Chromosomal Variation in Callus Lines and Regenerated Plantlets from Three Cultivars of *Allium fistulosum* L. ($2n=16$)

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Summary Three callus lines from 3 *Allium fistulosum* L. cultivars (Kaori, Fresh, Wede) and their regenerated plantlets were examined to elucidate the relationship between chromosomal aberration and morphogenetic capacity. In these callus lines, 9 karyotypes that were altered numerically and/or structurally were found in addition to the normal diploid karyotype (type S) identical to that of the mother plant. Structurally changed karyotypes occurred as a result of deletions and/or translocations. Of these, the occurrence of dicentric chromosomes arising through deletions and translocations between 2 a1 chromosomes was high. In regenerated plantlets from the callus lines, 3 structurally and/or numerically altered karyotypes were found in addition to the type S karyotype. Plantlets regenerated from callus cells were induced via shoot formation and subsequent root formation.

The original habitat of the welsh onion (*Allium fistulosum* L.) is known to be western China or Siberia, and it is one of the major vegetable crops cultivated in many countries because it is both cold- and heat-tolerant. Tissue culture of *Allium* species has mainly been done by regenerating plants from calli, protoplasts and meristematic tissues of *A. cepa* (Lu *et al.* 1989, Fellner and Havranek 1992, Van der Valk *et al.* 1992, Song and Peffley 1994), *A. sativum* (Havranek and Novak 1973, El-Gizawy and Ford-Lloyd 1987, Nagasawa and Finer 1988) and *A. tuberosum* (Pandey *et al.* 1992, Shuto *et al.* 1993, Matsuda and Adachi 1996, Xue *et al.* 1997). Several studies have reported raising *A. fistulosum* productivity through culture (Shahin and Kaneko 1986, Phillips and Hubstenberger 1987, Peffly 1992, Kim and Soh 1996). However, these reports did not mention any cytological details concerning chromosomal variations occurring during tissue culture.

In general, chromosomal aberrations such as numerical and structural changes are a common occurrence in calli and their regenerants. Furthermore, older calli are known to lose the capacity to regenerate plants because they either acquire high ploidy in the tissues or accumulate an increasing number of aneuploid cells that upset the balance of their chromosomal constitution (Evans and Reed 1981, Constantin 1981, Orton 1985). However, Ono *et al.* (1994) previously reported that callus cells of *Scilla scilloides* had a high regeneration capacity and that they showed various types of chromosomal aberration when subcultured for more than one year.

There have been several reports on chromosomal aberrations and plant regeneration in *A. fistulosum* callus cells (Joachimik *et al.* 1993, 1995), but they have not focused on changes in chromosome structure. In this paper we discuss the morphogenetic capacity in 3 *A. fistulosum* cultivars.

Materials and methods

Plant material

A. fistulosum L. cv. Wede from Korea, and cv. Fresh and cv. Kaori from Japan were used in this

study.

Tissue culture

The seeds of 3 *A. fistulosum* cultivars were surface sterilized, with 70% ethanol for 5 min and then with a 1% sodium hypochlorite solution containing 0.1% alconox. The seeds were then rinsed three times with sterile water. Surface sterilized seeds were cultured on a 10 cm petri dish containing 1 ml of a solid medium consisting of MS salts and vitamins (Murashige and Skoog 1962) diluted to one-tenth concentration with growth regulators and sugars omitted; the light condition was 2000 lx and the temperature was 25°C. Seedlings were aseptically cut into pieces about 5 mm in length and these pieces were used as explants for callus induction. The basal medium for callus induction consisted of MS medium containing 2–3% sucrose and 0.3% Gellan Gum (Kelco, Division of Merck and Co., Inc.). Various concentrations (0, 0.01, 0.1, 1, 10 mg/l) of 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin, and benzyladenine (BA) were added to the basal medium, individually or in different combinations. Induced calli were sub-cultured every 4 weeks on the same callus-induction medium. The medium for plant regeneration contained MS salts, vitamins, 0.5% sucrose and no growth regulators. This medium was also solidified with 0.3% Gellan Gum. The pH of all media was adjusted to 5.8 with 1 M KOH prior to autoclaving for 20 min.

Chromosome analysis

7-day-old callus cells were used for chromosome analysis. After being transferred to a new medium, root tip chromosomes from regenerated plantlets and mother plants were also examined. They were pretreated with 0.002 M 8-oxyquinoline solution for 4 h at 25°C and fixed in 45% acetic acid at 0°C for 20 min, then hydrolyzed in 1 N HCl at 60°C for 20 s. The samples were then stained and squashed in 2% aceto-orcein.

Results

Callus establishment

Approximately 2000 explants of the 3 cultivars were cultured with 48 concentration combinations of 2,4-D (0, 0.01, 0.1, 0.5, 1.0, 10.0 mg/l), kinetin (0, 0.01, 0.1, 1.0, 10.0 mg/l) and benzyladenine (BA) (0, 0.01, 0.1, 1.0, 10.0 mg/l). Of these combinations, callus formation was observed, after about one month, only in cultures with media supplemented with 0.1 or 0.5 mg/l 2,4-D. Callus formation rates were 35% (21 callus-formed explants/60 cultured explants) in the former and 29% (23 callus-formed explants/80 cultured explants) in the latter. Callus formation was not observed in cultures with media containing concentrations exceeding 1 mg/l 2,4-D, and explants died after browning in cultures with 10 mg/l 2,4-D. No callus formation occurred in cultures with kinetin- and BA-supplemented media, irrespective of their concentrations. The calli of the 3 cultivars were compact and friable (Fig. 1A). They were subcultured onto media supplemented with 0.1 mg/l 2,4-D and used for the analysis of morphogenesis and chromosomal aberrations.

Plant regeneration

When calli of cv. Fresh were transferred to MS medium without growth regulators, green spots appeared on the surface of the calli after about 10 days. They subsequently developed into shoot primordia, and eventually into shoots (Fig. 1B, C). About one month after the start of culture, plantlets were regenerated from shoots after rooting (Fig. 1D). Similar morphogenetic processes were also observed in the other 2 cultivars.

Variation in chromosome numbers of callus lines with subculture

As shown in Table 1, the proportion of normal diploid cells ($2n=16$) ranged from 85.8% to

Fig. 1. The process of plantlet development in *A. fistulosum* cv. Fresh. A) callus. B, C) shoot development after 15 days (B) and 21 days (C) of culture. D) regenerated plantlet after 1 month of culture. Bars indicate 1 cm in A, C, D, 5 mm in B.

95.9% in 5 Kaori callus lines (K1 to K5) after 3 months. The frequency ranged from 67.2% to 81.0% after 7 months. The frequency of tetraploid cells ($2n=32$) was from 3.0% to 27.6% after 3 to 7 months, while other aberrant cells, including aneuploids, accounted for 0.8% to 10.6% of the cells. Various ploidy levels were observed in the callus cells during the subculture period. Kaori callus cells were mainly diploid cells in the early stages of culture, but the frequency of tetraploid cells increased as the culture period lengthened.

In the case of Fresh, the frequency of diploid cells progressively increased with the length of the culture period in lines Fresh-1 and -2, but the occurrence of tetraploid cells increased over time in the other lines (Table 2). On the other hand, the Wede callus lines showed the same tendency as the Kaori lines, with increasingly frequent tetraploid cells over time (Table 3). This was especially evident in Wede-2 and -3, where the numbers of tetraploid cells increased suddenly after 6 months of culture. The frequency of chromosome number alterations in the callus cells of the 3 cultivars therefore differed.

Karyotype analysis of callus cells and regenerated plantlets

The chromosome complements of the 3 *A. fistulosum* cultivars ($2n=16$) consisted of 8 pairs of chromosomes, a1 to a8 (Fig. 2A); each a5 pair had a satellite on the distal part of the short arm. These results agree with those of Kurita (1952). Karyotype analysis of somatic metaphase chromosomes was performed using the callus lines Kaori-1, Fresh-2 and Wede-2 that had been induced from donor plants of each cultivar.

Table 1. The variation of chromosome numbers of calli with culture period in *Allium fistulosum* cv. Kaori

Cell lines	Culture periods after callus induction (months)	No. of cells of		
		2n=16 (%)	2n=32 (%)	others (%)
Kaori 1	3	119 (91.5)	10 (7.7)	1 (0.8)
	4	113 (85.6)	5 (3.8)	14 (10.6)
	5	173 (89.2)	13 (6.7)	8 (4.1)
	6	101 (71.6)	35 (24.8)	5 (3.6)
	7	207 (67.2)	85 (27.6)	16 (5.2)
Kaori 2	3	93 (95.9)	4 (4.1)	0 (0)
	4	97 (90.6)	8 (7.5)	2 (1.9)
	5	105 (89.7)	9 (7.7)	3 (2.6)
	6	125 (83.3)	15 (10.0)	10 (6.7)
	7	132 (81.0)	23 (14.1)	8 (4.9)
Kaori 3	3	87 (95.6)	3 (3.3)	1 (0)
	4	95 (94.0)	3 (3.0)	3 (3.0)
	5	103 (88.8)	8 (6.9)	5 (4.3)
	6	127 (84.7)	15 (10.0)	8 (5.3)
	7	97 (78.2)	20 (16.1)	7 (5.7)
Kaori 4	3	85 (91.4)	6 (6.5)	2 (2.1)
	4	79 (90.8)	5 (5.8)	3 (3.4)
	5	95 (85.6)	12 (10.8)	4 (3.6)
	6	108 (80.0)	19 (14.1)	8 (5.9)
	7	126 (76.4)	25 (15.1)	14 (8.5)
Kaori 5	3	103 (85.8)	14 (11.7)	3 (2.5)
	4	93 (85.3)	12 (11.0)	4 (3.7)
	5	127 (78.9)	23 (14.3)	11 (6.8)
	6	108 (78.8)	21 (15.3)	8 (5.9)
	7	112 (73.2)	28 (18.3)	13 (8.5)

In the Kaori-1 callus line, 4 karyotypes were observed: type S, Type I, type I-1 and type II. Fig. 2B shows type I (2n=15) and Fig. 2C shows type I-1 (2n=28). Fig. 2D shows type II (2n=32, tetraploid) mitotic metaphase chromosomes. The karyotypes observed in this callus line are summarized in Table 4, and representative karyotypes are shown in Fig. 3. The type S karyotype (2n=16) was identical to that of the mother plant. The type I karyotype (2n=15) arose from type S by a translocation and the elimination of a pair of a1 chromosomes, resulting in a dicentric chromosome. The type II karyotype was tetraploid (2n=32), which corresponds to double the type S chromosome complement. Secondary karyotype changes, resulting from alterations to previously altered karyotypes, were also found. Type I-1 (2n=28) arose from type I by a doubling of the chromosome complement of type I and the elimination of two a8 chromosomes. This was followed by a structural change involving the deletion of a segment of the long arm of chromosome a8 and its translocation to the short arm of a7. The type S karyotype was the most frequently found, with a rate of 54.0%; type II (tetraploid) was the second most frequent, with a rate of 20.3%. Type I and type I-1 were found at the relatively low rates of 10.8% and 14.9%, respectively. In the Kaori-1 callus line, chromosomes a1, a7 and a8 were the most likely to have undergone structural changes. In regenerated plantlets from the Kaori-1 callus line, two karyotypes of type S (2n=16) and type II (2n=32) were observed (Table 4, Fig. 3), with frequencies of 77.6% and 22.4%, respectively.

Four karyotypes were found in the Fresh-2 callus line: type S, type I, type II and type II-1 (Table 5, Fig. 4). Of these, type S (2n=16), type I (2n=15) and type II (2n=32) were the same as those found in the Kaori-1 callus line described above. Type II-1 (2n=31), which was a secondarily

Table 2. The variation of chromosome numbers of calli with culture period in *Allium fistulosum* cv. Fresh

Cell lines	Culture periods after callus induction (months)	No. of cells of		
		2n=16 (%)	2n=32 (%)	others (%)
Fresh 1	3	150 (58.6)	106 (41.4)	0 (0)
	4	128 (59.8)	86 (40.2)	0 (0)
	5	97 (68.3)	45 (31.7)	0 (0)
	6	135 (72.6)	47 (25.3)	4 (2.1)
	7	119 (90.2)	11 (8.3)	2 (1.5)
Fresh 2	3	97 (72.9)	36 (27.1)	0 (0)
	4	108 (71.0)	41 (27.0)	3 (2.0)
	5	123 (71.5)	45 (26.2)	4 (2.3)
	6	103 (79.2)	23 (17.7)	4 (3.1)
	7	137 (86.2)	17 (10.7)	5 (3.1)
Fresh 3	3	110 (94.0)	7 (6.0)	0 (0)
	4	105 (93.8)	7 (6.2)	0 (0)
	5	127 (93.4)	9 (6.6)	0 (0)
	6	94 (91.3)	7 (6.8)	2 (1.9)
	7	118 (86.1)	15 (11.0)	4 (2.9)
Fresh 4	3	103 (89.6)	12 (10.4)	0 (0)
	4	97 (90.7)	10 (9.3)	0 (0)
	5	114 (78.6)	27 (18.6)	4 (2.8)
	6	108 (76.1)	29 (20.4)	5 (3.5)
	7	126 (73.3)	38 (22.1)	8 (4.6)
Fresh 5	3	93 (87.7)	13 (12.3)	0 (0)
	4	126 (85.1)	18 (12.2)	4 (2.7)
	5	114 (79.7)	24 (16.8)	5 (3.5)
	6	123 (75.5)	27 (16.6)	13 (7.9)
	7	134 (74.0)	36 (19.9)	11 (6.1)

changed karyotype, resulted from type II by a translocation and elimination between 2 a2 chromosomes, resulting in the formation of a large chromosome. Of these karyotypes, type S was most frequently found with an occurrence rate of 48.0%; type I was the least observed, with a rate of 10.4%. The frequencies of type II and type II-1 were 26.0% and 15.6%, respectively. In this callus line, the structural chromosome changes occurred on chromosomes a1 and a2.

Three karyotypes, type S (2n=16), type II (2n=32) and type III (2n=18), were observed (Table 5, Fig. 4) in plantlets regenerated from the Fresh-2 callus line. The type III karyotype resulted from type S due to trisomies of chromosomes a5 (also containing a satellite) and a7. The frequencies of type S, type II and type III were 64.1%, 23.1% and 12.8%, respectively.

Seven karyotypes (type S, II, IV, V, VI, I-2, II-2) were observed (Table 6, Fig. 5) in the Wede-2 callus line. Of these, type IV (2n=15) and type VI (2n=15) arose from type S by the elimination of a chromosome: a1 in type IV and chromosome a2 in type V. Type VI (2n=17) arose from type S by a chromosome a2 trisomy and the deletion of a part of both arms of chromosome a8. Type I-2 and type II-2 were secondarily changed karyotypes. Type I-2 arose from type I by the translocation and elimination of a pair of a1 chromosomes and the deletion of part of a short arm of chromosome a8. Type II-2 arose from type II by a translocation and elimination between two a1 chromosomes. Of these 7 karyotypes, type S was the most frequently observed, as a rate of 36.2%, and type VI was the least observed, at a rate of 2.3%.

Despite various karyotypes being found in the Wede-2 callus line (Table 6, Fig. 5), regenerated Wede-2 plantlets consisted only of type S and type II-3. Type II-3 was derived from type II by a

Table 3. The variation of chromosome numbers of calli with culture period in *Allium fistulosum* cv. Wede

Cell lines	Culture periods after callus induction (months)	No. of cells of		
		2n=16 (%)	2n=32 (%)	others (%)
Wede 1	3	87 (97.8)	2 (2.2)	0 (0)
	4	95 (96.9)	3 (3.1)	0 (0)
	5	97 (96.0)	4 (4.0)	0 (0)
	6	134 (94.4)	6 (4.2)	2 (1.4)
	7	142 (92.2)	12 (7.8)	0 (0)
Wede 2	3	45 (91.8)	4 (8.2)	0 (0)
	4	102 (95.3)	5 (4.7)	0 (0)
	5	93 (95.9)	4 (4.1)	0 (0)
	6	3 (2.0)	110 (72.8)	38 (25.2)
	7	7 (4.0)	128 (72.3)	42 (23.7)
Wede 3	3	77 (97.5)	2 (2.5)	0 (0)
	4	97 (99.0)	1 (1.0)	0 (0)
	5	89 (97.8)	2 (2.2)	0 (0)
	6	67 (53.2)	59 (46.8)	0 (0)
	7	76 (43.9)	95 (54.9)	2 (1.2)
Wede 4	3	131 (98.5)	2 (1.5)	0 (0)
	4	125 (98.4)	2 (1.6)	0 (0)
	5	97 (90.7)	10 (9.3)	0 (0)
	6	105 (85.4)	18 (14.6)	0 (0)
Wede 5	3	87 (96.7)	3 (3.3)	0 (0)
	4	79 (96.3)	3 (3.7)	0 (0)
	5	103 (95.4)	5 (4.6)	0 (0)
	6	99 (82.5)	21 (17.5)	0 (0)
	7	136 (76.8)	35 (19.8)	6 (3.4)

translocation, the elimination of both pairs of chromosome a1, and a nondisjunction of an a3 chromosome leading to the appearance of 5 homologous chromosomes. The frequencies of type S and type II-3 were 77.4% and 22.6% respectively. In this callus line and the regenerated plantlets, the structural chromosome changes were found in chromosomes a1 and a8.

Discussion

There have been many studies on chromosomal aberrations in cultured plant cells and their regenerants using various plant materials (cf. Singh 1993). Most reports describe only aberrations in chromosome number, such as polyploidy and aneuploidy, and do not mention structural changes induced by deletions, translocations, duplications, inversions, etc. Structural chromosome changes were reported in detail for cultured cells of *Crepis capillaris* (2n=6) and *Haplopappus gracilis* (2n=4) with low chromosome number (Sacristan 1971, Sunderland 1977). In both cases, the extent of structural changes increased with multiplication of the ploidy level, and the only regenerants were *Crepis* plants with normal diploid karyotypes. In contrast, the morphogenetic capacities of few polyploid species, such as *Solanum tuberosum* (Gill *et al.* 1980) and *Triticum aestivum* (Karp and Maddock 1984), were not influenced by aneuploidy, polyploidy or structural chromosome changes. Our previous reports (Ono and Harashima 1983, Ono *et al.* 1994) using haploid cultured cells (n=5) from pollen culture of *Paeonia lactiflora* (2n=10) and diploid cultured cells of *Scilla scilloides* (2n=16) also revealed chromosomal aberrations in terms of both chromosome number and structure. Moreover, in *Scilla scilloides* callus cells, we reported regenerants with aneuploid chro-

Fig. 2. Metaphase chromosomes of *A. fistulosum* cv. Kaori. A) a root tip cell of mother plant (2n=16). B, C, D) callus cells (B: 2n=15, C: 2n=28, D: 2n=32), each of arrows indicate a dicentric chromosome. Bars indicate 10 μ m.

Table 4. Karyotypes of *Allium fistulosum* cv. Kaori

	Types		Chromosome constitution								No. of cells (%)	
Mother plants	S	2n=16	a1/a1	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a6/a7	a8/a8	20 (100.0)	
	S	2n=16	a1/a1	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8	40 (54.0)	
Calli				(Primary changes)								
	I	2n=15	a1 ^{a1} /—	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8	8 (10.8)	
	II	2n=32	a1/a1/	a2/a2/	a3/a3/	a4/a4/	a5/a5/	a6/a6/	a7/a7/	a8/a8/	15 (20.3)	
			a1/a1	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8		
					(Secondary changes)							
	I-1	2n=28	a1 ^{a1} /a1 ^{a1} /	a2/a2/	a3/a3/	a4/a4/	a5/a5/	a6/a6/	a7 ^{a8} /a7/	a8/a8 ⁻ /	11 (14.9)	
			—/—	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	—/—		
Total											74	
No. of plantlets (%)												
Regenerated plantlets	S	2n=16	a1/a1	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8	45 (77.6)	
	II	2n=32	a1/a1/	a2/a2/	a3/a3/	a4/a4/	a5/a5/	a6/a6/	a7/a7/	a8/a8/	13 (22.4)	
			a1/a1	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8		
Total											58	

Minus (-) indicate eliminations of chromosomes. Superior symbols indicate deletions (-) and translocations (a*) of chromosomes, respectively.

Fig. 3. Karyotypes of mother plant, callus cells and regenerated plantlets of *A. fistulosum* cv. Kaori.
Bar indicates $10\mu\text{m}$.

Table 5. Karyotypes of *Allium fistulosum* cv. resh

	Types	Chromosome constitution										No. of cells (%)
Mother plants	S	2n=16	a1/a1	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8		28 (100.0)
	S	2n=16	a1/a1	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8		46 (48.0)
Calli					(Primary changes)							
	I	2n=15	a1 ^{a1} /-	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8		10 (10.4)
	II	2n=32	a1/a1/	a2/a2/	a3/a3/	a4/a4/	a5/a5/	a6/a6/	a7/a7/	a8/a8/		25 (26.0)
			a1/a1	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8		
					(Secondary changes)							
	II-1	2n=31	a1/a1/	a2 ^{a2} /-	a3/a3/	a4/a4/	a5/a5/	a6/a6/	a7/a7/	a8/a8/		15 (15.6)
			a1/a1	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8		
Total												96
												No. of plantlets (%)
Regenerated plantlets	S	2n=16	a1/a1	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8		50 (64.1)
	II	2n=32	a1/a1/	a2/a2/	a3/a3/	a4/a4/	a5/a5/	a6/a6/	a7/a7/	a8/a8/		18 (23.1)
			a1/a1	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8		
	III	2n=18	a1/a1	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8		10 (12.8)
							a5		a7			
Total												78

Minus (-) indicate eliminations of chromosomes. Superior symbols indicate deletions (-) and translocations (a*) of chromosomes, respectively.

mosome numbers and structurally changed chromosomes.

There are very few reports on chromosome aberrations in *Allium fistulosum* callus cells. Joachimiak *et al.* (1993, 1995) recently reported on chromosome alterations in callus cells of this species, although changes in chromosome structure were not mentioned in detail. In the present study, we found cells with various ploidy levels during subculture of 3 *A. fistulosum* cultivar callus lines. This result agrees with that of Joachimiak *et al.* (1993, 1995) described above. We also observed various aneuploid karyotypes with structural chromosome changes that were not found in the mother plants *in vivo*. In *A. fistulosum* callus cells, Joachimiak *et al.* (1993, 1995) reported megachromosomes with several centromeres, which were induced by translocations and deletions involving two or more chromosomes. In this study, we also found a dicentric chromosome that was derived from translocation and deletion between two a1 chromosomes. Thus, the occurrence of dicentric chromosomes may be a common characteristic in *in vitro* *A. fistulosum* cultures. In addition, this study found that chromosomes a2, a7 and a8 were the most likely to have structural chromosome alterations.

In regenerated plantlets, numerically and structurally changed karyotypes were also observed, although the rates of diploids and tetraploids were remarkably high. These results indicate that even callus cells derived from a normal diploid and having changed karyotypes were able to maintain the ability to regenerate plants.

Studies on plant regeneration from *A. fistulosum* callus cells have been reported by several authors (Van der Valk *et al.* 1992, Song and Peffley 1994, Kim and Soh 1996). These reports concerned plant regeneration occurring through somatic embryogenesis, although detailed descriptions of the process of embryogenesis were not provided. In the present study, we observed plantlet regeneration from callus cells via shoot formation and could not find well-defined somatic embryoge-

Fig. 4. Karyotypes of mother plant, callus cells and regenerated plantlets of *A. fistulosum* cv. Fresh. Bar indicates 10 μm .

Fig. 5. Karyotypes of mother plant, callus cells and regenerated plantlets of *A. fistulosum* cv. Wede. Bar indicates 10 μ m.

Table 6. Karyotypes of *Allium fistulosum* cv. Wede

	Types	Chromosome constitution										No. of cells (%)
Mother plants	S	2n=16	a1/a1	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8	25 (100.0)	
	S	2n=16	a1/a1	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8	46 (36.2)	
				(Primary changes)								
	II	2n=32	a1/a1/ a1/a1	a2/a2/ a2/a2	a3/a3/ a3/a3	a4/a4/ a4/a4	a5/a5/ a5/a5	a6/a6/ a6/a6	a7/a7/ a7/a7	a8/a8/ a8/a8	25 (19.7)	
	IV	2n=15	a1/—	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8	10 (7.9)	
Calli	V	2n=15	a1/a1	a2/—	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8	14 (11.0)	
	VI	2n=17	a1/a1	a2/a2 a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8 ⁻	3 (2.3)	
				(Secondary changes)								
	I-2	2n=15	a1 ^{a1} /—	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8 ⁻	10 (7.9)	
	II-2	2n=31	a1 ^{a1} /—/ a1/a1	a2/a2/ a2/a2	a3/a3/ a3/a3	a4/a4/ a4/a4	a5/a5/ a5/a5	a6/a6/ a6/a6	a7/a7/ a7/a7	a8/a8/ a8/a8	19 (15.0)	
Total											127	
											No. of plantlets (%)	
	S	2n=16	a1/a1	a2/a2	a3/a3	a4/a4	a5/a5	a6/a6	a7/a7	a8/a8	48 (77.4)	
Regenerated plantlets	II-3	2n=32	a1 ^{a1} /a1/ a1/—	a2/a2/ a2/a2	a3/a3/ a3/a3	a4/a4/ a4/a4	a5/a5/ a5/a5	a6/a6/ a6/a6	a7/a7/ a7/a7	a8/a8/ a8/a8	14 (22.6)	
					a3							
Total											62	

Minus (—) indicate eliminations of chromosomes. Superior symbols indicate deletions (—) and translocations (a*) of chromosomes, respectively.

nesis. Further study is necessary to elucidate whether morphogenesis from callus cells of this species is due to somatic embryogenesis or shoot formation. We are now histochemically studying the initiation of morphogenesis in *A. fistulosum* callus cells.

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