, but was barely detected in male flower buds. Expression of the other five genes was detected in the ACASP but not in pistils of female flower buds. Expression of all six genes was elevated transiently following the application of 2-chloroethylphosphonic acid (ethephon), an ethylene-releasing agent, to the shoot apices. The correlation of expression of these genes with the formation of female flowers suggests they play a role in the arrest of stamens of female flowers in monoecious cucumber plants.

Keywords : area containing arrested pistil primordia (ACAPP), area containing arrested stamen primordia (ACASP), arrested stamen primordia (ASP), cyclin, ethephon

INTRODUCTION

Cucumber (*Cucumis sativus* L.) has been used as a model higher plant for the study of sex expression (Galun, 1961; Shifriss, 1961; Kubicki, 1969a; 1969b; Malepszy and Niemirowicz-Szczytt, 1991). Sex expression in cucumber plants is genetically controlled by the *F* and *M* genes. These genes interact to produce four different sex phenotypes: gynoeceious (*M-F*-), monoecious (*M-ff*), hermaphroditic (*mmF*-), and andromonoecious (*mmff*). Gynoeceious cucumber plants produce only female flowers; monoecious plants produce both male and female flowers; hermaphroditic plants produce bisexual flowers with both staminate and pistillate organs; and andromonoecious plants produce bisexual and male flowers. Of these various sex phenotypes, monoecious is the most common type of sex expression in cucumber plants.

Morphologically, all flower buds in monoecious cucumber plants contain both stamen and pistil primordia at the early stage of their differentiation, and later develop into male or female flowers. Pistil development is arrested in flower buds destined to become male flowers, whereas stamen development is arrested in flower buds that become female flowers (Kubicki, 1969c; Yamasaki et al., 2005). Sex differences are thus established by the selective arrest of sexual organ primordia in monoecious cucumber plants.

In cucumber, sex expression can be regulated not only by the genetic loci described above, but also by plant hormones and environmental conditions (Atsmon and Galun, 1960; Shifriss and George, 1964; Frankel and Galun, 1977; Takahashi et al., 1983; Durand and Durand, 1984). In particular, production of the plant hormone ethylene is highly correlated with femaleness in cucumber plants, for example, gynoeceious cucumber plants produce more ethylene than monoecious plants (George, 1971; Rudich et al., 1972; Trebitsh et al., 1987). In addition, inhibitors of ethylene biosynthesis or ethylene action suppress the development of female flowers and induce male flowers (Beyer, 1976; Atsmon and Tabbak, 1979; Takahashi and Suge, 1980; Takahashi and Jaffe, 1984; Yamasaki et al., 2000; Yamasaki and Manabe, 2011). Furthermore, application of ethylene to monoecious cucumber plants promotes the formation of female flowers (MacMurray and Miller, 1968; Iwahori et al., 1970; Takahashi and Suge, 1980; 1982; Yamasaki et al., 2000). These physiological phenomena were confirmed at the molecular level. Two genes (*CS-ACS1G* and *CS-ACS2*) which encode a key enzyme in the ethylene biosynthesis pathway, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, were identified as being related to sex expression in cucumber plants. The *CS-ACS1G* gene, which exists in gynoeceious cucumber plants but not in monoecious cucumber plants, was closely linked to the *F* gene (Trebitsh et al., 1997). The *CS-ACS2* gene

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was first isolated by Kamachi et al. (1997), who demonstrated that the expression of the *CS-ACS2* gene coincides with development of female flowers in monoecious and gynoeceous cucumber plants. Recently, the *CS-ACS2* gene was identified as the *M* gene (Boualem et al., 2009; Li et al., 2009). Thus, both *F* and *M* genes encode ACC synthase and ethylene is considered a sex hormone in cucumber plants.

Plant growth is driven by the process of cell division, coupled with subsequent cellular elongation and differentiation (Beemster et al., 2003; Jakoby and Schnittger, 2004). Cell division plays a role both in the developmental processes that create plant architecture and in the modulation of plant growth rates in response to the environment (Cockcroft et al., 2000; Beemster et al., 2002; De Veylder et al., 2011; Polyn et al., 2015). The mitotic cell cycle is characterized by a round of DNA replication (S phase) followed by mitosis and cytokinesis (M phase), which are separated by two gap phases (G_1 and G_2) (Inzé, 2005). Cyclin-dependent kinases (CDKs) and their cyclin partners regulate the G_1/S and G_2/M phase transitions as well as progression through, and exit from, the mitotic cell cycle (Menges et al., 2002; Beemster et al., 2005; Inzé and De Veylder, 2006; Berckmans and De Veylder, 2009; Harashima et al., 2013).

In higher plants, six distinct classes of CDKs, CDKA to CDKF, have been identified (Vandepoele et al., 2002). CDKA has been suggested to be an orthologue of yeast Cdc2/Cdc28p and a regulator of G_1/S and G_2/M phase progression (Magyar et al., 1997). *CDKA; 1* is the sole gene encoding CDKA in *Arabidopsis* and it is expressed throughout the mitotic cell cycle (Vandepoele et al., 2002; Menges et al., 2005). Similarly, the expression and translation pattern of CDKA is constitutive during the mitotic cell cycle in tomato (Joubès et al., 2000). CDKBs are plant-specific CDKs whose expression is restricted to G_2/M phase (Porceddu et al., 2001); therefore, CDKBs play a specific role in the regulation of the G_2/M phase transition. Of the cyclin classes identified to date, the A- and B-type cyclins accumulate during the G_2 and early M phases; A-type cyclins also accumulate during the S phase of the mitotic cell cycle (Mironov et al., 1999). D-type cyclins, also known as G_1 proteins, control the progression through the G_1 phase in response to environmental factors and nutrients, and therefore act as key regulators of entry into the mitotic cell cycle (Fuerst et al., 1996; Gutierrez et al., 2002; Shen, 2002; Trimarchi and Lees, 2002). Thus, agents regulating the mitotic cell cycle have been identified.

Although it is clear that ethylene regulates sex expression, the mechanism by which ethylene signals affect sexual organs, namely, the downstream mechanism of ethylene signaling during sex expression, remains unclear in monoecious cucumber plants. Given that sex differences are established by the selective arrest of sexual organ primordia, activity of the mitotic cell cycle must be affected during sex expression in such plants. Analyzing the expression of cell cycle-related genes in male and female flower buds of monoecious cucumber plants should help elucidate the roles of the mitotic cell cycle on sex differen-

tiation at the molecular level. We analyzed the expression of six cell cycle-related genes [*Cs Cyclin A* (*CsCycA*), *CsCycB*, *CsCycD3; 1*, *CsCycD3; 2*, *Cs Cyclin-dependent kinase A* (*CsCDKA*), and *CsCDKB*], previously isolated from cucumber (Fu et al., 2008; Zhang et al., 2009), in male and female flower buds of monoecious cucumber plants using semi-quantitative reverse transcription polymerase chain reaction (RT-PCR). We also analyzed expression of these genes in the shoot apices of plants treated with 2-chloroethylphosphonic acid (ethephon), an ethylene-releasing agent, using semi-quantitative RT-PCR. We discuss the roles of the activity of cell cycle in the sex expression of monoecious cucumber.

MATERIALS AND METHODS

Plant materials

A monoecious cucumber (*Cucumis sativus* L., 'Santo-suyo No. 2') line was used in this study. Seeds were purchased from Nakahara Seed Co., Ltd., Fukuoka, Japan. Seeds were germinated on wet filter paper in a Petri dish and incubated at 26°C in the dark for 2 to 3 d. Seedlings were then transferred to plastic pots containing a soil composite, Kumiai-Engei-Baido (0.4 g of N, 1.2 g of P, 0.2 g of K per kg; Seishin Sangyo Co., Kitakyushu, Japan). During the experiments, plants were provided with adequate water and supplied with fertilizer, 0.002% (v/v) Hyponex (Hyponex-Japan, Osaka, Japan). Plants were grown in a greenhouse at the University of Teacher Education Fukuoka under natural daylengths between mid-May and the end of July 2015.

Application of chemicals

To investigate the effect of ethylene on the expression of the six cell cycle-related genes (*CsCycA*, *CsCycB*, *CsCycD3; 1*, *CsCycD3; 2*, *CsCDKA*, and *CsCDKB*), 50 μ l of 0.35 mM ethephon, an ethylene-releasing agent (Iwahori et al., 1970), in H₂O containing 0.1% (v/v) Tween 20 was applied to the shoot apices of monoecious cucumber plants at the four-leaf stage (i.e., when the leaf blade of the fourth leaf was approximately 2 cm long) using a micropipette once a day for 3 d. This treatment induced the formation of female flowers in monoecious cucumber plants (Yamasaki et al., 2000). Each replicate contained ten cucumber plants. The shoot apices were cut using a razor blade 0.5, 6, and 24 h post-ethephon application for 3 d. The samples were frozen immediately in liquid nitrogen and stored at -80°C prior to the extraction of nucleic acids.

Semi-quantitative RT-PCR analysis

Male and female flower buds were classified from stage I to III based on the development state, as described in Table 1. Sexuality of the flowers could be determined by stage I due to the presence of an ovary. In male flowers, the area containing arrested pistil primordia (ACAPP) (Fig. 1A) is left after removing sepals, petals, and stamens. In female flowers, the area containing arrested stamen primordia (ACASP) (Fig. 1B) is left after removing sepals, petals, and pistils. Male flower buds at stage I were dissected into four floral organs (sepals, petals, stamens, and ACAPP) using a forceps and scalpel under a stereomicro-

Table 1 Developmental stages of male and female flower buds of cucumber, classified in accordance with a previous report (Yamasaki and Manabe, 2009).

	Length of flower buds (mm)	Morphological characters
Male flower buds		
Stage I	3–5	Mononuclear pollen formation
Stage II	8–10	Pollen maturation
Stage III	13–15	Anthesis (flower opening)
Female flower buds		
Stage I	5–10	Stigma and ovary development
Stage II	20–25	Embryo sac maturation
Stage III	35–40	Anthesis (flower opening)

Fig. 1 Dissected male and female cucumber flowers at stage III. A. Area containing arrested pistil primordia (ACAPP) in male flowers. B. Area containing arrested stamen primordia (ACASP) in female flowers. C. Arrested stamen primordia (ASP) in female flowers.

scope (SPZT-50FTM; Carton Optical Industries, Ltd., Tokyo, Japan). Female flower buds at stage I were also dissected into four floral organs (sepals, petals, ACASP, and pistils). These samples were frozen immediately in liquid nitrogen and stored at -80°C prior to the extraction of nucleic acids.

Total RNA was extracted from male and female flower buds at stage I, from the individual floral organs (sepals, petals, stamens, and ACAPP) of male flower buds at stage I, from the individual floral organs (sepals, petals, ACASP, and pistils) of female flower buds at stage I, and from shoot apices treated with ethephon, using TRI reagent (Sigma-Aldrich Co., Tokyo, Japan), according to the manufacturer's instructions. The concentration of total RNA was measured using spectrophotometry (V-630BIO; Jasco Co., Tokyo, Japan). cDNA was synthesized from total RNA using Rever Tra Ace[®] (Toyobo Co., Ltd., Osaka, Japan) and random hexamers in a 20 μl reaction volume. PCR was performed using Premix Taq (TaKaRa Ex Taq[™] Version, Takara Shuzo Co., Ltd., Shiga, Japan), 1 μl of cDNA, and gene-specific primers.

The following gene-specific primers were used: for *CsCycA* (accession number: EW968279), *CsCycA*-F2 5'-

TCG AAA TGA CAG CTC CAA CA-3' and *CsCycA*-R2 5'-TGC TGG CTG TAT TTT TCT CTG A-3'; for *CsCycB* (accession number: EW968280), *CsCycB*-F2 5'-AAG AAG CTG AGA ACG AGA GCA-3' and *CsCycB*-R 5'-TAC CAC TCC AAC TTC CCA AG-3'; for *CsCycD3; 1* (accession number: EW968283), *CsCycD3; 1*-F2 5'-TCT GTT TTG GCA ACT GCA AC-3' and *CsCycD3; 1*-R3 5'-CTG AAC TGA AAC CTG CGT CA-3'; for *CsCycD3; 2* (accession number: EW968284), *CsCycD3; 2*-F 5'-CAG AGA ATG GAG CTT TTG GTT C-3' and *CsCycD3; 2*-R 5'-ACA CAA TCG TCC CCA TAT CC-3'; for *CsCDKA* (accession number: EW968281), *CsCDKA*-F2 5'-TGT TGA AGG AAA TGC AGC AC-3' and *CsCDKA*-R2 5'-TCA TTT CGG CAA ATA TGC AA-3'; for *CsCDKB* (accession number: EW968282), *CsCDKB*-F3 5'-CAA TCC CTC TAT GTC GTT CG-3' and *CsCDKB*-R2 5'-GCT TGA GAT CAC GGT GAA GA-3'; and for *CsGlyceraldehyde-3-phosphate dehydrogenase (CsGAPDH)* (accession number: HQ156465), *CsGAP*-F 5'-TGG TGA GAA GGC TGT CAC TG-3' and *CsGAP*-R2 5'-TGG TCA TCA GAC CCT CAA CA-3'.

The PCR conditions were 94°C for 30 s, 55°C for 1 min, and 72°C for 1 min. The PCR products were separated using electrophoresis on a 2.0% agarose gel and stained with ethidium bromide ($0.5 \mu\text{g ml}^{-1}$) for 40 min. The gel was washed in distilled water for 5 min and photographed on a UV transilluminator (M-15, Funakoshi Co., Ltd., Tokyo, Japan). The intensity of the bands was measured using the public-domain NIH Image program (<http://rsb.info.nih.gov/ni-image/>, November 25, 2015). *CsGAPDH* was used as an internal control, and the expression levels of cell cycle-related genes were evaluated according to the ratio of the density of cell cycle-related genes/*CsGAPDH*.

Before performing semi-quantitative RT-PCR analysis, the number of cycles for each gene amplification was determined from the titration curves of the corresponding RT-PCR products. The signal intensities of the RT-PCR products were expressed in arbitrary units and plotted against cycle numbers (data not shown).

Analysis of epidermal cell number at stamens compared to petals in male and female flower buds

To investigate epidermal cell number in stamens compared to petals in male flower buds, petals and stamens were dissected from male flower buds at stage II using a forceps and scalpel under a stereomicroscope. Similarly, in female flower buds, petals and the ACASP were dissected

from female flower buds at stage II. These samples were immersed in 50% (v/v) bleach (Kitchen Kirei kirei Jokin and Hyohaku; Lion Co., Tokyo, Japan) until they were decolorized. After washing with distilled water, they were observed using a stereomicroscope or light microscope (ECLIPSE E600W; Nikon, Co., Tokyo, Japan). Epidermal cell area was calculated using photomeasure software (Kenis Ltd., Osaka, Japan) ($n=30$). Epidermal cell number per mm^2 was estimated through dividing 1.0 mm^2 by the mean area of 30 epidermal cells. This analysis was performed separately on three male flower and three female flower buds.

RESULTS AND DISCUSSION

To investigate how cell cycle activity of sexual organs correlated with sex expression, the expression of six cell cycle-related genes (*CsCycA*, *CsCycB*, *CsCycD3;1*, *CsCycD3;2*, *CsCDKA*, and *CsCDKB*) was analyzed in male and female flower buds at stage I from monoecious cucumber plants. Expression of *CsCycA* was not detected in four floral organs from male flower buds, whereas it was detected in female flower buds, mainly in the ACASP (Fig. 2A, B). Indeed, expression of *CsCycA* was detected in female flower buds but not in male flower buds at stage I (Fig. 2C). Expression of *CsCycB* was detected in all four floral organs in male flower buds (Fig. 2A), but, in female flower buds, *CsCycB* expression occurred mainly in the ACASP (Fig. 2B). *CsCycD3;1* expression was seen mainly in the petals of male flower buds (Fig. 2A) but in the sepals, petals, and the ACASP in female flower buds (Fig. 2B). *CsCycD3;2* expression was detected mainly in the stamens of male flower buds (Fig. 2A), in contrast to the petals and the ACASP in female flower buds (Fig. 2B). Expression of *CsCDKA* was observed mainly in petals and the ACAPP in male flower buds (Fig. 2A), but *CsCDKA* expression was seen in female flower buds in the sepals and in the ACASP (Fig. 2B). Expression of *CsCDKB* was detected mainly in sepals, petals, and stamens, with low expression in the ACAPP, of male flower buds (Fig. 2A), whereas, in female flower buds, it was detected in sepals, petals, and the ACASP (Fig. 2B). In summary, of the six genes, *CsCycA* showed specific expression in the ACASP in the female flower buds of monoecious cucumber plants. Expression of the other five genes (*CsCycB*, *CsCycD3;1*, *CsCycD3;2*, *CsCDKA*, and *CsCDKB*) in female flower buds occurred in the ACASP but was barely detectable in the pistils. It is therefore possible that, as well as *CsCycA*, these five genes are involved in the arrest of stamen primordia in female flower buds of monoecious cucumber plants. In male flower buds, expression of *CsCycB*, *CsCycD3;2*, and *CsCDKB* genes was detected in stamens and expression of *CsCycB*, *CsCDKA*, and *CsCDKB* genes was detected in the ACAPP. Thus, it is possible that *CsCycB*, *CsCycD3;2*, and *CsCDKB* genes are involved in the development of stamens and *CsCycB*, *CsCDKA*, and *CsCDKB* genes are involved in the arrest of pistil primordia.

Application of ethylene to monoecious cucumber

Fig. 2 Semi-quantitative RT-PCR analysis of expression of cell cycle-related genes in male and female cucumber flower buds. A. mRNA levels of six cell cycle-related genes (*CsCycA*, *CsCycB*, *CsCycD3;1*, *CsCycD3;2*, *CsCDKA*, and *CsCDKB*) in four floral organs (sepals, petals, stamens, and ACAPP) from male flower buds at stage I. The ratio of the density of each cell cycle-related gene to a reference gene (*CsGAPDH*) is shown. B. mRNA levels of six cell cycle-related genes (*CsCycA*, *CsCycB*, *CsCycD3;1*, *CsCycD3;2*, *CsCDKA*, and *CsCDKB*) in four floral organs (sepals, petals, ACASP, and pistils) of female flower buds at stage I. The ratio of the density of each cell cycle-related gene to a reference gene (*CsGAPDH*) is shown. C. mRNA levels of *CsCycA* in male and female flower buds at stage I. The ratio of the density of *CsCycA*/*CsGAPDH* is shown.

plants promotes the formation of female flowers (MacMurray and Miller, 1968; Iwahori et al., 1970; Takahashi and Suge, 1980; 1982; Yamasaki et al., 2000). To investigate the relationship between expression of the six cell cycle-related genes and formation of female flowers, we analyzed the effect of ethephon, an ethylene-releasing agent, on the expression of these genes.

Expression of the six genes was upregulated between 1.7- and 5.3-fold following ethephon treatment for 6 h (Fig. 3). Expression of *CsCycA* was upregulated 4.9-fold following ethephon treatment for 0.5 h (Fig. 3). Expression of *CsCycB* was upregulated 2.4- and 1.7-fold following ethephon treatment for 0.5 and 24 h, respectively (Fig. 3). Expression of *CsCDKB* increased 2.7-fold following

Fig. 3 The effects of application of ethephon on expression of cell cycle-related genes (*CsCycA*, *CsCycB*, *CsCycD3;1*, *CsCycD3;2*, *CsCDKA*, and *CsCDKB*) in the shoot apices of monoecious cucumber plants at the four-leaf stage (left-hand side). The ratio of the signal densities of each cell cycle-related gene to a control gene *CsGAPDH* is shown (right-hand side). The inset numbers in the graph on the right-hand side of the figure are the ratios of the signal densities of each cell cycle-related gene between ethephon-treated plants and control plants.

ethephon treatment for 24 h (Fig. 3). By contrast, *CsCycD3;1* expression decreased by half following ethephon treatment for 24 h (Fig. 3). In summary, expression of all six genes was upregulated within 6 h of ethephon treatment, with expression of *CsCycA* and *CsCycB* being upregulated especially fast (within 0.5 h of ethephon treatment). This suggests that these six cell cycle-related genes are involved in the formation of female flowers in monoecious cucumber plants.

Analysis of gene expression suggested that, at a molecular level, cell cycle activity was high in the ACASP in female flower buds of monoecious cucumber plants (Figs. 2, 3). In this case, the epidermal cell number in arrested stamen primordia (ASP) (Fig. 1C), compared with other organs, should increase in a later developmental stage of female flower buds. To test this hypothesis, the epidermal cell size and number in stamens and ASP was compared with that in another organ, the petals, in male and female flower buds at stage II.

In male flower buds at stage II, stamens were well developed (Fig. 4A, B; white and black arrowheads). Epidermal cell size in stamens (Fig. 4C; black arrowhead) did not differ significantly from that in petals (Fig. 4D; black arrowhead) in male flower buds. Stamen primordia were arrested in female flower buds at stage II (Fig. 4E, F; white and black arrowheads). Epidermal cells in ASP (Fig. 4G; black arrowhead) were smaller than those in petals (Fig. 4H; black arrowhead) in female flower buds.

The sizes of epidermal cells in petals and stamens (or ASP) in male and female flower buds were used to calculate the number of epidermal cells per mm² of tissue. In male flower buds, the epidermal cell number per mm² did not differ significantly between petals and stamens (Fig. 5); however, in female flower buds, the epidermal cell number per mm² in the ASP was much greater than that in petals (Fig. 5). As the epidermal cell number per unit area in ASP

was greater in female flower buds, the hypothesis was supported. These results strongly support the conclusion that, at the cellular level, cell division is maintained at a high rate in the ACASP in female flower buds. In general, cell division rates are high in places where cells are actively generated, such as the shoot and root apical meristems in higher plants (Grandjean et al., 2004; McCulloch and Till, 2005; Scheres, 2007; Morrison and Spradling, 2008). To our knowledge, these results are the first evidence that cell division is maintained at a high level in arrested tissue in higher plants. The comparison of cell sizes between ASP in female flower buds and developed stamens in male flowers (Fig. 4C, G) indicated that cell growth was restrained at ASP in female flower buds. Thus, the condition of the cells at ASP in female flower buds of monoecious cucumber plants was estimated as follows: although cell division rates remained high, cell growth was restrained.

In monoecious plants, the process of sex differentiation often involves cell death, resulting in the selective abortion of either stamen or pistil primordia (Wu and Cheung, 2000). Hao et al. (2003) demonstrated in cucumber that the arrest of stamen primordia involves programmed cell death (PCD) in female flowers; however, Bai et al. (2004) concluded that PCD might not be involved in the arrest of pistil primordia in male flowers. Isolation of the gene *Cs1-matrix metalloproteinase* (*MMP*) in cucumber showed its expression is involved in triggering or regulating PCD in cotyledons (Delorme et al., 2000). MMPs are a major group of enzymes that degrade the extracellular cell matrix (ECM) and are thought to play an important role in pathological processes such as arthritis, cancer and fibrotic diseases in animals (Woessner, 1998). We previously showed strong *Cs1-MMP* expression in the ACASP in female flower buds at stage I-III and in the ACAPP in male flower buds at stage I-III of monoecious cucumber plants (Yamasaki and Manabe, 2009). Such studies

Fig. 4 Cell sizes in monoecious cucumber flower buds. A, B. Developed stamens (white and black arrowheads) of male flower buds at stage II. C. Epidermal cells (black arrowhead) in developed stamens of male flower buds at stage II. D. Epidermal cells (black arrowhead) in petals of male flower buds at stage II. E, F. ASP (white and black arrowheads) in female flower buds at stage II. White square in figure E shows ACASP. G. Epidermal cells (black arrowhead) in ASP of female flower buds at stage II. H. Epidermal cells (black arrowhead) in petals of female flower buds at stage II.

Fig. 5 Epidermal cell number per mm² in petals and stamens of male flower buds and in petals and ASP of female flower buds at stage II. Values represent the average of 90 epidermal cells from three flower buds $\pm$ standard error. Statistically significant differences were determined using the Student's *t*-test (**: $P < 0.01$ vs. petals).

indicate that PCD is involved in the arrest of stamen primordia in female flower buds of monoecious cucumber plants. Taken together with the results of the current study, it appears that cells under division, restrained growth, and death coexist in the ASP in female flower buds of

monoecious cucumber plants. The following scenario is likely: although cell division activity is maintained at a high rate, cell growth is restrained and, finally, PCD occurs at ASP in female flower buds of monoecious cucumber plants. To understand the precise mechanism of sex differences in monoecious cucumber, it will be necessary to clarify the mutual relationships between cell division activity, restrained cell growth, and PCD in the future.

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