（別紙様式第３号）

学 位 論 文 要 旨

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題目: Biochemical and Genetic Studies on Functional Components in Allium AlienAddition Lines
（ネギ属異種染色体添加系統の機能性成分に関する生化学的および遺伝学的研究）

Allium cepa L., which includes bulb onion (*A. cepa* L. Common onion group) and shallot (*A. cepa* L. Aggregatum group, $2n = 2x = 16$, genomes AA), is an essential species cultivated in many parts of the world. Bulb onion is suitable for cultivation in high- and mid-latitude climates, while shallot is best for low latitudes. High accumulation of the functional chemical components, such as fructan and *S*-alk(en)yl-L-cystein sulfoxides (ACSO), is observed in *A. cepa*. These functional chemical compositions are potentially important trait for chemical composition breeding of *A. cepa* and related species. Detailed gene analyses of the upregulating genes for these chemicals in *A. cepa* are necessary for its efficacious utilization in the breeding of *Allium* crops. Alien chromosome addition lines are efficient for not only the chromosomal assignments of genes and DNA markers in donor species but also the introgression from donor species into recipient species. In the genus *Allium*, a complete set of Japanese bunching onion (*Allium fistulosum* L., $2n = 2x = 16$, FF) - shallot monosomic addition lines has been established ($2n = 2x + 1 = 17$, FF+1A - FF+8A) and utilized to determine chromosomal locations of genetic markers with a simple mode of inheritance. The present studies were conducted not only to reveal the complicated genetic system of the various functional chemical biosynthesis in *A. cepa* but also to apply the genetic systems in the chemical composition breeding of other *Allium* crops.

(1) Carbohydrate composition in leaf blade of monosomic addition lines and chromosomal locations of genes related to polysaccharide accumulation in *A. cepa*

In the measurement of carbohydrate content of eight monosomic additions, sucrose and fructan levels in leaves of FF+2A were significantly lower than FF throughout the year and springtime activity of acid invertase was also lower. FF+8A showed significantly higher winter sucrose accumulation and sucrose phosphate synthase (SPS) activity. Inbred high fructan (*Frc*) lines from the ‘W202A x Texas Grano 438’ onion population exhibited significantly higher sucrose levels prior to bulbing than low fructan (*frcfrc*) lines. SuSy activity in these lines was correlated with leaf hexose content but not with *Frc* phenotype. Markers for additional candidate genes for sucrose metabolism were obtained by cloning a major SPS expressed in onion leaf and exhaustively mining onion EST resources. SPS and SuSy loci were assigned to chromosome 8 and 6 respectively using monosomic additions and linkage mapping. Further loci were assigned to chromosomes 1 (sucrose phosphate phosphatase), 2 (SuSy and 3 invertases) and 8 (neutral invertase). The concordance between chromosome 8 localization of SPS and elevated leaf sucrose levels conditioned by high fructan alleles at the *Frc* locus in bulb onion or alien monosomic additions of chromosome 8 in *A. fistulosum* suggest that the *Frc* locus may condition variation in SPS activity.

(2) Effect of a single alien chromosome from shallot on pectin production in *A. fistulosum*

To determine the pectin contents, leaf blade tissues of eight monosomic additions were fractionated into 70% ethanol-soluble (EtOH-P) and alcohol-insoluble pectins. The high

accumulations of alcohol-insoluble pectin in September and its slightly decreasing in November were observed in FF+7A and FF+8A, whereas the EtOH-P contents drastically increased. This increase of EtOH-P in FF+7A and FF+8A was seemed to be caused by degradation of alcohol-insoluble pectin. A pectin methylesterase gene of shallot was allocated to chromosome 7A via the analysis of PCR-based marker. These results indicate that important genes related to pectin metabolism in shallot may be concentrated on chromosome 7A and 8A.

(3) Production of *S*-alk(en)yl-L-cysteine sulfoxides in monosomic addition lines

The determination of *S*-alk(en)yl-L-cysteine sulfoxides (ACSO) contents was carried out once every 3 months from August 2005 to May 2006. Every monosomic addition accumulated *S*-1-propenyl CSO as a major ACSO but hardly produced *S*-2-propenyl CSO throughout the year. Compared with *A. fistulosum*, FF+3A and FF+7A more greatly increased the proportions of *S*-methyl CSO in total ACSO, suggesting that anonymous genes related to the upregulation of *S*-methyl CSO production are located on chromosomes 3A and 7A of shallot. High accumulations of total ACSO in the monosomic additions FF+3A, FF+4A, FF+5A, and FF+8A were observed during different growth periods. Using PCR-based marker analysis, the adenosine 5'-phosphosulfate reductase (APSR) candidate gene, related to sulfur assimilation, was allocated to chromosome 2A. These results showed little association between the chromosomal locations of APSR and ACSO accumulation, suggesting that anonymous genes related to the upregulation of ACSO production are located on the 3A, 4A, 5A, and 8A chromosomes of shallot.

(4) Effect of a single alien chromosome from shallot on polyphenol production in *A. fistulosum*

The determination of polyphenol content was carried out monthly from January 2002 to December 2003. Throughout the 2-year period, every monosomic addition accumulated polyphenols in the leaf blades. Four monosomic additions (FF+2A, FF+5A, FF+6A, and FF+8A) caused a greater increase in the number of polyphenols than did *A. fistulosum*. These results indicate that the genes related to polyphenol production are located on the 2A, 5A, 6A, and 8A chromosomes of shallot.

(5) Production of alien addition lines in tetraploid *A. fistulosum* carrying 1A chromosome(s) of shallot and their application to breeding for a new vitamin C–rich vegetable

The determination of vitamin C (ascorbic acid) content revealed that the incorporation of alien chromosome 1A into a diploid background of *A. fistulosum* increased the internal ascorbic acid content of the leaf blade tissue. We produced a 1A disomic addition in the tetraploid of *A. fistulosum* ($2n = 4x + 2 = 34$) and demonstrated high-frequency transfer of the alien chromosome in crosses with *A. fistulosum*. Five plants of the 1A disomic additions were regenerated via apical meristem culture of the FF+1A on a Murashige and Skoog medium containing colchicine. These 1A disomic additions showed partial fertility for both female and male gametes. Most of the progenies from selfing of the 1A disomic additions and reciprocal crossing with *A. fistulosum* possessed chromosome 1A. Interestingly, 64% (18 of 28) of the plants obtained from the reciprocal crosses were 1A monosomic additions in a triploid background of *A. fistulosum* ($2n = 3x + 1 = 25$). These monosomic additions were more vigorous and vitamin C-rich than euploid plants of *A. fistulosum*.

The genetic information regarding the chromosomal locations of genes involved in the functional chemicals biosynthesis can be valuable for advancing the study of genetic systems yielding these treats. Moreover, a successful production of 1A disomic addition can become the breeding materials for the health promoting compound-rich Japanese bunching onion (*A. fistulosum*).