CYTOGENETICS OF INTERSPECIFIC HYBRIDS, *LYCOPERSICON ESCULENTUM* MILL. × *L. HIRSUTUM* HUMB. AND BONPL.¹

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THE genus *Lycopersicon* has been divided by MULLER (1940) into two subgenera:

- a. *Eulycopersicon*, to which *L. esculentum* Mill. (cultivated tomato) and *L. pimpinellifolium* Mill. (currant tomato) belong. These species are red-fruited and lack pseudostipules.
- b. *Eriopersicon*, to which *L. peruvianum* Mill., *L. cheesmanii* Riley, *L. glandulosum* C. H. Mull. and *L. hirsutum* Humb. & Bonpl. belong. These species are green-fruited and have pseudostipules.

LUCKWILL's (1943) classification is very similar to that of MULLER except that LUCKWILL recognizes an additional species, *L. pissisi*, which in MULLER's treatment is *L. peruvianum* var. *humifusum*.

The wild species, especially *L. hirsutum*, were intensively studied for disease resistance by ALEXANDER (1942); PORTE, DOOLITTLE and WELLMAN (1939); KIKUTA and FRAZIER (1947); and for flesh color by LESLEY and LESLEY (1947). MACARTHUR and CHIASSON (1947) studied the crossing relations of *L. esculentum* with the rest of the species and made preliminary observations of meiosis in pollen-mother cells of the F₁ hybrids.

In none of these, nor any other works, however, were segregating generations subjected to detailed genetical study, which is so important to breeding work. To understand the segregating generations it is necessary to study cytologically the chromosome behavior in the hybrid. These studies may furthermore contribute to our understanding of the cytotaxonomy and evolution of these species.

With these objectives in mind the present project was undertaken to investigate the relationships between *L. esculentum* and two botanical forms of *L. hirsutum*.

MATERIALS AND METHODS

The following stocks for these studies were provided by DR. CHARLES M. RICK.

Stocks of L. esculentum

- Line a. This is a line derived from the commercial variety San Marzano, with the marker genes Woolly (*Wo*), dwarf (*d*) and male-sterile₁₀ (*ms*₁₀), known to be linked on chromosome 2 (RICK and BUTLER 1956).

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Line b. This was derived from San Marzano also, and has the marker genes anthocyaninless (*a*), potato leaf (*c*), lutescent (*l*), and male-sterile₁₀ (*ms*₁₀).

Line c. This is the commercial variety Pearson, with the self-pruning (*sp*) and male-sterile₂ (*ms*₂) markers.

These three stocks were used as female parents because (1) the male-sterility eliminated the need for emasculation, and (2) the crosses cannot be made with *L. hirsutum* as the pistillate parent.

Stocks of L. hirsutum

Two forms of this species were used—the typical form, and *L. hirsutum* f. *glabratum*. The latter was represented by Rick's collection from Rio Pastaza at Baños, Ecuador, and the former by his collection from Rio Chillon, Peru. For the sake of simplicity these will be referred to hereafter by their respective locality names.

Chillon: This collection is of the typical form of *L. hirsutum*. It is very hairy, and has a thick, brittle, green stem. Chillon is sensitive to daylength and flowers only under 12-hour or shorter daylengths. When days are longer the flower buds dry and drop off at various stages. In the later part of May, June, July, and early August, it does not flower. It is self incompatible.

Baños: The collection from Baños is less hairy, and its stem is shorter and more elastic than that of Chillon. Young leaves and stems have a dark purplish color on the side exposed to sunlight while the shaded side is green. The inflorescence is not normally subject to this color variation. The leaves have a darker green color than those of Chillon. The leaflets vary in length, but are definitely shorter than those of Chillon. Baños is nonsensitive to daylength. It is self compatible and sets fruits abundantly both in the greenhouse and in the field at Davis, California. It is less vigorous than Chillon.

Hybridization of the species

The usual procedures were followed in making and tagging the crosses. One tenth of one percent Indolebutyric acid was used on pedicles and calyxes to induce fruit set, since all untreated crosses failed. In August and September 1951, 600 flowers were pollinated in each of the three *esculentum* lines—300 with Baños pollen and 300 with Chillon pollen.

Selfing and backcrossing

Flowers of F₁ *L. esculentum* × Baños were selfed in an insect-proof screen house. As controls eight pure *esculentum* plants heterozygous for *Wo* and *d* also were artificially selfed in the same screen house. Backcrosses were made on *esculentum* male-sterile plants in an isolation plot. If the male-sterile plants were in the main plot (in company with fertile plants), they were covered with 3' x 3' x 3' cages covered with fine cheesecloth to isolate them from contaminating pollen.

F₁'s of *L. esculentum* × Chillon were used for backcrosses on *esculentum* male-sterile plants both in the field and the greenhouse. These F₁ plants could not be selfed because they are self incompatible.

Chromosome morphology

Young buds of *L. esculentum*, Baños, Chillon, F₁ *L. esculentum* × Baños and F₁ *L. esculentum* × Chillon were fixed in 3:1 alcohol-acetic acid. After fixation at room temperature for twenty-four hours the buds were washed in several changes of distilled water, mordanted (method by MARTA WALTERS, quoted by BROWN 1949) for half an hour in four percent iron-alum solution, washed thoroughly in several changes of distilled water and stored at 32°F where they remained in good condition for more than a year.

Permanent slides were prepared according to the method of BROOKS, BRADLEY and ANDERSON (1950).

Chiasma frequency

Another group, comprising five or six plants each of *L. esculentum*, Baños, Chillon, F₁ *L. esculentum* × Baños and F₁ *L. esculentum* × Chillon, was raised under the uniform conditions of a greenhouse. Buds from three plants of each kind were fixed in the middle of March. Fifty or more cells were observed from each plant for determination of the chiasma frequency for the whole complement, and 40 or more were observed for chiasma frequency of the second chromosome.

Pollen study

Freshly opened flowers or those in the act of opening were collected between 9 A.M. and 10 A.M. from field grown plants of the parental species and their F₁'s. Particular care was taken to collect the material when the temperatures of the week before collection were not high enough to induce pollen abortion. Pollen was examined immediately after collection.

RESULTS

Fruit set

As mentioned earlier, no fruits set in these species crosses without the application of Indolebutyric acid. Even after such treatment fruit set was quite low, 6.3 to 14 percent in *L. esculentum* × Baños and 2.7 to 5.3 percent in *L. esculentum* × Chillon.

No control crosses were made in the field for comparisons; yet, it has been the experience that under comparable conditions at Davis *L. esculentum* has a fruit set above 70 percent. The *hirsutum* pollen—either of Baños or Chillon—was more successful in fruit set on Line c. A statistical analysis by the heterogeneity test showed that this differential receptivity to Baños pollen of Line c in contrast to Line a or b is highly significant. There was no significant difference when Chillon pollen was used; but the trend was in the same direction.

Furthermore, the difference in receptivity of the same lines of *L. esculentum* to pollen of the two *L. hirsutum* forms was highly significant. Under greenhouse conditions of 70°F during the day and 60°F at night, however, the percentage of fruit set was increased to 76.6 percent and there was no significant difference between interspecific and intraspecific crosses.

Seeds per fruit

The majority of fruits from interspecific crosses were found to contain seeds with aborted embryos. Only a few fruits had fully developed seeds, and these were present in small numbers. *L. esculentum* × Baños had 4 to 20 seeds per fruit while *L. esculentum* × Chillon had 4 to 11 seeds per fruit.

When field crosses were made in the summer of 1951, pure *L. esculentum* or *L. hirsutum* was not selfed for comparison. But in the following summer Baños was selfed, producing 50.4 ± 2.5 seeds per fruit. In Pearson (Line c) the average number of seeds per fruit varies from 100 to 250, while in San Marzano (Lines a and b) the average number of seeds per fruit is about 100. The trend of seeds per fruit is in the same direction as that of percentage fruit set in these interspecific crosses. Here again, the number of seeds per fruit in Line c × Baños or Line c × Chillon is higher than in the rest of the crosses; furthermore, when Baños is used as the pollen parent the seed set tends to be higher.

Under greenhouse conditions the interspecific crosses still have significantly lower seed set than either parent, though the yield is higher than that under field conditions.

Segregation of genes

Anthocyaninless (a) and *Potato-Leaf* (c): The segregation of these two characters was scored in the greenhouse during July and August. In order to avoid crowding seedlings were classified and removed as soon as they expressed these characters clearly. The backcross results are summarized in Table 1(a).

It is obvious that the χ^2 values calculated according to 1:1:1:1 expectation give a good fit with the expectation on the hypothesis of independent assortment of the two characters. However, when the families are combined the deviations from the expected 1:1:1:1 ratio are on the borderline of significance, the probability being .04. Genes *a* and *c* are definitely known to be located on different chromosomes (BUTLER 1952). The pollen abortion in the *L. esculentum* × Baños F_1 's, as noted in the cytological section, is not higher than that of either parent. Furthermore, germination in the backcross seeds is not lower than that of *L. esculentum* or Baños. A possible explanation for the above deviation, therefore, is selective elimination of the recombination types (+c) in an early zygotic stage.

The segregation of individual genes in all four F_2 families confirms the 3:1 expectation. At the same time the assortment of the two genes is independent, indicating no systematic disturbance [Table 1(b)].

Along with the above segregating progenies, the backcross *L. esculentum* × (*L. esculentum* × Chillon) was grown for the same gene segregation. This gave

TABLE 1

Segregation and assortment of a and c in the hybrids of L. esculentum × L. hirsutum

Family no.	a. Backcross <i>L. esculentum</i> × (<i>L. esculentum</i> × Baños)				Total	χ^2	P
	++	a +	+ c	ac			
BP ₂	26 (31.25)	29 (31.25)	30 (31.25)	40 (31.25)	125	3.54	.8-.3
BP ₃	79 (73.)	69 (73.)	61 (73.)	83 (73.)	292	4.05	.3-.2
BP ₄	22 (22.75)	22 (22.75)	19 (22.75)	28 (22.75)	91	1.88	.7-.5
BP ₅	44 (35.50)	37 (35.50)	26 (35.50)	35 (35.50)	142	4.65	.2-.15
Total	171 (162.50)	157 (162.50)	136 (162.50)	186 (162.50)	650	8.35	.04
b. F ₂ <i>L. esculentum</i> × Baños							
P ₁	124 (141.75)	53 (47.25)	59 (47.25)	16 (15.75)	252	5.85	.15-.1
P ₂	76 (76.50)	19 (25.50)	32 (25.50)	9 (8.50)	136	3.34	.3-.2
P ₄	54 (47.81)	13 (15.94)	12 (15.94)	6 (5.31)	85	2.41	.5-.3
P ₉	50 (51.75)	25 (17.25)	11 (17.25)	6 (5.75)	92	5.82	.2-.1
Total	304 (317.81)	110 (105.94)	114 (105.94)	37 (35.31)	565	1.45	.7-.5
c. Backcross <i>L. esculentum</i> × (<i>L. esculentum</i> × Chillon)							
BC ₁	165 (153.)	158 (153.)	117 (153.)	172 (153.)	612	11.93	<.01

Figures in parentheses are Mendelian expectations.

an opportunity to detect discrepancies, if any, between the two pollen parents—Baños and Chillon. In this progeny the assortment of *a* and *c* is not normal, since the probability of a fit as poor as that observed is less than .01. Interestingly enough, class *a c* is excess, while class + *c* is deficient, just as in the backcross *L. esculentum* × (*L. esculentum* × Baños). The deviations in the present case, however, are more extreme than those of the Baños backcrosses.

Self-pruning Habit (sp) and *Male-Sterile*₂ (ms₂): Of 600 plants of the *L. esculentum* × (*L. esculentum* × Chillon) backcross population in the field, 591 survived while in the *L. esculentum* × (*L. esculentum* × Baños) backcross, 177 plants out of 405 withered and died (SAWANT 1956). The same behavior was shown by the F₂ of *L. esculentum* × Baños, with 66 plants being lost in a total of 210. The results are summarized in Table 2a.

TABLE 2a

Assortment of sp and ms₂ in L. esculentum × L. hirsutum crosses

Family no.	++	sp ms ₂	sp +	+ ms ₂	Total	χ ²	P
BC ₁₀	140 (147.75)	142 (147.75)	182 (147.75)	127 (147.75)	591	11.55	< .01
BP ₂₀	45 (57.25)	47 (57.25)	97 (57.25)	40 (57.25)	229	37.25	< .001
P ₁₉	67 (81)	9 (9)	44 (27)	24 (27)	144	13.46	< .001

In *L. esculentum* × (*L. esculentum* × Chillon) backcross the recombination class *sp* + is in great excess, whereas the other recombination class, + *ms*₂ is deficient. It is interesting to note that the parental combinations, ++ and *sp ms*₂, are less than expected, though they do not show significant deviations. Nevertheless, these observations contradict the now classical hypothesis that recombination classes in species crosses tend to be deficient and parental classes excessive.

These results can be explained to some extent in terms of abnormal segregation of these individual genes (Table 2b). In the backcross *L. esculentum* × (*L. esculentum* × Baños) we find that the probability values are lower than those in Chillon backcross, and the deviations are in the same direction as in the Chillon backcross (Table 2b).

Woolly (Wo) and *Dwarf* (d): The results for these characters are tabulated in Table 3. P₃₇ is an exceptional F₂ family of the *L. esculentum* × Baños cross, which produced 70 homozygous woolly plants with a good fit to 3Wo:1+ ratio, the WoWo plants evidently being viable.

It is obvious that in these families assortment of the two characters is not independent, since the probability values are less than .001. When the χ²'s, are apportioned to the appropriate contributing factors, linkage proves to be the main source of the deviations. Families BWod-7 and Wod × acldry, being

TABLE 2b

Segregation of sp and ms₂ in L. esculentum × L. hirsutum crosses

Family no.	+	ms ₂	P χ ²	P	+	sp	χ ²	P
BC ₁₀ *	322 (295.50)	269 (295.50)	4.75	.05-.02	268 (295.50)	323 (295.50)	5.12	.05-.02
BP ₂₀ †	142 (114.50)	87 (114.50)	13.2	< .001	85 (114.50)	144 (114.50)	15.20	< .001
P ₁₉ ‡	106 (108)	38 (36)	.15	.7-.5	91 (108)	53 (36)	10.7	< .001

* BC₁₀—backcross *L. esculentum* × (*L. esculentum* × Chillon).

† BP₂₀—backcrosses *L. esculentum* × (*L. esculentum* × Baños).

‡ P₁₉—F₂ *L. esculentum* × Baños.

TABLE 3

Assortment of *Wo* and *d* in F_2 and backcross of *L. esculentum* $\times$ *L. hirsutum* and pure *L. esculentum*

Family no.*	++	<i>Wo d</i>	<i>Wo</i> +	+ <i>d</i>	Total	χ^2	P
B Wod-7	72 (50)	65 (50)	29 (50)	34 (50)	200	28.12	.001
Wod $\times$ acldry	224 (161.5)	212 (161.5)	114 (161.5)	96 (161.5)	646	80.51	.001
BP ₃₇	668 (490.5)	686 (490.5)	286 (490.5)	322 (490.5)	1962	285.30	.001
P ₃₇	147 (111.94)	127 (111.94)	308 (335.81)	15 (37.31)	597	28.65	.001
BC ₂₅	336 (225)	328 (225)	142 (225)	94 (225)	900	208.80	.001
BC ₂₇	335 (243.25)	330 (243.25)	140 (243.25)	148 (243.25)	973	163.40	.001

* B Wod-7 is backcross of $\frac{Wo d}{++}$ to double recessive $\frac{+ d}{+ d}$ female parent. It is pure *esculentum* family.

Wod $\times$ acldry is reciprocal of the above cross.

BP₃₇ is backcross of F_1 *L. esculentum* $\times$ Baños to double recessive *L. esculentum* female parent.

P₃₇ is F_2 of *L. esculentum* $\times$ Baños cross.

BC₂₅ and BC₂₇ are backcrosses of F_1 *L. esculentum* $\times$ Chillon to double recessive *L. esculentum* female parent.

homogeneous, are combined in one group; BC₂₅ and BC₂₇ form another group. From these, the crossing over values are calculated by means of the maximum likelihood method.

Table 4 shows that crossing over in *L. esculentum* $\times$ Baños hybrids is nearly the same when calculated from the backcross and F_2 data. Comparisons of linkage values in pure *L. esculentum*, Baños hybrids and Chillon hybrids made by the homogeneity test show that the comparison between *L. esculentum* $\times$ Chillon hybrid and *L. esculentum* is significant ($P=.05$); the other comparisons are of the same order.

TABLE 4

Summary of cytological observations of *L. esculentum*, *L. hirsutum* and their hybrids

	Crossover values		Average no. of chiasmata		
	Backcross	F_2	Whole complement	Second chromosome	Percent pollen abortion
<i>L. esculentum</i>	32.27 $\pm$ 1.61		17.91	1.77	7.09
Baños			17.14	...	6.79
Chillon			17.02	...	8.32
F_1 <i>L. esculentum</i> $\times$ Baños	30.98 $\pm$ 1.04	31.00 $\pm$ 2.03	17.19	1.74	7.41
F_1 <i>L. esculentum</i> $\times$ Chillon	27.98 $\pm$ 1.04		14.35	1.57	14.79
L.S.D.			.46*	.10*	1.67*
			.61†	.13†	2.21†

* At five percent level.

† At one percent level.

Cytology

The morphology of *L. esculentum* pachytene chromosomes has been described in detail by BARTON (1950) and BROWN (1949). The observations of these workers and those of the present study are in agreement. Mention should be made at this point that the tomato chromosomes are differentiated not only into chromatic and achromatic zones (BROWN 1949) but also that each zone is further differentiated into minute regions having differential stainability.

The meiotic divisions in the pollen mother cells of Baños and Chillon are essentially the same. No unusual structural configurations—translocations, inversions, multivalents, univalents or non-homologous pairings—are evident. The nucleolus in Chillon looks slightly smaller than that of *L. esculentum*, and the chromosomes of Chillon seem to stain lightly, indicating that they might differ from those of *L. esculentum* with respect to differentiation into chromatic and achromatic zones.

Cytology of F₁'s: No attempt was made to study leptotene and zygotene. In pachytene pairing was observed to be complete. No rings, cross-shaped configurations, nor loops were observed.

It is interesting to note that in the homologues of a bivalent in these F₁'s there is no difference in the stainability, such as is evident when the two parents are observed separately. Other workers have found evidence that the chromaticity of chromosomes is a gene controlled character but subject to the influence of the environment. Under ordinary temperature conditions the chromosomes of *Trillium* do not show any differentiation, but at 0°C and 5°C they clearly differentiate along their lengths into chromatic and heterochromatic regions (DARLINGTON and LA COUR 1940). Since there is cyclical correlation between gene reproduction and nucleic acid attachment, these authors conclude that staining ability—nucleic acid starvation or accumulation—is a gene controlled character. The difference in stainability in our case, then, presumably refers to physiological differentiation of the chromosome and not to the gene order. Its alteration in the F₁ shows that it does not necessarily represent a permanent structural difference.

Although the meiotic divisions are normal in gross aspects, when chiasma number for the whole complement as well as for the second chromosome are studied, certain significant differences are found, as noted in Table 4. The differences between mean chiasma number per full complement, of Baños, Chillon and F₁ *L. esculentum* × Baños are not significant, whereas the rest of the comparisons show significant differences. It is of particular interest to note that the mean chiasma number of F₁ *L. esculentum* × Baños is significantly lower than that of *L. esculentum* but not lower than that of Baños. The chiasma number of F₁ *L. esculentum* × Chillon, on the other hand, is very significantly lower than that of either *esculentum* or the Chillon parents. Comparing F₁ *L. esculentum* × Chillon with F₁ *L. esculentum* × Baños it is noticed that the former has a very significantly lower chiasma number.

The observations on the number of chiasma in the second chromosome sub-

stantiate, in part, the above results. In this case only *L. esculentum*, F_1 *L. esculentum* $\times$ Baños and F_1 *L. esculentum* $\times$ Chillón were studied. There is no significant reduction in chiasma number in F_1 *L. esculentum* $\times$ Baños as compared to pure *L. esculentum* but F_1 *L. esculentum* $\times$ Chillón has a significantly lower chiasma number than *L. esculentum* or F_1 *L. esculentum* $\times$ Baños.

It is clear from the comparisons of the differences in pollen abortion with the L.S.D. (Table 4) that the only significant difference is between F_1 *L. esculentum* $\times$ Chillón and the rest.

DISCUSSION

Nature of reproductive isolation between L. esculentum and L. hirsutum

Pollinations made under field conditions gave a very low set of fruit and seed in interspecific crosses. The reproductive isolating mechanism seems to be governed by several genes which interact disharmoniously, resulting in the abortion of a large portion of the embryos. Furthermore, there is an interaction between genotypes and the environment of the parental species, since under greenhouse conditions the seed set is improved and the fruit set is as good as that in the intraspecific crosses. The difference between field crosses and greenhouse crosses makes it difficult to infer the exact nature of the isolation mechanism operating under natural conditions. Neither field nor greenhouse conditions duplicate exactly the native habitat of these species in Ecuador and Peru. In the study of wheat hybrids LOVE (1941) has shown that unless definite criteria are established by intraspecific crossing relationships, it is difficult to decide on the relative significance of any individual criterion in determining the species relationships. The present study has revealed, moreover, that success in interspecific crosses can be influenced not only by the environment, but also by the genotype of the parents, for within *L. esculentum*, San Marzano and Pearson exhibit different crossability with Baños or Chillón. As a result, crossability under artificial conditions has to be considered with caution in deciding species relations.

The isolation barrier between *L. esculentum* and Baños appears to be comparatively weaker than that between *L. esculentum* and Chillón. This is true not only for crossing relations but also for chiasma frequency, pollen abortion, linkage value, seed fertility of F_1 's, and segregation in F_2 and backcrosses. In the present case the two processes of reproductive isolation and morphophysiological differentiation go hand in hand. The relationships revealed by cytogenetic methods are strengthened by physiological and morphological comparisons. Thus, Chillón is sensitive to daylength and it is self incompatible, while Baños, like *L. esculentum*, is nonsensitive to daylength and is self compatible. Morphologically Baños is like *L. esculentum* by being less hirsute than Chillón, and it resembles *L. esculentum* more than Chillón does in its unfolding of calyx, the size of the plant and the exertion of stigma. In conclusion, all the criteria that were applied consistently indicated a closer relationship between *L. esculentum* and Baños than between *L. esculentum* and Chillón.

Reproductive isolation between L. esculentum and Baños

The nature of reproductive isolation between these two entities is of special significance. Isolation barriers in most cases seem to be controlled by a series of genes. Thus, STEBBINS (1950) states: "The nature of interspecific isolation barriers precludes the possibility that species usually arise full-fledged at a single step barring the cataclysmic event of polyploidy." The withering in the *L. esculentum* x Baños hybrids and in their segregating generations, however, is controlled by a pair of complementary genes which have no effect in the pure species (SAWANT 1956). All the F_1 's and nearly half of the F_2 and backcross plants wither and die under field conditions. Such an isolation barrier can readily arise by two mutations to develop a pair of complementary factors. If the two entities involved have already diverged in their taxonomic characters, the incorporation of these complementary factors would provide an effective barrier to gene exchange between the entities, thereby providing further differentiation and full speciation.

The second difference between the *esculentum* hybrids of Chillon and Baños lies in the stage at which these isolation barriers operate. In the hybrids with Baños the latter operate in the sporophyte, whereas in Chillon hybrids they operate mainly in the gametophyte and to some extent in an early sporophytic stage (SAWANT 1956).

Segregating generations

The results of this investigation show that in backcrosses and F_2 progenies all six marker genes except *sp* and *ms₂* segregate normally when considered individually, the deviations being small and spurious. When combined segregation of these genes is studied, however, the deviations are significant and complex. In the backcross progeny, *L. esculentum* x (*L. esculentum* x *L. hirsutum*), *a*⁺ and *sp*⁺ are less frequent than expected though not significantly so. The segregation of *Wo* and *d* is of particular interest because these two genes are linked.

The crossing over value (genetic observation) between *Wo* and *d* in the F_1 *L. esculentum* x Chillon is lower than that of *L. esculentum* or the F_1 *L. esculentum* x Baños, and the difference may be accounted for by one or more of the following factors: A. Abnormal segregation of the individual genes; B. Selective elimination of recombination gametes; and C. Actual reduction in cytological crossing over or the chiasma frequency.

The first possibility is excluded since both *Wo* and *d* segregate normally. Selective elimination is known in other organisms such as corn, but none has yet been reported in *Lycopersicon*. The F_1 *L. esculentum* x Chillon has a higher pollen abortion (Table 4) than either of its parents or the F_1 *L. esculentum* x Baños. All the higher pollen abortion in this F_1 however, cannot be attributed to the recombination products of the first linkage group alone, considering that 11 other groups also are present in this hybrid. Besides, we have no positive evidence to show that the aborted pollen is all or almost all of recombination types and not of parental types, since in the parents pollen abortion is seven to eight percent as compared to 14.8 percent in the F_1 . The pollen abortion is then, pos-

sibly, caused by the interaction between complementary genes from both species. This hypothesis of interactions between genes of two species becomes more probable since there are also other complementary factors which control the withering phenomenon in the F_1 *L. esculentum* $\times$ Baños (SAWANT 1956).

Cytological studies show a distinct reduction in chiasma frequency in the F_1 *L. esculentum* $\times$ Chillon (Table 4) per full complement as well as for the second chromosome, on which *Wo* and *d* are located. Therefore, the most likely explanation of the low genetic crossing over value is the reduced chiasma frequency which, in turn, is gene controlled.

Phylogenetic relationships

Finally, taking into consideration the crossability, fertility of F_1 's the segregation in F_2 's and backcrosses, the crossing over value or linkage in F_1 , chiasma number in F_1 , and pollen abortion in F_1 , it becomes clear that Chillon is less closely related to *L. esculentum* than Baños is. Furthermore, as mentioned previously in the discussion, Baños resembles *L. esculentum* more than Chillon does. Then the question arises, "Do Baños and Chillon belong to the same species?" It is tempting from the above results, to separate them at least as subspecies. It should be mentioned at this point that from 97 flowers of Baños pollinated with Chillon pollen, under field conditions, only three fruits with a few seeds each were obtained. To arrive at a definite conclusion, however, the F_1 of this cross and the segregating generations (provided they can be obtained) must be studied.

SUMMARY

Studies were carried out to determine the relationships between *Lycopersicon esculentum* and the two forms of *L. hirsutum*, the typical form (Chillon) and the form *glabratum* (Baños) by means of crossing relations, sterility of F_1 hybrids, gene segregation, chiasma frequency and crossing over.

In field tests 9.8 percent of *esculentum* flowers set fruit after crossing with Baños and 3.4 percent with Chillon. The same crosses yielded much better in the greenhouse, the crosses of *L. esculentum* $\times$ Chillon being 76.6 percent successful—as high as the level of fertility of crosses within *L. esculentum*. Varieties of *L. esculentum* responded differently in the same crosses with *L. hirsutum*. Similarly in these interspecific crosses the number of seeds per fruit varied from 4 to 20, while in the intraspecific it is about 50. The seed set tended to be lower with Chillon than with Baños as pollen parent.

Six genes were introduced into the hybrids from *L. esculentum*, their normal alleles coming from *L. hirsutum*. The assortment of all these genes, however, is not normal. In progenies segregating for *a* and *c* the class *a c* is larger and the class *+ c* is smaller than expected on the basis of independent segregation, the deviations being more drastic when Chillon is used as the male parent. Similarly, in the segregation of *sp* and *ms₂*, the class *sp +* is larger and class *+ ms₂* is smaller than expected. In this case the large deviations occur in the families in which Baños is used as the pollen parent in the original cross. In the assortment of *Wo*

and *d* the recombination types *Wo* + and + *d* are less frequent since they are linked.

The genetic crossing over between *Wo* and *d* in the F_1 *L. esculentum* × Chillon was significantly lower than the one in F_1 *L. esculentum* × Baños or pure *L. esculentum*. The mean chiasma frequencies for the second chromosome of F_1 *L. esculentum* × Chillon were lower than those in *L. esculentum* or F_1 *L. esculentum* × Baños, showing the correspondence between the cytological and genetic crossing over values. Similarly, the mean chiasma frequencies for the full complement of F_1 *L. esculentum* × Chillon were lower than those in Baños, Chillon, F_1 *L. esculentum* × Baños, or pure *L. esculentum*.

The pollen abortion in F_1 *L. esculentum* × Chillon was higher than that in Baños, Chillon, F_1 *L. esculentum* × Baños or pure *L. esculentum*.

All of these cytological and genetical studies, therefore, confirm the separation of *L. esculentum* and *L. hirsutum* as distinct species. The studies reveal, furthermore, that Baños is more closely related to *L. esculentum* than is Chillon.

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LITERATURE CITED

- ALEXANDER, L. J., 1943 A survey of the genus *Lycopersicon* for resistance to the important tomato diseases occurring in Ohio and Indiana. Plant Disease Repr. Suppl. **136**: 49-85.
- BARTON, D. W., 1950 Pachytene morphology of the tomato chromosome complement. Am. J. Botany **37**: 639-643.
- BROOKS, R. M., M. V. BRADLEY, and T. H. ANDERSON, 1950 Plant microtechnique manual. Univ. of Calif. Davis.
- BROWN, S. W., 1949 The structure and meiotic behaviour of the differentiated chromosomes of tomato. Genetics. **34**: 437-461.
- BUTLER, L., 1952 The linkage map of the tomato. J. Heredity **33**: 25-35.
- DARLINGTON, C. D., and L. LA COUR, 1940 Nucleic acid starvation of chromosomes in Trillium. J. Genet. **40**: 184-213.
- KIKUTA, K., and W. A. FRAZIER, 1947 Preliminary report on breeding tomatoes for resistance to tobacco mosaic. Proc. Am. Soc. Hort. Sci. **49**: 256-262.
- LESLEY, J. W., and M. M. LESLEY, 1947 Flesh color in hybrids of tomatoes. J. Heredity **38**: 245-251.
- LOVE, R. M., 1941 Chromosome behaviour in F_1 wheat hybrids. I. Pentaploids. Can. J. Research Sec. C. **19**: 351-369.
- LUCKWILL, L. C., 1943 The genus *Lycopersicon*. Aberd. Univ. Stud. **20**.

- MACARTHUR, J. W., and L. P. CHIASSON, 1947 Cytogenetics of tomato species. *Genetics* **32**: 165-177.
- MULLER, C. H., 1940 A revision of the genus *Lycopersicon*. U.S. Dept. Agr. Misc. Publ. No. **382**.
- PORTE, W. S., S. P. DOOLITTLE, and F. L. WELLMAN, 1939 Hybridization of a mosaic tolerant, wilt-resistant *Lycopersicon hirsutum* with *L. esculentum*. *Phytopathology* **29**: 757-759.
- RICK, C. M., and L. BUTLER, 1956 Cytogenetics of tomato. *Advances in Genet.* **8**: 267-382.
- SAWANT, ANAND C., 1956 Semilethal complementary factors in a tomato species hybrid. *Evolution* **10**: 93-96.
- STEBBINS, G. L., 1950 *Variation and Evolution in Plants*. Columbia University. New York.