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https://doi.org/10.5109/22717

出版情報：九州大学大学院農学研究院紀要. 13 (1), pp.165-177, 1964-03. Kyushu University
バージョン：
権利関係：

Journal of the Faculty of Agriculture, Kyushu University, Vol. 13, No. 1
March 30, 1964

Cytogenetic studies in the Wakegi, *Allium fistulosum* var. *caespitosum**

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The Wakegi (*Allium fistulosum* var. *caespitosum*, or *A. wakegi*), an edible plant of quite ancient origin in Japan, has 16 somatic chromosomes (Morinaga and Fukushima, 1931), and karyotype analysis has disclosed that it is clearly heterozygous in its chromosome constitution, undoubtedly indicating the hybrid nature of its origin (Kurita, 1952, 1953a, b; Yamaura, 1961). As this plant does not flower excepting in some rare cases and is usually propagated vegetatively, the process of meiosis in its pollen mother-cells have not yet been described in detail by any author, though only certain meiotic irregularities accompanied have been touched upon by Kurita (1953b).

The author's observations in 1961-1962 of the meiosis in pollen mother-cells of this plant have disclosed that this form shows conspicuous meiotic irregularities and that those irregularities are likely to have resulted directly from the plant's genetic constitution and not from any adverse environmental conditions. The present paper deals with the results of those investigations carried out upon the meiotic and karyotypic aspects of the Wakegi plant.

MATERIAL AND METHODS

The material used was collected from a local form of the Wakegi cultivated in the suburbs of Fukuoka City, Kyushu. The meiosis was examined with 4 inflorescences in total, one raised in 1961 and three in 1962, all being obtained from the same single clone (Fig. 1). Flower buds were fixed in Newcomer's fluid (Newcomer, 1953) and preserved for observations. Meiosis was studied with the smear preparations of the pollen mother-cells in iron aceto-carmine. Somatic chromosomes

* Contribution from the Horticultural Laboratory, Faculty of Agriculture, Kyushu University.

were studied in root-tip squashes with 1 % acetic-orcein after the pre-treatment with the 0.002 mol 8-oxyquinoline solution according to the method used by Tjio and Levan (1950).

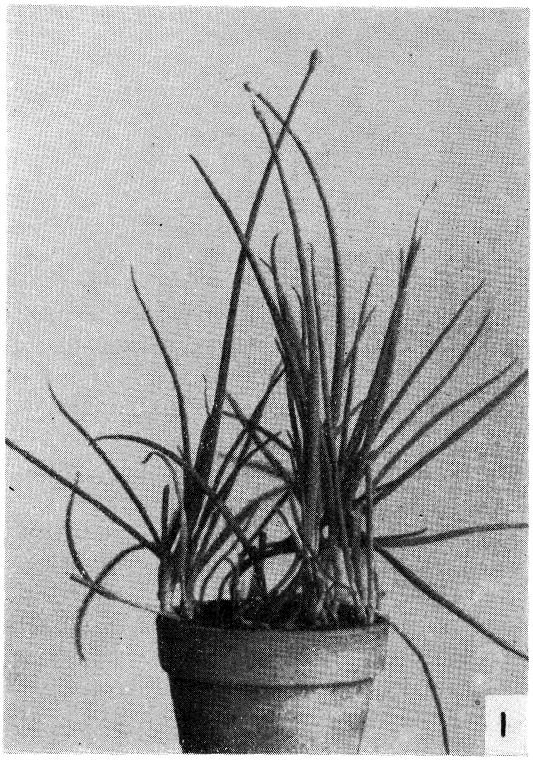


Fig 1. A local form of the Wakegi, *Allium fistulosum* var. *caespitosum*, grown in the suburbs of Fukuoka City, Kyushu.

RESULTS

1. Karyotype analysis

Somatic chromosomes in the Wakegi were notably heterozygous in their constitution and markedly different in this respect from those in the Japanese bunching onion, *A. fistulosum*. It could be easily found in the Wakegi that several somatic chromosomes, including SAT-chromosome, did not occur in pairs, e.g., as a-, b-, e-, *n*-, *o*-, and p-chromosomes, and even with each of the remaining ones it was rather difficult to identify clearly the respective partner (Figs. 2, 3). The karyotype

of the Wakegi will be represented by the schema: $K(2n)=13V+J_1+J_2+J_3^T$. This schema was obtained by laying the emphasis upon the *n*-chromosome because this chromosome has its centromere approximately in the subterminal position, while those having subterminal centromeres are exclusively the e- and u-chromosomes, as shown in Fig. 3.

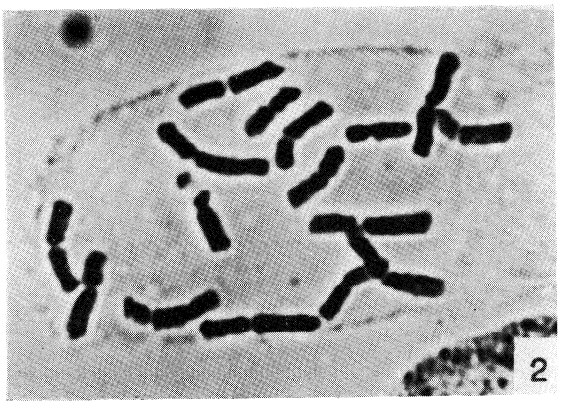


Fig. 2. Photomicrograph of the somatic chromosomes in the Wakegi. × 1000.

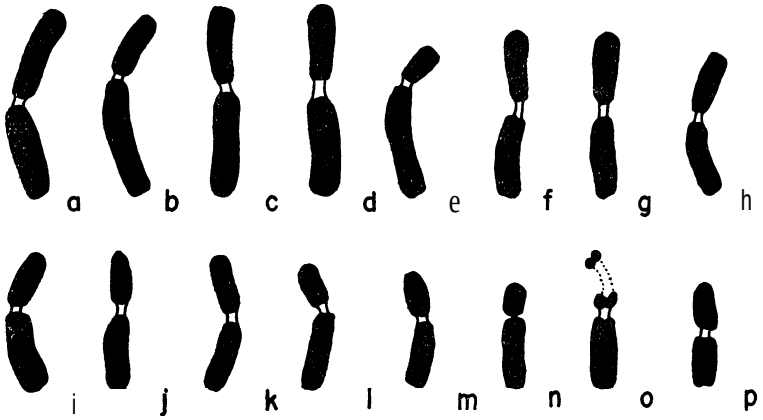


Fig. 3. Schematic representation to the karyotype in the Wakegi. x3500.

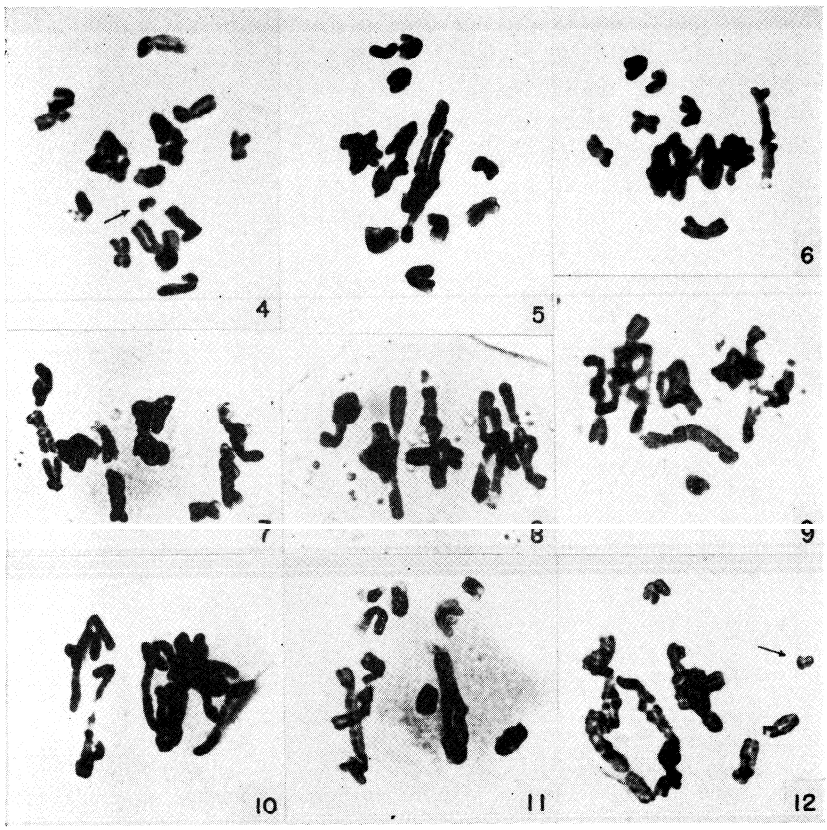
2. Meiotic divisions

(a) Chromosome pairings at metaphase-I : As shown in Table 1, the number of bivalents appearing at metaphase-I of PMCs varied between 2 and 8, but in the half of cells observed the chromosome configuration was $6_{II}+4_I$ or $5_{II}+6$, (Figs. 4–8). Heteromorphic bivalents, more particularly those of SAT-chromosomes, could be obviously noticed not

infrequently (Figs. 5-7). Certain SAT-chromosomes were paired with e-chromosomes to form bivalents, and such configurations were observed in 123 (62.8 %) out of 196 cells examined and in the rest cells the chromosomes occurred as univalents. Several multivalents which varied in their valency from tri- to pentavalents were found only in a small proportion (5.8 %) of the cells at metaphase-I, trivalents occurring most frequently (Table 1, Figs. 9–12).

Table 1. Chromosome pairings and occurrence of fragments at meiotic metaphase-I in pollen mother-cells.

Configuration	Frequency of PMCs with fragments				Total No. of PMCs	Per cent
	$+0_f$	$+1_f$	$+2_f$	$+3_f$		
8_{II}	9	2	1	- -	12	5.7
$7_{II}+2_I$	37	4	—	—	41	19.5
$6_{II}+4_I$	52	5	—	—	57	27.1
$5_{II}+6_I$	45	15	1	1	62	29.5
$4_{II}+8_I$	21	3	—	—	24	11.4
$3_{II}+10_I$	6	2	1	—	9	4.3
$2_{II}+12_I$	4	1	—	—	5	2.4
Total	174	32	3	1	210	100
$1_V+1_{IV}+2_{II}+3_I$	—	1	—	—	1	
$1_{IV}+1_{III}+1_{II}+7_I$	1	—	—	—	1	
$1_{IV}+4_{II}+4_I$	2	2	—	—	4	
$2_{III}+5_{II}$	1	—	- -	—	1	
$2_{III}+4_{II}+2_I$	—	1	—	—	1	
$1_{III}+4_{II}+5_I$	4	—	—	—	4	
$1_{III}+6_{II}+1_I$	1	—	—	—	1	
Total	9	4	—	—	13	
Total number of PMCs observed	183	36	3	1	223	
Per cent	82.1		17.9		100	

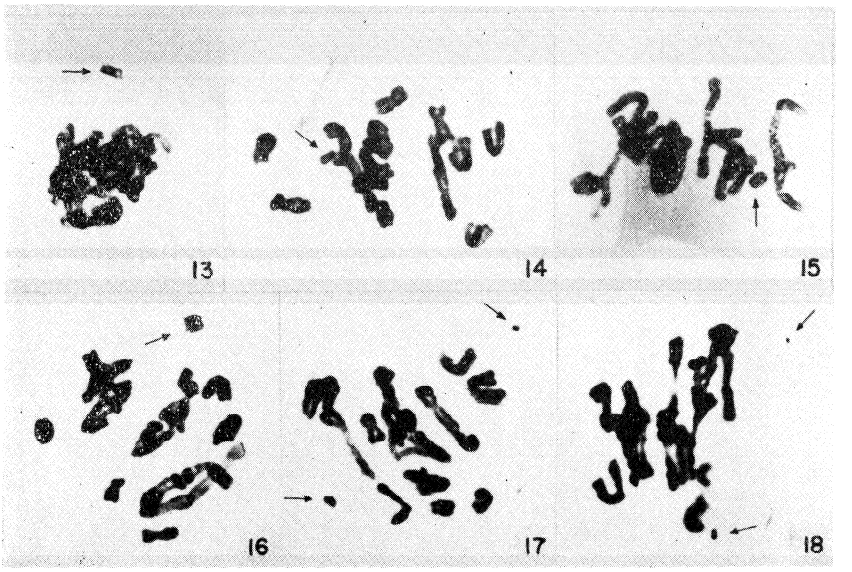


Figs. 4-12. Chromosome configurations at metaphase-I in the Wakegi. ×900. 4, $2_{II}+12_{I}+1r$. 5, $4_{II}+8_{I}$. 6, $5_{II}+6_{I}$. 7, $7_{II}+2_{I}$. 8, 8_{II}. 9 and 10, showing 1 and 2 trivalents respectively. 11, $1_{IV}+1_{III}+1_{II}+7_{I}$. 12, $1_{V}+1_{IV}+2_{II}+3_{I}+1r$. Arrows indicate the fragment.

(b) Occurrence of fragments : The occurrence of chromosome fragments was noteworthy phenomenon. As shown in Table 1, those fragments were found in 17.9 % of the cells examined. Number of fragments, showing continuous variation in their sizes, amounted to 3 per cell at the maximum. They were clearly distinguished from any of the intact parental chromosomes (Figs. 14–18). They made their first appearance at the diplotene stage and frequency appearances showed increasing towards the anaphase-I, extending to the maximum (Figs. 13-22). Fragments occurring before anaphase-I were double-structured, i.e., they were of the dyad-chromosomal origin.

(c) Anaphase-I and the later stages: Only a very small proportion of the cells under observation took somewhat normal appearance at

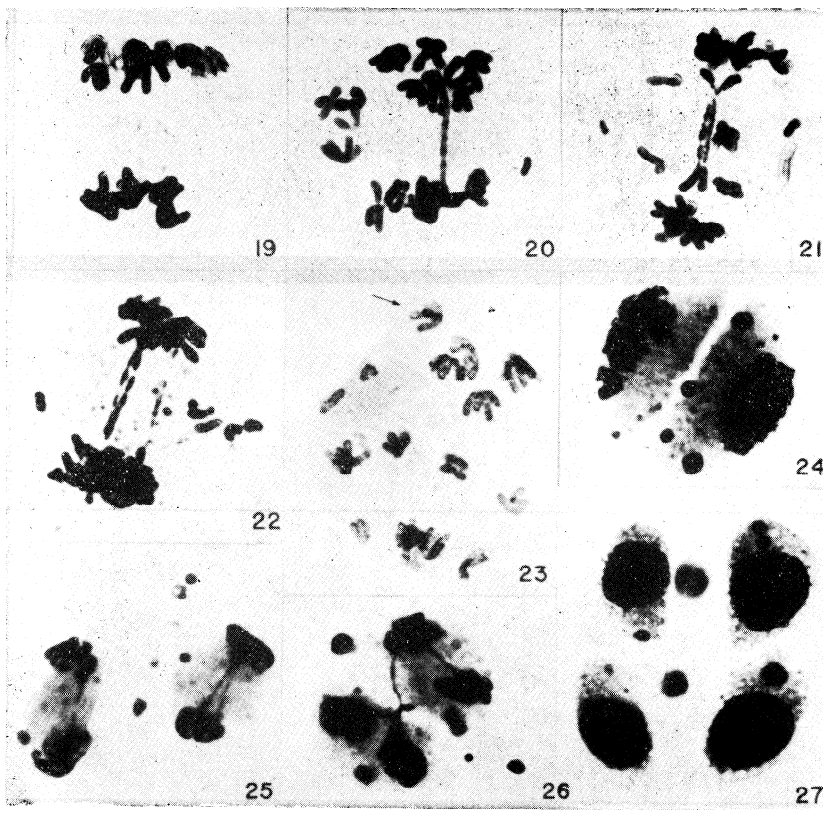
anaphase-I as shown in Fig. 19, and in the rest large number of cells there usually occurred a certain number of lagging chromosomes, chromosome bridges, and as well as of fragments (Figs. 20–23). Bridges formed during the first division were found in 57.1 % of the cells, and their maximum number per cell amounted to 4 (Table 2, Figs. 20, 22). Moreover, a number of loop-chromosomes, each containing 2 insertion regions, were found at anaphase-I (Fig. 23). In rare cases, double bridges were seen, as shown in Fig. 21 (cf. McClintock, 1938; Haga, 1946). At interkinesis there encountered with each of the 71.4 % of



Figs. 13-18. Occurrence of chromosome fragments, showing a continuous variation in size, at prophase (Fig. 13) and at metaphase-I (Figs. 14–18) respectively. Arrows indicate the fragment. x900.

the daughter cells a few mirconuclei, of which maximum number reached at 5 per cell (Table 3, Fig. 24). Those micronuclei were no doubt derived from the lagging univalents or from fragments. There observed the bridges in 39.8 % of the daughter cells at anaphase-II (Table 2, Fig. 25). Some of the bridges appeared at anaphase-I were found persisting throughout the second division. One of those persistent forms of bridges, as shown in Fig. 26, could be detected at terophase-II, and was reproduced diagrammatically in Fig. 28. Those four bridges interlocked were likely to be of two kinds, one formed at anaphase-I (1 and 2) and the other at anaphase-II (3 and 4). At the tetrad stage there appeared, in addition to normal tetrad, various kinds of sporads, i.e., monads, diads, triads, abnormal tetrads, pentads,

and hexads, and in 44.1 % of the microspores thus produced there could be noticed certain supernumerary micronuclei, the number of which varied between 1 and 4 per microspore (Table 3, Fig. 27).



Figs. 19–27. Meiotic irregularities at anaphase-I and its later stages. x 900. 19, an anaphase-I figure not containing any one fragment or bridge. 20, an anaphase-I clearly showing a chromatid bridge and a fragment. 21, an anaphase-I showing a chromatid double-bridge and some fragments. 22, a terophase-I showing 3 chromatid bridges and numerous fragments. 23, the arrow indicates an loop chromosome having 2 insertion regions at anaphase-I. 24, an interkinetic cell showing micronuclei. 25, chromatid bridges and fragments appeared at terophase-II. 26, a complex terophase-II figure showing interlocking of chromatid bridges (see Fig. 28). 27, a tetrad cell having numerous micronuclei.

Table 2. Frequency occurrence of chromatid bridges at the first and the second division.

Number of bridges appeared per cell	Number of cells observed			
	At first division		At second division	
0	72	42.9 %	53	60.2 %
1	74		30	
2	17	57.1 %	3	39.8 %
3	4		2	
4	1		—	
Total	168		88	
Calculated number of bridges per cell	0.738		0.477	
Expected number	0.810		0.405	

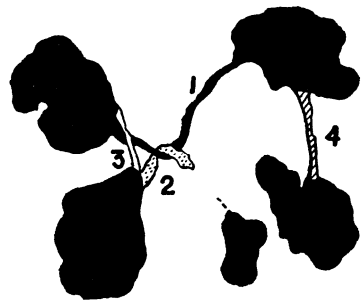


Fig. 28. Diagrammatic drowing of Fig. 25. ×1400.

Table 3. Frequency occurrence of micronuclei at the interkinesis and the pollen-tetrad stage.

Number of micronuclei per each daughter cell	Number of cells observed			
	At interkinesis		At pollen-tetrad stage	
0	62	28.6 %	162	55.9 %
1	86		108	
2	48		18	
3	19	71.4 %	1	44.1 %
4	1		1	
5	1		—	
Total	217		290	

3. Fertility

As the result of those irregular meiotic behaviors described in the preceding pages, all the young pollen-grains became to degenerate. When the flowers of the Wakegi were pollinated with the pollen-grains of A. *fistulosum*, the capsules could be set, but not obtaining any one viable seed.

DISCUSSION

The results of karyotype analyses of the Wakegi plants so far reported can be summed up as follows :

A. fistulosum var. *caespitosum*	$K(2n)=14V+J+J^t$	Kurita (1952, 1953b)
A. bouddhae var. *caespitosum*	$K(2n)=14V+2J^T$	(1953a)
A. wakegi, the western regional type	$K(2n)=13V+J_1+J_2+j^T$	Yatnaura (1961)
„ , Tokyo type	$K(2n)=13V+J_1{}^t+J_2+i$	„ („)

The Wakegi which has been dealt with in this report bears a close karyotypic resemblance to the plant studied by Kurita (1952, 1953b) and to the one by Yamaura (his western regional type ; 1961).

Those karyotype-analytic findings and the quite irregular meiotic phenomena in pollen mother-cells in the Wakegi seem to strongly support the assumption that this form may be of a product of hybridization. The chromosome associations at metaphase-I in this form as follows; the exact 8_{II} configuration appeared in only a small proportion of cells observed, but $(8—5)_{II}+(0—6)_I$ pairings took place in 81.8 % of them. It is, therefore, conceivable that the present form of the Wakegi may have been originated through certain interspecific hybridization between two more or less closely related forms. While examining the Wakegi cultured in the Shikoku district, Kurita (1953b) found that 16_I, $1_{II}+14_I$, and 2_{II} + 12, pairings take place in the pollen mother-cells and, moreover, that out of 3 kinds of pairings the first one (non-pairing) had occurred in some rare cases and the last in most cases. His data, though they were not quite sufficient to clarify fully the chromosome pairing behaviors in his form, seem to be somewhat different as a whole from the author's. On the other hand, several F_1 hybrids raised by different workers from the cross combination, *A. cepa* × A. *fistulosum*, revealed no less differences among each others in the frequency appearance of bivalents, multivalents, fragments, the chromatids with double insertion regions, and as well as in the crossability with each hybrid when used in backcrossings. Such differences noticed in those F, hybrids will no doubt be attributable to the differences in the geno-

typic differentiation of their parental forms concerned (Levan, 1936; Maeda, 1937; Emsweller and Jones, 1938). Those findings appear to suggest that Kurita's form is more or less different in its origin as compared with the author's

The chiasmata occurring in the meiosis were of the random type in this Wakegi form. According to some workers, A. *cepa* and A. ***ascalonicum*** had the randomized chiasmata, while ***A. fistulosum*** had the localized ones near the insertion region. In the F_1 hybrids of ***A.*** *cepa* × ***A.*** *fistulosum* (Levan, 1936 ; Emsweller and Jones, 1938) and of ***A. ascalonicum*** × ***A.*** *fistulosum* (Cochran, 1953), there occurred bivalents having randomized chiasmata. However, those two different types of chiasmata were detected in the same cell in some amphidiploid hybrids, i.e., in *A.cepa-fistulosum* (Jones and Clarke, 1942) and in ***A.*** *ascalonicum-fistulosum* (Jones and Kehr, 1957). Thus the foregoing findings will suggest that, if a certain *Allium* species with localized chiasmata had took part in the formation of this Wakegi form and, moreover, if the tetraploid Wakegi form could be produced, the tetraploid Wakegi thus produced and the progeny raised from their backcrosses will afford a valuable clue for solving the problem concerning the origin of preser Wakegi form and further will serve to provide us a material for futurt promising breeding of green onion varieties.

The appearance of fragments is a specific phenomenon to be noticed during meiosis in the Wakegi. Those fragments appeared first in some diplotene nuclei and were most frequently observed in anaphase-I. The causes of formation of those fragments before anaphase-I are not quite clear. It can hardly be attributed to the so-called crossing-over in the inverted section of chromosomes (McClintock, 1931, 1933, 1938; Haga, 1946). On the other hand, the similar chromosome breakage has been reported by several workers in various other plants (Upcott, 1937, in *Tulipa*; Emsweller and Jones, 1938, in *Allium*; Haga, 1953, in ***Paris***; etc.).

The existence of an intimate relationship between the fragmentation and the formation of chromatid bridges may be reasonably presumed from the fact that the chromosome breakage is usually accompanied by the fusion of break-ends (cf. Haga, 1953). If, regardless of the effect of crossing-overs, the breakage and the following fusion, which result in the formation of bridges and fragments, will take place between any two partners of the four chromatids (designated as ***a, a',*** *b*, and *b'*) at random, there may naturally occur two possible schemes of combination between sister chromatids ***(a-a'*** and b-b'), and four among homologous chromatids ***(a-b, a-b',*** *a'–b* and *a'–b'*), and, in consequence, at anaphase-I the former chromatids, each having two insertion region in it, will be transformed into loops and fragments

(and these loops will become bridges at anaphase-II) and the latter into bridges and fragments (Haga, 1953). In other words, the ratio of frequency in the bridge formations between A-I and A-II will be estimated to be 2 : 1. By the way, this A-I : A-II ratio can be experimentally demonstrable, as it has been done in the hands of Haga (1953) who has succeeded in calculating this frequency value of bridge formation on the data obtained by Upcott (1937) in *Tulipa* and on those obtained by Matsuura and Haga (1950) in their x-ray experiments in *Trillium kamtschaticum*. Considering from the foregoing point of view, the fact given in Table 2 is likely to indicate that most chromosome bridges occurring in the Wakegi are the product of the breakage-fusion mechanism operating between two related chromatids, and only a few others, if any, may be ascribable to the crossing-over in the inverted sections. Thus it seems quite probable that the chromosome fragmentation occurring in the Wakegi may be duly attributed to some genic unbalances that are likely to exist in this form.

SUMMARY

Karyological observation has disclosed that a local form of the Wakegi, *A. fistulosum* var. *caespitosum*, grown on the suburbs of Fukuoka City, is clearly heterozygous in its karyotype and is conspicuously irregular in the meiosis of its pollen mother-cells.

Karyotype analysis of this Wakegi form have revealed that the following formula will be assigned to the plant :—

$$K(2n) = 13V + J_1 + J_2 + J_3^T.$$

At meiotic metaphase-I in pollen mother-cells of this form, $(8—2)_{II} + (0—12)_I$ configuration was ascertained. In a small proportion of cells several pentavalent, tetravalent, and trivalent chromosomes were found. Thus the meiosis proceeded quite irregularly, and there usually appeared, in addition to some lagging univalents, the fragments and chromatid bridges, so that the formation of micronuclei was quite prevalent at the interkinesis and at the tetrad stage.

Young pollen-grains of the Wakegi soon degenerated without any further division of their nuclei. Artificial pollinations with the pollen-grains of *A. fistulosum* have been tried without obtaining any viable seeds.

The appearance of chromosome fragments was first observed at diplotene, and in the succeeding stages, there frequency appearance increased towards anaphase-I. Most of the fragments, showing continuous variation in their sizes and increasing in their number up to 3 per cell at metaphase-I, are considered to have been derived through the breakage-fusion mechanism operating between two related chromatids

and only a few others, if any, may be ascribable to the crossing-over within the inverted sections.

Observations in the Wakegi, carried out upon the morphological, ecological, and the karyological features, have made it quite conceivable that this plant form will be a certain product derived through the hybridization among A. *fistulosum*, A. *cepa*, and some other closely related species in the genus *Allium*.

ACKNOWLEDGEMENT

The author wishes to express his deep gratitude to Prof. Dr. E. Fukushima, Kyushu University, for his guidance throughout the course of this work, and also for his reading the manuscript.

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