134(S107)

ANALYSIS OF FLORAL HOMEOTIC GENE (*GTMADS4*) OF GENTIAN

Masahiro NISHIHARA, [1]Takahide YOKOI, Yoshiko ABE, Saburo YAMAMURA
(Iwate Biotechnology Research Center, Kitakami, Iwate 024-0003, [1]Hitachi, LTD., Life Science Group, Kawagoe, Saitama 350-1165)

We are studying on development of new gentian cultivars by genetic engineering. We have already cloned four homeotic genes (*GtMADS1~4*) containing MADS box domain from a gentian cultivars Maciry. Here we report the analysis of *GtMADS4* which shows about 60% homology with *AGL2* of *Arabidopsis* using tobacco transformants.

Tobacco transformation was done by *Agrobacterium*-mediated method with a binary vector having cDNA of *GtMADS4* under the control of the CaMV35S promoter. Twenty four primary transformants were screened and eight transgenic lines showing 3:1 segregation on kanamycin resistance were further analyzed. Four individuals of each line (T_1) were grown in a greenhouse and observed about effect on the flowering day, height and change of flower shapes. Seven out of eight lines had short flower stalks as compared with wild type. Some lines had a tendency to increase the number of flowers. Relationship between expression of *GtMADS4* gene and change of phenotypes is now being analyzed using homozygous T_2 plants.

135(S108)

CpMADS1, which is floral homeotic genes homologue in Charophycean unicellular green algae (_Closterium p-s-l_ complex), is specifically expressed in sexual reproduction.

Yoichi Tanabe, Mitsuyasu Hasebe1, Hiroyuki Sekimoto2, Motomi Ito2;(Grad. Schol.Sci.,Chiba Univ., 1Natl. Inst.Basic Biol., 2Grad. Schol.Arts and Sci.,Univ.Tokyo,)

The cloning of floral hometic genes had unveiled that most of them belong to the MADS-box gene family of transcription factors. To research the origin and evolution of floral homeotic genes (ABC-function genes), we had characterized a MADS-box gene from *Chara* and *Coleochaete* which is the member of multicellular green algae Charophytes inferred to be the closest to land plants. In this time, for the purpose of elucidating molecular evolution of MADS-box gene in plants, we characterized a MADS-box gene in Charophycean unicellular green algae. *Closterium* have a unicellular organization and a isogamete sexual reproduction system unlike *Chara*, *Coleochaete*, and land plants. Alignment of the deduced amino acid sequences with the MADS-box genes of land plants showed that the *Closterium* MADS-box gene (*CpMADS1*) has both MADS and K domains, similar to the MADS-box genes of other land plants. In the result of northern hybridization analyses, *CpMADS1* was specifically expressed in sexual reproduction and the transcripts of this gene increased in accord with increment in content ratio of gametangial cell.

The results of molecular phylogenetic analyses and northern hybridization expression analyses suggest that floral homeotic genes, which is TypeII MADS-box genes (M.Yanofsky et al.2000), evolved from a single gene around the time of first land plants and the ancestral function is presumably involved in cell differentiation, although the function of *CpMADS1* is unknown.

136(S109)

STRUCTURE AND EXPRESSION OF cDNAS ENCODING MADS PROTEINS IN SESAME (*Sesamum indicum* L.)

Yuu ICHIKAWA, Tatsuya WAKASUGI, Kyojiro MASUDA, Kyoji YAMADA (Dept. Biol. Sci.,Toyama Univ.)

Only one flower with two extrafloral nectaries is formed at the leaf axil in most strains of sesame. However, mutant strains that have three flowers per axil (3-capsule strains) have already been isolated. It was found that in such mutant strains, the additional flowers were derived from the extrafloral nectaries. As a first step toward elucidating the mechanism of conversion of extrafloral nectaries to flowers in sesame, we isolated cDNA clones of MADS-box genes that control flower development in angiosperms from sesame, and examined their expression patterns.

Total RNA was prepared from buds of 3-capsule sesame strain, 0311, and used as a template for PCR amplification. Seven different cDNA clones were isolated by the 3'RACE PCR. Each of the deduced amino acid sequences of these clones showed high homology (more than 80%) with that of the corresponding MADS-box gene of *Antirrhinum majus*, suggesting that the isolated clones are cDNAs coding for MADS proteins of sesame. According to the dendrogram of alignment, the seven cDNA clones are distributed in AG, AGL2, AP1, AP3 and PI groups. Full-length clones of the sesame cDNAs of MADS-box genes, except for the clone belonging to AGL2 group, were obtained by amplification of the 5'RACE PCR. Using a specific probe for each of the cDNA clones, analysis of expression patterns of sesame MADS-box genes are in progress.

137(S110)

ANALYSES OF THE FUNCTIONAL REGION OF 6b PROTEIN ON T-DNA OF *A.tumefaciens* AND THE ROLE OF ITS INTERACTING PROTEINS

Shinji TERAKURA, Saeko KITAKURA, Youko AZECHI, Yoshihisa UENO, Tomomichi FUJITA, Hiroetsu WABIKO[1], Yasunori MACHIDA (Div. Biol.Sci.,Grad.Sch.Sci., Nagoya Univ., [1]Biotech.Inst.Akita Coll.Agric.)

It is known that oncogene *6b* located on T-DNA region of *A. tumefaciens* can induce tumor on tobacco stem. The leaf discs of *6b*-transgenic tobacco form calli on phytohormone-free medium. We have already reported that 6b protein has transcriptional activity in the plant cell and localizes in nuclei. In order to decide the functional regions of 6b, we constructed 7 types of mutated 6b, most of which lacks one of regions homologous regions among plast gene family. The leaf-disc assay of tabacco transformed with these mutated 6b revealed that both the homologous regions and the 6b-specific region which is highly acidic and essential for the transactivation were indispensable. It is also confirmed that NtSIP2 which was cloned as one of 6b-interacting proteins localized in nuclei. These results and the preliminary result of yeast two-hybrid assay between NtSIP2 or NtSIP1 which contains tri-helix-like motif and preceding mutated 6b enabled us to discuss the function of 6b in the tabacco cell and the role of NtSIP1 and NtSIP2.