

Cytogenetical Study of the Interspecific Hybrid between *Capsicum annuum* and *C. baccatum*¹⁾

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To define the cytogenetical relationship between *Capsicum annuum* and *C. baccatum*, the pattern of chromosome pairing at meiotic metaphase in the F₁ hybrid was analysed. The average frequency was 0.02 for univalents, 8.93 for bivalents, 0.08 for trivalents, 0.72 for quadrivalents, 0.02 for quinquevalents and 0.49 for hexavalents. Occurrence of univalents was very rare. It is, thus, demonstrated that *C. annuum* and *C. baccatum* differ from each other by the presence of at least three reciprocal translocations. From the present results, it is concluded that *C. annuum* and *C. baccatum* share a basically homologous but structurally differentiated genome. The F₁ hybrid was sterile because of such a structural differentiation of chromosomes.

KEY WORDS : *Capsicum annuum*, *C. baccatum*, interspecific hybrid, chromosome pairing, genome, reciprocal translocation.

Introduction

Capsicum peppers (2n=24) originated in the New World. Their evolutionary aspects have been discussed by various investigators mainly on the basis of data from comparative morphology (ESHBAUGH 1964 ; PICKERSGILL 1966 ; YAMAMOTO 1978), karyotype analysis (OHTA 1962 ; SHOPOVA 1966 ; PICKERSGILL 1971), pollen stainability of F₁ hybrids (PICKERSGILL 1971 ; ESHBAUGH 1975 ; YAMAMOTO 1978), quantitative taxonomic study (PICKERSGILL *et al.* 1979) and electrophoretic studies (JENSEN *et al.* 1979 ; McLEOD *et al.* 1979 a, b, 1982, 1983). The pattern of meiotic chromosome pairing in the intra- and inter-specific hybrids of chili peppers, however, has been poorly documented so far. Meiosis of F₁ hybrids was observed only in the hybrids of *C. frutescens* × *C. annuum* (OHTA 1961). The data concerning the behaviour of chromosomes at meiosis in the F₁ hybrids are, thus, too scarce to allow a definition of intra- and inter-specific genome relationships among the *Capsicum* species.

We have already reported the intraspecific chromosomal structural variations in *C. annuum* (EGAWA and TANAKA 1984 b) and the pattern of meiotic chromosome pairing of interspecific hybrids between *C. frutescens* and *C. chinense* and between *C. frutescens* and *C. baccatum* (EGAWA and TANAKA 1984 a). The present paper reports the detailed results of cytogenetical study of the interspecific hybrid between *C. annuum* and *C. baccatum*.

Materials and Methods

The following parental strains are involved in the F₁ hybrid examined cytologically

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in the present study. The strain 4075 of *C. annuum* var. *minimum*, collected in Oaxaca, Mexico by Dr. Yamamoto in 1980 was used as a female parent. This strain belongs to the most common A type group regarding intraspecific chromosome structural variations in *C. annuum* (EGAWA and TANAKA 1984b). The male parent, *C. baccatum* var. *baccatum* (strain No. 3359), was obtained in Nuevo Gal, Dept. Beni, Volivia in 1973 during the ECAL (The Kyoto University Scientific Expedition to Latin America) (TANAKA ed. 1974; YAMAMOTO 1974, 1978). Both strains are wild types and produce small fruits on erect pedicels. These fruits are deciduous at the mature stage.

For the crossing, flowers were emasculated just before the buds opened and immediately pollinated in a glasshouse. Thereafter the flowers were bagged in order to prevent contamination.

The parental strains and F_1 plants were grown and maintained in pots in a glasshouse at the Plant Germ-plasm Institute, Faculty of Agriculture, Kyoto University.

For the cytological examination, young anthers were fixed in acetic alcohol (1:3). Chromosome pairing was observed at the first metaphase (MI) of pollen mother cells (PMCs) using the aceto-carmin squash method. Pollen stainability was assessed by the percentage of well-stained pollen grains by aceto-carmin.

Results

1. Morphology of F_1 hybrid

C. annuum has a pure white corolla and purplish blue anther. *C. baccatum* can be distinguished from *C. annuum* by the presence of greenish spots on the white corolla and a yellow anther. The flower of the F_1 plants has greenish spots on the white corolla characteristic of *C. baccatum* and a purplish blue anther typical of *C. annuum*. The results of the present morphological study of the interspecific hybrid between *C. annuum* and *C. baccatum* are consistent with those obtained by YAMAMOTO (1978).

2. Meiotic chromosome pairing of F_1 hybrid

Chromosome pairing at MI of F_1 (amphihaploid, $2n=24$) was very irregular. Out of 53 cells analysed cytologically, only one cell (1.9%) had the chromosome configuration of 12 bivalents. As listed in Table 1, the configurations of 9 bivalents+1 hexavalent (Fig. 1), 8 bivalents+2 quadrivalents (Fig. 2) and 7 bivalents+1 quadrivalent+1 hexavalent (Fig. 3) were observed in 18 (34.0%), 7 (13.1%) and 8 (15.1%) cells, respectively. Univalents were detected in only one cell. In this cell, a quinquevalent was formed simultaneously (Table 1). It

Table 1. Chromosome pairing at MI in interspecific hybrid of *C. annuum* \times *C. baccatum*

Chromosome pairing						No. of cells observed
I	II	III	IV	V	VI	
	12					1 (1.9%)
	10		1			16 (30.2%)
1	9			1		1 (1.9%)
	9	2				2 (3.8%)
	9				1	18 (34.0%)
	8		2			7 (13.1%)
	7		1		1	8 (15.1%)
total						53 (100.0%)

Note: I, II, III, IV, V and VI denote univalent, bivalent, trivalent, quadrivalent, quinquevalent and hexavalent, respectively.

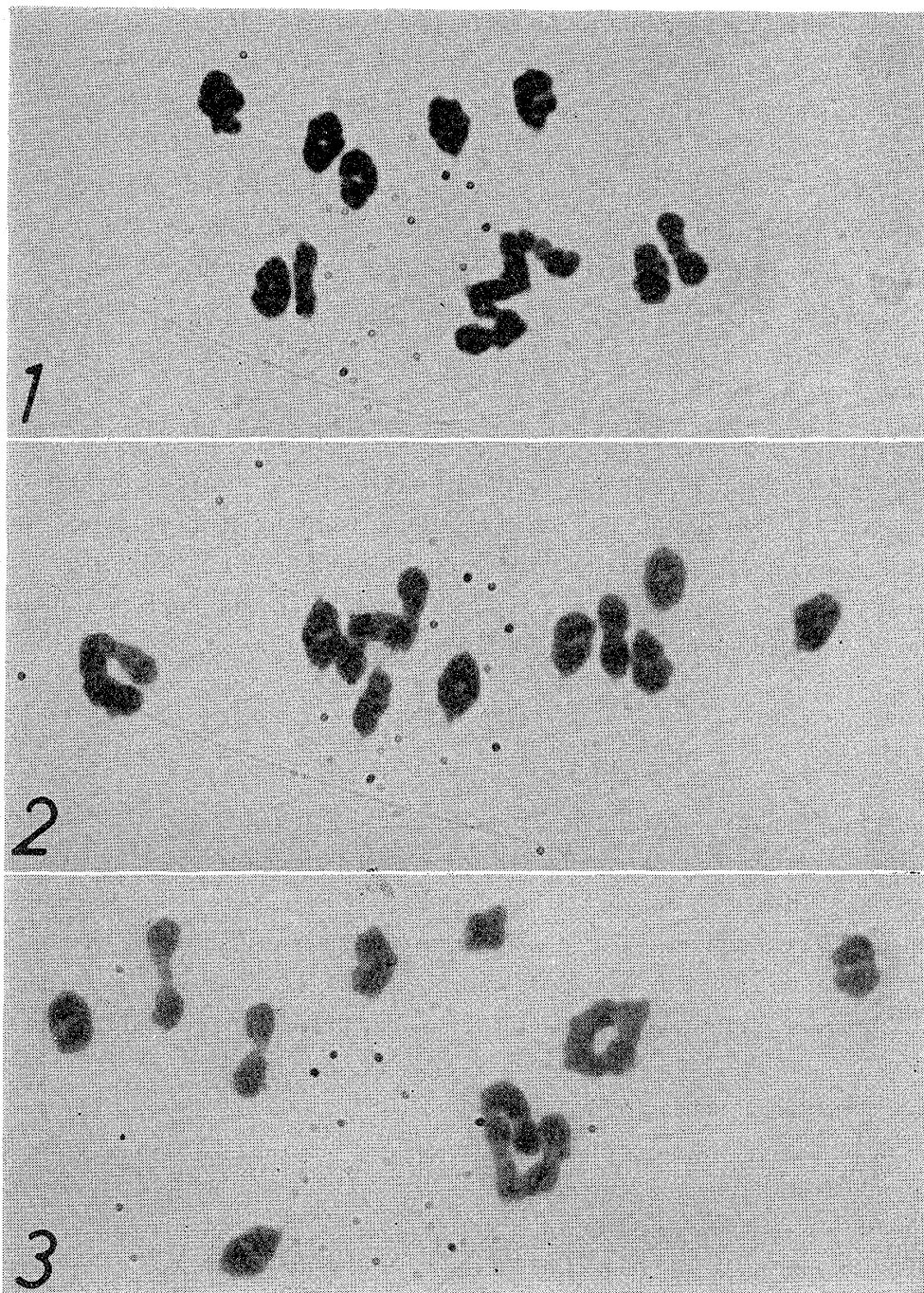


Fig.1-3. Chromosome pairing at MI in interspecific hybrid between *C. annuum* and *C. baccatum*. Fig.1: A chain of hexavalent and nine bivalents. Fig.2: Two chains of quadrivalents and eight bivalents. Fig.3: A ring of hexavalent, a ring of quadrivalent and seven bivalents.

is thus assumed that this univalent occurred due to the failure of chiasma formation in a hexavalent.

Average pattern and range of meiotic chromosome pairing at MI are presented in Table 2. The average frequency was 0.02 for univalents, 8.93 for bivalents (4.25 rings +4.68 rods), 0.08 for trivalents, 0.72 for quadrivalents, 0.02 for quinquevalents and 0.49 for hexavalents.

Table 2. Average frequency and range of chromosome pairing at MI and percentage of pollen stainability in interspecific hybrid of *C. annuum* × *C. baccatum*

No. of cells observed		Chromosome pairing								Pollen stain- ability
		I	II			III	IV	V	VI	
			ring	rod	total					
53	average	0.02	4.25	4.68	8.93	0.08	0.72	0.02	0.49	6.5%
	range	0~1	1~8	2~9	7~12	0~2	0~2	0~1	0~1	

Note : see Table 1.

3. Pollen stainability of F₁ hybrid

The percentage of stainable pollen in this interspecific hybrid was 6.5%, showing a high degree of sterility (Table 2). It was thus evident that reproductive isolation occurs between *C. annuum* and *C. baccatum*.

Discussion

Chromosome pairing at MI in the interspecific hybrid between *C. annuum* and *C. baccatum* was investigated. Meiosis of the F₁ hybrid was very irregular with multivalent formation. As clearly shown in Fig. 3, a quadrivalent and a hexavalent were observed at MI. It was thus demonstrated that *C. annuum* differs from *C. baccatum* by the occurrence of at least three reciprocal translocations. Occurrence of univalents was very rare. The average was 0.02 per cell. These results indicate that *C. annuum* and *C. baccatum* share a structurally differentiated but basically homologous genome.

As presented in Table 3, 32 (84.2%) of 38 quadrivalents examined were oriented in a zigzag manner at MI. It can be expected that the chromosomes involved in such quadrivalent associations are distributed alternately at the first anaphase (AI) so as to produce a balanced genetic constitution. Moreover, out of 26 translocated hexavalents, only 9 (34.6%) were distributed in a zigzag manner (Table 4). Most of the hexavalents, thus, would be expected to segregate adjacently or discordantly at AI and produce genetically unbalanced gametes. The considerable low pollen stainability of the F₁ hybrid (Table 2) which was observed may be ascribed primarily to such a structural differen-

Table 3. The frequency of various quadrivalent configurations detected in *C. annuum* × *C. baccatum*











Configuration			
Ring		Chain	
			
11	3	21	3
total 14		total 24	
Total no. of quadrivalent 38			

Table 4. The frequency of various hexavalent configurations detected in *C. annuum* × *C. baccatum*

Configuration			
Ring			
concordant		discordant	
			
4	5	3	7
total 19			
Chain			
concordant			
			
5	2		
total 7			
Total no. of hexavalent 26			

tiation of chromosomes through reciprocal translocation.

The cytogenetical relationship between *C. annuum* and *C. baccatum* detected in the present study was similar to that between the *C. frutescens*/*C. chinense* complex and *C. baccatum* as reported previously (EGAWA and TANAKA 1984a). The genomes of the *C. frutescens*/*C. chinense* complex and *C. baccatum* are also basically homologous but were differentiated structurally by the existence of at least two reciprocal translocations (EGAWA and TANAKA 1984a).

Considering the results of the present cytological analysis of interspecific hybrid between *C. annuum* and *C. baccatum* and between the *C. frutescens*/*C. chinense* complex and *C. baccatum*, it is strongly suggested that the rearrangements of the chromosome structure by reciprocal translocations played an indispensable role in the species differentiation of chili peppers.

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Capsicum annuum と *C. baccatum* の種間雑種の細胞遺伝学的研究¹⁾

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トウガラシ (*Capsicum* 属, $2n=24$) は, 新世界に起原した植物で, 4 種の栽培種を含み, かつその各々に近縁野生種が存在することが知られている. トウガラシの種内, 種間の類縁関係及び起原に関しては, 交雑可能性, 比較形態学, 雑種の花粉稔性, 核型分析, 電気泳動によるアイソザイム分析等の観点から多くの研究者により論じられてきた. しかし, 雑種の減数分裂における染色体の行動は, 殆んど報告されておらず, トウガラシ各種の種内及び種間のゲノムの相互関係を明らかにするには, この方面の研究からのデータは非常に不足している.

本研究では, *C. annuum* と *C. baccatum* のゲノムの類縁関係を調べるために, 種間雑種の花粉母細胞の減数分裂第一中期における染色体対合を観察した (Fig. 1~3). その結果, $9^{\text{II}}+1^{\text{VI}}$, $8^{\text{II}}+2^{\text{IV}}$ 及び $7^{\text{II}}+1^{\text{IV}}+1^{\text{VI}}$ の対合が細胞学的に調査した花粉母細胞のそれぞれ 34.0%, 13.1% 及び 15.1% に見い出された (Table 1). 染色体対合の平均頻度は, $0.02^{\text{I}}+8.93^{\text{II}}+0.08^{\text{III}}+0.72^{\text{IV}}+0.02^{\text{V}}+0.49^{\text{VI}}$ であった (Table 2). *C. annuum* と *C. baccatum* は, 3つの相互転座により異なることが証明された. また, 1 価染色体の出現頻度は, 平均で細胞当たり 0.02 と極めて低く, *C. annuum* と *C. baccatum* のもつゲノムは, 基本的には相同であると結論された.

雑種の花粉稔性は 6.5% で, 不稔の程度は高く明らかに両種間には生殖的隔離が発達している. この雑種におけるかなりの稔性の低下は, 4 価染色体や 6 価染色体の形成という成熟分裂における異常にその原因を帰することができると思われる (Tables 3 and 4).

本研究で見い出された *C. annuum* と *C. baccatum* 間の細胞遺伝学的関係は, 著者らが以前に報告した *C. frutescens* と *C. baccatum* 間の関係に類似している. この両種も基本的に共通のゲノムを有するが, 少なくとも 2つの相互転座の存在により構造的に分化していた (EGAWA and TANAKA 1984a).

以上の結果を考慮すると, トウガラシの種分化においては, 相互転座による染色体の再配列が, 極めて重要な役割を演じていることが強く示唆される.

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