

210(S203)

DYNAMIC ANALYSIS OF FLORIGEN-LIKE SUBSTANCES IN ARABIDOPSIS

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In *Arabidopsis* several floral genes are already identified, but the biochemical processes which might be involved floral induction are not clear. We reported in a previous meeting a new system for separation and detection of florigen-like substances in *Pharitis nil*. In this meeting, we will report the results of *Arabidopsis*, a long day plant.

Arabidopsis, ecotype Columbia is mainly used as the experimental material. The plants are grown under short-day condition for two months at 20°C. Then, the plants were grown long-day or short-day condition for various durations. Extraction was carried out at an end of light period. The procedures for extraction, separation and detection of florigen-like substances were similar to those reported in last meeting. We found many peaks which increased under long-day condition. We will refer to the results obtained in *fca*, *gigantea* and *ft*.

212(S205)

ANALYSIS OF THE TISSUE-SPECIFIC EXPRESSION OF ERAF17 THAT IS EXPRESSED DURING THE INDUCTION OF FEMALE FLOWER FORMATION IN CUCUMBER PLANTS BY ETHYLENE

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Ethylene regulates sex expression in cucumber plants (*Cucumis sativus* L.). To clarify the action of ethylene in the regulation of sex expression, we attempted to isolate genes whose expression changed during induction of the formation of female flowers at the apices of monoecious cucumber plants treated with ethephon, an ethylene-releasing compound. Using the differential-display method, we identified the *ERAF17* gene, whose expression was correlated with the development of female flowers. Sequence analysis of a cDNA fragment revealed that *ERAF17* has the sequence of a MADS-box gene.

In this study, we analyzed the tissue specificity of the expression of *ERAF17* in floral buds. Northern blot analysis and *in situ* hybridization revealed that *ERAF17* was expressed strongly in the pedicel of female flowers. Expression of *ERAF17* was also detected in the pedicel of male flowers, although it was weaker than in female flowers. In cucumber, the pedicel of female flowers is morphologically distinguishable from that of male flowers. Therefore, our results suggest that *ERAF17* affects the differentiation of female flowers by regulating pedicel development.

211(S204)

ACC SYNTHASE GENE, CS-ACS2, EXPRESSED AT PISTIL PRIMORDIA OF FLORAL BUDS IN CUCUMBER PLANTS

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In cucumber plants, immature floral buds contain stamen and pistil primordia, and sex differences are established by the arrested development of the inappropriate sex organs. Ethylene induces the development of female flowers. It is suggested that the ACC synthase gene, *CS-ACS2*, is involved in the synthesis of ethylene that induces the development of the female flowers. In order to understand the function of ethylene in the development of female flowers, we analyzed the expression of *CS-ACS2* in the floral buds by *in situ* hybridization. The *CS-ACS2* expressed only at the pistil primordia of immature floral buds. In more developed flowers, the expression of the *CS-ACS2* was detected at the ovary. These results suggest that ethylene acts on the development of the pistil, especially on the development of the ovary. Although ethylene induced the developmental arrest of the stamen primordia as well as the developmental promotion of the pistil primordia, we could not detect the expression of *CS-ACS2* at the stamen primordia. From these results, we consider that ethylene acts on the developmental arrest of the stamen primordia indirectly, and that the developmental arrest of the stamen primordia may be occurred as a result of the communication between the pistil primordia and the stamen primordia

213(S206)

ABA SIGNAL-INDUCED TRANSCRIPTION VIA A bZIP FACTOR TRAB1

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A rice bZIP factor, TRAB1 has been cloned based on its ability to interact with OsVP1. Previously, we have shown that TRAB1 can bind directly to ABA responsive elements (ABREs). Furthermore, we have demonstrated that TRAB1 can mediate ABA-inducible transcription. The latter conclusion came from the observation that a reporter gene containing GAL4 binding sites became ABA-inducible in plant cells when GAL4 DNA-binding domain (GBD):: TRAB1 fusion protein was co-expressed. Here, we further conducted molecular analyses of TRAB1 to elucidate what molecular change are brought about to TRAB1 as a result of ABA-signal transduction.

Analysis of deletion and amino acid substitution mutations in GBD::TRAB1 revealed that a specific Ser-to-Ala substitution resulted in the loss of ABA inducibility. In addition, *in vivo*-³²P-labeling experiment followed by immunoprecipitation demonstrated that TRAB1 is rapidly phosphorylated in response to ABA in suspension-cultured cells. TRAB1 protein was recovered predominantly in the crude nuclear fraction both before and after ABA-treatment.

These results indicate that the final or near final event of primary ABA-signal transduction which leads to gene activation is the phosphorylation of a specific serine residue of TRAB1 protein preexisting in the nucleus.