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# Use of an interspecific hybrid in identifying a new allelic specificity generated at the self-incompatibility locus after inbreeding in *Lycopersicon peruvianum*

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**Summary.** An interspecific hybrid between *Lycopersicon esculentum* (♀) and *L. peruvianum* has been raised by embryo rescue in vitro and used to confirm the presence of a new *S*-allelic specificity in its inbred *L. peruvianum* parent, a plant derived by enforced bud self-pollination of a self-incompatible clone with the genotype $S_1 S_2$. The inbred plant showed breeding behavior characteristic of both $S_2$ and a second specificity which was not $S_1$, $S_2$, $S_3$ or $S_f$. Two-dimensional gel electrophoresis of stylar proteins, however, showed only a single typical *S*-associated component with the $M_r$ and pI characteristic of $S_2$. The alteration in specificity, therefore, was not associated with a detectable change in an *S*-associated protein. The $F_1$ interspecific hybrid showed intermediacy of vegetative and reproductive characters, relatively high fertility and full self-incompatibility. Backcrossing to *L. esculentum* produced only abortive seeds requiring embryo culture. Backcrosses to *L. peruvianum* produced a very low proportion of filled germinable seeds. Pollen of the hybrid showed superior viability and tube growth rate compared with pollen of the two parent plants.

**Key words:** Self-incompatibility – Hybridization – Inbreeding – Embryo culture – Tomato – Heterosis – *Lycopersicon*

## Introduction

*Lycopersicon peruvianum,* a wild South American tomato, has been hybridized with the cultivated tomato, *L. esculentum,* a number of times for introduction of disease and pest resistance and the improvement of ascorbic acid content in tomato fruits (see review by Rick 1982).

Crosses between these species have generally been made unilaterally with *L. esculentum* as the female parent, owing to rejection of *L. esculentum* pollen by *L. peruvianum* styles. The cross is characterized by early endosperm abortion in developing ovules, and although occasional hybrids have been raised from rare mature seeds (Holmes 1939; Lesley and Lesley 1943; Porte and Walker 1945; Yeager and Purinton 1946; Nirk 1959), in vitro embryo rescue is normally required (Smith 1944; Alexander 1963; De Nettancourt et al. 1974; Thomas and Pratt 1981; Barbano and Topoleski 1984).

*L. peruvianum* is self-incompatible, with rejection of pollen tubes in the style controlled gametophytically by a typical multiallelic incompatibility (*S*) gene located on chromosome 1 (Lamm 1950; McGuire and Rick 1954; Tanksley and Loaiza-Figueroa 1985). *S*-allelic behavior is normally extremely stable in breeding programs, but changes of specificity have been reported to occur after enforced inbreeding (De Nettancourt et al. 1971; Hogenboom 1972) and in plants regenerated from anther culture (Sree Ramulu 1982). Interspecific crosses aimed at transfer of self-incompatibility from *L. peruvianum* to *L. esculentum* to facilitate the production of commercial hybrid tomato seed, have so far met with limited success. $F_1$ interspecific hybrids are generally self-incompatible, but the pollen/style rejection response becomes progressively weaker with continued backcrossing into *L. esculentum* (Martin 1961, 1968; Rick 1982).

In this paper we describe the characteristics and breeding behavior of an $F_1$ hybrid between *L. esculentum* and *L. peruvianum,* and its use in identifying a new *S*-allelic specificity produced after inbreeding.

## Materials and methods

*Lycopersicon esculentum* Mill. cv. 'Grosse Lisse' was grown from commercial seed. *Lycopersicon peruvianum* (L.) Mill. materials were clonal ramets from the population of defined *S*-genotypes described by Mau et al. (1986). Flowers of genotype $S_1 S_2$ were self-pollinated at the green bud stage using a

light coating of 1% NAA in crude lanolin smeared on the floral pedicels to prevent abscision of poorly filled fruits. A plant grown from a bud self-pollination of this type was tentatively identified as $S_2\,S_2$ on the basis of its acceptance of $S_1\,S_2$ pollen (i.e. not $S_1\,S_2$) and its compatibility with $S_1\,S_3$ styles (i.e. not $S_1\,S_1$).

*Production of a hybrid using embryo culture*

Pollen from several *S*-genotypes of *L. peruvianum* was used to pollinate emasculated flowers of *L. esculentum.* Fruits were produced, but these contained only abortive, partially developed seeds. Fruits were picked 40 days after pollination, surface sterilized 5 min in 1 : 1 (v/v) 4.5% sodium hypochlorite and 1.5% cetrimide, washed with sterile water and dissected aseptically. All ovules showed endosperm degeneration, and most also showed very little embryo development.

The hybrid was derived from a pollination by the inbred *L. peruvianum* plant which had been tentatively identified as $S_2\,S_2$. The embryo was only partially developed, with rudimentary cotyledons. It was cultured 16 days on MS medium (RM; Murashige and Skoog 1962) at 22 °C under continuous light (warm white fluorescent tubes; ca. 60 µmol $m^{-2}\,s^{-1}$), transplanted to sterile sand in a mist propagator for 15 days, and finally transferred to soil under normal greenhouse conditions.

*Gel electrophoresis of stylar proteins*

Styles were collected from freshly opened flowers of the hybrid, its inbred *L. peruvianum* male parent, the *L. esculentum* female parent and the original $S_1\,S_2$ clone of *L. peruvianum* from which the male parent was derived. Style extracts were prepared and examined by two-dimensional gel electrophoresis as described by Mau et al. (1986).

*Isoelectric focussing of leaf peroxidase isozymes*

Young leaves were taken from the hybrid, its inbred *L. peruvianum* male parent, and the *L. esculentum* female parent. Leaf extracts were prepared by grinding 0.3 g of leaf tissue in 0.7 ml of 0.5 M phosphate buffer, pH 7.0, and then centrifuging in a microfuge tube at 13,000 rpm for 30 min at 4 °C. Supernatant (15 µl per strip) was applied to an LKB Ampholine PAG plate (pH 3.5–9.5). Colored isoelectric markers (pI range 4.7–10.6; B.D.H., Poole, U.K.) were dissolved in water according to the manufacturers instructions, and applied to the gel in 5 µl aliquots. Isoelectric focussing was performed at constant power (15W) at 4 °C. The gel was equilibrated 10 min in Tris-saline buffer, pH 7.5, and stained for peroxidase activity using HRP color developing reagent containing 4-chloro-1-naphthol (Bio-Rad Laboratories, Richmond, California, U.S.A.; 50 mg in 20 ml methanol) and $H_2O_2$ (60 ml 100 volumes) in Tris-saline buffer pH 7.5 (100 ml) for 40 min at 30 °C in the dark. The reaction was stopped with distilled water.

*Fluorescence microscopy of pollen tube growth in styles*

For each of the crosses shown in Table 1 a minimum of 5 hand-pollinated styles were harvested 48 h after pollination of freshly opened flowers. Styles were fixed overnight in 1 : 3 v/v glacial acetic acid: ethanol, stored when necessary in 70% ethanol, cleared by autoclaving in 10% (w/v) aqueous sodium sulphite (15 psi, 104 KPa; 120 °C) for 15 min, and stained overnight in decolorized aniline blue (Merck Anilinblau WS 0.1% in aqueous 0.1 M $K_3PO_4$) (Williams and Knox 1982). For observation the styles were squashed gently in a drop of stain beneath a coverslip on a microscope slide. The coverslip was sealed with white petroleum jelly to prevent evaporation, and preparations were examined with epifluorescence optics using excitation wavelengths in the blue region (e.g. Zeiss filter combination KP490, KP500, RFl 510, LP 528; Olympus combination BP-490, DM–500 + 0–515).

**Table 1.** Pollen tube growth in styles after testcrossing to determine *S* genotypes. Genotypes of tester stocks are unbracketed. Bracketed genotypes were determined by this diallel. Boxes identify crosses distinguishing $S_2\,S_2'$ from $S_2\,S_2$. Le, *L. esculentum;* Lp, *L. peruvianum;* $F_1$, $F_1$ hybrid *L. esculentum* (♀) × *L. peruvianum*

| | ♀ \ ♂ | Le | $F_1$ | Lp | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $S_1S_f$ | $(S_2S_f)$ | $(S_2S_f')$ | $S_1S_2$ | $S_2S_3$ | $S_1S_3$ | $S_3S_3$ | $S_1S_1$ |
| Le | $S_1S_f$ | + | + | + | + | + | + | + | + |
| $F_1$ | $(S_2S_f)$ | - | - | + | + | + | + | + | + |
| | $(S_2S_f')$ | - | - | - | + | + | + | + | + |
| | $S_1S_2$ | - | - | + | - | + | + | + | - |
| Lp | $S_2S_3$ | - | - | + | + | - | + | - | + |
| | $S_1S_3$ | - | + | + | + | + | - | - | - |
| | $S_3S_3$ | - | + | + | + | + | + | - | + |
| | $S_1S_1$ | - | + | + | + | + | + | + | - |

*Pollen tube growth in vitro*

Freshly collected pollen samples from the hybrid, its inbred *L. peruvianum* male parent, and the *L. esculentum* female parent were germinated and grown on microscope slides coated with a semi-solid medium (Williams et al. 1982) modified from Brewbaker and Kwack (1963): 0.6% agarose, 12% sucrose, 1 mg $ml^{-1}$ $H_3BO_3$, 1 mg $ml^{-1}$ $KNO_3$, 3 mg $ml^{-1}$ $Ca(NO_3)_2 \cdot 4\,H_2O$, 2 mg $ml^{-1}$ $MgSO_4$, pH 6.0. Slides were incubated at 25 °C in closed Petri dishes lined with moist filter papers. Pollen tubes were vapour fixed at half hour intervals by adding 0.5 ml formaldehyde to the filter paper liners and reclosing the lids of dishes for at least 10 min. Three replicate slides of each pollen type were fixed at each time interval. For each slide the lengths of at least 100 pollen tubes of normal morphology were measured using a Zeiss compound bright-field microscope fitted with a camera lucida for operation over the magnetized tablet of a Zeiss Kontron Videoplan computerized image analysis system. Mean pollen tube lengths and variances were obtained. A weighted two-way analysis of variance was performed on mean pollen tube lengths, using variances as weights and adjusting for time to compare the three pollen tube groups. The experiment was repeated a second time after 4 months to check that the first result was not seasonal or transient.

*Scanning electron microscopy of surface hairs*

Small segments of young leaves and stems of the hybrid and its two parents were fixed 4 h in 4% paraformaldehyde in phosphate buffer, pH 7.0, washed twice in buffer, post-fixed 1 h in 1% $OsO_4$, washed again in buffer, dehydrated through an ethanol series, transferred from 100% ethanol to liquid $CO_2$ for critical point drying, sputter coated with gold and examined with a Phillips 505 scanning electron microscope.

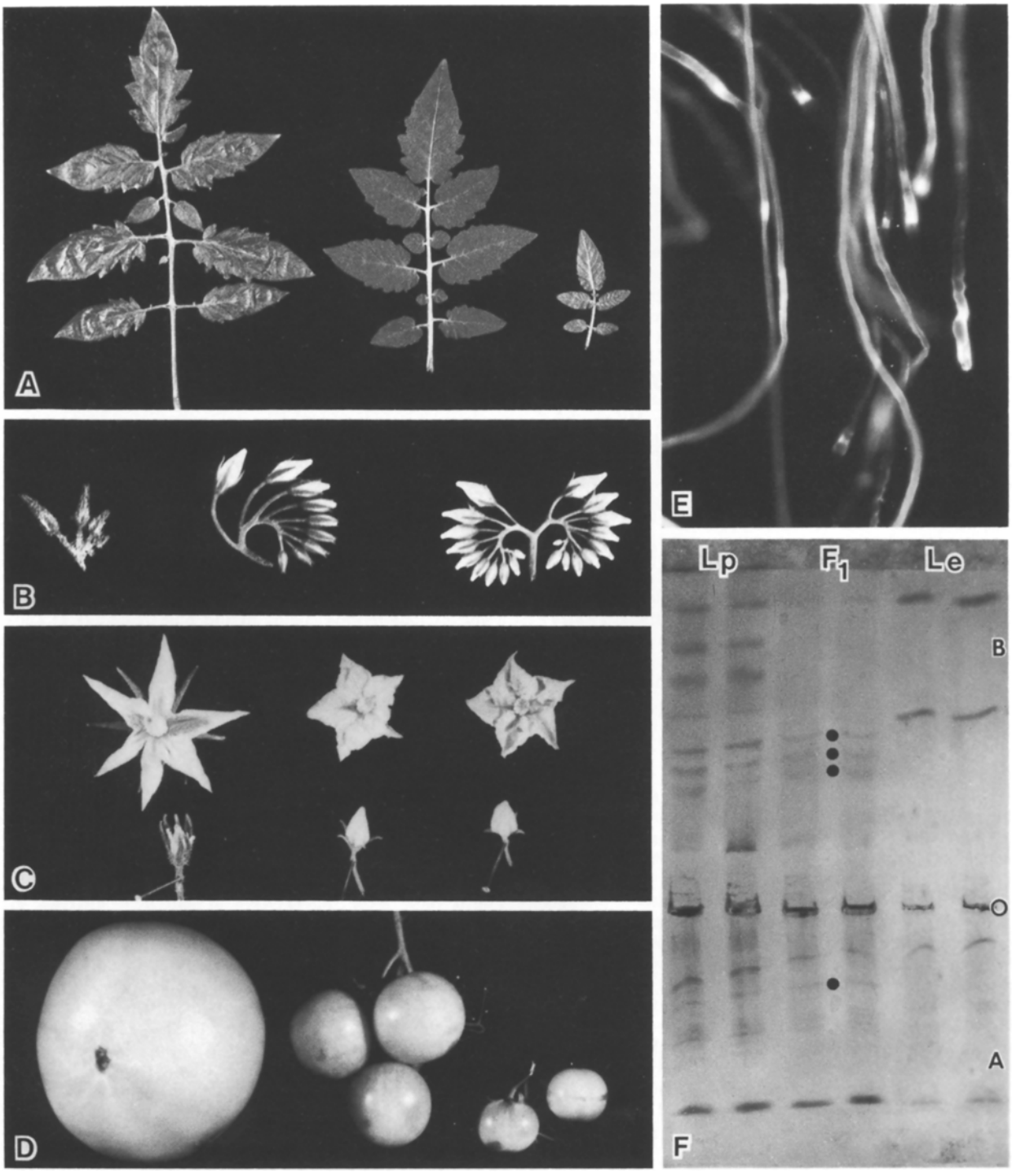


**Fig. 1.** **A-D** Comparisons between the $F_1$ interspecific hybrid (center), *L. esculentum* female parent (left) and *L. peruvianum* male parent (right): **A** leaves (× ca. 0.4); **B** young inflorescences (× ca. 0.8); **C** flowers (× ca. 0.7); **D** fruits (× ca. 0.7). **E** Arrested self pollen tube tips in the style of the $F_1$ hybrid showing sub-terminal callose deposition characteristic of the self-incompatibility response (× ca. 320). **F** Isoelectric focussing gel of leaf extracts stained for peroxidase activity. The hybrid ($F_1$, center two tracks) contains one acidic and 3 basic bands (black dots) characteristic of the male *L. peruvianum* parent (left-hand two tracks). *L. esculentum* parent, right-hand two tracks; O, origin; A, acidic; B, basic

*Pollen viability*

Pollen quality of the hybrid, its two parents and the $S_1 S_2$ clone of *L. peruvianum* was assessed as the percentage of brightly fluorescent grains in freshly collected pollen samples using the FCR procedure (fluorochromatic reaction) of Heslop-Harrison and Heslop-Harrison (1970).

*Seed set data*

Hand pollinated flowers were left on the plant to set fruit. Fruits were dissected to determine the percentage of ovules producing filled seeds.

## Results

*Confirmation of hybridity*

Once established in soil after embryo rescue the hybrid was vegetatively vigorous, and during early growth showed morphological intermediacy relative to the parents (Fig. 1 A). Scanning electron microscopy of young leaf and stem segments showed intermediacy of the hybrid with respect to the occurrence and distribution of trichomes, particularly glandular trichomes with 4-celled caps (Fig. 2). Analysis of leaf peroxidase isozymes showed that the hybrid had several major bands from the male *L. peruvianum* parent (Fig. 1 F). When the hybrid flowered the inflorescence comprised a single series of flowers as in the *L. esculentum* parent, but the flowers resembled those of *L. peruvianum* in number and morphology (Fig. 1 B, C). Stigmas were exserted beyond the anthers as in *L. peruvianum.* Fruits of the *L. esculentum* parent are red, while those of *L. peruvianum* are pale green. When compatibly pollinated the hybrid produced bright yellow fruits intermediate in size between those of the parents but closer to those of *L. peruvianum* (Fig. 1 D). The flesh had a sweet taste.

*Stylar proteins*

Two-dimensional gel electrophoresis (Fig. 3) confirmed the results of Mau et al. (1986) for *L. peruvianum* $S_1 S_2$. This clone showed $S_1$- and $S_2$-allele-associated proteins of $M_r$ ca. 28,000 and ca. 27,000 respectively, together with two proteins of $M_r$ ca. 30,000 common to all our *L. peruvianum* clones. The inbred plant tentatively identified as $S_2 S_2$ showed the two common components together with a single additional band at $M_r$ ca. 27,000 characteristic of the $S_2$ allele. The *L. esculentum* parent of the interspecific hybrid showed two major proteins at $M_r$ ca. 30,000 with pI similar to that of the $S_2$-allele-associated protein. The hybrid showed the $S_2$-allele-associated protein, the two common proteins from *L. peruvianum* and the two major components from *L. esculentum.* In addition, it showed two minor components at $M_r$ ca. 80,000 in the low pI range, also apparently derived from *L. esculentum.*

*Breeding behavior*

Pollinations were assessed as compatible or incompatible by examination of pollinated styles for the extent of pollen tube growth. Compatibly pollinated styles showed a large bundle of pollen tubes passing out of the style base, whereas in incompatibly pollinated styles all pollen tubes were arrested between one quarter and three quarters of the distance down the style. A mixture of swollen and burst tube tips, with or without large sub-terminal callose plugs, was seen in all incompatible pollinations. Most arrested tips showed heavy sub-terminal callose deposition (Fig. 1 E), usually in the form of a plug or collar-like wall thickening. Rejection of *L. esculentum* pollen tubes by *L. peruvianum* styles occurred closer to the stigma than rejection of self-incompatible *L. peruvianum* tubes, and showed very marked sub-terminal callose deposition. The intra-specific and interspecific arrest syndromes, however, were not qualitatively distinct.

Results of a diallel cross to determine the breeding behavior of the interspecific hybrid and its inbred male *L. peruvianum* parent are shown in Table 1. Tester clones of *L. peruvianum* were also intercrossed as a standard check. All tester stocks ($S_1 S_2$, $S_2 S_3$, $S_1 S_3$, $S_3 S_3$, $S_1 S_1$) behaved as expected in reciprocal crosses amongst themselves. All behaved as expected with respect to *L. esculentum,* rejecting *L. esculentum* pollen, and having their pollen accepted by *L. esculentum* styles. The $S_f$ allele of *L. esculentum* does not specify rejection of pollen by the style, but does confer on pollen a specificity such that it can be rejected by all styles carrying functional *S* alleles. The interspecific hybrid behaved as expected for the genotype $S_2 S_f$. It was strongly self-incompatible and unilaterally incompatible with *L. esculentum,* showing that the single $S_2$ allele was capable of rejecting both $S_2$ and $S_f$ pollen in the style. It was reciprocally compatible with tester genotypes not containing $S_2$, but showed unilateral incompatibility with $S_1 S_2$ and $S_2 S_3$ confirming the presence of a single $S_2$ allele. The inbred *L. peruvianum* parent of the hybrid was self-incompatible, unilaterally compatible with *L. esculentum,* and reciprocally compatible with tester genotypes $S_1 S_3$, $S_3 S_3$ and $S_1 S_1$ as expected. Its behavior was anomalous, however, in crosses to its hybrid offspring and the tester genotypes $S_1 S_2$ and $S_2 S_3$. As a putative $S_2 S_2$ homozygote it had been expected to show reciprocal incompatibility with hybrid offspring of genotype $S_2 S_f$, and unilateral incompatibility with $S_1 S_2$ and $S_2 S_3$. Instead it showed unilateral incompatibility with the hybrid and full reciprocal compatibility with $S_1 S_2$ and $S_2 S_3$ suggesting the presence of a second allelic specificity in addition to $S_2$ but not $S_1$ or $S_3$. These results were checked by additional pollinations and proved to be

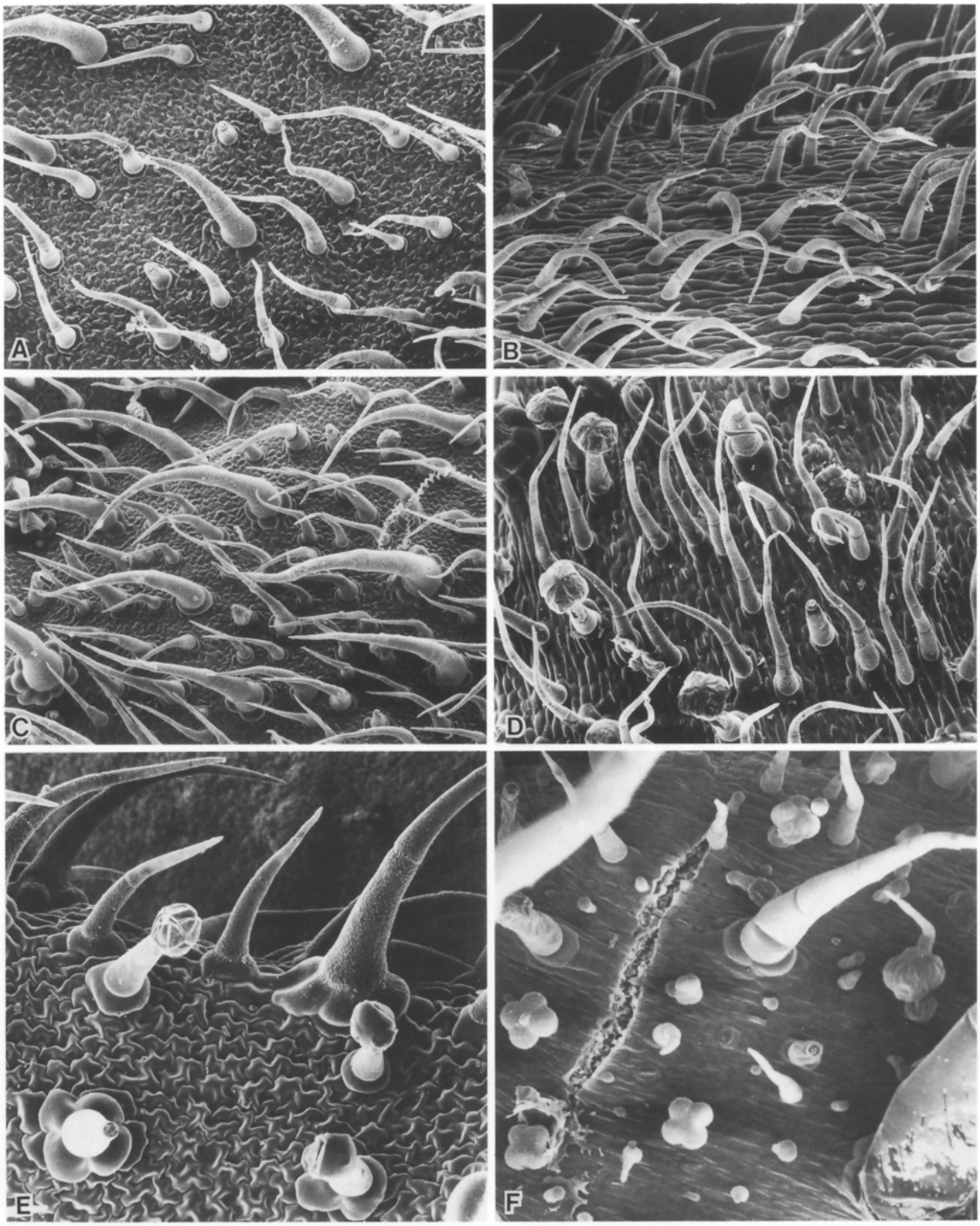


**Fig. 2.** Comparisons of surface trichomes **A, B** for the *L. peruvianum* male parent, **C, D** for the $F_1$ interspecific hybrid, and **E, F** for the *L. esculentum* female parent. **A, C** and **E** are leaf surfaces, **B, D** and **F** are stem surfaces. The hybrid shows an intermediate pilosity, particularly with regard to frequency of glandular trichomes with 4-celled caps. (**A, B** × ca. 200; **C** × ca. 150; **D** × ca. 450; **E** × ca. 400; **F** × ca. 350. The glandular trichomes are approximately the same size on all surfaces)

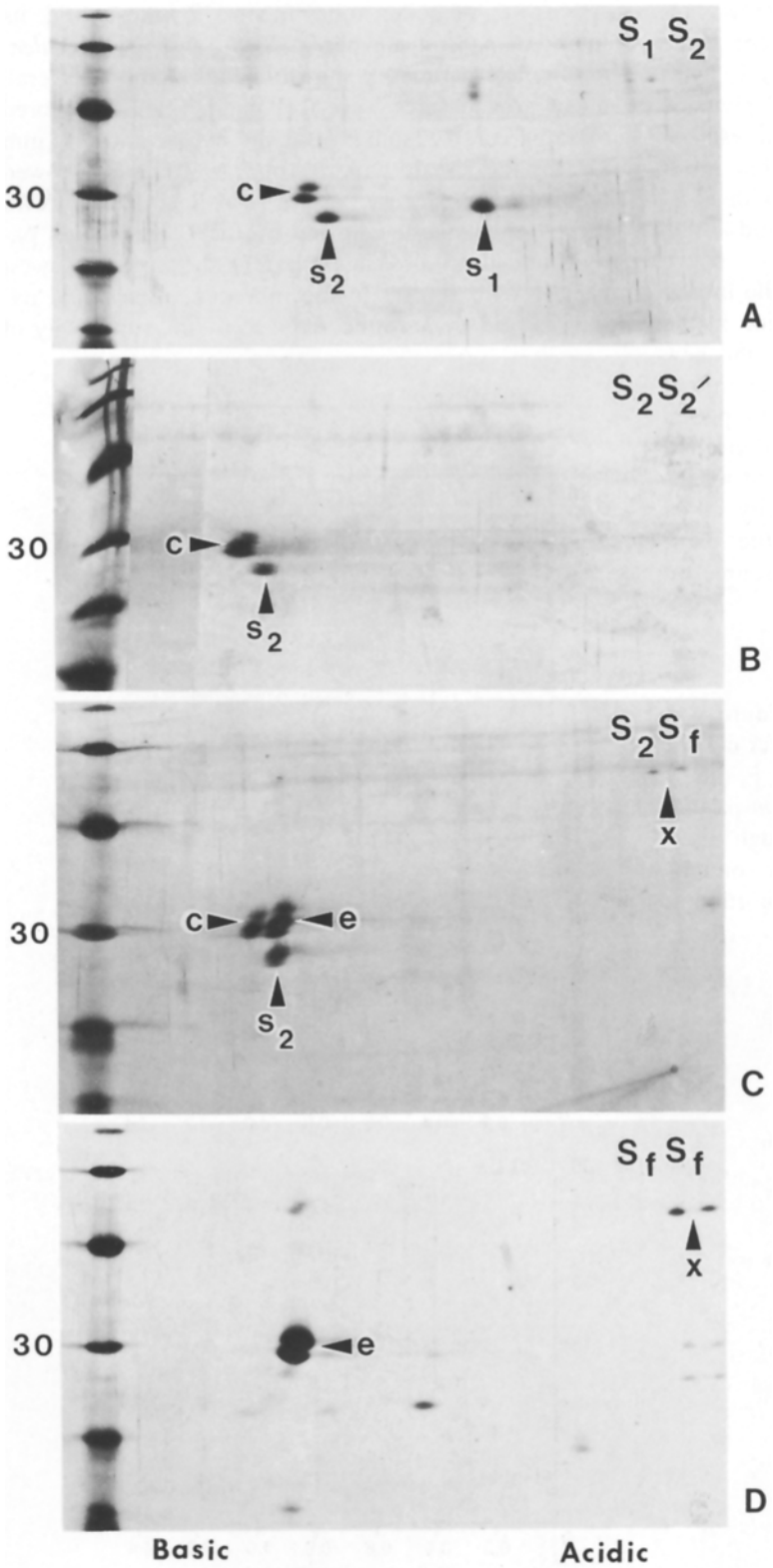


**Fig. 3.** Two dimensional gel electrophoresis of stylar proteins for **A** $S_1$ $S_2$; **B** the inbred *L. peruvianum* plant $S_2$ $S_2'$; **C** the $F_1$ interspecific hybrid $S_2$ $S_f$, and **D** the *L. esculentum* parent of the hybrid $S_f$ $S_f$. Molecular markers are $M_r$ 94,000 (top), 67,000, 43,000, 30,000, 20,100 and 14,400 (bottom) for each gel. $S_1$, $S_1$-allele-associated protein; $S_2$, $S_2$-allele-associated protein; c, two components common to all our *L. peruvianum* stocks; e, two major components characteristic of *L. esculentum;* x, minor proteins characteristic of the *L. esculentum* parent

consistent. Furthermore, normal fruits were set regularly by pollen from the inbred in these three anomalous pollinations, those on $S_1$ $S_2$ and $S_2$ $S_3$ styles giving full seed set, and those on the hybrid giving a mean of 11% filled seed. Reduced seed set as a result of post-fertilization abortion is expected in this interspecific backcross. Comparable pollinations with $S_1$ $S_2$ and $S_2$ $S_3$ pollen on the hybrid gave 12.0 and 11.8% filled seed, respectively.

Since the change in allelic specificity in the inbred was not accompanied by a detectable alteration in the $M_r$ or pI of the $S_2$-allele-associated protein, the new allelic specificity was designated $S_2'$ and the inbred genotype $S_2$ $S_2'$. The alteration affected both pollen and stylar specificities. If pollen specificity alone had been altered (genotype $S_2$ [$S_2$ ♀ $S_2'$ ♂]) the plant would have been self-compatible. If stylar specificity alone had been altered (genotype $S_2$ [$S_2'$♀$S_2$♂]) the plant would have been incompatible as a pollen source on $S_1$ $S_2$ and $S_2$ $S_3$.

*Pollen viability and pollen tube growth in vitro*

Pollen viability assessed by the FCR procedure was >99% for $S_1$ $S_2$ and other *L. peruvianum* tester clones, 59.5% for the inbred $S_2$ $S_2'$, 88.5% for the $F_1$ interspecific hybrid and 63.6% for the *L. esculentum* parent. For this last plant 9.9% of grains, although non-fluorescing (inviable), showed full cytoplasmic contents but were slightly reduced in size, suggesting abortion at a late stage of development.

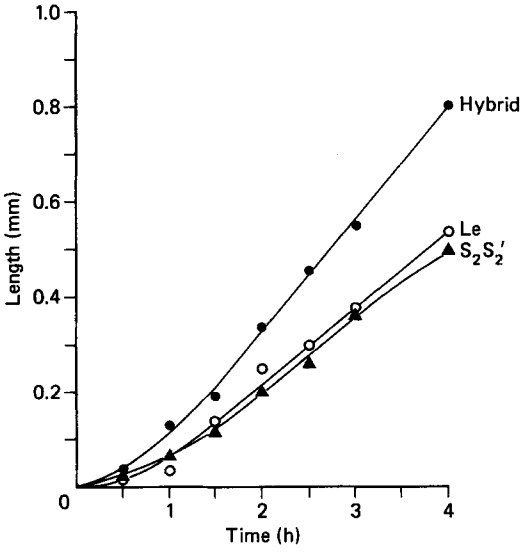


**Fig. 4.** Pollen tube growth in vitro for the $F_1$ interspecific hybrid (●), *L. esculentum* female parent (○) and inbred *L. peruvianum* male parent, $S_2$ $S_2'$ (▲)

Growth of pollen tubes in vitro for the hybrid, its inbred *L. peruvianum* parent $S_2$ $S_2'$ and its *L. esculentum* female parent are shown in Figs. 4 and 5. Overall mean growth rates were 0.12 mm $h^{-1}$ for the inbred male parent, 0.20 mm $h^{-1}$ for the hybrid and 0.14 mm $h^{-1}$ for the female parent. Tube growth rates showed the same comparative pattern as pollen viability, with the hybrid highest, followed by the *L. esculentum* parent, and the inbred male parent showing the lowest values. With respect to the inbred *L. peruvianum* parent, hybrid pollen tubes showed a 76% superiority of

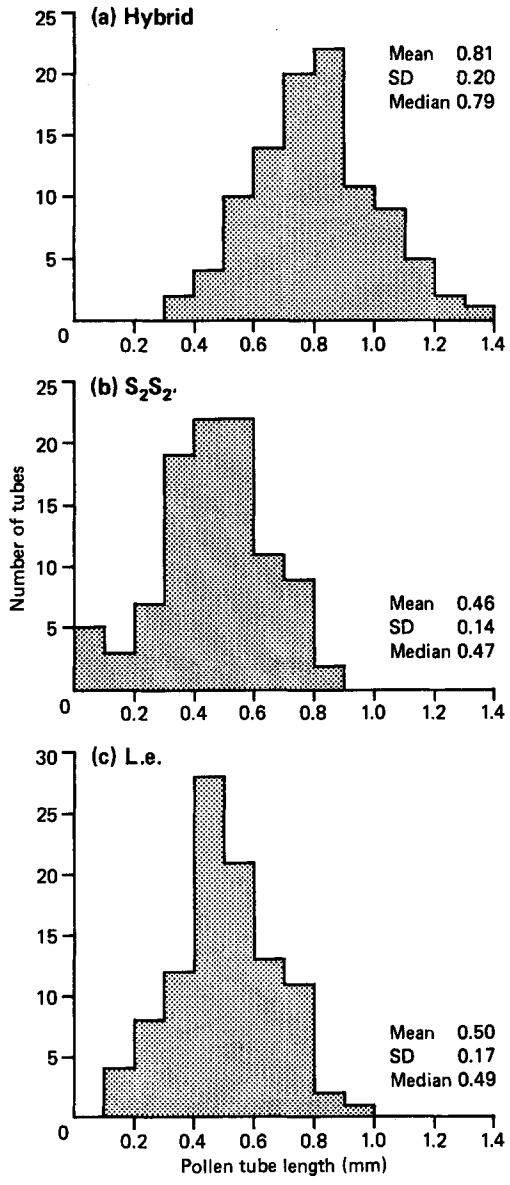


**Fig. 5a–c.** Frequency distributions of pollen tube length for representative single replicate slides of three pollen types fixed after 4 h growth in vitro. **a** $F_1$ interspecific hybrid; **b** inbred *L. peruvianum* male parent, and **c** *L. esculentum* female parent

growth rate after 4 h. Growth of hybrid pollen tubes was significantly more rapid than that of either parent group ($P < 0.05$ for the comparison with the inbred male parent, and $P < 0.01$ for the comparison with the *L. esculentum* parent). These results were confirmed by a second experiment performed 4 months after the first determination. For all three pollen types, all replicate slides in the two separate experiments gave essentially identical results, with replicate means differing by less than 0.04 mm.

*Backcrosses of the hybrid to parent species*

The hybrid rejected pollen of *L. esculentum*, and when used as a pollen parent on *L. esculentum* gave only partially developed seeds of the type produced in the original interspecific cross with *L. peruvianum* pollen on *L. esculentum*. Embryo culture has so far been used to rescue two backcross hybrids, but both succumbed to a suspected fungal infection shortly after transfer to soil. When pollinated with compatible *L. peruvianum* pollen the hybrid produced fruit with mostly abortive ovules but a small proportion of filled seed – i.e. 11.0% with $S_2\ S_2'$, 12.0% with $S_1\ S_2$, 11.8% with $S_2\ S_3$, 10.6% with $S_1\ S_3$, 7.5% with $S_3\ S_3$ and 4.8% with $S_1\ S_1$. A few seeds from the cross with $S_2\ S_2'$ pollen have been planted, and one plant is now growing vigorously in soil. The remaining seeds have not yet been planted. Reciprocal crosses with hybrid pollen on $S_1\ S_2$, $S_2\ S_3$ and $S_2\ S_2'$ were incompatible. On $S_1\ S_3$ pollen of the hybrid gave fruit with only 12.9% of filled seeds which have not yet been tested for germinability. Pollinations on $S_1\ S_1$ and $S_3\ S_3$ have not yet been tested.

## Discussion

An interspecific hybrid betwen *Lycopersicon esculentum* (♀) and *L. peruvianum* has been raised by embryo rescue in vitro and used to confirm the presence of a new *S*-allelic specificity in its inbred *L. peruvianum* parent, a plant derived by enforced bud self-pollination of a self-incompatible clone with the genotype $S_1\ S_2$. The inbred plant has been designated $S_2\ S_2'$ for the following reasons:

– it has transmitted a standard $S_2$ specificity to its hybrid offspring and therefore must carry a fully functional $S_2$ allele;
– it is compatible as male on $S_1\ S_2$ and $S_2\ S_3$ and must therefore also carry a pollen specificity which is not $S_1$, $S_3$ or $S_f$
– it is compatible as female with $S_1$ and $S_3$ pollen showing that its styles do not express either of these allelic specificities;
– two-dimensional gel electrophoresis of stylar proteins shows only a single typical *S*-associated component with $M_r$ and pI characteristic of $S_2$. Therefore the change of allelic specificity may be associated with either such a drastic change in protein structure that the analysis procedure has failed to detect the molecule, or with such a small change that the molecule runs together with the normal $S_2$-associated protein. We believe this second possibility to be more likely.

We do not know as yet whether the new allelic specificity will be transmissible to progeny and show normal behavior in outbreeding heterozygous plants. This aspect is being investigated. Our observations agree with reports by Denward (1963), Pandey (1970), De Nettancourt et al. (1971) and Hogenboom (1972) that new allelic specificities can arise during enforced inbreeding in gametophytic self-incompatibility systems. They are in contrast, however, to a report by Kheyr-Pour and Pernes (1985) who observed a distinctive new stylar protein associated with a new stable *S*-allelic specificity originating in a homozygous dihaploid of *Nicotiana alata*.

The $F_1$ interspecific hybrid, *L. esculentum* (♀) × *L. peruvianum*, reported here showed intermediacy of vegetative characters, flowers somewhat more like those of *L. peruvianum*, sweet yellow fruits of intermediate size but closer to those of *L. peruvianum*, relatively high fertility and full self-incompatibility. Backcrossing to *L. esculentum* produced only abortive seeds requiring embryo culture, while backcrossing to *L. peruvianum* produced a very low proportion of germinable seeds. These characteristics are in general agreement with previous observations for $F_1$ hybrids between these species (Lesley and Lesley 1943; Smith 1944; Porte and Walker 1945; Yeager and Purinton 1946; McGuire and Rick 1954; Nirk 1959; Alexander 1963; Martin 1961, 1968; Yamakawa 1971; De Nettancourt et al. 1974; Thomas and Pratt 1981). Exceptions are reports of self-fertile hybrids not requiring embryo culture in backcrosses to *L. esculentum* (Yaeger and Purinton 1946; Nirk 1959) and orange-red fruits (Yaeger and Purinton 1946).

The lowered pollen fertility of the inbred *L. peruvianum* plant $S_2\ S_2'$, compared with its parent clone $S_1\ S_2$ may be a function of inbreeding depression. Similarly, the superiority of hybrid pollen over the two parental pollen types with respect to both viability and tube growth may represent heterosis. Chase (1983) reported heterosis for heterozygous diploid pollen of tetraploid maize and potatoes. The reason for superiority of haploid hybrid pollen in the present study is unknown. It may represent: (1) heterotic interaction of allelic products of the same locus, based on transcription before separation of alleles to different cells at meiosis; (2) complementary interaction of the products of different loci, based on post-meiotic transcription; or (3) complementary interaction of nuclear factors with *L. esculentum* cytoplasm.

De Nettancourt et al. (1974) reported a 1 : 1 segregation of arrested self pollen tubes in $F_1$ interspecific hybrid styles, with burst callose-plugged tips representing $S_f$ tubes (equivalent to interspecific incompatibility), and swollen tips representing $S_i$ tubes (i.e. normal self incompatibility). We were unable to substantiate this report. All incompatible pollinations, including *L. peruvianum* (♀) × *L. esculentum*, *L. peruvianum* selfed and $F_1$ hybrid selfed, showed both types of arrested tips.

In the present work observation of a change of *S*-allelic specificity without a detectable gross alteration in $M_r$ or pI of a protein shown previously to be *S*-allele associated, raises fundamental questions as to how

specificity is determined. The change is either so small that it does not significantly affect the analytical properties of the protein, so large that the altered molecule has been missed altogether in the analytical procedure, or alternatively not associated with the protein moity at all but with some bound or unbound component which becomes involved during the incompatibility response in vivo.

The nature of the two major proteins of $M_r$ ca. 30,000 observed in *L. esculentum* styles requires further investigation. Presumably both components cannot be associated with $S_f$ function since both were transmitted to the hybrid which is assumed to have the genotype $S_2 S_f$. If one were found to be $S_f$-associated, sequence analysis and comparison with functional $S_i$ components might assist in determining the region(s) of $S_i$-associated proteins involved in specificity determination and/or the pollen tube rejection response. On the basis of breeding behavior, however, we consider it more likely that $S_f$ does not produce a stylar specificity protein. The two major proteins of *L. esculentum* styles may be homologous with the two common components of similar mobility and charge found in *L. peruvianum* styles.

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