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Genetical and Ultrastructural Aspects of Self and Cross Incompatibility in Interspecific Hybrids between Self-Compatible *Lycopersicum esculentum* and Self-Incompatible *L. peruvianum*[1]

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Summary. Cytological and genetical analyses were made of the breeding system of embryo-cultured interspecific tomato hybrids between *L. esculentum* and *L. peruvianum*. It was found that fluorescence techniques and electron microscopy allowed a distinction to be made between pollen tubes inhibited by a unilateral incompatibility reaction and pollen tubes inhibited by a self-incompatibility reaction, after self-pollination of the hybrids or after reciprocal crossing between the hybrid and the parental species. The observed differences, if real and reliable, demonstrate that unilateral incompatibility in *esculentum* pollen tubes is governed by a single gametophytic factor which is either linked or allelic to the S-locus. This finding is discussed with reference to recent reports that unilateral incompatibility is controlled, in *peruvianum* styles, by a number of different dominant genes and it is concluded that these dominant genes, the S-locus of self-incompatibility and the gametophytic factor regulating the unilateral reaction in *esculentum* pollen belong to the same linkage group. The strong sterility barriers which prevent practically all backcrosses between the hybrid and the parental species were shown to be independent of the factors regulating stylar incompatibility. *L. peruvianum* is heterozygous for the sterility genes which prevent fertilization or embryo formation when the interspecific hybrid is crossed, as pistillate parent, to different accessions of *L. peruvianum*. One *peruvianum* stock was found which, as a pollinator, was highly cross-fertile with the hybrids.

The presence of a concentric endoplasmic reticulum in inhibited pollen tubes was observed to be a constant feature of both the self- and the unilateral incompatibility reactions and was interpreted as an indication that incompatibility might lead to a general cessation of protein synthesis. Although incompatible tubes very much resemble, in this respect, the pollen tubes cultured *in vitro*, it seems probable, on theoretical grounds, that the inhibition of pollen tubes in incompatible styles does not result from an absence of growth promoting substances but from the presence of a metabolic inhibitor.

Introduction

The genetic system controlling self-incompatibility in *Lycopersicum* (Lamm, 1950; McGuire and Rick, 1954; Rick and Butler, 1956; Günther *et al.*, 1968; de Nettancourt *et al.*, 1971; Hogenboom, 1972c) and the mechanism of early embryo-abortion, which takes place when self-compatible species used as pistillate parents are crossed with certain self-incompatible species, have been identified but little information is available on the factors governing the incapacity of most self-incompatible species to yield viable seeds upon cross pollination with self-compatible species used as staminate parents. The isolating mechanism appears to be complex and to comprise two components: a pre-zygotic one which takes place in the style and can be equated to unilateral cross-incompatibility; and a second which prevents normal fertilization by pseudo-compatible pollen or leads to an early abortion of hybrid embryos. The detailed situation is, however, not known and even where the unilateral cross-incompatibility reaction takes place in the style, few data are available on the nature of the rejection process and on its relationship to the self incompatibility reaction. The work of Martin (1961a, 1961b, 1964, 1967, 1968), that of Hogenboom (1972a, 1972b, 1972d) and the general evidence accumulated from other plant genera (for a review, see Abdalla and Hermsen, 1972) convey the impression that either different genetic loci govern the two types of incompatibility reaction or that additional genes are involved in the manifestation of unilateral cross-incompatibility.

In view of the recent suggestion (de Nettancourt *et al.*, 1973b) that fluorescence techniques and electron microscopy permit a distinction to be made, in the genus *Lycopersicum*, between pollen tubes which

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are inhibited by the reaction of self-incompatibility and pollen tubes inhibited by the reaction of unilateral cross-incompatibility, it appeared worthwhile to use the distinctive features of each of these two types of inhibition for observing the transmissibility of self and unilateral incompatibility in tomato hybrids derived from crosses between a self-incompatible and a self-compatible species. Theoretically, and assuming the presence of a truly reliable marker, it should be possible with an analysis of this type to find out if unilateral incompatibility is governed by gametophytic or sporophytic factors and if the two types of reaction segregate independently of one another, either as unlinked loci or as alleles of the same genetic complex.

With such an aim in mind, the following research was carried out:

— production, by means of *in vitro* culture techniques, of an interspecific hybrid between the self-incompatible species (*L. peruvianum*) and the self-compatible species (*L. esculentum*), which had been used previously for comparing the ultrastructural features of self-incompatibility (de Nettancourt *et al.*, 1973a) with those of unilateral cross-incompatibility (de Nettancourt *et al.*, 1973b).

— analysis, by means of a crossing scheme identical to the one followed many years ago and for a similar hybrid by McGuire and Rick, in 1954, of the breeding system of this hybrid and of its compatibility relationships with the parental species.

— observations, by means of fluorescence techniques and electron microscopy, of the incompatible pollen tubes present in styles after self-pollination of the hybrid and after reciprocal cross-pollinations between the hybrid and various *esculentum* and *peruvianum* stocks.

— finally, in view of the recent report by Kroes (1973) that the inhibition of incompatible tubes resembles very closely the general cessation of growth occurring when pollen tubes are cultured *in vitro*, an attempt to analyse the ultrastructural features of tomato pollen tubes growing on artificial culture medium.

The results obtained in the course of this research are presented and discussed below.

Material und Methods

Production of interspecific hybrids between *L. esculentum* and *L. peruvianum*: pollinations were made on castrated flowers of *L. esculentum* cv. San Marzano Baldoni with mature pollen from the same S_1S_4 clone of *L. peruvianum* which had been used previously (de Nettancourt *et al.*, 1973a, 1973b). The *in vitro* culture technique used for by-passing the post-zygotic barrier which prevents viable hybrids to be formed when *L. esculentum* is the pistillate parent was that of Smith (1944).

Determination and analysis of self- and cross-compatibility relationships in the hybrid: self-pollinations on the hybrid and reciprocal cross-pollinations between the hybrid and tester stocks of the two parental species (*L. esculentum* San Marzano Baldoni and several *peruvianum* stocks all related to the accession used for producing the hybrids) were performed on flowers which had been bagged at the bud stage. In addition, reciprocal crosses were conducted between the hybrid and a completely unrelated accession of *L. peruvianum* (n° 959 in the Germplasm Bank at the Universidad Nacional Agraria in Lima, Peru) which had been kindly provided by Dr. M. Holle. Approximately 30 flowers were pollinated for each type of cross and about half of these were left on the plant to allow an estimation of fruit-setting and seed-set; the other flowers were harvested 24 hours after pollination and their styles prepared for analysis by means of fluorescence techniques or for observation under the electron microscope. As the two hybrids which were obtained in the course of the experiments (see section on Results) were both completely self-incompatible and cross-incompatible with one another, they were assumed to have received the same S allele from their *peruvianum* parent and were used without distinction, either as staminate or pistillate genitors, in the various series of backcrosses performed in this study.

In vitro culture of pollen: pollen grains from *L. esculentum*, *L. peruvianum* and from the interspecific hybrids were cultured on the medium recommended by Brewbaker and Kwack (1964) with 12% saccharose.

Electron microscopy: two types of fixatives were used for the styles and for the pollen tubes grown *in vitro*.

1. 5% glutaraldehyde in 0.075 M phosphate buffer, pH 6.9, at room temperature for 3–4 hours. The specimens were rinsed in the phosphate buffer solution for 3 hours, fixed in 1% OsO_4 in the same buffer during 2–3 hours, dehydrated in ethanol and embedded in Epon-Araldite.
2. In this case the fixative consisted of 1.2 g of paraformaldehyde and 5.5 ml of glutaraldehyde (25%) in 40 ml of phosphate buffer (0.15 M at pH 7.4). After three hours in the fixative, the styles were rinsed in the phosphate buffer and post-fixed with 1% OsO_4 in the phosphate buffer solution containing 5% sucrose. The material was dehydrated in acetone and embedded in Araldite.

Sections were cut with an LKB Ultrotome III, stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963), and observed with a Zeiss electron microscope EM 9A.

Fluorescence microscopy: the techniques involved were those which had been used previously (de Nettancourt *et al.*, 1973a, 1973b) and originally described by Linskens and Esser (1957) and by Martin (1958).

Results

Production of interspecific hybrids between *L. esculentum* and *L. peruvianum:* From 5 fruits which were harvested 40 days after interspecific pollinations between the two species, 24 embryos could be excised which were of sufficient size for transplantation and culture in the artificial medium of Smith (1944). Twenty-two of these embryos failed to grow on the medium while two developed normally and reached the seedling stage approximately 6 weeks after excision from the fruits. The plantlets were vigorous and displayed for several characters discreet but unmistakable signs (intermediate pilosity and intermediate stem pigmentation) of their hybrid origin, which was later confirmed by the appearance of the flower (very similar to that

Table 1. *Cross-compatibility and fertility relationships of the hybrids, as estimated by pollen tube growth analyses and seed-setting upon selfing and after crosses with L. esculentum and various peruvianum stocks*

Material	Types of pollen tube reactions in the style			Estimated seed-set		
	C	SI	USI	n⁰ of flowers pollinated	fruits obtained	seeds per fruit
(E × P) self		+	+	48	4	0.0
(E × P) × P.S_1S_2	+(*)	+		9	4	4.8**
(E × P) × P.S_1S_3				6	5	2.8**
(E × P) × P.S_1S_4	+	+		25	14	1.0**
(E × P) × P.S_2S_3	+			17	11	1.3**
(E × P) × P.S_4S_5				15	10	1.2**
(E × P) × $P_{\cdot 959}$	+	+(*)		23	19	18.0**
(E × P) × E			+	19	2	0.0
P.S_1S_2 × (E × P)		+	+	20	0	0.0
P.S_1S_3 × (E × P)				9	7	0.4
P.S_1S_4 × (E × P)		+	+	30	27	0.0.
P.S_1S_5 × (E × P)				22	0	0.0
P.S_2S_3 × (E × P)	+		+	7	6	≠
P.S_4S_5 × (E × P)	?	+(*)	+	16	7	2.8
$P_{\cdot 959}$ × (E × P)	+(*)	+(*)	+	36	21	0.4
E × (E × P)	+			10	10	0.0**

(*) only few tubes observed.
** very large number of aborted seeds or swollen ovules also present in the fruits.
≠ plant died before fruit maturity.
C: compatible growth; SI: self-incompatibility; USI: unilateral stylar incompatibility.

Fig. 1 a–b. Extremities of pollen tubes observed by means of fluorescence in incompatible styles. Fig. 1 a. Self-incompatible pollen tube showing a large apical vesicle practically devoid of callose. Fig. 1 b. *Esculentum* tube in *peruvianum* style; the pollen tube has opened and shows a thick callosic plug in the sub-apical region. ca. 1000 ×

of the staminate parent, *L. peruvianum*), the manifestation of self-incompatibility and, after compatible matings, the intermediate size and bright yellow coloration of the fruits. Pollen abortion, as estimated after staining with aceto-carmine, averaged 16%.

Compatibility relationships between the hybrids and the parental species: The observations on pollen tube growth, as analysed by means of the fluorescence techniques, and the data collected for fruit-setting and seed-set showed that the two hybrids were both completely self-incompatible and completely cross-incompatible with each other (table 1). After selfing or reciprocal cross-pollinations, the inhibited pollen tubes of the hybrids could be classified in two categories: one, which presented an accumulation of callose at the sub-apical portion of the apex, as in the case of the unilateral incompatibility reaction analysed previously (de Nettancourt *et al.*, 1973b; see fig. 1a), and one, which, as in the case of the self-incompatibility reaction (de Nettancourt *et al.*, 1973a; see fig. 1b), did not display such a feature but exhibited at the tube tip a large swollen vesicle practically devoid of callose.

When used as pistillate parents in crosses with the different tester-stocks of *peruvianum*, the hybrids consistently produced a large number of fruits (table 1): such fruits contained from 5 to 30 seeds in those cases where the male parent was the unrelated stock (clone 959) provided by Dr. Holle, and from 0 to 3 seeds after pollination with the related S_1S_2, S_1S_3, S_1S_4, S_2S_3 and S_4S_5 accessions. Observations by fluorescence showed that in the sterile crosses only S_1 had been inhibited in its growth through the style, the S_1S_4 and S_1S_2 staminate parents being the only ones to behave as semi-incompatible pollinators. It is, therefore, probable that the incompatibility allele transmitted to the hybrid by the S_1S_4 *peruvianum* parent was S_1. The inhibited tubes containing such an allele displayed the specific features of the self-incompatibility reaction which can be detected by the fluorescence technique. The fact that S_2, S_3, S_4 and S_5 pollen tubes had been able to reach the ovary of the hybrid was further demonstrated by the presence, in the fruits developed after pollination with tester plants containing such alleles, of many swollen ovules which had presumably been induced to swell by some kind of stimulus or had been fertilized and produced inviable zygotes.

In the reciprocal crosses to the *peruvianum* testers (*peruvianum* × hybrid) many extremely small aborted fruits with no, or, rarely, one or two normal-sized seeds were generally obtained. The highest seed yield was found after the crosses involving the S_4S_5 tester (more than one seed per pollinated flower) and clone 959. The lowest fruit-set and seed-set were estimated in the case of crosses with S_1S_2 and S_1S_4 testers. The fluorescence analysis revealed that all hybrid pollen tubes were inhibited in *peruvianum* styles containing an S_1 allele. In the vast majority of cases, the inhibited tubes, which could often be observed to have lost their extremities, presented the fluorescence pattern of tubes inhibited by the unilateral incompatibility reaction. For all crosses, however, even when the *peruvianum* tester did not contain the S-allele present in the hybrid, a number of tubes were found which appeared to display the features associated with the self-incompatibility reaction.

Electron microscopy analyses

a) Ultrastructural features common to all types of incompatibility reactions: In all types of incompatible pollen tubes (*esculentum* pollen tubes in hybrid styles, parental *peruvianum* pollen tubes in hybrid styles, hybrid pollen tubes in *peruvianum* styles, hybrid pollen tubes in hybrid styles), in addition to a great number of bipartite particles in the cytoplasm, a particular configuration of the rough endoplasmic reticulum was regularly observed (fig. 2a—2b). In section, the cisternae appeared as whorls of concentric parallel membranes, similar to those described by Shih and Rappaport (1971) who called them CER (concentric endoplasmic reticulum). A structure of this type had previously been shown in a microphotograph published in 1973a (de Nettancourt *et al.*) and was never observed in compatible pollen tubes (hybrid pollen in *esculentum* styles, non-parental *peruvianum* pollen in hybrid styles). It therefore appears that the presence of CER in a pollen tube shortly precedes or accompanies the manifestation of self- and cross-incompatibility.

Similarly, two other basic features of the self-incompatibility reaction in *L. peruvianum* and of unilateral incompatibility between *esculentum* and *peruvianum* (disappearance of the tube inner-wall and accumulation of bipartite particles in the tube cytoplasm) were also found to characterize the morphology of all the incompatible pollen tubes observed after back-crosses between the hybrids and the parental species or after self-pollination of the hybrids. It is therefore clear that these ultrastructural features are characteristic of all the types of incompatibility reactions analysed in the present study.

b) Ultrastructural features of pollen tubes cultured *in vitro*: The germination tests were relatively unsuccessful and, for all samples, only a small percentage of the pollen germinated. The length of the tubes usually did not exceed a few pollen diameters and, in most cases, the tubes opened and discharged their contents on to the medium. Although the experiments were not very satisfactory, it was interesting that, under such bad conditions, numerous bipartite particles, similar to those observed *in vivo* after self- or cross-incompatibility, were present in the pollen tubes and in the tube contents which had been discharged on to the medium.

Illustrations of bipartite particles in pollen germinated *in vitro* are presented in fig. 3a—b.

c) Ultrastructural features restricted to specific incompatibility relationships: As was outlined in a section above, the semi-compatible back-crosses between the hybrids, as maternal parents, and *peruvianum* stocks with an S_1 allele must lead, if S-alleles maintain their integrity and function in hybrid styles, to the rejection of S_1 pollen tubes by a mechanism identical to the one characterizing self-incompatibility. Similarly, the inhibition of hybrid pollen by *peruvianum* testers not bearing an S_1 allele or the inhibition of *esculentum* pollen by hybrid styles must be attributed to a rejection process analogous to that of unilateral incompatibility. In other words, if specific ultrastructural features do differentiate the self-incompatibility reaction from that of unilateral incompatibility, such variations ought to be observable between these two categories of matings.

Most of the observations made under the electron-microscope supported these assumptions. This was especially true for hybrid pollen (fig. 4a—b) which, after self-pollination, segregated for self-and cross-incompatibility features, and held equally well in the case of cross pollination between the hybrid as a maternal parent and the *peruvianum* accessions. After such crosses, the only cases of incompatible tubes were detected when the male progenitor produced S_1 pollen tubes and these tubes underwent the rejection process apparently similar to that of self-incompatibility. The expansion of the outer-wall was not, however, always as great as in the case of self-incompatibility and a number of tubes had to be classified for this character as intermediate between unilateral and self-incompatible ones. The situation was even more confusing after crosses between the *peruvianum* testers and the hybrids. In such cases, it appeared that a fraction of the hybrid pollen behaved like self-incompatible tubes even when the *peruvianum* styles did not carry the S-allele present in the hybrid plants.

In all cases where a reaction similar to that of unilateral incompatibility was expected, clear cases of "peeling-off" (degradation of the outer-wall which breaks down progressively) were recorded.

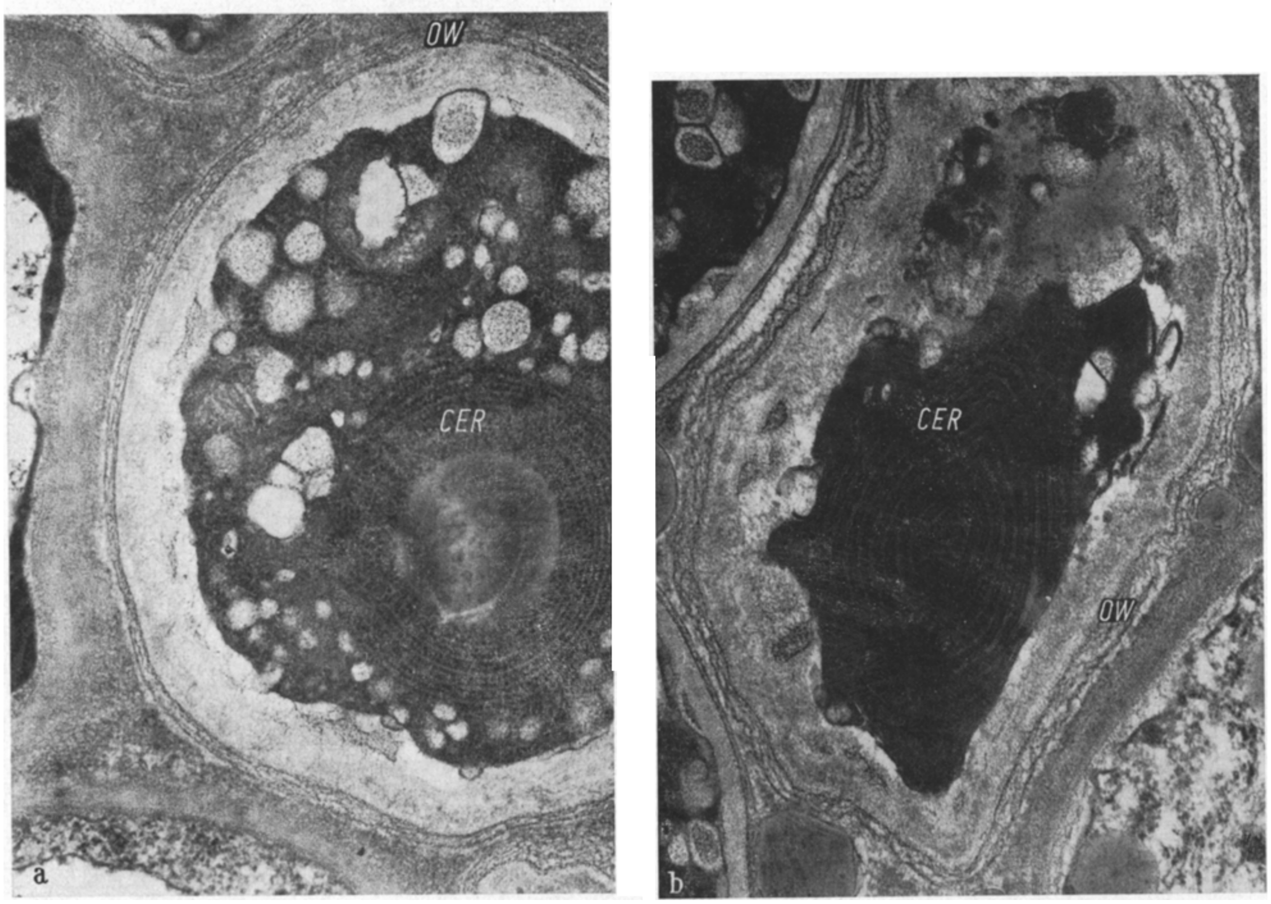

Fig. 2a—b. Concentric parallel configurations of the endoplasmic reticulum (CER) in cross-sections of *esculentum* pollen tubes inhibited in hybrid styles. Note the thin outer-wall typical of unilateral interspecific incompatibility (OW: outer-wall). Fig. 2a 41,000 ×, Fig. 2b 48,000 ×

All these observations made at the electron microscope corroborate those performed at the light microscope with the help of fluorescent techniques. With the exception of hybrid tubes which, in *peruvianum* styles, sometimes displayed unexpected self-incompatibility reactions, the predictable features of the self- and cross-incompatibility reactions could be traced back in the different crosses between the hybrids and the parental species. Hybrid styles obviously have the capacity to promote the two types of reaction and hybrid pollen appears to segregate, at least in hybrid styles and certain *peruvianum* styles, for both self- und unilateral incompatibility.

Discussion

1. Production of interspecific hybrids between L. esculentum and L. peruvianum

The production of interspecific hybrids between *L. esculentum* and *L. peruvianum* has been reported previously by Smith (1944), Alexander *et al.* (1942), Porte and Walker (1945), Yeager and Purinton (1946), Lincoln and Cummins (1949), Lesley (1950), Choudhury (1959), Nirk (1959), Majid *et al.* (1968), Günther *et al.* (1971) and Yamakawa (1971), and no claim for original research can be made in this respect in the present study. Yet, to our knowledge, this is the first time that an Italian cultivar of *L. esculentum* has been crossed successfully with *L. peruvianum*. For this reason, although the hybrids were produced with the main aim of analysing the cytological aspects of self- and cross-incompatibility, they, together with some of their advanced progenies, are now used in the plant breeding and disease resistance programme of the Casaccia Nuclear Centre in Italy.

The embryo-culture method (Smith, 1944) used proved to be very satisfactory if one considers that 2 hybrids were found after a single trial which involved the culturing of only 24 embryos. As underlined by Smith (l. c.), the limitation of the technique lies in the small number of hybrid embryos which are sufficiently developed (heartshape stage) to respond to *in vitro* culture.

As in the studies of Smith (l. c.) and of Lesley (1950), unmistakable signs were detected which demonstrated beyond any doubt the hybrid origin of the two plants obtained in our experiments. In our opinion, the most decisive characters for hybrid detection are the yellow coloration of the fruits and the complex incompatibility relationships of the hybrids to their parental species.

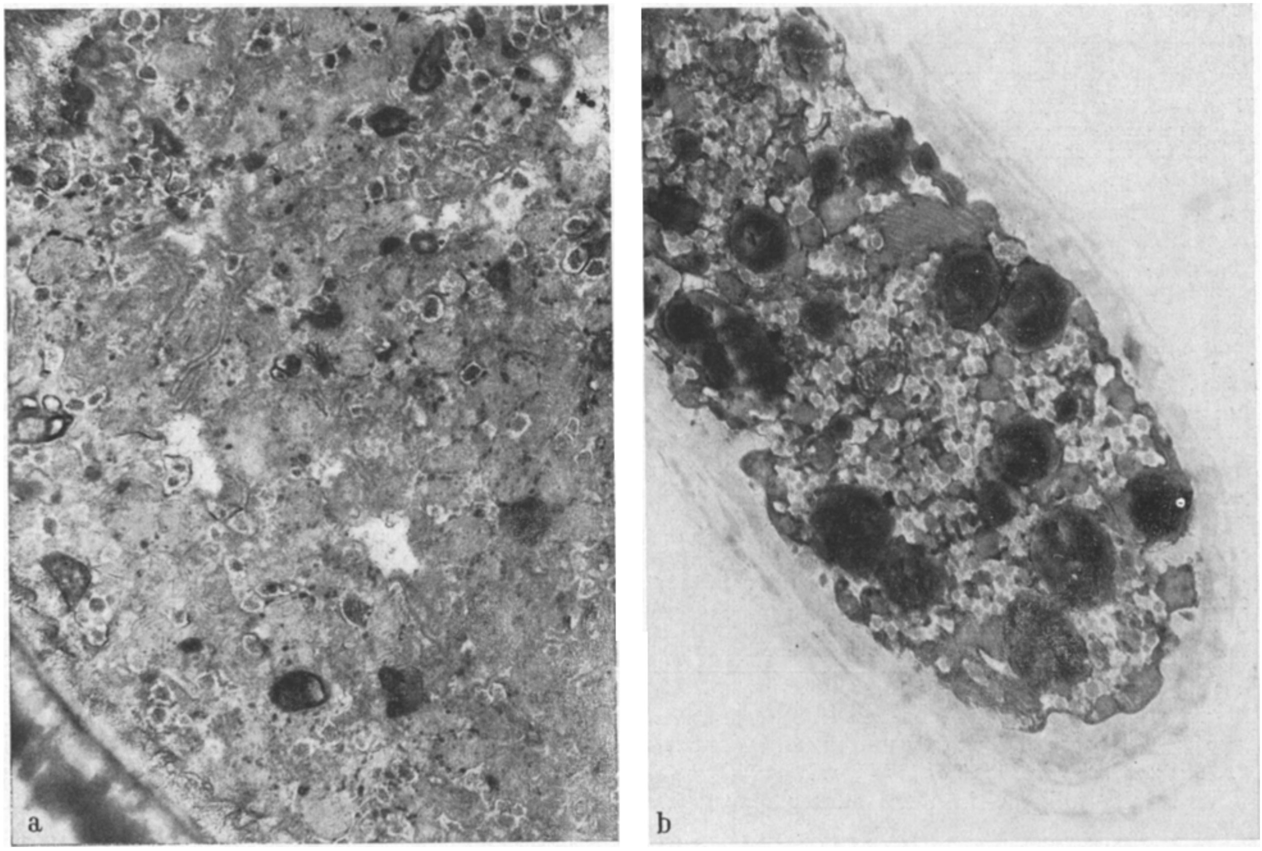

Fig. 3 a—b. Bipartite particles in pollen grains germinating *in vitro*. Fig. 3a. Germinating pollen of the hybrid, 24,000 ×; Fig. 3b. Pollen tube of *L. esculentum*, 18,000 ×

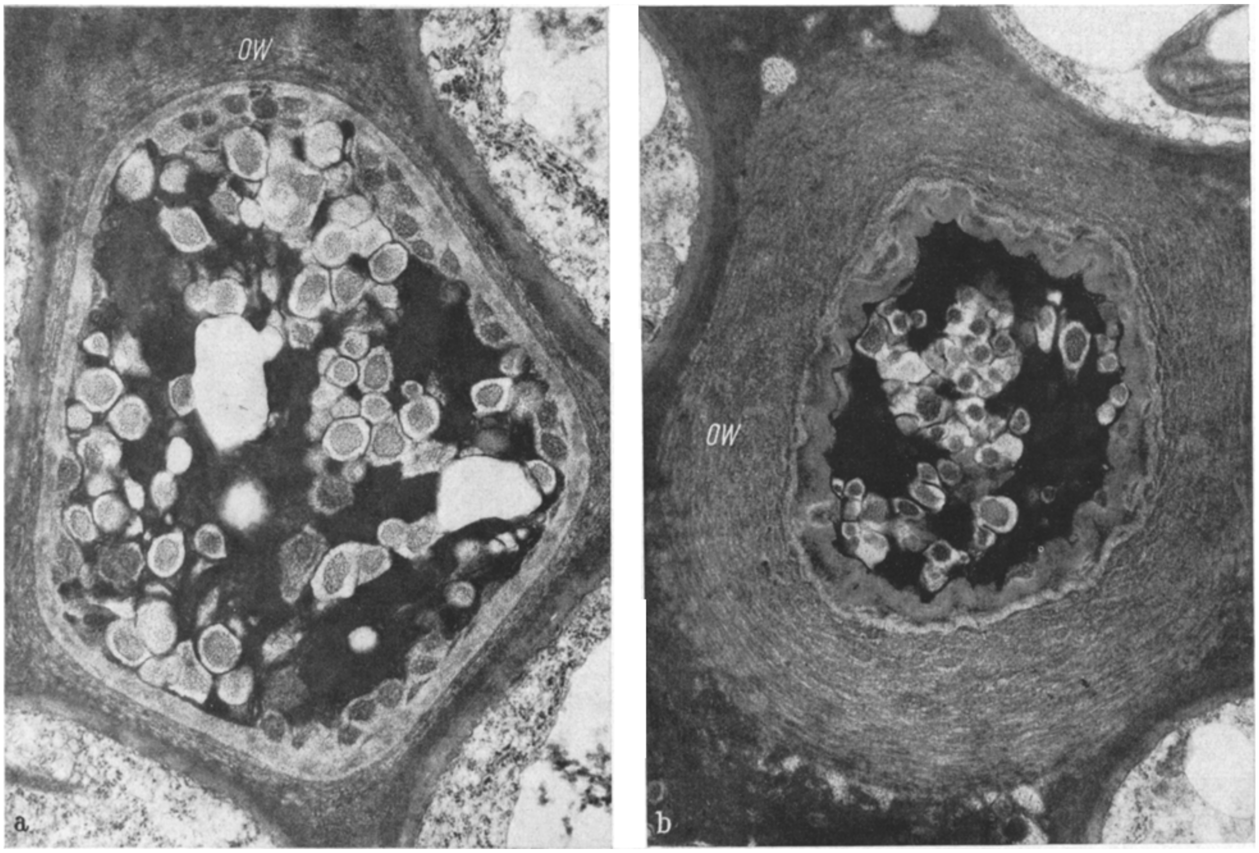

Fig. 4 a—b. Cross-sections of S_1S_4 styles of *L. peruvianum* after pollination with hybrid pollen. Fig 4a. An incompatible tube containing numerous bipartite particles presents the very thin outer-wall (OW) which is associated with unilateral interspecific incompatibility 26,400 × ; Fig. 4b. An incompatible tube presents the very thick outer-wall which is associated with self-incompatibility, 19,200 ×

♀				♂
E	L		I_c (USI)	E
$P.S_1S_2$	I_c (SI + USI)		L + I_p (SI)	$P.S_1S_2$
$P.S_1S_3$	I_c (SI + USI)		L + I_p (SI)	$P.S_1S_3$
$P.S_1S_4$	I_c (SI + USI)	♂ ♀ (E × P) × (E × P) I_c (SI + USI)	L + I_p (SI)	$P.S_1S_4$
$P.S_2S_3$	L + I_p (SI ? + USI)		L	$P.S_2S_3$
$P.S_4S_5$	L + I_p (SI ? + USI)		L	$P.S_4S_5$
$P._{959}$	L + I_p (SI ? + USI)		F + L	$P._{959}$

F	Fertility, normal-sized seeds are present in the fruits
L	fertilization failure or post-zygotic lethality as assumed from the presence of many aborted seeds or swollen ovules in the fruits
I_p	partial incompatibility reaction in the style
I_c	complete incompatibility reaction in the style
SI	self-incompatibility
USI	unilateral stylar incompatibility

Fig. 5. Classification of incompatibility and sterility barriers in the breeding system of the interspecific hybrids *L. esculentum* × *L. peruvianum*

2. Reproductive isolation of the hybrid

Our results fully confirm the earlier reports by McGuire and Rick (1954) and Rick and Butler (1956) that two main types of barriers control the breeding behaviour of interspecific hybrids between *esculentum* and *peruvianum*: one leads to the destruction of pollen tubes in the style and can be equated to stylar incompatibility; the other is restricted to the ovary and either prevents fertilization of leads to the early abortion of the embryo. It is the purpose of the present section to discuss and define further, in the light of the data summarized in fig. 5 and in table 1, the main characteristics of these two different types of isolating mechanisms.

Stylar incompatibility: It can be seen from table 1 and fig. 5 that stylar incompatibility may have two different causes, namely:

— the presence in the pollen and in the style of the same S allele (S_1 in the present study) which was transmitted by *L. peruvianum* to the hybrid plant;

— or the presence in the pollen tubes growing through *peruvianum* or hybrid styles of some *esculentum* factor which is not only present in *esculentum* itself but was also transmitted to the hybrids.

Although the fluorescence analyses and the observations made under the electron microscope were sometimes inconsistent and, for obvious reasons, could not be carried out on a very large scale, they indicate that these two types of stylar incompatibility differ from one another for the same morphological features which had been found previously (de Nettancourt *et al.*, 1973a, 1973b) to distinguish self-incompatibility (SI) in *L. peruvianum* from unilateral stylar incompatibility (USI) between *L. peruvianum* and *L. esculentum*. Such a finding, if confirmed unequivocally by more precise methods than the ones we have developed in the present study, is of particular importance because it leads to a set of deductions on the genetical control of unilateral incompatibility reactions in the style which may be summarized as follows:

— with no exceptions, the data recorded in table 1 show that, like SI, USI segregates as a gametophytic factor. This is particularly obvious in all crosses where hybrid pollen tubes could be subdivided into SI and USI classes.

— since, with few exceptions, only 2 different classes of inhibited pollen tubes could be found, USI, like SI, appears to be under monofactorial control in the pollen.

— although no evidence can be provided that SI and USI features are governed by different alleles of a same gene, it is clear from the regular segregations observed (especially noticeable in hybrid pollen, which, after self-pollination of the hybrids, formed approximately equal numbers of SI and USI tubes) that the two patterns of reaction are controlled by genetic elements located on the same chromosome and, possibly, within the same linkage group.

If our hypothesis is correct, stylar unilateral incompatibility in *esculentum* pollen and in the interspecific hybrid is governed gametophytically by a locus which is either identical with, or linked to, the locus governing self-incompatibility in *L. peruvianum*. Such a conclusion appears *a priori* to be at variance with that of Martin (1961a, 1967), who has found that the reproductive barrier isolating *L. esculentum* from its wild relatives, *L. chilense* and *L. hirsutum*, is of a complex nature and under the control of different genetic loci. As will be discussed in the next section, and has been suggested by Martin himself for hybrids between *L. esculentum* and *L. chilense*, stylar unilateral incompatibility is only one of the components of the complex barrier which controls the breeding system in our hybrids; the conclusion that this USI component is governed by a single gametophytic locus identical with, or linked to, the S gene is therefore in perfect accordance with the observations and hypotheses of Martin (1964, 1967). In addition, the postulate by Martin (1968), that hybrid tomato plants not only receive a self-incompatibility allele from the self-incompatible parent but also a dominant switch gene necessary for the expression of self-incompatibility, is by no means contradicted by our data because no attempts were made in the present study to find whether the advanced generations of the hybrids segregated for an eventual capacity to suppress activity of the S_1 allele transmitted by the *peruvianum* parent. As the switch gene hypothesized by Martin (1968) is sporophytic and dominant in action, all the pollen tubes produced by the hybrid when they contained the S_1 allele, evidently maintained their property to be rejected by a style with the same S_1 allele.

Since our observations clearly confirm or, in the case of an eventual switch-gene, do not contradict the hypotheses of Martin (l. c.), one could expect them to be in serious opposition to those of Hogenboom (1972d), who considers that self-incompatibility and interspecific growth inhibition are distinct phenomena. Hogenboom (l. c.), however, restricted his observations to an analysis of the capacities of different inbred styles of *peruvianum* to accept or to reject *esculentum* pollen, while our experiments essentially concerned the segregation in hybrid pollen of the pollen component which participates in the unilateral incompatibility reaction. Hogenboom (l. c.) finds that, for this reaction, the stylar component of *peruvianum* is sporophytic and multigenic; we find that in the pollen the *esculentum* component is monofactorial, gametophytic and either linked or allelic to the S-locus. These two conclusions are not necessarily opposed, and it is possible that a reaction mechanism between a single gametophytic factor in the pollen and one or more dominant genes in the style characterizes unilateral stylar incompatibility between *L. peruvianum* and *L. esculentum*. This is what has been postulated by Abdalla and Hermsen (1972) in their "two power competition hypothesis" of unilateral incompatibility, but the question remains as to why the populations of *peruvianum* which Hogenboom (l. c.) found to be in heterozygous condition for the loci governing unilateral incompatibility in the style maintain the presence of the dominant alleles if these are not involved in the control of self-incompatibility. Obviously, as implicitly stated by Abdalla and Hermsen (l. c.), one explanation could be that mutations from self-incompatibility to the type of self-compatibility characterizing *L. esculentum* frequently occur in the pollen at the S-locus of *L. peruvianum*, and that the dominant alleles have the selective advantage of preventing, by their capacity to reject self-compatible pollen, the breaking down of the self-incompatibility barrier and the inbreeding depression which follows such a process. The hypothesis appears particularly applicable to the present situation, since it accounts for the fact that self-compatible mutants of *L. peruvianum* have but very rarely, if ever, been obtained after mutagenic treatments (Davies and Wall, 1961; de Nettancourt and Ecochard, 1968; Hoffmann, 1969; Hogenboom, 1972b). The problem is, however, not so simple because the conclusions of Hogenboom (l. c.) suggest that in the F_2 progenies of interspecific hybrids a number of segregants should lack the dominant genes controlling unilateral incompatibility and express self-compatibility. As was shown by McGuire and Rick (1954), this is not the case in tomatoes, where the offspring from the self-incompatible interspecific hybrids which contained different self-incompatibility alleles did not form a single fruit upon selfing and was considered to be entirely self-incompatible. Since these F_2 individuals obviously contained at least one S-allele from their *peruvianum* parent, the most logical way to reconcile Hogenboom's findings and the data of McGuire and Rick is to assume that there is close linkage in *peruvianum* between the S-locus and the gene governing the pistil reaction to *esculentum* pollen. It has been shown above that the pollen component of *esculentum* which participates in the unilateral incompatibility reaction is either linked or allelic to the S-locus, so it may be concluded that the S-locus and most of the genetic elements governing unilateral incompatibility are located on the same chromosome and probably are closely linked. Hence, the model presented several years ago by Pandey (1962) of a complex incompatibility locus with different components governing self- and unilateral relationships still appears, at least in its main lines, to be valid.

Ovarian sterility: In the present article, ovarian sterility is defined as the failure of fertilization or of embryo development in all those cases where pollen tube growth through the style proceeded normally but shrunken seeds were collected in the mature fruit. It is clear from the results in table 1 that the occurrence of such a phenomenon does not depend upon the presence of identical S-alleles or of unilateral incompatibility factors in pollen and style but, with one exception, takes place after all matings compatible at the stylar level. This sterility barrier is stronger than the one found by McGuire and Rick (1954) since few or no seeds could be obtained by backcrossing the hybrid as staminate parent to *L. esculentum* or, as pistillate partner, to most of our *peruvianum* accessions. The fact that, in this last series of crosses, only the unrelated *peruvianum* stock from Dr. Holle proved to be a successful parent certainly demonstrates that it is not the S-genotype of the *peruvianum* pollen which governs the fertility of the backcross with the hybrid, but its genetic background. As a few seeds were obtained nevertheless when the *peruvianum* stocks which shared a common ancestor with the hybrid were used, it can be concluded that these stocks are heterozygous for the genes conditioning acceptance of the pollen in the hybrid ovary and that the occasional seed obtained resulted from a successful fertilization by rare recombinants. In other words, seed production in backcrosses between the hybrid and these related *peruvianum* stocks is conditioned, in the *peruvianum* pollen and independently of the compatibility relationships at the stylar level, by many unlinked loci or by at least two closely linked loci. Evidently, as can be seen in table 1, certain of these genes are also present in heterozygous condition in the P. 959 clone of Dr. Holle, because some embryo-abortion was also noted when pollen from this accession had been allowed to germinate through hybrid styles.

Whether or not the same series of genes which restricts successful fertilization or embryo development between hybrid plants as pistillate parents and *L. peruvianum* are also operating when the reciprocal backcrosses are concerned is still unknown, but nothing, *a priori*, excludes this possibility because few or no seeds were produced even in those cases where compatible pollen tube growth through the *peruvianum* styles had been observed.

3. Ultrastructural features of incompatibility

The ultrastructural analyses performed in this study offered the possibility, already taken into consideration in the previous section, of distinguishing between the two types of incompatibility reaction and demonstrated that pollen tube inhibition, after all types of incompatible mating, is correlated to several other phenomena, among which the most striking are not only the accumulation of particles and the absence of an inner-wall at the tube apex, but also the formation of CER in the cytoplasm of the tube. Although similar configurations of the endoplasmic reticulum have often been observed in various animal and plant tissues (for relevant literature, see Shih and Rappaport, 1971), their interpretation is difficult and often contradictory (Dereuddre, 1971). In most cases, however, the phenomenon has been recorded in temporarily inactive cells, such as those of resting potato tubers and *Betula* buds (Shih and Rappaport, l. c.; Dereuddre, l. c.), and seems to indicate the inactivation of some important biosynthetic mechanism. It is therefore tempting to suggest that one of the main actions of the incompatibility complex is to provoke the alteration of the endoplasmic reticulum and, thereby a general cessation of protein synthesis.

This proposition is still speculative, but gains some support from the first ultrastructural analyses of pollen tubes cultured *in vitro* under non-optimal conditions which showed that growth inhibition is, in this case also, accompanied by the formation of CER and of bipartite particles in the tube cytoplasm. The presence of CER has not yet been definitely demonstrated in *Lycopersicum* pollen tubes but was clearly observed, together with the bipartite particles in cultured pollen tubes of *Nicotiana alata* (Sarfatti *et al.*, in preparation). These features of pollen tubes grown *in vitro* are still under investigation and will be described more fully at a later date, but what seems clear at the moment is that the inhibition of pollen tubes grown *in vitro* and of pollen tubes in an incompatible style present many ultrastructural similarities. Although this resemblance certainly militates in favour of Kroes' interesting theory (1973), that incompatibility directly results from an absence in the pollen tubes of some of the enzymes necessary to break down stylar complexes, it must not be forgotten, at the same time, that such an hypothesis is not entirely consistent with our present knowledge on the nature of radiation-induced self-compatible mutations affecting only the stylar activity part or the pollen activity part of the S-locus. Besides, Kroes' theory is not altogether compatible with the fact that the pollen of self-incompatible species can grow through the styles of very unrelated species belonging to different families (Lewis and Crowe, 1958) and supposes, since hundreds of different alleles exist at the S-locus of incompatible species, that an amazingly large number of different enzymes are required for pollen tube growth.

Hence, the similar behaviour of pollen tubes growing through an incompatible style, *in vivo*, and on an artificial culture medium, *in vitro*, appears to be best interpreted by the assumption that the incompatibility complex acts as the specific inhibitor of a process (possibly protein synthesis) which, for other reasons related to an absence of appropriate nutrients, is also affected under *in vitro* conditions. Further analyses are, however, obviously required for demonstrating that the formation of CER and of bipartite particles in pollen tubes are relatively unspecific responses to different types of growth inhibition. It also remains to find the cause and meaning of the abundant particles which are observed, in some species, at the time the compatible pollen tube reaches the ovule and is on the point of opening in the degenerated synergid (Jensen and Fisher, 1968; Van Went, 1970; Vazart, 1971; de Nettancourt *et al.*, 1973a).

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