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GENETIC INSTABILITY AT THE S-LOCUS OF *LYCOPERSICON PERUVIANUM* PLANTS REGENERATED FROM *IN VITRO* CULTURE OF ANTHERS: GENERATION OF NEW S-SPECIFICITIES AND S-ALLELE REVERSIONS

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SUMMARY

Genetic analyses were made of plants regenerated from *in vitro* cultures of anthers and stem internodes in four different self-incompatible diploid genotypes of *Lycopersicon peruvianum*. Altogether 9 diploids, 15 tetraploids and 3 cytochimeric plants regenerated from stem internode cultures and 35 diploids, 10 tetraploids and 9 cytochimeras derived from anther culture, were analysed.

The plants regenerated from stem internode cultures had no alterations at the S-locus, whereas a high proportion of anther culture-derived regenerants carried sexually heritable changes, namely new S-specificities and S-allele reversions, the reverted S-allele showing a switch from gametophytic to sporophytic expression.

1. INTRODUCTION

WHEN nonmeristematic tissue explants or single cells are cultured *in vitro*, they display genetic instabilities of various kinds. These can occur at the time of callus induction and/or during further growth *in vitro*. Several factors appear to be associated with the degree of instability (D'Amato, 1975, 1977, 1978; Sunderland, 1977; Skirvin, 1978; Thomas *et al.*, 1979; Cappadocia and Sree Ramulu, 1981).

Many examples of genetic variability generated by plant cell cultures (somaclonal variation) have been discussed by Larkin and Scowcroft (1981). A majority of these deal with the occurrence of polyploidy, aneuploidy, chromosome number mosaicism and structural changes in cultures of cells, protoplasts or tissues, and in generated plants. In addition, some reports have appeared on the incidence of somatic mutations at loci affecting chlorophyll synthesis (reversion to a wild type allele or deletion) (Barbier and Dulieu, 1980), mitochondrial DNA variation (Gengenbach *et al.*, 1981) and sexually heritable changes in agronomic characters, such as yield, plant height, heading date, leaf characters, fertility, etc. (Mousseau, 1970; Popchistov and Zaganska, 1977; Cummings *et al.*, 1976; Oono, 1978).

The homomorphic gametophytic incompatibility system in *Lycopersicon peruvianum* is controlled by multiple alleles (S-alleles) at one locus (Lamm, 1950; McGuire and Rick, 1954; Gunther *et al.*, 1968). It has been proposed that the S-locus in monofactorial gametophytic systems has a tripartite structure: two parts controlling the reaction in the pollen and in the style, respectively, and one part common to both pollen and style which determines the specificity (Lewis, 1949, 1960). These parts of the S-locus are

apparently mutationally independent and functionally integrated (Lewis, 1951, 1958; Lewis and Crowe, 1954; Pandey, 1956). Thus, the identification of three distinct parts in the *S*-locus and the independent mutability of each of these parts provide valuable material for analysing the processes of gene regulation, metabolic pathways and their relationships to gene structure and gene mutation. In addition, *L. peruvianum* is an important species for tomato breeding, especially for disease resistance. Accordingly, several previous studies have been carried out with this species using *in vitro* culture techniques in order to investigate certain problems related to incompatibility (Devreux *et al.*, 1975; Sree Ramulu *et al.*, 1976a, b; Ancora *et al.*, 1977; Ancora and Sree Ramulu, 1981). In an earlier investigation on *in vitro* cultures of anthers and stem internodes of different self-incompatible diploid genotypes in *L. peruvianum* (Sree Ramulu *et al.*, 1976a), not only diploids, but also tetraploids and cytochimerae were regenerated.

The present study examines the occurrence, type and extent of genetic changes at the *S*-locus of these regenerated plants and explains the mechanism of origin of *S*-allele alterations.

2. MATERIAL AND METHODS

(i) *Plant material*

Plants regenerated *in vitro* from anther cultures of clones 9- S'_1S_3 and F13- $S_{12}S_{13}$ and from the stem internode cultures of P20- S_1S_5 and F9- S_4S_5 of *Lycopersicon peruvianum* Mill. ($2n=24$) (Sree Ramulu *et al.*, 1976a) were used in this study. Genetic analyses indicated that the genetic architecture of the S'_1 allele of clone 9- S'_1S_3 is different from that of S_1 of clone 1- S_1S_1 . The details are later given in Results.

Table 1 gives details on the frequency and type of plants analysed in each of the clones. For details on *in vitro* culture procedures and cytological techniques, reference is made to Sree Ramulu *et al.* (1976a, 1977). The plants were grown under controlled conditions (16 h day, 13 000 lx, 23°C; 8 h night, 17°C, relative humidity 60–70 per cent).

(ii) *Pollination procedures*

Self- and cross-pollinations were performed in strict isolation for the detection of changes at the *S*-locus of regenerated plants. The plants were selfed manually, and in all the experiments self-pollinations and crosses between two individuals were made on 10–20 or more flowers, preferably at two different periods in the life cycle of the plants.

The progeny tests were made to ascertain the transmissibility of altered *S*-alleles and their functional behaviour in pollen and style. Several tester-stocks were used for determining *S*-genotypes: S_1S_1 , S_2S_2 , S_2S_3 , S_1S_4 , S_2S_4 , S_2S_5 , S_1S_6 , S_2S_6 , S_1S_7 , S_2S_7 , and S_6S_7 .

Observations on pollen tube growth in styles of pollinated flowers were made by fluorescent microscopy (Martin, 1958). Wherever necessary, the data on fruit- and seed-set were supplemented by observations on pollen tube growth. Pollen abortion was ascertained by staining tests with acetocarmine.

3. RESULTS

(i) *Analysis of plants regenerated from stem internode cultures of clones P20-S₁S₅ and F9-S₄S₅*

Altogether 15 tetraploids, 9 diploids and 3 cytochimeras (4n-2n-2n) were analysed (table 1). The data on fruit- and seed-set after self-pollinations, and test-crosses with the respective mother clones showed that no alterations occurred at the *S*-locus of the diploids and cytochimeric regenerants (table 2). The testing at tetraploid level for altered *S*-alleles requires several tester-stocks with all necessary combinations of *S*-alleles. The data in table 2 can only reveal the compatibility behaviour, but not altered *S*-alleles. As can be seen from the results, all the tetraploids behaved as normal autotetraploids with regard to compatibility.

TABLE 1

Types of plants regenerated from in vitro cultures of anthers and stem internodes in self-incompatible diploid genotypes of Lycopersicon peruvianum (2n = 24)

Genotypes	Diploids	Tetraploids	Cytochimeras*	
			4n-2n-2n	2n-4n-4n
Anther culture				
9-S ₁ S ₃	32	0	5	1
F13-S ₁₂ S ₁₃	3	10	3	0
Stem internode culture				
P20-S ₁ S ₅	0	7	1	0
F9-S ₄ S ₅	9	8	2	0

* 4n-2n-2n or 2n-4n-4n condition refers to the ploidy of L₁, L₂ and L₃ layers.

TABLE 2

Compatibility behaviour after self-pollinations and test-crosses of plants regenerated from the stem internode cultures of clones P20-S₁S₅ and F9-S₄S₅

Clone	Regenerated plants		Self-pollinations	Test-crosses	
	Type	No.		♀ ×	♂ ×
P20-S ₁ S ₅	Tetraploid	7	+	—	+
	Cytochimeras (4n-2n-2n)	1	—	—	—
F9-S ₄ S ₅	Tetraploid	8	+	—	+
	Diploid	9	—	—	—
	Cytochimeras (4n-2n-2n)	2	—	—	—

+ compatible

— incompatible

(ii) *Analysis of plants regenerated from anther cultures of clone 9-S₁S₃*(a) *First generation plants*

In all, 32 diploids and 6 cytochimeric plants were analysed. In 5 cytochimeric plants of type 4n-2n-2n (tables 1 and 3) the tetraploid L₁ layer had no influence on seed-set, as verified by test-crosses with cross-compatible tetraploids. The remaining plant with 2n-4n-4n condition behaved like a normal autotetraploid (self-compatible) (date not given in table 3). As can be seen from the data on fruit and seed yields presented

TABLE 3
Fruit and seed yields after self-pollinations and test-crosses of first generation plants regenerated from the anther culture of clone 9-S₁S₃

Group No.	Regenerants Plants	Self-pollinations		Test-crosses			
		Fruits per pollinated flower	Average no. Seeds per fruit	Fruits per pollinated flower	Average no. Seeds per fruit	Fruits per pollinated flower	Average no. Seeds per fruit
1	16 plants*	0	0	0	0	0	0
2	5 plants*	0.42	6.52	0	0	0	0
3	Plant 2†	0		0.53	12.90	0.95	8.50‡
4	Plant 10	0.08	4.0	0	0	0.60	9.50
	Plant 12	0	0	0	0	0.70	4.57
	Plant 15	0	0	0	0	0.75	15.77
	Plant 16	0	0	0	0	0.59	14.80
	Plant 17	0	0	0	0	0.60	8.00
	Plant 18	0	0	0	0	0.50	1.40
	Plant 19	0.06	3.0	0	0	0.41	4.66
	Plant 20	0.14	1.0	0	0	0.30	6.50
	Plant 21	0	0	0	0	0.75	5.00
	Plant 22	0	0	0	0	0.50	8.50
	Plant 24	0	0	0	0	1.00	17.66
	Plant 25	0	0	0	0	0.83	10.00
	Plant 26	0	0	0	0	0.50	8.60
	Plant 34	0.66	5.05	0	0	0.75	5.33
	Plant 36	0.73	6.00	0	0	0.30	6.00

* Two among these plants are cytochimeras (4n-2n-2n).

† This plant is 4n-2n-2n in type.

‡ The seeds were very small, flat, yellowish in colour and inviable.

in table 3, not a single case of (viable) fruit-set occurred in 16 (group 1) out of 37 regenerants after self-pollinations, or test-crosses with the parental clone ($9-S'_1S_3$) indicating that no changes were generated at the *S*-locus. These plants had high male and female fertility as determined by tests on pollen staining and tube growth in styles, and fruit- and seed-set after crosses with tester-stocks.

Five other regenerants (group 2) gave relatively high fruit-set and regular seed-set after self-pollinations, but the reciprocal crosses with the parental clone $9-S'_1S_3$ were incompatible. The analyses of 125 progeny plants derived from the selfed seeds showed that all were self-incompatible and cross-incompatible with $9-S'_1S_3$, thus indicating that these regenerants displayed "pseudo self-compatibility".

The fruit- and seed-set data of the remaining 16 regenerated plants showed that 15 (group No. 4 and 5) set seed when crossed as males and 1 (plant 2 of group 3) when used as a female also with $9-S'_1S_3$, (table 3). Besides showing cross-compatibility as pollen parents with $9-S'_1S_3$, plants No. 34 and 36 (group 5) gave relatively high fruit-set and regular seed-set after self-pollinations. All the plants had high pollen and ovule fertility as verified by crosses with cross-compatible tester-stocks. They were also normal in karyotype and meiotic behaviour. These results, thus indicate that alterations occurred at the *S*-locus of regenerated plants and that they are expressed only in the pollen of 15 regenerants, and both in the pollen and pistil of one regenerant (plant 2).

(b) *Second and third generation plants*

In order to ascertain the nature of these changes, their transmissibility and their functional behaviour in pollen and style, the progenies that were obtained from 12 different crosses between $9-S'_1S_3$ and regenerated plants were analysed. Besides self-pollinations, reciprocal crosses were performed with $9-S'_1S_3$ (table 4). The results showed that all of the 112 progeny were

TABLE 4

Compatibility behaviour after self-pollinations and test-crosses of the second generation progeny

	No. of progeny analysed	Self- pollinations	Test-crosses	
			$\text{♀} \times 9-S'_1S_3$	$\text{♂} \times 9-S'_1S_3$
$9-S'_1S_3 \text{ ♀} \times \text{plant 24}$	9	—	+	+*
$9-S'_1S_3 \text{ ♀} \times \text{plant 15}$	8	—	+	+*
$9-S'_1S_3 \text{ ♀} \times \text{plant 16}$	20	—	+	+
$9-S'_1S_3 \text{ ♀} \times \text{plant 17}$	14	—	+	+†
$9-S'_1S_3 \text{ ♀} \times \text{plant 22}$	12	—	+	+*
$9-S'_1S_3 \text{ ♀} \times \text{plant 34}$	13	—	+	+*
$9-S'_1S_3 \text{ ♀} \times \text{plant 19}$	6	—	+	+
$9-S'_1S_3 \text{ ♀} \times \text{plant 26}$	7	—	+	+
$9-S'_1S_3 \text{ ♀} \times \text{plant 18}$	5	—	+	+
$9-S'_1S_3 \text{ ♀} \times \text{plant 21}$	4	—	+	+
$9-S'_1S_3 \text{ ♀} \times \text{plant 25}$	4	—	+	+
$\text{Plant 2 ♀} \times 9-S'_1S_3$	10	—	+†	+†

* Two progeny plants were sterile.

† One progeny plant was sterile.

— Cross-incompatible.

+ Cross-compatible (in almost all cases >20 seeds/fruit).

both self-incompatible, and reciprocally cross-compatible with $9-S'_1S_3$, which indicate that these changes are heritable.

Further, in order to determine the S -genotypes in pollen and styles, some progeny plants selected from each of 7 different crosses between $9-S'_1S_3$ and the regenerants, were analysed. These test-crosses were performed with tester-stocks S_1S_1 , S_2S_2 , S'_1S_3 and S_2S_3 . It can be seen from the data in table 5 that a new allele, namely S_2^* occurred in pollen and styles of all 63 progeny plants. Further, the data on S -genotypes in pollen and styles of the third generation progeny (table 6) confirm the transmissibility and stability of the new S_2^* allele. It should be mentioned here that the clone $9-S'_1S_3$ used in this study has been obtained previously after inbreeding of clone 006- S_1S_2 , the S_3 being derived from S_2 (de Nettancourt *et al.* 1971, 1975). The occurrence of a new S_2^* allele in the plants regenerated from the anther culture of $9-S'_1S_3$ indicates that the S_3 allele reverted to a new S_2^* allele.

(c) *Sporophytic action of new S_2^* allele*

The progenies of crosses between regenerants carrying the new S_2^* allele and the parental clone $9-S'_1S_3$ regularly segregated as $1 \times S_2^*S_2^* : 1 \times S_2^*S_3$ instead of the expected $1 \times S'_1S_2^* : 1 \times S_2^*S_3$ (tables 5 and 6). This suggests that the new S_2^* allele is dominant over the S'_1 allele of clone $9-S'_1S_3$ and that plants of S_2^* phenotype are of $S'_1S_2^*$ genotype. This type of switch from gametophytic to sporophytic action of the S_2^* allele is transmitted from one generation to the other.

(d) *Difference between S'_1 allele of clone $9-S'_1S_3$ and S_1 of clone $1-S_1S_1$*

The new S_2^* allele shows no dominance over the S_1 allele of clone $1 \times S_1S_1$. The test crosses indicate that when $1 \times S_1S_1$ is crossed with plants stabilized for the new S_2^* specificity, *i.e.*, $S_2^*S_3$ genotype, or plants of S_2^* phenotype ($S'_1S_2^*$ genotype), the progeny segregated approximately as $1 \times S_1S_2^* : 1 \times S_1S_3$, or $1 \times S_1S'_1 : 1 \times S_1S_2^*$, but contained no S_2^* phenotype. Thus, it appears that the S_1 allele of clone $1-S_1S_1$ is different from that of clone $9-S'_1S_3$. As the clone $9-S'_1S_3$ has previously been obtained after inbreeding of clone 006- S_1S_2 (de Nettancourt *et al.*, 1971), it is likely that, besides the generation of S_3 , some change might have occurred in the S'_1 allele. Therefore, it is probable that the genetic architecture of the S'_1 allele of clone $9-S'_1S_3$ is different from that of S_1 of clone $1-S_1S_1$.

(iii) *Analysis of plants generated from anther culture of clone F13- $S_{12}S_{13}$*

The results of self-pollinations and test-crosses with the parental clone F13- $S_{12}S_{13}$ show that among the ten tetraploids, nine behaved as normal autotetraploids with regard to compatibility (table 7). The other tetraploid was not only self-incompatible, but also cross-incompatible as male with F13- $S_{12}S_{13}$ as determined from fruit- and seed-set and pollen tube growth. The pollen abortion was 90 per cent. However, test-crosses with cross-compatible tetraploids reveal that this plant was both male and female fertile. These results indicate that the self-incompatibility in this tetraploid is due to the S -homozygous condition (quadruplex: $S_{12}S_{12}S_{12}S_{12}$ or

TABLE 5
Classification and genotypes of genotypic frequencies in the second generation progeny

Parents	No. of progeny classified	Self-pollinations	Test-crosses performed and results obtained										Genotypes detected	
			$\times$ S_1S_1 $\varnothing$	$\times$ S_2S_2 $\varnothing$	$\times$ $S_1'S_3$ $\varnothing$	$\times$ S_2S_3 $\varnothing$	$\times$ S_1S_1 δ	$\times$ S_2S_2 δ	$\times$ $S_1'S_3$ δ	$\times$ S_2S_3 δ	$\varnothing$	δ		
9- $S_1'S_3$ $\varnothing$ $\times$ Plant 24	3	—	+	+	+	—	+	—	+	—			$S_2'S_3$	$S_2'S_3$
	4	—	+	—	+	—	+	—	+	+			$S_2'S_2$	$S_2'S_2$
	1	—	..	..	..	..	+	—	+	—			sterile	$S_2'S_3$
	1	—	..	..	..	..	+	—	+	+			sterile	$S_2'S_2$
9- $S_1'S_3$ $\varnothing$ $\times$ Plant 15	3	—	+	+	+	—	+	—	+	—			$S_2'S_3$	$S_2'S_3$
	3	—	+	—	+	—	+	—	+	+			$S_2'S_2$	$S_2'S_2$
	1	—	..	..	..	..	+	—	+	—			sterile	$S_2'S_3$
	1	—	..	..	..	..	+	—	+	+			sterile	$S_2'S_2$
9- $S_1'S_3$ $\varnothing$ $\times$ Plant 16	1	—	+	+	+	—	+	—	+	—			$S_2'S_3$	$S_2'S_3$
	1	—	+	+	+	—	+	—	+	+			$S_2'S_3$	$S_2'S_3$
	3	—	+	—	+	—	+	—	+	+			$S_2'S_2$	$S_2'S_2$
	2	—	+	+	—	—	+	+	+	+			S_3S_3	S_3S_3
Plant 2 $\varnothing$ $\times$ 9- $S_1'S_3$	3	—	+	+	+	—	+	—	+	—			$S_2'S_3$	$S_2'S_3$
	1	—	+	+	+	—	+	—	+	+			$S_2'S_3$	$S_2'S_3$
	1	—	+	—	+	—	+	—	+	—			$S_2'S_2$	$S_2'S_2$
	1	—	+	—	+	—	+	—	+	+			S_3S_3	S_3S_3
9- $S_1'S_3$ $\varnothing$ $\times$ Plant 17	1	—	..	..	..	..	+	—	+	—			sterile	$S_2'S_2$
	1	—	+	—	+	—	+	—	+	+			$S_2'S_2$	sterile
	3	—	+	+	+	—	+	—	+	—			$S_2'S_3$	$S_2'S_3$
	1	—	+	+	+	—	+	—	+	+			$S_2'S_3$	$S_2'S_3$
9- $S_1'S_3$ $\varnothing$ $\times$ Plant 22	5	—	+	—	+	—	+	—	+	—			$S_2'S_2$	$S_2'S_2$
	1	—	..	..	..	..	+	—	+	—			sterile	$S_2'S_3$
	3	—	+	+	+	—	+	—	+	—			$S_2'S_3$	$S_2'S_3$
	3	—	+	—	+	—	+	—	+	+			$S_2'S_2$	$S_2'S_2$
9- $S_1'S_3$ $\varnothing$ $\times$ Plant 34	2	—	..	..	..	..	+	—	+	—			sterile	$S_2'S_2$
	4	—	+	+	+	—	+	—	+	—			$S_2'S_3$	$S_2'S_3$
	1	—	+	+	+	—	+	—	+	+			$S_2'S_3$	$S_2'S_3$
	4	—	+	—	+	—	+	—	+	+			$S_2'S_2$	$S_2'S_2$
9- $S_1'S_3$ $\varnothing$ $\times$ Plant 34	4	—	+	+	+	—	+	—	+	—			$S_2'S_3$	$S_2'S_3$
	1	—	+	+	+	—	+	—	+	+			$S_2'S_3$	$S_2'S_3$
	4	—	+	—	+	—	+	—	+	+			$S_2'S_2$	$S_2'S_2$
	2	—	..	..	..	..	+	—	+	+			sterile	$S_2'S_3$

— cross-incompatible.
+ cross-compatible (in almost all cases >20 seeds/fruit).
.. sterile.

TABLE 6
Classification of genotypes and genotypic frequencies in the third generation progeny

Parents	No. of progeny classified	Self-pollinations	Test-crosses performed and results obtained										Genotypes	
			$\times$	S_1S_1 ♀	S_2S_2 ♀	$S_1'S_2$ ♀	$S_1'S_3$ ♀	S_2S_3 ♀	S_1S_1 ♂	S_2S_2 ♂	$S_1'S_3$ ♂	S_2S_3 ♂	♂	♀
Plant 3 ($9-S_1'S_3 \times$ Plant 22) $\times$ $9-S_1'S_3$	1	—	+	+	+	+	+	—	—	+	—	$S_1'S_3$	S_2S_3	
	1	—	+	+	+	+	+	—	—	+	+	$S_1'S_3$	S_2S_3	
	8	—	+	—	—	+	—	—	—	+	+	$S_1'S_3$	S_2S_3	
	1	—	..	..	..	..	..	+	—	+	+	sterile	S_2S_2	
	1	—	..	..	..	..	..	+	+	+	+	sterile	S_2S_3	
Plant 5 ($9-S_1'S_3 \times$ Plant 17) $\times$ $9-S_1'S_3$	3	—	+	+	+	+	+	—	—	+	—	$S_1'S_3$	S_2S_3	
	2	—	..	..	..	..	..	+	—	+	—	$S_1'S_3$	S_2S_3	
												sterile	S_2S_3	

— cross-incompatible.
+ cross-compatible (in almost all cases >20 seeds/fruit).
.. sterile.

TABLE 7

Compatibility behaviour after self-pollinations and test-crosses of plants regenerated from the anther culture of clone F13-S₁₂S₁₃

Regenerated plants Type	No.	Self- pollinations	Test-crosses	
			♀ × F13-S ₁₂ S ₁₃	♂ × F13-S ₁₂ S ₁₃
Tetraploid	9	+	—	+
Tetraploid	1	—	+	—
Diploid	2	—	—	—
Diploid (Plant 15)	1	—	—	+
Cytochimeras (4n-2n-2n)	3	—	—	—

+ compatible.

— incompatible.

$S_{13}S_{13}S_{13}S_{13}$). The abnormal morphological features expressed by vegetative and floral parts and the reduced vigour and fertility seem to be associated with the homozygous condition.

Among the other regenerated plants, only one plant, *i.e.*, plant 15 showed cross-compatibility as male with F13-S₁₂S₁₃ (0.8 fruits/flower and 12.3 seeds/fruit) (table 7). The results of the analysis of 17 progeny plants obtained from crosses between plant 15 (♂) and F13-S₁₂S₁₃ show that all were selfincompatible and reciprocally cross-compatible with F13-S₁₂S₁₃. Plant 15 as well as its progeny were normal in karyotype and meiotic behaviour. These results indicate that in plant 15 a new *S*-specificity was generated which expressed itself in the pollen in the first generation and was then transmitted to progeny where it stabilized both in the pollen and pistil.

The new *S*-allele was cross-compatible with all the diploid tester-stocks of *L. peruvianum* (*S*₁ to *S*₁₃ alleles) available at our institute.

4. DISCUSSION

The results obtained in the present study show that many of the regenerants (16 out of 38 plants) from the anther culture of clone 9-S₁'S₃ carried a heritable change at the *S*-locus. The testing of *S*-genotypes performed for three generations in order to determine the type of *S*-gene alterations in the regenerants revealed the following features.

- All the 16 regenerants carried a new *S*₂^{*} allele.
- The *S*₃ allele of the parental clone 9-S₁'S₃ reverted to a new *S*₂^{*} allele.
- The new *S*₂^{*} allele is dominant over *S*₁' allele of clone 9-S₁S₃, and this switch from gametophytic to sporophytic action is heritable.

Extensive studies with radiations and chemical mutagens have shown that none generated new *S*-alleles (Lewis, 1949, 1951; Lewis and Crowe 1954; Pandey, 1956, 1965; Brewbaker and Natarajan 1960; de Nettancourt and Ecochard, 1968; Hoffman, 1971; van Gastel and de Nettancourt, 1974, 1975), thus suggesting that new *S*-specificities probably do not result from point mutations at the *S*-locus (see Sree Ramulu, 1980). Inbreeding, the only method which so far has generated new *S*-alleles leads to some processes which result under favourable conditions in the generation of new *S*-specificities (Denward, 1963; de Nettancourt and Ecochard, 1969;

de Nettancourt *et al.*, 1971; Pandey, 1970a, 1972, 1977; Hogenboom, 1972; Anderson *et al.*, 1974; Sree Ramulu, 1982). The *S*-alleles are stable under the conditions of a relatively high degree of heterozygosity and the *S*-allele dependent polygenic system is presumably drastically modified by inbreeding.

Fisher (1961) formulated a hypothesis of new *S*-allele generation through recombination between two parental allele complexes. This hypothesis has been elaborated by Lewis (1962) and discussed by Lundqvist (1965) and Pandey (1970b). In previous studies on the generation of new *S*-alleles by inbreeding in *L. peruvianum*, de Nettancourt (1977) also discussed the implications of Fisher's model in relation to the rare cases of reversion of the S_3 allele to S_2 . These occurred among the progeny of crosses between the inbred plants stabilized for the new *S*-specificity (S_2S_3 or S_1S_3) and the original clone (S_1S_2) (see de Nettancourt *et al.* 1971, 1975). According to the model, the new *S*-specificity generated through recombination would not be able to bring about compatibility on selfing and remains incompatible to each of the two parental alleles. The recombinant allele produces a mixture of the two parental antigens and leads to cross-reactions with the pistil containing both original alleles. However, if the new *S*-allele produces a qualitatively different antigen from the two parental alleles, it can bring about compatibility on selfing. The various *S*-alleles considered as identical and classified within the same group may, in fact, be genetically different. The reversions can occur through recombination and the reverted allele need not be similar in structure to the parental allele, but can simply display an identical phenotype.

The results obtained in the present study on the features characterizing the new S_2^* specificity produced in plants regenerated from anther culture of clone 9- $S_1'S_3$, seem to fit with this hypothesis.

- (a) The new S_2^* allele is presumably different in structure from that of its ancestral allele S_2 .
- (b) The new S_2^* expressed in the pollen of regenerants of group 4 was not able to bring about compatibility on selfing. The same appears to be true in plant 15 regenerated from the anther culture of clone F13- $S_{12}S_{13}$ in which a new *S*-specificity expressed in the pollen could not bring about self-compatibility.
- (c) However, in two plants (group 5) regenerated from clone 9- $S_1'S_3$, the new S_2^* expressed in pollen could bring about moderate self-compatibility. The progeny obtained from selfed seeds segregated approximately as $1 \times S_2^*S_2^* : 1 \times S_2^*S_3$ as did the progeny from crosses between the regenerants (δ) and the parental clone 9- $S_1'S_3$.

The possibility that the regenerants carrying the new S_2^* allele originated from microspore (or pollen)-derived calli can not be excluded.

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