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RESEARCH ARTICLE

Evolution of chromosome 6 of *Solanum* species revealed by comparative fluorescence in situ hybridization mapping

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Abstract Comparative genetic linkage mapping using a common set of DNA markers in related species is an important methodology in plant genome research. Here, we demonstrate a comparative fluorescence in situ hybridization (FISH) mapping strategy in plants. A set of 13 bacterial artificial chromosome clones spanning the entire length of potato chromosome 6 was used for pachytene chromosome-based FISH mapping in seven distantly related *Solanum* species including potato, tomato, and eggplant. We discovered one paracentric inversion and one pericentric inversion within specific lineages of these species. The comparative FISH mapping data revealed the ancestral structure of this chromosome. We demonstrate that comparative FISH mapping is an efficient and powerful methodology to study chromosomal evolution among plant species diverged for up to 12 million years.

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Introduction

The Solanaceae are a large family of angiosperms including 98 genera and approximately 2.700 species (Olmstead and Bohs 2006). About half of the species in Solanaceae belong to the genus *Solanum*, which is the economically most important genus containing several major crops, including potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*), and numerous wild relatives of these crops (Knapp et al. 2004). Lack of fossil records in the Solanaceae makes calculations of divergence times in the family problematic. However, Wikstrom et al. (2001) suggested that the genus *Solanum* diverged from its closest related genus about 12 million years ago, and Nesbitt and Tanksley (2002) suggested that tomato and potato may have diverged for seven million years. The majority of the species in *Solanum* share the same basic chromosome number of x=12.

The phylogenetic relationships of *Solanum* have been studied extensively based on comparative analysis of morphology (D'Arcy 1972), crossability and chromosome pairing (Matsubayashi 1991), and most recently, DNA restriction site and sequence data (Debener et al. 1990; Spooner et al. 1993; Spooner and Castillo 1997; Olmstead and Bohs 2006; Rodriguez and Spooner 2009). Comparative genetic linkage mapping using common sets of genetic markers has been conducted in a number of *Solanum* species. For example, comparative genetic mapping revealed five whole arm paracentric inversions between tomato and potato chromosomes 5, 9, 10, 11, and 12 (Tanksley et al. 1992), in spite of a highly similar karyotype shared by the two species (Barton 1950; Yeh and Peloquin 1965). Similarly, eggplant and tomato were differentiated by a minimum of 24 inversions and five translocations (Doganlar et al. 2002; Wu et al. 2009).

Although several phylogenetic and comparative genetic mapping studies have been completed, there is limited information available for the evolution on individual chromosomes among the *Solanum* species. Comparative fluorescence in situ hybridization (FISH) mapping between potato and tomato chromosome 6 was conducted previously using common sets of potato or tomato bacterial artificial chromosome (BAC) clones (Iovene et al. 2008; Tang et al. 2008). The comparative FISH mapping effort revealed an inversion involving the euchromatic portions of the short arms of potato and tomato chromosome 6 (Iovene et al. 2008; Tang et al. 2008). We expanded this effort to five additional *Solanum* species. The comparative mapping data revealed the ancestral structure of chromosome 6 of the *Solanum* species and two chromosomal inversions in specific lineages. We demonstrate that comparative FISH mapping is an efficient and powerful methodology to study chromosomal evolution among plant species.

Results and discussion

Cross-species FISH of potato BACs in different *Solanum* species

To select potato BACs for comparative FISH mapping, we tested the hybridization of 33 BAC clones, which were previously mapped to potato chromosome 6, to chromosomes of five *Solanum* species, including eggplant and one of its related species *Solanum caripense* (Tzimbalo), *Solanum etuberosum* (E genome), and two wild potato species *Solanum bulbocastanum* (B genome) and *Solanum chromatophilum* (P genome; Fig. 1). Most of the BAC clones mapped to the euchromatic regions of potato chromosome 6 generated distinct FISH signals in other species. However, most BACs mapped to the heterochromatic regions of potato chromosome 6 did not produce unambiguous signals in other species, thus could not be used in comparative mapping. By adjusting the amount of the C_ot-1 blocking DNA in the hybridization mixture, we were able to map two moderately repetitive clones, RH 069B12 (clone 4) and RH 188N15 (clone 5), which were mapped in the euchromatin–heterochromatin boundary of potato chromosome 6 (Iovene et al. 2008), in most of the *Solanum* species analyzed. We selected a total of 13 potato BACs for comparative FISH mapping (Table 1). All these BAC clones, except clone 13, were precisely mapped on the pachytene chromosome 6 of potato (Iovene et al. 2008). Clone 13 is located at the distal end of the long arm and was added in this experiment to cover the entire length of chromosome 6 (Table 1).

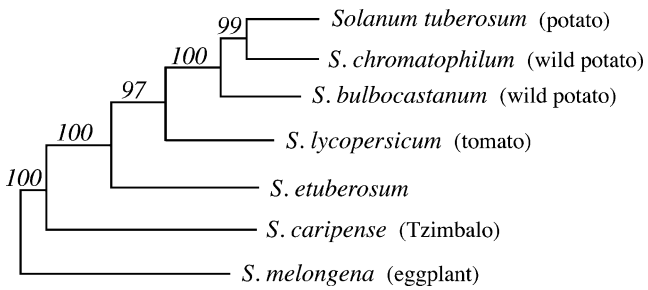

Fig. 1 Phylogenetic relationship of the seven *Solanum* species used in comparative FISH mapping based on Spooner et al. (1993) for *S. melongena* and Rodriguez and Spooner (2009) for the other species. Bootstrap values are placed *above the branches*. This phylogenetic estimate uses *Solanum albornozii* and *Solanum andreanum* as placeholders for *S. chromatophilum*, present on the same clade, and *Solanum brevicaule*, *Solanum raphanifolium*, and *Solanum verrucosum* as placeholders for *S. tuberosum* based on Spooner and Castillo (1997)

The intensities of the FISH signals derived from the 13 selected potato BACs varied among different *Solanum* species. For examples, distinct signals were detected on *S. etuberosum* chromosome 6 from all 13 BACs. However, relatively weak signals and enhanced hybridization background were observed on chromosome 6 of eggplant and *S. caripense*. The background signals can be reduced by adding C_ot-1 blocking DNA for these two species. Several other approaches were applied to increase the signal strength of the potato BACs on eggplant and *S. caripense* chromosomes, including reducing the concentration of deionized formamide in hybridization mixtures from the regular 50% in 2X SSC to 40% in 2X SSC and extending the hybridization incubation time from 12 to 48 h. We were able to map a total of 11 and 9 BACs on chromosome 6 of eggplant and *S. caripense*, respectively (Table 1). Successful cross-species FISH mapping of BAC clones has previously been reported between *Arabidopsis thaliana* and *Brassica* species (Jackson et al. 2000; Ziolkowski and Sadowski 2002; Howell et al. 2005), as well as between sorghum and maize (Zwick et al. 1998; Koumbaris and Bass 2003; Amarillo and Bass 2007).

The structure and rearrangements of chromosome 6 in *Solanum* species

Chromosome 6 in all species was identified using BAC clone 39P07 (BAC clone 2), which is located near the end of the short arm (Dong et al. 2000). The relative chromosomal positions of individual clones in each *Solanum* species were first determined by co-FISH mapping of two adjacent clones. Multiprobe FISH cocktails containing up to 11 different BAC probes were then hybridized to the same pachytene chromosome to obtain more accurate measurements.

The pachytene chromosome 6 in the seven species analyzed showed a similar morphology. Pericentromeric heterochromatin was observed in chromosome 6 of all

Table 1 Measurements of chromosomal locations of 13 BAC clones in seven *Solanum* species

Clone no.	BAC	Relative physical location (%)											
		Potato[a]	Tomato[a]	*S. bulbocastanum*	*n*	*S. chromatophilum*	*n*	*S. caripense*	*n*	*S. melongena*	*n*	*S. etuberosum*	*n*
1	RH160K03	1.2±0.4	10.1±0.7	1.2±0.1	12	0.9±0.2	11	2.1±0.2	13	1.7±1.9	8	1.3±0.2	10
2	39P07	4.2±0.7	6.9±1.1	4.1±0.1	12	3.5±0.2	10	3.9±0.5	10	3.4±2.1	8	3.6±0.3	8
3	RH084L03	5.6±0.9	5.7±0.8	5.0±0.2	5	5.4±0.4	3	5.1±0.9	3	4.5±2.0	4	4.3±0.7	8
4	RH069B12	11.6±1.0		10.0±0.2	4	10.3±0.4	3	9.6±1.0	3	9.9±1.4	3	57.9±2.1	3
5	RH188N15	45.7±1.9		42.5±1.0	6	42.3±1.2	4					19.0±1.6	8
6	RH094G20	52.5±1.6	45.2±2.7	50.1±0.9	11	50.4±0.7	8	58.7±1.9	8	54.6±5.9	7	14.2±0.6	11
7	RH102I10	57.7±1.4	51.2±4.2	54.8±0.9	14	55.4±0.8	13	61.2±1.4	10	59.0±4.5	7	10.0±0.7	12
8	RH051B02	58.6±1.7		55.8±1.6	4							59.2±2.4	6
9	RH103A21	70.9±2.2		69.5±1.0	10	71.7±1.1	8	73.0±2.5	7	75.9±5.0	5	68.2±1.5	8
10	RH087B02	77.3±1.6		77.9±1.1	9			78.8±4.8	5			76.8±1.3	8
11	RH200K19	86.4±1.0	84.7±2.7	86.8±1.2	12	86.5±1.4	10	87.7±5.2	4			86.2±1.6	7
12	RH204G08	91.5±1.0	92.0±2.8	91.6±1.3	12	91.5±1.3	12	90.7±1.2	4	90.6±5.5	6	91.6±1.9	7
13	RH070E17	97.7±0.1		99.9±1.4	12	99.4±1.5	10	99.6±1.4	3	99.8±2.0	5	99.0±2.4	6

The position of each BAC on pachytene chromosome 6 was measured as $(S/T)\times 100$, where S is the distance (in micrometers) from the FISH site to the end of the short arm of the chromosome and T is the total length of the chromosome (in micrometers). Only data points with a minimum of three measurements are listed

[a] Data from potato (except for BAC RH070E17) and tomato are from Iovene et al. (2008)

seven species analyzed (Figs. 2 and 3). However, the size of the heterochromatin domain relative to the entire chromosome and the intensity of 4′,6-diamidino-2-phenylindole (DAPI) staining of the chromosome 6 varied among the seven species. The centromeres, which were embedded in the middle of the heterochromatin domain, were clearly less stained than the flanking heterochromatin in some species, such as potato and tomato (Iovene et al. 2008), but cannot be unambiguously and/or consistently identified in every species, especially *S. etuberosum*. Identification of the centromeres was further complicated by the presence of multiple DAPI-faint domains in the pericentromeric heterochromatin in most *Solanum* species. Thus, the position of the centromeres was not specified on the diagrams in Fig. 3.

A characteristic heterochromatic knob was observed in the middle of the long arm of potato chromosome 6 but was absent in tomato chromosome 6 (Iovene et al. 2008) (Fig. 3). This knob was also observed on chromosome 6 from the wild potato species with P and B genomes and was located at the same position (Fig. 2a, b). We observed two successive interstitial knobs with different sizes on the long arm of *S. caripense* chromosome 6. Interestingly, the position of these two knobs was different from the position of the single knob observed in potato. Both knobs were proximal to BAC clone 6 on *S. caripense* chromosome 6 (Fig. 2c, d), whereas the knob in potato was located between BAC 6 and BAC 7 (Fig. 2a, b). Thus, a small inversion in this region on *S. caripense* chromosome 6 may explain the different positions of these knobs (Fig. 3). FISH mapping of additional BACs in this region will be necessary to confirm this possible inversion.

Complete synteny of all 13 clones was observed among *S. caripense*, *S. melongena*, potato, and the wild potato species containing the B and P genome (Figs. 2e, f and 3). The 13 clones were mapped on similar positions of chromosome 6 from five *Solanum* species (Fig. 3). However, we detected a large inversion spanning the entire pericentromeric heterochromatin domain in *S. etuberosum*. The order of BAC clones 4, 5, 6, and 7 in *S. etuberosum* was reversed compared to the order of these clones in

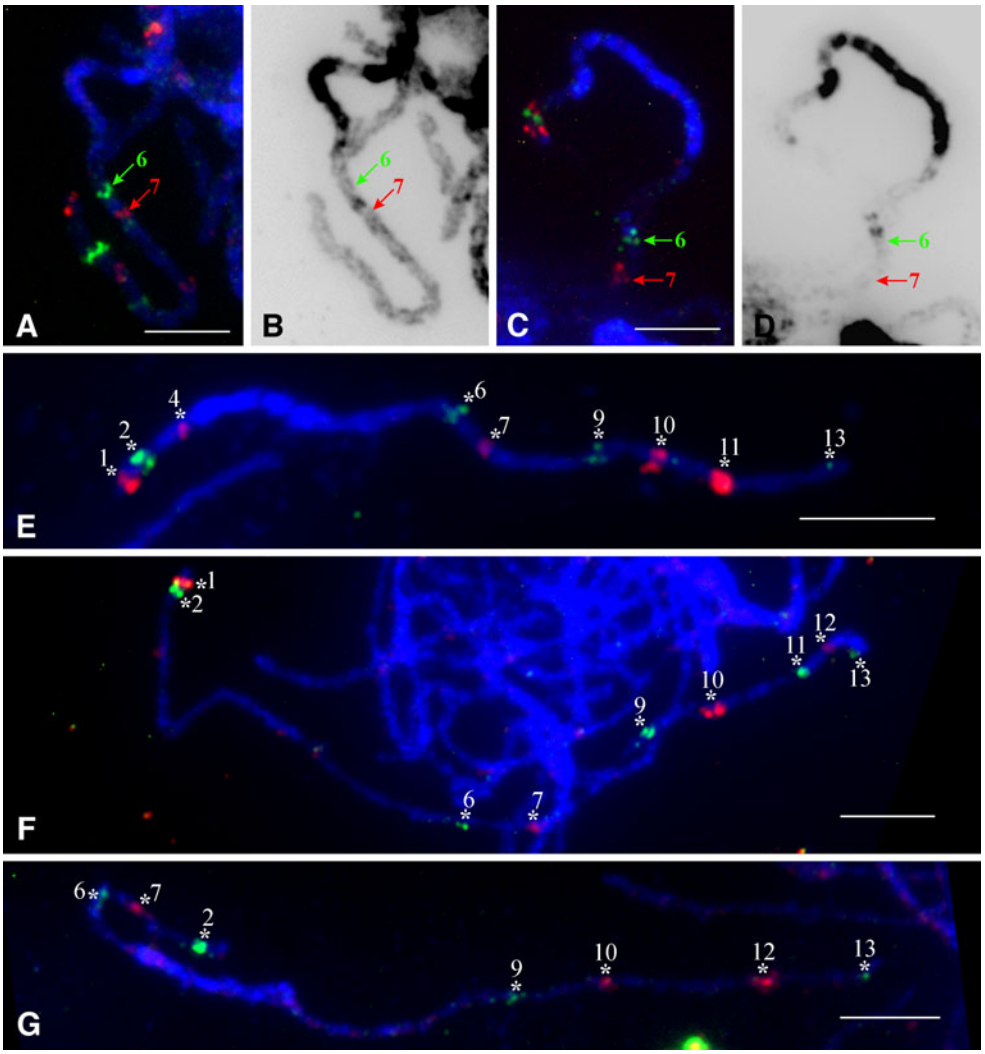

Fig. 2 FISH mapping of potato chromosome 6-specific BACs on the pachytene chromosome 6 of different *Solanum* species. **a** A heterochromatic knob on the long arm of *S. bulbocastanum* chromosome 6 was mapped between BAC clone 6 (*green*) and 7 (*red*). **b** The DAPI-stained chromosomal image was converted as a black–white image to enhance the visualization of the knob. **c** Two successive heterochromatic knobs on the long arm of *S. caripense* chromosome 6 were mapped to proximal to BAC clone 6. **d** The DAPI-stained chromosomal image was converted as a black–white image to enhance the visualization of the two knobs. **e** FISH mapping of nine BACs on the pachytene chromosome 6 of *S. bulbocastanum*. **f** FISH mapping of nine BACs on the pachytene chromosome 6 of *S. caripense*. **g** FISH mapping of seven BACs on the *S. etuberosum* chromosome 6. Note: Clones 7 and 6 are in a reversed order and are located on the short arm of the chromosome. *Bars*=5 μm

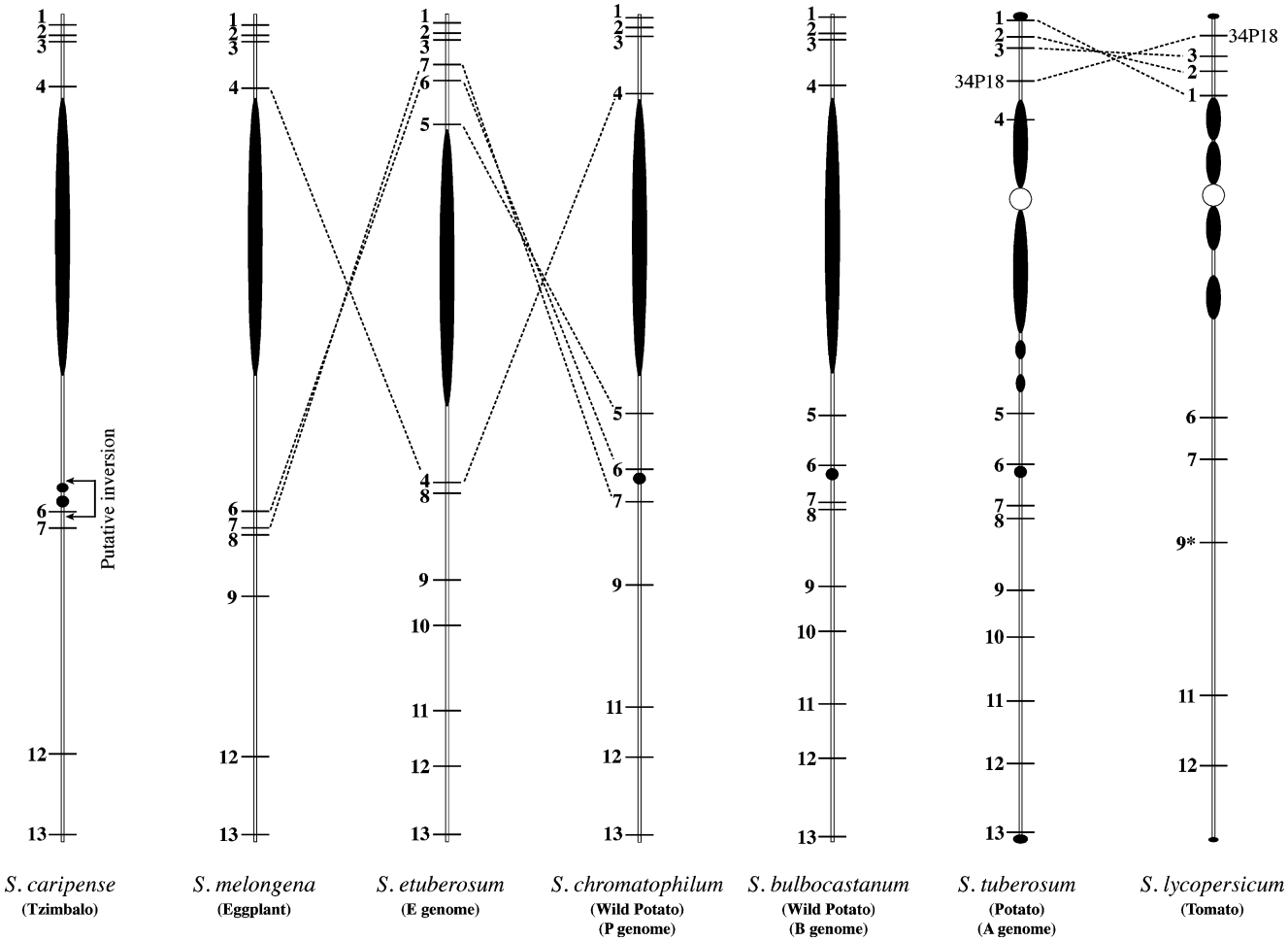

Fig. 3 Diagrams illustrated the locations of 13 BACs specific to chromosome 6 on seven different *Solanum* species. A paracentric inversion associated with tomato chromosome 6, a pericentric inversion associated with *S. etuberosum* chromosome 6, and a putative small inversion on the long arm of *S. caripense* chromosome 6 are marked. Heterochromatic regions are represented by *solid/shaded thickenings*. *Shaded thickenings* indicate regions that were less stained by DAPI than the regions marked by *solid thickenings*. *Hatched thickenings* indicate regions that are separated into multiple small knobs on early pachytene chromosomes. The *open circles* on potato and tomato chromosome 6 represent the centromere. The centromeric positions of chromosome 6 from the other five *Solanum* species are not determined. The position of BAC clone 34P18 is marked on potato and tomato chromosome 6 to depict the size of the paracentric inversion. This BAC was not included in the comparative FISH mapping in other *Solanum* species. The exact position of BAC clone 9 on tomato chromosome 6 is not determined, but it is located between BAC clones 7 and 11

eggplant and all potato species (Figs. 2g and 3). The breakpoint of this inversion on the long arm is located between BAC 7 and BAC 8, which accounts for approximately 1% of the potato pachytene chromosome 6 (Table 1). It is interesting to note that this is the first pericentric inversion detected in the *Solanum* species, as all previously reported inversions appeared to be paracentric (Tanksley et al. 1992; Iovene et al. 2008; Tang et al. 2008).

The ancestral structure and evolution of chromosome 6 of *Solanum* species

The pachytene chromosome 6 from eggplant and its distantly related potato share synteny with all 13 BACs along the entire length of the chromosomes. Thus, the ancestral structure of chromosome 6 for the species listed in Fig. 1 should resemble to chromosome 6 of eggplant. A paracentric inversion involving the euchromatic portion of the short arm emerged during the evolution of the tomato genome. Similarly, a pericentric inversion occurred during the evolution of *S. etuberosum*. These two inversions either did not include or include the entire pericentromeric heterochromatin. Thus, the overall morphology of pachytene chromosome 6 remained similar for all seven *Solanum* species (Fig. 3).

Doganlar et al. (2002) reported a "paracentric inversion" in eggplant chromosome 6 based on comparative genetic mapping between *S. melongena* and tomato. These authors concluded that this inversion is unique to eggplant because the same rearrangement was not previously detected in other *Solanum* species (Doganlar et al. 2002). However, the failure

to detect the paracentric inversion associated with tomato chromosome 6 in the previous potato/tomato/pepper comparative genetic mapping was possibly due to the lack of markers in the inverted region (Tanksley et al. 1992; Livingstone et al. 1999). We now clearly demonstrate that this paracentric inversion is distinct to tomato chromosome 6, not to eggplant chromosome 6. Similarly, the pericentromeric inversion associated with *S. etuberosum* was not detected in a comparative genetic mapping effort between the A and E genome potato species (Perez et al. 1999), which is also possibly due to the lack of markers in the inverted region.

Comparative FISH mapping in plants

There are two different approaches for comparative FISH mapping among related species. The first approach is to develop a DNA probe from an entire chromosome of one species and to map this probe in related species. Such a comparative chromosome-specific painting has been a very powerful technique to study chromosomal evolution in mammalian species (Wienberg and Stanyon 1997). However, chromosome-specific painting probes are difficult to develop in most plant species due to the presence of large amount of highly repetitive DNA sequences. Thus far, comparative chromosome-specific painting has only been achieved in *A. thaliana* and its related species, which contain relatively small genomes thus less repeats (Lysak et al. 2001; Lysak et al. 2006).

The second approach is to develop a multiple probe cocktail that contains DNA clones distributed along the length of a chromosome and to map the probes in related species (Jiang and Gill 2006). DNA clones containing mainly single copy sequences can be selectively included in the cocktails. Although such multiple probe cocktails do not cover the entire chromosome, any chromosomal rearrangements involving more than one probe can be readily identified. Deletion and duplication may also be discovered based on the presence and absence of the probes. This approach has also been extensively used in chromosomal evolution studies in mammalian species (Eder et al. 2003). Recent applications of this approach in plant species have yielded exciting results, including the first centromere repositioning event in plant species (Han et al. 2009). The current study further demonstrates the power and efficiency of comparative FISH mapping among distantly related plant species diverged up to 12 million years.

Comparative genetic linkage mapping using a common set of DNA marker in different species has been one of the most popular approaches in comparative plant genome research (Devos and Gale 2000). This approach, however, relies on the availability of appropriate mapping populations and is a time-consuming process. Comparative FISH mapping provides an alternative approach to study synteny and chromosome rearrangements among related plant species. The FISH-based methodology does not rely on a mapping population and reveals the physical characteristics associated the chromosomal rearrangements such as translocations and inversions (Lysak et al. 2006; Iovene et al. 2008; Tang et al. 2008). Other cytological targets, such as DNA fibers (Fransz et al. 1996; Jackson et al. 1998) and mechanically stretched chromosomes (Valarik et al. 2004; Koo and Jiang 2009), can be used to increase the resolution for comparative FISH mapping. We expect that the comparative FISH mapping approach will be more widely employed due to the abundant BAC resources developed in various plant species in recent years.

Materials and methods

Plant materials

Five *Solanum* species together with potato and tomato were selected for comparative FISH mapping, including *S. melongena* (eggplant) and a related species *S. caripense* (Tzimbalo, PI 243342), *S. etuberosum* (E genome, PI 558306), and two wild potato species *S. bulbocastanum* (B genome, PI 498223) and *S. chromatophilum* (P genome, PI 365339; Fig. 1). *S. etuberosum*, *S. chromatophilum*, and *S. bulbocastanum* represent the basic E, P, and B genome species that were previously characterized based on the degree of chromosome pairing in the hybrids derived from the diploid species (Matsubayashi 1991). All these species are diploids with a chromosome number of 24. Seeds of all *Solanum* species were obtained from USDA/ARS, Potato Introduction Station, Sturgeon Bay, Wisconsin, USA.

Fluorescence in situ hybridization

Young flower buds were harvested and fixed in freshly prepared 3:1 Carnoy's solution for at least 1 day. The buds with anthers containing pollen mother cells at the pachytene stage were selected for preparation. The anthers from the selected buds were rinsed for 5 min in distilled water for three times and then were transferred into enzyme mixtures containing 4% cellulose, 2% pectinase, and 0.2% pectolyase for 2.5 h at 37°C. The enzyme solution were replaced using distilled water and left on ice for 30 min before transferring the digested anthers into the fixative solution (glacial acetic acid/methanol, 1:3). The anthers were macerated with 45% glacial acetic acid on slides and covered with a 22×22-mm cover glass and then immediately stored in −80°C freezer until use.

A total of 13 potato BAC clones was used in FISH mapping in different *Solanum* species. In order to reduce the nonspecific hybridization background, C_ot-1 DNA was

isolated from eggplant and *S. caripense* according to published protocols (Zwick et al. 1997) and added in the hybridization mixtures. FISH was conducted essentially according to published procedures (Jiang et al. 1995). The BAC DNA was labeled with either biotin-16-UTP or digoxigenin-11-dUTP and detected by a fluorescein isothiocyanate-conjugated antibiotin antibody and a rhodamine-conjugated anti-digoxigenin antibody (Roche Diagnostics, Indianapolis, IN), respectively. Chromosomes were counterstained with DAPI in an antifade solution VectorShield (Vector Laboratories, Burlingame, CA), and images were captured using a SenSys CCD camera attached to an OlympusBX60 microscope. The CCD camera was controlled using IPLab Spectrumv3.1 software (Signal Analytics, Vienna, VA) on a Macintosh computer. High-quality pachytene chromosome preparations were used for reprobing using published protocols (Cheng et al. 2001).

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