Abbreviations

ACC	1-aminocyclopropane-1-carboxylate
AVG	aminoethoxyvinylglycine
bp	base pairs
cDNA	complementary deoxyribonucleic acid
cv.	cultivar
Da	dalton
dCTP	deoxy cytidine 5' triphosphate
Dnase	deoxyribonuclease
GA	gibberellic acid
IAA	indole-3-acetic acid
MMLV	moloney murine leukemia virus
mRNA	messenger ribonucleic acid
PCR	polymerase chain reaction
RACE	rapid amplification of cDNA ends
RT-PCR	reverse transcription-polymerase chain reaction
SDS	sodium dodecyl sulfate
STS	silver thiosulphate
v/v	volume/volume
w/v	weight/volume

Abstract

Sex determination systems in plants, leading to unisexuality as monoecy and dioecy, are one of the strategies to promote outcrossing and avoid inbreeding. In several species with unisexual flowers, it is suggested that the sex of an individual or of a flower is determined by the regulation of the levels of endogenous plant hormones. However, molecular mechanisms of the regulation of the levels of hormones are not clarified yet.

The sex expression in cucumber (*Cucumis sativus* L.) plants is determined by genotype and the environmental conditions under which plants are grown. The sex expression in flowers of cucumber plants is also regulated by levels of ethylene at the apex of cucumber plants. In the study described in this thesis, I examined the regulatory mechanisms of the levels of ethylene at the apices of cucumber plants and showed that the expression of genes for 1-aminocyclopropane-1-carboxylate (ACC) synthase that is a key enzyme in the pathway of ethylene biosynthesis plays an important role for the regulation of sex expression in cucumber plants.

I have cloned a novel cDNA (CS-ACS2) for ACC synthase from the apices of monoecious cucumber plants. The sequence of CS-ACS2 was different from those of cDNA fragments for auxin-induced and wound-induced ACC synthase, respectively. Among of these, only *CS-ACS2* mRNA was detected at the apices of gynoecious cucumber in which female flowers were developing. The expression of the *CS-ACS2* at the apices of three cucumber cultivars with different patterns of sex expression in flowers was examined by RNA gel blot analysis. In these cultivars, both the timing and the levels of expression of the *CS-ACS2* transcript were correlated with the development of female flowers at the nodes.

Furthermore, the timing of the induction of expression of the *CS-ACS2* at the apex corresponded to that of the action of ethylene in induction of the first female flower at the apex of gynoecious cucumber plants. These results suggest that the development of female flowers is regulated by the level of *CS-ACS2* mRNA at the apex.

The expression of *CS-ACS2* was localized in the apices of cucumber plants. The *CS-ACS2* transcript was expressed in limited floral buds that would develop into female flowers. Since ethylene acts on a floral bud to induce the development of a female flower, these results suggested that the expression of *CS-ACS2* transcript at floral buds induces the development of female flowers *via* ethylene production.

Above mentioned results suggest that the *CS-ACS2* is involved in the regulation of sex expression in cucumber plants. On the other hand, Trebitsh *et al.* (1997) reported the identification of another ACC synthase gene (*CS-ACS1*) and showed that monoecious cucumber possesses a single copy of this gene, whereas gynoecious line possesses one additional copy designated *CS-ACS1G*. Furthermore, they showed that the *CS-ACS1G* is closely linked to the *F* locus at which the partially dominant allele controls female sex expression. However, they did not show the expression of *CS-ACS1G* at intact shoot apices of gynoecious cucumber and the difference of the expression of *CS-ACS1* between the monoecious and the gynoecious.

To elucidate whether the *CS-ACS1G* is expressed at the apices of gynoecious cucumber plants, expression analysis was examined at the apices of isogenic gynoecious (*FF*) and monoecious (*ff*) lines with the use of CS-ACS1 probe, because the CS-ACS1 probe was reported to hybridize to both *CS-ACS1* and *CS-ACS1G* gene. Although the transcript was detected only at the apices of the isogenic gynoecious line, it was not detected at the apices of isogenic monoecious line. Furthermore, I showed that the quantity of the transcript detected at the apices of F1 (*Ff*) seedlings

between the isogenic gynoecious and monoecious lines is one half that of the isogenic gynoecious (*FF*) line. Since the gynoecious-specific ACC synthase gene (*CS-ACS1G*) is closely linked to the F locus, it is possible that the transcript detected at the apices of gynoecious line with the use of CS-ACS1 probe is the transcript of the *CS-ACS1G*.

I also examined the expression of *CS-ACS2* at the apices of isogenic gynoecious (*FF*) and monoecious (*ff*) lines. The transcripts of *CS-ACS1G* and *CS-ACS2* were detected at the apices of gynoecious line. On the other hand, only the *CS-ACS2* transcript was detected at the apices of monoecious line. Although the transcript of *CS-ACS2* was detected at the apices of both lines, the levels of transcript of *CS-ACS2* were higher at the apices of gynoecious line than at those of monoecious line. Furthermore, I showed that the expression of *CS-ACS2* was induced by ethylene. These results indicate that the expression of *CS-ACS1G* transcript at apices of the gynoecious line hasten the timing of expression of the *CS-ACS2* transcript and increase the levels of expression of the transcript *via* ethylene production at the apices compared to the isogenic monoecious line.

Although it is suggested that the sex expression in flowers is primarily regulated by the levels of ethylene at the apices of cucumber plants, gibberellin regulates the sex expression in flowers and promotes the development of male flowers. To elucidate whether gibberellin regulates the sex expression through an inhibition of ethylene synthesis, effects of gibberellin on the expression of *CS-ACS1G* and *CS-ACS2* were examined. Although the treatment of the apices with GA_4 did not affect the expression of *CS-ACS1G* at apices of gynoecious line, the treatment with GA_4 induced the development of male flowers following decrease in the level of *CS-ACS2* transcript at the apices of monoecious line.

Based on the results obtained in this study, I proposed a working hypothesis for the regulatory mechanisms of sex expression in monoecious

and gynoecious cucumber plants as follows. In monoecious cucumber plants, sex expression is regulated by the level of *CS-ACS2* transcript at floral buds. A high level of gibberellin at the apices causes a decrease in the level of ethylene *via* suppression of the expression of *CS-ACS2* transcript at floral buds and the floral buds develop into male flowers. Once the expression of *CS-ACS2* mRNA is induced developmentally, the expression of *CS-ACS2* transcript is subject to positive feedback regulation *via* ethylene production. In consequence, the increased level of ethylene induces the development of female flower. On the other hand, gynoecious cucumber plants possess the *CS-ACS1G*. The expression of *CS-ACS1G* at the apices of gynoecious cucumber was detected at the early stage of development. Therefore, it is considered that the expression of *CS-ACS1G* transcript may cause an increase in the level of ethylene and the ethylene produced induces the expression of *CS-ACS2* mRNA at all floral buds. The increased level of ethylene initiates a developmental process of the formation of female flowers.

This is a first report for the identification of genes involved in the biosynthesis of plant hormones that regulate the sex expression in unisexual plants. Furthermore, I could show a possibility that the diversity of sex phenotype is brought about by a combination of the genes that are involved in the biosynthesis of plant hormone.