Genes Genet. Syst. (2004) **79**, p. 263–269

Production and characterization of alien chromosome additions in shallot (*Allium cepa* L. Aggregatum group) carrying extra chromosome(s) of Japanese bunching onion (*A. fistulosum* L.)

Tran Thi Minh Hang[1], Masayoshi Shigyo[1,2,*], Naoki Yamauchi[1,2] and Yosuke Tashiro[3]

[1]*The United Graduated School of Agricultural Sciences, Tottori University, Tottori 680-8553, Japan*
[2]*Department of Biological and Environmental Science, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753-8515, Japan*
[3]*Department of Biotechnology and Plant Breeding, Faculty of Agriculture, Saga University, Saga 840-8502, Japan*

(Received 29 June 2004, accepted 29 October 2004)

First and second backcrosses of amphidiploid hybrids ($2n = 4x = 32$, genomes AAFF) between shallot (*Allium cepa* Aggregatum group) and *A. fistulosum* were conducted to produce *A. cepa* – *A. fistulosum* alien addition lines. When shallot (*A. cepa* Aggregatum group) was used as a pollinator, the amphidiploids and allotriploids set germinable BC_1 and BC_2 seeds, respectively. The 237 BC_1 plants mainly consisted of 170 allotriploids ($2n = 3x = 24$, AAF) and 42 hypo-allotriploids possessing 23 chromosomes, i.e., single-alien deletions ($2n = 3x–1 = 23$, AAF-nF). The single-alien deletions in the BC_1 progeny showed dwarfing characteristics and were discriminated from the allotriploids ($2n = 24$) and hyper-allotriploids ($2n = 25$) by means of flow cytometric analysis. The chromosome numbers of 46 BC_2 seedlings varied from 16 to 24. Eight monosomic additions ($2n = 2x+1 = 17$, AA+nF) and 20 single-alien deletions were found in these BC_2 seedlings. Consequently, six kinds of *A. cepa* – *A. fistulosum* alien chromosome additions possessing different chromosome numbers ($2n = 17, 18, 20, 21, 22, 23$) were recognized in the BC_1 and BC_2 populations. A total of 79 aneuploids, including 62 single-alien deletions, were analyzed by a chromosome 6F-specific isozyme marker (*Got-2*) in order to recognize its existence in their chromosome complements. This analysis revealed that two out of 62 single-alien deletions did not possess 6F. One (AAF-6F) out of the possible eight single-alien deletions could be identified at first. The present study is a first step toward the development of a useful tool, such as a complete set of eight different single-alien deletions, for the rapid chromosomal assignment of genes and genetic markers in *A. fistulosum*.

Key words: alien addition line, *Allium cepa, Allium fistulosum*, flow cytometry, isozyme

INTRODUCTION

Japanese bunching onion (*Allium fistulosum* L., $2n = 2x = 16$, genome FF) is a most economically important crop in East Asia, i.e., Japan, China, Taiwan and South Korea (Inden and Asahira, 1990). Genetic studies in *A. fistulosum* are challenging because of cross-pollination, biennial generation, and severe inbreeding depression. A limited number of genetic studies have been published on *A. fistulosum*, including the mode of inheritance of cytoplasmic male sterility (Moue and Uehara, 1985) and polymorphic isozyme loci (Haishima and Ikehashi, 1992; Haishima et al., 1993; Mangum and Peffley, 1994).

Allium fistulosum contains several characteristics attractive to the breeding of *A. cepa* (bulb onion and shallot), such as resistance not only to pink root disease (Netzer et al., 1985), caused by the fungus *Pyrenochaeta terrestris*, but also to onion leaf blight (Currah and Maude, 1984), caused by *Botrytis squamosa.* Among all the interspecific crosses in *Allium*, hybridization between *A. fistulosum* and *A. cepa* has been carried out the most

Edited by Kiyotaka Okada
* Corresponding author. E-mail: shigyo@yamaguchi-u.ac.jp

extensively because *A. fistulosum* shows desirable agronomic traits. Interspecific F_1 hybrids between these two species have been readily produced and shown to be resistant to several kinds of *A. cepa* diseases (Brewster, 1994).

The F_1 hybrids are completely sterile because of chromosomal rearrangements between the two species. The development of alien addition lines, therefore, has proved to be a practical approach for introgressing genes from the related species. Sears (1956) transferred genes for leaf rust resistance from *Aegilops* into wheat. Recently in *Allium*, a similar trial via *A. fistulosum – A. cepa* monosomic addition lines was applied in transferring rust resistance from chromosome 1A of *A. cepa* into *A. fistulosum* (Wako et al., unpublished data).

Allium cepa – A. fistulosum chromosome addition lines seem to be beneficial for the study of genome organization in *A. fistulosum* as well as for the practical breeding of *A. cepa*. Peffley et al. (1985) reported the production of four *A. cepa – A. fistulosum* monosomic addition lines ($2n = 17$) and several hypo-triploids ($2n = 20, 22, 25$). However, these lines still do not cover all the genomes of *A. fistulosum*. Further, Shigyo et al. (1994) proposed using hypo-allotriploids ($2n = 3x - 1 = 23$, AAF - nF) together with the *A. cepa – A. fistulosum* monosomic additions ($2n = 2x + 1 = 17$, AA + nF) in allocating the genes and genetic markers to the *A. fistulosum* chromosome.

The aims of the present study were to find the monosomic additions and hypo-allotriploids in the backcross progeny of amphidiploids between *Allium cepa* and *A. fistulosum* and to characterize them by means of several methods of analysis.

MATERIALS AND METHODS

Production of backcross progenies. Our previous study using fertility restorers (tetraploids) of Wakegi onion (*Allium* x *wakegi* Araki), a natural hybrid between *A. fistulosum* and shallot (*A. cepa* Aggregatum group), revealed that a considerable number of hypo-allotriploids resulted from the crossing of the tetraploid *A.* x *wakegi* and *A. cepa* Aggregatum group (Tashiro et al., unpublished data). Therefore, the amphidiploids AAFF ($2n = 4x = 32$) as well as the allotriploids AAF were backcrossed to shallot (*A. cepa* L. Aggregatum group) as the pollinator in this study. The pedigree of backcross progenies is given in Fig. 1. The parts surrounded by the frames in this figure were carried out in this study. As seed parents, one F_2 (SUA392) and three F_1BC_1 (SUA393, SUA394, SUA395) lines were provided by Saga University. During the 2000–2002 flowering season (March to May) in Yamaguchi, Japan, each plant used for seed and pollen parents was planted in a nursery pot, enclosed in

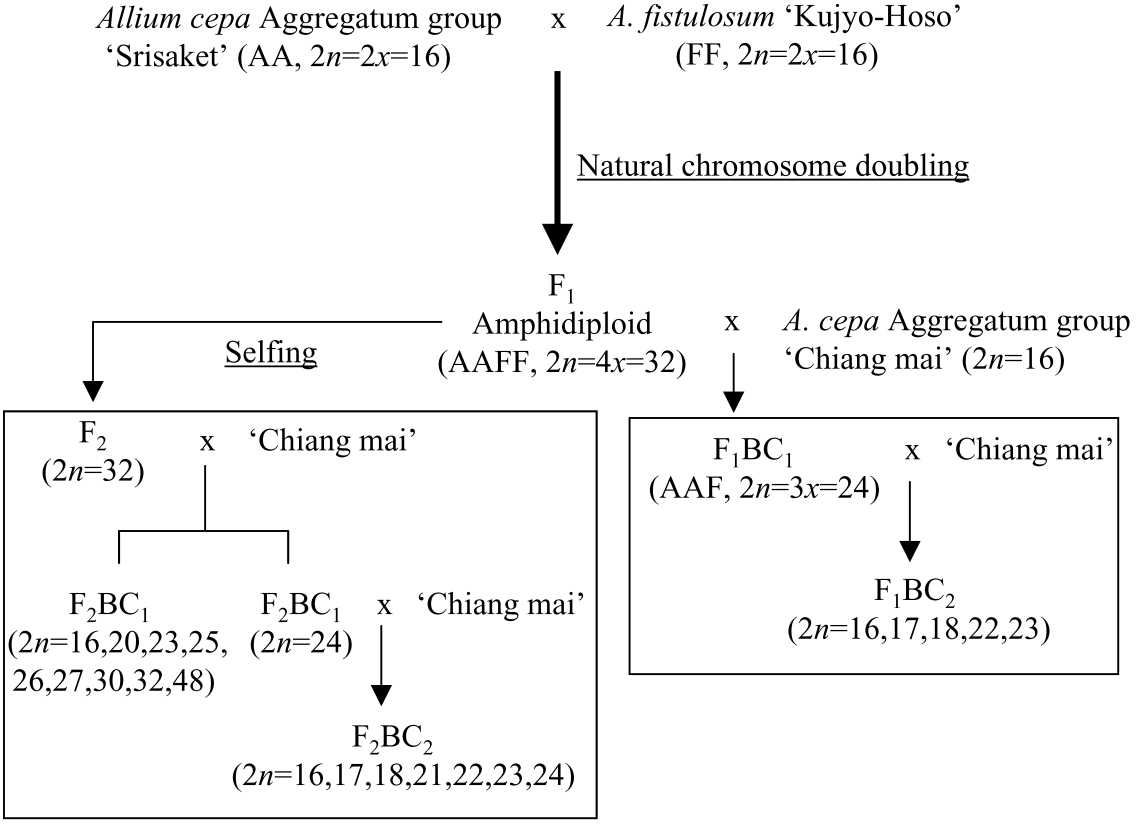


Fig. 1. Pedigree of plant materials obtained in this study. The crossings surrounded by the frame in this figure were conducted in this study.

a glass house, and covered with mosquito nets to prevent unexpected crossing. All crossings in this study were done by hand pollination. The capability of seed set was evaluated by the percentage of ovules that developed into seeds (PODS) and the germination rate, as described in our previous study (Shigyo et al., 2003).

Chromosome observation, morphological evaluation, flow cytometry analysis, and isozyme assay. In all the backcross progenies in every generation, somatic chromosomes in squashed root tip cells were observed by Feulgen reaction staining.

All 224 plants of the F_2BC_1 (194 plants), F_1BC_2 (eight plants) and F_2BC_2 (22 plants) progenies obtained in 2002 were morphologically studied six months after sowing. Three characteristics of plantlets--number of tillers, number of expanded leaves per tiller and length of leaf blade --were evaluated to determine the morphological effects of different numbers of extra chromosomes in an *Allium cepa* diploid background.

In order to develop a rapid method for the identification of hyper- and hypo-allotriploids, flow cytometric analysis of three allotriploids with different numbers of chromosomes ($2n$ = 23, 24, 25) was performed with a PA flow cytometer (Partec, Münster, Germany). The assay plants consisted of six hypo-allotriploid plants, six allotriploids, and two hyper-allotriploids. These were selected from each chromosome group at random. Between eight and 10 months after sowing, a young leaf blade was collected from the assay plant, cut with a razor-blade, and placed in DAPI staining solution (Partec) with a leaf blade of *Allium vavilovii* M. Pop. Et Vved., a diploid wild relative for *A. cepa*, which was used as the internal standard. Nuclei were counted for every fluorescence intensity (FI) at the G1 peak, and the extra chromosome index (EI; the mean FI of the test plant in relation to that for the internal standard, expressed as percentage) described in our previous report (Shigyo et al., 2003) was calculated for three different allotriploids. The measurement was repeated three times for each assay plant.

In our previous study (Shigyo et al., 1994), a monosomic addition of AA + 6F and hypo-allotriploid AAF - 6F ($2n = 3x - 1 = 23$) was employed to assign an isozyme gene locus *Got-2* to chromosome 6F of *Allium fistulosum*. In this study, the chromosome 6F-specific isozyme marker, *Got-2*, was used to identify seedlings carrying this chromosome in every backcross progeny. All procedures for the isozyme analysis of glutamate-oxaloacetate transaminase

Table 1. Variation of chromosome numbers in BC_1 and BC_2 generations

Crossing combination	Backcross generation	Number of plants observed	Frequency of plants													
			Chromosome numbers ($2n$)													
			16	17	18	20	21	22	23	24	25	26	27	30	32	48
AAFFxAA	F_2BC_1	237	3	0	0	1	0	0	42	170	12	1	1	1	5	1
AAFxAA	F_1BC_2	22	1	4	1	0	0	4	12	0	0	0	0	0	0	0
	F_2BC_2	24	6	4	1	0	1	2	8	2	0	0	0	0	0	0

Table 2. Morphological characteristics of plantlets obtained in 2002

No. of chromosomes	F_2BC_1				F_1BC_2				F_2BC_2			
	No. of lines	No. of tillers[a]	No. of expanding leaves per tiller[a]	Leaf length (mm)[a]	No. of lines	No. of tillers[a]	No. of expanding leaves per tiller[a]	Leaf length (mm)[a]	No. of lines	No. of tillers[a]	No. of expanding leaves per tiller[a]	Leaf length (mm)[a]
16	3	1.0±0.0	5.3±0.5	176.3±4.1	1	1.0	5.0	101.0	5	1.8±0.2	6.3±0.5	122.0±6.6
17	0	–	–	–	3	1.3±0.3	3.3±0.3	149.5±11.8	3	1.7±0.3	3.5±0.2	123.5±4.1
18	0	–	–	–	0	–	–	–	1	3.0	3.0	142.9
21	0	–	–	–	0	–	–	–	1	1.0	4.0	121.0
22	0	–	–	–	0	–	–	–	2	1.0	3.0	103.8
23	33	1.0±0.0	3.7±0.1	140.2±4.9	5	1.2±0.2	3.8±0.5	160.9±22.6	7	1.0±0.0	5.3±0.5	189.9±13.8
24	143	1.1±0.0	5.1±0.1	183.3±2.3	0	–	–	–	2	2.0	5.9	155.7
25	10	1.8±0.6	5.2±0.5	178.9±10.7	0	–	–	–	0	–	–	–
26	1	1.0	3.0	142.0	0	–	–	–	0	–	–	–
27	1	1.0	3.0	122.1	0	–	–	–	0	–	–	–
32	2	1.0	5.0	185.5	0	–	–	–	0	–	–	–
48	1	1.0	3.0	128.2	0	–	–	–	0	–	–	–

[a]: Data are shown with mean ± standard error.

(GOT; EC 2.6.1.1) through native-PAGE with the crude extract of enzyme from a young expanding leaf were conducted according to the procedure of Shigyo et al. (1994).

RESULTS

Production of alien additions. The amphidiploids and allotriploids were crossed with shallot as the male to produce BC_1 and BC_2 seeds, respectively. The amphidiploids had higher seed fertility [PODS = (1114 / 16140) × 100 = 6.9] than the allotriploids [PODS = (254 / 50520) × 100 = 0.5]. The germination rates of the BC_1 seeds reached 38.4% [= (377 / 981) × 100]. The rates of the BC_2 seeds were nearly the same [(86 / 220) × 100 = 39.1%]. Finally, 237 BC_1 and 46 BC_2 seedlings survived. The F_2BC_1 progenies were mainly allotriploids and hypo-allotriploids with 23 chromosomes. The frequency of two different allotriploids accounted for approximately 89% of all the F_2BC_1 seedlings (Table 1). The chromosome numbers of the BC_2 seedlings varied from 16 to 24 for two different generations (F_1BC_2, F_2BC_2). Hypo-allotriploids (23 chromosomes) were the most frequent (43.5%), followed by monosomic additions (17.4%). Thus, several kinds of *Allium cepa* – *A. fistulosum* chromosome additions were generated from the first or second backcross.

Morphological characteristics of BC_1 and BC_2 progenies in the initial growing period. Several morphological characteristics of plantlets were evaluated in all the generations obtained in 2002 (Table 2). In the F_2BC_1 population, great differences among seedlings possessing different numbers of chromosomes were observed only in the length of the expanding leaf. Regarding the two major types of derivatives, the leaf lengths of the hypo-allotriploids (mean ± SE = 140.2 ± 4.9 mm) were clearly shorter than those of the allotriploids (183.3 ± 2.3). Except for a few cases in this generation, there was a tendency for the euploids to be larger and more vigorous than the aneuploids. In the BC_2 population composed of the two generations, F_1BC_2 and F_2BC_2, clear differences appeared only in the leaf length. However, the euploids were smaller and less vigorous than the aneuploids in this population, contrary to our expectations.

Characterization of three types of allotriploids by means of flow cytometric analysis. Fig. 2 shows typical histograms of flow cytometric analyses for three materials with different numbers of chromosomes in comparison with the internal standards. Bimodal peaks were formed when test plants were measured together with the internal standard for G1- and G2-phase nuclei. All three cases with different numbers of chromosomes had fluorescence intensities (FI) higher than the internal standard. The results from the G1 peak are summarized in Table 3. The FI value of test plants ranged from

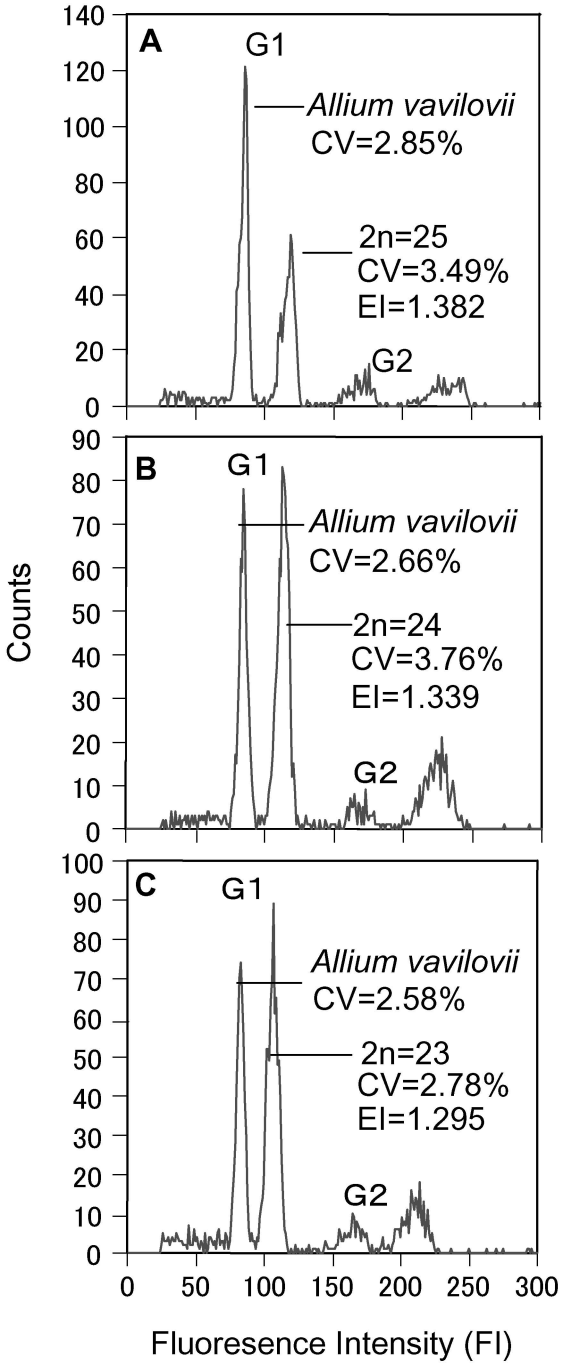


Fig. 2. Representative histograms of nuclei isolated from mixtures of leaves of three plants with different numbers of chromosomes. (**A**) *Allium vavilovii* – hyper-allotriploid ($2n = 3x + 1 = 25$); (**B**) *A. vavilovii* – allotriploid ($2n = 3x = 24$); (**C**) *A. vavilovii* – hypo-allotriploid ($2n = 3x - 1 = 23$).

111.66 to 115.3, whereas those for the internal standard ranged from 83.3 to 86.5. The P value (*t*-test) obtained when comparing the means of the FI in the test plants with those in the internal standard showed, in all three cases, significant differences ($P < 0.001$). There were significant differences in the extra chromosome index

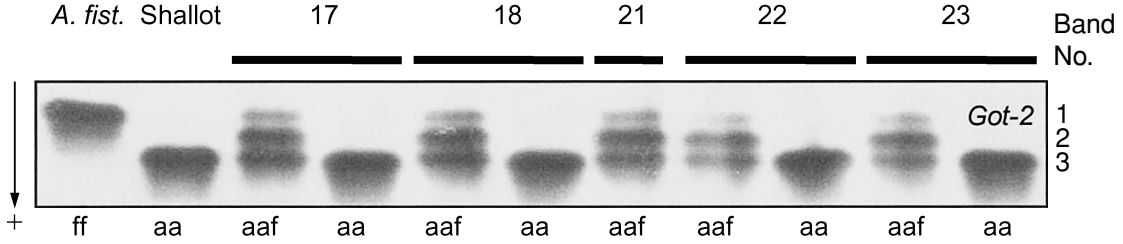
Table 3. Flow cytometric analysis for G1 peak of fourteen plant materials with different numbers of chromosomes in comparison with an internal standard, diploid *Allium vavilovii*

Chromosome numbers (2*n*)	Number of test plants	Fluorescence intensity		Extra chromosome index
		Test plant	Internal standard	
23	6	109.7±2.6 (3.5±0.1)	84.9±2.0[a] (3.3±0.2)[b]	1.292±0.002a[c]
24	6	116.6±2.2 (4.1±0.3)	83.4±1.6 (3.6±0.3)	1.338±0.001b
25	2	115.3±1.3 (3.9±0.3)	83.3±1.0 (3.5±0.4)	1.384±0.002c

[a]: All data are shown with mean ± standard error.
[b]: Numerical values in parentheses indicate coefficient of variation.
[c]: Mean separation within each column by Tukey's multiple range test, $P < 0.05$.

(EI) among three different kinds of allotriploids, and the mean values of EI rose as the number of chromosomes increased. Consequently, it was possible to distinguish three kinds of plant material with different numbers of chromosomes by flow cytometric analysis.

GOT analysis. *Allium fistulosum* possesses a pair of distinctive sub-telocentric chromosomes 6F (Jones, 1990). In this study, banding patterns for *Got-2* were observed to determine whether the chromosome 6F existed in hyper-diploids (2*n* = 17–18) and hypo-allotriploids (2*n* = 21–23) (Fig. 3). According to Shigyo et al. (1994), *Got-2* is a dimeric enzyme in *A. fistulosum* and *A. cepa*, and each of the two species has different alleles (*f* and *a*, respectively). In the backcross progenies produced in this study, there were two types of band patterns: a triplicate band pattern for both two alleles (*f* and *a*) and a single band pattern for one allele (*a*). A dosage effect of the GOT genes was clearly observed on zymograms of the triplicate band pattern: bands 2 and 3 were



F_2BC_1	0	0	0	0	0	0	0	42	0
F_1BC_2	1	3	0	1	0	4	0	10	2
F_1BC_2	2	2	1	0	1	1	1	8	0
Total	3	5	1	1	1	5	1	60	2

Fig. 3. Glutamate-oxaloacetate transaminase zymograms. Lanes (left to right): *Allium fistulosum*, shallot, monosomic additions (2*n* = 17), double monosomic additions (2*n* = 18) and hypo-allotriploids (2*n* = 21, 22, 23). aa and ff indicate homozygous genotypes. aaf shows the heterozygous genotype. The frequencies of chromosome numbers in respective generations are shown in the bottom half of the figure.

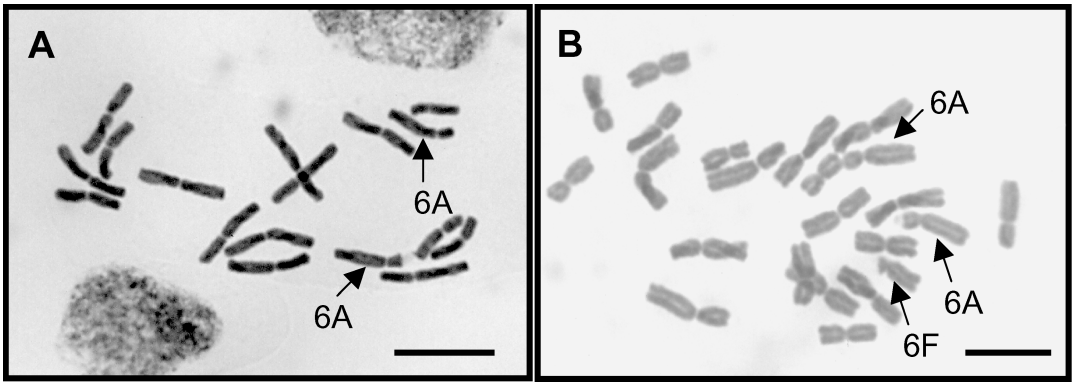


Fig. 4. Typical somatic metaphase chromosomes of monosomic addition (2*n* = 17) (**A**) and hypo-allotriploid (2*n* = 23) (**B**). Arrows point to the sub-telocentric chromosomes (6A, 6F). The monosomic addition does not possess the chromosome 6F. The hypo-allotriploid possessed the chromosome 6F. Scale bar = 10 μm.

stained more intensely than band 1. These results indicated that genotypes of the triplicate and single band patterns showed, respectively, *aaf* and *aa*. This result allowed us to identify the alien chromosome assortment of hyper-diploids and hypo-allotriploids. Seventy out of seventy-nine aneuploids examined (88.6%) carried chromosome 6F. We were able to confirm this result by means of a simple karyotypic observation (Fig. 4).

DISCUSSION

It was impossible to complete eight possible *Allium cepa* – *A. fistulosum* monosomic additions due to the insufficient number found in the BC_2 progeny. The low seed fertility in the second backcross added to the difficulty. The main factor contributing to the low fertility could be the existence of deleterious alleles on a diploid background of shallot (*A. cepa* Aggregatum group). Because shallot has been asexually propagated for centuries, it is likely that mutation has produced deleterious alleles that are maintained in the heterozygote state. After sexual propagation, progeny plants homozygous for these deleterious alleles should show reduced vigor or perish, as shown in garlic, *A. sativum* L. (Jenderek, 2004), which is another *Allium* asexually propagated plant. To avoid this risk, the seed-propagated cultivar of bulb onion (*A. cepa* Common onion group) can be effectively substituted for shallot (*A. cepa* Aggregatum group) in the next backcrossing to produce the monosomic additions.

On the other hand, production of the hypo-allotriploids AAF-nF was easier than that of the monosomic additions. An increase in the number of alien chromosomes eases the effect of the deleterious gene found in shallot. Surprisingly, more hypo-allotriploids were obtained in the BC_1 than in the BC_2 progenies. We can offer the following interpretation of this phenomenon. Tashiro et al. (1981) found that abnormal chromosome pairings were among PMCs at metaphase-I in reciprocal amphidiploid hybrids between *Allium fistulosum* and shallot (*A. cepa* Aggregatum group): More than 90% of the pollen mother cells had 16 bivalents, though two univalents and one quadrivalent were also found in rare cases (< 6%). Chromatid non-disjunction might occur in the process of female gamete formation in amphidiploids since such an unexpected chromosome pairing is supposed to be also observed in embryosac mother cells. Several aneuploids ($2n$ = 20, 23, 25, 26, 27, 30) found in the F_2BC_1 could result from fertilization between the aneuploid female gamete produced via abnormal pairings (e.g. n = 15) and the normal male gamete of *A. cepa* (n = 8) by chance. Thus, the amphidiploids have a great deal of flexibility in regard to the occurrence of backcross progeny with an unexpected chromosome number, including hypo-aneuploids with 23 chromosomes. We coined the term *single-alien deletion* (e.g., $2n = 3x\text{-}1 = 23$) to designate a hypo-allotriploid in which one of the alien chromosomes are missing from the *A. fistulosum* complement in the diploid background of *A. cepa*. A complete set of all eight possible single-alien deletions would be very useful for locating the genes to specific chromosomes of *A. fistulosum*. Further cytogenetic studies including flow cytometric analysis and PCR-based DNA markers will be used to identify the complete single-alien deletion set among the remaining 60 F_2BC_1 plants. The rapid system for chromosomal identification with the help of flow cytometric analysis would be applicable not only for detecting numerical chromosome changes but also for identifying missing chromosomes in the alien deletions.

In the monosomics of several diploid species, the loss of a chromosome has a very drastic, sometimes lethal, effect on plant morphology (Khush, 1973; Singh, 2003). We observed that, in the juvenile stage, the loss of one chromosome at the triploid level was not as severe as the same loss at the monosomic level. Since this result is due to non-numerical changes of the *Allium cepa* chromosome complement consisting of a diploid background, it is unnecessary to consider the phenotypic expressions caused by recessive deleterious genes located on *A. cepa* chromosomes. Such an alien deletion is recommended as a tool for the chromosomal assignment of genes in the donor parent when the vegetative propagation plant is made to be the recipient parent. Furthermore, evaluating several effects of deleted chromosomes on the morphology and physiology of allotriploids could reveal the chromosomal locations of quantitative trait loci (QTL) in *A. fistulosum*. The simplicity of this technique can be compared with conventional QTL assignment by mapping. The unlinked QTL can be efficiently assigned to a chromosome using the single-alien deletions, although we may not be able to distinguish the linked QTLs due to their cumulative effect.

The authors wish to thank Miss S. Shinozaki, Mr. T. Utsunomiya and Miss N. Matsubara for their contributions to this study. The authors would like to thank Dr. M. J. Havey of USDA and the University of Wisconsin for critically reviewing this manuscript. The authors further express their gratitude to Ms. Lynn Hofstad of Hot Line for reviewing the manuscript regarding the English language.

REFERENCES

Brewster, J. L. (1994) The genetics and plant breeding of *Allium* crops. In: Onions and other vegetable Alliums. (Ed.: Brewster, J. L.), pp. 41–62. CABI Publishing.

Currah, L. and Maude, R. B. (1984) Laboratory tests for leaf resistance to *Botrytis squamosa* in onion. Annals of Applied Biology **105**, 277–283.

Haishima, M. and Ikehashi, H. (1992) Identification of isozyme genes in native varieties of Japanese bunching onion (*Allium fistulosum* L.). Japan. J. Breed. **42**, 497–505.

Haishima, M., Kato, J. and Ikehashi, H. (1993) Isozyme polymorphism in native varieties of Japanese bunching onion

(*Allium fistulosum* L.). Japan. J. Breed. **43**, 537–547.

Inden, H and Asahira, T. (1990) Japanese bunching onion (*Allium fistulosum* L.). In: Onion and Allied Crops. Vol. III (Eds.: Rabinowitch, H. D. and J.L. Brewster), pp. 159–78. CRC Press, Inc. Boca Raton, Florida.

Jenderek, M. M. (2004) Development of S_1 families in galic. Acta. Hort. **637**, 203–206

Jones R. N. (1990) Cytogenetics. In: Onion and Allied Crops. Vol. I (Eds.: Rabinowitch, H. D. and J.L. Brewster), pp. 199–212. CRC Press, Inc. Boca Raton, Florida.

Khush, G. S. (1973) Cytogenetics of alien additions and substitutions. In: Cytogenetics of Aneuploids (Ed.: Khush, G.S), pp. 238–249. Academic Press.

Mangum, P. D. and Peffley, E. B. (1994) Inheritance of ADH, 6-PGDH, PGM and SKDH in *Allium fistulosum* L. J. Amer. Soc. Hort. Sci. **119**, 335–338.

Moue, T. and Uehara, T. (1985) Inheritance of cytoplasmic male sterility in *Allium fistulosum* L. (Welsh onion). J. Japan. Soc. Hort. Sci. **53**, 432–437. (*in Japanese*)

Netzer, D., Rabinowitch, H. D. and Weintal, C. (1985) Greenhouse technique to evaluate pink root disease caused by *Pyrenochaeta terrestris*. Euphytica **34**, 385–391

Peffley, E. B., Corgan, J. N., Horak, K. E. and Tanksley, S. D. (1985) Electrophoretic analysis of *Allium* alien addition lines. Theor. Appl. Genet. **71**, 176–84.

Sears, E. R. (1956) The transfer of leaf-rust resistance from *Aegilops umbellutaca* to wheat. Brookhaven Symp. Biol. **9**, 1–22

Shigyo, M., Tashiro, Y. and Miyazaki, S. (1994) Chromosomal locations of glutamate oxaloacetate transaminase gene loci in Japanese bunching onion (*Allium fistulosum* L.) and shallot (*A. cepa* L. Aggregatum group). Jpn. J. Genet. **69**, 417–424.

Shigyo, M, Wako, T., Kojima, A., Yamauchi, N. and Tashiro, Y. (2003) Transmission of alien chromosomes from selfed progenies of a complete set of *Allium* monosomic additions: the development of a reliable method for the maintenance of a monosomic addition set. Genome **46**, 1098–1103.

Singh, R. J. (2003) Chromosome aberrations – Numerical chromosome changes. In: Plant cytogenetics (Ed.: Singh, R. J.), pp. 157–276. CRC press, Boca Raton, Florida.

Tashiro, Y. (1981) Cytogenetic studies on the origin of *Allium wakegi* Araki. II. Fertility and meiotic behavior of the tetraploid *A. wakegi* and the amphidiploid hybrid between *A. ascalonicum L.* and *A. fistulosum L.* Bull. Fac. Agr., Saga Univ. **50**, 85–95