Multivalent Chromosomes in Garlic, *Allium sativum* L.

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Introduction

Cytogenetic studies in garlic, *Allium sativum* L., have been made by many workers. It was reported by Katayama[4], Morinaga and Fukushima[13], Levan[10], Takenaka[18], and Ono[15] that garlic had 16 somatic chromosomes, and the karyotype analyses were done by Mensinkai[12], Kurita[8,9], Krivenko[7] and Battaglia[1]. Takenaka observed multivalent[18], and hexavalent and quadrivalent chromosomes[19] at meiosis in the pollen mother cells; while Krivenko[7], and Koul and Gohil[6] observed eight normal bivalent chromosomes. Gohil and Koul[3] observed desynapsis in a clone of garlic. Katayama[5] observed multivalent, chiefly hexavalent, chromosomes in two clones and eight bivalent chromosomes in another clone, simultaneously. From the facts mentioned above, some different types of meiotic irregularities in garlic may be expected.

Materials and Methods

The material used here was cultivar Shanhai-wase, originally introduced from China and grown extensively in Japan. In total, seven inflorescences were used as materials, one in 1973, three in 1974 and three in 1977. They were picked at random out of the population of cultivar Shanhai-wase, respectively, and were raised in the respective year. Flower buds were fixed in Newcomer's fluid or in Farmer's fluid and preserved for observations. The anthers were smeared in iron aceto-carmine solution, and the meiotic behavior of chromosomes in pollen mother cells was examined. At metaphase-I the chromosomes of garlic were frequently complicated and contracted, so that they were also observed at diplotene.

Results

Chromosome pairings at meiosis in the pollen mother cells of garlic, cultivar Shanhai-wase, were shown in Table 1. In every inflorescence there was recognized almost the same occurrence-frequency of the chromosome configurations in the pollen mother cells.

As shown in Table 1, no pollen mother cell had eight bivalent chromosomes, and multivalent chromosomes were observed in all of the pollen mother cells examined. The occurrence-frequency of octovalent chromosomes was greater than that of any other multivalent chromosomes. Octovalent chromosomes were observed in 105 of 131 (80.2%) pollen mother cells. Most of the octovalent chromosomes showed firm

Table 1. Chromosome pairings at meiosis in pollen mother cells

Configuration	Number of pollen mother cells		
	Ring multivalent	Chain multivalent	Total
$1_{X}+3_{II}$		1	1
$1_{VIII}+4_{II}$	56	48	104
$1_{VIII}+3_{II}+2_{I}$		1	1
$1_{VII}+4_{II}+1_{I}$		7	7
$1_{VI}+5_{II}$		4	4
$1_{VI}+4_{II}+2_{I}$		1	1
$1_{V}+1_{III}+4_{II}$		3	3
$2_{IV}+4_{II}$		8	8
$1_{IV}+1_{III}+4_{II}+1_{I}$		1	1
$2_{III}+4_{II}+2_{I}$		1	1
Total			131

pairings (Figs. 1, 3, 4, 6, 7), though some of them showed pairings weaker than that shown in Fig. 5. Ring octovalent chromosomes were observed in 56 of 104 pollen mother cells having octovalent chromosomes (Figs. 1–4), while all of the rest presented chain octovalent chromosomes (Figs. 5–7).

At metaphase-I, multivalent chromosomes may give rise to several arrangements, and, since the arrangements determine the mode of anaphase disjunction, each arrangement may have its own genetic consequences, presumably. However, it was impossible in the present observations to determine whether the multivalent chromosomes resulted in the alternate (zigzag) metaphase arrangement or in the adjacent metaphase arrangement, because the chromosomes were quite complicated and contracted at metaphase-I.

Ring multivalent chromosomes were never observed, excepting octovalent chromosomes. Heptavalent chromosomes were observed in seven pollen mother cells, and all of them were constantly accompanied by one univalent chromosome. Hexavalent chromosomes were observed in five pollen mother cells, and were accompanied by no other multivalent chromosomes. Pentavalent, quadrivalent or trivalent chromosomes were also observed, but the total chromosome numbers with relation to multivalents in a pollen mother cell were eight or less in every pollen mother cell that included pentavalents, quadrivalents or trivalents (Fig. 8). Pentavalent chromosomes were constantly accompanied by one trivalent, though they were observed in only three pollen mother cells.

The occurrence-frequency of four bivalent chromosomes in a pollen mother cell was 95.4% (125 of 131 PMCs), and the number of bivalent chromosomes in a pollen mother cell was restricted to from three to five without exception. There were observed univalent chromosomes in 11 of 131 PMCs (8.4%).

The distribution of chromosomes after metaphase-I was as follows. In about 80% of the pollen mother cells examined, sixteen chromosomes were distributed equal to each pole, at anaphase-I. In the rest of the pollen mother cells, chromosomes were distributed unequal, mainly in 9-7.

Most of the pollen mother cells accomplished their meiotic division; and after being released from the pollen tetrads, all the microspores aborted.

Discussion

There were observed multivalent chromosomes at meiosis in every pollen mother cell of garlic, cultivar Shanhai-wase. About four fifths of all the examined pollen mother cells had the chromosome configuration, which may be represented by a schema, ($1_{VIII}+4_{II}$). The other chromosome configurations observed here seemed to be derived from this schema ($1_{VIII}+4_{II}$), excepting one chromosome configuration ($1_{X}+3_{II}$). In the configuration ($1_{VIII}+3_{II}+2_{I}$), two univalents might be derived from the one bivalent of ($1_{VIII}+4_{II}$). In the configuration ($1_{VII}+4_{II}+1_{I}$), one heptavalent and one univalent might be derived from the octovalent. In the configuration ($1_{VI}+5_{II}$), one hexavalent and one bivalent might be derived from the octovalent. In the configuration ($1_{VI}+4_{II}+2_{I}$), one hexavalent and two univalents might be derived from the octovalent. In the configuration ($1_{V}+1_{III}+4_{II}$), one pentavalent and one trivalent might be derived from the octovalent. In the configuration ($2_{IV}+4_{II}$), two quadrivalents might be derived from the octovalent. In the configuration ($1_{IV}+1_{III}+4_{II}+1_{I}$), one quadrivalent, one trivalent and one univalent might be derived from the octovalent. In the configuration ($2_{III}+4_{II}+2_{I}$), two trivalents and two univalents might be derived from the octovalent. Some of those configurations mentioned above might result from the configuration ($1_{VIII}+4_{II}$) during the procedure of the smear method. The chromosome configuration, anyway, at meiosis of cultivar Shanhai-wase appeared to be chiefly represented by ($1_{VIII}+4_{II}$). This was confirmed by the fact that ring multivalents existed only as octovalents in the pollen mother cells examined here. And moreover, it may be confirmed by the fact that four bivalents in a pollen mother cell were observed in 95.4% of the examined pollen mother cells.

The chromosome configuration which was observable here most frequently was {1_{VIII}(ring, ⑧)$+4_{II}$} (Figs. 1–4). The chromosome configuration (1⑧$+4_{II}$) in the clone examined here seemed to result from the reciprocal translocations. And the reciprocal translocations were assumed to be symmetrical (eucentric), because the distributions of the chromosomes at anaphase-I were almost normal in most of the pollen mother cells. In individuals with the symmetrical reciprocal translocations, the behavior of the chromosomes at meiosis depends upon the frequency and location of the chiasmata and upon the mode of the centromere orientation[16]. If crossing-over and chiasma formation take place in all the pairing segments, chromosomes must result in a ring. If chiasma formation is absent in any one of the pairing segments, chromosomes may result in a chain. If one more chiasma formation is absent in another part of the pairing segments, the octovalent may become one heptavalent and one univalent, two quadrivalents, one hexavalent and one bivalent, or one pentavalent and one trivalent. One heptavalent and one univalent or two quadrivalents were observed here more frequently than one hexavalent and one bivalent or one pentavalent and one trivalent. The octovalent chromosomes in cultivar Shanhai-wase may probably possess two or three weak pairings.

The results of the investigations on the chromosome configuration of garlic at meiosis hitherto reported by the workers are given in Table 2.

Table 2. Chromosome pairings in various clones of garlic reported by the workers

Clone	Source	Chromosome configuration	Author
Market	Seoul (Korea)	multi- and bivalents	Takenaka (1931) [18]
Shirokuki	Korea	8_{II}	Katayama (1936) [5]
Akakuki	Korea	mainly $1_{VI}+5_{II}$	
Zairai	Tokyo (Japan)	$1_{VI}+5_{II}$	
————	Turkistan (U.S.S.R.)	8_{II}	Krivenko (1938) [7]
Market	Mishima and Tokyo (Japan)	hexa- or quadri- and bivalents	Takenaka (1953) [19]
Three cultivated populations	Srinagar, Agra and Jaipur(India)	8_{II}	Koul and Gohil (1970) [6]
Wan ruban	Bhadarwah (India)	desynapsis	Gohil and Koul (1971) [3]
Shanhai-wase	China	mainly $1_{VIII}+4_{II}$	present authors

Multivalent chromosomes have also been reported by other workers, while eight bivalents of 16 chromosomes have been reported. It is obvious that garlic includes different clones with various chromosome configurations at meiosis. It is confirmed by Katayama's report showing the existence of multivalents in two clones and that of eight normal bivalents in another clone, simultaneously[5]. The fact that garlic includes different clones with various chromosome configurations at meiosis may require a discussion debating whether garlic was originated from one line or more than one line.

If garlic was originated from one line, eight bivalents may be regarded as the original chromosome configuration at meiosis. It is difficult to assume that the clones with eight bivalents, as reported by some workers, were derived from the clone with multivalent chromosomes. A clone with $(1_{IV}+6_{II})$ might be derived from the original clone with eight normal bivalents by one reciprocal translocation between nonhomologous chromosomes. And the second or the third reciprocal translocation might lead the chromosome configuration from $(1_{IV}+6_{II})$ to $(1_{VI}+5_{II})$ or to $(1_{VIII}+4_{II})$. The clones with the chromosome configuration such as $(1_{IV}+6_{II})$ may persist in garlic because of the vegetative propagation, though such clones have not been found, yet. In *Oenothera* having one ring multivalent, the reciprocal translocation is presumed to take place most easily by becoming interlocked at meiotic prophase[17]. There may be a possibility that the reciprocal translocation occurs also in the somatic cells of garlic, basing on the fact that Yamashita[20,21] synthesized the artificial multivalent chromosomes in *Triticum* by X-radiation applied on to the dormant seeds. Once meiotic irregularity occurred, it might be easily maintained, owing to the fact that the crop was propagated vegetatively and selected artificially. And the meiotic irregularity might be easily accumulated on account of the same reasons. Different clones with the various chromosome configurations might be derived for the reasons mentioned above.

Takenaka[19] assumed that "Garlic is a hybrid originated from a natural or artificial cross between two related species whose chromosome complements have become differentiated by translocations, inversions or deficiencies in the course of evolution.", after having observed multivalents at meiosis in garlic. However, the existence of the clones with eight normal bivalents reported by Katayama[5], Krivenko[7], and Koul and Gohil[6] may be inconsistent with this assumption of his. Chromosome aberrations at meiosis are usually discussed in relation to the fertility of the plant. Garlic is a completely sterile plant, and the cause of the sterility has been left indistinct. Meiotic irregularity might cause the sterility in the clones as examined here. On the other hand, *Oenothera* or *Rhoeo* with a ring multivalent is not completely sterile. In garlic, any clone with eight normal bivalents is sterile as well as the clones with multivalents are[5,6,7]. Therefore, the cause of the sterility of garlic is still left indistinct.

The original clone with eight normal bivalents might be fertile, provided that garlic was originated from one line. And the clone may have obtained sterility or meiotic irregularity through the long historical process of vegetative propagation.

If garlic was originated from more than one line, each clone of garlic may have possessed their original chromosome configurations at meiosis, respectively. All the clones of garlic may be divided into two groups, one with eight normal bivalents and the other with the meiotic irregularity. The meiotic irregularity may result from hybridity, polyploidy, translocations, or some other chromosome mutation, and this meiotic irregularity may cause sterility.

Multivalent chromosomes frequently occur in the polyploid, and polyploid plants are likely to become less fertile being propagated vegetatively[14]. In *Allium paniculatum* group, polyploid plants are likely to become viviparous[11]. From the viewpoint mentioned above, it may be assumed that there is a possibility that garlic is polyploid, but in reality this is improbable on account of the fact that there is no plant with eight chromosomes as 2n in the genus *Allium*[2].

Summary

The chromosome pairings were observed at meiosis in garlic, *Allium sativum* L., cultivar Shanhai-wase. The chromosome configurations at meiosis were ($1_{VIII}+4_{II}$) in about four fifths of all the pollen mother cells examined. More than half of the octovalent chromosomes observed were ring octovalents, while the rest of them were chain octovalents. The occurrence-frequency of four bivalent chromosomes in a pollen mother cell was 95.4% (125 of 131 PMCs). Those chromosome configurations were observed more sharply at diplotene than at metaphase-I. The results were discussed in comparison with the various chromosome configurations, including eight normal bivalents, hitherto reported by the workers.

References

1) Battaglia, E.: Mutazione cromosomica e cariotipo fondamentale in *Allium sativum* L. *Caryologia* (in Italian with English summary), **16**, 1–46 (1963)
2) Darlington, C. D. and Wylie, A. P.: *Chromosome atlas of flowering plants.* George Allen and Unwin Ltd., London (1955)
3) Gohil, N. and Koul, A. K.: Desynapsis in some diploid and polyploid species of *Allium. Can. J. Genet. Cytol.*, **13**, 723–728 (1971)
4) Katayama, Y. : The chromosome number in *Phaseolus* and *Allium*, and an observation on the size of stomata in different species of *Triticum. J. Sci. Agr. Soc. Tokyo*, **303**, 52–54 (1928)
5) Katayama, Y.: Chromosome studies in some Alliums. *J. Coll. Agr. Tokyo Imp. Univ.*, **13**, 431–441

(1936)
6) Koul, A. K. and Gohil, R. N. : Causes averting sexual reproduction in *Allium sativum* LINN. *Cytologia*, **35**, 197–202 (1970).
7) Krivenko, A. A. : A cytological study of garlic (*Allium sativum* L.). *Biologicheskij Zhurnal* (in Russian with English summary), **7**, 47–68 (1938)
8) Kurita, M. : Karyotype of *Allium scorodoprasum* L. *var. viviparum* REGEL. *La Kromosomo* (in Japanese with English summary), **11**, 407–409 (1951)
9) Kurita, M.: On the karyotypes of some *Allium*-species from Japan. *Mem. Ehime Univ.* Sect.II (Sci.), **1**, 179–188 (1952)
10) Levan, A. : Cytological studies in *Allium* (a preliminary note). *Hereditas*, **15**, 347–356 (1931)
11) Levan, A.: Cytological studies in *Allium paniculatum* group. *Hereditas*, **23**, 317–370 (1937)
12) Mensinkai, S. W.: Cytogenetic studies in the genus *Allium*. *J. Genet.*, **39**, 1–45 (1939)
13) Morinaga, T. and Fukushima, E.: Chromosome numbers of cultivated plants III. *Bot. Mag. Tokyo*, **45**, 140–145 (1931)
14) Müntzing, A.: The evolutionary significance of autopolyploidy. *Hereditas*, **21**, 263–378 (1936)
15) Ono, Y.: Chromosome numbers in *Allium*. *Jap. J. Genet.* (in Japanese with English summary), **11**, 238–240 (1935)
16) Rieger, R., Michaelis, A. and Green, M. M.: *A glossary of genetics and cytogenetics*. Springer-Verlag, Berlin (1968)
17) Stebbins, G. L.: *Variation and evolution in plants*. p.380–441, Columbia Univ. Press, New York (1950)
18) Takenaka, Y.: Further reports of the cytological investigations on the sterile plants. *J. Chosen Natural Hist. Soc.* (in Japanese with English summary), **12**, 25–41 (1931)
19) Takenaka, Y.: Ring formation in the meiosis of *Allium scorodoprasum var. viviparum*. *Ann. Rep. Nat. Inst. Genet.* (Japan), **4**, 41–42(English ed.), 42–43(Japanese ed.) (1953)
20) Yamashita, K.: X-ray induced reciprocal translocations in *Triticum aegilopoides* and *T. monococcum*. *Jap. J. Genet.*, **22**, 34–37 (1947)
21) Yamashita, K.: H. Kihara (ed.), *Saibō-idengaku* [*Cytogenetics*—in Japanese] vol. 1. p. 123–146 Yōkendō, Tokyo (1951)

Figs. 1–8. Chromosome configurations at metaphase-I (Figs. 1, 5) and at diplotene (Figs, 2–4, 6–8). × ca. 750.
1–4, a ring octovalent and four bivalents
5–7, a chain octovalent and four bivalents
8, two chain quadrivalents and four bivalents.