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Genomic structural differentiation in *Solanum*: comparative mapping of the A- and E-genomes

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Abstract A genetic map was constructed from an F_2 population of 76 individuals for the purpose of comparing the arrangement of loci in the A and E *Solanum* genomes. This progeny was derived from an interspecific cross between the species *Solanum palustre* × *Solanum etuberosum*, both of which are E-genome species. Two hundred and eighty one probes previously mapped in tomato and potato (A-genome, as postulated for diploid cultivated potato species by Matsubayashi 1991) disclosed 109 segregating loci in this population. Of these, 80 loci were linked in 19 linkage groups covering a total of 720.4 cM, with an average of 9 cM between markers. Although the genetic map of the E-genome showed conservation for most linkage groups with those of tomato and the A-genome, various translocations and possible inversions and transpositions were detected. It is evident that the accumulation of these structural changes in the E-genome is sufficient to cause the observed hybrid sterility. The major rearrangements in the E-genome included multiple translocations involving mosly linkage groups 2 and 8. Also a transposition was detected on group 9, with the same group-10 inversion distinguishing potato from tomato. Definitively groups 2, 8, 9 and 10, and possibly groups 1, 4 and 12, in the E-genome are structurally different from their homologues in the A-genome. In general, recombination values were larger in the E- than in the A-genome. The extensive structural differentiation of the E-genome with respect to that of potato and tomato justifies its present designation as a different genome, which is supported by previous chromosome-pairing studies. The difficult introgression of desirable traits from the *Etuberosum* species into potato can be explained by these structural differences.

Key words Chromosome structure · Restriction fragment length polymorphism · Linkage · Molecular markers · Genomics

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Introduction

The potato and its wild *Solanum* relatives are classified under the section *Petota*, with 21 series covering over 200 species (Hawkes 1990). The study of genomic relationships through chromosome pairing and the degree of fertility has led to the postulation of five basic genomes in this section: A, B, C, D, and E. All the cultivated potatoes, which range from diploid to pentaploid, are believed to share the same genome which is known as the A-genome (Matsubayashi 1991). Crosses involving *Solanum* tuber-bearing and the non-tuber-bearing species, *Solanum fernandizianum* Phil., *Solanum palustre* Schltdl. (syn. *Solanum brevidens* Phil., Spooner et al. 1993, 1996) and *Solanum etuberosum* Lind., result in a failure of chromosome pairing and high levels of sterility in the hybrids (Ramanna and Hermsen 1979, 1981; Watanabe et al. 1995). Based on these observations Ramanna and Hermsen (1979) proposed the genomic symbols E^1 for *S. etuberosum*, E^2 for *S. brevidens* (*S. palustre*) and E^3 for *S. fernandizianum* to distinguish them from the A-genome tuber-bearing species.

Comparative mapping using common RFLP (restriction fragment length polymorphism) probes in

potato and tomato species (*Lycopersicon* spp.) discloses that the genomes of these two crops, in spite of being classified in different genera, are essentially homosequential. The only major rearrangements known to distinguish these genomes are five chromosomal inversions near the centromeres on chromosomes 5, 9, 10, 11 and 12 (Tanksley et al. 1992). These structural differences, however, seem to be the cause of the high sterility observed in tomato and potato hybrids, which have been obtained only by protoplast fusion (Melchers et al. 1978; Shepard et al. 1983; Jacobsen et al. 1993). The similarity of the potato and tomato genomes presents a paradox, since it would imply that the existence of different genomes in the genus *Solanum* is not warranted. This assertion clearly contradicts the cytological observations on chromosome behavior in intergenomic hybrids. Recent circumstantial evidence based on molecular phylogeny further supports the genomic divergence of the tuber-bearing and non-tuber-bearing *Solanum* groups (Hosaka et al. 1984; Debener et al. 1990; Spooner et al. 1993). Based on chloroplast DNA restriction-site phylograms, Spooner et al. (1993) separated the *Solanum* E-genome species and the tuber-bearing species into two different clades which they recognized as sections *Etuberosum* and *Petota*, respectively. Previously, all these species were included in the section *Petota* though separated in different taxonomic series. Furthermore, the chloroplast DNA study of Spooner et al. (1993) suggested that the tuber-bearing species are phylogenetically closer to the species of the genus *Lycopersicon* than to non-tuber-bearing *Solanum* species.

The present work was aimed to shed light on the structure of the *Solanum* genomes by comparative mapping of the A- and E-genomes. An E-genome map was constructed with a set of common RFLP probes used to generate the existing tomato and potato maps in an F_2 progeny generated by crossing the species *S. etuberosum* and *S. palustre*.

Materials and methods

Plant material

The E-genome *Solanum* accessions involved in this study were *S. palustre* (PI 245764) and *S. etuberosum*, (PI 245924). They were used to generate an F_2 mapping population consisting of 76 plants. For this purpose, a plant from each accession was crossed, using *S. palustre* as the female parent. A single F_1 plant was selfed to generate the F_2 seed.

The plants were grown in 1-gallon pots under standard greenhouse conditions.

DNA extraction

DNA isolation was done using the procedure based on McGrath et al. (1990) as modified by Hu and Quiros (1991).

Evaluation of polymorphism

Ten micrograms of total genomic DNA from the parental species and five F_2 plants were digested with 15 units of restriction enzyme in the appropriate 10 × reaction buffer and incubated for 3 h at 37°C. The enzymes used to survey polymorphism were: *Eco*RI, *Eco*RV, *Bam*HI, *Hin*dIII, *Pst*I, *Xba*I and *Xho*I.

Southern blotting and probe hybridization

Following agarose-gel electrophoresis of the digested DNA (Sambrook et al. 1989) it was transferred to Hybond N neutral membranes (Amhersham Life) by Southern blotting, following the manufacturer's protocol. The DNA was then cross-linked to the membranes by UV irradiation in a BioRad GS Gene Linker using the C3 program.

The probes employed in this study originated from S. D. Tanksley's laboratory at Cornell University. All of them have been previously mapped in potato and tomato (Tanksley et al. 1992). The probes were labeled using digoxigenin 11-dUTP (Boehringer Mannheim), with PCR-amplification according to the protocol of Emanuel (1991).

RFLP detection was done following the protocol of Panaud et al. (1993) with the following modifications. Membranes were pre-hybridized for 3 h in 250 ml of hybridization solution (5 × SSC, 0.1% sarcosyl, 0.01% SDS, 1% non-fat dried milk) at 65°C. Hybridization was performed overnight in 10 ml of hybridization solution containing 200–400 ng of denatured probe at 65°C in 125-ml hybridization bottles in a Robbins Scientific model 400 hybridization oven. The membranes were washed twice at room temperature with 250 ml of 5 × SSC, 0.1% SDS, followed by two washes at 65°C for 15 min with 250 ml of 0.5 × SSC, 0.1% SDS. The membranes were equilibrated in 100 ml of buffer 1 (0.1 M Tris-HCl pH 7.5, 0.15 M NaCl) for 2 min and then incubated in buffer 2 (buffer 1 plus 1% non-fat dried milk) for 30 min. The enzymatic detection was carried out on 20 ml of alkaline phosphatase anti-digoxigenin antibody conjugate (Boehringer Mannheim) with 37.5 mU per ml in buffer 2. Excess of conjugated alkaline phosphatase was eliminated by washing two times in buffer 1 plus 0.1% SDS, and two times in buffer 1 for 15 min each time at room temperature. Final equilibration was done in buffer 3 (0.1 M Trizma base pH 9.5, 0.15 M NaCl, 0.05 M $MgCl_2$.6H20) for 5 min. Membranes were soaked in CDP Star solution (Boehringer Mannheim) with a 1 in 100 dilution in buffer 3, the solution being re-used multiple times, then wrapped in Saran Wrap and exposed to a X-ray film for 8–12 h.

Membranes were re-used up to 10-times. Stripping of the probe was accomplished by washing membranes in de-ionized water for 5 min at room temperature, two washings in stripping solution (0.1 M NaOH, 0.1% SDS) at 42°C, and a final wash in 2 × SSC.

Other markers

In addition to RFLPs, RAPD and AFLP™ markers were used in an attempt to consolidate small sub-groups with the main linkage groups, but not for intergenomic comparisons. For RAPD markers, the protocol reported by Quiros et al. (1993) was employed, using Operon Technology (Alameda, Calif.) 10-mer primers A7, C10, C12, E6, E7, and E8. For AFLP markers, we used a Gibco BLR-Life Technology (Gaithersburg, Md.) kit with *Eco*RI-*Mse*I restriction enzymes, following the manufacturer's instructions. The primers were labeled using ^{33}P and the gels exposed to Kodax Biomax MR X-ray film.

F_2 population mapping

Ten micrograms of DNA from each of 76 F_2 plants, plus the F_1 hybrid and the parents (*S. palustre* and *S. etuberosum*), were

digested using the enzymes that disclosed polymorphism in the previous phase.

Linkage analysis of the segregating loci was carried using the program Mapmaker (Lander et al. 1987) initially at a LOD score of 3.0 and with the Kosambi mapping function. This same function was previously used for the construction of the existing tomato and potato maps (Tanksley et al. 1992). Unexpected linkages with respect to the A-genome, were re-tested using a LOD score of 5.0 to minimize the possibility of spurious associations and to confirm possible structural rearrangements. For this reason, the linkage groups were drawn in the final map using a LOD score of 5.0, unless otherwise indicated in the text. When comparing the linkage arrangement of marker loci mapped in the E-genome with those reported for the A-genome and tomato, single-copy loci were considered homologous across genomes. In the case of duplicated-copy loci, those showing the same linkages in the E-genome with the expected flanking markers observed in tomato and potato were considered homologous. The linkage of flanking markers to single-copy loci was also used to confirm their homology across species. The tomato and potato maps were employed to tentatively align subgroups of the E-map linkage groups, for which direct genetic evidence was not available.

Results

E-genome map

Approximately one-third (102) of the 281 probes tested in this experiment, which were previously mapped in tomato and/or potato, showed polymorphism between the parental *Solanum* species with one or more enzymes. Therefore, the level of polymorphism in this interspecific cross is estimated to be only 36%. A total of 1791 probe/enzyme combinations revealed only 140 polymorphic sites. The enzymes disclosing higher polymorphism were *Hin*dIII, *Eco*RV and *Eco*RI, which in combination accounted for 62% of the total polymorphism observed in the F_2 population.

Fifty clones (50%) were single-copy sequences, in that each produced only two allelic fragments in the F_2 individuals. Thirty three (32%) revealed a non-segregating extra band in addition to the bands from the polymorphic locus, whereas 18 clones (18%) had two or more non-segregating additional bands. The parental plants were homozygous for all markers used to construct the map (data not shown). This was expected based on the previous high level of homozygosity observed in these species, and their autogamous nature. None of the alleles for a given probe/enzyme combination segregating in the F_2 displayed more than one band. Based on these observations it is safe to assume that each of the non-segregating bands observed correspond to a duplicated locus where the same allele is shared by both parents.

The 102 probes showing polymorphism in the *S. palustre* × *S. etuberosum* F_2 population produced a total of 109 segregating loci that were evaluated for linkage. Under a LOD-5 threshold, 80 loci were assigned to 19 linkage groups (Fig. 1) while 29 remained unlinked. The linkage groups covered a total of 720.4 cM with an average distance of 9 cM between markers.

In addition to the RFLP markers, 14 AFLP and 11 RAPD markers were mapped in an attempt to bridge the large distances between some of the RFLP markers and in order to consolidate the small linkage groups and unlinked RFLPs. Only five AFLPs and nine RAPDs were useful for this purpose.

The goodness-of-fit test revealed that only two loci, TG28 and TG261, deviated from expected Mendelian segregation ratios (data not shown). These two markers were not eliminated since they linked to the expected flanking markers in their respective linkage groups.

Comparative mapping of the E- and A-genomes

Linkage group 1

A total of seven RFLP loci were mapped corresponding to linkage group 1, five of which matched markers on the same linkage group in both potato and tomato (Fig. 1 A). These five markers in common to the three genomes were separated into two subgroups in the E-genome, since no linkage was observed between them. The arrangement of the loci in both subgroups was well conserved among the three genomes. There were, however, two unexpected loci on the E-genome subgroups, CT241 and TG208, located on groups 8 and 4, respectively, in tomato and the A-genome. These are presumably single-copy loci, thus indicating that this discrepancy is not due to the scoring of different duplicated loci. Ancillary AFLP and RAPD markers failed to link the two E-genome sub-groups, even when the LOD score was reduced to 2.0.

Linkage group 3

Seven loci were mapped on this linkage group in the E-genome, corresponding to the same markers mapped in both the A-genome and tomato linkage group 3 (Fig. 1 B). In the E-genome, however, this linkage group was split into two subgroups due perhaps to the large distance between loci TG135 and TG129, which is greater than 50 cM in tomato. Two RAPD markers and a LOD score reduced to 2.4, were necessary to link TG129 and TG134. Besides these discrepancies, the locus arrangement across the three genomes was well conserved, except for some of the distances that tended to be larger in the E-genome map.

Linkage group 4

Five loci were mapped on two subgroups in the E-genome, corresponding to group 4 in both the A-genome and tomato (Fig. 1 C). Only four of the five

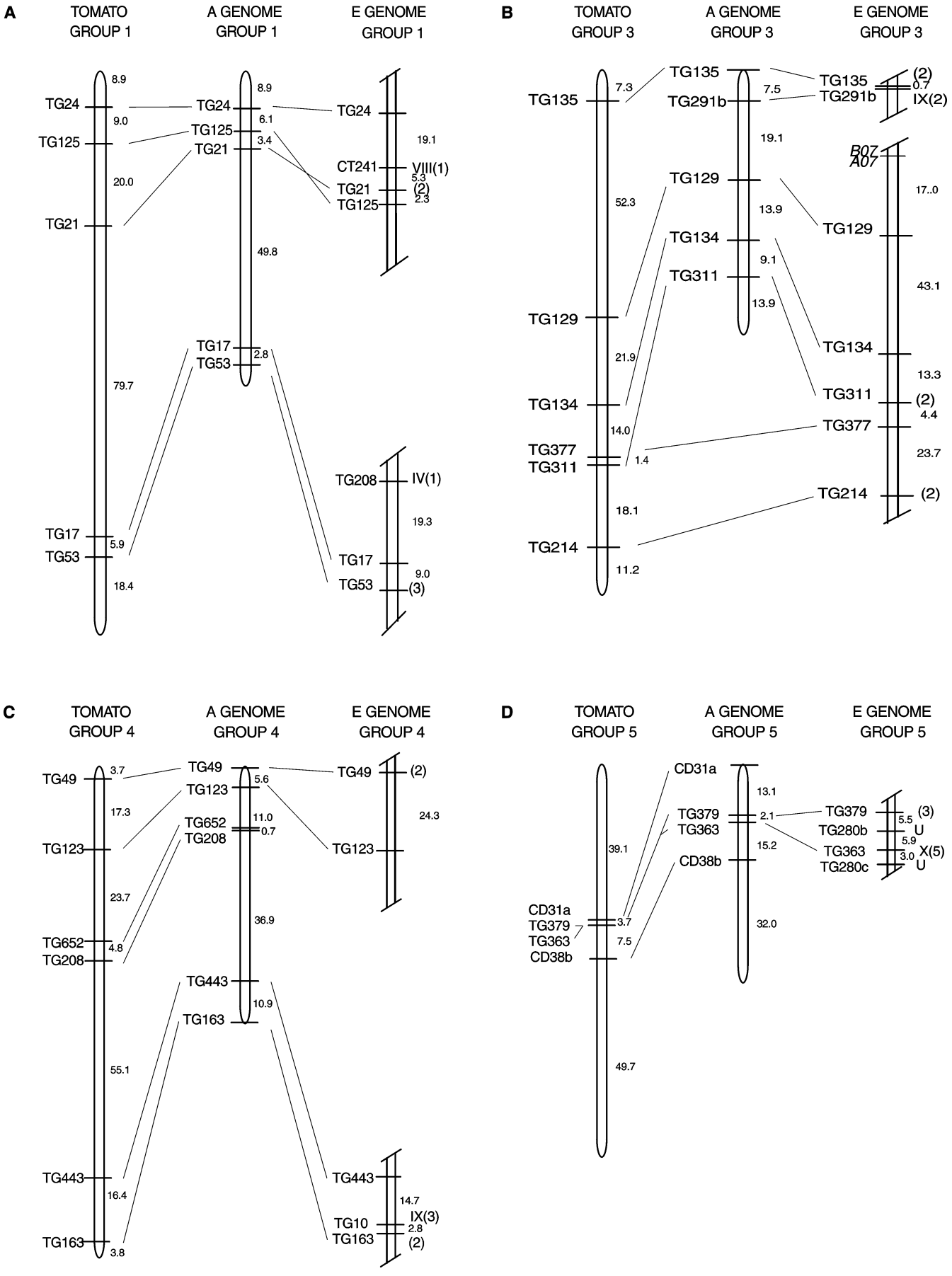
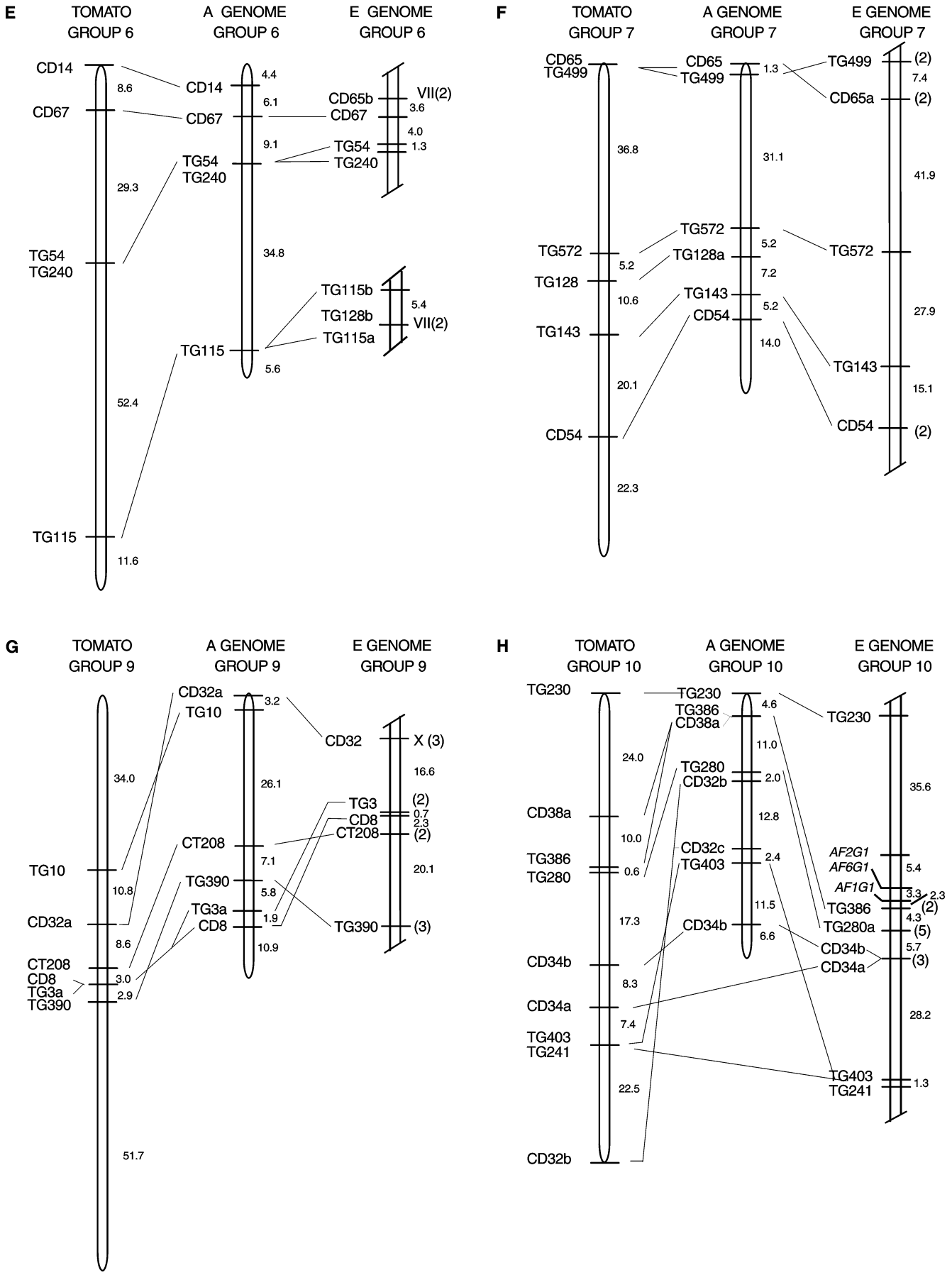


Fig. 1 See page 1189 for legend



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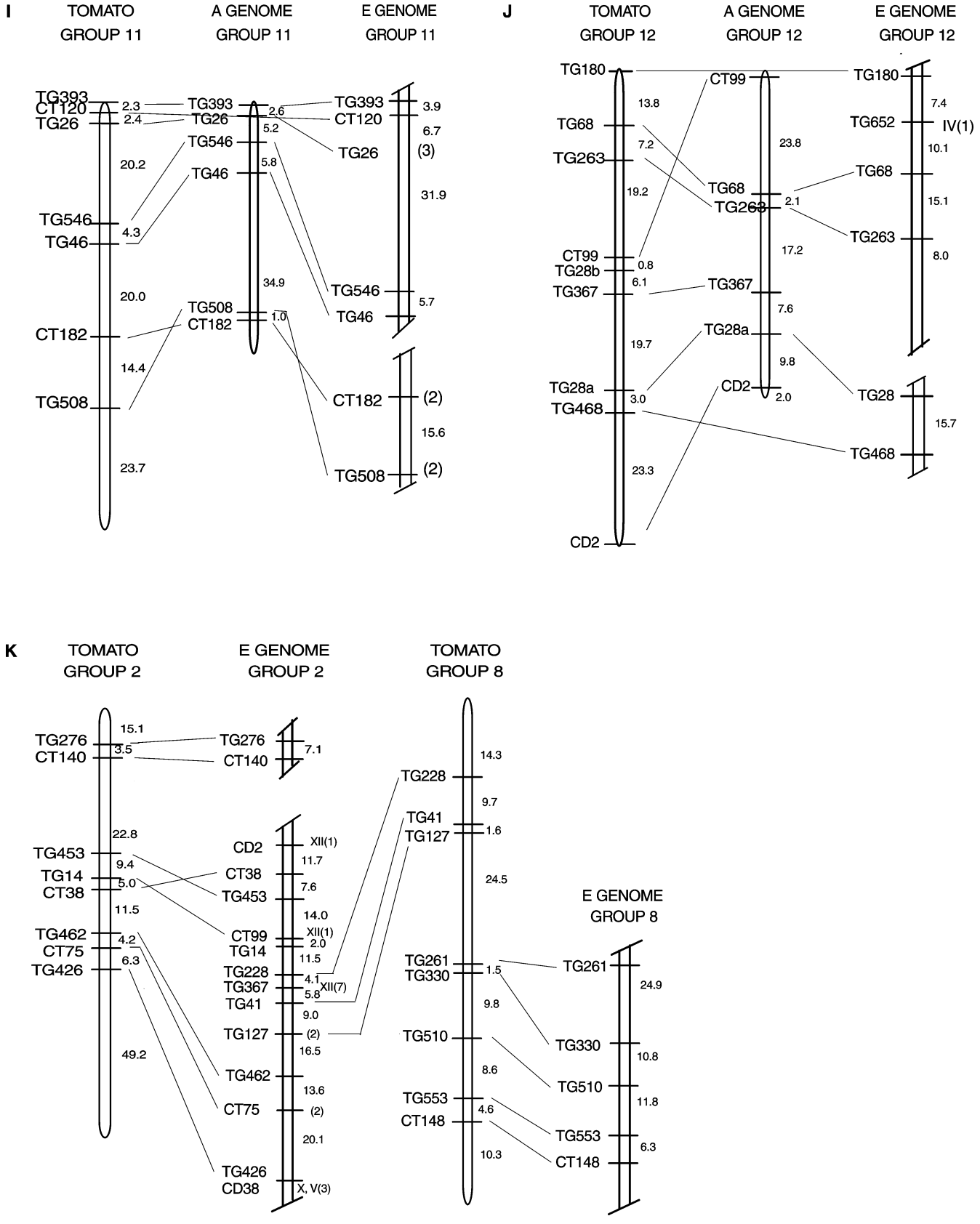

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markers matched those expected for group 4. The fifth one, TG10, is a multiple-copy marker, so it is possible that the mapped locus in the E-genome does not correspond to the mapped locus in tomato and the A-genome, which is located on group 9. The two subgroups in the E-genome could not be consolidated either with ancillary makers or lower LOD scores. In any case, the distance between the markers at the ends of the subgroups TG123 and TG443 is higher than 50 cM in tomato and close to this value in the A-genome. Another discrepancy observed for this group was the absence of locus TG208, which was present instead on group 1. Besides these alterations, the order of the other markers was well-conserved.

Linkage group 5

Only four loci were available for comparing the three genomes for this group (Fig. 1 D). There were only two markers in common with the A-genome and tomato groups, namely TG379 and TG363. The other two corresponded to duplicated loci TG280b and TG280c, whose locations have not been reported in the existing tomato and potato maps.

Linkage group 6

In the E-genome this group consisted of two subgroups including seven markers, five of which were in common with the corresponding markers of the tomato and A-genome group 6 (Fig. 1E). The unexpected markers, CD65b and TG128b, have duplicated loci, so it is likely that the loci which mapped on E-genome group 6 do not correspond to the loci mapped in potato and tomato. This is supported by the fact that the second locus for CD65, CD65a, maps as expected to group 7.

Linkage group 7

Five of six probes previously mapped on A-genome linkage group 7 produced markers segregating in the E-genome mapping population (Fig. 1 F). The arrangement of these loci was well conserved in all three genomes. The only possible discrepancy was the position of locus CD65a, which was linked to the expected flanking markers for group 7, but inverted with respect to locus TG499.

Linkage group 9

Five out of the six markers tested for group 9 mapped, as expected, in the E-genome; the exception was TG10 which remained unlinked (Fig. 1 G). The order of four of the loci, however, was changed. All three genomes showed a unique order for these markers, reflecting the presence of inversions and/ or transpositions.

Linkage group 10

Six of seven probes revealing seven loci previously located on the tomato and A-genome linkage group 10 segregated in the E-genome population (Fig. 1 H). In tomato and potato this group differs by an inversion. The arrangement of corresponding loci in the E-genome followed the order of loci observed in tomato. The order of the rest of the markers was well-conserved, although the distances tended to be shorter in the E-genome compared to those in the other species crosses. Three interstitial AFLP markers were necessary to link TG230 and TG386.

Linkage group 11

All seven markers which mapped on two subgroups in the E-genome matched those found on the tomato and A-genome group 11. The two markers in the smaller subgroup are included in an inversion that differentiates the A-genome from the tomato genome and shortens the distance from 14 cM in tomato to 1.0 cM in potato. It was not possible to determine whether the E-genome segment conserved the tomato or the potato arrangement; however, the larger distance between the two markers was similar to that observed in tomato. The order of the rest of the markers agreed in all three genomes. The two subgroups in the E-genome failed consolidation by RAPD and AFLP markers.

Linkage group 12

Five markers corresponding to tomato and A-genome linkage group 12 mapped in two sub-groups in the

Fig. 1A–K Corresponding linkage groups for tomato, potato (A-genome) and the E-genome. The locus name is depicted on the left side of the groups. Distances in centimorgans are indicated on the right in *small numbers*. *Roman numerals*, when present, indicate the linkage group location(s) of that locus or its duplicated members in tomato and the A-genome. The *number in parenthesis* to the right side of the marker indicates the number of duplicated loci observed in the E-genome for that marker. For simplicity, only RFLP markers are shown on the maps, except for groups 3 and 10 were RAPD and AFLP markers (*in italics*) necesary to coalesce subgroups are shown. **A and B**: linkage groups 1 (*left*) and 3 (*right*). **C and D**: linkage groups 4 (*left*) and 5 (*right*), (*U* means unmapped in tomato and potato). **E and F**: linkage groups 6 (*left*) and 7 (*right*). **G and H**: linkage groups 9 (*left*) and 10 (*right*). **I and J**: linkage groups 11 (*left*) and 12 (*right*). **K**: groups 2 and 8. A-genome linkage groups were omitted for simplicity since they conserve the tomato marker arrangement but are less informative because they contain fewer markers

E-genome, following the expected order (Fig. 1 J). A sixth marker, TG652, was linked in the larger subgroup, which was unexpected since this single-locus marker is normally found on group 4 in tomato and potato. Another discrepancy was the absence of loci CD2 and CT99 which were unexpectedly linked to markers of chromosomes 2 and 8 (Fig. 1 K). These two markers are single-copy, which suggests possible translocations involving chromosome 12 in the E-genome.

Linkage groups 2 *and* 8

Applying a LOD score of 5 to reduce the possibility of spurious linkages, a total of 19 markers were linked in a complex group of three subgroups in the E-genome. The main subgroup, with 13 loci, consisted mainly of markers from groups 2 and 8 from tomato and the A-genome (Fig. 1 K) The segment carrying the chromosome-8 markers was flanked by two segments carrying mostly chromosome-2 markers. In addition, two markers from group 12, CD2 and CT99, were also present in this subgroup. The order of loci on the segment carrying markers TG453, TG14 and CT38 was disrupted by the group-12 marker CT99 and by at least one inversion. A locus for TG367 was also located on chromosome 12 in tomato and potato, but since there are multiple copies of this sequence, it is likely that the locus mapped in the E-genome is different from those mapped in tomato and potato. The same can be said for marker CD38.

The small subgroup of two loci was tentatively placed on top of the main subgroup to align it to the corresponding markers in tomato group 2. Lower LOD scores and ancillary markers failed to consolidate this subgroup to any of the other ones. On the other hand, the third subgroup (which consisted only of linkage group-8 markers), at a LOD score of 3, was linked in an inverted position to the main subgroup. In such a scenario, markers CD38 (and TG426) and CT148 were linked at a distance of 33.3 cM. Although this may represent a true rearrangement, we have drawn the chromosome-8 group separately from group 2 until further evidence is generated to support it.

Discussion

Comparative mapping of tomato, the A-genome and the E-genome

Comparative mapping between potato and tomato demonstrates that both genomes are largely homosequential, differing only by five inversions (Tanksley et al. 1992). The genetic map of the E-genome shows a general conservation for most linkage groups with those of tomato and the A-genome, though various major translocations and several possible inversions were detected. The magnitude of these rearrangements could prove to be more extensive in view of our unsuccessful attempts to coalesce subgroups into the main linkage groups, which may represent true additional rearrangements. In any case, it is evident that the accumulation of the structural changes observed so far in the E-genome is sufficient to cause the observed sterility and disturbed meiosis in A-genome × E-genome species hybrids (Matsubayashi 1991). Therefore, it is justifiable to classify the genomes of the non-tuber-bearing species *S. etuberosum* and *S. palustre* differently. Furthermore, these changes result in linkage relationships between some chromosomal segments not found in either the A-genome or tomato.

Although 19 linkage groups of various sizes were detected in the E-genome population, most of the smaller segments could be tentatively aligned to their corresponding groups in the A-genome and tomato, based on the distances observed in the tomato and potato map. This was done on the assumption that the E- and A-genomes share the same chromosomal structure in these segments. Although this assumption is risky, it at most underestimates additional rearrangements to those documented by this work.

Linkage-group conservation

Disregarding ambiguous markers produced as multiple-locus markers by a single probe, practically all groups, except for 2 and 8, were well conserved in the E- and A-*Solanum* genomes, as well as in tomato. The same may be true for groups 1 and 7, differing only from their corresponding groups in the other two species by single inversions. The short distances between markers in these segments, however, does not make reliable the judgment as to whether these are indeed true inversions or mapping artifacts. On the other hand, the gene order in the E-genome for group 10 served to confirm the potato rearrangement reported for the segment defined by markers TG403 and CD34. The E-genome conserved the tomato arrangement, since it lacked the inversion distinguishing the latter from the A-genome group 10 (Tanksley et al. 1992). The presence of this inversion only in the A-genome indicates that such a rearrangement is specific to potato, and perhaps other tuber-bearing species as well.

Major rearrangements

Two main types of major rearrangements in the E-genome were observed, translocations and inversions. (1) *Inversions.* Group 9 in all three genomes has undergone extensive rearrangements due to the incidence of

various inversions. This group differs in potato and tomato by a complex inversion. In the E-genome, a transposition may have taken place along the length of the existing group. Marker rearrangement for this segment in all three genomes suggests that it may be susceptible to a high breakage rate.

(2) *Translocations.* Most of the rearrangements in the E-genome were located on linkage groups 2 and 8, due to multiple translocations involving also segments from chromosome 12. Other possible translocations were those observed on group 1, which displayed markers from groups 4 and 8, and the translocation of marker TG652 from group 12 into group 4. So, apparently, groups 1, 2, 4, 8, and 12 in the E-genome are structurally different from their homologues in the tomato and A-genome due to translocations, and in group 9 due mostly to a transposition. At this point, it is impossible to determine the extent of the rearrangements for chromosomes 1, 4 and 12, if any, but certainly chromosomes 2 and 8 are extensively rearranged.

Based on the existing evidence, it is possible to speculate on the events leading to the present structure of the E-genome. Using the phylogram of Spooner et al. (1993), constructed by chloroplast DNA analysis, it is fair to assume an original ancestor branching out into two major lineages. The first lineage giving rise to the *Solanum* tuber-bearing species (Section *Petota*) and the *Lycopersicon* species, which later separate into sub-lineages due to the accumulation of chromosomal inversions. The second lineage originated the E-genome species of the Section *Etuberosum*, which have a more southern range of geographic distribution (central Chile, including the Juan Fernandez island; Correll 1962). At this juncture, it is not possible to ascertain the structure of the ancestral genome. It would first be necessary to study the genomic structure of other sections, such as *Basarthrum* and *Dulcamara* (Spooner et al. 1993). Since all the species in both lineages have a genome of $x = 12$, it is clear that the *Solanum* members of the subgenus *Potatoe* have undergone extensive chromosomal rearrangements, based mostly on translocations and inversions. The prevalence of these structural changes could not be previously appreciated from the comparative mapping of tomato and potato genomes because of their taxonomic proximity. The presence of extensive rearrangements in *Capsicum* (Lefebvre et al. 1995), however, hinted that translocations have also played a pivotal role in the evolution of solanaceaeous genomes.

A single E-genome?

Ramanna and Hermsen (1981) distinguished three E-genomes, denoted by superscripts 1 to 3, one genome type for each *Etuberosum* species. These were renamed more descriptively by Matsubayashi (1991) as E^b, E^e and E^f, corresponding to *S. brevidens* (now *S. palustre*), *S. etuberosum* and *S. fernandizianum*, respectively. The fact that, in the interspecific *S. palustr* × *S. etuberosum* F_2 population used in our study, only 3% of the segregating loci presented distorted segregation, suggests that structurally both *S. palustre* and *S. etuberosum* share the same E-genome. Although our genome coverage was not complete, it seems unnecessary to distinguish two E-genomes in the accessions of these two species. The percentage of markers showing skewed segregation in this cross was much lower than the percentages of segregation distortion previously reported for other interspecific crosses in *Solanum* (Bonierbale et al. 1988; Gebhardt et al. 1991) and *Lycopersicon* (Bernatzky and Tanksley 1986), which were above 20%.

The relative low level of polymorphism, 35.5%, detected in our mapping population compared to those reported for other interspecific *Solanum* populations, 81 and 98% by Bonierbale et al. (1988), is another indication of the close taxonomic proximity of *S. palustre* and *S. etuberosum*. This is further supported by the trend for a higher recombination frequency observed for a large number of chromosomal segments, as well as the full fertility and regular meiosis of the F_1 hybrids (Perez 1996).

E-genome introgression

The species belonging to the *Etuberosum* group have been considered by potato breeders as sources for disease resistance. Indeed, through somatic hybridization it has been possible to transfer resistance to bacterial rot from *S. palustre* to potato (Ehlenfeldt and Helgeson 1987; Austin et al. 1988; Fish et al. 1988; Pehu et al. 1989; Novy and Helgeson 1994). Furthermore, homoeologous pairing and possible intergenomic recombination for some of the chromosomes have been reported in addition lines derived from the somatic hybrids between *S. palustre* and *S. tuberosum* (Williams et al. 1990, 1993; McGrath et al. 1994, 1996). For some of the chromosomes, however, there were unexpected associations of markers normally located on other linkage groups. These anomalies were attributed by the authors to intragenomic recombination events. However, in light of the new data provided herein, it is possible that some of these associations are a reflection of the pre-existing linkages inherent to the E-genome.

In summary, the present study revealed that the genomic structure of the *Etuberosum* species is differentiated from the potato and tomato genomes, justifying its present E-genome designation. It is likely that the number of rearrangements in the E-genome with respect to the A-genome have been underestimated. This is due to the following limitations: the lack of polymorphism to fill existing gaps in some of the linkage groups,

including the coalescence of sub-groups into major groups, and to the presence of duplicated loci which in some situations made orthology inferences disputable. In any case, structural differences between the *Solanum* genomes have been demonstrated in this study. Therefore, future work aimed at the introgression of traits from the *Etuberosum* species to tomato or potato should be carefully contemplated and realistically evaluated, especially for genes present in largely rearranged groups, such as groups 2, 8 and 9, and possibly 1, 4, 7 and 12. Problems in the recovery of recombinants for segments of at least half of the chromosomes in hybrids between the A-genome and the *Etuberosum* species are evident due to their structural differences.

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