Part II. Analysis of the Regulatory Mechanisms of the Sex Expression by Levels of Expression of 1-Aminocyclopropane-1-Carboxylate Synthase Genes.

Introduction

I have cloned a cDNA (CS-ACS2) for ACC synthase, whose expression was correlated with the sex expression in cucumber plants, as described in part I. On the other hand, Trebitsh *et al.* (1997) reported the cloning of another ACC synthase gene, designated as *CS-ACS1* from cucumber plants. Although no *CS-ACS1* mRNA was detected at intact apices of monoecious cucumber line, auxin induced the expression of *CS-ACS1* transcript at apices as well as the production of female flowers in a monoecious line. A similar expression pattern of *CS-ACS1* by the auxin treatment was also observed at apices of a gynoecious line. Furthermore, they reported that the CS-ACS1 probe hybridized to a single band in the monoecious line, and to a pair of homologus bands in the gynoecious line by southern blot analysis. It was interpreted as a duplication of the *CS-ACS1* gene in the gynoecious genotype and the gynoecious-specific putative copy of *CS-ACS1* was designated as *CS-ACS1G*. Furthermore, they showed that the *CS-ACS1G* was mapped on the same position as the *F* locus (Trebitsh *et al.* 1997).

The partially dominant allele at the *F* locus controls femaleness (Pierce and Wehner 1990). The intensity of the female phase increases as the dosage of dominant alleles at the *F* locus increases from zero to two (Shifriss 1961, Shifriss *et al.* 1964). It is suggested that a similarity exists between the action of the *F* gene and the ethylene action. A high correlation exists between ethylene evolution and female sex expression (Rudich *et al.* 1972). Inhibitions of ethylene action or biosynthesis repress expression at the *F* locus (Atsmon and Tabbak 1979). Based on the physiological involvement of ethylene in the regulation of female sex expression in cucumber described above, Trebitsh *et al.* (1997) suggested a possibility

that the *CS-ACS1G* gene is the *F* locus itself. However, the correlation between *CS-ACS1G* expression and the sex phenotype remained to be explained. Although the CS-ACS1 probe hybridized to both the *CS-ACS1* and *CS-ACS1G* genes, they could not detect the expression of both of them at intact shoot apices of gynoecious cucumber and the difference of the expression of *CS-ACS1* between the monoecious and the gynoecious by RNA blot analysis with total RNA (Trebitsh *et al.* 1997)

To elucidate the relationship between the expression of *CS-ACS1G* and the sex phenotype, I have examined the expression of *CS-ACS1/CS-ACS1G* transcripts at intact apices of cucumber plants using isogenic monoecious (*ff*) and gynoecious (*FF*) lines by RNA blot analysis with poly $(A)^+$ RNA and DNA blot analysis of a RT-PCR reaction. Furthermore, I compared the expression of *CS-ACS1/CS-ACS1G* with that of *CS-ACS2* at intact apices of isogenic monoecious (*ff*) and gynoecious (*FF*) lines to clarify the relationship between the expression of the different ACC synthase genes and the development of female flower.

In the first of this part, I show that both *CS-ACS1/CS-ACS1G* and *CS-ACS2* transcripts were expressed at the apices of gynoecious line and only *CS-ACS2* transcript was expressed at the apices of monoecious line, and discuss a possibility that the transcript detected with the use of CS-ACS1 probe in this experiment is the transcript of the *CS-ACS1G* gene.

Next, I examined the relationship between gibberellin and ethylene in the regulation of sex expression in cucumber plants. Treatment with gibberellin promotes the formation of male flowers (Wittwer and Bukovac 1958, Galun 1959). Inhibitors of gibberellin synthesis suppress male flower development and promote the formation of female flowers (Frankel and Galun 1977, Mitchell and Wittwer 1962, Yin and Quinn 1995). Although the mechanisms of regulation of sex expression by gibberellin

are not known, it is speculated that gibberellin acts more upstream than ethylene on the sex determination in cucumber plants (Yin and Quinn 1995). Therefore, I examined effects of gibberellin on the expression of *CS-ACS1G* and *CS-ACS2* at apices of gynoecious (*FF*) and monoecious (*ff*) lines to examine whether gibberellin regulates the endogenous production of ethylene *via* the expression of ACC synthase genes.

Materials and Methods

Plant Materials

The seeds of isogenic monoecious (*Cucumis sativus* L.; *ff*) and gynoecious (*C. sativus* L.; *FF*) cucumber lines were derived from cucumber plants (*C. sativus* L., cv. Rensei; *Ff*) at Tohoku Seed Co. (Utsunomiya, Japan). The seeds of F1 (*Ff*) progeny resulting from these two isogenic lines were also prepared at the Tohoku Seed Company. The F1 line had a higher degree of female sex expression. The seeds of monoecious cucumber cultivar (*C. sativus* L., cv. Shimoshirazu) and gynoecious cucumber cultivar (*C. sativus* L., cv. Rensei) were obtained from a local market, all seeds were germinated and grown in soil-filled pots in a greenhouse at 25 °C with 12 h of light per day. After growth of the seedlings, the sex of each flower on the first 25 nodes was examined and classified as male or female. A node was designated male if it had at least one male flower, and it was designated female if only female flowers were present on it.

To analyze the gene expression at intact apices of the isogenic monoecious, gynoecious and F1 lines, the apices were cut off from the seedlings just below the youngest leaf at indicated stages of growth, frozen immediately in liquid nitrogen, and stored at - 80°C prior to extraction of nucleic acids. For studying the localization of expression of *CS-ACS2* transcript in the apex of a cucumber plant, the apices of 25-day-old Rensei and Shimoshirazu plants were prepared as described above, and floral buds and unexpanded leaves from the eighth node to the sixteenth node were excised from the apices under a light-microscope, frozen immediately in liquid nitrogen, and stored at - 80°C prior to extraction of nucleic acids.

For studying the effect of treatment with gibberellin on the expression of *CS-ACS1/CS-ACS1G* and *CS-ACS2* transcripts at the apices of monoecious and gynoecious isogenic lines, the apices were treated with distilled water or solution with various GA_4 concentrations (10^{-6} M, 10^{-5} M and 10^{-4} M) for 8 days at 10 days after planting. The treated apices of 18-day-old plants were prepared as described above, frozen immediately in liquid nitrogen, and stored at - 80°C prior to extraction of nucleic acids.

Sex Expression in Flowers

To study the timing of sex conversion of the first flower from the female to the male after treatment with AVG, the apices of the gynoecious line were treated with 100 μM AVG in 0.2% (v/v) Tween 20 for a day at 12, and 15 days after planting, respectively. The effects of gibberellin on the sex expression of monoecious and gynoecious lines were examined by treating the apices of both lines with 10^{-4} M GA_4 for 8 days at 10 days after planting, respectively. After growth of the seedlings, the sex of each flower on the nodes was then examined and classified as male or female. A node was designated male if it had at least one male flower and it was designated female if only female flowers were present on it.

Isolation of RNA

The conditions for the isolation of RNA were the same as described in Materials and Methods of Part I.

Preparation of a cDNA Probe

Because the sequence of CS-ACS3 obtained in this study (Part I) is identical to that of *CS-ACS1* (Trebitsh *et al.* 1997), the insert of CS-ACS3 was used as the CS-ACS1 probe. The inserts of CS-ACS2 and CS-ACS3 were isolated from the plasmid and labeled as described in Materials and Methods of Part I.

RNA Gel Blot Analysis

Poly (A)+RNA (2 μg per lane) was subjected to electrophoresis on a formaldehyde gel and transferred to a GeneScreen Plus membrane (Du Pont, Boston, MA, USA) as described in Materials and Methods of Part I. The membrane was hybridized with ^{32}P-labeled CS-ACS1 probe, and conditions for the hybridization and wash were the same as described in Part I. The membranes were washed with boiling 0.01x SSC (1x SSC is 0.15 M NaCl, 15 mM sodium citrate) and 0.01% SDS to dehybridize the probe. The membrane was rehybridized with ^{32}P-labeled CS-ACS2 probe. Autoradiographs were obtained using a Bio-Imaging Analyzer (BAS 5000; Fuji Photo Film Co., Tokyo, Japan). Equal amounts of mRNA in each lane were verified by hybridization with ^{32}P-labeled ubiquitin.

Expression Analysis by RT-PCR

The following pairs of oligonucleotides were used: *CS-ACS1*-specific primers CS1-S1 (5'-GGGTCTTGCCGAGAATCAACTAACA-3') spanning positions 15 to 33 and 146 to 151 of the CS-ACS1 genomic sequence, and CS1-A1 (5'-GTTGGGTGACTTGGAAGCCGTTGGA-3') spanning position 619 to 643 of the *CS-ACS1* genomic sequence (Trebitsh *et al.* 1997); *CS-ACS2*-specific primers CS2-S334 and CS2-A776 that

sequences were shown in part I. A detailed description of the RT-PCR condition was given in the part I. The PCR products were analyzed by 2% agarose gel electrophoresis and blotted to GeneScreen Plus membranes (Du Pont, Boston, MA, USA). The blots were hybridized with the CS-ACS1 probe and the CS-ACS2 probe, respectively, as previously described.

Results

Time Course of the Expression of ACC Synthase Genes at the Apices of Isogenic Gynoecious and Monoecious Cucumber Lines

A pair of isogenic gynoecious (*FF*) and monoecious (*ff*) cucumber lines were used to elucidate the relationship between the expression of ACC synthase genes (*CS-ACS1/CS-ACS1G* and *CS-ACS2*) and sex expression in flowers of cucumber plants. Figure II-1 shows the patterns of sex expression in flowers of these lines. The gynoecious line produced only female flowers, whereas the monoecious line produced male flowers on several lower nodes, followed by a mixed phase of male and female flowers (Fig. II-1). As shown in Figure II-2, no male flowers were induced on gynoecious line that was treated with AVG at 12 days after planting. The application of AVG to the apices of 15-day-old gynoecious line induced male flowers on lower nodes including the first flower (Fig. II-2). These results suggest that the floral primordia was not formed at the apices of 12-day-old seedlings and floral buds which sexes were not determined yet were first formed at the apices of 15-day-old.

Figure II-3 shows the time course of the expression of *CS-ACS1/CS-ACS1G* and *CS-ACS2* transcripts at the apices of these isogenic gynoecious and monoecious lines. Although the transcript of *CS-ACS1/CS-ACS1G* was not detected at the apices of monoecious line, the transcript was detected at the apices of gynoecious line at all stages examined by RNA blot analysis with poly $(A)^+$ RNA (Fig. II-3). The same results were also obtained by DNA blot analysis of a RT-PCR reaction on total RNA isolated from the apices of isogenic gynoecious and monoecious lines (Fig. II-4). To elucidate the physiological function of the *CS-*

ACS1/CS-ACS1G, the transcript was examined at the apices of F1 seedlings that were generated from a mating between the isogenic gynoecious and monoecious lines. As shown in Figure II-4, the *CS-ACS1/CS-ACS1G* transcript was also detected at the apices of F1 seedlings at all stages examined. The levels of *CS-ACS1/CS-ACS1G* transcript were one half those of the isogenic gynoecious line (Fig. II-4). As shown in Figures II-3 and II-4, the *CS-ACS2* transcript was not detectable at the apices of 12-day-old seedlings of the gynoecious, monoecious and F1 cucumber lines. The *CS-ACS2* transcript was detected at the apices of 15-day-old seedlings of gynoecious line and the levels increased up to 21 days after planting. Although the *CS-ACS2* transcript was detected at the apices of 18-day-old and 21-day-old seedlings of monoecious line, the levels of the transcript were low compared to those of the gynoecious line. The timing and the levels of expression of the *CS-ACS2* transcript at the apices of F1 seedlings were nearly the same as those of the gynoecious line (Fig. II-4).

Effects of Silver Thiosulphate on the Expression of *CS-ACS1/CS-ACS1G* and *CS-ACS2* at the Apices of Gynoecious and Monoecious Lines

The apices of gynoecious and monoecious lines were treated with 1 mM of STS, an inhibitor of ethylene action, for 8 days beginning at 10 days after planting. Although the level of *CS-ACS1/CS-ACS1G* at the apices of gynoecious lines was not affected by the treatment of STS, the levels of the *CS-ACS2* transcript at the apices of both gynoecious and monoecious lines at 18 days after planting decreased by the application of STS to the apices (Fig. II-5).

Effects of Gibberellin on the Expression of *CS-ACS1/CS-ACS1G* and *CS-ACS2* at the Apices of Gynoecious and Monoecious Lines

The apices of gynoecious and monoecious lines were treated with 10^{-6} M, 10^{-5} M and 10^{-4} M GA_4, respectively, for 8 days beginning at 10 days after planting. The treatment of monoecious line with 10^{-4} M GA_4 increased in a proportion of male flowers on lower nodes (Fig. II-6). However, the treatment of gynoecious line with 10^{-4} M GA_4 resulted in the abortion of floral buds on lower nodes (Fig. II-6). The dose-dependent decrease in the expression of *CS-ACS2* transcripts was observed at the apices of both gynoecious and monoecious lines at 18 day after planting (Fig. II-7). However, the level of *CS-ACS1/CS-ACS1G* at the apices of gynoecious lines was not affected by the treatment of GA_4 (Fig. II-7).

Discussion

Sex expression in cucumber plants is mainly determined by two loci, namely, *F* and *m*. The *F* gene is a partially dominant gene that controls femaleness (Pierce and Wehner 1990). The dominant allele at the m locus specifies unisexual flowers. A line dominant for *F* (gynoecious) has a higher degree of female sex expression than an isogenic line recessive for this gene (monoecious), as in *MM* or *Mm* plants. As shown in Figure II-1, the isogenic gynoecious line (*FF*) used in this experiment had a high degree of female expression than the isogenic monoecious line (*ff*).

Trebitsh *et al.* (1997) have reported the identification of an ACC synthase genomic sequence in cucumber (*CS-ACS1*) that is auxin-inducible in both monoecious and gynoecious cucumber species. Monoecious cucumber possesses a single copy of this gene, whereas gynoecious line possesses at least one additional copy (*CS-ACS1G*) and the *CS-ACS1G* gene is closely linked to the *F* locus (Trebitsh *et al.* 1997). However, they did not show the expression of *CS-ACS1G* transcript at intact apices of gynoecious cucumber. Although they could not detect the expression of *CS-ACS1* transcript at intact apices of gynoecious cucumber plants by RNA blot analysis with total RNA (Trebitsh *et al.* 1997), I could detect the gynoecious-specific expression of the *CS-ACS1/CS-ACS1G* transcript by both RNA blot analysis with poly $(A)^+$ RNA (Fig. II-3) and DNA blot analysis of a RT-PCR reaction on total RNA prepared from the apices of the isogenic gynoecious cucumber line (Fig. II-4). The transcript of *CS-ACS1/CS-ACS1G* was detected at the apices of isogenic gynoecious line and not detected at those of isogenic monoecious line at all stages examined. The reason for this discrepancy may be due to the difference in detectable level of the RNA by blot analysis used in the experiments.

As shown in Figures II-3 and II-4, the *CS-ACS1/CS-ACS1G* transcript was detected only at the apices of the isogenic gynoecious cucumber line. In these experiments, I used CS-ACS1 as the probe for the detection of the transcript by RNA gel blot analysis and DNA blot analysis of a RT-PCR reaction. However, Trebitsh *et al.* (1997) reported that the CS-ACS1 probe hybridized to both the *CS-ACS1* and *CS-ACS1G* genes. Therefore, it is not clear whether the detected transcript by the CS-ACS1 probe is the *CS-ACS1* transcript or the *CS-ACS1G* transcript. However, the following results suggested the possibility that the transcript detected with the use of the CS-ACS1 probe at the apices of isogenic gynoecious line was the transcript of the *CS-ACS1G* gene. (i) Trebitsh *et al.* (1997) reported that the monoecious cucumber line dose not possess the *CS-ACS1G* gene, though both isogenic gynoecious and monoecious lines commonly possess the *CS-ACS1* gene. (ii) I could not detect the *CS-ACS1/CS-ACS1G* transcript at intact apices of the isogenic monoecious line but could detect it at those of the isogenic gynoecious line (Figs. II-3 and II-4). (iii) Trebitsh *et al* (1997) reported that the *CS-ACS1G* gene is closely linked to the *F* locus. (iv) I showed that the quantity of the transcript detected at the apices of F1 (*Ff*) seedlings is one half that of the isogenic gynoecious (*FF*) line (Fig. II-4). Therefore, I consider that the transcript detected with the use of the CS-ACS1 probe at the apices of both isogenic gynoecious and the F1 seedlings is the transcript of the *CS-ACS1G* gene (Figs. II-3 and II-4).

The transcript of *CS-ACS2* was detected at the apices of the isogenic monoecious cucumber line and it was also detected at those of the isogenic gynoecious and the F1 seedlings (Figs. II-3 and II-4). It is interesting to note that the levels of transcript of *CS-ACS2* were higher at the apices of gynoecious line than at those of monoecious line (Figs. II-3

and II-4). I used isogenic gynoecious (*FF*) and monoecious (*ff*) lines that differ in alleles of a *F* locus but share a similar genetic background in this experiment. Therefore, it is suggested that the expression of *CS-ACS2* at apices of gynoecious line is regulated by the *F* locus. Furthermore as described above, it is considered that the *CS-ACS1G* is expressed at the apices of gynoecious line. From these results, there is a possibility that the expression of *CS-ACS2* transcript is induced by ethylene that is produced by the expression of *CS-ACS1G*. To examine this possibility, I studied the effect of STS, an inhibitor of ethylene action, on the expression of *CS-ACS2* at the apices of gynoecious line. The levels of transcript of *CS-ACS2* decreased as a result of STS treatment at the apices of gynoecious line (Fig. II-5). These results indicate that *CS-ACS2* is an ethylene-inducible gene and that the expression of *CS-ACS1G* transcript at apices of the gynoecious line hastens the timing of expression of the *CS-ACS2* transcript and increases the levels of expression of the transcript *via* ethylene production at the apices compared to the isogenic monoecious line.

At the apices of monoecious line, only the transcript of *CS-ACS2* was detected and the levels of *CS-ACS2* mRNA were developmentally regulated (Figs. II-3 and II-4). In Part I, I showed that the timing and the levels of expression of the *CS-ACS2* transcript at the apices of monoecious cucumber cultivars were correlated with the development of female flowers on the nodes, and that *CS-ACS2* transcript is expressed only in limited floral buds that will develop into female flowers. These results suggest that the sex expression of a monoecious line is regulated by the level of *CS-ACS2* mRNA at the apex. The levels of transcripts of *CS-ACS2* decreased as a result of STS treatment at the apices of monoecious line as well as isogenic gynoecious line (Fig. II-5). These results suggest that the expression of *CS-ACS2* transcript at flowers that will develop into female

flowers is enhanced by ethylene. Several papers reported the positive feedback of ethylene on the expression of genes encoding ACC synthase (Rottmann *et al.* 1991, O'Neill *et al.* 1993, Park *et al.* 1992, Woodson *et al.* 1992, Nakatsuka *et al.* 1997). From these results, it is considered that the expression of *CS-ACS2* transcript is subject to positive feedback regulation *via* ethylene production to make the distinct difference in the level of ethylene at flowers that will develop into female flowers from that at flowers that will develop into male flowers.

I showed the dose-dependent decrease in the levels of *CS-ACS2* transcripts at the apices of monoecious and gynoecious lines by treatment with gibberellin (Fig. II-7). These results suggest that gibberellin is one of the regulator of the expression of *CS-ACS2* transcript. The treatment of gibberellin promotes the formation of male flowers (Galun 1959), and the treatment of inhibitor of gibberellin synthesis promotes the formation of female flowers (Mitchell and Wittwer 1962, Frankel and Galun 1977). In fact, the treatment of monoecious line with 10^{-4} M GA_4 increased in a proportion of male flowers (Fig. II-6). Furthermore, Yin and Quinn (1995) suggested that gibberellin acts more upstream than ethylene. The expression of *CS-ACS2* correlated with the sex expression in monoecious line as shown in Part I. These results suggest that gibberellin acts on the regulation of sex expression by suppressing the expression of *CS-ACS2* transcript. Furthermore, an inverse correlation exists between the level of endogenous gibberellin and the level of *CS-ACS2* transcript at apices of monoecious cucumber plants. Hemphill *et al.* (1972) extracted gibberellin from shoot apices of monoecious plants at various developmental stages and observed that the level of gibberellin increased at apices 1 week after germination and then decreased. The expression of *CS-ACS2* at apices of monoecious line was low at early developmental stages (12-15 days after

planting) and then increased (Fig. II-3). The monoecious line produced only male flowers on lower nodes (Fig. II-1). These results suggest that the high level of endogenous gibberellin suppressed level of *CS-ACS2* transcript, which, in turn, decreased ethylene production from apices of monoecious line at early developmental stages and then decreased level of gibberellin induced expression of *CS-ACS2*. On the other hand, the level of *CS-ACS1G* at the apices of gynoecious line was not affected by the treatment of gibberellin (Fig. II-7). In this study, I could not evaluate the effects of gibberellin on the sex expression of gynoecious line, since the treatment with 10^{-4} M GA_4 resulted in the abortion of floral buds (Fig. II-6).

Figure II-1. The patterns of sex expression in flowers of the isogenic gynoecious (*FF*) and monoecious (*ff*) cucumber lines. Plants were grown in soil-filled pots in a growth chamber with 12 h of light per day at 25 °C. The node number indicates the position of individual nodes along the main shoot. Closed circles, nodes with female flowers; open circles, nodes with male flowers; no circles, vegetative nodes. Data from five plants are presented in each case.

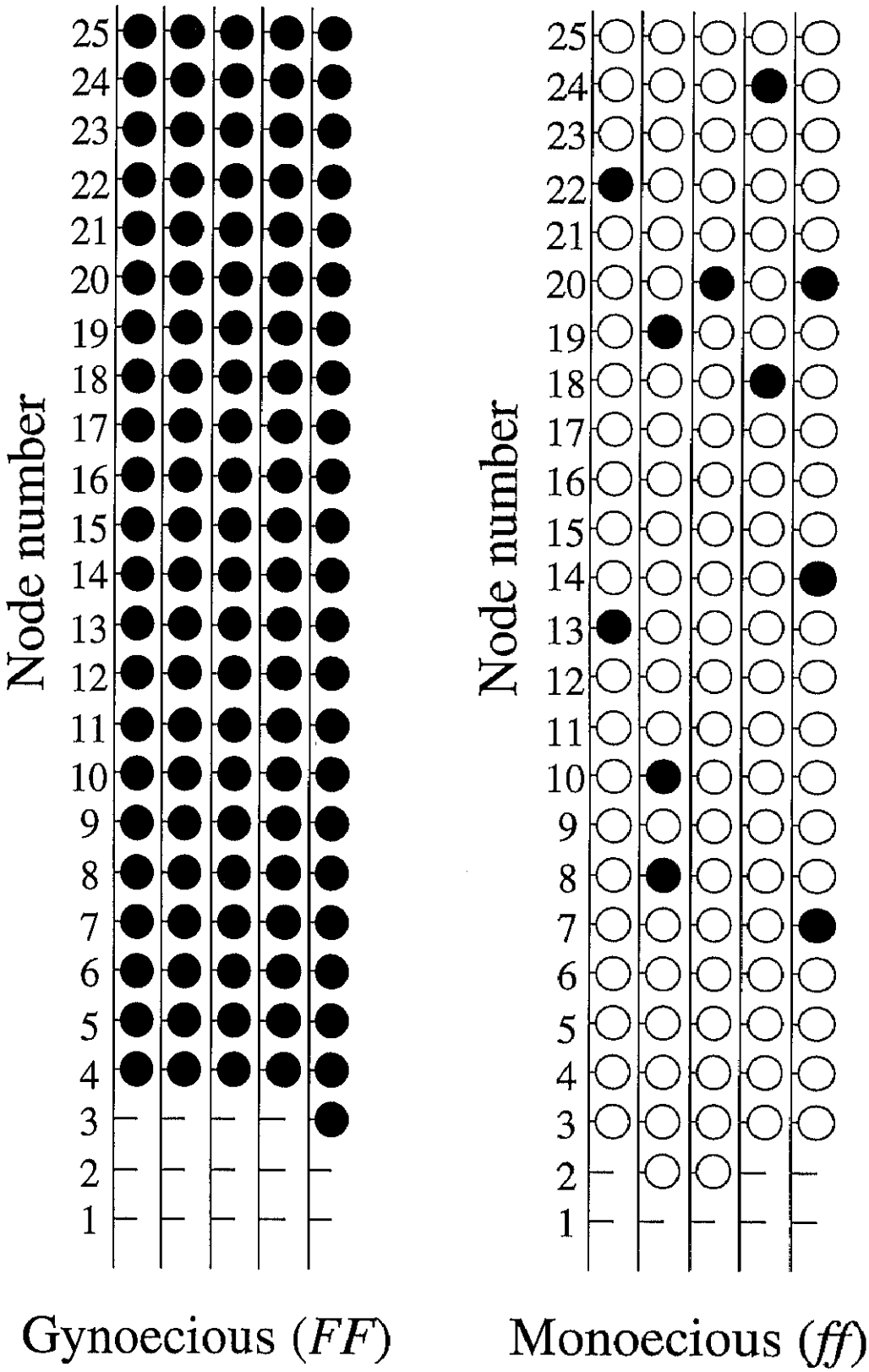

Node number
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Gynoecious (FF)
Node number
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Monoecious (ff)

Figure II-2. The timing of sex conversion of flowers from female to male by treatment of apices with AVG. AVG (100 μM) was applied to the apices of gynoecious line of indicated ages for a day. The node number indicates the position of individual nodes along the main shoot. Closed circles, nodes with female flowers; open circles, nodes with male flowers; no circles, vegetative nodes.

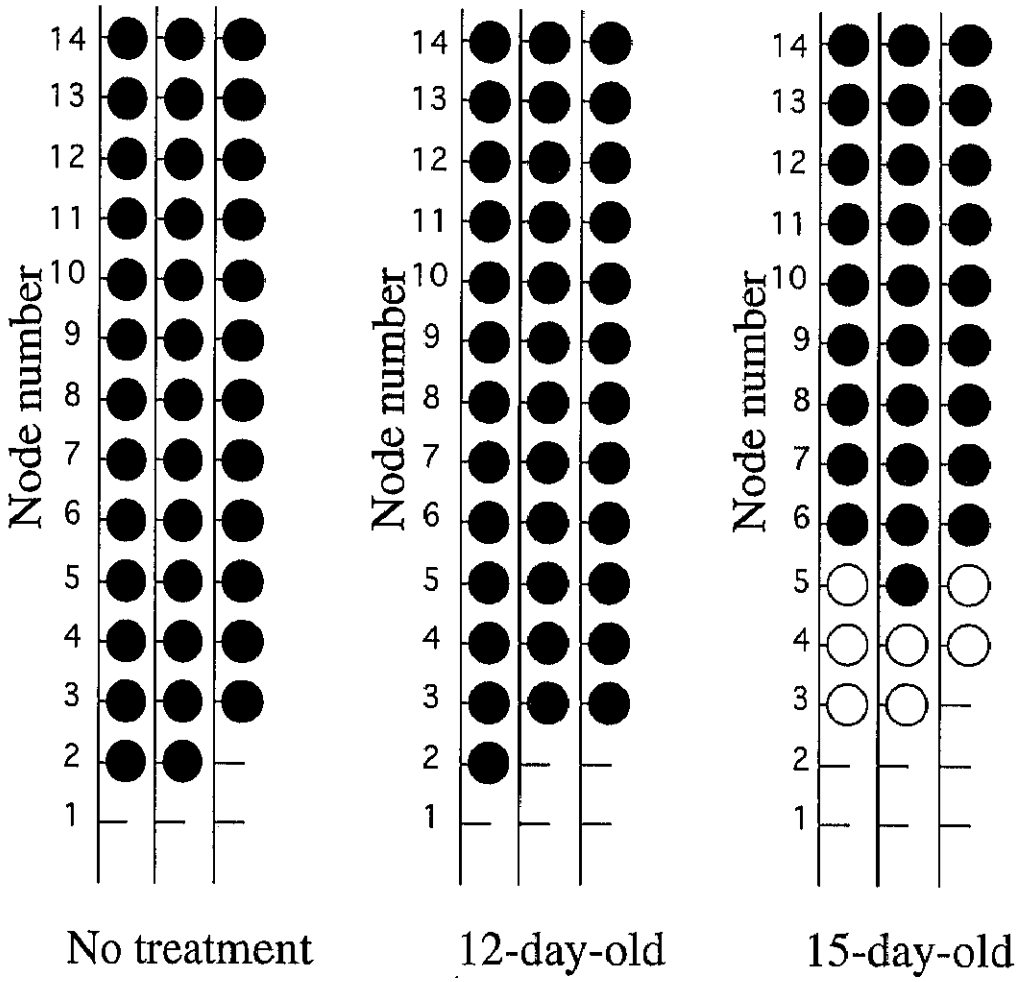

Node number
14
13
12
11
10
9
8
7
6
5
4
3
2
1
No treatment
12-day-old
15-day-old

Figure II-3. Time course of the expression of *CS-ACS1*/*CS-ACS1G* and *CS-ACS2* transcripts at the apices of the isogenic gynoecious (*FF*) and monoecious (*ff*) cucumber lines. The plants were grown under the conditions described in Fig. 1. Poly (A)+ RNA was extracted from the apices of gynoecious and monoecious lines when the plants were 12-day-old (i.e., at the first leaf stage), 15-day-old (i.e., at the two-leaf stage), 18-day-old (i.e., still at the two-leaf stage) and 21-day-old (i.e., at the three-leaf stage). Extracted poly (A)+ RNA (2 μg) was separated on a formaldehyde-containing agarose gel, transferred to a nylon membrane and allowed to hybridize with the CS-ACS1, CS-ACS2 and ubiquitin probes, respectively.

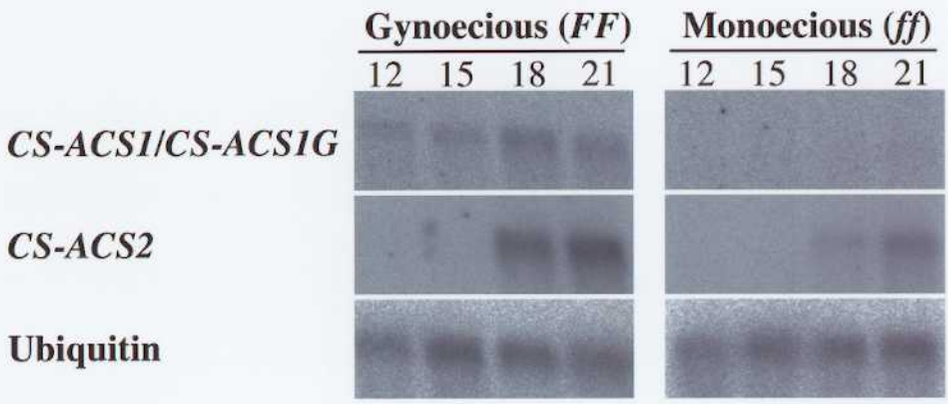

Gynoecious (FF)
Monoecious (ff)
12 15 18 21
12 15 18 21
CS-ACS1/CS-ACS1G
CS-ACS2
Ubiquitin

Figure II-4. Time course of the expression of *CS-ACS1/CS-ACS1G* and *CS-ACS2* transcripts at the apices of the isogenic gynoecious (*FF*), monoecious (*ff*) and F1 cucumber lines. The plants were grown under the conditions described in Fig. 1. Total RNA was extracted from the apices of these lines when the plants were 12-day-old (i.e., at the first leaf stage), 15-day-old (i.e., at the two-leaf stage), 18-day-old (i.e., still at the two-leaf stage) and 21-day-old (i.e., at the three-leaf stage). CS-ACS1 and CS-ACS2 cDNAs were amplified from the total RNA by RT-PCR using a pair of CS-ACS1-specific primers (CS1-S1 and CS1-A1) and a pair of CS-ACS2-specific primers (CS2-S334 and CS2-A776), respectively, under the conditions described in "Materials and Methods". The PCR products were separated on agarose gels, transferred to nylon membranes and allowed to hybridize with the CS-ACS1 and CS-ACS2 probes, respectively.

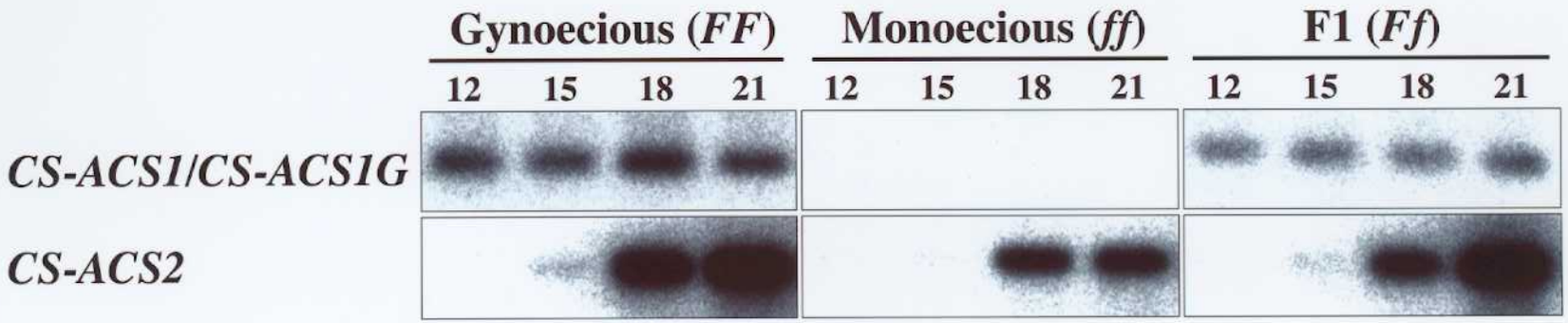

Gynoecious (FF)
Monoecious (ff)
F1 (Ff)
12 15 18 21
12 15 18 21
12 15 18 21
CS-ACS1/CS-ACS1G
CS-ACS2

Figure II-5. Effects of treatment with silver thiosulphate (STS) on the levels of *CS-ACS1/CS-ACS1G* and *CS-ACS2* mRNA at the apices of isogenic gynoecious (*FF*) and monoecious (*ff*) cucumber lines. The plants were treated as described in "Materials and Methods". Total RNA was extracted from the apices of 18-day-old plants. CS-ACS1 and CS-ACS2 cDNAs were amplified from the total RNA by RT-PCR using a pair of CS-ACS1-specific primers (CS1-S1 and CS1-A1) and a pair of CS-ACS2-specific primers (CS2-S334 and CS2-A776), respectively, under the conditions described in "Materials and Methods". The PCR products were separated on agarose gels, transferred to nylon membranes and allowed to hybridize with the CS-ACS1 and CS-ACS2 probes, respectively.

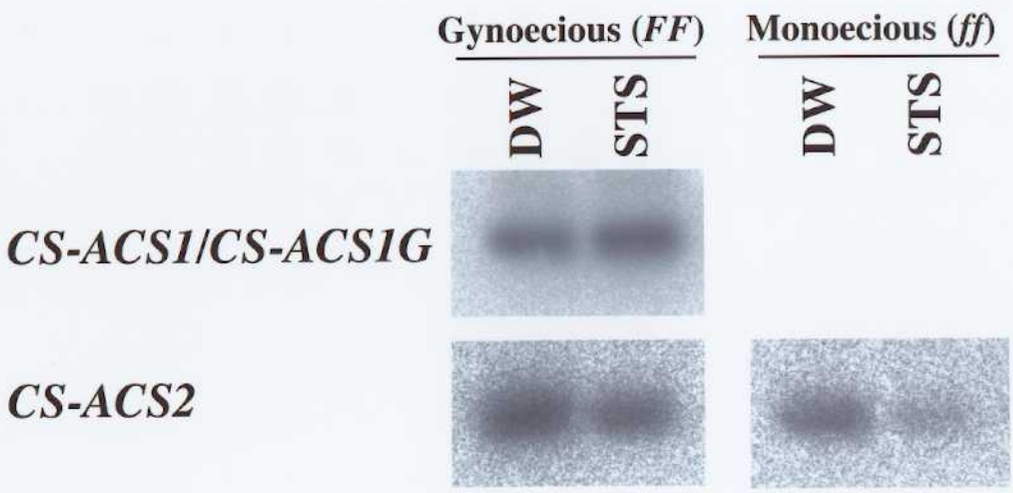

Gynoecious (FF)
Monoecious (ff)
DW
STS
DW
STS
CS-ACS1/CS-ACS1G
CS-ACS2

Figure II-6. Effects of treatment with gibberellin (GA_4) on the sex expression of isogenic (a) monoecious (*ff*) and (b) gynoecious (*FF*) cucumber lines. The plants were treated as described in "Materials and Methods". The node number indicates the position of individual nodes along the main shoot. Closed circles, nodes with female flowers; open circles, nodes with male flowers; no circles, vegetative nodes.

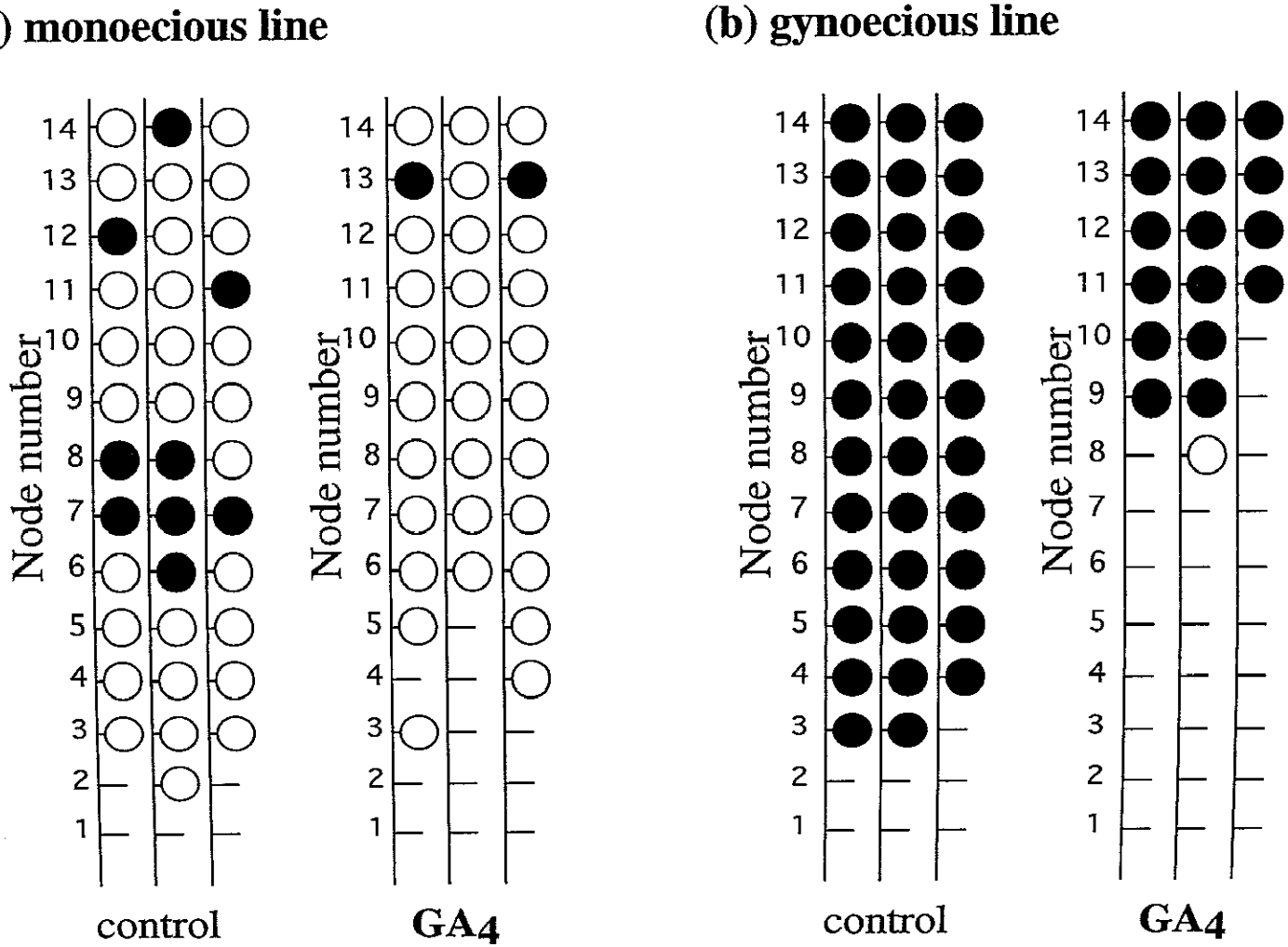
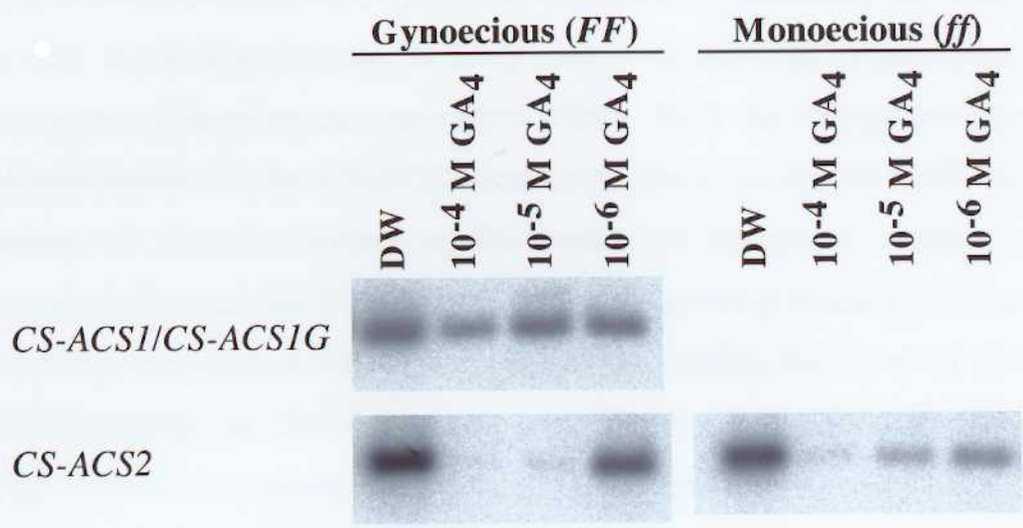

(a) monoecious line
(b) gynoecious line
Node number
14
13
12
11
10
9
8
7
6
5
4
3
2
1
control
GA4
control
GA4

Figure II-7. Effects of treatment with gibberellin (GA_4) on the levels of *CS-ACS1/CS-ACS1G* and *CS-ACS2* mRNA at the apices of isogenic gynoecious (*FF*) and monoecious (*ff*) cucumber lines. The plants were treated as described in "Materials and Methods". Total RNA was extracted from the apices of 18-day-old plants. CS-ACS1 and CS-ACS2 cDNAs were amplified from the total RNA by RT-PCR using a pair of CS-ACS1-specific primers (CS1-S1 and CS1-A1) and a pair of CS-ACS2-specific primers (CS2-S334 and CS2-A776), respectively, under the conditions described in "Materials and Methods". The PCR products were separated on agarose gels, transferred to nylon membranes and allowed to hybridize with the CS-ACS1 and CS-ACS2 probes, respectively.


Gynoecious (FF)
Monoecious (ff)
DW
10-4 M GA4
10-5 M GA4
10-6 M GA4
DW
10-4 M GA4
10-5 M GA4
10-6 M GA4
CS-ACS1/CS-ACS1G
CS-ACS2