園学雑. (J. Japan. Soc. Hort. Sci.) 68 (3) : 494-498. 1999.

Fertility of Alien Monosomic Addition Lines of Japanese Bunching Onion (*Allium fistulosum* L.) with Extra Chromosomes from Shallot (*A. cepa* L. Aggregatum group)

Masayoshi Shigyo*, Mitsuyasu Iino and Yosuke Tashiro
Faculty of Agriculture, Saga University, Honjyo 1, Saga 840-8502

Summary

In a series of alien monosomic addition lines (AMALs; FF+1A-FF+8A) of Japanese bunching onion (*Allium fistulosum* L.) with extra chromosomes from shallot (*A. cepa* L. Aggregatum group), seed and pollen fertility were assessed to reveal the effect of the alien chromosomes on the fertility of the recipient species. Data for seed fertility which were derived from three styles of pollination (open pollination, selfing, and backcrossing with *A. fistulosum*) yielded considerable variations among types of the AMALs. FF+8A had high seed fertility, whereas FF+3A had very low one. Pollen grains with normal number and shape of nuclei were observed in all types of the AMALs, but pollen fertility varied among the types. Particularly, FF+4A had high pollen fertility (approx. 80 %), whereas FF+1A expressed very low pollen fertility (approx. 31 %). These results indicate that alien chromosomes remarkably influence both seed and pollen fertility of the recipient species. A regression analysis revealed that there was no correlation between seed and pollen fertility in the AMALs. This indicates that genes related to the destinies of seed and pollen in the AMALs are located respectively on different chromosome of *A. cepa* Aggregatum group.

Key Words: *Allium*, shallot, Japanese bunching onion, alien monosomic addition line, fertility.

Introduction

In edible Alliums, shallot (*Allium cepa* L. Aggregatum group, $2n$=16, genomes AA), which has the highest adaptability to tropical and sub-tropical zones, is an important crop in these zones. Therefore, detailed genetic analyses of this species are required for the efficient breeding of *Allium* crops suitable for low latitudinal regions. Since a series of alien monosomic addition lines (AMALs) of Japanese bunching onion (*A. fistulosum* L., $2n$=16, FF) with extra chromosomes from shallot was established previously (Shigyo et al., 1996), several trials have been conducted to determine the chromosomal locations of genes and genetic markers of *A. cepa* Aggregatum group. Chromosomal locations of isozyme genes (Shigyo et al., 1994, 1995a, 1995b, 1996), rRNA gene (Shigyo et al., 1996), genes for flavonoid and anthocyanin production in leaf sheaths (Shigyo et al., 1997a), and RAPD markers (Shigyo et al., 1997c) of *A. cepa* Aggregatum group were determined using this series. Moreover, a study on morphological characteristics of AMALs revealed that several phenotypic expressions of the recipient species, *A. fistulosum*, are considerably influenced by alien genes on the extra chromosomes (Shigyo et al., 1997b). It seems that the alien chromosomes also affect sexual reproduction of *A. fistulosum*.

To investigate the effects of alien chromosomes on the reproductive cycle of *A. fistulosum*, we examined pollen and seed fertility/germinability of each type in a series of AMALs.

Materials and Methods

Plant materials

A series of AMALs of *A. fistulosum* with extra chromosomes from *A. cepa* Aggregatum group ($2n$=17, FF+1A-FF+8A) (Shigyo et al., 1996) was used as plant materials (Table 1). *A. fistulosum* cv. Kujyo, which had been used in developing AMALs, was used as the control and pollen parent in backcrosses.

Estimation of seed fertility

Seed fertility of the plants mentioned above were examined by open pollination, selfing, and backcrossing with the pollen of *A. fistulosum*. The seed fertility was estimated by the percentage of ovules that developed

Received; December 4, 1997. Accepted; August 17, 1998.
The research was supported in part by Grant-in-Aid for Research Fellow of the JSPS (No. 0058) from the Ministry of Education, Science, Sports, and Culture of Japan.

* Present address : Faculty of Agriculture, Yamaguchi University, Yoshida 1677-1, Yamaguchi 753-8515, Japan

into seeds (PODS; see Table 1). For open pollination, two to 10 plants of each type of AMAL (total 36 plants) and one plant of *A. fistulosum* were grown in the field of Saga University (Table 1). Sixteen out of the 36 plants (two plants for each type of AMAL) were grown in pots at a green house and used for selfing and backcrossing. All plants examined originated from different seed. Umbels were bagged and hand-pollinated except open pollination. In backcrossing, the stamens were removed to avoid selfing. The germination capacity was examined on seeds obtained from open pollination. About 100 seeds produced in each AMAL plant were sown onto double filter papers in five petri dishes (containing about 20 seeds each) and moistened with tap water. These petri dishes were placed in an incubator in the dark at 25 ℃. Germinated seeds were counted twelve days after sowing.

Table 1. Seed fertility and seed germination in open-pollinated alien monosomic addition lines (AMALs) and *A. fistulosum*.

Plant material	FF+extra chromosome	No. of plants	Percentage of ovules that developed into seeds (PODS)[z,y]	Percentage of seeds that germinated[y]
AMALs of	FF+1A	5	10.1 ± 4.5	4.1 ± 1.3
A. fistulosum	FF+2A	2	14.1 ± 6.4	36.0 ± 3.8
	FF+3A	4	3.1 ± 1.9	3.0 ± 1.7
	FF+4A	8	5.0 ± 1.0	31.6 ± 9.0
	FF+5A	3	18.8 ± 10.0	56.7 ± 17.9
	FF+6A	2	28.5 ± 6.3	70.5 ± 2.5
	FF+7A	10	8.4 ± 1.4	19.0 ± 7.1
	FF+8A	2	45.7 ± 8.0	74.5 ± 4.0
A. fistulosum	FF	1	25.7	84.0

[z] Percentage of ovules that developed into seeds= $\frac{\text{No. of seeds produced}}{\text{No. of flowers pollinated} \times \text{No. of ovules per flowers (6)}} \times 100$

[y] All data except *A. fistulosum* are shown with mean ± SE.

Table 2. Seed fertility in open-pollianated, selfed, and backcrossed alien monosomic additon lines (AMALs) and *A. fistulosum* and their pollen fertility.

Plant meterial	FF+extra chromosome	Plant No.	Percentage of ovules that developed into seeds (PODS)[z]			Pollen fertility[z,y] (%)
			Open-pollinated[x]	Selfed	Backcrossed	
AMALs of	FF+1A	11	5.7 (763)[w]	2.3 (359)	29.2 (220)	21.9
A. fistulosum		130	8.1 (912)	10.0 (277)	27.6 (271)	39.5
	FF+2A	132	7.7 (742)	4.8 (533)	13.5 (189)	65.7
		141	20.4 (958)	5.0 (642)	11.3 (363)	70.2
	FF+3A	5	2.0 (835)	5.9 (515)	16.0 (256)	72.2
		42	1.2 (363)	0.5 (205)	6.6 (294)	66.0
	FF+4A	10	4.9 (1609)	8.6 (455)	40.3 (342)	81.7
		50	7.8 (1663)	12.1 (516)	27.6 (130)	95.5
	FF+5A	26	32.1 (623)	9.2 (280)	28.4 (210)	48.6
		71	20.4 (888)	6.0 (340)	29.6 (162)	54.2
	FF+6A	120	34.7 (722)	4.3 (342)	17.6 (195)	83.1
		308	22.2 (696)	2.2 (202)	20.2 (138)	59.4
	FF+7A	246	10.1 (1102)	12.8 (875)	24.6 (285)	57.4
		324	6.9 (680)	5.5 (286)	28.8 (211)	53.3
	FF+8A	65	53.7 (1405)	23.2 (1584)	51.4 (400)	85.5
		240	37.7 (812)	2.0 (293)	26.8 (175)	45.4
A. fistulosum	FF	1	25.7 (2231)	36.4 (609)	36.4 (609)	94.8

[z] All data in 16 plants were used for ANOVA in Tables 3 and 5 and for regression analysis in Fig.1.

[y] Data on pollen fertility were also used to calculate mean ± SE shown in Table 4.

[x] PODS data in open pollination were also used to calculate mean ± SE shown in Table 1.

[w] Numerical values in parentheses indicate no. of flowers pollinated.

Table 3. Analysis of variance for seed fertility (PODS) of alien monosomic addition lines (AMALs) in three pollination styles.

Source	Sum of squares	Degree of freedom	Mean square	F value
Pollination style (A)	2554.555	2	1277.277	53.54**
Type of AMAL (B)	2674.185	7	382.026	16.01**
Plant (C)	194.005	1	194.005	8.13*
A × B	2020.875	14	144.348	6.05**
A × C	10.090	2	5.045	-
B × C	582.730	7	83.247	3.49*
A × B × C (Error)	334.000	14	23.857	
Total	8370.440	47		

* and ** significant at the 1 % and 0.1 % levels, respectively.

Estimation of pollen fertility

Pollen fertility was determined using the field-grown materials. About 1,500 pollen grains, collected equally from three florets at full bloom per plant, were observed with no replication. Acetocarmine preparations of fresh pollen grains were made, and the number and shape of nuclei in respective pollen grains were observed. Each pollen grain was classified into one of four classes: 0, no nucleus; 1, one nucleus; 2, one vegetative nucleus and one round sperm nucleus; and 3, one vegetative nucleus and one crescent-shaped sperm nucleus. Class 3 pollen grains were considered fertile. The percentage of them in all the pollen grains observed was used to indicate pollen fertility.

Statistical analyses

Data on seed and pollen fertility in 16 plants (Table 2, two plants per AMAL type) were used for statistical analyses. The PODS data were used for two-way analysis of variances (ANOVA) for styles of pollination, types of the AMAL, and plants of the same type of the AMAL. The pollen fertility estimated were applied for one-way ANOVA for types of extra chromosomes and plants with same extra chromosome. A regression analysis of seed fertility (PODS) in backcrossing and pollen fertility was performed to seek the relationship between them.

Results and Discussion

Seed fertility

All 36 open-pollinated plants of the AMALs produced germinable seeds (Table 1). Seed fertility (PODS) in open pollination showed considerable variations both within and among types of the AMALs (FF+1A - FF+8A). The PODSs varied from 3.1 to 45.7. That of *A. fistulosum* was 25.7. The AMAL FF+8A had the highest seed fertility, whereas FF+3A and FF+4A had very low seed fertility. The germination percentages ranged from 3.0 to 74.5 among the types. That of *A. fistulosum* was 84.0.

Table 4. Pollen fertility of alien monosomic addition lines (AMALs) and *A. fistulosum*.

Plant material	FF + extra chromosome	No. of plants	Pollen fertility (%)[z]
AMALs of *A. fistulosum*	FF+1A	5	31.4 ± 8.9
	FF+2A	2	68.0 ± 2.3
	FF+3A	4	62.7 ± 15.4
	FF+4A	8	80.5 ± 5.9
	FF+5A	3	50.1 ± 2.5
	FF+6A	2	71.3 ± 11.9
	FF+7A	10	49.0 ± 6.8
	FF+8A	2	65.5 ± 20.1
A. fistulosum	FF	1	94.8

[z] All data except *A. fistulosum* are shown with mean ± SE.

Sixteen out of the 36 plants used for open pollination also formed seeds by selfing and backcrossing (Table 2). The means of PODSs in the 16 plants decreased in the following order: backcrossing (mean ± SE; 25.0 ± 3.2), open pollination (17.2 ± 4.4), and selfing (7.2 ± 1.6). With the exception of two cases (backcross in plant No. 5 and selfing in plant No. 240), FF+3A had very low PODS whereas FF+8A had fairly high PODS in all styles of pollination. Plant No. 65 of FF+8A showed obviously higher PODS than *A. fistulosum* in open pollination and backcrossing.

There were significant differences in seed fertility among styles of pollination (A), among types of the AMAL (B), and between plants of the same type of the AMAL (C) (Table 3). Furthermore, interactions between pollination style (A) and extra chromosome (B) and between extra chromosome (B) and plants with same extra chromosome (C) were apparent.

These results reveal significant variations in seed fertility among the AMALs attributable to alien chromosomes.

Pollen fertility

Stained pollen grains with normal number and shape of nuclei were found in the 36 plants examined (Table 4). There were considerable variations in pollen fertility

Table 5. Analysis of variance for pollen fertility in alien monosomic addition lines (AMALs).

Source	Sum of squares	Degree of freedom	Mean square	F value
Type of AMAL (A)	4050.610	7	578.659	3.06
Plant (B)	66.423	1	66.423	-
A × B (Error)	1321.958	7	188.851	
Total	5438.991	15		

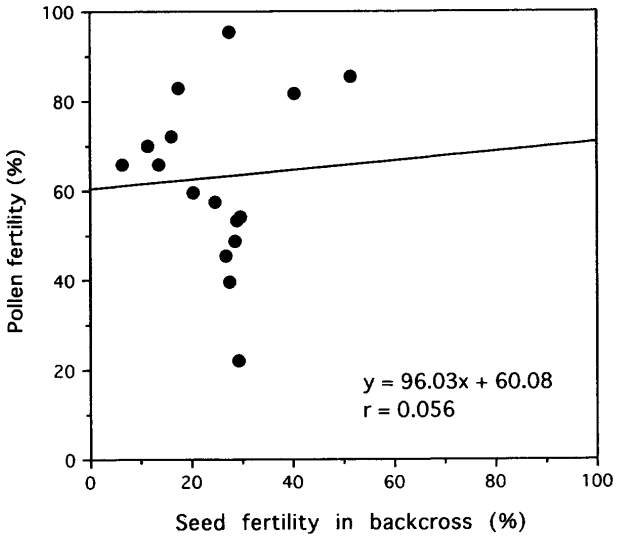

Fig. 1. Scatter diagram showing the lack of relationship between seed fertility (PODS) of alien monosomic addition lines in backcross and their pollen fertility.

both within and among types of the AMALs (FF+1A - FF+8A). The pollen fertility of the AMALs ranged from 31.4 to 80.5 % and was lower than that of *A. fistulosum* (94.8 %). ANOVA for pollen fertility (Table 5) did not show significant differences among the types. However, FF+4A obtained high pollen fertility (approx. 80 %), whereas FF+1A possessed very low pollen fertility (approx. 31 %). The results mentioned above indicate that alien chromosomes greatly influence both seed and pollen fertility of the recipient species.

Correlation between seed and pollen fertility

The results of a regression analysis using the 16 plants shown in Table 2 reveal that there is no correlation (r=0.056) between seed and pollen fertility (Fig. 1). This result could lead us to assume that phenotypic expressions on seed and pollen fertility in the AMALs are dominated by different alien gene. Furthermore, the genes related to seed and pollen fertility may be located respectively on different chromosome of *A. cepa* Aggregatum group.

Efforts have been made to develop AMALs of several diploid species (Singh, 1993). Successful attempts to establish complete series of AMALs have been reported in rice (Jena and Khush, 1989; Yasui, 1997) and sugar beet (van Geyt et al., 1988; Reamon-Ramos and Wrick, 1992). Jena and Khush (1989) investigated both seed and pollen fertility in the AMALs of *Oryza sativa* with extra chromosomes from *O. officinalis*. They also recognized considerable variations in seed and pollen fertility among the AMAL types and found 0 % in one plant. In our study, we found no plant completely lacking fertility. This result demonstrates that extra chromosomes from *A. cepa* Aggregatum group do not have a strong effect on the fertility of AMALs in *A. fistulosum*.

All eight types of AMALs produced seeds and formed fertile pollens, which indicates that seed propagation is a useful maintenance method in addition to vegetative propagation conventional in our AMALs. Further cytogenetic studies on chromosome number in the selfed and backcrossed progenies of AMALs are necessary to confirm this proposition.

Acknowledgment

Masayoshi Shigyo acknowledges the support of JSPS Research Fellowships for Young Scientists.

Literature Cited

Jena, K. K. and G. S. Khush. 1989. Monosomic alien addition lines of rice: production, morphology, cytology, and breeding behavior. Genome 32: 449-455.

Reamon-Ramos, S. M. and G. Wricke. 1992. A full set of monosomic addition lines in *Beta vulgaris* from *Beta webbiana*: morphology and isozyme markers. Theor. Appl. Genet. 84: 411-418.

Shigyo, M., Y. Tashiro and S. Miyazaki. 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., Y. Tashiro, S. Isshiki and S. Miyazaki. 1995a. Chromosomal locations of five isozyme gene loci (*Lap-1*, *Got-1*, *6-Pgdh-2*, *Adh-1* and *Gdh-1*) in shallot (*Allium cepa* L. Aggregatum group). Jpn. J. Genet. 70: 399-407.

Shigyo, M., Y. Tashiro, S. Isshiki and S. Miyazaki. 1995b. Chromosomal locations of isocitrate dehydrogenase and phosphoglucoisomerase gene loci in shallot (*Allium cepa* L. Aggregatum group). Jpn. J. Genet. 70: 627-632.

Shigyo, M., Y. Tashiro, S. Isshiki and S. Miyazaki. 1996. Establishment of a series of alien monosomic addition lines of Japanese bunching onion (*Allium fistulosum* L.) with extra chromosomes from shallot (*A. cepa* L.

Aggregatum group). Genes Genet. Syst. 71: 363-371.
Shigyo, M., Y. Tashiro, M. Iino, N. Terahara, K. Ishimaru and S. Isshiki.1997a. Chromosomal locations of genes related to flavonoid and anthocyanin production in leaf sheath of shallot (*Allium cepa* L. Aggregatum group). Genes Genet. Syst. 72: 149-152.
Shigyo, M., M. Iino, S. Isshiki and Y. Tashiro. 1997b. Morphological characteristics of a series of alien monosomic addition lines of Japanese bunching onion (*Allium fistulosum* L.) with extra chromosomes from shallot (*A. cepa* L. Aggregatum group). Genes Genet. Syst. 72: 181-186.
Shigyo, M., T. Miyazaki, S. Isshiki and Y. Tashiro. 1997c. Assignment of randomly amplified polymorphic DNA markers to all chromosomes from shallot (*Allium cepa* L. Aggregatum group). Genes Genet. Syst. 72: 249-252.
Singh, R. J. 1993. Chromosomal aberrations - Numerical chromosome changes (Heteroploidy). p. 111-254. In: R. J. Singh (ed.). Plant cytogenetics. CRC Press, Boca Raton.
van Geyt, J. P. C., M. Oleo, W. Lange and Th. S. M. de Bock. 1988. Monosomic additions in beet (*Beta vulgaris*) carrying extra chromosomes of *Beta procumbens*. Theor. Appl. Genet. 76: 577-586.
Yasui, H. 1997. Cytogenetical studies on alien chromosome addition lines in rice (*Oryza sativa* L.), each carrying a single chromosome(s) of *O. punctata* Kotschy. Ph. D. Thesis. Kyusyu Univ., Fukuoka, Japan.

シャロット由来単一異種染色体を添加したネギ系統の稔性

執行正義*・飯野益康・田代洋丞

佐賀大学農学部　840-8502　佐賀市本庄町1番地

摘　要

8種類のシャロット由来単一異種染色体をそれぞれ添加したネギ系統を用いて，外来染色体が染色体受容親の稔性に及ぼす影響を調査した．三種類の受粉様式(放任受粉，自家受粉，ネギの花粉を用いた戻し交雑)における添加系統の種子稔性は8種類の添加型間でいろいろな程度を示した．特に，シャロットの第8染色体をもつ添加系統(第8添加型)は高い種子稔性を示し，また，第3添加型はかなり低い稔性を示した．花粉稔性に関しては，調査したすべての添加系統が稔性花粉を有したが，その程度は添加型間で異なっていた．特に，第4添加型は高い稔性(約80%)を示し，第1添加型は低い花粉稔性(約31%)を示した．以上の結果は外来染色体が染色体受容親の種子および花粉稔性に影響を及ぼすことを強く示唆した．さらに，各添加型の種子稔性と花粉稔性との間には相関はなく，種子および花粉稔性を支配する遺伝子はそれぞれ別の染色体に座乗していることが示唆された．

*現在：山口大学農学部 753-8515 山口市吉田 1677-1