Korean Jour. Botany
Vol. 18, No. 3, 92-100(1975)

Karyotypic Analyses Based on Heterochromatin Distribution in *Allium fistulosum* and *Allium ascalonicum*

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*Allium fistulosum*과 *Allium ascalonicum*에서 헤테로크로마틴 分布에 의한 核型分析

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ABSTRACT

The present study demonstrates karyotype based on H-patterns of *A.fistulosum* and *A. ascalonicum* using Giemsa technique. The results obtained in this study are summarized as follows: i). Karyotypic analysis of *A. fistulosum* is $6V_{II}+J_{II}^{t}+J_{II}$ and that of *A. ascalonicum* collected from a local farm in the suberbs of Taegu city clearly heterozygous as $13V+J_1^t+J_2+i$. ii). The heterochromatin of both species is generally located distally in both arms of chromosomes and each chromosome type possesses some variations on H-patterns. iii). The percentage of heterochromatin to total chromosome length in cell is about 14.6% in *A. fistulosum*, 12.8% in *A. ascalonicum*. The number of bands is revealed about 38 in *A. fistulosum* and 33 in *A. ascalonicum*. Also in the amounts of chromocenters per nucleus, the former is somewhat more than the latter.

INTRODUCTION

It has been shown previously that the heterochromatin reveals singular reaction by cold treatment (Darlington and La Cour, 1962), but the most recent, the method of preferential staining of heterochromatin of the chromosome has been known and numerous method of treatment about this were presented (Yosida and Sakai, 1972; Ray and Hamerton, 1973; Vosa, 1973; Yamaski, 1973; Schweizer, 1973). It has suspected that Giemsa banding will be facilitated to study the similarities between related species.

Karyotypic analysis of *Allium ascalonicum* was reported that the somatic chromosome (2n=16) were clearly heterozygous and pairing behaviours of chromosomes in PMCs meiosis I were irregular (Kurita, 1953; Iwasa, 1964). *Allium fistulosum* (2n=16) is exactly consists of eight homologues, but these two species are very similar to each other in external features. Therefore, the author attempted to disclose karyotypic analyses of above two species based on H-patterns using Giemsa technique and to compare the similarities of chromosome between these species by H-patterns.

MATERIALS AND METHODS

The plant materials, *Allium fistulosum* (stone-leek) and *Allium ascalonicum* (shallot) were collected from a local farm in the suberbs of Daegu city in Korea. As pretreatment for scattering chromosomes the method of photodissociated treatment of potassium ferricyanide solution under sterilizing lamp (Kim, 1974) was employed. All roots were fixed in ethanol-glacial acetic acid (3 : 1) for 6-12 hours and were stored in absolute alcohol for up to 2-3 days in the refrigerator (about 6°C). They were transferred direct from ethanol to 45% acetic acid and left for 2-3 hours at room temperature prior to preparation. Then root-tips were placed in a drop of 45% acetic acid on a slideglass, gently heated, chopped, covered with coverglass, and gently heated and squashed. The coverglass was prized off on dry ice, and the preparation air-dried.

The materials squashed in 45% acetic acid were immersed in a saturated barium hydroxide at room temperature for 5-10 minutes and the slide was rinsed in distilled water, and then incubated in 4×SSC (1×SSC is 0.15M NaCl plus 0.015M sodium citrate *p*H 7.0, McKay, 1973) at 60°C for 30-60 minutes. The preparations were rinsed in distilled water, stained with Giemsa for 5-10 minutes, rinsed and air-dried. Giemsa stain was prepared with stock diluted 20X with M/15 Sörensen phosphate buffer *p*H 6.9. All observations were made under oil immersion with Olympus EHCRTR-3 microscope with greenfilter combination. Photographs were taken on Kodak high contrast copy panchromatic film.

RESULTS AND DISCUSSION

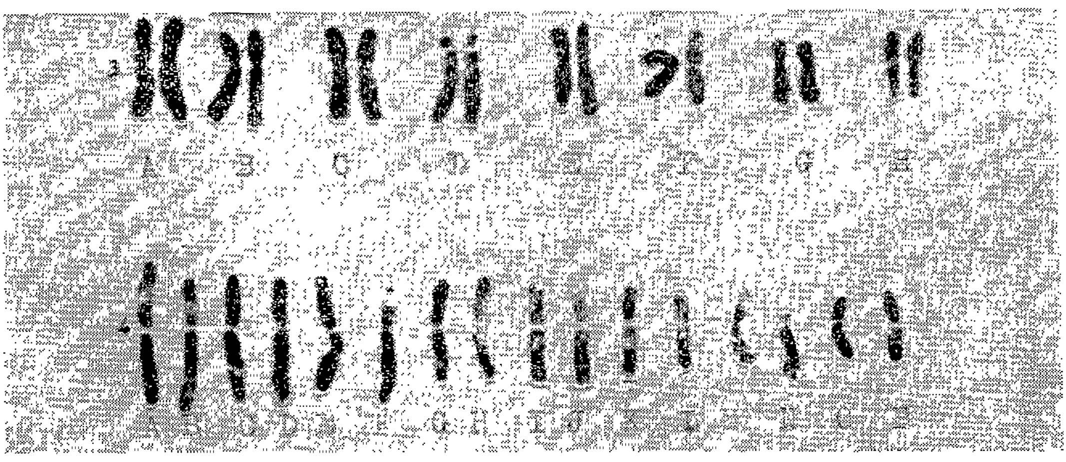
General Karyotype

General karyotypic analyses of *A. fistulosum* and *A. ascalonicum* can be assigned as following formulae;

A. fistulosum $(2n=16)=6V_{II}+J_{II}^{t}+J_{II}$.

A. ascalonicum $(2n=16)=13V+J_1^{t}+J_2+i$.

Figs. 1 and 2. Photographs of the somatic chromosome in *A. fistulosum* (1) and *A. ascalonicum* (2). The bar indicated 10 μ distance.



Figs. 3 and 4. Idiograms of *A. fistulosum* (3) and *A. ascalonicum* (4).

It is noticeable mark that the chromosome of *A. ascalonicum* does not have homologous chromosome. Iwasa (1964) reported that *A. ascalonicum* does flower in some rare cases and usully propagated vegetatively, and that this plant grown on the suberbs of Fukuoka city in Japan was disclosed clearly heterozygous in its karyotype. The karyotype of the present study is shown in Fig.4. Kryological observation of Iwasa was $13V+J_1+J_2+J_3^t$, having satellite in N-chromosome. On the other hand in materials of the present observation, satellite was found in F-chromosome (Fig. 4). It is supposed that karyological difference reveals according to the growing districts.

H-pattern

1). *Allium fistulosum*

Fig. 5 shows the mitotic metaphase chromosome stained with Giemsa. Bands are mostly located in the end of both arms of each chromosome, and in addition to these bands, the other band near the centromere of the long arm formed in a pair of G-chromosome (Fig. 6). Fig. 6 shows diagrammatic representation of H-patterns in metaphase chromosomes from different plants (Nos. 3 and 11 in Table 1).

Intra-specific banding variations on each chromosome were found. The eight chromosome types and their banding variants are shown in Fig. 7. All 20 plants under study are unique in their chromosome band endowment and the polymorphism

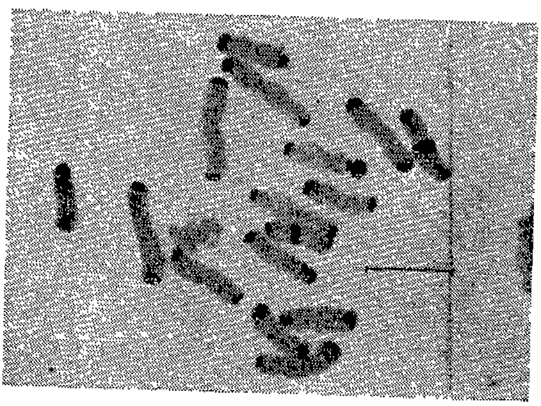


Fig. 5. Mitotic metaphase of *A. fistulosum* from plant No, 1 (see Table 1). The bar indicated 10μ distance.

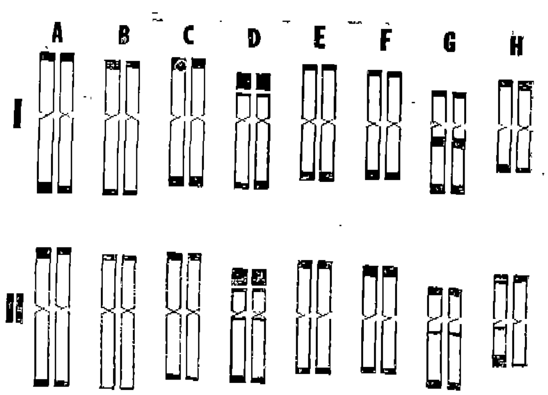


Fig. 6. A diagrammatic representation of the chromosome complement of plant No. 3 (I) and No. 11 (II) in *A. fistulosum* (see Table 1).

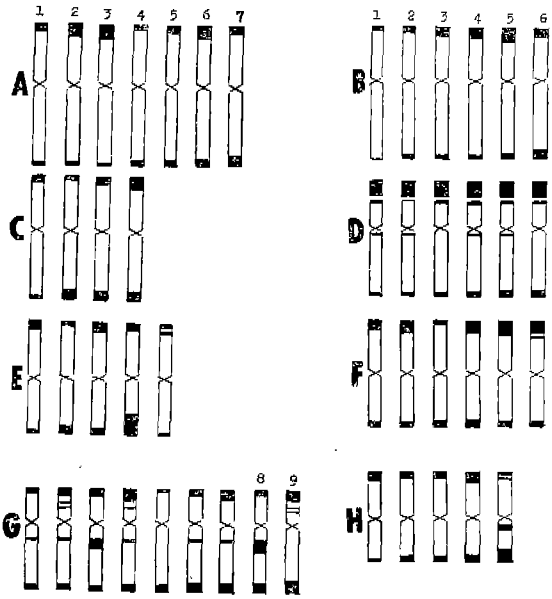


Fig. 7. A diagrammatic representation of the eight chromosome types and all their variants found in a population of *A. fistulosum*.

involves all chromosomes of the complement. Chromosome A is the polymorphic of the complement with seven variants. It is submetacentric and the heterochromatic segments are located only distally in both arms. Chromosome B possesses six variants. Among them, five variants have bands which are located distally in both arms and one has band in the short arm. Chromosome C is the least polymorphic of the complement with only four variants according to the amounts of heterochromatin in the end of both arms. Chromosome D is sat-chromosome which possesses six variants. Satellite is contituted with completely heterochromatin. Their bands are located distally in both arms and near the centtromere of the long arm (3 variants), in the end of both arms (2 variants), in the end of the long arm and near the centromere of the long arm (1 variant). Chromosome E possesses five variants, and among them there are one variant which reveals very large segment in the long arm and one variant which reveals two segments into the short arm. Chromosome F possesses six variants constituting five variants that are located distally in both arms and one variant with two different size of H segment in the short arm. Chromosome G is the most polymorphic of the complement with the nine variants and they are divided into two groups; one variant which is only located distally in both arms and those which are located in various positions besides the end of both arms. The latter is divided that the band are located near the centromere of the long arm (5 variants), one band is located near the centromere of the long arm and two thin bands are located in the middle of the short arm (1 variant), one band near the centromere of the long arm and one band in the middle of the short arm (1 variant), two thin band are located in the middle of the short arm (1 variant). H-chromosome possesses five variants, four variants which have bands located distally in both arms and one variant which has band in each arm besides the end of both arms. The frequency of the various chromosome variants is given is given in Table 2. Table 1 shows the heterochromatin percentage per chromosomal total length and number of band in 20 plants under study. The percentage of the total length of the diploid complement varies from 11.7% to 18.0%

Table 1. Chromosome constitution (H-percentage) and numbers of band in 20 plants of *A. fistulosum*

Plant No.	A	B	C	D	E	F	G	H	Mean %(H)	No. of bands
1	13.2	15.9	15.1	25.9	17.7	17.9	23.3	2.31	18.6	40
2	15.3	9.8	11.0	21.9	12.9	14.7	25.7	23.3	16.3	40
3	11.0	9.3	15.9	20.3	11.8	10.0	23.8	16.5	16.4	36
4	9.8	10.3	10.0	21.5	15.9	15.7	19.8	21.2	16.1	40
5	13.8	13.1	12.8	23.0	14.9	21.4	22.9	18.1	16.4	40
6	8.3	8.3	13.5	27.0	11.7	12.6	13.3	10.4	12.1	38
7	13.5	9.8	10.5	19.2	8.1	13.0	24.3	15.7	15.3	39
8	11.9	10.9	14.0	20.7	10.6	23.6	16.7	15.8	15.2	40
9	7.2	11.3	13.1	16.0	11.0	15.5	12.0	13.6	12.0	42
10	7.8	9.2	10.7	17.4	11.9	12.6	12.3	14.1	11.8	39
11	10.1	4.0	9.6	19.5	11.9	15.3	15.3	16.5	12.0	44
12	14.3	9.7	12.3	22.0	11.5	14.0	16.9	11.5	13.9	36
13	10.7	10.0	10.5	23.5	15.7	15.0	22.0	15.3	15.0	38
14	9.3	12.6	9.9	21.9	16.0	20.4	18.6	17.5	15.3	38
15	13.7	10.9	11.1	19.1	12.8	13.8	17.4	23.4	14.8	37
16	10.8	11.2	11.6	18.2	15.4	12.9	21.0	13.7	14.4	40
17	13.8	12.5	15.8	25.5	12.2	18.6	20.0	18.6	17.1	41
18	11.3	8.5	9.6	15.0	18.4	16.1	21.2	16 7	14.2	39
19	11.2	11.1	10.3	20.7	11.7	12.3	12.2	19.8	13.4	34
20	10.1	8.0	9.0	16.7	12.2	10.3	16.6	14.1	11.7	36

Table 2. Frequency of chromosome variants in *A. fistulosum*

Chromosome type	Variants								
	1	2	3	4	5	6	7	8	9
A	4	4	4	5	13	3	7	—	—
B	2	12	15	5	3	3	—	—	—
C	15	8	12	5	—	—	—	—	—
D	2	2	10	13	9	4	—	—	—
E	7	14	9	7	3	—	—	—	—
F	9	5	12	5	7	2	—	—	—
G	2	7	2	2	2	11	8	4	2
H	5	16	7	11	1	—	—	—	—

Table 3. Percentage of heterochromatin in each arm on total length chromosome type variants in *A. fistulosum*

Chromosome type	Variants								
	1	2	3	4	5	6	7	8	9
A	4.8/3.0	8.8/3.7	10.0/2.7	3.9/4.9	6.8/5.2	9.2/5.6	6.1/7.4	—	—
B	4.0/0	4.5/4.7	6.5/3.9	6.9/5.8	8.0/3.2	11.0/4.4	—	—	—
C	5.7/4.3	3.0/7.2	5.5/6.8	8.9/6.9	—	—	—	—	—
D	15.3/2.8	14.3/3.4	15.3/4.0	15.3/4.4	15.3/5.5	15.3/6.6	—	—	—
E	6.6/4.7	3.9/7.3	8.6/6.4	6.5/17.7	8.9/4.2	—	—	—	—
F	7.3/5.9	9.2/5.2	4.8/8.0	9.7/8.1	13.0/7.0	12.8/5.9	—	—	—
G	6.1/7.2	10.7/3.7	8.3/1.1	14.2/9.4	4.9/7.3	6.1/10.2	9.6/10.4	6.3/15.6	13.3/11.1
H	6.7/4.3	8.0/7.2	9.6/7.5	13.2/9.0	6.6/18.3	—	—	—	—

%(H) in short arm/%(H) in long arm.

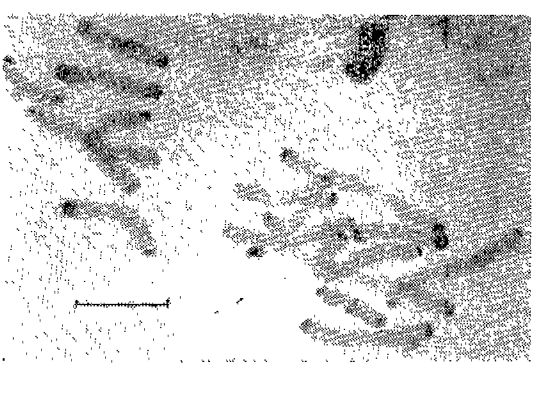


Fig. 8. Mitotic metaphase of *A. ascalonicum* from root-tip No.2. (see Table 4). The bar indicated 10μ distance.

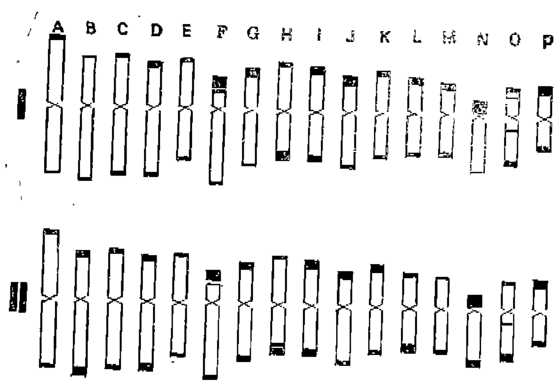


Fig. 9. A diagrammatic representation of the each chromosome of root-tip No. 3(I) and No. 13(II) in *A. ascalonicum.*

and about 14.6% of the total chromosomal length of 20 plants is banded. The number of bands of the diploid complement varies from 34 to 44 and about 38 bands are revealed in diploid chromosome. The percentage of heterochromatin for each chromosome type variants is shown in Table 3.

2). *Allium ascalonicum*

Fig. 8. shows the mitotic metaphase chromosome stained with Giemsa. Bands are mostly located distally in both arms, in addition to these bands, the other band near the centromere of the long arm formed in O-chromosome (Fig.9). Fig. 9 shows diagrammatic representation of H-patterns in metaphase chromosomes from different root-tips (Nos. 3 and 11). This plant show some variations according to the heterochromatin distribution. The 16 chromosome types and their banding variants on the position and amounts of heterochromatin found in the population under study are shown in Fig. 10. Each of chromosome A,C,E,K,L, and M is divided into two groups; band which is located distally only in the one arm and those which are in both arms. In chromosome B,D,G,I, and P, the bands are all located distally in both arms. Chromosome F is sat-chromosome and satellite is stained darkly with Giemsa. Among them bands are located distally in both arms (2 variants), only in the long arm (1 variant), in the end of both arms and near the centromere of the long arm (1 variant). Chromosome H is metacentric and possesses six variants.

Bands are located distally in both arms (4 variants), in the one arm (1 variant), towards the end of one arm besides the end of both arms (1 variant). Chromosome N is telocentric. Short arm is generally constituted with heterochromatin and the end of the long arm is banded (4 variants). Chromosome O possesses four variants and bands are located distally in both arms (1 variant), near the centromere of the long arm and near the end of the short arm besides the end of both arms (1 variant), near the centromere of the long arm

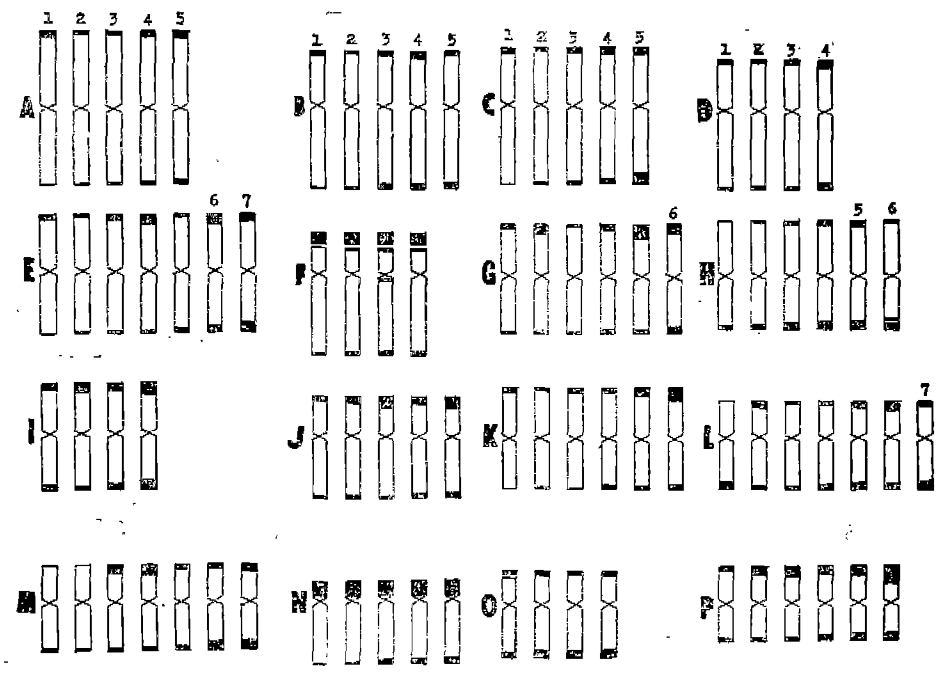


Fig. 10. A diagrammatic representation of the 16 chromosome types and all their variants found in a population of *A. ascalonicum*.

Table 4. Chromosome constitution (H-percentage) and number of band in 20 root-tipse of *A. ascalonicum*

Plant No.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Mean %(H)	No. of bands
1	3.1	1.8	2.4	5.1	6.2	10.0	10.0	12.2	11.9	11.6	13.4	11.5	5.8	20.7	19.6	22.2	9.5	34
2	2.8	6.1	4.1	3.8	6.5	8.3	14.3	6.4	10.0	17.1	11.4	18.2	14.7	17.4	20.0	19.4	9.7	36
3	3.4	2.2	5.3	7.7	5.7	9.2	11.3	13.1	15.0	12.7	9.2	13.5	14.0	22.3	21.6	20.3	10.1	35
4	8.9	7.2	4.1	13.1	14.1	18.3	14.2	8.9	22.5	11.3	9.0	20.2	25.0	25.0	22.6	29.6	15.6	34
5	3.0	3.7	4.7	8.3	7.3	13.3	11.3	8.0	11.7	8.0	5.9	10.0	14.8	11.8	19.0	8.6	8.6	33
6	5.0	4.4	8.2	5.5	6.7	6.5	14.7	6.9	16.9	13.5	6.6	17.0	21.2	6.7	22.1	21.9	10.6	35
7	3.8	4.7	6.1	7.9	3.3	13.1	12.8	10.2	15.3	17.6	8.6	20.0	15.6	9.2	17.3	16.0	17.0	33
8	5.9	8.8	7.0	7.0	7.1	13.7	20.7	16.7	19.2	14.5	13.1	17.9	18.9	17.3	13.6	25.0	13.0	34
9	5.9	9.0	6.9	12.9	5.2	13.3	15.8	9.7	19.8	15.8	10.7	15.1	16.7	9.2	26.7	32.6	13.0	34
10	9.4	4.2	4.7	9.8	13.1	4.1	12.5	13.3	15.4	14.5	12.3	14.5	16.4	4.4	20.0	28.3	11.8	31
11	4.1	8.8	6.0	9.8	15.0	14.3	13.9	4.0	21.8	21.2	14.3	10.9	21.7	22.2	17.8	21.3	13.3	31
12	7.4	8.7	5.3	5.6	16.0	15.8	19.4	10.4	25.5	17.2	7.6	21.3	26.0	26.2	26.2	21.1	15.3	33
13	4.2	7.5	7.8	11.4	5.4	14.5	12.2	12.2	17.1	11.8	15.3	16.2	7.0	25.8	17.2	20.0	12.0	34
14	6.7	8.6	11.5	6.0	5.5	14.5	15.0	14.8	18.2	11.5	15.3	21.6	18.6	20.5	21.1	21.1	13.3	33
15	5.0	3.8	3.3	12.2	6.8	18.3	13.2	12.5	13.1	14.9	11.3	4.7	20.9	20.0	20.5	18.2	11.4	33
16	7.6	7.5	14.9	7.5	7.7	17.3	10.4	15.5	20.2	1.5	18.8	9.3	22.9	22.2	27.5	23.7	14.1	32
17	6.8	7.4	6.2	15.9	14.6	5.6	13.7	18.2	22.9	19.2	16.0	10.6	18.2	34.4	21.1	37.2	15.1	34
18	5.2	6.0	8.8	11.3	10.5	11.0	10.5	16.3	16.1	13.1	6.9	18.6	18.6	19.2	21.7	21.7	12.3	34
19	4.0	6.5	4.6	5.0	9.8	10.5	15.0	7.0	14.1	11.0	4.2	13.3	18.6	14.3	17.7	16.4	9.4	33
20	8.6	5.9	5.7	5.8	4.5	10.4	9.5	11.8	9.0	10.6	11.1	15.7	4.7	10.7	16.2	14.7	8.8	29

Table 5. Frequency of chromosome variation in *A.ascalonicum*

Chromosome type	Variants						
	1	2	3	4	5	6	7
A	3	2	6	6	3	—	—
B	3	3	8	1	5	—	—
C	2	7	7	2	2	—	—
D	5	6	3	6	—	—	—
E	1	2	8	2	2	2	3
F	12	4	2	2	—	—	—
G	4	4	3	3	2	4	—
H	1	4	4	5	5	1	—
I	4	8	2	6	—	—	—
J	5	5	4	2	4	—	—
K	2	2	6	4	5	1	—
L	1	5	2	2	3	5	2
M	1	2	2	2	4	6	3
N	4	4	4	7	1	—	—
O	1	2	10	7	—	—	—
P	2	2	3	7	4	2	—

Table 6. Percentage of heterochromatin in each arm on total lenght of each chromosome type variants in *A. ascalonicum*

Chromosome type	Variants						
	1	2	3	4	5	6	7
A	5.0/0.2	2.6/0.6	2.3/1.4	4.0/1.9	4.6/4.0		
B	2.9/1.0	0.8/2.1	2.7/3.7	5.2/3.5	3.4/5.2		
C	5.3/0	2.6/1.6	4.4/2.1	4.7/3.7	5.7/7.6		
D	3.7/1.2	4. /1.4	5.8/3.3	6.7/5.8			
E	4.5/0	2.4/2.1	3.9/2.4	7.6/2.5	1.6/5.3	9.4/6.0	6.2/7.8
F	11.3/3.1	13.3/4.2	13.3/6.2	13.3/5.0			
G	3.4/2.9	7.8/2.9	11.6/2.0	5.8/6.0	13.1/4.8	8.9/6.7	
H	0/3.3	2.2/4.8	2.9/6.9	5.7/7.0	5.9/10.0	2.4/9.8	
I	5.5/4.9	10.0/4.2	8.8/7.5	11.9/10.2			
J	5.8/3.7	9.0/3.7	11.7/3.6	8.9/6.1	12.2/6.5		
K	5.9/0	4.1/2.2	6.7/2.3	6.8/5.8	9.5/5.4	12.5/6.3	
L	0/9.3	8.0/4.9	1.5/6.0	5.7/6.6	8.6/8.6	11.6/7.8	6.0/12.0
M	0/4.7	2.4/4.0	11.9/4.6	12.8/7.0	6.8/7.9	6.8/13.3	9.6/13.6
N	18.2/0	18.2/2.0	18.2/4.8	18.2/7.5	18.2/10.6		
O	4.1/6.8	9.0/11.1	5.6/11.4	5.3/11.8			
P	7.2/4.8	11.0/5.1	15.0/7.3	11.5/8.7	14.8/10.7	24.4/10.3	

%(H) in short arm/%(H) in long arm.

besides the end of both arms (2 variants). Table 4 shows the heterochromatin percentage per chromosomal total length and the number of bands in 20 root-tips under study. The percentage of the total length of the each plate varies from 8.6 to 17.0% about 12.8% of the total chromosome length of 20 root-tips are banded. The number of bands of a metaphase plate varies from 29 to 36 and about 33 bands are revealed. The frequency of the various chromosome variants is shown in Table 5. The percentage of heterochromatin of both arms for each chromosome type variants is shown in Table 6. Fig. 11 shows the nuclei showing Giemsa stained chromocentres of *A. fistulosum* and *A. ascalonicum*. Marks and Schweizer (1974) reported that there was a close parallel between the number and sizes of chromocentres per nucleus and the extent of banding in metaphase chromosome of *Anemone* and *Hepatica*. Observing the number and size of chromocentres would seem to be a reliable means of serving species of Giemsa banding in their metaphase chromosomes. In number and size of chromosomes. In number and size of chromocentres of both species *A. fistulosum* reveals about 38, 14.6% respectively, while *A. ascalonicum* reveals about 33 and 12.8%. Therefore, in Fig. 11 interspecific difference of chromoncentres these two species didn't be shown distinctly but *A. fistulosum* was a little much more than *A. ascalonicum*.

Schweizer(1973) reported that the heterochromatin distribution from preparation of cold treatment

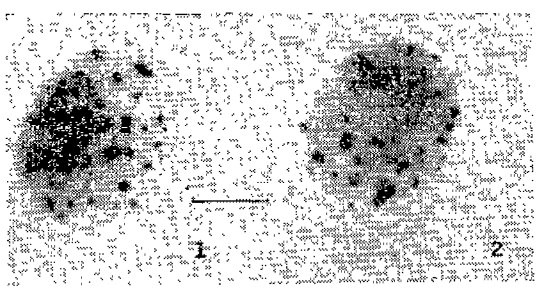


Fig. 11. Nuclei showing Giemsa stained chromocentres of *A. fistulosum*(1) and *A. ascalonicum*(2). The bar indicated 10μ distance.

and Giemsa technique was shown to be identical with each other in chromosome of root-tip cells from *Trillium grandiflorum*. Sumner (1972) reported that aquous barium hydroxide improved both speed and reliability of band forming. In preliminary experiment, when the saturated barium hydroxide process omitted, bands were not formed and stain was as faint as chromosome cannot be recognize, in spite of 4×SSC was incubated for 24 hours. In the preparation air dried left for 2 days or over at room temperature, the bands are not formed as a rule. As treatment for scattering chromosome, photodissociated treatment of potassium ferricyanide solution was identical with colchicine treatment in the location and size of Giemsa bands.

The data discussed in these two species so far show that bands are generally located in the end of both arms of each chromosome. Satellite is constituted with completely heterochromatin. There can be found the similarity between G-chromosome of *A. fistulosum* and O-chromosome of *A. ascalonicum*; another band besides the end of both arms is formed near the centromere of the long arm, though the size of bands varies more or less.

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(Received May 12, 1975)