Studies on the Haploid Plants of *Solanum tuberosum*

II. Meiotic chromosome pairing in the polyhaploid plants

Motokazu MATSUBAYASHI

(Hyōgo Agricultural College)

As regards the polyploid nature of *S. tuberosum*, it has been considered to be autotetraploid by some researchers (FUKUDA, 1927; MÜLLER, 1930; LUNDEN, 1937; CADMAN, 1942, 1943; LAMM, 1945 and SWAMINATHAN, 1954a, 1954b, 1956), though it has been regarded as allotetraploid by the others (LONGLEY & CLARK, 1930; KOMAROV, 1931; MEURMAN & RANCKEN, 1932; BAYLIS, 1936; ELLISON, 1936; and IVANOVSKAJA, 1936). This problem is still left undecided up to present. In investigating the origin of *S. tuberosum* as well as in establishing a reasonable program of potato breeding, it is of considerable importance to know whether this species is an auto- or allo-tetraploid. In such a case, valuable informations will be given from cytogenetic studies of polyhaploid plants derived from this species.

Several true polyhaploids of *S. tuberosum* have already been reported by earlier researchers, as described in the previous paper (KAWAKAMI & MATSUBAYASHI, 1960). Their cytological behavior has also been reported preliminarily by IVANOVSKAJA (1939), MATSUBAYASHI & KAWAKAMI (1958), PELOQUIN & HOUGAS (1958), and TAGUCHI & IRIKURA (1959). Our knowledge of this respect, however, is rather fragmentary even at present.

Thus, an attempt was made by the author to examine in detail the pairing behavior of and the meiotic chromosomes pollen fertility in the polyhaploid *S. tuberosum* (variety Deodara) under two different seasonal conditions, and these results were compared with those for the closely related diploid species, *S. stenotomum*, grown under the same conditions. In the present paper an account is given of these results and the author's view on the polyploid nature of *S. tuberosum* discussed on the basis of the results.

Further studies on the chromosome behaviors at prophase, anaphase I, and subsequent stages will be reported later.

Material and Methods

Of the materials used in this study, the polyhaploid plants ($2n=24$; DE2-001) of *S. tuberosum* (variety Deodara) were the same as those described in the previous paper (KAWAKAMI & MATSUBAYASHI, 1960) and *S. stenotomum* ($2n=24$) used for comparison with them came from the selected lines whose original forms had been provided through the Inter-regional Potato Introduction Station at Sturgeon Bay, Wisconsin, U.S.A. The tubers of these materials were planted on March 10th, 1958, and thereafter the plants grown from them were cultured under similar natural conditions in the experimental field.

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The pollen mother cells were collected from several individuals of each species on two different days, June 12th and July 15th, to evaluate the response of pairing behavior of chromosomes to seasonal conditions, especially to climatic temperature. These cytological materials were observed using the acetocarmine technique modified by MATSUBAYASHI (1955). The average climatic temperatures for the three nights preceding each date of fixation were 16.7 and 24.6°C respectively. The pollen grains were, in each case, collected from several mature anthers on the tenth day after fixing the pollen mother cells, and stained with dilute acetocarmine solution. Pollen fertility was estimated by calculating the percentage of stainable grains with normal appearance.

Results

Chromosome pairing at metaphase I

In the pollen mother cells fixed in June, as shown in Table 1, the polyhaploids exhibited various meiotic configurations as follows: Out of a total of 140 cells analysed, 12 bivalents were observed in 92 cells (65.7%), 11 bivalents +2 univalents in 35 (25.0%), 10 bivalents +4 univalents in 11 (7.9%), and 1 trivalent +10 bivalents +1 univalent in the remaining 2 (1.4%). Most of the bivalents were of rod-type paired very loosely and a few of them were of ring-type (Fig. 2a, b and c). *S. stenotomum* formed regularly 12 bivalents in all the cells fixed on the same day. Each arm of these bivalents was closely associated with each other and the majority of them were of ring-type (Fig. 2d).

On the other hand, in the pollen mother cells fixed in July, there was a remarkable difference in meiotic configuration between the two species (Tab. 1). At metaphase I in *S. stenotomum* 12 bivalents were almost invariably formed, whereas in the polyhaploids bivalent formation was considerably reduced, showing a wide variation in pairing frequency. In the latter, of the 181 cells examined, only 9 (5.0%) had 12 bivalents and the rest showed various numbers of bivalents ranging from 5 to 11 and univalents in numbers corresponding to those of the bivalents. A single trivalent formation was also found in 11 cells (6.1%) and this frequency was significantly higher than

Table 1. Chromosome pairing at metaphase I

Date of fixation	Polyhaploid					<i>S. stenotomum</i>				
	III	II	I	No. of cells	%	III	II	I	No. of cells	%
12/VI	—	12	—	92	65.7	—	12	—	78	100.0
	—	11	2	35	25.0	—	—	—	—	—
	—	10	4	11	7.9	—	—	—	—	—
	1	10	1	2	1.4	—	—	—	—	—
Total Mean	2 0.01	1619 11.56	116 0.83	140		— —	936 12.00	—	78	
15/VII	—	12	—	9	5.0	—	12	—	67	97.1
	—	11	2	26	14.4	—	11	2	2	2.9
	—	10	4	43	23.8	—	—	—	—	—
	—	9	6	57	31.5	—	—	—	—	—
	—	8	8	22	12.1	—	—	—	—	—
	—	7	10	7	3.9	—	—	—	—	—
	—	6	12	5	2.8	—	—	—	—	—
	—	5	14	1	0.5	—	—	—	—	—
	1	9	3	5	2.8	—	—	—	—	—
	1	8	5	3	1.7	—	—	—	—	—
	1	7	7	1	0.5	—	—	—	—	—
	1	6	9	2	1.1	—	—	—	—	—
Total Mean	11 0.06	1685 9.31	941 5.20	181		— —	826 11.97	4 0.06	69	

the case in June. These meiotic features appear to be almost similar to what PELOQUIN & HOUGAS (1958) have observed in a polyhaploid of *S. tuberosum* (US-W3). The mean pairing frequency in the present case is thus represented as $0.06_{III} + 9.31_{II} + 5.20_I$ and the mode as $9_{II} + 6_I$ (Fig. 3a, b and c).

Such pairing behavior in the polyhaploids shows a marked contrast with that in *S. stenotomum* which had a complete pairing of the chromosomes in most (97%) of the cells examined (Fig. 3d). Figure 1 graphically represents the frequency of bivalents formed in the pollen mother cells of the polyhaploids and *S. stenotomum* for the materials fixed at two different dates.

Fig. 1. Comparison of bivalent frequency at metaphase I between the polyhaploid (H) and *S. stenotomum* (S). The figures given in brackets indicate dates of fixation.

Chiasma frequency at metaphase I

Chiasma frequency per bivalent as well as per cell was found to be significantly lower in the polyhaploids than in *S. stenotomum*, in both cases of fixation (Tab. 2). In the polyhaploids, it was noted that, while the chiasma frequency per cell was considerably reduced in the cells fixed in July as compared with that in the cells fixed in June, the chiasma frequency per bivalent was even high in the same cells. The former case is obviously due to a more frequent occurrence of unpaired chromosomes in the cells fixed in July. The latter case may be explained as in the following. In the cells fixed in July, many of the bivalents paired loosely with a single chiasma may dissociate to become univalents and consequently the bivalents with two chiasmata form a larger proportion.

Table 2. Chiasma frequency at metaphase I

		No. of cells observed	Bivalent				Trivalent				Chiasma freq. per cell	Half chiasma freq.
			No. of biv.	No. of biv. with 1x. 2xta.	Total No. of xta.	Chiasma freq. per biv.	No. of triv.	No. of triv. with 2xta. 3xta.	Total No. of xta.	Chiasma freq. per triv.		
12/VI	Poly-haploid	140	1617	1189 428	2045	1.265	2	1 1	5	2.500	14.643	1.220
	<i>S. stenotomum</i>	78	936	412 524	1460	1.559	—	— —	—	—	18.718	1.559
15/VII	Poly-haploid	181	1687	1171 516	2203	1.306	11	5 6	28	2.545	12.326	1.027
	<i>S. stenotomum</i>	69	826	427 399	1225	1.483	—	— —	—	—	17.754	1.479

of the paired chromosomes. Thus, it would be considered that the bivalents which readily fail to pair under unfavorable conditions, especially such as high temperature, are involved in the bivalents with a single chiasma.

Fig. 2. Meiosis in PMC's fixed in June (ca. $\times 1500$)
a, b, and c—Metaphase I in the polyhaploid showing 12_{II} , $11_{II}+2_I$,
and $10_{II}+4_I$ respectively. d—Metaphase I in *S. stenotomum*, 12_{II} .

Fig. 3. Meiosis in PMC's fixed in July (ca. $\times 1500$)
a, b and c—Metaphase I in the polyhaploid showing $10_{II}+4_I$, $9_{II}+6_I$,
and $7_{II}+10_I$ respectively. d—Metaphase I in *S. stenotomum*, 12_{II} .

Fig. 4. Pollen grains fixed in June (ca. $\times 200$)
a—The polyhaploid, showing no good pollen. b—*S. stenotomum*

Pollen fertility

In pollen analysis, normal-shaped grains well stained with acetocarmine were considered as "good pollen". The pollen data obtained in this way are given in Table 3. It is clearly seen from the table that, for both dates of fixation, *S. stenotomum* shows a relatively high percentage of good pollen, whereas the polyhaploids have no good pollen except for a very rare case of middle-sized and giant grains which appear to be sexually non-functional (Fig. 4).

Table 3. Pollen analysis

			Stainable					Abortive	Total
			Normal form			Abnormal form			
			Giant	Middle	Small	Middle	Small		
22/VI	Poly-haploid	{ Freq. %	2 0.1	— —	— —	28 1.1	28 1.1	2410 97.7	2468
	<i>S. stenotomum</i>	{ Freq. %	— —	4 0.3	1020 68.3	1 0.1	26 1.7	441 29.6	1492
25/VII	Poly-haploid	{ Freq. %	1 0,03	2 0,05	— —	10 0.2	125 3.4	3565 96.3	2703
	<i>S. stenotomum</i>	{ Freq. %	— —	2 0.2	700 54.3	— —	30 2.3	556 43.2	1288

Discussion

It is evident from the data presented above that, although the polyhaploids reported here have fairly regular meiotic pairing under favorable conditions, they show a frequent occurrence of unpaired chromosomes, a low chiasma frequency in the paired chromosomes, and an occasional formation of multivalent at metaphase I, under unfavorable conditions such as relatively high climatic temperature and aging. Such pairing behavior was found neither in *S. stenotomum* nor in several other diploid *Solanum* species, except in a clone of *S. schickii* (MATSUBAYASHI, unpub.). The pairing behavior of the present polyhaploids is somewhat different from that observed by IVANOVSKAJA (1939) and PELOQUIN & HOUGAS (1958) in polyhaploid plants derived from the varieties Aurora and Katahdin respectively, but quite similar to that found by the latter researchers and TAGUCHI & IRIKURA (1959)

in such plants derived from the selection Minn. 15-2-10-1-2 and Norin No. 1 respectively. Particularly, the observations made by Taguchi and Irikura, who have found a high frequency of univalents at a high climatic temperature, agree with the results obtained in the present study. These facts seem thus to indicate that the chromosomes in the two genomes of polyhaploid *S. tuberosum* have a fairly weaker ability to pair with each other than those of naturally occurring diploid *Solanum* species.

Most of the data reported by the above researchers indicate that pollen fertility of the polyhaploid *S. tuberosum* is extremely low. The same result is also obtained in this study. Such pollen sterility is liable to be taken as a plausible evidence to suggest non-interchangeability of chromosomes between the two genomes. This interpretation would, however, become doubtful owing to the finding of a pollen fertile polyhaploid plant from the variety Katahdin (PELOQUIN & HOUGAS, 1958) and also due to the fact that the pollen sterile polyhaploids mentioned above are functional as female parents in crosses with some diploid *Solanums* (PELOQUIN & HOUGAS, 1958; HOUGAS & PELOQUIN, 1959; TAGUCHI & IRIKURA, 1959; and MATSUBAYASHI, unpub.). Although such is the case, it also seems to be a fact that both pollen and seed fertilities of the polyhaploids are generally low, when compared with those of diploid species in the same genus.

On the basis of the above considerations, it is presumed that even if the chromosomes in the two genomes of the polyhaploids are interchangeable to some extent, affinity between them is not so intimate. A similar view has also been suggested from the observations of pairing behavior in the tetraploid hybrids obtained by crossing *S. tuberosum* (variety Deodara) with induced autotetraploid *S. saltense* (KAWAKAMI & MATSUBAYASHI, 1957). In addition, the occasional occurrence of multivalent formation at metaphase I and of chromatin bridges at anaphase I (IVANOVSKAJA, 1939; PELOQUIN & HOUGAS, 1958; and MATSUBAYASHI, unpub.) is interpreted as due to structural differences existing between the two chromosome sets. Thus, it seems highly probable that the two genomes in the polyhaploids are differentiated to a certain degree. It would be more appropriate, therefore, to consider that the original species, *S. tuberosum*, from which the polyhaploids arose, in its current evolutionary step, is neither autotetraploid such as artificially doubled diploid *Solanum* species nor allotetraploid like *S. longipedicellatum*, but it is a "segmental allotetraploid" in the sense proposed by STEBBINS (1947).

This view supports SWAMINATHAN's suggestion (1954) that "the current commercial varieties can be considered as segmental allotetraploids", though it is not in agreement with his opinion (1956) that "*S. tuberosum* originally arose as an autotetraploid" and hence "a single diploid species is responsible for its origin". As HAWKES (1956) has stated, it is more probable to consider that this species may have originated as an amphidiploid hybrid between two certain diploid species which were not genomically quite identical.

Summary

- 1) In this paper, meiotic chromosome pairing and pollen fertility of polyhaploid plants

($2n=24$) of *S. tuberosum* (variety Deodara), under two different seasonal conditions, are described in comparison with those of the closely allied diploid species *S. stenotomum* ($2n=24$).

2) The polyhaploids were found to show a frequent occurrence of unpaired chromosomes and an occasional formation of trivalents under unfavorable conditions, in spite of the fact that *S. stenotomum* had regular meiotic pairing under the same conditions. The pollen fertility of the former plants was extremely low, in contrast with that of the latter.

3) On the basis of these results, affinity between the two chromosome sets of the polyhaploids is discussed and consequently it is suggested that *S. tuberosum* can be considered as a segmental allotetraploid.

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バレイショの半数体に関する研究

第2報 二基半数体における染色体対合

松 林 元 一

(兵庫農科大学)

S. tuberosum の原型はなく、従てその起源を究明することはバレイショ育種の基礎問題として重要な課題とされている。それには先ず本種のゲノム構成を明かにすることが必要である。従来この点についての見解は数多く出されているが、いずれも本来の四倍体のレベルにおける研究に基いているため、研究者によつてその見解に開きがあつた。半数体の研究は、かかる場合に重要な意義をもっている。

著者は *Deodara* の半数体につき、異つた季節的条件のもとで染色体の対合行動と花粉稔性を調べ、これを本種に最も近縁な二倍種 *S. stenotomum* のそれらと比較して次の如き知見を得た。

還元分裂中期における対合は、気温によつて著しく影響され、低温時では約 66% の細胞に 12II が形成される

が、高温時では 5% 前後の細胞にそれがみられるにすぎない。他はいずれも前者で 2~4、後者で 2~14 にわたる一価染色体を示す。しかも二価染色体の多くは対合が極めてゆるく、キアズマ頻度もかなり低い。更に若干の細胞には三価接合もみられる。花粉稔性は極度に低い。しかるに *S. stenotomum* はいずれの場合においても常に規則正しい対合を示し、花粉稔性も半数体に比べてはるかに高い。

これらの事実は、*S. tuberosum* のもつ 2 組のゲノムが時に対合はなし得ても、その親和力は近縁性二倍種に比べるとかなり弱く、その間には染色体の交換も自由にはゆるされない程度の構造的差異が存在することを示唆する。従て本種を同質四倍体とする見解には同調しがたく、部分的異質四倍体とみるのがより妥当と考える。