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学 位 論 文 要 旨
SUMMARY OF DOCTORAL THESIS

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題目 Title: **Applied Cytogenetic Studies of *Allium* Alien-Chromosome Addition Lines toward Elucidation of Genetic Systems in Bulb Formation of Shallot (*Allium cepa* L. Aggregatum group)**

〔シャロットのりん茎形成に関する遺伝系の解明に向けたネギ属異種染色体添加系統の応用細胞遺伝学的研究〕

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. Bulb formation, an agronomic trait of *A. cepa,* consists of both bulb pigmentation and bulb enlargement due to the accumulation of flavonoids and fructo oligosaccharides, respectively. The functional chemical compositions of both compounds make bulb formation a remarkable trait of *A. cepa* and related species. 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 use of the monosomic additions is not applicable to the chromosomal assignment of genes related to phenotypic expression with a complex mode of inheritance. The present studies were conducted to reveal the complicated genetic system of flavonoid biosynthesis and bulb enlargement in *A. cepa*.

1) Development of *Allium cepa* chromosome-specific DNA markers

To determine the chromosomal locations of genes related to bulb formation in *A. cepa,* it is necessary to use *A. fistulosum* – shallot multiple alien addition lines carrying chromosome 5A with a gene(s) involved in regulating the expression of bulb formation and other extrachromosome(s) ($2n = 2x + 2 – 2x + 7 = 18 – 23$). Reproducible DNA markers are necessary for the complete identification of extrachromosomes in the multiple additions. In the present study, 21 microsatellite markers were assigned to each chromosome of *A. cepa* via a simple PCR assay using the primer pairs to amplify microsatellites of bulb onion (AMS). The results of this study assigned at least two chromosome-specific DNA markers on each chromosome.

2) Production of multiple additions of *Allium fistulosum* with chromosome 5A

After the second backcross of an amphidiploid between *A. fistulosum* and shallot was conducted to produce the multiple additions, 113 plants obtained from the crossing were

analyzed by a chromosome 5A-specific phosphoglucoisomerase isozyme marker of shallot. Thirty plants, the chromosome numbers of which ranged from 18 to 23, were preliminarily selected for an alien addition carrying 5A. The genomic constitution in 19 of the 30 plants was completely identified based on the results from seven other chromosome markers of shallot including the above-mentioned microsatellite markers (1A, AMS16-261; 2A, *Got-1*^{A}; 3A, AMS23-218; 4A, AMS20-372; 6A, *Got-2*^{A}; 7A, Ac5SL; 8A, *Gdh-1*^{A}) and karyotype analyses.

3) Biochemical and morphological characterizations in the basal part of the leaf sheath of the multiple additions

In high-performance liquid chromatography analyses to detect flavonoids, the multiple additions were divided into two initial groups: one group with a high content of kaempferol and another group with an excess amount of quercetin. In a comparison of the genomic constitution of the each group, chromosome 7A was found only in the latter group. The results revealed that an F3'H gene for the synthesis of quercetin was located on 7A. An anonymous glucosyltransferase gene for quercetin was allocated to 3A or 4A, and a 3GT gene was allocated to 4A through similar direct comparisons between the genomic constitution and flavonoid contents of the multiple additions. In the examination of morphological characteristics, four multiple additions in which chromosome 2A was deleted showed obvious bulb enlargement and no tillering. This result indicated that anonymous genes related to bulb enlargement and the tillering were located on chromosome 2A.

4) Chromosomal assignment of genes involved in bulb formation

In the phenotypic analyses of the multiple additions, it was difficult to assign flavonoid biosynthetic and bulbing genes to individual chromosomes except in the cases of F3'H and 3GT. SCAR analyses for several genes using two complete sets of monosomic additions resulted in the assignment of CHS-A, CHS-B, CHI, F3H, F3'H, DFR, FLS, and ANS genes, as well as a sucrose transporter, to chromosomes 2A, 4A, 3A, 3A, 7A, 7A, 4A, 4A, and 5A, respectively. All the structural genes involved in flavonoid biosynthesis influencing bulb color were assigned to the chromosomes of *A. cepa*.

5) Development of tetraploid *A. fistulosum* with a pair of chromosomes 5A

Although it would be beneficial to use the monosomic addition FF+5A in the development of high-flavonoid *A. fistulosum,* the direct use of monosomic additions in breeding is difficult because the reduction of chromosomal stability in the germ lines leads to a gradual loss of extrachromosomes in successive generations. The production of tetraploid *A. fistulosum* with a pair of chromosomes 5A was conducted to obtain a means for the high-frequency transfer of the alien chromosome to other genotypes of *A. fistulosum*. Of the 12 plants regenerated from shoot tip culture of FF+5A, 3 could be objective tetraploids based on the results of the ploidy analysis of the first, second, and third germ layers.

The genetic information regarding the chromosomal locations of genes involved in the flavonoid biosynthesis and bulb enlargement of *A. cepa* can contribute to the identification of complex genetic systems yielding these traits. Moreover, a successful production of tetraploid *A. fistulosum* carrying a pair of extrachromosomes suggests a de novo breeding line of Japanese bunching onion (*A. fistulosum*).