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Trisomics from Triploid-diploid Crosses in Self-Incompatible *Lycopersicum peruvianum*

I. Essential Features of Aneuploids and of Self-Compatible Trisomics*

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Summary. An attempt was carried out to produce trisomics of the wild tomato *L. peruvianum*, to define their essential features, and to detect relationships between trisomy and the expression of self-compatibility.

Triploid-diploid crosses in *L. peruvianum* yielded nearly 40% aneuploids. Of these, 18% were single trisomics, and the rest had 2, 3 and 4 extra chromosomes. Almost all the trisomics occurred in crosses where the triploid was used as female parent. Vigour and fertility of trisomics were not much different from those of disomics, and morphologically they were very similar.

The extra chromosome was identified in three self-compatible trisomic plants through somatic and pachytene chromosome morphology. One of these plants was trisomic for chromosome 1, while the other two were trisomic for chromosome 3. In these trisomics a positive correlation was found between chromosome length and trivalent formation, but no relationship between chromosome length and frequency of laggards was observed.

A series of test-crosses revealed that the capacity of the trisomics to produce seed upon selfing always resulted from alterations of the incompatibility phenotype of the style and not from competitive interaction in the pollen. Progeny analyses showed that the self-compatibility features of the trisomics were not transmitted from one generation to the next. The implications of these findings are discussed.

Key words: *Lycopersicum peruvianum* - Aneuploids - Trisomics - Self compatibility - Specificities

Introduction

Although trisomics of the cultivated tomato *(Lycopersicum esculentum)* have often been produced in the past (Rick and Barton 1954, 1956; Rick and Notani 1961; Ecochard and Merx 1972) no attempts have been made to construct trisomic lines of the self-incompatible wild species of *Lycopersicum* which constitutes a very important source of germplasm for the improvement of tomato varieties. This absence of experimental material is regrettable because the use of the trisomic method for associating genes and linkage groups to their chromosomes could be of great value for exploring the genetic structure of wild tomato species and for establishing the cytogenetic basis of gametophytic monofactorial incompatibility in the genus.

In particular, since the incompatibility character is expected from the competition theory (see Lewis 1947, 1949; Brewbaker and Natarajan 1960; Pandey 1967) to break down in pollen grains containing two different S-alleles, it should be possible to detect rapidly, upon selfing, the trisomic for the S-locus and to identify, thereafter, the chromosome bearing the self-incompatibility gene. As the action of each individual S-allele remains independent in the style, the presence of three different S-alleles in the self-compatible trisomics suspected of carrying three S-bearing chromosomes can, in theory, be confirmed by means of appropriate crosses with diploid tester stocks used as staminate partners.

In an attempt to carry out such investigations and at the same time to accumulate basic information on the trisomics of wild tomato species, a research programme has been executed, in the framework of the Association EURATOM-ITAL. The programme cen-

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tered essentially on the production and analysis of trisomics in the self-incompatible species *Lycopersicum peruvianum*. The results obtained are presented and discussed below.

Material and Methods

Production of triploid and trisomic lines

Five self-incompatible diploid genotypes, namely clone 006-S_1S_2, 10-S_1S_4, F9-S_4S_5, F12-$S_{10}S_{11}$ and F13-$S_{12}S_{13}$ and one autotetraploid ($S_1S_1S_2S_2$) obtained among the inbred progenies of clone 006-S_1S_2, were used in the present study. The details of the origin and source of the different diploid S-genotypes were published in an earlier report (Sree Ramulu et al. 1976).

Triploid material was produced by crossing the autotetraploid ($S_1S_1S_2S_2$) to a diploid genotype, S_1S_4. Although the triploid expressed high male and female fertility, it failed to yield seeds upon selfing. The trisomics were thus obtained through crosses between the triploid, as staminate or pistillate parent, and various cross-compatible diploid S-genotypes.

Characterization of trisomic plants

1. Detection of self-compatible trisomics

The seedlings grown from the seeds of triploid-diploid crosses were screened for aneuploids by making chromosome determinations in root-tip meristems. Root-tips were treated with bromonaphtalene for 75 min., fixed in acetic alcohol (1:3), hydrolised in 1N HCl for 7 min. at 60°C, stained with Feulgen reagent and squashed in 1% acetocarmine. All the trisomic plants detected were selfed by hand pollination under conditions of complete isolation.

2. Morphology and cytology of self-compatible trisomics

Morphological characters of leaf, flower, fruit and seed were recorded for those trisomics which showed some fruit development and/or seed-set after self-pollination. For meiotic studies in trisomics, young anthers were fixed in a mixture of propionic acid and absolute alcohol (1:3) saturated with ferric acetate. The standard acetocarmine smear method was used in preparing the slides.

The determination of S-genotypes in the styles of triploid and of the self-compatible plants found in the course of the study was carried out by means of crosses with a range of diploid tester stocks bearing the various S-alleles (S_1, S_2, S_4) present in the original parental lines and alleles (S_5, S_{10}, S_{11}, S_{12}, S_{13}) from unrelated accessions. In all, the following tester stocks were used: S_1S_2, S_1S_4, S_1S_5, S_2S_4, S_4S_5, $S_{10}S_{11}$, $S_{12}S_{13}$.

Results

Essential features of the triploid obtained from the cross $4\underline{n}\ (S_1S_1S_2S_2) \times 2\underline{n}\ (S_1S_4)$

The various crosses carried out with the diploid tester stocks used as staminate parents clearly indicate (Table 1) a relatively high rate of ovule fertility in the ovary of the triploid. The rejection, fully confirmed by means of fluorescent microscopy, of S_1, S_2 and S_4 pollen by the triploid styles and the acceptance of pollen from S_4S_5, $S_{10}S_{11}$ and $S_{12}S_{13}$ testers establish beyond any doubt the S-genotype of the triploid plant as $S_1S_2S_4$.

Table 1. Fruit formation and seed yields obtained in test-crosses between triploid and various diploid S-genotypes in *Lycopersicum peruvianum*

Cross	No. of flowers crossed	No. of fruits developed	No. of Seeds set
Triploid × 006-S_1S_2	49	0	0
Triploid × 10-S_1S_4	48	0	0
Triploid × F9-S_4S_5	33	31	253
Triploid × F12-$S_{10}S_{11}$	19	19	54
Triploid × F13-$S_{12}S_{13}$	42	42	189

Pollen viability, as determined by acetocarmine staining, amounted to 41.78% in the triploid plant which, however, regularly failed to set any seed upon selfing. Observations under the fluorescent microscope revealed that the pollen of the triploid failed to grow through the style and displayed the features which are typical of the self-incompatibility reaction (see de Nettancourt et al. 1973).

In crosses with the diploid testers as pistillate parents, a small quantity of seed was produced which indicates that at least a certain proportion of the pollen yielded by the triploid is functional and fertile (Table 2). The numbers of fruits and of seeds formed were, however, always much higher in the reciprocal crosses, i.e. when the triploid was used as pistillate partner.

Table 2. Seed germination, plant survival and percentage aneuploids among the progeny derived from crosses between triploid and various diploid S-genotypes in *Lycopersicum peruvianum*

Cross	No. of flowers crossed	No. of fruits formed	No. of seeds set	No. of seeds sown	No. of seeds germinated	% germination	No. of plants survived to maturity after germination	% survival	No. of plants observed cytologically	Aneuploids obtained	
										No.	%
Triploid ♀ × F_9-S_4S_5	33	31	253	70	59	84.3	37	62.7	30	17	56.7
F_9-S_4S_5 × Triploid	51	2	4	4	4	100.0	3	75.0	3	-	-
Triploid × F_{12}-$S_{10}S_{11}$	19	19	54	46	40	86.9	28	70.0	25	7	24.0
F_{12}-$S_{10}S_{11}$ × Triploid	48	3	5	5	2	40.0	2	100.0	2	-	-
Triploid × F_{13}-$S_{12}S_{13}$	42	42	189	45	43	85.6	35	81.4	27	17	62.9
F_{13}-$S_{12}S_{13}$ × Triploid	85	12	18	18	7	38.9	6	85.7	5	1	20.0
006-S_1S_2 × Triploid	98	2	2	2	2	100.0	2	100.0	2	1	50.0
10-S_1S_4 × Triploid	76	9	25	25	24	96.0	24	95.8	24	4	16.7
Total	452	120	550	215	181	84.18	137	75.1	118	47	39.8

Frequencies of aneuploids and of trisomics in the progenies derived from crosses between the triploid and diploid testers

Two hundred and ten seeds were sown from the total of 550 seeds produced from the different crosses listed in Table 2. Out of these, 181 germinated and 136 plants survived to maturity of which 118 were analysed cytologically. The chromosome counts performed on these 118 plants (Table 3) show that 47 (39.8%) were aneuploids and 71 (66.2%) diploids.

Among the aneuploids, single trisomics (2n + 1) were found to occur at a frequency of 18.6% while individuals with 2, 3 and 4 extra chromosomes were detected in, respectively, 11.9, 5.9 and 2.5% of the cases. One tetraploid plant was identified among the progeny of a cross between the triploid used as female parent and the diploid tester stock $S_{10}S_{11}$. The data presented in Table 3 also indicate that almost all the aneuploids were detected in the progenies of crosses where the triploid had been used as pistillate partner.

Growth and fertility of the trisomics

The data on frequency distribution for plant height revealed little difference between disomics and single trisomics in the distribution under different classes of heights or in mean height; the plants with 2 or

Table 3. Frequency of plants with different chromosome numbers among the progenies derived from crosses between triploid and diploid S-genotypes

Cross	Chromosome numbers					
	24	25	26	27	28	48
Triploid × F9-S_4S_5	13	6	5	4	2	-
F9-S_4S_5 × triploid	3	-	-	-	-	-
Triploid × F12-$S_{10}S_{11}$	18	4	2	-	-	1
F12-$S_{10}S_{11}$ × triploid	2	-	-	-	-	-
Triploid × F13-$S_{12}S_{13}$	10	8	5	3	1	-
F13-$S_{12}S_{13}$ × triploid	4	-	1	-	-	-
006-S_1S_2 × triploid	1	1	-	-	-	-
10-S_1S_4 × triploid	20	3	1	-	-	-
Total	71	22	14	7	3	1

Table 4. Frequency distribution for plant height in disomics and trisomics obtained from triploid-diploid crosses

Material	Height of plants (cm)									
	1 month-old plants					2 months-old plants				
	1.0-3.0	3.1-6.0	6.1-9.0	9.1-15.0	Mean ± S.E.	10-20	21-30	31-40	41-60	Mean ± S.E.
Disomics (2n)	8	23	20	20	6.93 ± 0.42	7	28	21	15	33.86 ± 1.51
Single trisomics (2n + 1)	2	8	7	5	6.45 ± 0.62	-	8	11	3	32.11 ± 1.68
Double trisomics (2n + 2)	3	7	3	1	5.99 ± 0.63	2	7	5	-	28.27 ± 2.22

Table 5. Frequency distribution for pollen viability in disomics and trisomics derived from triploid-diploid crosses

Material	Pollen viability (%)							
	31-40	41-50	51-60	61-70	71-80	81-90	91-100	Mean ± S.E.
Disomics (2n)	-	-	5	8	4	20	25	83.32 ± 1.63
Single trisomics (2n + 1)	2	1	1	3	3	5	7	75.41 ± 4.37
Double trisomics (2n + 2)	1	1	1	1	4	-	2	68.68 ± 7.50

Table 6. Fruit formation and seed yields after self-pollination in the trisomics derived from triploid-diploid crosses

Plant No.	Chromosome No.	Origin	No. of flowers pollinated	No. of fruits formed	No. of seeds set	Average No. of seeds per fruit	Average No. of seeds per pollinated flower	% pollen viability
220	25	Triploid ♀ × F_9-S_4S_5	28	5	560	112.00	20.00	72.64
172	25	Triploid × F_{13}-$S_{12}S_{13}$	57	32	315	9.84	5.53	95.28
145	25	Triploid × F_{13}-$S_{12}S_{13}$	23	17	73	4.29	3.17	62.05
269	25	Triploid × F_{12}-$S_{10}S_{11}$	8	1	7	7.00	0.88	87.03
142	25	Triploid × F_{13}-$S_{12}S_{13}$	25	3	14	4.66	0.56	65.31
132	25	Triploid × F_{12}-$S_{10}S_{11}$	30	3	1	1.00	0.03	82.81
147	26	Triploid × F_{13}-$S_{12}S_{13}$	17	2	-	-	-	92.94
160	26	Triploid × F_{13}-$S_{12}S_{13}$	9	3	-	-	-	53.68
261	26	Triploid × F_{12}-$S_{10}S_{11}$	22	5	-	-	-	67.96
276	26	Triploid × F_{12}-$S_{10}S_{11}$	23	1	-	-	-	95.91

more extra chromosomes showed some reduction in the mean height (Table 4). The results on frequency distribution and mean of pollen viability indicated that the trisomics produce a rather high percentage of fertile pollen (Tables 5 and 6) which was only slightly lower than in the disomics. Almost all the trisomic plants flowered. The mean flowering time of 71 disomic plants was 70.36 days, as compared to 79.31 days in single trisomics and to 81.42 days in double trisomics.

Self-compatibility in trisomics

With the hope of detecting trisomics for the self-incompatibility locus, the 47 aneuploids were screened, through self-pollination tests, for the self-compatibility character. This character should express itself in plants with three S-bearing chromosomes if self-incompatibility really breaks-down in heteroallelic digenic pollen grains. In all, 800 flowers were self-pollinated and 6 of the 22 single trisomics produced a certain amount of fruits and seeds upon selfing (Table 6). However, only 4 of these (plants 145, 172, 220 and 269) set seed regularly enough to be tabulated as self-compatible. Among the 14 double trisomics, no individual yielded seeds upon selfing and only 4 plants occasionally developed fruits. Out of the 7 plants with 3 extra-chromosomes, one failed to flower while the remaining six expressed self-incompatibility. Finally, in the group of plants with 4 additional chromosomes, two individuals which showed slower growth, stunted habit and leaf and floral abnormalities did not reach anthesis and failed to survive. The remaining one plant was self-incompatible.

Among the 70 disomics analysed from the same progenies for control purposes, only two individuals (plant 119, plant 183) expressed a certain level of weak and sporadic self-compatibility.

Cytological identification of the self-compatible trisomic (plant 220) and of trisomics setting some seed upon selfing

Karyotype of the diploid mother clone F_{13} - $S_{12}S_{13}$

The karyotype of the diploid mother clone and the idiogram of the four longest chromosomes are presented in Figs.1 and 2, respectively. Based on arm lengths,

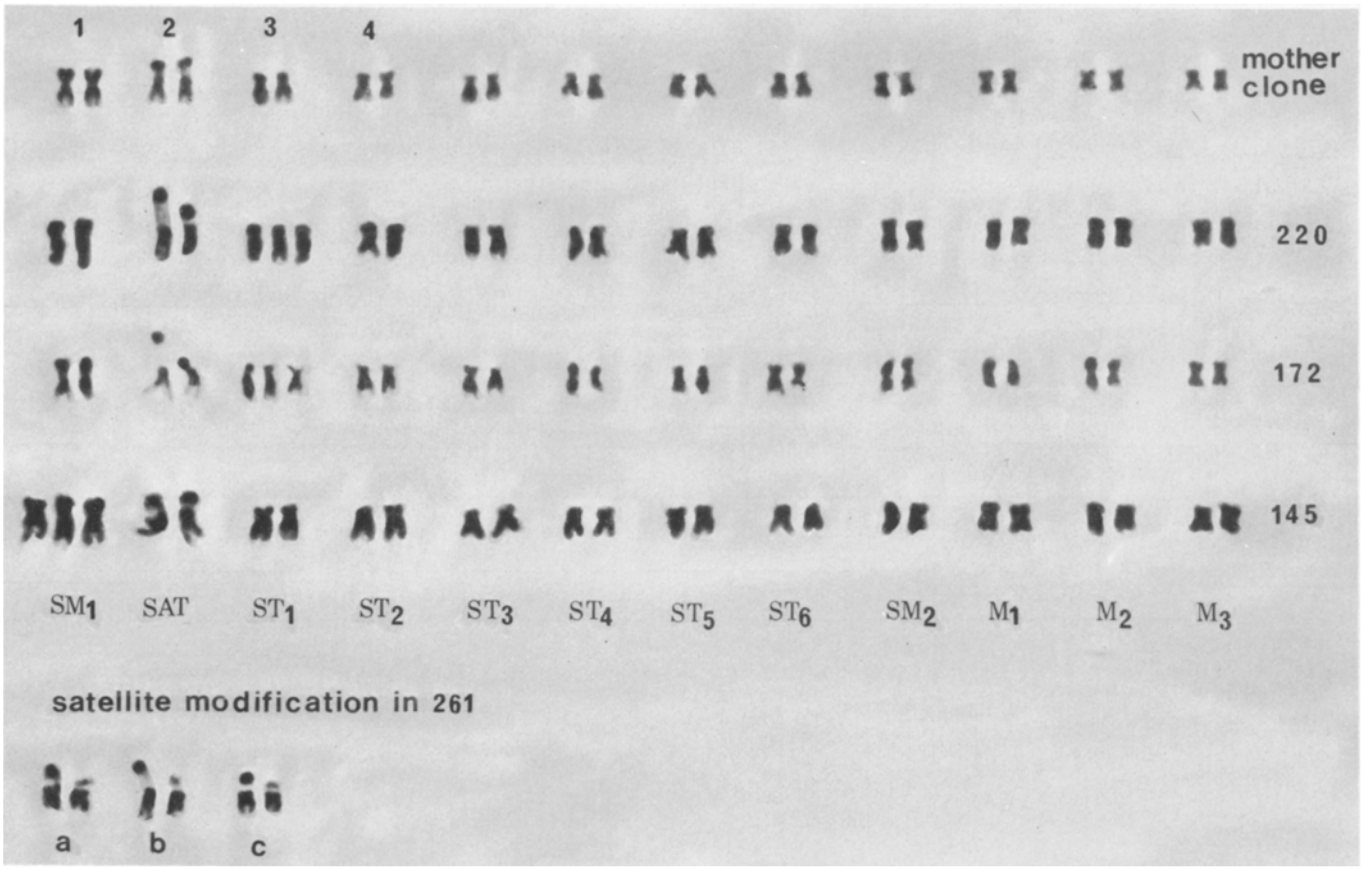

Fig.1. Somatic metaphase chromosomes in diploid mother clone (F13-$S_{12}S_{13}$) and single trisomics for chromosome 1 (plant 145) and chromosome 3 (plants 220 and 172); satellite modification in trisomic plant 261 (a, b, c refer to the satellite chromosome pair in 3 different cells in which one homologue shows 60% deletion in satellite part). SM, SAT, ST and M refer to respectively sub-median, satellite, sub-terminal and median chromosomes

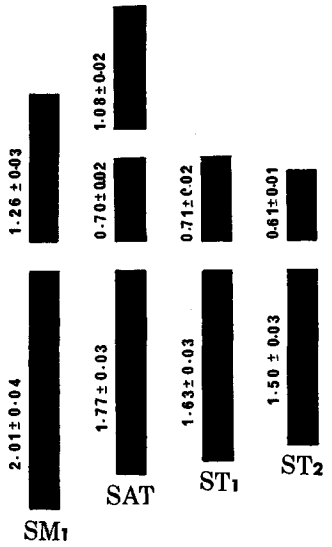

Fig.2. Idiogram of chromosome 1, 2, 3 and 4 (mean length of chromosome arms in mμ)

arm ratio and presence or absence of satellite, the karyotype can be subdivided into 3 median, 2 sub-median, 6 sub-terminal and 1 satellited pairs of chromosomes.

Measurements performed on the four longest chromosomes (Fig.2) revealed that chromosome 1, with a total length of 3.27 mμ, is the second longest chromosome. It is asymetric with a sub-median centromere. Chromosome 2, the longest of the complement (3.55 mμ), is the nucleolar chromosome. The satellite is attached to the short arm and greatly facilitates its identification. Chromosomes 3 and 4, almost equal in length (2.34 and 2.11 mμ), are subterminal.

Karyotype of the self-compatible trisomics

Chromosome identification was carried out only in the case of the trisomic plants which yielded a certain amount of fruits and seeds upon selfing and was thus essentially restricted to plants 172, 220 and 145. The examinations, summarised in Fig.1, reveal that plant 145 is trisomic for chromosome 1 while plants 220 and 172 display three copies of chromosome 3.

Meiosis in the self-compatible trisomic (plant 220) and in the trisomics setting some seed upon selfing

Chromosome association in diakinesis and metaphase I and the distribution of chromosomes in anaphase I and II were studied in trisomics for chromosome 1 (plant 145) and for chromosome 3 (plants 220 and 172). Cytological analysis at diakinesis (Table 7) indicated that no association of more than three chromosomes nor any ring trivalents were formed, and that these trisomics were therefore of primary type.

At diakinesis and metaphse I in the PMC's of both triplo-1 and triplo-3, the chromosome associa-

Table 7. Chromosome associations in diakinesis and first metaphase of meiosis in single trisomics

		Diakinesis								
			% PMC's with					Mean association per PMC		
Plant No.	Trisomic	Total no. of PMC's	12II+1I	1III+11II	11II+3I	10II+5I	9II+7I	8II+9I	III	II	I
145	Triplo-1	36	66.67	19.44	13.89	-	-	-	0.19	11.67	1.09
220	Triplo-3	61	70.50	13.47	10.42	5.61	-	-	0.13	11.61	1.38
172	Triplo-3	40	67.48	16.32	16.20	-	-	-	0.16	11.60	1.30
	Mean of the 2 triplo-3	101	68.99	14.89	13.31	2.80	-	-	0.14	11.61	1.34
Metaphase											
145	Triplo-1	56	50.00	14.29	35.71	-	-	-	0.17	11.50	1.57
220	Triplo-3	118	51.69	6.38	25.83	8.48	5.08	2.54	0.06	11.25	2.32
172	Triplo-3	64	59.38	9.50	18.62	12.50	-	-	0.09	11.58	1.77
	Mean of the 2 triplo-3	182	55.53	7.94	22.23	10.49	2.54	1.27	0.07	11.41	2.04

tion of 12 II + 1 I (Fig.3c) was more frequent than the association of 1 III + 11 II (Figs.3a, b), 11 II + 3 I, or of other types (Table 7). The frequency of cells with 1 trivalent varied between the two plants of the same trisomic (220 and 172); this was also true with respect to other configurations. When the data of both plants 220 and 172 of triplo-3 were pooled together, and the mean frequencies of trival-

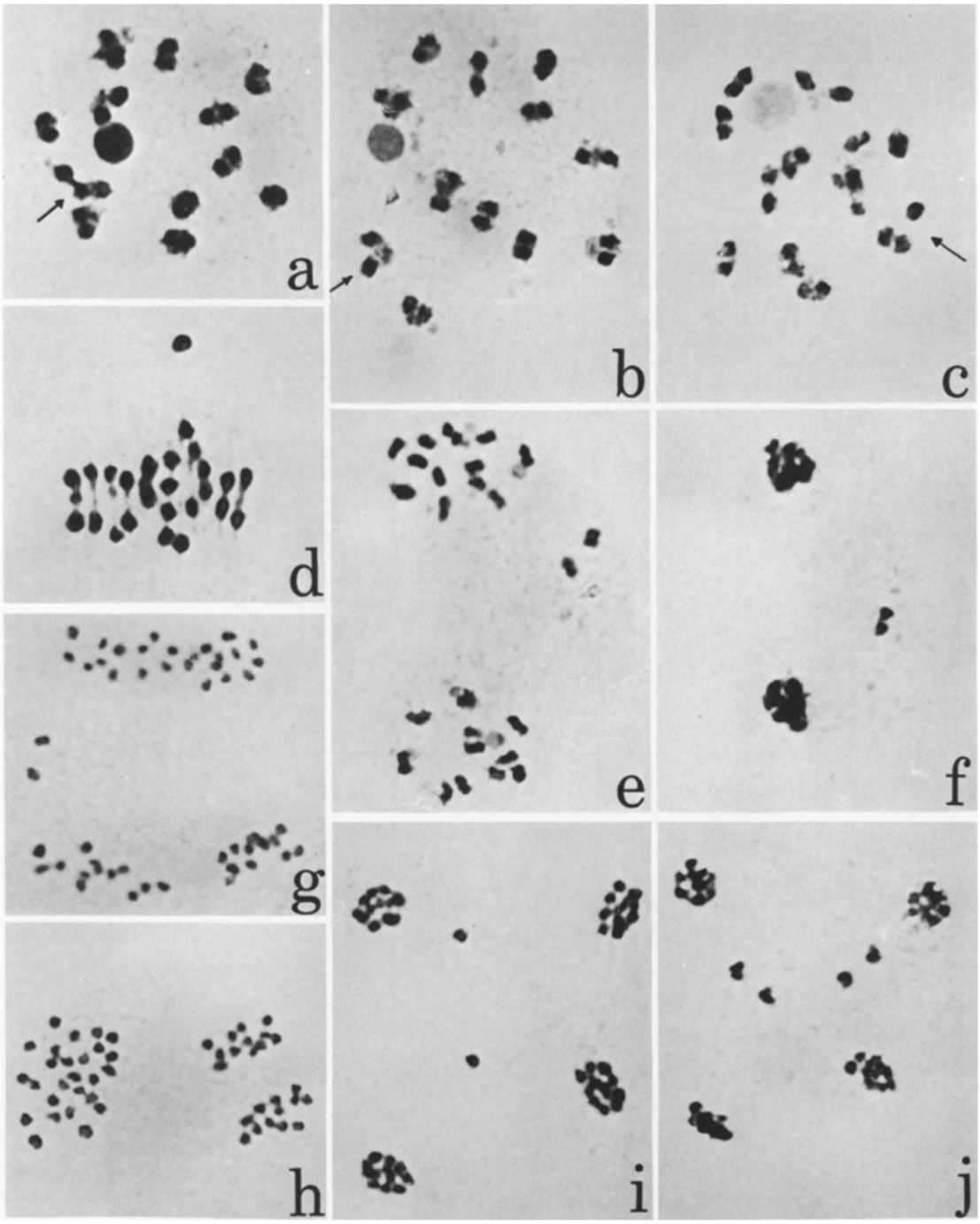

Fig.3. Chromosome associations at diakinesis and MI, and chromosome distribution in AI and AII of meiosis in single trisomics: diakinesis with 1 III + 11 II (a, b) and 12 II + 1 I (c); early disjunction of bivalents in MI (d); AI distribution 12-11 + 2 laggards (e); TI with 1 laggard (f); A II distribution 12-12-12-12+2 laggards (g); 12-12 and 26 (h); T II (early) with 2 laggards (i) and 4 laggards (j)

Table 8. Distribution of chromosomes at first and second anaphase of meiosis in single trisomics

Plant No.	Triscmics	% cells showing distribution of chromosomes				
		Anaphase I				
		total no. of cells	12-13	12-12+ 1 laggard	12-12+ 2 laggards	12-11+ 3 laggards
145	Triplo-1	144	57.29	27.08	11.46	4.17
220	Triplo-3	143	66.43	24.47	5.60	3.50
172	Triplo-3	212	48.58	43.39	6.13	1.19
	Mean of the 2 triplo-3	355	57.50	33.93	5.86	2.34

Table 9. Salient morphological characters of single and double trisomics derived from triploid-diploid crosses

Trisomics	Leaf	Flower			
		Color of buds	Shape of buds	Flower production	Flowering time
220(2n+1)	Margin not dentate	Normal	Tips blunt	Very low	Normal
172(2n+1)	More dentate	Bud tips rose	Tips blunt	Normal	Normal
145(2n+1)	Very less dentate	Yellowish green line on petals	Depression at top	Normal	Late
269(2n+1)	Cup shaped, elongate, not dentate	Normal	Blunt	Very low	Late
261(2n+2)	Not dentate, leaflets overlapped	Normal	Blunt	Low	Late
276(2n+2)	Long, narrow, tips pointed, not dentate	Buds top portion brownish, yellowish green line on petals	Long, narrow pointed	Normal	Late
160(2n+2)	Long, narrow and small leaflets, tips pointed, less dentate	Normal	Pointed	Low	Late

Late: by 8-10 days

ents were compared between them and triplo-1, a trend of variation was observed; at diakinesis the association, 1 III + 11 II occurred in 19.44% of the cells in triplo-1, whereas in triplo-3 it occurred in only 14.89% of the cells. At metaphase I also, triplo-1 showed a higher percentage of cells with one trivalent than triplo-3. It was observed that the frequency of cells with trivalents was relatively lower at MI than at diakinesis in both the trisomics. The most common trivalent configuration was V-shaped (Fig.3b), but chain configurations were also observed (Fig.3a).

Both the percentage of cells with univalents and the mean univalent frequency per cell were higher at MI than at diakinesis. In triplo-3, univalent frequency was higher than in triplo-1, particularly in plant 220 (triplo-3) where a maximum of 9 to 11 univalents per cell were observed at MI. In this plant as a result of an early disjunction of bivalents (Fig.3d), 6.56% of the cells in diakinesis and 16.10% of the cells in MI showed more than 3 univalents. The univalents were more frequently out of the metaphase plate.

The AI distribution of the extra chromosome in the trisomics was, in the majority of cases (57%), 12-13 (Table 8). In 27.08% and 33.93% of the cells respectively in triplo-1 and triplo-3, the chromosomes separated 12-12 with one chromosome lag-

Anaphase II						% cells with laggards	
Total No. of cells	12-12-13-13	12-12-13-12+ 1 laggard	12-12-12-12+ 2 laggards	12-12-12-12+ 3 laggards	12-12-12-12+ 4 laggards	Anaphase I	Anaphase II
149	71.81	8.72	17.45	1.34	0.67	42.71	28.18
116	87.55	3.89	7.00	-	1.56	33.57	12.45
120	96.00	4.00	-	-	-	50.71	4.00
236	91.77	3.94	3.50	-	0.78	42.14	8.22

Fruit				Seeds
Shape	Skin color	Size (circumference in mm)	Calyx	Size (100 seeds weight in gm)
Round	Light purple	Large (64)	Normal	Medium (0.10)
Round	Very light purple	Large (60)	Normal	Medium (0.10)
Round	Green, but with purple patches	Small (41)	Normal	Small (0.06)
Oval, stylar end pointed	Light green	Small (35)	Long, narrow sepals enclosed	Large (0.13)
Oval, stylar end pointed	Light green	Small (32)	Long, narrow sepals enclosed	No seeds
Round	Green but with brown patches	Medium (57)	Long, narrow sepals enclosed	No seeds
Oval	Light green	Small (42)	Long, narrow sepals enclosed	No seeds

ging. After the bivalents had completed their anaphase movement, the univalents remaining behind began to separate equationally (Fig.3f). The frequency of cells with 2 laggards was higher in triplo-1 (Fig. 3e) than in triplo-3. In both trisomics a low percentage of cells with 3 laggards was observed.

At AII (Table 8) the most regular distribution of chromosomes, i.e. 12-12-13-13, was found very frequently (71.81% in triplo-1 and 91.77% in triplo-3). There were also cells with 2, 3 or 4 laggards (Figs. 3g, i). It was observed that in some anaphase poles that had 26 chromosomes, anaphase movement seemed to occur later at the poles where 26 instead of 24 chromosomes were present.

Morphology of the trisomics

As *L. peruvianum* is an allogamous species and since several different stocks, with variable morphology, were used for producing the trisomics, there is little hope for any significant relationship in the trisomics between karyotype and phenotype. It is, however, interesting to note that plants 220 and 172, both of which are trisomics for chromosome 3, exhibited similar features for the colour, shape, and size of their fruits (Table 9). Also the size and weight of the seed were practically identical. The resemblance between the two plants, which differed for other characters such as leaf morphology (Fig.4, B) and

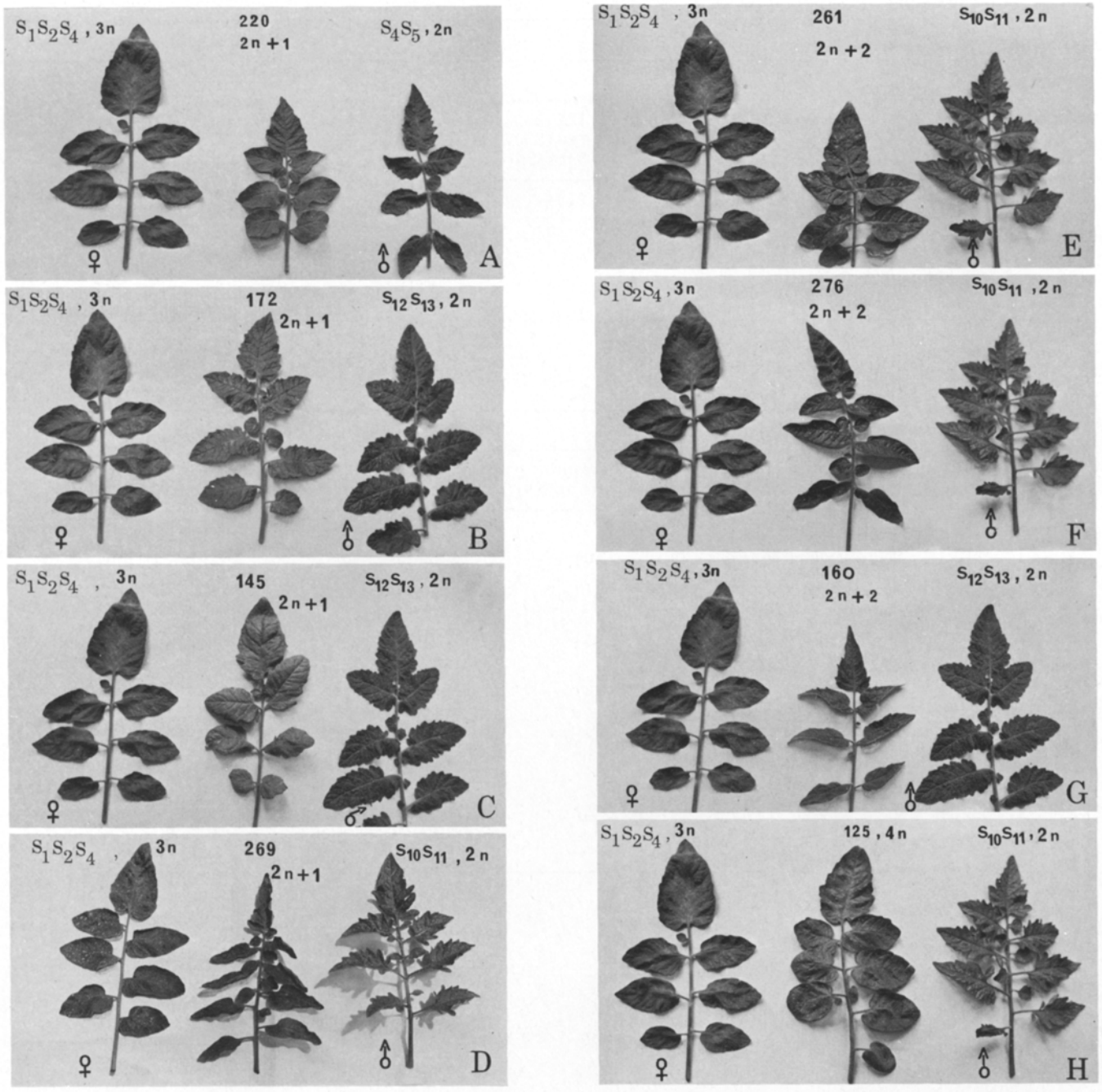

Fig.4. Leaf phenotype in single and double trisomics and in the tetraploid obtained from triploid-diploid crosses; each figure shows the leaf phenotype of the two parents and of the trisomics

flowering capacity, undoubtedly must have a meaning because plant 145 (triplo-3), although it derived from the same crosses as plant 172, expressed a completely different phenotype.

Among the other trisomics which were submitted to detailed morphological analysis, one must note the plants 269 and 261, with respectively one and two extra chromosomes and characterized by modifications of the satellite (complete deletion of the satellite in 269 and deficiency of 60% in plant 261; Fig.1). These two trisomics originated from the same cross and presented similar fruit phenotypes, namely an

ovate shape with pointed stylar ends. However, the leaf characters and the general habit of these plants were different (Figs.4D, E; Table 9).

The remaining two double-trisomics (276 and 160) displayed long and narrow leaves with pointed tips (Figs.4F, G).

Nature of the self-compatibility character in the self-compatible trisomic (plant 220)

With the aim of ascertaining the site (pollen or style) of the self-compatibility character expressed by plant 220 (trisomic for chromosome 3 originated from the cross 3n - $S_1S_2S_4 \times$ 2n - S_4S_5), a series of reciprocal crosses were carried out with the testers S_1S_2, S_2S_4, S_2S_5, S_1S_4 and S_1S_5. The outcome of these test-crosses (Table 10) demonstrates beyond doubt that:

- plant 220 produces functional S_2 and S_5 pollen and does not yield any compatible pollen;

- the style is the site of the self-compatibility character expressed by plant 220 which behaves as if at least one of the two S alleles active in the pollen failed to function in the pistil.

Not a single self-compatible plant could be found among the progenies of the cross, plant 220 × 006 - S_1S_2 (Table 11).

The search for three S-specificities in trisomics expressing weak self-compatibility

The two primary trisomics (plant 145: triplo-1; plant 172: triplo-3) which, in addition to plant 220, had been selected in this study on the basis of their capacity to set seed upon selfing, were searched by means of appropriate test-crosses for the presence of 3 different specificities in the style. The results (Table 12) show that not a single case of crossing behaviour was observed which could suggest heteroallelism in the pistil of plant 145 or 172.

It is thus apparent, in view of these observations and of these modes for plant 220 and other plants, that neither chromosome 1 nor chromosome 3 can be considered as the bearer of the S-locus.

Not a single self-compatible plant could be found among the progenies of the crosses between plants

Table 10. Cross-compatibility relationships of the self-compatible trisomic (plant 220:triplo-3) to the tester stocks

Cross performed	No. of flowers pollinated	No. of fruits formed	Average no. of seeds per fruit
220 × S_1S_2	10	10	50.00
220 × S_1S_4	9	8	75.50
220 × S_1S_5	9	7	81.43
220 × S_2S_5	8	6	69.00
220 × S_2S_4	8	8	91.75
220 × S_4S_5	9	6	63.33
S_1S_2 × 220	8	8	Above hundred
S_1S_4 × 220	8	8	Above hundred
S_1S_5 × 220	9	9	Above hundred
S_2S_5 × 220	21	-	None
S_2S_4 × 220	8	8	Above hundred
S_4S_5 × 220	8	8	Above hundred

Table 11. Compatibility behaviour of F_1 plants derived from crosses between the trisomics and the diploid clone 006-S_1S_2

Cross	No. of F_1 plants selfed	Compatibility behaviour of F_1s	
		No. of SC plants	No. of SI plants
145 × 006-S_1S_2	19	0	19
172 × 006-S_1S_2	18	0	18
220 × 006-S_1S_2	20	0	20

Table 12. Crossing behaviours of triplo-1 (plant 145) and of triplo-3 (plant 172) with marker-stocks used as staminate parents

Cross made	Flowers pollinated	Fruits formed	Average no. of seeds per fruit
145 × 006-S_1S_2	15	9	12.10
145 × P10-S_1S_4	15	15	62.33
145 × P9-S_2S_4	7	3	17.33
145 × F13-$S_{12}S_{13}$	12	10	43.60
172 × 006-S_1S_2	11	6	60.66
172 × P10-S_1S_4	8	8	104.50
172 × P9-S_2S_4	11	9	126.33
172 × F13-$S_{12}S_{13}$	11	11	38.88

145 and 172, used as pistillate partners, and the diploid tester stock 006 - S_1S_2 (Table 11).

As in the case of the offspring of plant 220, all the progenies from crosses between plants 145 and 172, used as pistillate partners, and the diploid tester stock 006 - S_1S_2 were completely self-incompatible.

Discussion

The occurrence of aneuploidy and trisomy in the progenies of crosses between triploid and diploid plants of *L. peruvianum*

Aneuploids were recovered among the progenies of triploid × diploid crosses with an overall frequency of 40% and almost all of them occurred in crosses where the triploid was used as pistillate parent. It is thus evident that the transmission of n + 1 and n + 2 gametes essentially takes place, as reported for other plants (see for instance Tsuchiya 1967; Gill et al. 1970), through the embryosac and not via the pollen.

As aneuploid plants with one extra-chromosome occurred in higher frequencies than plants with more than one extra-chromosome, it is clear that the distribution of aneuploidy resulting from triploid - diploid crosses in *L. peruvianum* belongs to the type defined by Ising (1969) as trisomic (only single, double and triple trisomics are produced) and not to the aneuploid type (chromosome numbers from 2n to 3n) nor the bi modal type (maximum near the euploid numbers). The aneuploid type has been detected in *Solanum* (Vogt and Rowe 1968) and in other genera of higher plants (see Khush 1973).

Types of primary trisomics found

Since only the aneuploid plants expressing a certain degree of self-compatibility were selected for the cytological identification of their extra-chromosomes, it is highly probable that only a fraction of the viable trisomics of *L. peruvianum* was detected in the present study. The 12 primary trisomics can be obtained in the cultivated tomato, *L. esculentum*, (Rick and Barton 1954) and it is more than likely that they can also be produced in *L. peruvianum* because we detected in this species cases of aneuploidy characterized by the presence of 3 or 4 extra-chromosomes which are not tolerated in *L. esculentum*.

Whatever the exact situation may be, it is clear from our results that triplo-1 and triplo-3 can be obtained with ease in *L. peruvianum* and that these trisomics are perfectly viable and relatively fertile as pistillate and staminate parents.

Morphological distinctness of single and double trisomics

Khush (1973) reviewed the morphological features of trisomics in 29 different species and concluded that the trisomics of diploid species with few duplications in their genomes were morphologically distinct and, in contrast with the situation in polyploid species, could be distinguished on the basis of their phenotypes. This conclusion is particularly well supported by the situation in *Datura* where the trisomics can be identified at all stages of growth and in cultivated tomatoes where each chromosome in triplicate is associated with a distinct syndrome of morphological changes (Rick and Barton 1954). The observations made for *L. esculentum* by Rick and Barton (loc. cit.) in the case of triplo-1 and triplo-3 do, to some extent, also apply to triplo-1 and triplo-3 in *L. peruvianum*. As in the trisomic for chromosome 1 in *L. esculentum*, triplo-1 in *peruvianum* displays smaller flowers and smaller fruits but its leaf segments, stems and other plant - parts resemble wild type and do not show the reduction in size characterizing the *esculentum* trisomic. The same observation applies in the case of triplo-3 which, exhibiting in *peruvianum* the larger fruits and seeds typical of triplo-3 in *esculentum*, failed to produce the elongate leaves, stem internodes and inflorescenses of its *esculentum* counterpart. In addition, clear morphological differences were found in the present study between the two different *peruvianum* plants of the same trisomic (triplo-3).

Vigour and fertility of the trisomics

Khush (1973) in his review on the morphology of trisomics showed that the unbalance caused by the pre-

sence of an extra chromosome in diploid species usually leads to reduction in vigour and fertility. Such an effect is not observed in polyploids which obviously tolerate duplications in their genomes.

Differences in tolerance to additional chromosomes also appear to distinguish wild species from cultivated forms. The thesis is supported by the fact that the trisomics of wild species such as *Clarkia unguiculata* (Mooring 1960), *Collinsia heterophylla* (Dhillon and Garber 1960), and *Solanum chacoense* (Hermsen et al. 1970) are vigorous, fertile and indistinguishable from each other and from the disomics. In trisomics of wild *Hordeum spontaneum*, vigour, fertility and the transmissibility of trisomy were higher than in the cultivated barley (Tsuchiya 1960; Burnham 1962). Rick and Notani (1961) reported that the trisomics in the primitive tomato variety 'red cherry' were as vigorous and fertile as the disomics and concluded that wild or primitive forms display tolerance to trisomy as a part of their plasticity and ability to withstand various unfavourable situations. The observations made in the present study clearly confirm such a conclusion since the average vigour and fertility of the trisomics detected in *L.peruvianum* were not much different from those of their disomic sibs. Furthermore, the survival in *L.peruvianum* of plants with three and four chromosomes definitely demonstrates the tolerance of *L.peruvianum* to certain types of aneuploidy which are not accepted by the cultivated tomato.

Cytology of the trisomics

The analysis of diakinesis and MI in triplo-1 and triplo-3 clearly revealed that these were of the primary type. The frequency of various types of chromosome configurations differed between triplo-1 and triplo-3, and the trivalent frequency was lower at MI than that at diakinesis. Several authors demonstrated that trivalent frequency is chromosome dependent and is lower at MI than at earlier stages of meiosis. The frequency of various types of chromosome associations may be variable for the trisomics of different species, and is generally different for the trisomics of the same species (see review in Khush 1973). The frequency with which a trisomic forms a trivalent depends on the total length, since longer chromosomes have a higher chiasma frequency than shorter ones (Einset 1943; Rick and Barton 1954). The different members of the chromosome complements of barley (Tsuchiya 1960) and *Nicotiana sylvestris* (Goodspeed and Avery 1939) are almost the same size, and no significant differences in the frequency of cells with trivalents were observed. In wild *L.peruvianum*, where the various chromosomes of the complement differ in their total length, there appears to be some relationship between the frequency of trivalents and the length of chromosomes; slightly higher trivalent frequency was found in triplo-1 than in triplo-3. It was observed that the trivalent frequency of 15% (triplo-3) and 20% (triplo-1) at diakinesis of *L.peruvianum* trisomics is comparatively much lower than that observed in *L.esculentum* trisomics for chromosome 1 (64%) and chromosome 3 (70%) (Rick and Barton 1954). The reason for the low trivalent frequency in the wild species of tomato is however not clear at present, and a detailed analysis on pairing and terminalisation in the other primary trisomics might give a clue in this respect.

Chromosome distribution at anaphase I was not very regular in either triplo-1 or triplo-3; about 40% of PMC's showed laggards in varying numbers. The extra chromosome when present as a univalent behaves irregularly:

i) it may pass to one of the poles at AI undivided, and divide normally at AII,

ii) it may lag and fail to be included in any of the TI nuclei, or

iii) it may divide equationally, and sister chromatids pass to opposite poles, or one or both may lag and fail to be included in TI nuclei.

The frequency of cells with laggards at AI was similar in both triplo-1 and triplo-3. At AII, the frequency of cells with laggards was higher in triplo-1 than in triplo-3. Einset (1943) working with trisomics of maize found a positive correlation between the length of trisomics and the frequency of trisomics. He also observed a lower frequency of lagging chromosomes at AI in trisomics involving longer chromosomes and a higher frequency of lagging chromosomes in trisomics involving shorter chromosomes. In *L.peruvianum* there was a somewhat higher frequency of trivalents in the trisomic for the longer chromosome, but no positive relationship between chromo-

some length and the frequency of cells with laggards was observed. Chen and Grant (1968a, b) reported that in *Lotus pedunculatus* there was more trivalent formation in trisomics involving longer chromosomes, but no correlation was observed between the chromosome length and frequency of laggards.

The influence of trisomy on the incompatibility character in the pollen

One of the objectives of the present study was to detect individuals trisomic for the S-bearing chromosomes and producing, through competition effects in hetero-S-allelic pollen, a certain amount of pollen compatible upon selfing. As the self-compatibility character of the self-compatible trisomics detected in the present study did not originate from a change in the pollen phenotype but from alterations in the style, the conclusion must be reached that either trisomy for the S-bearing chromosome is not tolerated in *L.peruvianum* or that competition effects do not occur in the pollen of plants which are trisomic for the S-chromosome. The second alternative is probably the correct one because the triploid stock, which gives rise to the trisomic progenies and which is known to display three different S-alleles, completely failed to produce the slightest amount of self-compatible pollen and yet regularly set some seed as staminate partner in compatible crosses. It is however possible that the fertile pollen yielded by the triploid never contained more than a single copy of the S-bearing chromosome.

Origin of stylar compatibility in triplo-3 and in the other plants setting a certain amount of seed upon selfing

The origin of stylar compatibility in triplo-3 (plant 220) and in the other trisomics which yielded some seed after self-pollination is not known at this time. From the fact that self-compatibility was found in only a few trisomics and, to some extent in certain disomics, it is probable that there is no causal relationship between trisomy and the expression of stylar self-compatibility. It is also clear, since the character could not be transmitted to the progenies of the trisomics, that stylar self-compatibility did not result from a permanent alteration of the S-locus. It thus seems that the observed breakdown of the incompatibility phenotype corresponds to a temporary state of pseudo-compatibility similar to the one previously described by Pandey (1959). Another explanation, however, could be that new S-alleles were generated, in substitution for one of the parental alleles, in the pistil of certain individuals, such as plant 220, which thus became receptive to half of their own pollen and yet transmitted a fully functional system of self-incompatibility to their progenies. This possibility, in complete agreement with what is known about the generation of new incompatibility alleles in inbred populations of *L.peruvianum* (de Nettancourt et al. 1971), is presently being tested through a series of progeny analyses with marker stocks.

The detection of trisomics with 3 specificities in the style

Since all the trisomics detected in the present study had been selected on the basis of their capacity to set a certain amount of seed upon selfing, and since this tendency towards self-compatibility unfortunately turned out to originate from modifications in the style rather than in the pollen, it is not possible to know if the cross-compatibility of the trisomics with the tester-stocks resulted from the stylar compatibility character alone or from the absence in their styles of the S-alleles present in the testers. It is thus impossible to reach any conclusion on the occurrence in *L.peruvianum* of trisomics expressing three different S-specificities in their pistils.

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