

## General Introduction

Most animal species are unisexual, with male and female gametes produced directly by meiosis in different individuals. By contrast, the great majority of flowering plants produce hermaphroditic flowers. These flowers contain the male organs (the stamens) and the female organs (the pistils). Sexual reproduction is the major mechanism by which genetic variation is resorted and redistributed, and in plants the basic flower pattern has been modified such that outcrossing is favored (Darwin 1876). About 10% of plant species produce unisexual flowers that have only stamens or pistils, and separate the male and female flowers spatially to promote allogamy (Dellaporia and Calderon-Urrea 1993). The two main sexual conditions in unisexual plants are monoecy, in which male and female flowers are carried on the same individual; and dioecy, in which individuals carry either male or female flowers. In addition to these major classes, plant species exhibit a range of other sexual states (andromonoecious, gynomonoecious and trimonoecious). These types of sexuality are almost equally distributed among monocotyledonous and dicotyledonous genera.

Sex determination in flowering plant occurs during floral development. Floral buds of plants with unisexual flowers are initially bisexual and usually contain stamen and pistil primordia and then they exhibit stage-specific arrest of preformed organ primordia (Dellaporia and Calderon-Urrea 1993). However, such arrest occurs at different stages of floral morphogenesis in various species (Atsmon and Galun 1960, Durand and Durand 1991, Lazarte and Palser 1979, Mohan Ram and Nath 1964, Ye *et al.* 1991). The application of plant hormones induces reversion or modification of sex in some species of dioecious and monoecious plants (Durand and Durand 1984, Frankel and Galun 1977). The sex reversion by

hormonal treatment suggests that the floral primordia are still sexually bipotent and that plant hormones regulate the program for the development of stamen or pistil.

Participation of the endogenous hormones in the regulation of sex has been studied on several species, e.g. *Mercurialis annua*, *Zea mays* and *Cucumis sativus*. In *Mercurialis annua* which is a dioecious species, the sex expression is modified by exogenously applied plant hormones. Cytokinins have a feminizing effect and auxins have a masculinizing effect. High levels of endogenous cytokinin, *trans*-zeatin, accumulates in female shoot apices and *trans*-zeatin riboside rather than the free base accumulates in male shoot apices (Louis *et al.* 1990). Levels of auxin, indole-3-acetic acid, were higher in male flowers than female flowers (Louis *et al.* 1990). *Zea mays* is a monoecious plant, and produces male flowers in the tassel and female flowers in the ear. Mutations affecting the gibberellic acid (GA) biosynthesis pathway (*dwarf* mutations) resulted in shortened internodes in the plant, and many of these mutations also perturb the normal process of stamen abortion in the ear without affecting the gynoecia (Emerson and Emerson 1922). These results suggest that GA may be involved in the process of stamen abortion. In *Zea mays*, feminization of tassels can be induced by short days and by cool nights (Heslop-Harrison 1961). Such feminized tassels have 100 times the normal level of gibberellin (Rood *et al.* 1980). These studies suggest 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 plant hormones are not clarified yet.

Cucumber plants (*Cucumis sativus* L.) have been the subject of extensive research in sex expression. Most traditional cucumber plants are monoecious, although a wide range of sex type (e.g. gynoecious, androecious and hermaphrodite) can be found in the various cucumber

genotype (Malepszy and Niemirowicz-Szczytt 1991). Monoecious plants produce male flowers at the base of the main stem, then they produce male and female flowers on the middle part, and finally female flowers are produced at the top. The gynoeceous type of cucumber plant produces only female flowers. All floral buds have both stamen and carpel primordia at an early developmental stage (Atsmon and Galun 1960) and they develop subsequently into either male or female flowers as shown in Figure 1.

As summarized in Table 1, the sex expression in cucumber plants is determined mainly by three genes, namely, *F*, *a* and *m*. The *F* is a partially dominant gene that controls female expression. The recessive allele at the *a* locus increases male tendency. The dominant allele at the *m* locus specifies unisexual flowers. However, characterizations of these genes have not been achieved.

In cucumber plants, the expression of sex is also influenced by the application of plant hormones; ethylene and inhibitor of gibberellin synthesis promote the formation of female flowers, and gibberellins and inhibitors of both ethylene action and ethylene biosynthesis promote the formation of male flowers (Galun 1959, Mitchell and Witter 1962, McMurray and Miller 1968, Rudich *et al.* 1969, Iwahori *et al.* 1970, Bayer 1976, Frankel and Galun 1977, Atsmon and Tabbak 1979). Based on the opposite effects of the two hormones, gibberellin and ethylene, two alternative hypotheses could be advanced to explain their mechanisms of action. (i) Two-hormone balance hypothesis: Each of the two hormones promotes the development of one sex type and the relative levels of the two hormones will determine the sex of floral organs at a particular site on the plant. (ii) One-hormone hypothesis: The one hormone inhibits the formation of one type of sex organ and, at the same time, promotes that of the other sex organ. Yin and Quinn (1995) reported that ethylene was involved directly in the sex determination in cucumber plants and they

supported the one-hormone hypothesis. They speculated that gibberellin might negatively regulate the levels of endogenous ethylene. In fact, there are some reports to suggest that sex expression in flowers is regulated by levels of ethylene at the apex of cucumber plants. Rudich *et al.* (1972, 1976) reported that a high correlation existed between the evolution of ethylene from apices and the formation of female flowers. Furthermore, Trebitsh *et al.* (1987) reported that the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, a key enzyme in the pathway of ethylene biosynthesis, was higher at the apices of gynoecious plants than those of monoecious plants. These results suggest that levels of enzymes in the ethylene biosynthetic pathway may be important to the regulation of sex expression in cucumber plants.

As mentioned above, cucumber plants are one of the best studied unisexual plants for the regulation of sex expression in higher plants. The genetic system responsible for sex determination is well studied (Malepszy and Niemirowicz-Szczytt 1991) and many methods for molecular biological approach are established in cucumber plants. Therefore, I started a study for the regulatory mechanisms of sex expression in cucumber plants. In this study, I identified and characterized an ACC synthase gene (*CS-ACS2*) that is involved in the development of female flowers in cucumber plants. This finding clarified the role of endogenous ethylene in the regulation of sex expression in cucumber plants. In cucumber plants, another ACC synthase gene (*CS-ACSI*) has been cloned and it is suggested the existence of gynoecious-specific ACC synthase gene (*CS-ACS1G*) that is closely linked to the *F* locus on genotype of gynoecious cucumber plants (Trebitsh *et al.* 1997). Therefore, I examined the expression of two ACC synthase genes (*CS-ACSI* and *CS-ACS2*) at the apices of isogenic monoecious (*ff*) and gynoecious (*FF*) cucumber lines and clarified the roles of these genes on the sex expression in cucumber plants.

In this thesis, I describe the identification and characterization of a cDNA (CS-ACS2) for ACC synthase from the apices of cucumber seedlings in Part I and the analysis of the regulatory mechanisms of the sex expression by levels of expression of ACC synthase genes in Part II. Based on the results reported in this thesis, I propose a working hypothesis for the regulatory mechanisms of sex expression in cucumber plants.

Table 1. The relationship between sex phenotype and genotype in cucumber plants.

| Sex phenotype                 | Genotype   | Sex of flowers      |
|-------------------------------|--|---------------------|
| monoecious                    | <i>ff, MM/Mm, AA/Aa</i>  | ♂ + ♀               |
| gynoecious<br>(subgynoecious) | <i>FF</i> <sup>1)</sup> , <i>MM/Mm, AA/Aa/aa</i><br><i>Ff, MM/Mm, AA/Aa/aa</i> | ♀<br>(♂ + ♀, ♂ ≪ ♀) |
| andromonoecious               | <i>ff, mm</i> <sup>2)</sup> , <i>AA/Aa</i><br><i>Ff, mm, AA/Aa/aa</i>          | ♂ + ♂               |
| hermaphrodite                 | <i>FF, mm, AA/Aa/aa</i>  | ♀                   |
| androecious                   | <i>ff, MM/Mm/mm, aa</i> <sup>3)</sup>  | ♂                   |

1) The *F* locus: Shifriss (1961) and Kubicki (1969a)

2) The *m* locus: Galun (1961) and Kubicki (1969b)

3) The *a* locus: Kubicki (1969c)

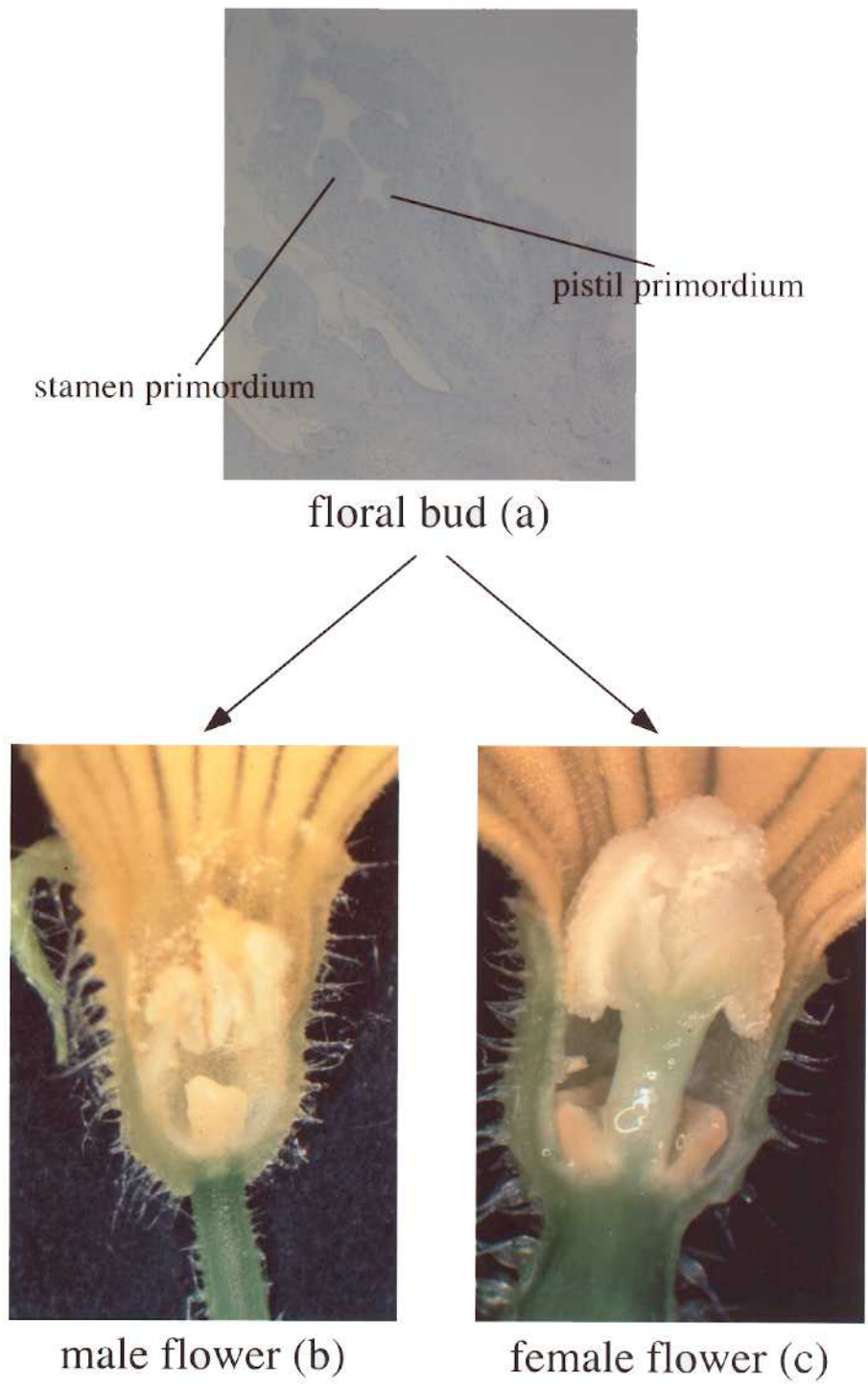


Fig. 1 Flowers of cucumber plants. (a) Floral bud. (b) Male flower. (c) Female flower.