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The Application of Alien-Chromosome Addition Lines and Cytoplasmic Substitution Lines to Studies on Genetics and Breeding in *Allium cepa*

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ABSTRACT

In edible Alliums, shallot (*Allium cepa* L. Aggregatum group, 2*n*=2*x*=16, genomes AA) is an important vegetable crop in South-East Asia and has the highest adaptability to tropical and sub-tropical zones. In diploid *Allium* species, we established eight monosomic additions, representing eight different chromosomes of shallot in a background of Japanese bunching onion (*A. fistulosum* L., 2*n*=2*x*=16, FF). The monosomic additions (2*n*=2*x*+1=17, FF+nA) were used for genetic analyses of shallot and for improving *A. fistulosum* cultivars. Genetic analyses identified 48 chromosome-specific genetic markers in shallot. The effect of extra shallot chromosomes, on morphology and fertility of *A. fistulosum,* was also identified. Furthermore, onion linkage groups were successfully assigned to *A. cepa* chromosomes by using the *A. fistulosum* – shallot monosomic additions. To develop shallot CMS lines with wild species cytoplasm, we conducted cytoplasmic substitution between *A. galanthum* and shallot by continuous backcrossing. In a BC_1 generation, male sterile plants appeared, and the male sterility was maintained in a BC_3 generation stably. RFLP analysis of chloroplast DNA confirmed that the lines inherited cytoplasm from *A. galanthum*. Our results demonstrated that the development of shallot CMS lines was possible by this genetic approach. We also induced haploid and doubled haploid plants of F_1 hybrids between the CMS shallot and bulb onion by using unpollinated flower culture. This review paper was prepared, primarily, to introduce the results so far obtained for the gene analyses of *A. cepa* using a complete set of *A. fistulosum* – shallot monosomic additions and for development of shallot CMS lines.

† Ken-ichiro Yamashita and Masayoshi Shigyo contributed equally to this publication.

Keywords: alien-chromosome addition line, *Allium*, cytoplasmic substitution line, onion, shallot
Abbreviations: RAPD, random amplified polymorphic DNA; **rDNA**, ribosomal DNA; **RFLP**, restriction fragment length polymorphism; **SNP**, single nucleotide polymorphism; **SSR**, simple sequence repeat

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INTRODUCTION

Of the edible Alliums, *Allium cepa* L. is one of the fastest-growing species in the food market. Bulb onion (*A. cepa* Common onion group) is an especially important commodity worldwide as it ranks second place in economically important horticultural crops. Shallot (*A. cepa* Aggregatum group) is one of the more important crops in South-East Asia and has the highest adaptability to tropical and sub-tropical zones in all edible alliums. Though several differences on physiological and ecological characteristics are recognized between these two crops (Tashiro *et al*. 1982), there is the compatibility in the genetic information. In this paper, we introduce studies on genome analyses of *A. cepa*

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Invited Review

using a complete set of *A. fistulosum* – shallot alien monosomic addition lines.

The establishment of a true seed shallot and the development of F_1 cultivars are expected to lead to many horticultural advantages such as high efficiency of propagation, high storage ability and shipping quality of seeds. Since the emasculation at a large scale is not practical in commercial F_1 seed production of shallot, the development of cytoplasmic male sterile (CMS) lines is essential. We also mention studies on the development of shallot CMS lines possessing cytoplasm of *Allium galanthum* Kar. *et* Kir., a wild species in section *Cepa* of genus *Allium*.

STUDIES ON GENETICS AND BREEDING IN *ALLIUM* ALIEN-CHROMOSOME ADDITION LINES

Establishment of a complete set of *Allium* monosomic addition lines and development of *Allium* multiple alien addition lines

A method for production of *A. fistulosum* – shallot alien additions is presented in **Fig. 1**. Alien additions were selected from second backcross progenies (BC_2) of amphidiploid hybrids between *A. fistulosum* and shallot (Shigyo *et al.* 1996). After backcrossing to *A. fistulosum*, the triploid hybrid AFF of BC_1 showed a very low seed set. Nevertheless, 274 BC_2 plants were ultimately obtained. The chromosome count of 253 BC_2 plants revealed that the chromosome numbers varied from 16 to 24. Forty-seven plants had 17 chromosomes. At meiotic metaphase-I, all pollen mother cells (PMCs) of 43 plants formed 8II +1I. The bivalents formed exhibited localized chiasmata characteristic of *A. fistulosum*. Forty-one plants were consequently selected as promising monosomic additions since one plant showed an abnormal karyotype and one another was lost. The monosomic additions were applied for elaborate karyotypic observations with the calculations of relative chromosome length and centromeric index in each extra chromosome. Two complete sets (FF+1A-FF+8A) of the monosomic additions were independently generated in the BC_2 population (**Fig. 2**). Another karyotype analysis of somatic chromosomes by means of genomic *in situ* hybridization (GISH) confirmed the previous results (Shigyo *et al.* 1998). Dozens of shallot chromosome-specific genetic markers were developed through the monosomic additions (see below "Isozyme and DNA markers"). Further, *A. fistulosum* carrying two to seven chromosomes of shallot, i.e. *A. fistulosum* – shallot multiple alien addition lines ($2n=2x+2-2x+7=18-23$), were produced, and extra chromosomes from shallot in the BC_2 population could be identified using the chromosome-specific markers (Masuzaki *et al.* 2006b; **Fig. 3**).

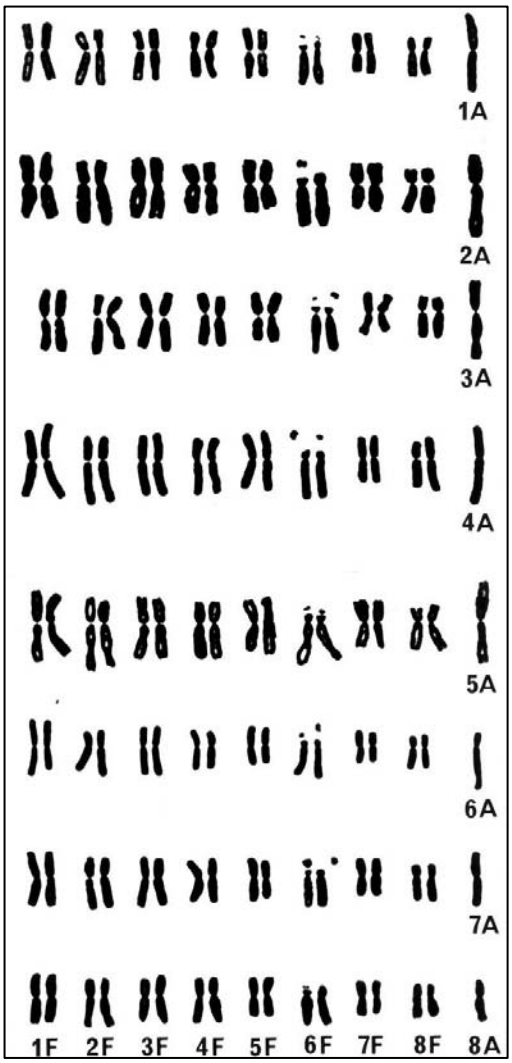

Fig. 2 Somatic metaphase chromosomes in a complete set of *Allium fistulosum* – shallot monosomic additions. (FF+1A, 130; FF+2A, 132; FF+3A, 5; FF+4A, 32; FF+5A, 71; FF+6A, 120; FF+7A, 23; FF+8A, 65). 1F-8F: Chromosomes from *A. fistulosum*. 1A-8A: Extra chromosomes from shallot. (From **Shigyo *et al.*** (1996) *Genes and Genetic Systems* **71**, 363-371, with kind permission from The Genetics Society of Japan.)

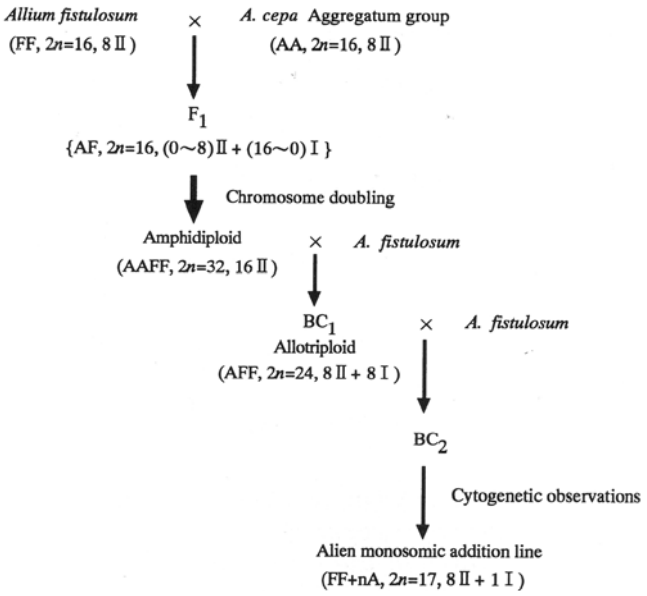

Fig. 1 Procedure for producing Japanese bunching onion (*Allium fistulosum*) – shallot (*Allium cepa* Aggregatum group) monosomic additions. (From **Shigyo *et al.*** (1996) *Genes and Genetic Systems* **71**, 363-371, with kind permission from The Genetics Society of Japan.)

Morphological and physiological characteristics of *Allium* alien-chromosome additions

To study the effect of the extra chromosomes on morphological characters in both vegetative and reproductive stages, a set of *A. fistulosum* – shallot monosomic additions was grown in an experimental field (Shigyo *et al.* 1997a). Several morphological characters of the monosomic additions were found to be specific to the respective alien chromosomes from shallot (**Fig. 4**). The most distinctive characteristics in each monosomic addition were as follows; spheroidal spathe in FF+1A, bloomless leaf blade in FF+2A, slow expansion of leaf in FF+3A, acuminate spathe in FF+4A, reddish-yellow leaf sheath in FF+5A, arch-like leaf blade in FF+6A, fast expansion of leaf in FF+7A, and intensely yellow anther in FF+8A. The results indicate that these character expressions are deeply related to alien genes on extra chromosomes from shallot. In the examination of morphological characteristics in the multiple additions, four

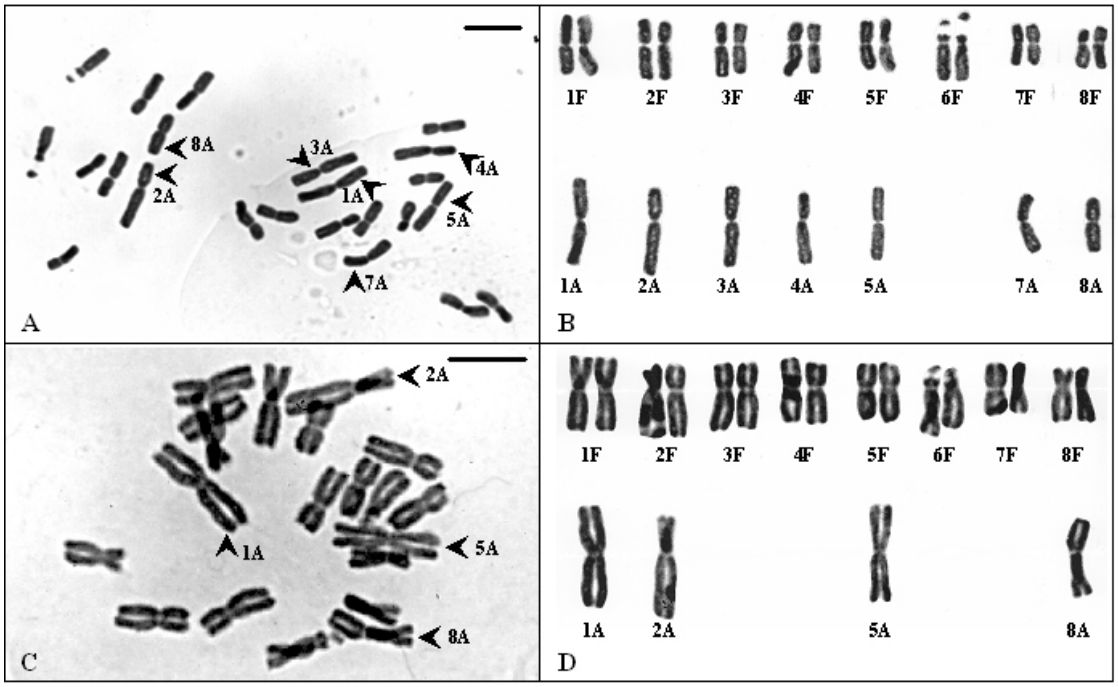

Fig. 3 Somatic metaphase chromosomes of *Allium fistulosum* – shallot multiple alien addition lines [A-B $S_2$27 (2*n* = 23), C-D $S_2$9 (2*n* = 20)]. A, C: Arrowheads point to the extra chromosomes. Scale bar = 10 μm. **B, D:** Eight pairs of *A. fistulosum* chromosomes (1F-8F), showing similar sizes and shapes, and solitary chromosomes of shallot (1A-8A) are distinguished. (From **Masuzaki *et al.*** (2006b) *Theoretical and Applied Genetics* **112**, 607-617, with kind permission from Springer Science and Business Media.)

Fig. 4 Plants of a complete set of *Allium fistulosum* – shallot monosomic additions (1-8) and *A. fistulosum* (C) in vegetative stage. Each Arabic numeral (1-8) corresponds to the extra chromosomes (1A-8A) of the monosomic additions. (From **Shigyo *et al.*** (1997a) *Genes and Genetic Systems* **72**, 181-186, with kind permission from The Genetics Society of Japan.)

multiple additions in which chromosome 2A was deleted showed obvious swelling of their leaf sheaths and no side-shoots formation (Masuzaki *et al*. 2007). This result indicated that anonymous genes related to bulbing were located on chromosome 2A.

Reproduction system of monosomic additions

Considerable attention which has been devoted to maintaining this complete set of monosomic additions is important for both practical and fundamental studies of them. We have maintained the complete set of *Allium* monosomic additions since 1991 through vegetative propagation. However, the risk of losing this precious material owing to plant death is high, so it is important to maintain the complete set via other methods, such as generative reproduction. One method, suggested by comparisons between male and female transmission rates of various alien chromosomes, is the crossing of a monosomic addition (♀) with *A. fistulosum* (♂) (Shigyo *et al*. 1999). However, there is still a need for an improved maintenance protocol for a set of *Allium* monosomic additions. For example, after monosomic addition × *A. fistulosum*, low female transmission values (less than 10%) of the extra chromosomes were observed. Selfed progeny plants of a complete set of the monosomic additions were produced to examine the transmission rates of respective alien chromosomes (Shigyo *et al*. 2003). All eight types of the selfed monosomic additions set germinable seeds. The numbers of chromosomes (2*n*) in the seedlings were 16, 17 or 18. The eight extra chromosomes varied in transmission rate (%) from 9 (FF+2A) to 49 (FF+8A). The complete set of monosomic additions was reproduced successfully by self-pollination. A reliable way to maintain a set of *Allium* monosomic additions is to combine the two crossing methods, selfing and female transmission.

The use of monosomic additions for assignment of genes and genetic makers to respective chromosomes of shallot

Isozyme and DNA markers

By introducing a single chromosome from shallot into the nucleus of *A. fistulosum*, it is possible to determine chromosomal locations of various genetic markers of shallot. Be-

cause of codominance, expression of individual isozyme alleles from the extra chromosome can be readily detected by comparing the zymograms of monosomic additions with those of the normal controls. Such a principle also could be applied to DNA markers to determine the chromosomal locations of rDNA, RAPD and SSR markers of shallot. Chromosomal locations of 10 isozyme genes, one rDNA locus, 16 RAPD markers and 21 SSR markers have thus far been determined in *A. cepa* (Shigyo *et al*. 1994, 1995, 1996, 1997b; Masuzaki *et al*. 2006a). We have so far completed the assignment of at least four chromosome-specific genetic markers to each chromosome of shallot.

Genes related to production of flavonoids and anthocyanins

In morphological analyses of the set of the monosomic additions, pigmentation in the basal part of leaf sheath was observed only in a monosomic addition carrying chromosome 5A of shallot (FF+5A) (Shigyo *et al*. 1997a). This result indicated that the genes related to pigmentation might be located on the chromosome 5A. The pigments produced in leaf sheath were identified via high-performance liquid chromatography (HPLC) analyses. Five anthocyanin and four flavonoid compounds were detected in shallot but no anthocysnins and flavonoids in *A. fistulosum*. In the set of the monosomic additions, all the compounds detected in shallot were observed only in FF+5A, whereas no compounds in the other monosomic additions. These results revealed that anonymous genes for anthocyanin and flavonoid production in bulbs of shallot were located on 5A (Shigyo *et al*. 1997c). However, anthocyanin and flavonoid compounds produced were different in the number and compositions between shallot and FF+5A. For example, in flavonoids, although shallot included a lot of contents of quercetin as bulb onion, FF+5A showed a much higher content of kaempferol, the precursor of quercetin, rather than quercetin. To reveal the chromosomal location of the gene related to the synthesis of quercetin, quercetin and kaempferol in the skin and outer scale were compared by HPLC analyses among the 19 *A. fistulosum* – shallot multiple alien addition lines carrying chromosome 5A and the other chromosome(s). Ten multiple additions with chromosomes 5A and 7A showed the higher content of quercetin rather than kaempferol as same as shallot. On the other hand, nine multiple additions deleting 7A showed the same excess content of kaempferol as FF+5A. Consequently, these results demonstrated that the flavonoid 3'-hydroxylase (F3'H) gene involved in the quercetin biosynthesis might be located on chromosome 7A (Masuzaki *et al*. 2006b). In addition, an UDP-glucose:flavonoid 3-*O*-glucosyltransferase gene to convert anthocyanidin into anthocyanin was allocated to 4A through a similar direct comparison between the genomic constitution and anthocyanin contents of the multiple additions (Masuzaki *et al*. 2006c). Sequence characterized amplified region (SCAR) analyses for several genes using two complete sets of monosomic additions resulted in the assignment of chalcone synthase (CHS)-A, CHS-B, chalcone isomerase, flavanone 3-hydroxylase, F3'H, dihydroflavonol 4-reductase, flavonol synthase and anthocyanidin synthase genes, to chromosomes 2A, 4A, 3A, 3A, 7A, 7A, 4A and 4A, respectively (Masuzaki *et al*. 2006b, 2006c). All the structural genes involved in flavonoid biosynthesis influencing bulb color were assigned to the chromosomes of *A. cepa*.

Genes related to production of carbohydrates

Sugar content is regarded as one of important factors to enhance food quality of Japanese bunching onion. We observed the sugar dynamics in leaf tissues of monosomic addition lines through two years (Hang *et al*. 2004). The characteristic difference was revealed in FF+2A and FF+8A (**Fig. 5**). Except for FF+2A, every monosomic addition line accumulated non-reducing sugars in winter leaf

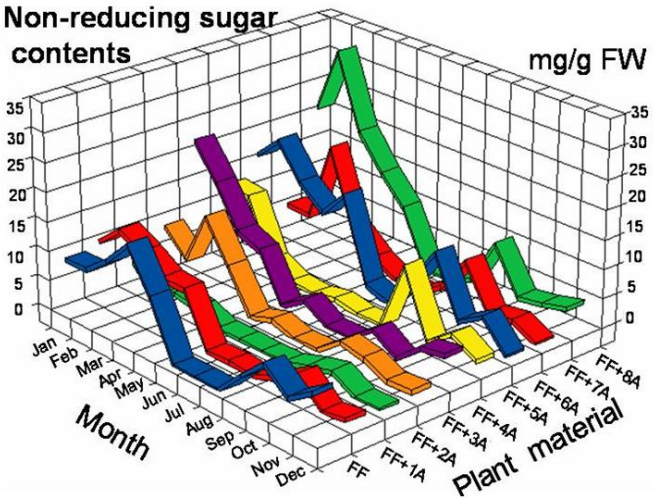

Fig. 5 The year-round variation of non-reducing sugar contents in a complete set of monosomic additions (FF+1A-FF+8A) compared with *Allium fistulosum* (FF). The mean value in each month from Jan. 2002 to Dec. 2003 is shown in this figure. (From **Hang *et al*.** (2004) *Genes and Genetic Systems* **79**, 345-350, with kind permission from The Genetics Society of Japan.)

blades. FF+8A specially caused an increase of non-reducing sugars in winter. FF+2A hardly produced non-reducing sugars through the two-year study. These results indicated that genes related to non-reducing sugar metabolisms were located on the chromosomes 2A and 8A. Using thin-layer chromatography and HPLC analyses, it was revealed that several types of oligosaccharides were accumulated as non-reducing sugars in leaf blade tissues. Quantitative trait locus (QTL) analysis of total bulb fructan content in the intraspecific onion mapping population 'Brigham Yellow Globe (BYG) 15-23' x 'Alisa Craig (AC) 43' using a complete molecular marker map revealed only one significant QTL in the chromosomal region of 8A (McCallum *et al*. 2006).

Onion linkage group assignment to individual chromosomes via monosomic additions

Two genetic maps have been constructed in *A. cepa*. One is an AFLP linkage map generated from an interspecific backcross between *A. cepa* and *A. roylei* (van Heusden *et al*. 2000b). The second is the RFLP, SSR and SNP linkage map from an intraspecific cross between onion inbreds 'BYG15-23' x 'AC43' (King *et al*. 1998; Martin *et al*. 2005). The set of *A. fistulosum* – shallot monosomic additions was useful for assignment of the linkage groups to their responding chromosomes by determining the chromosomal locations of several constituent DNA markers on the linkage maps (**Fig. 6**). Thus, 10 linkage groups from the interspecific crossing (van Heusden *et al*. 2000a) and bulb onion linkage groups from the intraspecific crossing (Martin *et al*. 2005) were successfully assigned to chromosomes of *A. cepa* using the monosomic additions.

STUDIES ON GENETICS AND BREEDING IN *ALLIUM* CYTOPLASMIC SUBSTITUTION LINES

Production of cytoplasmic substitution lines of shallot possessing *Allium galanthum* cytoplasm by continuous backcrossing

CMS is a maternally inherited trait that prevents production of viable pollen grains (Edwardson 1970). Since maturation of female gametes is not disturbed in CMS plants, they can serve as seed parents in hybrid seed production. Section *Cepa* in which shallot and Japanese bunching onion belong also contains following some wild species, *A. galanthum* Kar. *et* Kir., *A. altaicum* Pall., *A. oschaninii* O. Fedtsch., *A.*

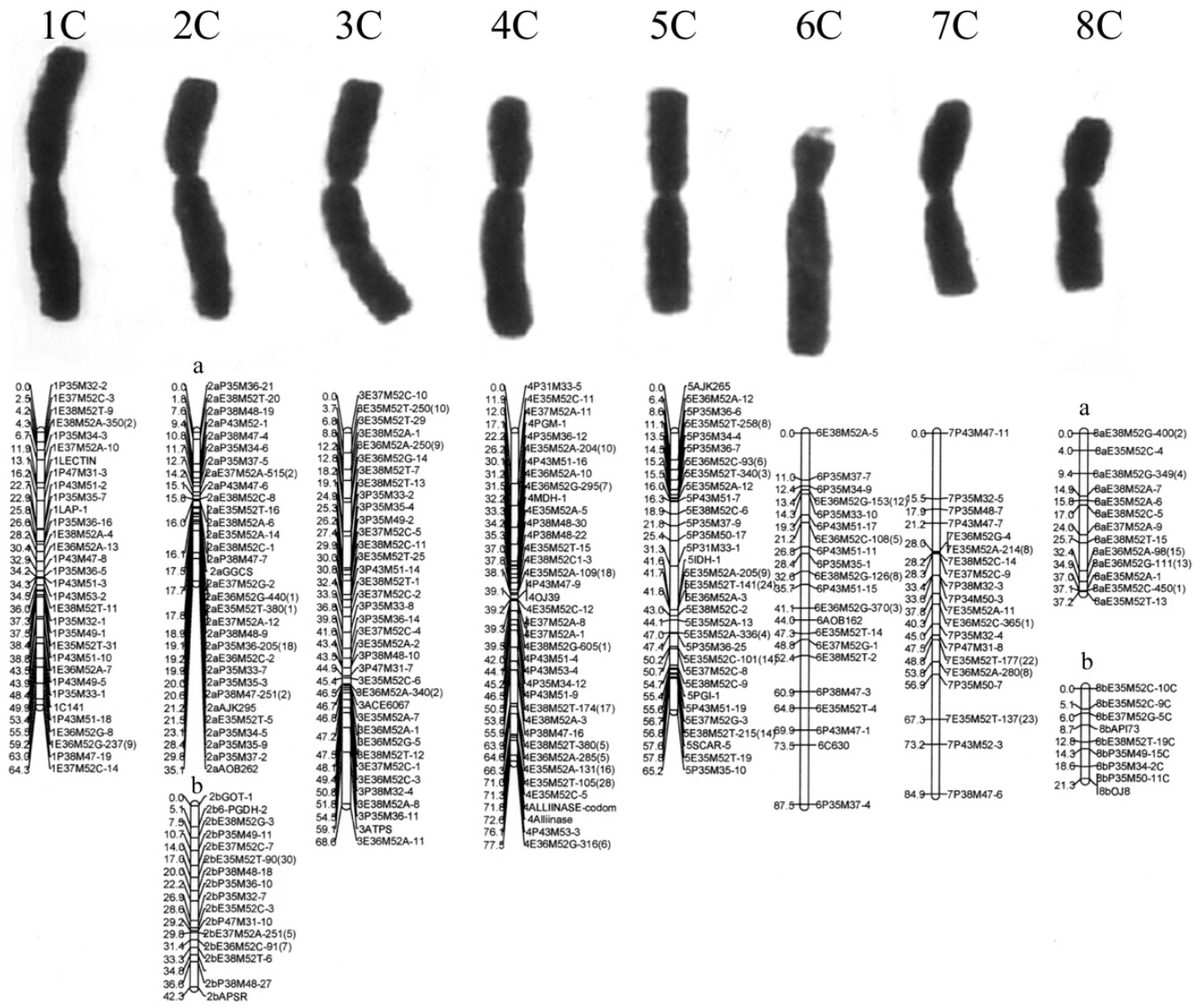

Fig. 6 Linkage map of *Allium cepa*. Genetic distances shown in centiMorgans are on the left. (Revised and redrawn from **Shigyo** (2007) pp 245-269, with kind permission from CRC Press.)

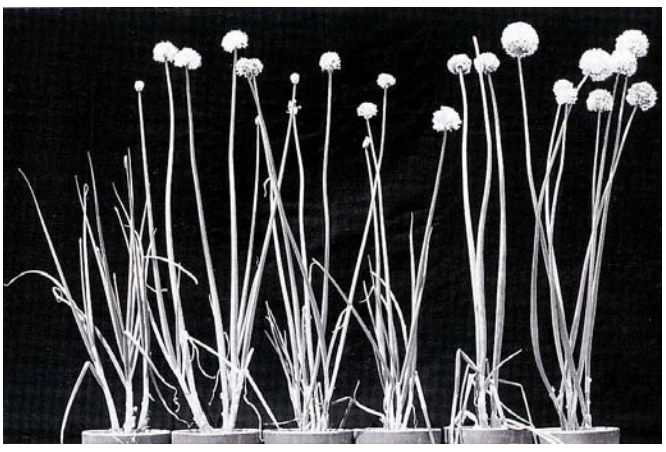

Fig. 7 Plants of *Allium galanthum*, F_1, BC_1, BC_2, BC_3 and shallot (left to right). (From **Yamashita and Tashiro** (1999) *Journal of the Japanese Society for Horticultural Science* **68**, 256-262, with kind permission from The Japanese Society for Horticultural Science.)

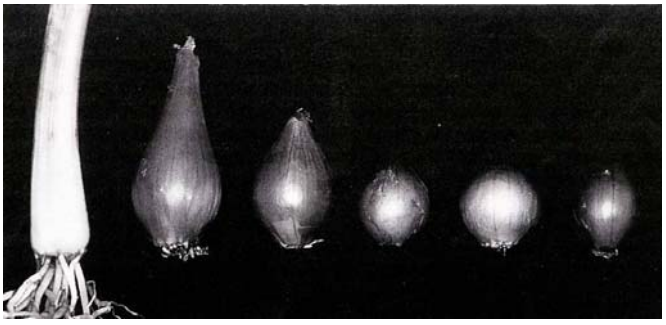

Fig. 8 Basal portion of leaf sheath of *Allium galanthum*, bulbs of F_1, BC_1, BC_2, BC_3 and shallot (left to right). (From **Yamashita and Tashiro** (1999) *Journal of the Japanese Society for Horticultural Science* **68**, 256-262, with kind permission from The Japanese Society for Horticultural Science.)

vavilovii M. Pop *et* Vved., *A. pskemense* B. Fedtsch. (Hanelt 1990). We regarded the wild species as CMS sources of shallot. The cytoplasm of *A. galanthum* was substituted for that of shallot by continuous backcrossing in which *A. galanthum* was used as a cytoplasm donor and shallot was as a recurrent parent. *A. galanthum* was crossed as a seed parent with shallot to obtain interspecific F_1 hybrids, followed by continuous backcrossing of the F_1 hybrids to shallot. Consequently, BC_3 progeny plants were produced (**Figs. 7, 8**). By RFLP analysis of chloroplast DNA (cpDNA), it was confirmed that the backcross progeny plants surely inherited cytoplasm from *A. galanthum* (Yamashita and Tashiro 1999).

Influence of *Allium galanthum* cytoplasm on fertility of shallot

Pollen fertility of the interspecific hybrids and backcross progeny plants was evaluated based on pollen morphology and stainability with acetocarmine. Seed fertility of them was measured by crossings with shallot. The interspecific hybrids showed low pollen fertility (8.8% or less) and seed sets (0.04% or less). In a BC_1 generation, male sterile plants appeared and the male sterility was maintained stably in a BC_3 generation. Although seed fertility varied considerably among the progeny plants in each backcross generation, it was restored gradually with the progress of backcrossing. The male sterility observed was attributed to incompatibility between shallot nucleus and *A. galanthum* cytoplasm because high frequency of bivalent chromosomes was observed in most of the backcross progeny plants at meiotic metaphase-I (**Fig. 9A**). Microsporogenesis in the male sterile plants was observed to reveal the process of unviable pollen production. After the tetrad stage (**Fig. 9B**), the protoplasm of pollen grains gradually degenerated (**Fig. 9C**), finally resulting in empty tetrads or empty pollen grains in the anthers (**Fig. 9D, 9E**). From these results, it was confirmed that the development of shallot CMS lines was possible by using this genetic approach (Yamashita and Tashiro 1999). For the efficient cytoplasmic substitution, it is desirable to select the plants in which the nucleus substitution has advanced earlier in each backcross generation. By application of GISH to somatic metaphase chromosomes of the backcross progeny plants between *A. galanthum* and shallot, the nucleus substitution process was successfully visualized. Since degree of nucleus substitution varied remarkably among plants in each backcross generation, we concluded that GISH was an effective means to select such plants at the juvenile stage (Yamashita *et al*. 2000).

Haploid induction from F_1 hybrids between CMS shallot and bulb onion by unpollinated flower culture

The crossing between shallot and bulb onion is an effective breeding strategy to improve bulb size of shallot and adaptability of bulb onion to tropical and sub-tropical zones. After the successful production of shallot CMS lines possessing *A. galanthum* cytoplasm (Yamashita and Tashiro

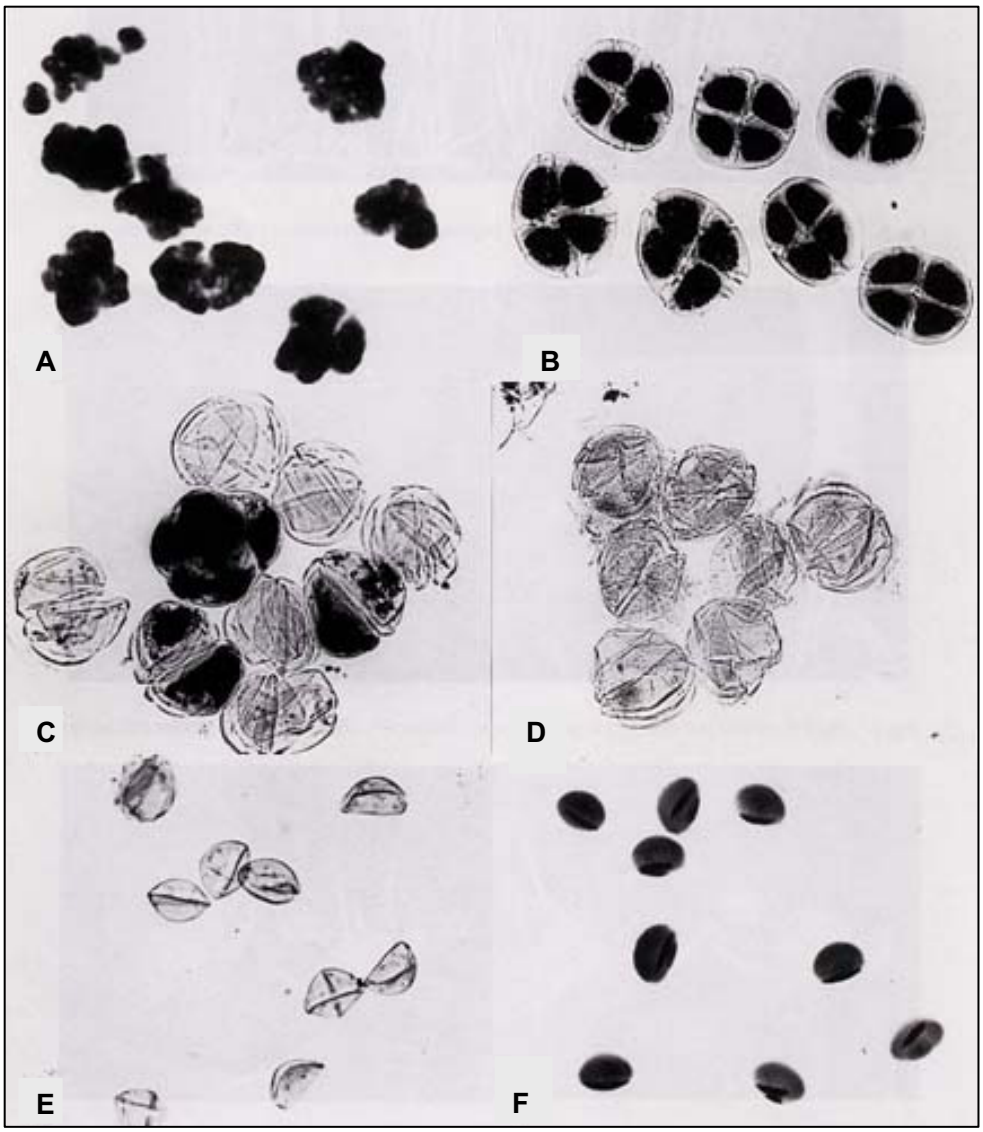

Fig. 9 Chromosome pairing at metaphase-I in a PMC of BC_3 plant (A), tetrads (B), degeneration of protoplasm in microspores (C-E), and normal pollen grains of shallot (F). (From **Yamashita and Tashiro** (1999) *Journal of the Japanese Society for Horticultural Science* **68**, 256-262, with kind permission from The Japanese Society for Horticultural Science.)

1999), we continued the further backcrossing to shallot, resulted in BC_6 generation. Then, F_1 hybrids were produced between the CMS shallot and bulb onion. The F_1 hybrids exhibited male sterility and intermediate morphological and physiological characters. To fix useful gene combinations from both the species, haploid plants of the F_1 hybrids were induced via *in vitro* gynogenesis by unpollinated flower culture (Endang *et al*. 2002). Eleven out of 13 seedlings that survived had a haploid number of chromosomes ($2n=x=8$), while other 2 plants had a doubled haploid number of chromosomes ($2n=2x=16$). All the haploid and doubled haploid plants were confirmed to inherit the cytoplasm from *A. galanthum* by PCR analyses of cpDNA and mitochondrial DNA. The haploid and doubled haploid plants exhibited variation for morphological and physiological characters, i.e. number of shoots, leaf morphology, bulb color and bulb size, which indicated that these plants induced had various gene combinations from both shallot and bulb onion (Endang *et al*. 2002).

CONCLUSION AND FUTURE DIRECTIONS

As mentioned above, the complete set of *Allium* monosomic additions was successfully used for the several kinds of genome analyses in *A. cepa*. Nowadays the genetic studies identified more than one hundred of chromosome-specific genetic markers in bulb onion as well as shallot. As a result of these maker analyses, Dutch and US onion linkage maps were successfully assigned to *A. cepa* physical chromosomes. Furthermore, the monosomic additions were proved to be very effective in revealing the effects of each single alien chromosome from *A. cepa* on the production of several functional chemical compounds, i.e. organo sulfur compounds, nondigestible carbohydrates, polyphenols, etc. in the leaf tissue of *A. fistulosum*. As the most successful results, the assignment of all structural genes concerning the flavonoid biosynthesis to individual chromosomes of *A. cepa* could be accomplished by both SCAR analyses of candidate genes in the monosomic additions and direct comparisons between the chromosomal constitution and flavonoid contents in scarly leaves of *A. fistulosum* – shallot multiple addition lines ($2n=2x+2$-$2x+7=18$-23) (Masuzaki *et al*. 2006b, 2006c). At present, the complete set of the monosomic additions has been used for improving *A. fistulosum* cultivars. We are under developing a novel breeding program for introducing disease resistance into *A. fistulosum* via a specific type of the monosomic additions. Furthermore, a disomic addition line carrying chromosomes 8A of shallot with diploid *A. fistulosum* background ($2n=2x+2=18$, FF+8A8A) could be regarded as a promising line with high production of non-reducing sugars (Shigyo *et al*. 2003). We are also executing such approaches in following three species-combinations, *A. cepa* – *A. fistulosum*, *A. cepa* – *A. roylei* and *A. fistulosum* – *A. roylei*.

Shallot CMS lines were successfully developed by cytoplasmic substitution using *A. galanthum* as a CMS source. The shallot CMS lines would be useful for commercial F_1 seed production by crossing with various shallot strains, bulb onion cultivars or related species. On the other hand, shallot was reported to be in heterozygous state because its selfed progeny plants showed variations for morphological and physiological characters (Tashiro *et al*. 1982). This genetic heterozygosity also means the presence of possibility for developing various shallot cultivars with unique characteristics. Haploid and doubled haploid plants with various characteristics were induced from F_1 hybrids between CMS shallot and bulb onion by unpollinated flower culture (Endang *et al*. 2002). By combining the crossing under the CMS system with fixation of desirable characteristics by unpollinated flower culture, it is expected that true seed and F_1 hybrid cultivars of shallot with desirable gene combinations will be released to markets. *A. galanthum* cytoplasm was demonstrated to induce CMS in bulb onion (Havey 1999) and Japanese bunching onion (Yamashita *et al*. 1999a, 1999b, 2002; Yamashita and Tashiro 2004; Yamashita *et al*. 2005) as well as shallot (Yamashita and Tashiro 1999). Interestingly, alleles to restore male fertility for bulb onion possessing S-cytoplasm showed no male fertility restoration for the CMS onion with *A. galanthum* cytoplasm (Havey 1999), which shows that the genetic background of these two CMS systems quite differs each other. Although we also produced other shallot alloplasmic lines possessing *A. vavilovii* cytoplasm, the *A. vavilovii* cytoplasm scarcely influenced on both male and female fertility in shallot (data not shown). It is sure that influence of wild species cytoplasm on fertility of shallot varies with the source of cytoplasms within *Allium*. Actually, it is desirable to utilize plural and diverse CMS for F_1 seed production because the application of a single or a few cytoplasms leads to a great reduction of genetic variation and vulnerability to diseases in F_1 cultivars as the case of CMS-T in maize (Hooker *et al*. 1970; Levings III 1991). If the cytoplasmic substitution using more wild species would be accomplished for shallot, the elucidation of nuclear-cytoplasmic incompatibility toward the development of various shallot CMS lines should well progress.

Thus, both alien-chromosome addition lines and cytoplasmic substitution lines have been contributed greatly to studies on genetics and breeding in *Allium* species.

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