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The Generation of New *S* Alleles at the Incompatibility Locus of *Lycopersicum peruvianum* Mill.*

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Summary. A detailed analysis has been made of *S* genotypes in progenies derived from induced and spontaneous inbreeding processes in a clonal population of *Lycopersicum peruvianum* Mill. The results indicate that, in certain genetic backgrounds, induced inbreeding leads to the generation of a new *S* allele which usually first appears in the pistil of individuals otherwise homozygous for one of the parental specificities. When the change in specificity occurs in *S* heterozygotes, spontaneous self-compatibility is promoted and the new allele can be transmitted, via selfing, to the following generation.

The factors and mechanisms which may be involved in the generation of new specificities at the *S* locus of higher plants are discussed and preliminary evidence is provided which suggests that the hypothesis of mutation by equal crossing-over is not applicable to the present study.

I. Introduction

Very little information is available on the factors and mechanisms which contribute new S alleles to the multi-allelic series of self-incompatible species with a one-locus gametophytic system of incompatibility. The work of Lewis (1954), Lewis and Crowe (1954), Pandey (1956, 1965) and Brewbaker and Natarajan (1960) has shown that changes in S specificity cannot be induced by radiations, but it is apparent from three reports (Denward, 1963; de Nettancourt and Ecochard, 1969; Pandey, 1970a) that simple inbreeding techniques bring about the spontaneous generation of new S alleles in a number of allogamous species. In these circumstances, inbreeding seems to be a far more efficient mutagen than ionizing rays which obviously cannot reconstruct within the *S* locus. Yet, the conclusion may appear premature because Denward was not able to eliminate completely the risk of pollen contamination during his experimental work, and because the observations of de Nettancourt and Ecochard and those of Pandey were restricted, at the time, to only very limited material.

It is the purpose of the present article, which summarizes five years of experimental work, to demonstrate that the generation of a new *S* specificity during inbreeding is, in the complete absence of pollen contamination risks, a reproducible phenomenon which can be observed regularly in several inbred progenies derived from a single diploïd clone.

2. Material and Methods

Test-species

All experiments were carried out with the self-incompatible species *Lycopersicum peruvianum* Mill., for which the presence of a monofactorial gametophytic system of homomorphic self-incompatibility has been clearly demonstrated by Lamm (1950), McGuire and Rick (1954) and Günther *et al.* (1968). The same clonal population (*L. peruvianum* 006, derived from a single S_1S_2 plant), which had been previously analysed in radiation experiments (de Nettancourt and Ecochard, 1968), was used. To avoid the risk of pollen contamination by foreign pollen, no other *Peruvianum* genotype has been introduced since 1966 to our laboratories, which are 3 miles away from any other research station and completely surrounded by woods and dwellings.

Inbreeding Techniques

A) Spontaneous inbreeding. In order to study as many inbred lines as possible, intensive use was made of sporadic spontaneous pseudo-compatibility processes which occurred in the advanced progenies of a plant derived from a previous radiation experiment (de Nettancourt and Ecochard, 1968). In all, three inbred progenies from I_1 and I_2 generations (I_1-1200, I_2 1200-53 and I_2 1200-87), each comprising 30 to 130 individuals, were screened for new specificities.

B) Induced inbreeding. Attempts to induce a temporary break-down of self-incompatibility in the mother clone and in advanced progenies were made using hormone treatment (Davies and Wall, 1961) and heat shock (Townsend and Danielson, 1968). In the hormone treatment, five different concentrations of alpha-naphthalene acetic acid were tested by spraying the mature flowers with aqueous solutions or, for the highest concentration (1%), by application in lanolin around the calyx. All plants were kept in controlled climate rooms (12 hrs day, 12,000 Lux, 23 °C; 12 hrs night, 17 °C; relative humidity 60–70%). Two temperature treatments were tried in the heat-shock experiments, namely 32 °C for two days at the time of self-pollination and 32 °C for one week. A third temperature test was conducted by placing the flowering plants for one week in the open under the conditions of Dutch autumn (night temperature around 5 °C). In addition, synergistic effects were looked for by combining hormone treatments and temperature shocks. All pollination was done manually with fresh pollen harvested from control plants.

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Detection of new specificities

A sample of 15 individuals from the first progeny analysed (spontaneous inbreeding in I_1–1200) was submitted, together with the mother clone ($S_1 - S_2$), to a diallelic test. Once the various genotypes present in this sample population had been classified by means of identity tests in back-cross progenies, four-point tests were carried out on the remaining population (119 individuals) using the collection of testers obtained in the diallelic test. For all other progenies obtained via spontaneous or induced inbreeding, four-point tests were made routinely. In addition, every single plant studied was selfed manually and, in all experiments, selfing tests and crosses between two individuals were made on at least 10 flowers, and usually 20, at two different periods in the life-cycles of the plants.

After back-crossing individuals with a new specificity to the mother clone, progeny tests were performed in several cases to estimate: 1) the transmissibility of the new specificity from one generation to the next; 2) its functional behaviour in pollen and style; 3) its capacity to segregate as a single genetic factor allelic to the *S* locus.

In all cases, pollen-style relationships were termed compatible when, both male and female partners being normally fertile, an average of 12 seeds per pollinated flower was obtained. The relationship was considered incompatible when this value was less than 4. Borderline cases (seed-set per pollinated flower ranging from 4 to 11) are referred to, in this article, as weakly self-compatible.

3. Results

A) Generation of a new specificity during spontaneous inbreeding

1. Description of plants selfing spontaneously. As stated in the section on Material and Methods, 3 plants in the 1st and 2nd inbreeding generations of the mother clone were found to set, at specific periods, very high numbers of seeds upon selfing. These 3 plants are codified here as I_1-1, I_2-53 and I_2-87.

I_1-1 was a very weak plant which belonged to a population of 700 individuals obtained from selfing the mother clone (S_1 S_2) during chronic exposure to low dosages of γ-rays (de Nettancourt and Ecochard, 1968). The plant collapsed a few days after anthesis, which was considerably delayed, and before any crosses to the mother clone could be attempted. Yet, selfing under bag had been carried out on 12 flowers from 2 inflorescences, which yielded the amazingly high average number of 109 seeds per pollinated flower (with a range of 36 to 171 seeds per flower).

Plants I_2-53 and I_2-87 were detected among 134 individuals from the selfed progeny of plant I_1-1

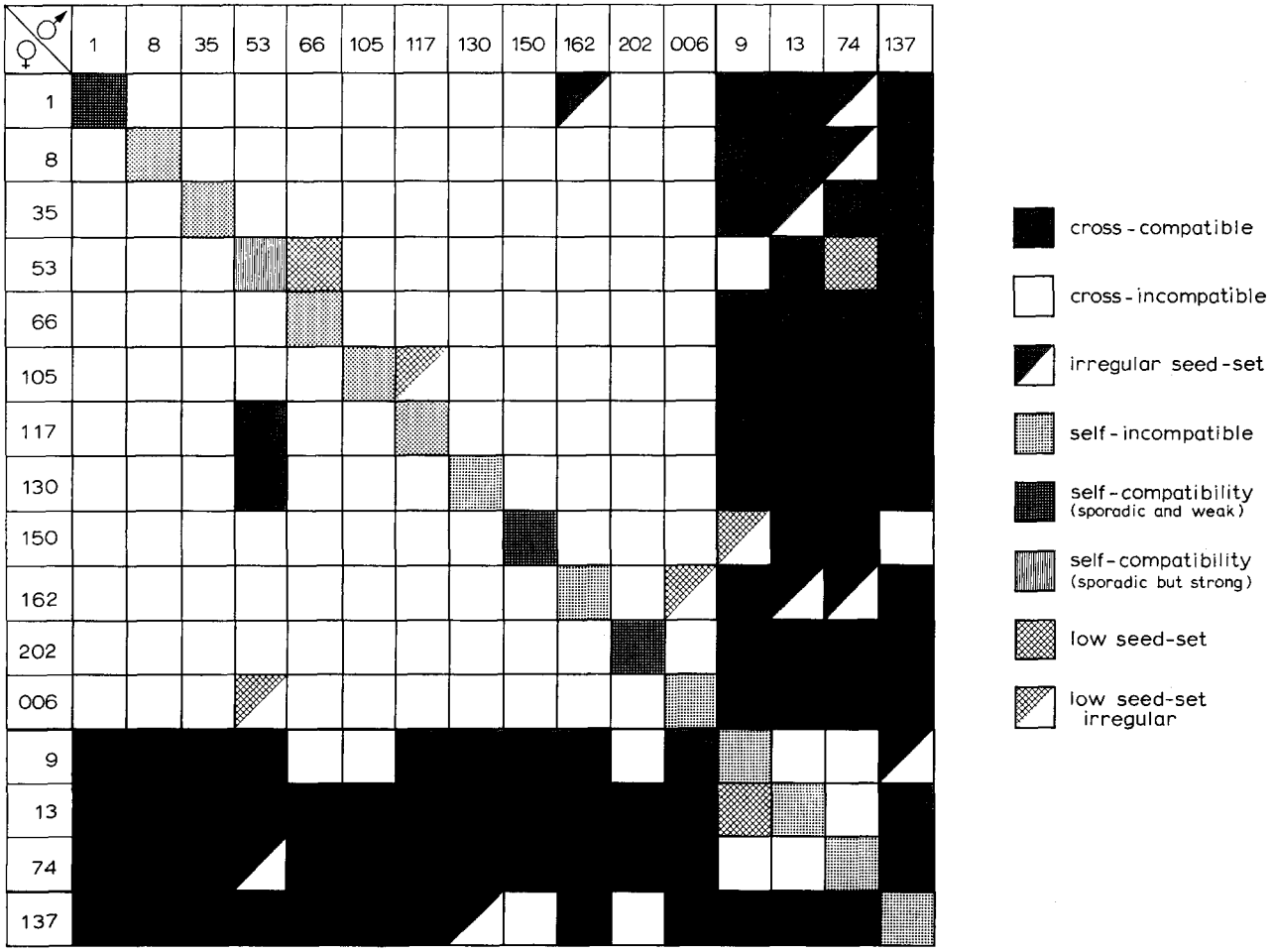

Fig. 1. Results of diallel crosses in I_2 and back-crosses to the mother clone (006)

described above. I_2-53 was vigorous and produced more than 95% stainable pollen. In the diallelic crosses, summarized in Fig. 1, the plant was classified as genotypically identical to the mother clone (S_1S_2). As a rule, I_2-53 was strictly self-incompatible. However, on one occasion self-pollination of 8 flowers under strictly controlled isolated conditions resulted into an average of 62 seeds per flower (with a range of 18 to 141).

Plant I_2-87, on the other hand, was a weak plant with more than 40% aborted pollen, and always set a limited number of seeds (0 to 10) after selfing and after test-crosses with different markers. Hence, the plant cannot be classified with regard to its genotype at the *S* locus and, if one agrees with the estimation standards of Hoffmann (1969), may eventually be considered as regularly self-compatible.

Both I_2-53 and I_2-87 were propagated by cuttings and are available in the collection of this laboratory.

2. Analysis of progenies obtained via selfing. Progeny from plant I_1-1: 15 plants taken at random from the 134 individuals which constituted this progeny were analysed, together with the mother clone (006, genotype S_1S_2), in a diallel cross. The results, in Figure 1, indicate that three groups of plants were involved, corresponding to three different genotypes, namely S_1S_2 (11 plants), S_aS_b (3 plants) and S_cS_d (1 plant). However, a small number of contradictions were noticed in some of the diallel matings where the results of crosses between groups and within groups did not always conform to the classification proposed above. While such anomalies may, in the case of negative exceptions (− instead of +), be attributed to sudden changes in pollen fertility, which was found to vary enormously in certain inbred plants from one week to the next, it is more difficult to account for the positive exceptions (+ instead of −) in Figure 1. These were very rare, however, and possibly related to specific signal reactions between pollen and styles which eventually switched off activity at the *S* locus or inhibited the formation of the incompatibility complex. In this connection, it must be noted that a detailed statistical analysis of the distribution of positive and negative anomalies (kindly performed by the TNO statistics bureau in Wageningen) suggested that all anomalies were distributed at random and could not be consistently related to any given class of *S* genotype nor to any given cross-combination between and within groups. It therefore appears that the anomalies must be attributed to experimental errors or to factors which are not located on the S chromosome.

It must also be observed, from Figure 1, that the plants involved in positive anomalies did not display any particular tendency towards self-compatibility which, with one exception (plant I_1-53), was always extremely low. Hence the self-compatibility character in plant I_1-1 is not hereditary and must be attributed to the formation of a new specificity at the time of selfing.

Once the presence of at least one new specificity had been established in the offspring of plant I_1-1, tests of progeny from reciprocal backcrosses to the original S_1S_2 clone and from crosses between the S_aS_b and S_cS_d groups were made to ascertain the number of new alleles generated. At the same time, representative individuals from each group were used as tester stocks for a complete genotypic classification of the 134 individuals constituting the progeny of I_1-1.

The results in Table 1, which summarizes the back-cross data, clearly indicate that only one new specificity, together with the original alleles S_1 and S_2, was segregating in the population submitted to diallelic testing. This new specificity, arbitrarily referred to as S_3 in the remaining portion of this article, corresponds therefore to S_b and S_d in the original classification of groups, whereas S_a and S_c are equivalent to S_1 and S_2, respectively. In other words, the three groups of plants in Figure 1 correspond, respectively, to S_1S_2 (genotype of the original clone), S_1S_3 (formerly S_aS_b) and S_2S_3 (formerly S_cS_d). Another observation which can be made from Table 1, and which will be discussed at a later stage, is the regular occurrence among the back-cross progenies of a small fraction of S_2S_2 and, more rarely, S_1S_1 homozygotes. In most instances, these cannot be explained by accidental selfing with incompatible pollen and must therefore be attributed to reversion events of an unknown nature.

It can be seen from Table 2 that the classification into S_1S_2, S_1S_3 and S_2S_3 genotypes holds true for the entire progeny of plant I_1-1 which was found to segregate, on the basis of the test-crosses made, into 61 S_1S_2:59 S_1S_3:1S_2S_3. Because of inconsistent behaviour, which was often due to sterility, a few plants in Table 2 do not fit this classification. With two exceptions, the plants I_2-53 and I_2-87 which were described earlier, all individuals were highly self-incompatible and did not yield more than a few seeds after repeated hand-pollination.

Progeny from plant I_2-53: 38 individuals in this progeny were submitted to four-point tests using the tester stocks selected previously and the mother clone 006. The results in Table 3 clearly show that plant I_2-53, which the diallelic test classified as S_1S_2, had been segregating at the time of spontaneous selfing for three specificities, namely S_1, S_2 and S_3. Again, the new specificity, S_3, was found to be perfectly functional in both pollen and style. The segregation ratio of 25 S_1S_3 to 8 S_2S_3 suggests that I_2-53 was S_1S_2 in styles and ovules and produced S_3 pollen at the time the plant became, for a short period, highly self-compatible. Another explanation, which appears less likely however, is that the plant remained S_1 and S_2 in the pollen but all styles and ovules generated S_3 instead of the original specificities. All plants in the

Table 1. *Identity tests for S_a, S_b, S_c and S_d. It can be seen that S_a and S_c correspond to the original specificities S_1 and S_2 respectively; S_b and S_d are identical and represent a new specificity (S_3). Hence, S_aS_b is S_1S_3 and S_cS_d corresponds to S_2S_3*

Parents	Progeny plants tested	Test-crosses performed on progeny plants				Plants classified	Classification in groups	Genotype
		♀ × S_1S_2	♀ × S_aS_b	♀ × S_cS_d	♂ × S_1S_2			
$S_1S_2 \times S_aS_b$	63	+	−	+	+	30	S_aS_b	S_1S_3
		+	+	−	+	26	S_cS_d	S_2S_3
		+	+	+	−	3	S_cS_c*	S_2S_2
$S_aS_b \times S_1S_2$	54	−	+	+	−	30	S_1S_2	S_1S_2
		+	+	−	+	18	S_cS_d	S_2S_3
		+	+	+	−	4	S_cS_c*	S_2S_2
		+	+	+	−	1	S_aS_a*	S_1S_1
$S_1S_2 \times S_cS_d$	19	+	−	+	+	9	S_aS_b	S_1S_3
		+	+	−	+	10	S_cS_d	S_2S_3
$S_cS_d \times S_1S_2$	15**	−	+	+	−	2	S_1S_2	S_1S_2
		+	−	+	+	2	S_aS_b	S_1S_3
$S_aS_b \times S_cS_d$	20	−	+	+	−	11	S_1S_2	S_1S_2
		+	+	−	+	6	S_cS_d	S_2S_3
		+	+	+	−	1	S_cS_c*	S_2S_2

* Homozygosity confirmed by means of reciprocal backcrosses to S_aS_b and to S_cS_d.
** 11 plants in this progeny were sterile or gave inconsistent results suggesting instability at the *S* locus.

Table 2. *Classification of genotypes and genotypic frequencies in the progeny of plant I_1-1*

Test cross performed				Number of plants classified	Genotype detected
♀			♂		
× S_1S_2	× S_1S_3	× S_2S_3	× S_1S_2		
−	+	+	−	62	S_1S_2
+	−	+	+	59	S_1S_3
+	+	−	+	1	S_2S_3
−	−	−	−	4	sterile*
−	−	−	+	1	S_1S_3 (female sterility)
+	−	+	−	2	S_1S_3 (S_3-pollen sterile)**
±	+	+	±	1	S_1S_2 (sporadic self-comp.)
+	+	+	+	1	self-compat.
+	−	−	+	2	unknown
−	−	+	−	1	unknown
−	−	+	+	1	unknown

* also sterile in crosses with S_1S_3 as maternal parent.
** as detected in crosses with S_2S_3 as maternal parent; possibly S_1 homozygous for the pollen.

progeny from I_2-53 were self-pollinated several times but no evidence was obtained that self-compatible individuals were present in the population. A number of plants (5) were sterile or appeared to be unstable at the *S* locus.

Progeny from plant I_2-87: 34 individuals were tested in this progeny. Most of the plants were extremely weak and sterile so that results from the test-crosses were chaotic, but it was obvious in at least 5 individuals that the new specificity S_3 had been produced by I_2-87 and segregated with the original S_1 and S_2 alleles. One plant sporadically produced a few seeds upon selfing but all the other individuals were strictly self-incompatible (0 seed per pollinated flower). Several leaf and flower syndromes were observed which must probably be ascribed to inbreeding effects.

B) Generation of a new specificity after induced inbreeding

1. Efficiency of the techniques used for promoting seed-set after selfing. The results obtained after treat-

Table 3. *Classification of genotypes and genotypic frequencies in the progeny of plant I_2-53 (38 flowering individuals)*

Test-crosses performed					Number of plants classified	Genotype
♀ × I_2-53	♀ × S_1S_3	♀ × S_2S_3	♀ × S_1S_2	♂ × S_1S_2		
+	−	+	+	+	25	S_1S_3
+	+	−	+	+	8	S_2S_3
−	−	−	−	−	3	sterile
−	−	+	−	+	1	?
+	−	−	−	−	1	?

Table 4. *Effects of hormone and temperature treatments on fruit formation and seed-set upon selfing in the mother clone*

Treatment	Total number of flowers treated	Number of fruits formed per 100 flowers			Number of fruits in distribution classes for seed-set per fruit									Average no of seeds per treated flower	Average no of seeds per seeded fruit
		Normal	Aborted	Total	0	1—5	6—10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60	61 to 70		
Water (control)	49	—	—	—	—	—	—	—	—	—	—	—	—	—	—
N.A.A. 10^{-3}	72	77	5	82	44	12	—	—	—	—	—	—	—	0.28	0.87
N.A.A. 10^{-4}	83	17	4	21	6	8	—	—	—	—	—	—	—	0.16	1.50
N.A.A. 10^{-5}	37	22	—	22	—	7	1	—	—	—	—	—	—	0.65	3.00
N.A.A. 10^{-6}	67	19	4	23	3	10	—	—	—	—	—	—	—	0.33	2.20
N.A.A. 1%	56	4	0	4	—	2	—	—	—	—	—	—	—	0.04	1.0
32 °C-2 days	233	9	0.4	9.4	1	4	6	5	1	—	—	—	1	0.99	12.83
32 °C-2 days + N.A.A. 10^{-3}	219	45	34	79	81	26	2	—	—	1	—	—	—	0.35	4.47
32 °C-2 days + N.A.A. 1%	63	2	—	2	1	—	—	—	—	—	—	—	—	—	—
32 °C-1 week	59	—	—	—	—	—	—	—	—	—	—	—	—	—	—
32 °C-1 week + N.A.A. 10^{-3}	94	35	64	99	32	1	—	—	—	—	—	—	—	0.01	1.0
Field test	33	6	—	6	—	1	—	1	—	—	—	—	—	0.48	8.0

ment of the mother clone (S_1S_2) with hormone, heat-shock or a combination of the two are summarized in Table 4. It is obvious that hormone treatment, especially at the 10^{-3} concentration, increased fruit-setting and that the optimum concentration probably lies between 10^{-3} and 10^{-2}, the latter having only slight effects. Temperature shocks alone had a very weak action on fruit formation but when used with the hormone resulted in the setting of a high number of normal and aborted fruits. For seed-set, the results were almost reversed, temperature being much more effective than hormone in inducing seed formation in the limited number of fruits obtained. Furthermore, distribution data on seed-set per fruit indicate that a higher seed-set could be obtained in some fruits after temperature treatments or field tests than was ever recorded in the hormone series.

It is tempting to postulate, in view of these results, that different mechanisms are involved after NAA treatment (fruit-setting) and heat-shock or cold treatment (seed-setting). However, only weak synergistic action, if any, could be obtained in combined treatments, and additional studies are obviously needed for a real understanding of the situation. Yet, for all practical purposes concerned, the experiment fulfilled its aims and sufficient quantities of selfed-seeds were available for an analysis of inbreeding effects.

Hormones and temperature treatments on a second S_1S_2 individual (I_2-8) obtained in the progeny test of plant I_1-1 yielded comparable results and provided sufficient quantities of seeds for extensive progeny testing.

In contrast, and in spite of the fact that more than 700 flowers were handled, absolutely no seed from selfing after either hormone applications or heat-shock could be obtained from I_3-42 (S_1S_2 individual in the third generation of inbreeding).

2. Analyses of progenies obtained via selfing

Progeny from the original clone (S_1-S_2): 81 plants were grown, of which 62 flowered sufficiently for genotypic analyses by four-point tests (Table 5). Ten individuals were highly sterile (more than 90% pollen abortion) and could not be classified coherently. Three plants, which showed good pollen stainability, were compatible as females with only one of the tester stocks; in a repetition of the test-crosses, two of these 3 individuals refused all pollen and behaved as though they had the three specificities present in the style, whereas the third turned out to be compatible, as pistil-parent, with one of the genotypes which it had previously rejected. The pollen of that plant was incompatible with S_1S_2 styles.

All other plants were found to yield reproducible results from one pollination date to the next. Among them, 28 individuals were clearly S_1S_2 in pollen and styles, 14 were S_2S_2 homozygotes and only 2 could be classified S_1S_1. As can be seen from Table 5, 5 plants were regularly compatible with S_1S_2 male-testers and rejected S_3 pollen. The pollen from 4 of these plants (1-22-1, 1-22-3, 1-46-2, 1-51-5) behaved as S_2, whereas pollen in the fifth individual (1-26-4) clearly appeared to be S_1. Hence, these 5 plants may be classified as S homozygous for the pollen and S heterozygous (the original specificity displayed in the pollen and the new

Table 5. *Classification of genotypes and genotypic frequencies in the progeny of clone 006 (S_1S_2) submitted to hormone treatments and heat shocks*

Test-crosses performed				Number of plants classified	Genotype	
♀ × S_1S_3	♀ × S_2S_3	♀ × S_1S_2	♂ × S_1S_2		♂	♀
+	+	−	−	28	S_1S_2	S_1S_2
+	+	+	−	2	S_1S_1*	S_1S_1*
+	+	+	−	14	S_2S_2*	S_2S_2*
−	+	+	−	1	S_1S_1*	S_1S_3*
+	−	+	−	4	S_2S_2*	S_2S_3*
+	−	−	−	1	?	?
−	+	−	−	1	?	?
−	−	+	−	1	?	?
−	−	−	−	10	sterile	sterile

* ascertained and/or confirmed in reciprocal back-crosses to S_1S_3 and S_2S_3.

specificity S_3) in the style. Whether or not the ovules in these 5 exceptional 'mutants' also carry S_3 is not yet known and will be ascertained in the coming months. Preliminary data on fruit formation in one back-cross to S_1S_2 clearly suggest, however, that S_3 is present in the ovules and segregates normally.

With one exception (23 seeds for 26 flowers pollinated), not one individual in the progeny yielded seed upon selfing.

Progeny from plant I_2-8(S_1S_2): testing of this progeny is not yet complete but it is worth mentioning that one individual yielded large numbers of seeds upon selfing at specific periods of its life cycle. The situation encountered here appears comparable to that of plants I_1-1 and I_2-53 and definitely justifies further testing in the next generation. Other points of interest which are emerging from the progeny analysis of plant I_2-8 are that S_1S_1 and S_2S_2 homozygotes are recovered in the expected frequencies, and that no individual appears to be homozygous in the pollen and heterozygous in the style. Hence, there seem to be basic differences between plant I_2-8 and the original clone (both of which are S_1S_2) with regard to the occurrence of S_3 after selfing. Such variations may perhaps be attributed to different levels of inbreeding, I_2-8 being a second generation offspring of the mother clone.

Progeny from plant I_3-42 (S_1S_2): all attempts to induce seed-set upon selfing by means of heat-shock and hormone treatments have failed, although more than 700 flowers were treated and hand-pollinated. Part of this failure may be attributed to the fact that plant I_3-42 produced only 5% stainable pollen (which proved functional in crosses with S_1S_3 and S_2S_3 testers), but it is also tempting to conclude that the induction of selfing after the third generation of inbreeding is a very difficult task.

C) Transmission, function, segregation and permanence of the new specificity

It can be seen from Table 2, which summarizes the progeny tests from various crosses between S_1S_2, S_1S_3 and S_2S_3 plants taken at random from the offspring of plant I_1-1, and from Table 3, that the new specificity S_3 is normally transmitted from one generation to the next, is usually functional in both pollen and style, and segregates from the original specificities as a single gametophytic factor allelic to S_1 and S_2. In only one cross ($S_1S_3 \times S_1S_2$) did the segregation ratio (30:18) significantly deviate from a 1:1 distribution. A repeat of this cross with another S_1S_3 as pistil-parent yielded a ratio of 44 S_1S_2 to 32 S_2S_3 which fits more closely the expected segregation frequency. Yet it is possible that a selection mechanism operates, in this particular cross, against the formation of S_2S_3 genotypes.

The stability of S_3, after it is established in a progeny, is unchallengeable considering that our S_1S_3 and S_2S_3 tester stocks have now been in use for more than 4 years and have never been found to revert to the original specificities. However, the occurrence of S_2S_2 homozygous individuals in back-cross progenies (Table 1), and certain chaotic or irregular responses in the inbred progenies of the mother clone (Table 5) and of plant I_2-86, may be taken as an indication that: S_3, when transmitted in certain genetic backgrounds, can revert; S_3, which appears to be first produced in the pistil, is usually not functional in the pollen nor completely stabilised before one further generation of breeding. In this respect, the analogy with Denward's observations (1963) on *Trifolium* is particularly striking.

Discussion

A) The generation of a new specificity by inbreeding

There appears to be little doubt that inbreeding is a very powerful agent for generating a new allele at the *S* locus of *L. peruvianum*. In this connection, the recent suggestion by Pandey (1970b), that S_3 may have originated in our experiments from the radiation treatment which was applied at the first generation of some of our material, does not appear completely valid for several reasons:

1. Previous work by Lewis (1954), Lewis and Crowe (1954), Pandey (1956, 1965) and Brewbaker and Natarajan (1960) has shown that specificity mutations at the *S* locus were not induced by ionizing rays. The argument may be fallacious, however, because one wonders, in view of the fact that inbreeding alone can generate a new specificity, if the screening tests applied by these workers could discriminate between the expected *S* homozygotes and

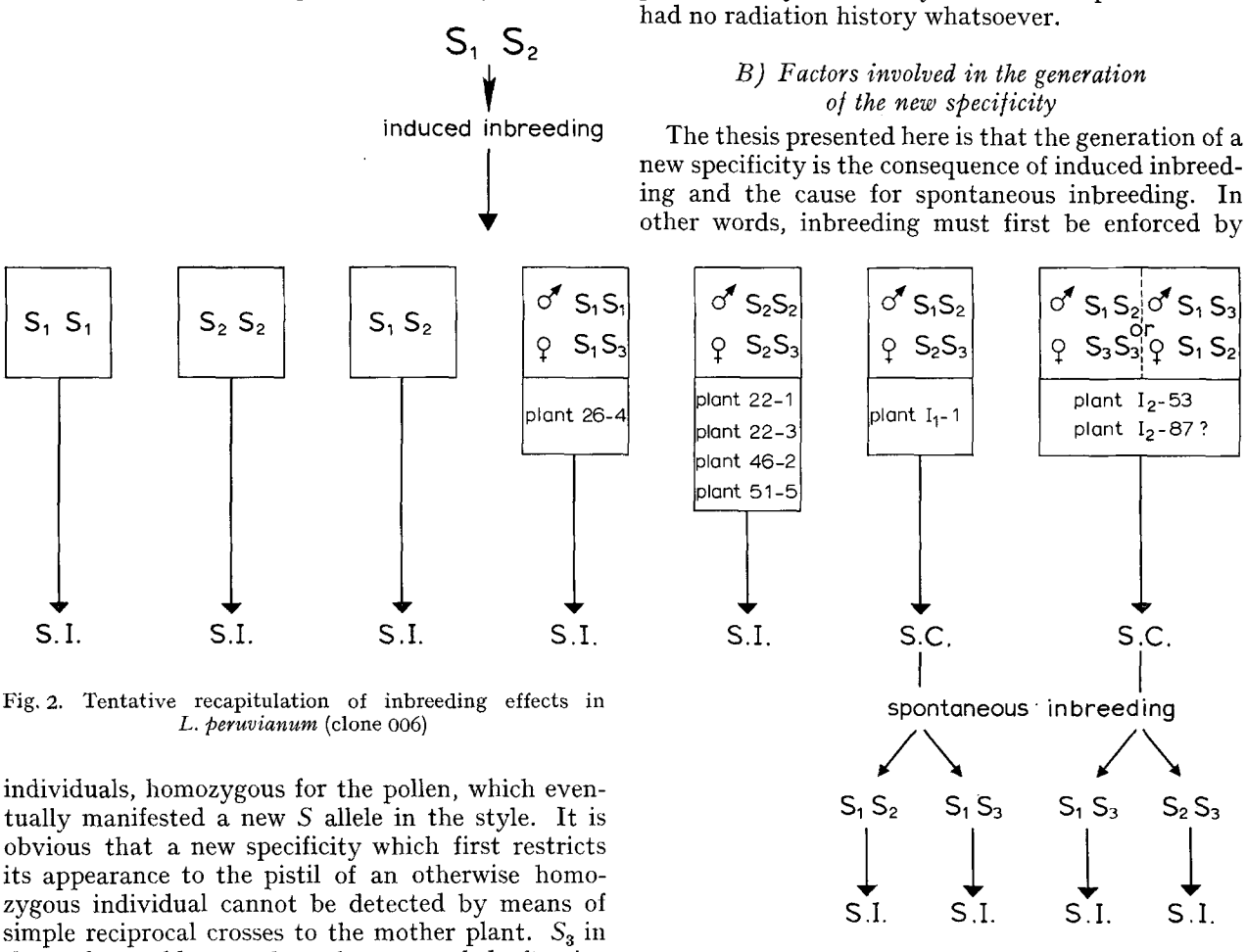

Fig. 2. Tentative recapitulation of inbreeding effects in *L. peruvianum* (clone 006)

individuals, homozygous for the pollen, which eventually manifested a new *S* allele in the style. It is obvious that a new specificity which first restricts its appearance to the pistil of an otherwise homozygous individual cannot be detected by means of simple reciprocal crosses to the mother plant. S_3 in the style would never have been revealed after induced inbreeding if S_1S_3 and S_2S_3 testers had not been made available, in the first place, from the experiments on spontaneous inbreeding. Lewis (1951) possibly anticipated this contingency when he observed that new specificities could perhaps not be detected by conventional methods.

2. Reports by Denward (1963) and, very recently, by Pandey himself (1970a) indicate that inbreeding alone, in the complete absence of radiation treatment, generates new *S* alleles at the *S* locus.

3. In the present study, when the material presented a radiation history, the new specificity not only appeared in I_1 progenies but was also generated in I_2, and, possibly, in I_3 segregants. Granted that delayed effects may be involved, one must then assume that they are first expressed, after two complete cycles of inbreeding, only in some segregants and only at certain ontogenetic stages of the plants. Unless one assumes a complex system for transmission of mosaics, these plants cannot have been chimeric for the genetic factors which Pandey suspects of promoting crossing-over at the *S* locus.

4. The new specificity was also observed, in the present study, in the styles of inbred plants which had no radiation history whatsoever.

B) Factors involved in the generation of the new specificity

The thesis presented here is that the generation of a new specificity is the consequence of induced inbreeding and the cause for spontaneous inbreeding. In other words, inbreeding must first be enforced by various techniques, such as bud pollination (Shivanna and Rangaswamy, 1969), acute irradiation of styles (Linskens *et al.*, 1960), chronic irradiation of flowering plants (de Nettancourt and Ecochard, 1968), hormone treatment (Pandey, 1967; Martin, 1968) or heat shock (Ascher and Peloquin, 1966), which either inhibit floral abscission or prevent the incompatibility reaction from taking place. Then, in the resulting progeny, certain genetic backgrounds enhance the formation of a new specificity which, judging from our results in Table 5, is essentially restricted to the pistil of *S* homozygotes.

The relationship between homozygosity at the *S* locus and the appearance of S_3 makes it tempting to conclude that the genetic factors responsible for the formation of a new *S* allele are recessive and located

on the *S*-bearing chromosome. This condition is, however, neither sufficient (several *S* homozygotes did not express the new specificity) nor completely necessary, because at least one heterozygous individual (plant I_2-53) was found, at specific periods and either in the pollen or in the style, to generate a new specificity. In this last case, the formation of a new *S* allele in only the male or the female part of an *S* heterozygote naturally leads, as observed in the present study, to self-compatibility and to spontaneous inbreeding. The phenomenon lasts as long as the new allele continues to be formed and, at the next generation, with the new specificity being active in the pollen and style, self-incompatibility is reinstated in the entire progeny. It is probable that this hypothetical scheme (summarized in Figure 2) is also representative of the situation which occurred in plant I_1-1. This plant, assumed to be S_1S_2, possibly changed into S_2S_3 in the pistil so that S_1 pollen tubes were able to cross the style barrier and to fertilize S_2 and S_3 ovules. The single S_2S_3 individual recovered out of 134 individuals in the progeny would then have resulted from accidental selfing by S_2 pollen.

C) Mechanisms

Before any discussion of the mechanisms which generate new *S* alleles, the following 3 elements which emerge from the present study must be underlined:

Only one new specificity (S_3), and always the same one, was detected after either spontaneous or induced inbreeding.

In at least two cases (plant I_1-1 and plant I_2-53), the new *S* allele was generated in sudden waves and at very high frequencies.

In back-cross progenies the new specificity appeared, in a few but regular instances, to revert to the original type.

Bearing these facts in mind one may perhaps speculate on the nature of the mechanisms which generated S_3 in the *peruvianum* material studied at our laboratory.

1. Mutation by equal crossing-over: it is possible that, as suggested by Pandey (1970a, 1970b), somatic crossing-over within the *S* locus is responsible for the formation of S_3. Only one cross-over product may have been functional, and consequently only one new specificity would be detectable in the progeny. It is also possible that, as conceived by Pandey, somatic crossing-over is restricted to certain tissues in the plant and leads, when it occurs, to the production of many gametes with the new specificity. It is, however, difficult to reconcile Pandey's hypothesis with the observation, in the present study, that S_3 in certain genetic backgrounds seems to revert regularly to the original form. Obviously, reversion of a recombinant towards one parental type cannot be attributed to equal crossing-over between the recombinant and the second parental type. Yet this is exactly what would have had to happen if the formation of S_2S_2 genotypes in the progeny from $S_1S_3 \times S_1S_2$ (Table 1) is ascribed to equal crossing-over. It may be argued, of course, that S_3 was produced by crossing over, whereas reversion in the back-cross progenies took place via some other mutational mechanism or was simulated by the parthenogenetic or androgenetic development of gametes which afterwards became diploid. The hypotheses appear to us somewhat far-fetched in view of the relatively high frequency of recorded reversions. There are four methods available for testing the theory of mutation by equal crossing-over: the analysis of amino acid sequences in the polypeptides coded by the parental and the recombinant alleles; the use of completely homozygous lines obtained from ex-haploïds and where equal crossing-over cannot generate genetic variability; the study of asynaptic mutants where crossing-over cannot take place; the reversion test.

The first two approaches are among the distant goals at our institute and elsewhere in Wageningen and Roma; the last one is actually being continued on a large number of selfed progenies and of offspring from crosses between S_1S_3, S_2S_3 and S_1S_2 genotypes. Meanwhile, on the basis of the very preliminary evidence obtained on possible reversion events, one is forced to assume that S_3 probably does not result from equal crossing-over within the incompatibility locus. This conclusion is not really damaging to Pandey's theory since this author uses the term recombination in a broad sense which may include phenomena such as gene conversion.

2. Mutation by non-equal crossing-over or by deletion-duplication events: deletion or duplication of genetic material within the *S* locus, as induced by unequal crossing-over or by any other mechanism, could lead to the generation of a new specificity in both forward and backward directions. Under this hypothesis, S_3 would probably correspond to a frameshift mutation (or to a terminal change) if only one cistron constitutes the specificity locus, or could derive from a change in the number of cistrons present if a more complex architecture is assumed at the *S* locus. In his reference to the intracistronic recombination hypothesis, Pandey did not indicate if equal or unequal events had to be involved and it might therefore be considered that his hypothesis also includes mutation of the *S* locus by non-equal crossing-over. Again, one may wonder if this exchange process can take place at only specific periods and at very high frequencies in inbred plants which, in all probability, were not chimeric. Furthermore, the fact that the apparently reverted allele was found in homozygous condition renders the hypothesis of reversion by unequal somatic crossing-over practically impossible. Hence, once more, one would have to assume that forward mutations and reversions are not governed by the same mechanism.

3. Mutation by substitutions or inversions: these mechanisms, which are known to operate at the nucleotide level in mutagenic processes (Heslot, 1965) may have generated S_3 in the present study. However, one would not expect such mutations to occur very often in non-irradiated material nor to revert regularly in certain genetic backgrounds.

4. Mutation by activation-inactivation phenomena: it is known that self-incompatibility in many allogamous species is governed by a two loci system (Lundquist, 1965). With interspecific relationships, Pandey (1962) has shown that primary and secondary specificities are involved which express different strengths and different activities. Even if one rejects the Edström theory on master and slave genes (previously discussed by de Nettancourt, 1969), it is not inconceivable that inbreeding, via the elaboration of new repressing and de-repressing substances, inactivates the *S* locus and reactivates a closely linked unit which had been previously repressed. The hypothesis certainly fails to explain the origin of genetic polymorphism at the *S* locus of higher plants but, with regard to the generation of S_3 in our *peruvianum* material, provides an answer which is no more speculative than any of the other possibilities discussed above. Recent work by Martin (1968) has demonstrated the presence of a gene in the tomato which can switch-in and -off the *S* locus and which may possibly be involved, together with the genetic recombination induced by inbreeding, in activation-inactivation processes on different segments of the *S* locus.

Conclusion

This study, which has required the handling of several hundred plants and of more than 50,000 flowers, indicates that the generation of a new *S* specificity is a regular phenomenon in the inbred progenies derived from the S_1S_2 mother clone of *L. peruvianum* used at our laboratories.

Sterility, inbreeding depression and occasional instability of the breeding system rendered difficult the analysis of *S* genotypes in a number of advanced progenies. Yet, it clearly appears that the new specificity S_3 was usually generated in the pistil of inbred individuals which were otherwise homozygotes for one of the parental specificities. This finding suggests that some of the genetic factors governing the generation of S_3 are recessive and located on the *S*-bearing chromosome. Under these circumstances, it is practically impossible, unless individual back-cross progenies are screened one by one, to detect a new specificity if marker lines with the new allele are not available for further test crossing. Hence, it is understandable that new specificities originating in this manner have not been reported earlier by various investigators who were usually obliged to restrict their screening tests to reciprocal crossing with the mother plant.

Nevertheless, it is evident that changes in specificity do also occur in *S* heterozygotes. In this case, the formation of such specificities appears to be only temporary and leads, via sporadic self-compatibility, to the transmission of the new allele to the following generation. It is certainly not implied that all cases of self-compatibility in inbred lines of *L. peruvianum* (Hogenboom, 1968; Hoffmann, 1969) are caused by the generation of new specificities.

Little can be said, at the moment, about the mechanisms involved in the generation of *S* alleles. The hypothesis of mutation by equal crossing-over does not appear to apply to the present analysis, but specific tests which can discriminate between accidental selfing and reversion will have to be carried out in still larger numbers before a conclusive statement can be made. The possibility that inactivation-reactivation processes of two closely linked loci are involved is considered reasonable, in view of the fact that self-incompatibility by two-loci systems is frequent in nature and because a few plants, classified as female sterile in this study, may eventually be suspected of expressing 3 specificities in the style. Such plants, which possibly represent cases of reactivation of a previously inert locus not associated with inactivation of the original allele, will be submitted, in the coming months, to fertility tests by means of crosses with pollen from unrelated *peruvianum* stocks and, eventually, to detailed back-cross analysis. Meanwhile, the hypothesis of a switch mechanism is at least partly supported by the findings of Martin (1968) that the *S* gene in the tomato can be turned on and off by a switch gene and that, as suggested by Denward (1963), the genetic action of the *S* allele depends on a highly organized polygenic system which is drastically modified by inbreeding.

Zusammenfassung

Es wurde eine ausführliche Analyse der *S*-Genotypen in Nachkommenschaften einer geklonten Population von *Lycopersicon peruvianum* Mill. nach induzierter und spontaner Inzucht gemacht.

Die Ergebnisse deuten an, daß induzierte Inzucht bei einem bestimmten genotypischen Milieu zum Entstehen eines neuen *S*-Allels führt. Dieses erscheint gewöhnlich zuerst in den Griffeln einzelner Individuen, die im übrigen für eines der elterlichen Allele homozygot sind. Wenn die Änderung in *S*-Heterozygoten auftritt, dann wird die spontane Selbstkompatibilität gefördert. Das neue Allel kann durch Selbstung in die nächste Generation übertragen werden.

Die Faktoren und Mechanismen, die am Entstehen neuer Allele am *S*-Locus höherer Pflanzen beteiligt sein können, werden diskutiert. Ein vorläufiger Hinweis wird dafür erbracht, daß die Hypothese der Mutation durch equal crossing-over für die vorliegende Untersuchung nicht zutrifft.

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