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MORPHOLOGICAL AND CYTOLOGICAL ANALYSIS
OF AN INTERSPECIFIC HYBRID EGGPLANT,
SOLANUM MELONGENA L. X SOLANUM TORVUM SW.

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Kenneth R. McCammon

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Kenneth R. McCammon

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ABSTRACT

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OF AN INTERSPECIFIC HYBRID EGGPLANT,
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An interspecific hybrid between *S. melongena* L. cv. Millionaire and *S. torvum* Sw. was produced by means of unilateral sexual hybridization. *S. torvum* was used as the pollen parent. The cross was made in an effort to transfer resistance to *Verticillium* wilt (*Verticillium dahliae*) from *S. torvum* into the cultivated species. The progenies were determined to be hybrids based on morphological observations. Attempts to self and backcross the hybrid to the parents were unsuccessful. Observations of the pollen from the F_1 plants indicated low viability. Meiosis in the parents appeared normal. Cytological observations of hybrid PMCs showed gross abnormalities in all stages of meiosis. It appears that the sterility may be due to lack of homology and genic imbalances of the parental genomes. This results in abnormal pairing at metaphase I and altered chromosome distributions at anaphase I and subsequent stages, producing pollen with an abnormal complement of chromosomes.

This thesis is dedicated to my grandmother, Mrs. B. O. LeBlanc, and to my parents, Mr. Frederick V. McCammon, Sr., and the late Joyce L. McCammon.

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INTRODUCTION

Commercial production of eggplant (Solanum melongena L.) in Michigan is limited by a high incidence of Verticillium wilt. This soil borne disease is caused by the fungus Verticillium dahliae Klebahn, (referred to as V. albo-atrum Reinke and Berth. in earlier literature), and results in severe wilting, stunted growth, vascular discoloration, reduced yields, and premature death. This problem is compounded by the lack of genetic resistance within S. melongena, and the absence of chemical or physical methods for the control of Verticillium wilt.

In an effort to solve this problem, Solanum torvum Sw., a wild species of eggplant, was crossed with S. melongena L., in order to transfer its resistance to V. dahliae into commercial eggplant. An interspecific hybrid between the two species has been produced that is sterile. The purpose of this paper is two-fold: (1) to report on the morphological characteristics of the hybrid, and (2) to present a probable explanation of the sterility cytologically.

LITERATURE REVIEW

Burton and deZeeuw (1958) conducted a series of studies to determine the importance of seed transmission of V. albo-atrum as a causative agent in Verticillium infection and determined that the disease was not seed transmitted. Wilhelm (1955) found the pathogen had a wide range of hosts, enabling it to survive in soil for long periods of time. Due to the lack of efficient means of soil treatment to eliminate the pathogen, Lockwood and Markarian (1961) attempted to breed cultivars of eggplant resistant to Verticillium, utilizing germplasm sources within S. melongena L., with limited success.

According to Bhaduri (1951), the genus Solanum contains approximately 2000 species, the majority of which are of the non-tuberiferous type. They are mostly herbs and shrubs, though some may attain the size of small trees. Within the non-tuberiferous types are two types, with and without spines on the leaves and stems.

Yamakawa, et al (1978) screened a number of species related to S. melongena for resistance to Verticillium and other diseases which attack eggplant. They found Solanum torvum Sw. to be resistant not only to Verticillium wilt, but Fusarium and Pseudomonas wilts as well. Both of these

Solanum species are spined.

Rai (1959) reported that S. melongena L. originated in either India or tropical Africa, and is cultivated throughout the warmer regions of the globe. According to Rao (1972) and Deb (1979), S. torvum Sw. is indigenous to India and southeast Asia, but extends through Malaysia and the Phillipines, to Australia, Africa and tropical America.

The general morphological features of S. melongena L. have been elucidated by Khan (1979), while S. torvum Sw. was described morphologically by Hossain (1972). A review of cytogenetic studies in the genus Solanum was conducted by Magoon, et al (1961), in which they reported a chromosome number of n=12 for both species. Two previous attempts to hybridize these two species appear in the literature. The first, reported by Pearce (1975), involved the crossability of S. melongena L. with related species. He was able to produce healthy F_1 progeny from a cross between S. melongena and S. torvum, however, they were sterile and exhibited few stainable pollen. Yamakawa, et al (1978) also produced a hybrid between these species, but neither F_2 nor backcross progenies were obtained. To the author's knowledge, the morphological characteristics of the hybrid or the cytological bases for their sterility have not been reported.

Efforts to hybridize S. melongena and other related species appear in the literature. Tatebe (1936) made a cross between S. integrifolium Poir. and S. melongena L., in which a hybrid was only produced when S. melongena was used

as the pollen parent. The hybrid showed a closer resemblance to S. integrifolium than to S. melongena, and was sterile. Although cytological observations suggested that meiosis was normal through the tetrad stage, shortly after liberation of the microspores from the tetrad, they were observed to disintegrate, resulting in inviable pollen production.

Khan, et al (1978) produced a semi-fertile hybrid with S. integrifolium, using S. melongena L. var. Pusa Purple Long as the female parent. Meiosis appeared normal, though various meiotic abnormalities were noted in about 15% of the pollen mother cells (PMCs). They concluded that the two genomes had been differentiated by cryptic structural changes of the chromosomes.

In a study involving the hybridization of S. melongena L. and S. cumingii Dunal, Campinpin, et al (1963) were able to produce a fertile hybrid. Cytological observations indicated that there was considerable similarity between the two parental genomes. In the F_1, pairing was found to be normal, and the percentage of viable pollen was approximately the same as that of the parents.

In an effort to incorporate resistance to Verticillium wilt into S. melongena, Nasrallah and Hopp (1963) made crosses with S. gilo and S. indicum. Reciprocal F_1's were produced, and all F_1 plants were highly sterile. The hybrid S. melongena X S. gilo could be backcrossed to S. gilo, but not to S. melongena. In this hybrid, pairing appeared to be

normal at metaphase I, however, irregular chromosomal distributions as well as the formation of bridges were noted in anaphase I.

Rajasekaran (1970) conducted cytogenetic analyses of the F_1 hybrid S. indicum X S. melongena L. and its amphidiploid. The cross was only successful when S. indicum was used as the pistillate parent. The hybrid exhibited normal meiosis, although it was semi-sterile, with 49% stainable pollen. They were able to produce a fertile amphidiploid and concluded that the sterility was due to small segmental differences existing in the chromosomes of the parental genomes.

Cytological studies were also conducted by Rajasekaran (1971), on the F_1 hybrid S. xanthocarpum Schrad. and Wendl. X S. melongena L. and its colchicine derived amphidiploid. The hybrid was produced only by using S. xanthocarpum as the female parent. The resulting F_1 was sterile, despite normal meiosis. Fertility was restored in the amphidiploid, suggesting cryptic structural hybridity.

A similar study by Rajasekaran (1971) utilized S. melongena var. insanum Prain. A hybrid was produced only when S. xanthocarpum was used as the female parent. The resulting hybrid was sterile, and efforts at selfing and backcrossing to either parent were unsuccessful. The most common chromosomal association noted at metaphase I involved the formation of 10 bivalents and 1 quadrivalent. Only about one-third of the cells observed exhibited normal

pairing of 12 bivalents at metaphase I. Subsequent stages appeared normal. The pairing in the hybrid indicated a close affinity between the two parental genomes, and the differences appeared to be due to segmental interchange, as indicated by the formation of a quadrivalent at metaphase I, followed by normal anaphase I segregation.

The breeding behavior of S. zuccagnianum Dun. with S. melongena L. was reported by Rajasekaran and Sivasubramanian (1971) using S. zuccagnianum as the female parent. As in previous studies, the reciprocal cross was unsuccessful. The hybrid was sterile, and backcrosses to the parents were unsuccessful. Examination of the pollen indicated sterility. Metaphase I associations of 10 bivalents and 1 quadrivalent were most commonly observed, with a small percentage of cells showing 12 bivalents. Subsequent stages generally appeared normal. The parental genomes appeared to possess some homology, and the sterility was explained on the basis of segmental interchanges and small cryptic differences of the chromosomes.

Solanum macrocarpon L. was used as the pollen parent and was crossed with two varieties of S. melongena L. indigenous to India, by Wanjari (1975). While most PMCs showed regular bivalent formation at metaphase I, some univalents and multivalent formations were noted, with corresponding irregularities in subsequent stages. Sterility was postulated as due to cryptic structural differences between the genomes.

Schaff, et al (1980) also utilized S. macrocarpon in interspecific hybridizations with eleven genotypes of S. melongena. When S. melongena was used as the female parent, five of the eleven combinations of crosses set fruit. Using S. macrocarpon as the female, nine of the eleven combinations were successful. The fertility of the F_1 progenies was variable, ranging from complete absence of flowers to the production of fruits with few seeds.

Rao, et al (1979) conducted a study to determine the inter-relationship between S. melongena L. and S. hispidum Pers. They utilized an Indian cultivar of S. melongena, var. Pusa Purple Long, as the female parent. The reciprocal cross was unsuccessful. The resulting hybrid was highly sterile, with less than 2% pollen fertility. Hybrid meiosis was irregular. Few bivalents and large numbers of univalents were noted. The chromosomes appeared to be very lossely paired in the bivalents. Anaphase I exhibited erratic behavior of the univalents with varying numbers of laggards. Delayed separation of the bivalents, as well as bridges and fragments, were also noted in anaphase I. Micronuclei were observed in both telophase I and II, with most sporads containing multiple clumped and degenerating pollen grains.

Based on the meiotic abnormalities observed, the parental genomes were considered dissimilar. The high frequency of univalents, coupled with loose associations of the bivalents, suggest that the differences in the parental

genomes were due to structural changes within the chromosomes which may have occured during the evolution and differentiation of the species.

Rao (1980) obtained similar results from the hybridization of S. melongena L. var. Baromishi X S. hispidum Pers. The resulting hybrid was sterile with 1.4% pollen viability. Hybrid meiosis was irregular, and anomalies were observed at every stage. He concluded that structural changes of the chromosomes, coupled with disharmonious interaction of the parental genes were responsible for the sterility of the hybrid.

Attavian, et al (1980) reported the crossability of several Solanum species and classified the crosses into three groups: A, species reciprocally crossable (S. gilo Raddi X S. integrifolium Poir.; S. nodiflorum Jacq. X S. integrifolium), capable of producing fully fertile F_1 plants, B, species unilaterally crossable (S. indicum L. X S. incanum L.; S. gilo X S. nodiflorum), and C, species reciprocally noncrossable (S. gilo X S. indicum). Several varieties of S. melongena L. were used to investigate the possibility of gene transfer between species as a method of varietal improvement, and they found that many species of Solanum are crossable inter se.

MATERIALS AND METHODS

The parents utilized in the hybridization were S. melongena L. cv. Millionaire, (Takii Seed Company of Kyoto, Japan), and a selection of S. torvum Sw., supplied by Professor K. Yamakawa of the Vegetable and Ornamental Crops Research Station, Tsu-City, Japan.

Morphological Study

S. melongena L. is an erect, branched shrub, about one meter tall, with spines confined to the flower pedicel and calyx. The leaves are large, oblong-ovate, shallowly sinuate-lobed, nearly glabrous above but densely tomentose beneath (Khan, 1979). In S. melongena L. cv. Millionaire, the flowers are large, solitary, or occasionally, in clusters of 2 to 3. The calyx is spiny, purple, deeply lobed and persistent. The corolla is about 3.7 cm in diameter. The fruit is a large elongate berry, an average of 20 cm long and 4.5 cm in diameter, dark purple in color.

Hossain (1973) reports that S. torvum Sw. is usually 2 to 3 meters tall, a much branched and moderately spined shrub, with simply lobed to lobate-sinuate leaves. Its inflorescences represent a form of a cyme. The calyx is deeply five-lobed, white and deciduous. The fruit is a smooth, spherical berry, 10 to 13 mm in diameter, green when young, yellowish green to yellow when ripe.

Plants of each species were grown in the greenhouse. After anthesis of the initial flowers, bud pollinations were made reciprocally by emasculating the female parents, followed by hand pollination. Repeated pollinations were made for each cross, and were successful only when S. melongena was used as the female parent. The fruit, upon harvest, yielded very few seeds. The seeds, when grown, produced both selfed and hybrid seedlings.

The F_1s were identified from the selfs by several morphological characters. The following hybrid and parental information were recorded: flower size and color, fruit size, shape and color, leaf width, length and margin type, amount and location of spines, number of flowers per inflorescence, and the presence of stem anthocyanin. Data on flower size, fruit size, leaf width, leaf length, and flowers per inflorescence were obtained by averaging 10 measurements per character. All other data were based on visual observations.

Cytological Study

In order to determine the proper stage for the collection of flower buds for cytological analysis, samples were collected from greenhouse grown plants of both parents and the hybrid, at two hour intervals, beginning at 9 AM and ending at 5 PM. Cytological observations indicated that buds collected between 9 and 11 AM and 3 and 5 PM, were most suited for analysis. However, buds collected at 10 PM yielded better cells for observing metaphase I chromosomes

for the hybrid.

Buds were collected and fixed in a 1:3 acetic acid-absolute alcohol solution. After 24-48 hours, they were transferred to 70% alcohol and stored in a refrigerator until used. In preparation for analysis, buds were hydrolyzed in 1N HCl for 6 minutes. After hydrolysis, buds between 8 and 10 mm long, with anthers 4 to 5 mm long, were selected and the anthers removed and smeared in 1% acetocarmine. All observations were made on pollen mother cells. Photomicrographs were made to record the meiotic process for both parents.

For the hybrid, data on meiotic abnormalities were recorded at diakinesis, metaphase I and II, anaphase I and II, and the tetrad stage. Photomicrographs were made to record the anomalies at the various meiotic stages. The presence of micronuclei was determined by observing the tetrad stage. Pollen viability estimates were based upon the percent of pollen stainable with potassium iodide (I_2KI). 1000 pollen grains were counted for the parents and the hybrid and those stained darkly were considered viable.

For the study of hybrid mitosis, root tips were collected from actively growing roots and pretreated in 0.02% orthodichlorobenzene (ODB) for 45 minutes. After pretreatment they were fixed in a 1:3 acetic acid-absolute alcohol solution for 24 hours, hydrolyzed in 1N HCl for 10 minutes, and smeared in 1% acetocarmine.

RESULTS

Morphological observations of the parents and the F_1 hybrid are summarized in Table 1. Leaf length, leaf width and number of flowers per inflorescence were greater for S. torvum than for S. melongena, while flower diameter and fruit size were larger for S. melongena than for S. torvum. Due to sterility, the fruiting characteristics of the hybrid are not available.

The hybrid plant more closely resembled S. torvum in flower diameter, spininess, leaf margin, inflorescence type and flowers per inflorescence. The lavender colored flowers resembled those of S. melongena. The leaves of the F_1 exhibited the presence of anthocyanin as in S. melongena, but were spined and deeply lobed as in S. torvum (Figure 1). All other traits were intermediate between the parents.

Cytological Study

The parents and the F_1 had 2n=24 chromosomes. No mitotic irregularities were noted in the hybrid. Meiosis in the parents appeared normal, with 12 bivalents at metaphase I, followed by normal meiosis in subsequent stages (Figure 2). The pollen appeared well developed and exhibited a viability of 87.5% for S. melongena, and 96.9% for S. torvum.

The size of the hybrid pollen varied greatly, viability

TABLE 1. Comparison of certain morphological characteristics of the parents and F_1 hybrid.

Characteristic	*S. melongena*	*S. torvum*	F_1 hybrid
Leaf Size (cm)			
Length	17.4 ± .41	20.8 ± .45	18.8 ± .42
Width	10.9 ± .33	20.4 ± .45	15.7 ± .40
Leaf Margin	sinuate-lobed	deeply lobed	deeply lobed
Flower Diameter (cm)	3.7 ± .19	2.1 ± .14	2.4 ± .15
Flower Color	lavender	white	lavender
Fruit Shape	elongate	globular	-
Fruit Size (cm)			
Length	20.0 ± .45	1.2 ± .11	-
Width	4.5 ± .21	1.0 ± .10	-
Fruit Color	dark purple	green	-
Spine Location	calyx and pedicel	stems, leaves and petioles	stems, leaves and petioles
Inflorescence Type	simple	corymbose cyme	corymbose cyme
Flowers/Inflorescence	1.1 ± .10	47.9 ± .69	21.3 ± .46
Anthocyanin	present	absent	present

Figure 1. Morphology of leaves and inflorescences of S. melongena, S. torvum and their F_1 hybrid.

A. Leaf morphology. Left: S. melongena; Right: S. torvum; Center: F_1 hybrid (.20X).

B. Individual flowers. Left: S. melongena; Right: S. torvum; Center: F_1 hybrid (.75X).

C. Inflorescnece types. Left: S. melongena; Right: S. torvum; Center: F_1 hybrid (.60X).

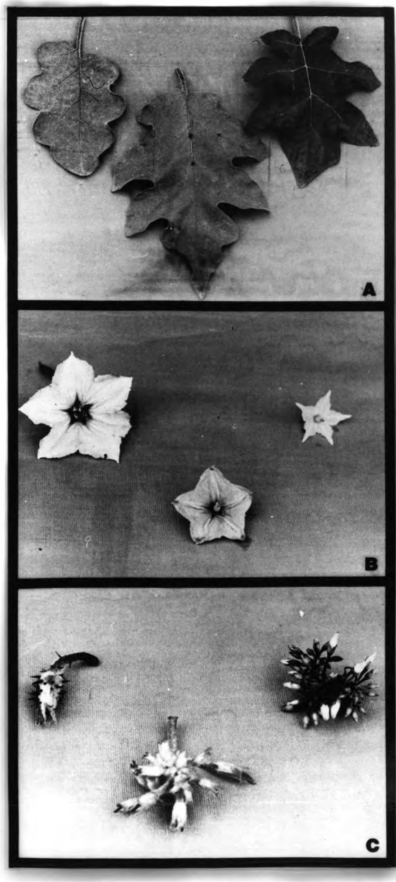

A
B
C

Figure 2. Meiosis in *S. melongena* and *S. torvum*.

A. Diakinesis in *S. melongena* showing 12 bivalents (1050X).

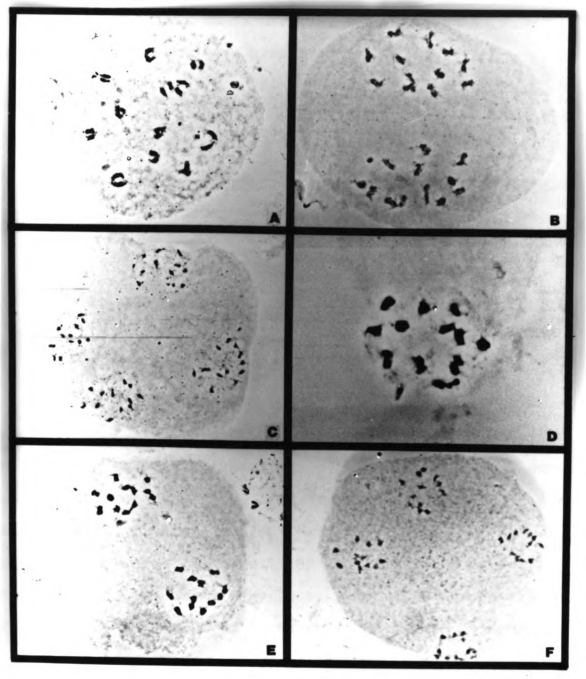
B. Anaphase I in *S. melongena* (1155X).

C. Anaphase II in *S. melongena* (975X).

D. Metaphase I in *S. torvum* showing 12 bivalents (2575X).

E. Anaphase I in *S. torvum* (1050X).

F. Anaphase II in *S. torvum* (935X).


A
B
C
D
E
F

was estimated at 1.7%. Meiosis in the hybrid was highly irregular. The data on chromosomal associations at diakinesis of the hybrid are presented in Table 2.

TABLE 2. Chromosome associations at diakinesis in *S. melongena* X *S. torvum*.

Association			Cells Observed	
Univalents	Bivalents		No.	%
8	8		3	11.1
12	6		3	11.1
16	4		13	48.2
20	2		8	29.6
$\bar{x}$ 15.85	4.07	Total	27	100.0

A total of 27 PMCs were analyzed at diakinesis, in which the formation of 4 bivalents and 16 univalents was observed in 48.2% of the cells. Complete pairing of all of the chromosomes was not observed. A maximum of 8 bivalents was observed in 11.1% of the cells. Mean numbers of univalents and bivalents observed were 15.85 and 4.07, respectively. The bivalents exhibited very loose pairing, characterized by wide separation with a thin strand of chromatin joining the two chromosomes (Figure 3A).

Data on chromosome associations at metaphase I of the hybrid are presented in Table 3. A total of 75 PMCs were observed. A maximum of 4 bivalents were observed in 28% of the cells, while 29.3% of the cells showed 24 univalents (Figure 3B). The mean number of univalents and bivalents

Figure 3. Meiosis of S. melongena X S. torvum hybrid.

A. Diakinesis with loosely paired bivalents and many univalents (915X).

B. Metaphase I showing 24 univalents (1045X).

C. Anaphase I with 15/9 disjunction (1125X).

D. Anaphase I with 15/8 disjunction and 1 lagging chromosome (arrow) (1340X).

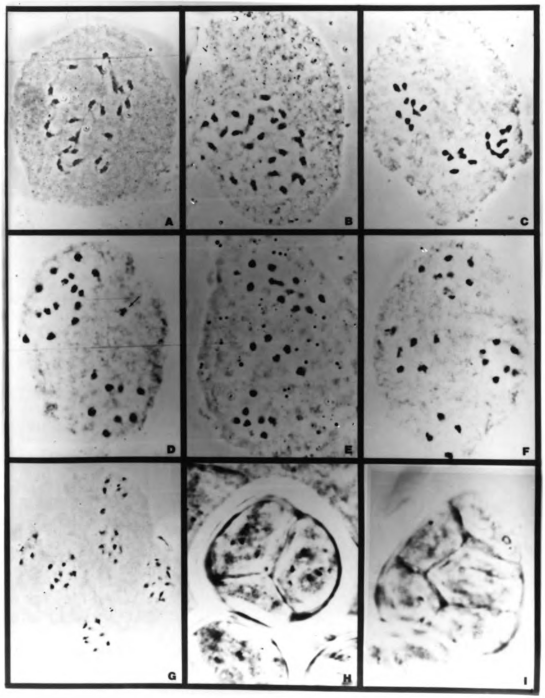
E. Anaphase I with 12/7 disjunction and 5 lagging chromosomes (1105X).

F. Anaphase I with quadripolar distribution of chromosomes (1315X).

G. Anaphase II with hexapolar distribution of chromosomes (950X).

H. Sporad stage showing 2 large cells and 1 small cell (1560X).

I. Sporad stage showing a pentad (1670X).


A
B
C
D
E
F
G
H
I

for all of the cells observed, were 19.71 and 2.15, respectively.

TABLE 3. Chromosome associations at metaphase I in S. melongena X S. torvum.

Association			Cells Observed	
Univalents	Bivalents		No.	%
16	4		21	28.0
18	3		17	22.7
20	2		11	14.7
22	1		4	5.3
24	-		22	29.3
$\bar{x}$ 19.71	2.15	Total	75	100.0

A total of 143 PMCs were observed at anaphase I, and both dipolar and multipolar chromosome associations were noted. Data on anaphase I dipolar distributions and the occurence of lagging chromosomes are presented in Table 4. Segregation of 12 chromosomes to each pole was noted in 8.4% of the cells, while 14.7% showed a 14-10 distribution, which was followed by 13-11 in 12.6% and 15-9 in 11.2% of the cells (Figure 3C). The number of lagging chromosomes varied from 1 to 6 (Figure 3D,E).

Spindle abnormalities were observed occasionally, probably due to split or multiple spindles, which resulted in multipolar distributions. Data for multipolar distributions at anaphase I are presented in Table 5. Seven percent of the PMCs observed showed these anomalies, with 5.6% showing tripolar segregation and 1.4% with quadripolar segregation

TABLE 4. Frequency of laggards and dipolar distributions at anaphase I in S. melongena X S. torvum.

Distribution	Number of Laggards	Cells Observed	
		No.	%
12-12	-	12	8.4
18- 6	-	3	2.1
16- 6	2	1	0.7
16- 7	1	1	0.7
16- 8	-	8	5.6
15- 9	-	16	11.2
15- 8	1	4	2.8
14-10	-	21	14.7
14- 9	1	4	2.8
14- 8	2	3	2.1
13-11	-	18	12.6
13-10	1	4	2.8
13- 9	2	6	4.2
12-11	1	7	4.9
12-10	2	10	7.0
12- 7	5	4	2.8
11-11	2	5	3.5
11-10	3	3	2.1
11- 9	4	1	0.7
9- 9	6	2	1.4
	Total	133	93.0

TABLE 5. Frequency of multipolar chromosome distributions at anaphase I in S. melongena X S. torvum.

Distribution	Cells Observed	
	No.	%
Tripolar		
14-6-4	2	1.4
14-5-5	1	0.7
12-6-6	1	0.7
10-9-5	2	1.4
10-7-7	2	1.4
Quadripolar		
11-6-6-1	1	0.7
7-7-6-4	1	0.7
Total	10	7.0

TABLE 6. Frequency of chromosome distribution at metaphase II in S. melongena X S. torvum.

Number of bivalents on metaphase plates	Cells Observed	
	No.	%
12-12	3	20.0
13-11	4	26.7
14-10	4	26.7
15- 9	2	13.3
12-10-2	2	13.3
Total	15	100.0

(Figure 3F).

The frequency of chromosome distribution observed at the second metaphase was determined from observing 15 PMCs (Table 6). Unequal distribution of chromosomes at metaphase II was probably due to the unequal distributions noted in anaphase I. The most frequent distribution types were 13-11 and 14-10, each occuring in 26.7% of the cells, followed by a normal 12-12 distribution in 20.0% of the cells.

Quadripolar and multipolar distributions of the chromosomes were noted when 57 PMCs were observed at anaphase II. Data on the frequency of quadripolar chromosome distribution at anaphase II are presented in Table 7. Segregation of 12 chromosomes to each of the poles was observed in 7.0% of the cells. Seven percent of the cells also showed distributions of 14-14-10-10 and 15-15-9-9, while 5.3% showed distributions of 13-13-11-11 and 14-12-11-11. Multipolar distributions were observed in 26.25% of the cells (Table 8, Figure 3G).

Microspore formation at the tetrad stage is presented in Table 9. Examination of 69 sporads showed that the number of microspores formed from each microsporocyte was irregular, with 4.3% forming single cells, 18.8% dyads, and 18.8% triads (Figure 3H). Only 15.9% of the cells appeared to have normal tetrad formation. The remaining sporads contained 5 or 6 microspores (Figure 3I). Micronuclei formation varied from 1 to 4, with 37.7% of the cells producing 1 micronuclei and 18.9% producing two. A maximum of 4 micronuclei per cell

TABLE 7. Frequency of quadripolar chromosome distribution at anaphase II in S. melongena X S. torvum.

Distribution	Cells Observed	
	No.	%
12-12-12-12	4	7.0
20-20- 4- 4	1	1.75
20-16- 9- 3	1	1.75
18-12-12- 6	1	1.75
17-17- 7- 7	1	1.75
17-12- 8- 7	1	1.75
16-16- 8- 8	2	3.5
16-15- 9- 8	2	3.5
16-14-11- 7	1	1.75
16-14- 9- 9	2	3.5
16-13-10- 9	2	3.5
16-12-12-10	1	1.75
15-15- 9- 9	4	7.0
15-12-11-10	1	1.75
14-14-10-10	4	7.0
14-13-12- 9	1	1.75
14-13-11-10	1	1.75
14-12-12-10	2	3.5
14-12-11-11	3	5.3
13-13-12-10	1	1.75
13-13-11-11	3	5.3
13-12-12-11	2	3.5
Total	42	73.75

TABLE 8. Frequency of multipolar chromosome distributions at anaphase II in S. melongena X S. torvum.

Distribution	Cells Observed	
	No.	%
Pentapolar		
15-13- 9-7-2	1	1.75
14-11-10-8-5	1	1.75
14-11- 9-9-5	1	1.75
13-12-11-8-4	1	1.75
13-12- 8-8-7	1	1.75
11-10-10-9-8	1	1.75
Hexapolar		
19-17-4-4-2-2	1	1.75
14-12-8-8-3-3	1	1.75
13-13-7-6-5-4	1	1.75
13-11-9-5-5-5	1	1.75
12-12-8-7-5-4	1	1.75
10-10-9-8-6-5	1	1.75
9- 9-9-9-6-6	1	1.75
9- 9-8-8-8-6	1	1.75
Heptipolar		
11-11-8-5-5-5-3	1	1.75
Total	15	26.25

were observed in 2.9% of the cells.

TABLE 9. Microspore formation at the "tetrad" stage in *S. melongena* X *S. torvum*.

Cells/"Tetrad"	Cell Size		Cells Observed	
	Large	Small	No.	%
1	1	-	3	4.3
2	2	-	13	18.8
3	1	2	2	2.9
3	2	1	11	15.9
4	2	2	4	5.8
4	3	1	1	1.5
4	4	-	11	15.9
5	5	-	1	1.5
5	3	2	2	2.9
5	4	1	14	20.3
6	2	4	2	2.9
6	4	2	5	7.3
		Total	69	100.0

Determination of the presence of micronuclei was by visual observation, with no assurance that a "normal" appearing cell contained the proper chromosomal complement to insure fertility in the mature pollen.

DISCUSSION

The hybridity of the interspecific F_1 from the cross of S. melongena X S. torvum was determined on the basis of morphological characteristics and from cytological observation of the meiotic abnormalities exhibited by the hybrid.

The "loose" associations observed at diakinesis and metaphase I may be due to asynapsis, a failure of the chromosomes to pair at prophase, or desynapsis, a premature separation of bivalents prior to these stages. Univalents were observed in all of the cases. Short chromosomes have been reported to have a lower chiasma frequency than long chromosomes, and thus a tendency towards early separation (Kostoff, 1940). Short chromosomes are characteristic of many Solanum species (Swaminathan, et al, 1954). The low chiasma frequency in the hybrid chromosomes may have contributed to the "loose" associations and occurence of univalents at diakinesis and metaphase I.

Lack of homology between the two genomes may also be a cause of univalent formation. The degree of homology between the chromosomes of two species is generally considered as an indication of the evolutionary relationship between them. However, pairing appears to be based upon a balance between chromosome development and the stage of meiotic development. Rao, et al, (1979) suggest that these

timing relationships may be controlled in such a manner that slight changes in genetic constitution and genic balances may inhibit pairing of chromosomes which may otherwise possess enough homology to pair.

Bivalents resulting from allosyndetic pairing between chromosomes derived from the two parents, would suggest that the parental chromosomes possess homologous segments. However, the occurence of 24 univalents in some of the PMCs, coupled with the "loose" associations of bivalents in the other cells, would suggest that the species may have differentiated by structural changes of the chromosomes.

Based on observations at anaphase I, the aberrant behavior of the chromosomes would probably not allow for the development of viable pollen grains. The abnormal segregations noted in anaphase I was a continuation of the failure of the chromosomes to pair at metaphase I. When bivalent formation is limited, the resulting univalents are scattered throughout the cell. As the bivalents begin to divide, the univalents either move to the equatorial plate, or gather about the pole nearest their former position (Li, et al, 1945). The univalents which fail to reach the equatorial plate are seen to lag behind at anaphase I, and depending upon their proximity to the poles, are either included in the daughter nuclei, or are lost in the cytoplasm. These excluded chromosomes may, in part, be the micronuclei observed in the tetrads of the hybrid.

The anomalies observed in anaphase I were present in

the subsequent stages of meiosis. Abnormal chromosome distributions at anaphase II, and the occurence of multipolar meiosis at both anaphase I and II, may result in the production of gametes with unbalanced chromosome complements. According to Tai (1970), non-homology between genome-specific spindle organizers can result in multipolar meiosis and each genome possesses a genome-specific spindle organizer. During fertilization, the male spindle organizer enters the egg cell and either the spindle organizers fuse, or one degenerates in favor of the other.

The behavior of the chromosomes may be due to an interaction of chromosome homology and the homology between chromosomes and their spindle organizers. Thus, if either the male or female spindle organizer disintegrates, the affinity between the spindle organizer of one species and the chromosomes of another may be limited or non-existent. In such a case, chromosomes released from the control of their spindle organizer will move randomly within the cell, and may be observed as laggards. In this species hybrid, both male and female spindle organizers may be present, causing the separation of genomes into different groups by means of multipolar meiosis.

The abnormal microspore formation observed at the tetrad stage of the hybrid was most likely due to multipolar meiosis occuring in the PMCs. Cytokinesis appears to result from an interaction of spindle organizers, such that whenever an organizer is present, cytokinesis occurs, cleaving

the cytoplasm and maintaining a constant ratio of one spindle organizer per cell (Tai, 1970). As the number of spindle organizers increases, so does the number of cytokineses, resulting in the formation of more microcells. Cells containing more than four microspores would arise from this process.

Cells with only one or two microspores were observed in the hybrid. Apparently in these cells, normal nuclear envelope formation and cytokinesis were hindered by multipolar divisions and unpaired chromosomes, resulting in the formation of one or two microspores. According to Machado (1978), this is a common occurence in interspecific hybrids. The formation of a restriction nucleus, which results from the inability to separate the chromosomes into individual nuclei, can result in a polyploid nucleus which would exhibit normal meiosis and give rise to a viable gamete.

Although meiotic irregularities appear to be responsible for the sterility of this hybrid, it is very difficult to distinguish between genic and chromosomal sterility cytologically (Stebbins, 1958). Thus, genic imbalances and lack of homology of the parental chromosomes may be acting jointly to produce sterility in this hybrid.

BIBLIOGRAPHY

1. Attavian, B. N., B. L. Pollack and G. Jelenkovic. 1980. Crossability of selected Solanum species. Abstr. 341. J. Amer. Soc. Hort. Sci. 15:3:2:87.

2. Bhaduri, P. N. 1951. Inter-relationship of non-tuberiferous species of Solanum with some consideration on the origin of brinjal (Solanum melongena L.). Indian J. Genet. and Plant Breeding. 11:75-82.

3. Burton, C. L. and D. J. deZeeuw. 1958. Studies on transmission of Verticillium wilt of eggplant in Michigan. Plant Dis. Reporter. 42:4:427-436.

4. Campinpin, J. M., M. Lande and J. V. Pancho. 1963. Cytogenetics of interspecific hybrid between Solanum melongena Linn. and S. cumingii Dunal. Phillipine J. Sci. 92:169-178.

5. Deb, D. B. 1979. Solanaceae in India. In, Eds. J. G. Hawkes, R. N. Lester, A. D. Skelding. The biology and taxonomy of the Solanaceae. Linn. Soc. Symp. Ser. No. 7, Linnean Society of London. Academic Press.

6. Hossain, M. 1973. Observations on stylar heteromorphism in Solanum torvum Sw. (Solanaceae). Bot. J. of the Linnean Society. 66:291-301.

7. Khan, R., G. G. Rao and S. Baksh. 1978. Cytogenetics of Solanum integrifolium and its possible use in eggplant breeding. Indian J. Genet. and Plant Breeding. 38(3):343-346.

8. Khan, R. 1979. History of Solanum melongena L. In, Eds. J. G. Hawkes, R. N. Lester, A. D. Skelding. The biology and taxonomy of the Solanaceae. Linn. Soc. Symp. Ser. No. 7, Linnean Society of London. Academic Press.

9. Kostoff, D. 1940. Fertility and chromosome length. J. of Hered. 31:33-34.

10. Li, H. W., W. K. Pao and C. H. Li. 1945. Desynapsis in the common wheat. Amer. J. Bot. 32:92-101.

11. Lockwood, J. L. and D. Markarian. 1961. Breeding eggplants for resistance to Verticillium wilt. Mich. Agr. Exp. Sta. Quart. Bull. 44:1:15-20.

12. Machado, M. 1978. Cytogenetic analysis of the interspecific hybrid, Vigna radiata X V. umbellata. MS Thesis. Michigan State University.

13. Maggoon, M. L., S. Ramanujam and D. C. Cooper. 1962. Cytogenetical studies in relation to the origin and differentiation of species in the genus Solanum L. Carylogia. 15:1:151-252.

14. Nasrallah, M. E. and R. J. Hopp. 1963. Interspecific crosses between Solanum melongena L. (Eggplant) and related Solanum species. Proc. Amer. Soc. Hort. Sci. 83:571-574.

15. Pearce, K. G. 1975. Solanum melongena L. and related species. Ph. D. Thesis. University of Birmingham, U. K., cited in, Eds. J. G. Hawkes, R. N. Lester, A. D. Skelding. The biology and taxonomy of the Solanaceae. pg. 625. Linn. Soc. Symp. Ser. No. 7. Linnean Society of London. Academic Press.

16. Rai, U. K. 1959. Cytogenetic studies in Solanum melongena L. I: Chromosome morphology. Carylogia. 12:2:299-315.

17. Rajasekaran, S. 1970. Cytogenetic studies of the F_1 hybrid Solanum indicum L. X S. melongena L. and its amphidiploid. Euphytica. 19:217-224.

18. _____. 1971. Cytological studies on the F_1 hybrid (Solanum xanthocarpum Schrad. and Wendl. X S. melongena L.) and its amphidiploid. Carylogia. 24:3: 261-267.

19. _____. 1971. A cyto-morphological study of the F_1 hybrid, Solanum xanthocarpum Schrad. and Wendl. X S. melongena var. insanum Prain. Madras Agr. 58: 308-309.

20. _____ and V. Sivasubramanian. 1971. Cytology of the F_1 hybrid of Solanum zuccagnianum Dun. X S. melongena L. Theor. and Appl. Genet. 41:85-86.

21. Rao, B. G. S. 1972. Morphology of the pachytene chromosomes of Solanum torvum. Econ. Bot. 26:2:189-196,

22. Rao, G. R., R. Khan and S. Baksh. 1979. Inter-relationship between Solanum melongena and Solanum hispidum. Indian J. Genet. and Plant Breeding. 39:3:446-450.

23. Rao, G. R. 1980. Cytogenetic relationshop and barriers to gene exchange between Solanum melongena L. and Solanum hispidum Pers. Carylogia. 33:429-433.

24. Schaff, D. A., C. H. Boyer and B. L. Pollack. 1980. Interspecific hybridization of Solanum melongena L. X S. macrocarpon L. Abstr. 342. J. Amer. Soc. Hort. Sci. 15:3:2:87.

25. Stebbins, G. L. 1958. Hybrid inviability, weakness, and sterility of interspecific hybrids. Advances in Genet. 9:147-215.

26. Swaminathan, M. S., M. L. Magoon and K. L. Mehra. 1954. A simple propionic carmine PMC smear method for plants with small chromosomes. Indian J. Genet. and Plant Breeding. 14:87-88.

27. Tai, W. 1970. Multipolar meiosis in diploid crested wheatgrass, Agropyron cristatum. Amer. J. Bot. 57(10): 1160-1169.

28. Tatebe, T. 1936. Genetic and cytological studies on the F_1 hybrid of scarlet or tomato eggplant (S. integrifolium Poir.) X eggplant (S. melongena L.). Bot. Mag. (Tokyo). 50:457-462.

29. Wanjari, K. B. 1975. Cytogenetic studies on F_1 hybrids between Solanum melongena L. and S. macrocarpon L. Hort. Res. 15:77-82.

30. Wilhelm, S. 1955. Longevity of Verticillium wilt fungus in the laboratory and the field. (Abstr.). Phytopathology. 45:180-181.

31. Yamakawa, K. and H. Mochizuki. 1978. Studies on the use of disease resistance of wild non-tuberous Solanum species in the breeding of eggplants. Abstr. 1674. 20th Int. Hort. Congr. Sydney, Australia.