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ORIGINAL PAPER

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Fine mapping of the *parthenocarpic fruit* (*pat*) mutation in tomato

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Abstract The *parthenocarpic fruit* (*pat*) gene of tomato is a recessive mutation conferring parthenocarpy, which is the capability of a plant to set seedless fruits in the absence of pollination and fertilization. Parthenocarpic mutants offer a useful method to regulate fruit production and a suitable experimental system to study ovary and fruit development. In order to map the *Pat* locus, two populations segregating from the interspecific cross *Lycopersicon esculentum* × *Lycopersicon pennellii* were grown, and progeny plants were classified as parthenocarpic or wild-type by taking into account some characteristic aberrations affecting mutant anthers and ovules. Through bulk segregant analysis, we searched for both random and mapped AFLPs linked to the target gene. In this way, the *Pat* locus was assigned to the long arm of chromosome 3, as also confirmed by the analysis of a set of *L. pennellii* substitution and introgression lines. Afterwards, the *Pat* position was refined by using simple sequence repeats (SSRs) and conserved ortholog set (COS) markers mapping in the target region. The tightest COSs were converted into CAPS or SCAR markers. At present, two co-dominant SCAR markers encompassing a genetic window of 1.2 cM flank the *Pat* locus. Considering that these markers are orthologous to *Arabidopsis* genes, a positional cloning exploiting the tomato-*Arabidopsis* microsynteny seems to be a short-term objective.

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Introduction

Tomato, *Lycopersicon esculentum* Mill., has a self-fertilizing mode of reproduction with wind as the primary pollination stimulus. Normally, the tomato plant produces seeded fruits after pollination and fertilization. However, these processes depend on narrow environmental constrains (Picken 1984). Good pollen production is permitted by night temperatures ranging between 15 and 21°C, and air circulation is necessary to ensure pollen shedding. Such conditions are not often met in unheated greenhouses or tunnels during winter or early spring cultivations. Parthenocarpy, which is the formation of seedless fruits in the absence of functional pollination or other stimuli (Gustafson 1942), offers a method of dealing with the problem of poor fruit set by circumventing the temperature and wind-dependent fertilization process.

Growth regulators, such as synthetic auxins, are used by tomato growers to induce parthenocarpic fruit set in some production regions (Rubatzky and Yamaguchi 1995). However, these agricultural practices add extra costs to the production, and fruit defects may be a problem when auxins or other growth regulators are applied (Schwabe and Mills 1981). The drawbacks connected with chemical treatments can be overcome by exploiting genetic parthenocarpy, a trait that is currently widely diffused in several crops, such as banana, grape, watermelon and cucumber (Varoquaux et al. 2000). On the one hand, genetic parthenocarpy can be artificially achieved in tomato with exogenous genes, which, through ovary and/or ovule specific promoters, drive the oversensitisation to or the accumulation of auxins in carpel tissues before anthesis (Carmi et al. 1997; Ficcadenti et al. 1999). On the other hand, different tomato lines carrying mutations for parthenocarpy have been discovered or selected (reviewed by Lukyanenko 1991); among these genes, the most interesting for breeding are *parthenocarpic fruit* (*pat*; Soressi and Salamini 1975) and *parthenocarpic fruit-2* (*pat-2*; Philouze and Maisonneuve 1978). The former gene, the object of this work, was obtained by mutagenesis with ethyl methanesulfonate

(Bianchi and Soressi 1969). This mutant is characterized by a parthenocarpic phenotype with high penetrance and expressivity, that also entails earlier ripening and enhanced fruit quality (Falavigna et al. 1978). Anyway, the expressivity of the mutation can be reduced by high night temperatures and by certain genetic backgrounds (Mazzucato et al. 1999). As a pleiotropic effect, *pat* flowers have aberrant androecia and ovules, and therefore reduced male and female fertility (Mazzucato et al. 1998). Notwithstanding the interest for these mutations and the research activity carried out at the physiological level on both *pat* (Mapelli et al. 1978; Mazzucato et al. 1999) and *pat-2* (Fos and Nuez 1997; Fos et al. 2000), almost no advances have been made towards the genetic characterization, mapping and cloning of the underlying genes. To date, the only gene for parthenocarpy having been cloned is *MdPI* of *Malus domestica* (Yao et al. 2001), which corresponds to the apple ortholog of the class B MADS-Box *PISTILLATA* of *Arabidopsis thaliana* (Goto and Meyerowitz 1994).

As an initial effort toward the map-based cloning of the *pat* gene and an essential step for developing marker-assisted selection tools, the aim of the present work was the establishment of a local map in the *Pat* locus region and its integration into the tomato molecular map. We searched for AFLP molecular markers (Vos et al. 1995) tightly linked to the target locus applying a bulk segregant analysis (BSA; Michelmore et al. 1991) strategy. The map position of the makers linked to the *Pat* locus was determined by comparison with the AFLP-RFLP tomato map published by Haanstra et al. (1999), and with other maps reported on the Solanaceae Genomic Network web site (http://www.sgn.cornell.edu/).

Materials and methods

Plant materials

A BC_1F_1 population was derived from the interspecific cross between a *L. esculentum* line homozygous for the *pat* mutation (cv Chico III genetic background) and a *Lycopersicon pennellii* LA716 plant (homozygous for the wild-type allele, WT). A single F_1 plant was used as male parent for the backcross to the Chico III-*pat* line. In order to obtain good seed yields, backcrosses were performed under conditions that minimize *pat* expression: plants were cultivated at Viterbo (42°26'N, 12°04'E), Italy, during the autumn season and upper inflorescences were used for hand pollination as described by Mazzucato et al. (1999). A BC_1F_2 population was obtained by selfing a single heterozygous BC_1F_1 individual. All plants from the mapping populations were grown at the same location in an unheated tunnel and under ambient light in spring. Such conditions maximize the expressivity of the *pat* mutation.

To validate the map position of markers putatively linked to the target locus, we employed a set of *L. pennellii* alien substitution lines (ASLs) for chromosome 1, 2, 3, 4, 6, 8 and 11 (accession number LA2091, LA1639, LA1640, LA3469, LA3142, LA1642 and LA1643, respectively; Rick 1969) and a set of *L. pennellii* introgression lines (ILs; Eshed and Zamir 1994).

Screening for the *pat* mutation

To classify plants segregating for the *pat* mutation, 4–6 flowers were collected at anthesis from the second and fourth inflorescence, and the occurrence of aberrant anthers and ovules was determined. Anthers were analysed under a stereomicroscope after dissecting the anther cones. Anther aberrancy was recorded as the percentage of short or carpelloid anthers over the total number of anthers observed. To count aberrant ovules, dissected ovaries were opened and pieces of placenta with ovules cut off. Specimens were observed under either a stereo, bright field or fluorescence microscope as described (Mazzucato et al. 1999). Ovule aberrancy was expressed as the percentage of abnormally developed ovules over a total of 40–60 observed per ovary. For comparison, observations on anthers and ovules were also made on WT and *pat* mutant flowers in the original, *L. esculentum* background. The *pat* phenotype was assigned to plants concurrently showing more than 2% of short and/or carpelloid anthers, and 2% of aberrant ovules.

DNA extraction and molecular marker analysis

Total DNA was extracted according to Doyle and Doyle (1990), using about 200 mg of fresh tissue (leaves or inflorescences) collected in a 1.5-ml Eppendorf tube. To identify random AFLP markers (Vos et al. 1995) linked to the *Pat* locus, a BSA (Michelmore et al. 1991) was carried out using two DNA pools. Each bulk represented eight BC_1F_1 plants displaying either the mutant or the WT phenotype. To identify AFLPs reported on the integrated RFLP-AFLP tomato map (Haanstra et al. 1999), the BSA was performed on eight DNA pools, each representing four mutant or four WT BC_1F_2 plants.

AFLP marker analysis was conducted essentially as described by Vos et al. (1995), starting from about 250 ng of DNA for each plant. Random markers were amplified by 146 combinations of *Pst*I/*Mse*I primers with three additional nucleotides: mapped markers were amplified using the published primer sequences (Haanstra et al. 1999). After PCR, the amplified products were added to an equal volume of formamide-loading buffer [98% deionised formamide, 10 mM EDTA pH 8.0, 0.25% bromophenol blue and 0.25% xylene cyanol (w/v)] and denatured at 94°C for 5 min. Eight microliters of the denatured PCR products were loaded on 6% polyacrilamide gels (37.5:1 acrylamide:bis-acrylamide) containing 7 M urea, 1 × TBE buffer. Electrophoresis was carried out at a constant temperature (45°C) and wattage (45 W) for 120–150 min. Bands were scored after having been visualized through the silver staining method (Bassam et al. 1991). The size of the amplified bands was estimated by comparison with suitable DNA ladders. AFLP markers were named according to the standard list for AFLP primer nomenclature [Keygene, The Netherlands, (http://www.keygene.com/html/index_research.htm) followed by the marker length (bp)].

Primers for microsatellites SSR300 and SSR601 were synthesized from published sequences (Tomato-EXPEN 2000 map, http://www.sgn.cornell.edu) and used in amplification as reported in Table 1. PCR reactions, polyacrylamide gel electrophoresis, and silver staining were processed with the same procedure employed for AFLPs. Up to three reactions were loaded onto the same well with a time lag of 25 min.

Primers to amplify the conserved ortholog set (COS) markers (Fulton et al. 2002) T796, T1143 and T1283 were designed from the published sequences (http://www.sgn.cornell.edu). Primer sequences and PCR reaction conditions are reported in Table 1. The cleaved amplified polymorphic sequence (CAPS) technique was performed by digesting 10 μl of the PCR reaction with 5 U of the appropriate enzyme (Table 1). Restriction fragments were resolved on 2.5% (w/v) agarose gels.

Table 1 Primer sequences and PCR reaction parameters for SCAR, SSR and COS markers

Marker type	Marker name	Primer sequence (5′–3′)	PCR product length (bp)	Annealing temp. (°C)	No. of cycles
SCAR	*E35M61-186*	*F GAATTCACAACGTGATAGTA* *R TTAACTGATTTTTTTATGACTGCAAG*	161	53	32
SCAR	*P44M31-386*	*F TGCAGATCAAACATCCATCTG* *R TAAAAACTCAAAAATGACTTTGC*	386, 425[a]	55	30
SCAR	*P39M37-260*	*F TAAACGCTCGACTTAGTGTAC* *R GCAAATCGCAAATATCAAGGC*	150	55	30
SSR	SSR300[b]	*F AATGGCAGCTATGATGAGCC* *R ACCCGACTTCATTTCACCTG*	525	60	30
SSR	SSR601[b]	*F TCTGCATCTGGTGAAGCAAG* *R CTGGATTGCCTGGTTGATTT*	166	55	30
COS/CAPS	T796[c]	*F TCGGAGTATTGATGGCCTGTC* *R TCAACATGAGGACCACGGC*	~1,800	60	32
COS/SCAR	T1143	*F GGAGAATGGGCATCTACAA* *R CCTTTAGGATGGATTCCG*	~1,100, ~900[a]	55	32
COS/CAPS	T1283[d]	*F TCGGAGTATTGATGGCCTGTC* *R TCAACATGAGGACCACGGC*	510	55	32

[a] Product length on *L. pennellii* and *L. esculentum* respectively
[b] Primers published in the Solanaceae Genomics Network web site (<exref type="“URL”">http//:www.sgn.cornell.edu/</exref>)
[c] Polymorphic after digestion with *Eco*RI
[d] Polymorphic after digestion with *Mse*I

Conversion of AFLPs into SCAR markers

To sequence AFLP markers that were converted in sequence characterized amplified regions (SCARs), we eluted the target DNA from the gel matrix. A sharp, clean razor blade was used to excise the piece of gel containing only the selected band. The gel slice was placed into a 1.5-ml Eppendorf tube with 100 μl of sterile water, boiled for 15 min and centrifuged for 2 min at 11,400 rpm. The supernatant was transferred to a new tube and 10 μl of 3 M Na-acetate (pH 5.2), 5 μl of glycogen and 450 μl of 100% ethanol were added to precipitate the DNA. Tubes were shaken and incubated at −20 or −80°C for at least 30 min, then centrifuged for 15 min at 11,400 rpm. The supernatant was removed and pellets were washed twice with 85% cold ethanol. Finally, pellets were dried for 5 min under vacuum, re-suspended in 10 μl of sterile water and stored at 4°C.

To amplify the eluted products, a standard pre-amplification PCR protocol (50-μl reaction volume; Vos et al. 1995) with the corresponding +1 or +3 AFLP primers was performed on each sample. To check the purity and size of each band, a 10-μl sample of each PCR product was electrophoresed on a 2% (w/v) agarose gel. The remaining amplified DNA was eluted using the QIAquick PCR purification kit (QIAGEN, Hilder, Germany). The quality and concentration of the eluted product was checked on a 2% agarose gel. If clean, the eluted DNA was directly used as a template for sequencing by an ABI PRISM 310 automatic sequencer (Applied Biosystems, Foster City, Calif.).

All the sequenced AFLPs contained the *Eco*RI or *Pst*I adapter sequence at one end and the *Mse*I adapter sequence at the other one. Based on the sequence, suitable internal primers were designed taking care of including the restriction sites. SCAR primer sequences and amplification parameters are shown in Table 1. SCAR analysis was performed on leaf tissue following Klimyuk et al. (1993). Briefly, 2 to 4 mm^2 of young leaf tissue were collected in a 1.5 Eppendorf tube containing 40 μl of 0.25 N NaOH. Tubes were boiled from 40 to 50 s. The solution was neutralized with 40 μl of 0.25 N HCl and then added with 20 μl of Tris-HCl 0.5 M pH 8 and 0.25% (v/v) NONIDET P-40. Tubes were boiled for a further 2 min. The tissue explant was placed in a PCR tube and stored at −20°C until used as a template for standard PCR. SCAR markers derived from AFLPs were reported with the same name of the corresponding AFLP written in italics.

Data analysis

Chi-square (χ^2) tests were performed to examine the goodness-of-fit between the expected Mendelian ratio for the BC_1F_1 and BC_1F_2 populations, and the segregation data for the markers and the parthenocarpic/wild-type trait. Clearly readable AFLP bands were scored as dominant markers.

AFLP sequences were compared with those in GenBank/EMBL/DDBJ/PDB non-redundant sequence databases using the BLAST2 package and the BLASTX and BLASTN algorithms (Altschul et al. 1990). Additional search was done in the GenBank/EMBL/DDBJ EST Division and in the TIGR Tomato Gene Index (http://www.tigr.org/).

The software package JoinMap 3.0 (van Ooijen and Voorrips 2001) was used to perform the linkage analysis between the molecular markers and the *Pat* locus, and to integrate the map distances of the two mapping populations: a LOD score of 3.0 or above was specified. The Kosambi mapping function was used to convert recombination frequencies into map distances.

Results

Screening and segregation of the *pat* gene

F_1 plants from the interspecific cross *L. esculentum* × *L. pennellii* were confirmed to be hybrid, based on intermediate leaf morphology, vegetative vigour and indeterminate growth habit. Moreover, accordingly with the recessive nature of the *pat* mutation, F_1 flowers were wild-type and showed intermediate traits between the two parents (Fig. 1A). The classification of the segregating progenies based on the expression of parthenocarpy proved to be inadequate, because this phenotype had incomplete penetrance in this material. Only about 20% of those BC_1F_1 plants otherwise assessed to be a *pat* mutant clearly expressed the parthenocarpic phenotype (data not shown), i.e. a high fruit set (Fig. 1B) and/or seedlessness. In addition, since we worked with interspecific hybrids the rate of sterile BC_1 plants was rather high

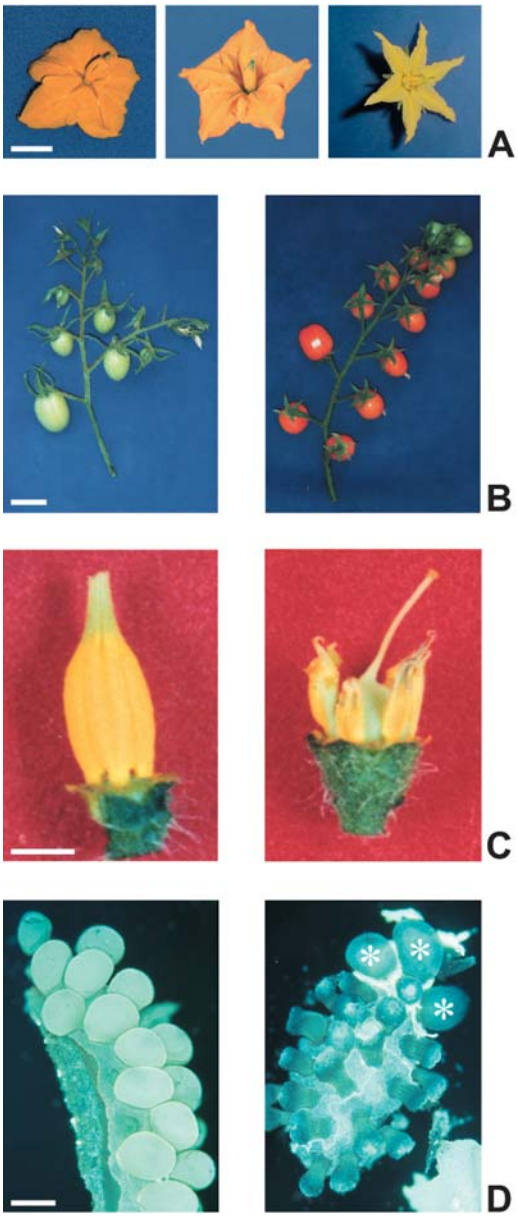

Fig. 1A–D Expression of the *pat* syndrome affecting different organs in the plant material used in the crossing scheme. **A** From left to right, representative flowers from *L. pennellii* (staminate parent, homozygous for the WT allele at the *Pat* locus), from the F_1 (heterozygous, with a WT flower phenotype) and from *L. esculentum* (seed parent, homozygous for the *pat* allele, some floral aberrations are visible). **B** Inflorescence of a WT BC_1 segregant (left) compared with the inflorescence of a *pat* BC_1 segregant (right) at the same time point after anthesis: the latter displays a complete and anticipated fruit set. **C** Comparison between WT (left) and *pat* (right) androecium morphology at anthesis; *pat* anthers are short and dyalitic and the ovary has already started to increase in size. **D** Comparison between WT (left) and *pat* (right) ovaries with ovules. Mutant ovules are mostly undeveloped and irregularly shaped; a few normally developed ovules are visible (a*sterisks*). *Bars* are 10, 30, 3 and 0.1 mm in A to D respectively

(above 50%), thus limiting a classification based on fruit development. Consequently, we classified the plants belonging to the segregating populations by taking into account the aberrations induced by the *pat* allele to anthers and ovules. As previously described (Mazzucato et al. 1998), in *pat* mutant plants a certain percentage of the anthers are short and dialytic (Fig. 1C), seldom bearing carpel-like structures and ovules on their adaxial surface. Moreover, in the ovary, a certain percentage of mutant ovules remain undeveloped and fail to increase in size, the integument ceases to develop and the micropyle does not bend downwards (Fig. 1D). Such aberrations were clearly expressed in interspecific progenies and, therefore, could be scored unambiguously. The *pat* phenotype was assigned to plants concurrently showing an aberrant phenotype on anther and ovule development. Allowance was made for a maximum 2% aberrancy in WT plants. This threshold was necessary because, even though the wild allele of *Pat* can be considered as completely dominant, heterozygous plants may show a little degree of floral organ aberration. The reliability of this rationale was confirmed by growing a number of BC_1F_2 progenies and scoring the *pat* phenotype (data not shown).

Both the BC_1F_1 and the BC_1F_2 populations showed undistorted segregation at the *Pat* locus. In the BC_1F_1, we scored 185 *pat* versus 150 WT plants that fitted a 1:1 Mendelian ratio (χ^2=3.6; P>0.05), while 15 (4.1%) plants remained undetermined, showing WT anthers and *pat* ovules or vice versa. Within the BC_1F_2, we classified 58 *pat* and 151 WT plants that fitted a 1:3 distribution (χ^2=0.45; $P \geq 0.50$). Although the classification criterion based on anther and ovule aberrations turned out to be easy and reliable; it should be noted that the distribution of *pat* expressivity on the floral organs of progeny plants was highly variable, ranging from 3 to 100% (Table 2).

AFLP-BSA and SCAR analysis

Using 146 *Pst*I/*Mse*I primer combinations, approximately 3,800 selectively amplified DNA fragments were analysed on the two parents and on two bulks of the BC_1F_1 plants. Depending on the primers, we scored 20–70 bands per gel. On the whole, the primer combinations assayed exhibited 55% polymorphism between parents. Out of 42 putatively linked markers, 25 were analysed in detail on the single plants composing the bulks and five of them (P44M31-386, P39M37-260, P39M44-222, P39M40-211 and P39M44-270) resulted tightly linked to the target locus, since they showed no recombination with *Pat* alleles. To draft the map of the linked markers with respect to the *Pat* locus, we screened a sub-set of 48 BC_1F_1 plants (24 *pat* and 24 WT). With regard to *Pat*, all the markers mapped on the same side, the closest one being P44M31-386, which was esteemed at a distance of 2.1 cM. The other four markers were evenly distributed up to a distance of 10.8 cM from *Pat*.

Table 2 Distribution of *pat* expressivity on anther and ovule aberrant development in 185 BC_1F_1 mutant plants

Trait	Frequency classes				
	>2–20%	21–40%	41–60%	61–80%	81–100%
Aberrant anthers					
No. of plants	32	22	36	32	63
Percentage (%)	17.3	11.9	19.5	17.3	34.0
Aberrant ovules					
No. of plants	43	52	51	22	17
Percentage (%)	23.2	28.1	27.6	11.9	9.2

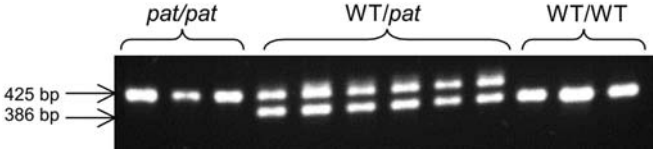

Fig. 2 Co-dominant SCAR marker *P44M31-386* tested on a set of 12 BC_1F_2 plants segregating for the *pat* allele

After sequencing, BLAST searches revealed significant similarity with database entries for three out of five AFLP sequences (data not shown), but this information did not help the integration of our local map into the tomato genetic map.

Afterwards, the two closest AFLPs (P44M31-386 and P39M37-260) were converted into SCAR markers so that they could be easily assayed on a larger number of plants. Two hundred and seventy eight BC_1F_1 plants were characterized at these two loci that finally turned out to be 0.5- and 4.1-cM distant from *Pat* respectively. The closest marker, SCAR *P44M31-386*, resulted to be co-dominant since it amplified only one band (425 bp) on *pat* plants but two bands (386 and 425 bp) on WT, heterozygous individuals (Fig. 2). After sequencing, the alignment of the two amplification products revealed that they were highly homologous, differing mainly for a 39-bp insertion/deletion (data not shown).

Localization of the *Pat* locus on the integrated RFLP-AFLP tomato genetic map

To determine the map position of the *Pat* locus, we analysed with the linked markers the set of 50 *L. pennellii* introgression lines developed by Eshed and Zamir (1994). Unfortunately, neither the five AFLPs, nor the two SCARs gave a positive signal, i.e. a *L. pennellii* allele, in any line. Therefore, we addressed the integrated RFLP-AFLP tomato map published by Haanstra et al. (1999). A BC_1F_2 population was grown in order to select plants homozygous at the *P44M31-386* co-dominant marker locus (Fig. 2), which served to compose four couples of DNA pools (four plants each). These bulks were amplified with the five *Pst*I/*Mse*I and the 22 *Eco*RI/*Mse*I primer combinations employed for the construction of the RFLP-AFLP map (Haanstra et al. 1999). Markers showing polymorphism in at least two couples of bulks were assayed on single plants. In this way, we identified two *L. pennellii*-derived AFLPs, E33M47-433 and E35M61-186, tightly linked to the *Pat* locus since only one plant out of 32 was recombinant in that region. On the AFLP-RFLP map, these markers were placed on the long arm of chromosome 3, separated by 4.1 cM. Moreover, the linkage analysis showed that other markers mapping on chromosome 3 were linked to *Pat*, although the association was loose, being these markers located at more than 20 cM from the target locus. AFLPs P14M49-332, P14M49-331 and E35M60-106 instead, were scored as monomorphic in our material although they mapped close to E33M47-433 and E35M61-186 (Haanstra et al. 1999). Overall, we could roughly estimate that about one-fourth

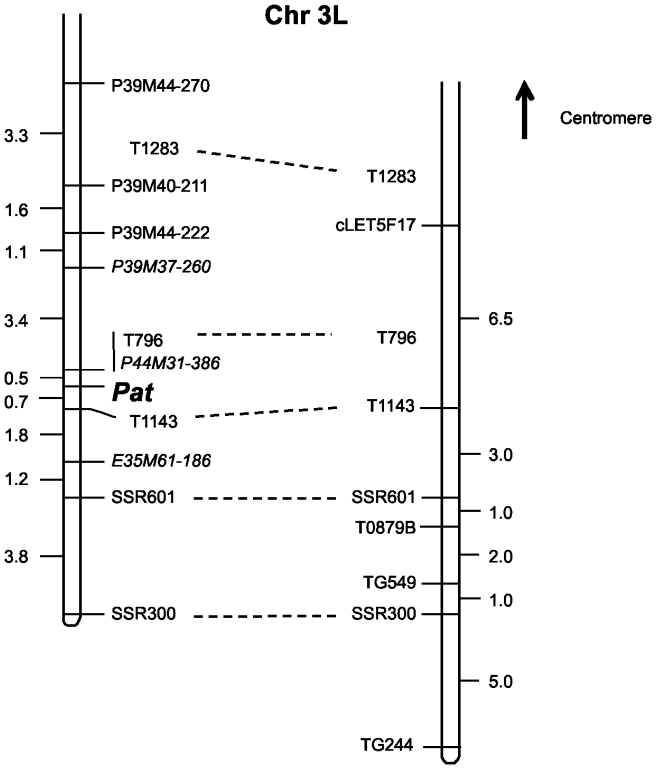

Fig. 3 Genetic linkage map surrounding the *Pat* locus including AFLP, SSR and COS markers (left) and its integration with the genetic map of the long arm of tomato chromosome 3 (right) published in the Solanaceae Genomics Network (http://www.sgn.cornell.edu/). AFLPs written in *italics* have been converted into and used as SCARs. Genetic distances are expressed in centimorgans

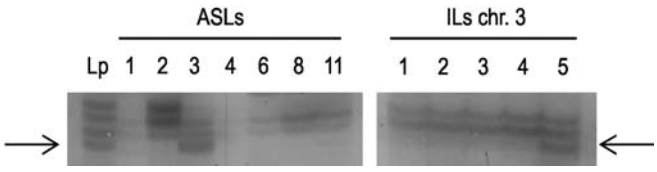

Fig. 4 AFLP marker E35M61-186 (*arrows*) amplified on *L. pennellii* (Lp), seven alien substitution lines (ASLs) covering chromosomes 1, 2, 3, 4, 6, 8 and 11, and on the five introgression lines (ILs) covering chromosome 3

of the markers reported on the map could be recognized in our Materials and with our methods.

E35M61-186 was sequenced and SCAR primers were designed, whereas E33M47-433, being difficult to score, was left out from further investigations. The SCAR analysis of 195 BC_1F_1 and 118 BC_1F_2 plants positioned *E35M61-186* at 2.5 cM from the *Pat* locus, on the opposite side with respect to the previously described linked markers (Fig. 3).

Validation of the map position and fine mapping

To confirm that the markers scored in our material corresponded to those mapped on chromosome 3 by Haanstra and co-workers (1999), we screened the seven ASLs available for chromosomes 1, 2, 3, 4, 6, 8 and 11. Each of these lines has a chromosome pair from *L. pennellii* replacing the *L. esculentum* homoeolog (Rick 1969). Along with the ASLs, the ILs covering chromosomes 5, 7, 9, 10 and 12 were analysed in order to scan the whole genome. As expected, E35M61-186 was detected in the ASL carrying chromosome 3 from *L. pennellii* (Fig. 4). When we addressed the five ILs harbouring the chromosome 3 introgressions, only IL 3–5 gave a positive signal, confirming that our marker corresponded to E35M61-186 and that finally the *Pat* locus mapped on the telomeric region of the long arm of chromosome 3 (Fig. 4).

The region covered by IL 3–5 contains two SSR markers, SSR300 and SSR601 (http://www.sgn.cornell.edu). The assay of these microsatellites on our mapping populations revealed that SSR300 and SSR601 were located distally from the *Pat* locus at 7.5 and 3.7 cM respectively. Differently from the length reported in the Solanaceae Genomic Network web site, which refers to cDNA, SSR601 on genomic DNA gave amplicons of approximately 525 bp (data not shown).

In order to better integrate our local map with those published and to develop co-dominant CAPS markers, we designed primers internal to the COS sequences T796, T1143 and T1283 (http://www.sgn.cornell.edu). The amplicons corresponding to T796 and T1283 showed different restriction patterns between *L. pennellii* and *L. esculentum* after having being digested with *Eco*RI and *Mse*I respectively. The amplicon corresponding to T1143, instead, turned out to be a co-dominant SCAR marker, because the designed internal primers amplified a sequence approximately 200 bp longer in *L. esculentum* than in *L. pennellii.* Therefore, we applied the CAPS procedure and the SCAR analysis to the BC_1F_1 and BC_1F_2 individuals showing recombination with the previously identified *Pat*-linked markers. This analysis allowed us to locate T1283 between P39M44-270 and P39M40-211, T1143 between *E35M61-186* and the *Pat* locus, 0.7 cM distal from this latter; whereas T796 co-segregated with *P44M31-386* (Fig. 3).

Discussion

In this work, we report the molecular mapping of the *parthenocarpic fruit* (*pat*) gene (Bianchi and Soressi 1969), a recessive mutation non-allelic to other known tomato genes for parthenocarpy. At present, this is the first gene for parthenocarpy of the *Solanaceae* family that has been mapped. The importance of the *pat* mutation is due to its possible use in breeding programmes, as well as in studies aimed to the comprehension of mechanisms underlying the fruit set. An understanding of the molecular events underlying parthenocarpy, in fact, would provide information on factors regulating fruit and seed formation, and thus open new perspectives for yield improvements by biotechnological means.

Accurate genetic mapping relies on the ability to score unambiguously the phenotype of the target gene. For this reason, we classified our segregating populations according to the aberrations of anthers and ovules induced by *pat* (Mazzucato et al. 1998), rather than taking into account seedlessness itself. In this way, we overcame the effect that the environment and the genetic background have on parthenocarpy, and were able to score sterile plants. Although even the floral aberrations are affected by the environment and the genetic background, we can conclude that this classification criterion is reliable, since only 4.1% of the plants remained undetermined. As shown in Table 2, the expressivity of floral organ aberrations was highly variable, most likely due to the effect of genetic modifiers, since within the original *pat* line the variability is lower (Mazzucato et al. 1999). More extreme phenotypes in populations segregating after the interspecific cross, in fact suggest an interaction with the genetic background (Ku et al. 1999). The interspecific cross could also be responsible for the slight skewness of the BC_1F_1 population toward the *L. esculentum pat* allele, an observation consistent with the finding that in interspecific backcrosses the alleles of the recurrent parent may segregate in excess (Mutschler and Liedl 1994).

Two segregating populations were raised; the BC_1F_1 allowed the selection and mapping of random AFLPs, whereas the BC_1F_2 was grown after we were able to quickly select plants homozygous in the *Pat* region. Random AFLPs were developed using *Pst*I as a rare cutter enzyme, because this nuclease is likely to generate markers more evenly distributed over the genome, in contrast to the *Eco*RI that supply markers tending to

group together in centromeric clusters (Haanstra et al. 1999; Vuylsteke et al. 1999). Five markers linked to the target gene were identified, but when we assayed them on the *L. pennellii* ILs no polymorphism was scored in any line. This result implies that a degree of polymorphism exists within the *L. pennellii* LA716 accession that reduce marker transferability across different mapping populations.

Being unable to map the *Pat* locus with the ILs, we addressed the integrated RFLP-AFLP tomato map (Haanstra et al 1999) searching for mapped AFLPs on a BC_1F_2 population. The choice of employing a BC_1F_2 population was taken in order to detect markers also derived from the recurrent parent (*L. esculentum*), because they represent approximately one half of the mapped markers. Using the BSA strategy on more bulks composed by a small number of homozygous plants, we were confident to identify even markers not tightly linked to *Pat*, dealing in the meantime with a limited number of PCR reactions. Actually, we could score at least ten of those markers mapped on chromosome 3 (data not shown), together with new markers not reported on the map. Two markers, E35M61-186 and E33M47-433, were found tightly linked to *Pat*, both mapping on the long arm of chromosome 3. The identity and position of E35M61-186 was checked on both the ASLs and the ILs. According with the expected map position, only the ASL carrying the *L. pennellii* chromosome 3 and the IL 3–5 showed this marker. This result demonstrates the suitability of the RFLP-AFLP map for genetic mapping, even considering that we worked with plant material and an AFLP detection method rather different from that used for the map construction.

To extend the integration of our AFLP map and to refine the localization of *Pat*, we assayed markers mapping in our target region: SSR300 and SS601, the CAPS markers derived from COS T796 and T1283, and the SCAR marker obtained from COS T1143. The two SSRs mapped distal to *Pat*, and they defined on our populations a genetic window of 3.8 cM, distant 3.7-cM from *Pat*. Since an RFLP marker, TG549, is flanked by these two SSRs, we could also locate the *pat* gene on the classical RFLP map (Tanksley et al. 1992). The COS markers T796 and T1143 turned out to be the closest to *Pat*: in particular T1143 allowed us to narrow the genetic window surrounding the *Pat* locus down to 1.2 cM. Having available co-dominant SCAR markers flanking the target locus, we can now select from a large population the recombinant individuals for one of the two markers at a very early stage of development.

The availability of markers orthologous to Arabidopsis genes also represents a starting point for comparative mapping, a powerful approach for enriching of molecular markers the region surrounding a gene of interest, or for attempting a candidate gene strategy by utilizing the chromosomal microsynteny between the target and the model species (Bennetzen and Freeling 1993; Moore et al. 1995; Schmidt 2000; Ku et al. 2001; Oh et al. 2002).

The present information about the map position of the *Pat* locus does not indicate any suitable candidate gene for the mutation. Known loci mapping on the long arm of chromosome 3, such as *TM8*, *divaricata* and *solanifolia*, have been interlaced with the molecular map and they all fall outside the region of interest (Lifschitz et al. 1993; van der Biezen et al. 1994, 1996). Moreover, none of the COS markers flanking *Pat* co-segregated with the target locus, nor did they reveal any microsyntenic region in the Arabidopsis genome that could directly be suitable for comparative mapping.

Finally, it is worth noting that the physical/genetic distance ratio in a genomic region of chromosome 3 proximal to the *Pat* locus, was estimated to be 123 kb/cM on average (Mesbah et al. 1999), six times lower than the mean calculated for the tomato genome (750 kb/cM; Tanskley et al. 1992). If such an estimation proves to be reliable in the distally located 1.2-cM *Pat* region, a chromosome landing approach (Tanksley et al. 1995) may be feasible by employing those markers described in this work.

The information acquired so far, the higher mapping resolution achievable with the premises reported here, and the increased knowledge on the physiology of the trait (Mazzucato et al. 1999; Testa et al. 2002), should prompt the positional cloning efforts and indicate the candidate genes to finally identify the sequence responsible for the *pat* mutation.

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