Cytogenetics of Potato and Tomato Wild Relatives

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Abstract

Cytogenetics has historically contributed to the taxonomy, genetics, and breeding of cultivated and wild *Solanum* species. This chapter summarizes the contributions of cytogenetic research to our understanding of genome structure and evolution of potato and tomato wild relatives. We focus on the advances in cytogenetics, going from the classical chromosome morphological analysis of species and their hybrids to the recent oligonucleotide-based chromosome paints, which are helping to identify and compare chromosomes and genomes of the wild *Solanum* relatives, detect large-scale changes among these species, and clarify the parental origin of polyploid potatoes. Given the large number of species, comparative fluorescence in situ hybridization (FISH) mapping and genome size data are still sparse. However, these studies are helping uncover the karyotypic differences among cultivated and wild *Solanum* species, a diversity with a significant impact on introgression and pre-breeding programs, and characterize their rich repertoire of tandem satellite sequences. In addition, this chapter summarizes how the analysis of the centromeres of several *Solanum* species has provided a new model system to study the centromere evolution and the accumulation of satellite repeats in these specialized chromosomal regions.

2.1 Introduction

Solanum is one of the most abundant genera of the Angiosperms and comprises nearly half of the species of the Solanaceae family. The genus includes many landraces and wild crop relatives, which are an invaluable resource of genes and allelic variants critical for the genetic improvement of *Solanum* crops such as potato and tomato (see Chaps. 3, 4, 5, 6). The introgression of useful traits into the cultivated *Solanum* crops from their wild relatives is often challenging due to various pre- and postzygotic reproductive barriers among these species (reviewed in Camadro et al. 2004; Bedinger et al. 2011; Spooner et al. 2014; Bethke et al. 2017; Chetelat et al. 2019). Chromosomal rearrangements and other large-scale changes between parental genomes may represent an additional barrier to introgression, by compromising meiotic

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chromosome pairing and disjunction, and/or by decreasing the fitness of the interspecific hybrids (Seah et al. 2004; van der Knaap et al. 2004; Anderson et al. 2010; Verlaan et al. 2011; Gaiero et al. 2018). Cytogenetic research can contribute to point out the karyotypic differences among related species.

Historically, cytogenetics has had a profound impact on *Solanum* taxonomy, genetics, and breeding, especially for potatoes and tomatoes. Early cytological studies in potatoes contributed to the discovery of the widespread occurrence of unreduced (2n) gametes in the tuber-bearing Solanums and its underlying cytogenetic mechanisms (den Nijs and Peloquin 1977; Peloquin et al. 1999; Carputo et al. 2000), and to the endosperm balance number (EBN) hypothesis related to the fundamental role of endosperm in the outcome of crosses among *Solanum* species (Johnston et al. 1980; Hawkes and Jackson 1992; reviewed in Carputo et al. 1999; Bethke et al. 2017). These seminal studies, along with an efficient method to generate potato maternal haploids (Hougas et al. 1964), led to new potato breeding strategies based on ploidy level manipulation, thus facilitating crosses with wild species and diploid landraces (reviewed in Carputo et al. 2000; Bethke et al. 2017). Tomato cytogenetic stocks, including primary trisomics and radiation-induced deletion lines, have had a key role in the development of the first genetic linkage maps of tomato and the assignment of linkage groups to individual pachytene chromosomes (reviewed in Harper and Cande 2000; Chetelat and Ji 2007). These studies indicated an overall nonlinear relationship between genetic and cytological distances and that cytological chiasmata do not form in the heterochromatic regions of tomato (Barton 1951; Khush and Rick 1968).

Despite these advances, *Solanum* is not an amenable genus for cytogenetic studies. The wild relatives of potato (sect. *Petota*) and tomato (sect. *Lycopersicon*) and their close outgroup species (sects. *Etuberosum*, *Juglandifolium* and *Lycopersicoides*) all share the same base chromosome number x = 12 (Rodríguez and Spooner 2009; reviewed in Gavrilenko 2011; Grandillo et al. 2011). Most species are diploid (2n = 2x = 24), with polyploids restricted to the potato clade. Similar to cultivated potato (2n = 4x = 48) and tomato (2n = 2x = 24), most wild Solanums have small and condensed mitotic chromosomes that are poorly differentiated in morphology and size, and not suitable for a detailed cytogenetic analysis. Therefore, the identification of the chromosomes of cultivated potato and tomato and the initial comparisons with their wild relatives have been based on the morphology of their pachytene chromosomes (Barton 1950; Marks 1955, 1969; Sawant 1958; Rick 1960; Khush and Rick 1963; Yeh and Peloquin 1965; Ramanna and Prakken 1967; Ramanna and Wagenvoort 1976; Wagenvoort 1988; Matsubayashi 1991), an analysis feasible only for diploid genotypes.

A major breakthrough came from the development of chromosome-specific markers based on libraries of bacterial artificial clones (BAC) of potato and tomato species (reviewed in Szinay et al. 2010; Gavrilenko 2011) and, more recently, from chromosome paint probes based on collections of synthesized oligonucleotides that cover entire chromosomes or regions (Braz et al. 2018; He et al. 2018; reviewed in Jiang 2019; Pham et al. 2019). The chromosome-specific BACs were initially used to identify the chromosomes of cultivated *Solanum* crops, integrate genetic linkage maps of these species with their chromosomes, and contribute to the sequencing efforts of these crop species. In addition, chromosome markers and paints are currently helping to elucidate the genome organization of both wild and cultivated Solanums and the extent of synteny and large-scale chromosome rearrangements among these species. Given the large number of wild potatoes and tomatoes, these comparative studies are sketchy, that is, focused on a few species and/or a few chromosomes. Besides, we lack a comprehensive dataset on the genome size values and repeat contents, especially across the numerous wild potato relatives.

The present chapter summarizes the contributions of cytogenetic research to our understanding of the genome structure and evolution of potato and tomato wild relatives, with a focus on the extent of chromosomal rearrangements

and differences in composition and chromosomal location of repetitive DNA among *Solanum* species. Emphasis is also given to centromeric DNA because the potato and its close relatives have recently emerged as a model system to study the evolution of centromere-associated sequences. Prospective applications of cytogenetics to comparative studies in *Solanum* are briefly discussed.

2.2 Chromosome Identification in *Solanum*

Chromosome identification is at the basis of any cytogenetic investigation and is a useful tool for plant breeding and comparative studies. This section summarizes resources and tools that were initially established to identify potato and tomato chromosomes, going from chromosome morphological analysis to the recent oligonucleotide-based chromosome paints, and that were subsequently applied to study wild *Solanum* relatives.

Meiotic pachytene chromosomes provide sufficient morphological variations in length, arm ratio, and amount and distribution of heterochromatin to distinguish all 12 chromosome pairs of tomato and diploid potato clones (Barton 1950; Yeh and Peloquin 1965; Ramanna and Prakken 1967; Marks 1969; Ramanna and Wagenvoort 1976; Wagenvoort 1988). The pachytene analysis was extended to several wild tomato relatives and interspecific hybrids as well as a few wild potatoes (Sawant 1958; Menzel 1962; Khush and Rick 1963; Marks 1969; Hermsen and Ramanna 1973). However, this classical analysis had limited power to detect structural rearrangements, and, in addition, it was not easy to perform on polyploid potatoes.

Fluorescence in situ hybridization (FISH) and the use of large-insert genomic libraries have provided a robust tool for chromosome identification, karyotyping, and integration of the chromosomal features in the genetic linkage maps of both potato and tomato (Fuchs et al. 1996; Dong et al. 2000; Song et al. 2000; Tang et al. 2009; Choudhary et al. 2020). Various BAC libraries are available for potato (de Boer et al. 2011; Yang et al. 2015; Chen et al. 2019), tomato (Fulton et al. 2002; Budiman et al. 2004), and several wild relatives, e.g. *S. bulbocastanum* (Song et al. 2000), *S. habrochaites* (Wolters et al. 2015), and *S. pinnatisectum* (Chen et al. 2004). For each potato and tomato chromosome, a considerable number of BACs were selected by screening these libraries with genetically mapped molecular markers. In turn, many of these potato and tomato map-anchored BACs were FISH-mapped on the chromosomes of the corresponding species. Because the initial sequencing efforts for these crops used a BAC-by-BAC approach, BAC FISH provided valuable support to validate the assemblies of both species genomes (The Potato Genome Sequencing Consortium 2011; The Tomato Genome Consortium 2012). Chromosome-specific BACs have also provided a powerful tool to reveal collinearity and chromosome rearrangements between *Solanum* crops and their wild relatives in comparative FISH mapping studies. This is because most BACs located in either potato or tomato euchromatic regions generate distinct FISH signals not only within *Solanum* genus but also in related genera such as *Capsicum*. In addition, the availability of many stable fluorochromes has enabled the mapping of multiple probes at once, avoiding the need for time-consuming re-probing experiments of the same slides (Peters et al. 2009; Szinay et al. 2010). However, potato and tomato BACs located in heterochromatic regions generally work exclusively in close relatives (Iovene et al. 2008; Tang et al. 2008; Lou et al. 2010; Peters et al. 2012; Szinay et al. 2012; Gaiero et al. 2016), thus limiting the detection of rearrangements with breaks in these regions.

Similarly, several satellite repeats generate different hybridization patterns in terms of abundance, distribution, and number of FISH signals among closely related species and even at intraspecies level among accessions (Tek et al. 2005; Torres et al. 2011; Gong et al. 2012; Zhang et al. 2014). Comparative FISH mapping using these satellite repeats has often provided insights into the evolutionary dynamics of these repetitive elements rather than highlighting rearrangements

per se. This topic is reviewed in the last part of the chapter.

The availability of reference genomes of various *Solanum* crops and the technical advances in DNA synthesis have opened to a new strategy to paint individual chromosomes (Beliveau et al. 2012; Han et al. 2015; Braz et al. 2018; reviewed in Jiang 2019). This new strategy relies on FISH probes made of pools of thousands of custom-synthesized oligonucleotides, which are designed based on single-copy sequences associated with a specific chromosome or chromosome region (Beliveau et al. 2012; Han et al. 2015; Braz et al. 2018; Pham et al. 2019; do Vale Martins et al. 2019). Braz et al. (2018) selected as FISH probes a large set of oligos from the single-copy sequences associated with 26 specific chromosome regions in the potato genome. These oligo-probes produced 26 distinct FISH signals that uniquely labeled each of the twelve potato chromosomes with a sort of barcode/banding pattern, which, in turn, allowed the karyotyping of all mitotic metaphase chromosomes at once in diploid, tetraploid, and hexaploid potatoes. Along with oligo-based whole chromosome paints, these probes were successfully used for comparative FISH mapping among distantly related *Solanum* species (Braz et al. 2018). The authors showed that the oligo-FISH barcode approach enables to pinpoint rearranged chromosomes among related species even by using low-resolution mitotic karyotypes (Braz et al. 2018; see below). In addition, He et al. (2018) demonstrated that oligo-based FISH is a robust tool to visualize specific chromosomes of *Solanum* polyploids during various meiotic stages, which opens new opportunities for cytogenetic investigations in polyploids and hybrids (see next section).

Finally, genomic in situ hybridization (GISH), alone or in combination with FISH, has been extensively applied in *Solanum* to identify specific genomes or alien chromosomes in interspecific hybrids and natural polyploids, as well as to study their meiotic behavior (Parokonny et al. 1997; Garriga-Calderé et al. 1998; Dong et al. 2001; Ji and Chetelat 2003; Ji et al. 2004; Pendinen et al. 2012; Rakosy-Tican et al. 2020). The next sections of this chapter will summarize the main findings of these cytogenetic investigations.

2.3 Chromosome Number, Ploidy, and Genome Differentiation

All species of sect. *Petota*, *Lycopersicon,* and their closely related outgroups (sects. *Etuberosum*, *Juglandifolium,* and *Lycopersicoides*) share the same basic chromosome number x = 12. Polyploidy is confined to potatoes, whereas all 13 wild tomato taxa are diploid. Ploidy level has been one of the most important taxonomic characters for the identification of cultivated potatoes; these show various ploidies, from the diploid (2n = 2x = 24) to the pentaploid (2n = 5x = 60) level (reviewed in Spooner et al. 2014). Chromosome number has been determined for most of the 107 wild potato species recognized by Spooner et al. (2014). Hijmans et al. (2007), in a comprehensive survey of ploidy reports in sect. *Petota*, concluded that over 60% of the wild potatoes exist exclusively at diploid level. Tetraploids are the most common polyploids among wild potatoes, followed by hexaploids and triploids, whereas pentaploids are rare. In addition, many species [19–21, depending on the taxonomic treatment; see Hijmans et al. (2007); Spooner et al. (2014)] have multiple cytotypes.

The determination of the type of polyploidy (that is, auto- or allopolyploids) has traditionally relied on the analysis of meiotic chromosome configurations in natural polyploids and interspecific hybrids, as well as on interspecific crossability and on hybrid fitness (Matsubayashi 1991; reviewed in Gavrilenko 2007; Gavrilenko 2011). Clearly, this analysis had to face several challenges due to the large number of small chromosomes, the difficulty to trace various types of meiotic configurations, and the diverse origin and cytological behavior of the polyploids of sect. *Petota* (see He et al. 2018). Matsubayashi (1991) proposed a five-genome hypothesis to explain the cytological and crossability data and to distinguish the different ploidy types across

sect. *Petota* (reviewed in Gavrilenko 2007, 2011; Spooner et al. 2014). According to this hypothesis, all diploid potato species shared a similar genome denoted with an A, which represented the main genomic group and included slightly different variants to account for minor structural differences (denoted with superscript letters). A different genome E was hypothesized for the distantly related diploid species of sect. *Etuberosum* (Matsubayashi 1991). Allopolyploid potatoes always contained one genome A component and differed from each other for the second genome denoted as B, C, D, or P (Matsubayashi 1991; reviewed in Gavrilenko 2007, 2011; Spooner et al. 2014). However, the origin of the allopolyploid species has been much debated because, while the diploid *S. verrucosum* (or its progenitor) was indicated as a donor for the genome A, the other genomes had no known extant diploid species representatives (Spooner et al. 2008; reviewed in Gavrilenko 2007, 2011; Spooner et al. 2014). GISH helped clarify the controversial origin of several allopolyploid potatoes (Pendinen et al. 2008, 2012), by testing candidate donor species, which were identified through extensive molecular phylogenetic studies (reviewed in Spooner et al. 2014). GISH confirmed that the North and Central American *S. hjertingii* and *S. stoloniferum* (2n = 4x = 48) derived from two different genomes (genome A of *S. verrucosum* and genome B of diploid Mexican species; Pendinen et al. 2008). Based on the observation of exclusively intragenomic pairing at meiosis, *S. hjertingii* and *S. stoloniferum* should be considered strict allotetraploids (Pendinen et al. 2008). In addition, GISH revealed a complex genomic constitution for the Mexican allohexaploids *S. hougasii, S. iopetalum,*, and *S. schenckii* (2n = 6x = 72), which involved the contributions of at least three genomic components (A, B, and P genomes; Pendinen et al. 2012).

Conversely, GISH indicated a different origin for the Mexican hexaploid *S. demissum* (2n = 6x = 72), a species often used in potato breeding as a source of disease resistance. Various authors postulated different genome formulae for *S. demissum*, although there was a general agreement that *S. demissum* had two similar genomes differing from the third one (reviewed in Matsubayashi 1991). However, the GISH results of Pendinen et al. (2012) supported an autopolyploid origin of *S. demissum*, containing three chromosome sets similar to the basic A genome. Previous sequencing data indicated that *S. demissum* likely comprises two types of slightly differentiated genome A (Spooner et al. 2008; Rodríguez and Spooner 2009). Additional support came from the karyotype analysis of this species using oligo-FISH barcode approach (described above) which indicated that *S. demissum* contains six copies of each of the 12 potato chromosomes because the FISH signal pattern of the six homeologous chromosomes was identical to those of the reference potato species (Braz et al. 2018). Moreover, He et al. (2018) used oligo-based chromosome painting probes to monitor the chromosome pairing of four different *S. demissum* chromosomes (namely, chromosome 2, 4, 7, and 11) at meiotic prophase I. The authors demonstrated that during male meiosis, these *S. demissum* chromosomes have a diploid-like pairing behavior (Fig. 2.1). No hexavalent pairing was detected (He et al. 2018). Indeed, the analysis of chromosome pairing at pachytene using chromosome 7 and 11 probes demonstrated three independent bivalents in 80% and 98% of the cells observed, respectively (Fig. 2.1a1–c1; He et al. 2018). In addition, the prevalent configuration at diakinesis/metaphase I was of three independent bivalents for each of the four *S. demissum* chromosomes analyzed (He et al. 2018). Therefore, other mechanisms, independent from genome differentiation, are at the base of the bivalent pairing of the putative autohexaploid *S. demissum* (He et al. 2018).

The work of Pendinen et al. (2008; 2012) provided implicit evidence for a significant genome differentiation among the diploid representatives of various genome groups, that is, among *S. verrucosum* (genome A); the Mexican *S. cardiophyllum*, *S. ehrenbergii*, *S. jamesii*, and *S. bulbocastanum* (all genome B); and *S. andreanum* and *S. piurae* (both genome P). Similar indirect evidence comes from the GISH analysis of various hybrids involving both potatoes and

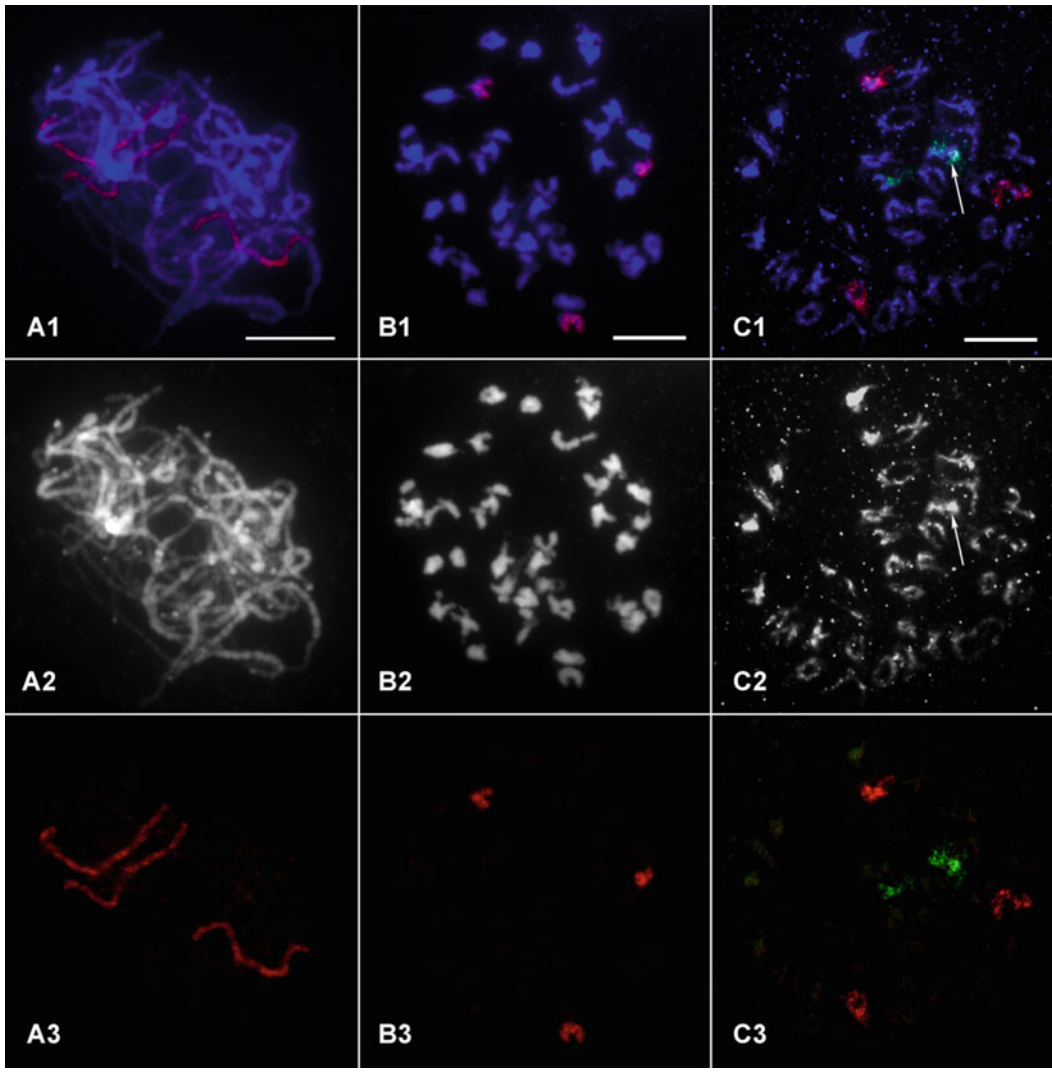

Fig. 2.1 Chromosome painting in meiotic cells from *Solanum demissum* (2n = 6x = 72; He et al. 2018). Photographs by He L. and Jiang J. **a1** Three chromosome 11 bivalents are observed after hybridization of a pachytene cell with chromosome 11 specific probe. **b1** Three chromosome 11 bivalents are observed after hybridizaton of a diakinesis cell with chromosome 11 probe. **c1** Six copies of chromosome 7 paired as three bivalents and six copies of chromosome 2 paired as one quadrivalent (white arrow) and one bivalent after hybridization of a diakinesis cell with chromosome 2 (green) and chromosome 7 (red) probes, respectively. **a2–c2** Chromosome images that were digitally separated from **a1–c1**, respectively. **a3–c3** FISH signals that were digitally separated from **a1–c1**, respectively. Bars = 10 μm

tomatoes. Apart from wide hybrids between cultivated potato and tomato (A and L genomes, respectively; Garriga-Calderé et al. 1997), cultivated potato (or tomato) and E genome species from sect. *Etuberosum* (Dong et al. 1999, 2001; Gavrilenko et al. 2001, 2002), and between tomato and species from sect. *Lycopersicoides* (Pertuzé et al. 2003; Ji et al. 2004), GISH differentiated the parental chromosomes of hybrids between cultivated tomato and *S. pennellii* (Haider Ali et al. 2001) and *S. peruvianum* (Parokonny et al. 1997), as well as between cultivated

potato and *S. bulbocastanum* (A and B genomes, respectively; Iovene et al. 2007). Conversely, GISH could not distinguish the parental genomes of hybrids between cultivated potato and *S. commersonii*. This result suggested poor divergence between the bulks of the repetitive sequences of these species (Gaiero et al. 2017).

2.4 Nuclear Genome Size of Wild and Cultivated *Solanum* Species

The nuclear genome size of a species is an important taxonomic character with several practical and predictive applications (Bennett and Leitch 2011). Its knowledge contributes to identify species and uncover polyploidization/aneuploidization/diploidization events as well as large-scale differential repeat amplification among close relatives. Nuclear genome size estimates are available for a limited number of potato and tomato wild relatives (http://data.kew.org/cvalues/). However, because *Lycopersicon* is a relatively small section, the available data provide an idea of the extent of variation in nuclear genome size among wild tomatoes. The sizes of their genomes are about 1 pg/1C, with a variation among species of up to about 30%. DNA content of cultivated tomato is estimated at 0.94–1.03 pg/1C based on different studies and cultivars (Arumuganathan and Earle 1991; Michaelson et al. 1991; Valkonen et al. 1994), equal to approximately 907–1000 Mb/1C. The closely related *S. cheesmaniae* and the more distant *S. habrochaites* (=*Lycopersicon hirsutum*) have estimates comparable to that of tomato (Bennett and Smith 1976; Arumuganathan and Earle 1991). Two different values (0.85 pg/1C and 1.15 pg/1C) are reported for *S. pimpinellifolium*, which is another close relative of cultivated tomato (Bennett and Smith 1976; Barow and Meister 2002), whereas *S. peruvianum* and *S. pennellii* have larger genomes of 1.15 pg/1C (1095 Mb) and 1.23–1.38 pg/1C (1192–1337 Mb), respectively (Arumuganathan and Earle 1991). The genome sizes of the outgroup species from sect. *Juglandifolia* are comparable to that of tomato, whereas the genomes of *S. lycopersoides* and *S. sitiens* (sect. *Lycopersicoides*) are about 30% larger than tomato (Chetelat 2009). The larger genome size of *S. pennellii* and *S. lycopersicoides* are consistent with earlier cytological observations of the pachytene complements of both species, which indicated that several *S. pennellii* chromosomes have longer heterochromatic regions than those of tomato (Khush and Rick 1963) and that the pachytene karyotype length of *S. lycopersicoides* is 1.5 fold longer than in tomato (Menzel 1962).

Repetitive sequences may underlie the genome size variation observed among some *Solanum* species. Repeat abundance and genome size are correlated in representative species of the potato and tomato clade. The tomato relatives with larger genomes contain different amounts of some satellite repeats and a significantly higher proportion of unclassified elements consisting of degraded or truncated elements (Gaiero et al. 2019). On the other hand, although the intraspecific variation in nuclear DNA content may reflect real variability (Doležel and Greilhuber 2010), the different values reported for *S. pimpinellifolium* (as well as for other *Solanum* species) could be due to different methodologies and reference standards (Bennett and Smith 1976; Barow and Meister 2002).

In contrast, the genome size estimates for sect. *Petota* are sparse in proportion to the large number of species of this section. Several estimates rely on a single study and accession. In addition, a reassessment of the genome size data is highly desirable, considering the revised taxonomy of the section. Haploid potato clones have a genome size estimated at 0.8–0.88 pg/1C (that is, 831–856 Mb). The genomes of several wild diploid species, including *S. berthaultii*, *S. pinnatisectum*, *S. sparsipilum*, *S. vernei*, and the recently sequenced *S. commersonii* and *S. chacoense* are in the same size range of the haploid potato clones (Anderson et al. 1985; Arumuganathan and Earle 1991; Bennett and Smith 1991; Valkonen et al. 1994; Aversano et al. 2015; Leisner et al. 2018). However, there are also reports of diploid species with larger genomes, such as 1.1 pg/1C for the Mexican *S. polyadenium* and 1.2 pg/1C for the landrace

S. stenotomum (Anderson et al. 1985; Bennett and Smith 1991). For comparison, species of sect. *Etuberosum* have genomes of similar or slightly smaller size than that of haploid potato clones (Valkonen et al. 1994).

2.5 Collinearity and Rearrangements Revealed by Meiotic Analyses

Disturbances in pairing between homeologous in interspecific hybrids may provide evidence for chromosomal rearrangements. Early studies on the meiosis of several interspecific tomato hybrids suggested no large-scale structural rearrangements between parental chromosomes and minor differences in the lengths of the heterochromatic regions of some chromosomes of certain species (e.g., tomato and *S. pennellii*; Sawant 1958; Khush and Rick 1963; reviewed in Chetelat and Ji 2007). Thanks to the higher resolution, electron microscope analyses of pachytene spread synaptonemal (SC) complexes from five F_1 tomato interspecific hybrids revealed numerous synaptic irregularities occurring mostly in heterochromatin but also in euchromatin (Anderson et al. 2010). The irregularities consisted primarily of mismatched kinetochores, paracentric inversion loops, and a large reciprocal translocation (exclusively in the tomato x *S. chmielewskii* hybrid). Mismatched kinetochores were the most common irregularity observed in all hybrids and were interpreted as the result of pericentric inversions that occurred in some of the lineages and/or of differences in genome size (Anderson et al. 2010). The F_1 hybrid tomato x *S. chmielewskii* (one of the closest tomato relatives) had the highest number of irregularities. However, in general, the synaptic irregularities increased with the phylogenetic distance of the wild parent from cultivated tomato (Anderson et al. 2010). Preferential pairing was observed in the allohexaploids produced by doubling 3 × fusion hybrids between tomato, potato, and *S. pennellii,* whereas tomato chromosomes readily paired with their homeologues from *S. pennellii* before doubling (Haider Ali et al. 2001). Preferential pairing was also observed in hybrids and substitution lines with more distantly related species *S. lycopersicoides* and *S. sitiens* (Ji and Chetelat 2003; Ji et al. 2004). However, in most cases, tomato chromosomes show regular homoeologous pairing with chromosomes from its wild relatives.

Analysis of pairing and segregation has also been performed in interspecific hybrids within the potato clade. The analysis of several interspecific potato hybrids provided evidence for cryptic structural differences as well as "definite structural differences" (such as translocations) among diploid wild potatoes genomes (reviewed in Matsubayashi 1991). For example, the genomes of *S. jamesii* and *S. bulbocastanum* were denoted by different superscript letters because their diploid F1 hybrids formed ten bivalents and one tetravalent in metaphase I, indicative of a reciprocal translocation (Matsubayashi 1991). Gaiero et al. (2017) reported that the pachytene chromosomes of 3 × hybrids between *S. commersonii* and *S. tuberosum* Group Phureja were paired both as bivalents and trivalents (Fig. 2.2a1). Some pairing breakpoints were observed, which could be evidence of small-scale rearrangements (Fig. 2.2a1 and inset). At diakinesis/metaphase I of these triploids, configurations of 7III + 5II + 5I suggested a near autotriploid behavior (Fig. 2.2a2–3). The univalents occurred at random, as indicated by BAC FISH chromosome identification (Fig. 2.2b). In backcross (BC) progenies, homoeologous pairing was maintained, as evidenced by the formation of multivalents (Fig. 2.2c). Analysis of fertile BC1 pentaploid/near-pentaploid *S. commersonii–S. tuberosum* hybrids obtained from a different breeding scheme also indicated intergenomic pairing with multivalent associations of up to five chromosomes, even though most chromosomes paired as bivalents (Barone et al. 1999). Similarly, analysis of tetraploid somatic hybrids between a haploid potato clone and *S. bulbocastanum* detected multivalent pairing at pachytene as well as at diakinesis. However, most chromosomes formed bivalents, likely an indication of preferential intragenomic pairing (Iovene et al. 2012). Comparative FISH mapping studies can shed light on the underlying reasons for these pairing behaviors.

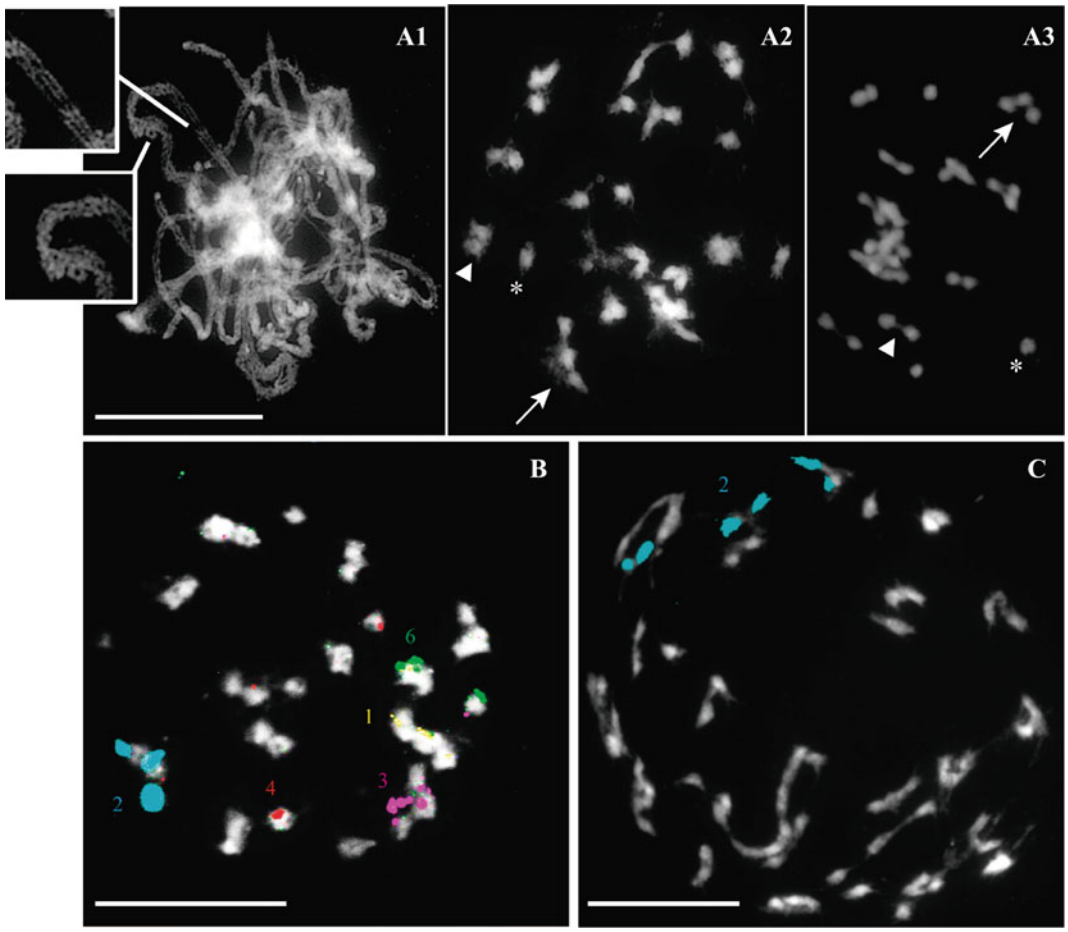

Fig. 2.2 **a** Homoeologous pairing in pollen mother cells (PMC) from *Solanum commersonii* and *S. tuberosum* Group Phureja 3 × hybrids (2n = 3*x* = 36). **a1** Pachytene complement with bivalents and trivalents and a loop (see insets). **a2** Diakinesis with five trivalents (arrow), seven bivalents (arrowhead), and seven univalents (asterisk). **a3** Metaphase/early anaphase I complement showing migration of trivalents (arrow), bivalents (arrowhead), and univalents (asterisk). **b** Homoeologous pairing in pollen mother cells (PMC) from the same 3 × hybrids hybridized with rDNA and potato chromosome-specific BAC probes specific to chromosomes: 1 (yellow, 5S rDNA), 2 (blue, 18S-25S rDNA), 3 (purple), 4 (red), and 6 (green). Chromosome 1, 2, and 3 paired as trivalents; chromosomes 4 and 6 paired as bivalents/univalents. **c** Homoeologous pairing in pollen mother cells (PMC) at diplotene/diakinesis of a genotype (2n = 5*x* + 5 = 65) derived from the backcross progeny of the same 3 × hybrid using 18S-25S rDNA (blue) as probe. The six identified chromosomes are forming multivalents (a quadrivalent + a bivalent). Scale bars represent 10 μm. Adapded from Gaiero et al. (2017)

2.6 Collinearity and Rearrangements Revealed by Comparative FISH

Synteny and collinearity among Solanaceae crops have been mainly studied through comparative genetic linkage mapping. In addition, considerable efforts have been devoted to integrating genetic, cytogenetic and high-throughput sequencing approaches in the assessment of collinearity. These studies showed that tomato and potato are differentiated by nine major inversions involving five whole arm paracentric inversions on chromosomes 5, 9, 10, 11 and 12, one inversion encompassing the euchromatic

portion of 6S, two additional inverted chromosome segments on the long arm of chromosome 2, and another one on the long arm of chromosome 12 (Bonierbale et al. 1988; Tanksley et al. 1992; Iovene et al. 2008; Tang et al. 2008; The Potato Genome Sequencing Consortium 2011; The Tomato Genome Consortium 2012; Peters et al. 2012; Szinay et al. 2012).

Comparative FISH mapping has been extended to wild potato and tomato relatives, and it is bringing to light several previously undescribed rearrangements (see Table 2.1, Achenbach et al. 2010; Lou et al. 2010; Peters et al. 2012; Szinay et al. 2012). However, relatively few species and/or few chromosomes have been analyzed, especially among wild potatoes. One of such comparative FISH studies analyzed the order of several potato and tomato BACs along seven chromosome arms (5S, 6S, 7S, 9S, 10L, 11S, and 12S) of potato, tomato as well as selected wild relatives and outgroups (Szinay et al. 2012). The authors noted that potato and its wild relatives (*S. bulbocastanum*, *S. pinnatisectum*, *S. tarijense*, *S. megistacrolobum*) had an identical hybridization pattern on those chromosome arms, and therefore these species were regarded as syntenic group A (Szinay et al. 2012). Similarly, tomato and its wild relatives *S. pimpinellifolium*, *S. peruvianum* and the distantly related *S. habrochaites* had identical BAC order and were thus regarded as syntenic group B. Based on the meiotic pairing analysis of hybrids *S. habrochaites*-tomato (Anderson et al. 2010; see the previous section), it is likely that *S. habrochaites* differs from tomato for rearrangements involving chromosome arms not studied by Szinay et al. (2012). Other distantly related wild tomatoes showed synteny with group B in some chromosome arms but not in others (Table 2.1). For example, *S. pennellii*, *S. chilense* and the outgroups *S. ochrantum* (sect. *Juglandifolia*) and *S. lycopersicoides* (sect. *Lycopersicoides*) were collinear with the rest of the tomato species in the short arm of chromosome 5 (5S), as well as in 9S, 10L, and 11S. However, there was a small inversion close to the pericentromeric heterochromatin on 12S that differentiated *S. chilense*, while a small terminal inversion on 6S separates *S. pennellii* from syntenic species B (Table 2.1, Szinay et al. 2012). Non-tuber-bearing *S. etuberosum,* in many cases, shared chromosome collinearity with potato and its relatives. However, *S. etuberosum* had large inversions in 7S and 9S compared to both potatoes and tomatoes, whereas it was collinear with syntenic species B for 10L (Table 2.1, Szinay et al. 2012). High resolution cytogenetic mapping was also employed to detect potential rearrangements among selected wild and cultivated *Solanum* species along the entire length of chromosome 6 (Lou et al. 2010). The authors were able to elucidate the ancestral structure of this chromosome and the different steps in chromosomal evolution through cross-species BAC FISH. The ancestral chromosome 6 should resemble that of *S. melongena* (eggplant), and it is conserved across potato and wild relatives *S. bulbocastanum* and *S. chromatophylum*. The non-tuber-bearing *S. etuberosum* displays a large pericentric inversion, while tomato differs in the previously-reported paracentric inversion in the short arm (Table 2.1, Lou et al. 2010).

Additional smaller inversions between tomato and its wild relatives were uncovered because these structural rearrangements had a negative impact on breeding (see Table 2.1; van der Knaap et al. 2004; Verlaan et al. 2011). BAC FISH revealed two rearrangements between tomato and *S. chilense* in the pericentromere of the long arm of chromosome 6, that is the region where the resistance gene *Ty-1* is introgressed, causing suppression of recombination and linkage drag (Verlaan et al. 2011). The locus *sun*, which controls the tomato fruit shape, was accurately located on the short arm of tomato chromosome 7 (van der Knaap et al. 2004) through FISH on extended DNA fibers. The authors suggested that because *sun* is located in a highly dynamic region of the tomato genome, the allelic variation found at this locus may be due to an insertion/deletion event. Therefore, comparative FISH helped clarify the causes of suppressed recombination around the gene of interest. On the other hand, Gaiero et al. (2016) found high collinearity at the chromosomal scale between potato and its wild relatives *S. commersonii* and

Table 2.1 Overview of chromosome rearrangements between tomato, potato, and their wild relatives discovered through fluorescent *in* situ hybridization (FISH). Tomato is compared to its wild relatives from *Solanum* Sect. *Lycopersicon* and its more distant relatives from Sect. *Lycopersicoides* (*S. lycopersicoides)* and Sect. *Junglandifolia* (*S. ochrantum*), as well as to *S. etuberosum*. Potato and its wild relatives from Sect. *Petota* for which there are reports (*S. bulbocastanum*, *S. chromatophylum* only for chr 6, *S. chacoense, S. commersonii, S. megistacrolobum, S. pinnatisectum*, *S. tarijense*) are taken as a group and compared to both tomato and the outgroup species *S. etuberosum.* Chromosome (Chr) arms involved in the rearrangements are indicated by S (short) or L (long). Superscripts indicate source

Chr	Tomato versus					Potato and wild relatives versus	
	S. pennellii	*S. chilense*	*S. lycopersicoides*	*S. ochrantum*	*S. etuberosum*	Tomato	*S. etuberosum*
2	nd				Reciprocal translocation between 2L and 7S [d]	Large distal 2L inversion [f]	Reciprocal translocation between 2L and 7S [d]
5	–				Large 5S inversion [a]	Large 5S inversion [a] and small 5L inversion [g]	–
6	Small distal 6S inversion.[a]	Small proximal 6L inversion [c]	Small proximal 6S inversion [a]	Large 6S inversion [a]	Large pericentric inversion [e]	Large 6S inversion [a, e, f, h, i]	Large pericentric inversion [e]
7	Small 7S inversion [a, b]	–	Small 7S inversion [a]	–	Large 7S inversion [a]	–	Large 7S inversion [a]
9	–				Small proximal 9S inversion [a]	Large 9S inversion [a]	Small distal 9S inversion [a]
10	–				–	Large 10L inversion [a, f]	Large 10L inversion [a]
11	–				Large distal 11S inversion [a]	Large distal 11S inversion [a]	–
12	–	Small proximal 12S inversion [a]	–	–	Large distal 12S inversion [a]	Large distal 12S inversion [a]	–

[a]Szinay et al. 2012; [b]van der Knapp et al. 2004; [c]Verlaan et al 2011; [d]Braz et al. 2018; [e]Lou et al. 2010; [f]Peters et al. 2012; [g]Achenbach et al. 2010; [h]Tang et al. 2008; [i]Iovene et al. 2008; nd = not determined; – = no rearrangement detected by FISH

S. chacoense when they compared the cytogenetic positions of potato BACs previously located on RH potato (Tang et al. 2009). Altogether, the few comparative BAC FISH studies available for tuber-bearing Solanums reported no evidence for large-scale chromosome rearrangements between potato and its wild relatives (Lou et al. 2010; Szinay et al. 2012; Gaiero et al. 2016; Braz et al. 2018).

All the genetic and cytogenetic studies have indicated that inversions are the main mode of chromosome differentiation among potatoes and tomatoes. However, a comparative oligo-based chromosome painting study showed that *S. etuberosum* differs from potato and its wild relative *S. bulbocastanum*, tomato, and eggplant for a reciprocal translocation between 2L and 7S (Fig. 2.3; Braz et al. 2018). Similarly, *S.*

caripense, a more distant relative of tomato and potato, differs from the same set of species for another reciprocal translocation between 4L and 11S (Braz et al. 2018). On the other hand, the oligo-FISH pattern on *S. bulbocastanum* chromosomes was identical to that on potato (Braz et al. 2018). Therefore, the chromosomes of the potato species analyzed seem not to be affected by gross rearrangements, differently to tomatoes (Lou et al. 2010; Peters et al. 2012; Szinay et al. 2012; Braz et al. 2018). However, additional comparative mapping studies comprising more tuber-bearing species are needed to confirm collinearity between potato and its wild relatives.

2.7 Cytogenetics of Satellite Repeats in *Solanum*

A significant portion of the potato and tomato genomes is occupied by satellite DNA, which consists of long arrays of nearly identical tandem repeat units (called monomers) spanning up to several megabases. Cytogenetics has contributed significantly to the characterization of the satellite repeats repertoire in *Solanum*, which is the focus of this last section. Satellite families isolated from various potatoes and tomatoes are typically located in the heterochromatin, at subtelomeric and (peri)centromeric regions but also at interstitial chromosomal sites. Many of these repeats are widespread throughout potatoes and tomatoes (Stupar et al. 2002; Tek and Jiang 2004; Jo et al. 2009; Torres et al. 2011; Tang et al. 2014). However, many satellite families display a remarkable inter- and even intraspecific variation, especially in their abundance and chromosomal distribution, with extreme patterns of presence/absence, which have suggested that these sequences evolve rapidly (Tek et al. 2005; Gong et al. 2012; Wang et al. 2014; Zhang et al. 2014). For this reason, several repeats that were initially identified provided useful species-specific RFLP-based markers to identify *Solanum* species and their interspecific somatic hybrids (Pehu et al. 1990; Schweizer et al. 1993; Stadler et al. 1995). The following paragraphs provide an overview of the main satellite repeats among potato and tomato wild relatives, with the satellite repeats grouped for their similarity with "universal" repeats (rDNA and telomeric sequences) and/or for their chromosomal distribution.

2.7.1 rDNA Gene Clusters and Related Satellite Repeats

The ribosomal DNA gene clusters are among the best-characterized satellite arrays in eukaryotes. In potato, tomato, and their wild relatives, the 18S-25S rDNA cluster was mapped at the end of the short arm of chromosome 2, whereas the 5S rDNA was located interstitially on the short arm and next to the centromere of chromosome 2 (Ganal et al. 1988; Visser and Hoekstra 1988; Lapitan et al. 1989; Xu and Earle 1996; Dong et al. 2000; Stupar et al. 2002; Chang et al. 2008; Jo et al. 2009; Gaiero et al. 2016; Choudhary et al. 2020). Minor rDNA sites have been reported on other chromosomes in various species or accessions (Xu and Earle 1996; Brasileiro-Vidal et al. 2009). Conversely, two of the six copies of chromosome 2 in the autohexaploid *S. demissum* (2n = 6x = 72) lacked the 18S-25S rDNA sites (Braz et al. 2018), whereas in the allotetraploid *S. stoloniferum* the two pairs of 18S-25S rDNA sites derived from two different parental genomes had a very different size, possibly a result of the allopolyploidization process (Pendinen et al. 2008).

Ribosomal DNA gene clusters may be a source of novel satellite families. Indeed, satellite repeats made of tandem monomers with high sequence similarity to the intergenic spacer (IGS) of the 18S-25S rDNA are widespread across potatoes and tomatoes (Table 2.2; Stupar et al. 2002; Jo et al. 2009). These sequences were initially isolated from *S. bulbocastanum* and tomato BAC libraries. Some of these sequences had similarity to both the IGS and portions of the rDNA coding sequences (Stupar et al. 2002; Jo et al. 2009), which could explain the minor interstitial 18S-25S rDNA signals reported for tomatoes (Xu and Earle 1994; Brasileiro-Vidal

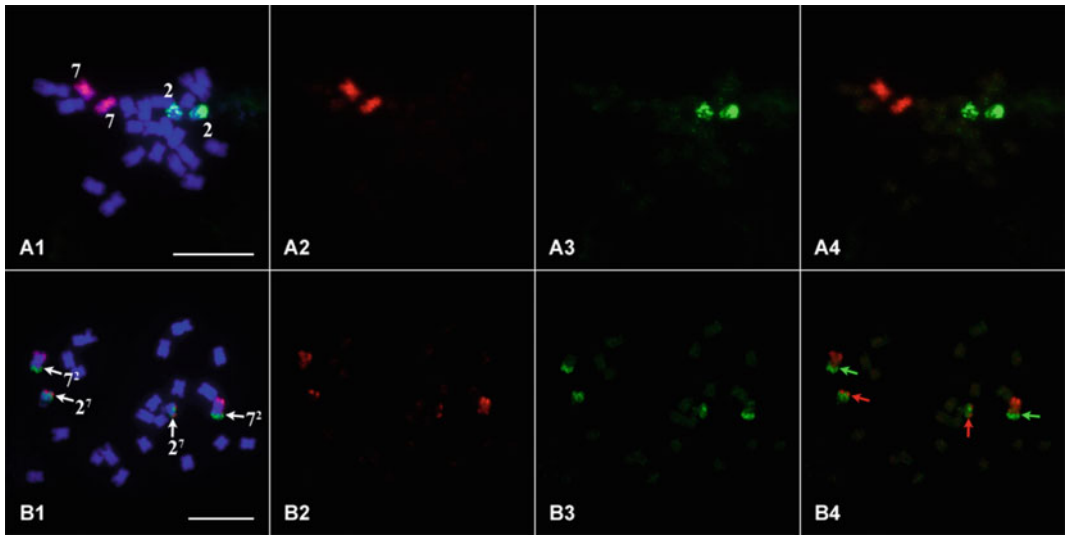

Fig. 2.3 A chromosomal translocation between potato and *Solanum etuberosum* detected by chromosome painting (Braz et al. 2018). Photographs by Braz G. T., He L., and Jiang J. **a1–a4** Painting of chromosome 2 (green) and 7 (red) of the diploid potato clone DM. Red (**a2**), green (**a3**), and both red and green (**a4**) fluorescence signals were digitally separated from **a1**. **b1–b4** Painting of chromosomes 2 (green) and 7 (red) in *S. etuberosum*. Red (**b2**), green (**b3**), and both red and green (**b4**) fluorescence signals were digitally separated from **b1**. Red arrows in **b4** point to the breakpoint where a small chromosome 7 fragment attached to chromosome 2 (2^7). Green arrows in **b4** point to the breakpoint where a large chromosome 2 fragment attached to chromosome 7 (7^2). Bars = 10 μm

et al. 2009). FISH using these IGS-related repeats in potatoes revealed a variable number of pericentromeric sites in hemizygous condition (Stupar et al. 2002). In addition, FISH indicated that different IGS-related repeats occupied adjacent and distinct pericentromeric heterochromatic domains. Southern blot analysis indicated that IGS-related DNA sequences are present in a wide range of *Solanum* species and that this repeat family is evolutionary dynamic and capable of rapid structural and copy number changes (Stupar et al. 2002). Similar to what found in potatoes, IGS-related repeats are associated with the heterochromatic pericentromeric regions of several pachytene chromosomes of tomato and close wild relatives (Table 2.2; Jo et al. 2009).

2.7.2 Telomeric, Subtelomeric, and Related Repeats

The chromosomal ends of *Solanum* spp. contain typical Arabidopsis-type telomeric tandem repeats (TT[T/A]AGGG), which are organized in long arrays at the end of the chromosomes in association with subtelomeric satellite repeats (Ganal et al. 1991; Zhong et al. 1998; Torres et al. 2011).

A number of subtelomeric satellite sequences have been identified in *Solanum* (Table 2.2), including TGRI in cultivated tomato, CL14/PRG1 and CL34 in potato, and Sb4/2 in *S. brevidens* (Ganal et al. 1988; Preiszner et al. 1994; Torres et al. 2011; Tang et al. 2014). Four repeats (TGR1, Sb4/2, Cl14, and PRGI) show sequence similarity to each other and are widespread across tomatoes and potatoes (Table 2.2). TGRI, one of the most abundant satellites of the tomato genome, is located at almost all chromosome ends in association with canonical telomeric repeats to form arrays up to 1.3 Mb long, as well as at interstitial sites on some chromosomes (Ganal et al. 1988; Zhong et al. 1998). A Southern analysis indicated that TGRI is widespread across the tomato clade (Ganal et al. 1988). On the other hand, CL14/PRG1,

Table 2.2 Partial list of *Solanum* satellite repeats characterized by cytogenetic tools. Repeats are grouped according to their chromosomal distribution and for their similarity with "universal" repeats (rDNA and telomeric sequences). References are indicated with superscripts

Repeat type	Repeat name (monomer length, bp)	Species	Chromosome distribution	Similarity with other repeats	Hybridization in another species
Subtelomeric	TGRI (162)[a,b,c]	*S. lycopersicum*	Most chrs; few interstitial sites	Partial with Sb4AX	Widespread among wild tomatoes
	Sb4AX (1728)[d]	*S. brevidens*	Most chrs	Partial with TGRI	nd
	CL14 (182)[e]; PGR1 (182)[f]	*S. tuberosum*	Most chrs	Partial with TGRI and Sb4AX	*S. verrucosum, S. cardiophyllum, S. chomatophilum, S. lycopersicum* (only CL14)
	CL34 (339)[e]	*S. tuberosum*	Most chrs	nd	*S. verrucosum*
Pericentromeric	Sobo (4700)[g]	*S. bulbocastanum*	1 hemizygous site on chr 7	LTR *Sore1*	No hybridization
Pericentromeric, IGS-related	2D8 (5900)[h]	*S. bulbocastanum*	4 hemizygous sites on 4 chrs	IGS of 18S-25S rDNA	Widespread among wild potatoes and tomatoes
	26J19 (5900)[h]	*S. bulbocastanum*	2 hemizygous sites on 2 chrs	IGS	nd
	4A4 (nd)[h]	*S. bulbocastanum*	1 hemizygous site	IGS and 18S-25S rDNA coding sequences	nd
	pIGS (nd)[i]	*S. lycopersicum*	Sites on 3 pachytene chrs	IGS and 18S-25S rDNA coding sequences	*S. lycopersicum* var. *cerasiforme*, *S. pimpinellifolium*
Pericentromeric, ITRs	pSbTC1 (2800)[j]; oligonucleotide telomeric probe[k]	*S. bulbocastanum* –	Most chrs	Perfect and degenerated telomeric motifs	Cultivated potato and many wild potato relatives
Centromeric	St24 (979)[l]; Sv161.5 (nd)[m]	*S. tuberosum; S. verrucosum*	Cen1	nd	*S. verrucosum* Cen7; weak or no signals in other species
	St3-58 (2957)[l]	*S. tuberosum*	Cen2	Ty3/gypsy, Chromovirus	No signals
	St3-294 (5390)[l]	*S. tuberosum*	Cen3 and Cen9	Ty3/gypsy, Chromovirus	Weak or no signals
	St3-238 (3814)[l]	*S. tuberosum*	Cen8	Ty3/gypsy, Chromovirus	Weak or no signals
	St49 (2754)[l]; Sv209 (nd)[m]	*S. tuberosum*; *S. verrucosum*	Cen5	ITR	Multiple centromeres of various wild potatoes*:* telomeric signals in tomato

(continued)

Table 2.2 (continued)

Repeat type	Repeat name (monomer length, bp)	Species	Chromosome distribution	Similarity with other repeats	Hybridization in another species
	St57 (1924) [l]; Sv161.6 (nd) [m]	*S. tuberosum*; *S. verrucosum*	Cen7	nd	*S. verrucosum* Cen7; no signals in other species
	St18 (1180) [l]	*S. tuberosum*	Cen9	Ty3/gypsy, Chromovirus	Unidentified Cen of *S. verrucosum*; no signals in other species
	Sv14 (nd)[m]	*S. verrucosum*	Cen4	LTR retrotransposon chromodomain	No signals
	Sv44 (nd)[m]	*S. verrucosum*	Cen4	nd	No signals
	Sv54 (nd)[m]	*S. verrucosum*	Cen2 and Cen10	nd	*S. tuberosum* Cen9; *S. chomaophilum* Cen2; no signals in other species
	Sv123 (nd)[m]	*S. verrucosum*	Cen2 and Cen10	nd	Cen1, Cen2 and unknown Cen of *S. chomatophilum*; no signals in other species
	Sv98 (nd)[m]	*S. verrucosum*	Most centromeres	Ty3/gypsy, Chromovirus	Most centromeres of *S. tuberosum*; no signals in other species
	Sv43 (nd)[m]	*S. verrucosum*	Weak signals in most centromeres	nd	Weak signals in most centromeres of *S. tuberosum* and *S. chomaophilum*; no signals in other species
	Sv132 (nd)[m]	*S. verrucosum*	Weak signals in most centromeres	Ty3/gypsy retrotransposon	Weak signals in most centromeres of *S. tuberosum* and *S. jamesii*; no signals in other species
	TGRIV (7000)[c]	*S. lycopresicum*	All centromeres	GYPSODE1 retrotransposon	nd

[a] Ganal et al. 1988; [b] Zhong et al. 1998; [c] Chang et al. 2008; [d] Preiszner et al. 1994; [e] Torres et al. 2011; [f] Tang et al. 2014; [g] Tek et al. 2005; [h] Stupar et al. 2002; [i] Jo et al. 2009; [j] Tek and Jiang 2004; [k] He et al. 2013; [l] Gong et al. 2012; [m] Zhang et al. 2014; nd = not determined; Chr = chromosome; IGS = intergenic spacer of the 18S-25S rDNA; ITR = interstitial telomeric repeats

identified in potato, is located exclusively at the subtelomeric regions of about half of the potato chromosomes (Fig. 2.4; Torres et al. 2011; Tang et al. 2014). CL14 repeat generated similar hybridization patterns even among *Solanum* species distantly related to potato, although few non-subtelomeric FISH signals were observed in several species (Torres et al. 2011). These findings suggested that CL14/PRG1, along with the related TGRI and Sb4/2, belong to an ancient repeat family that has maintained its (predominant) subtelomeric positions in all *Solanum* species (Torres et al. 2011; Tang et al. 2014). By contrast, a FISH survey of CL34, also identified in potato, indicated that this repeat had emerged recently, since it hybridized to about half of the chromosome ends of cultivated potato and its close relative *S. verrucosum*, whereas it generated very weak or no signals in more distantly related wild potato species (Fig. 2.4; Torres et al. 2011).

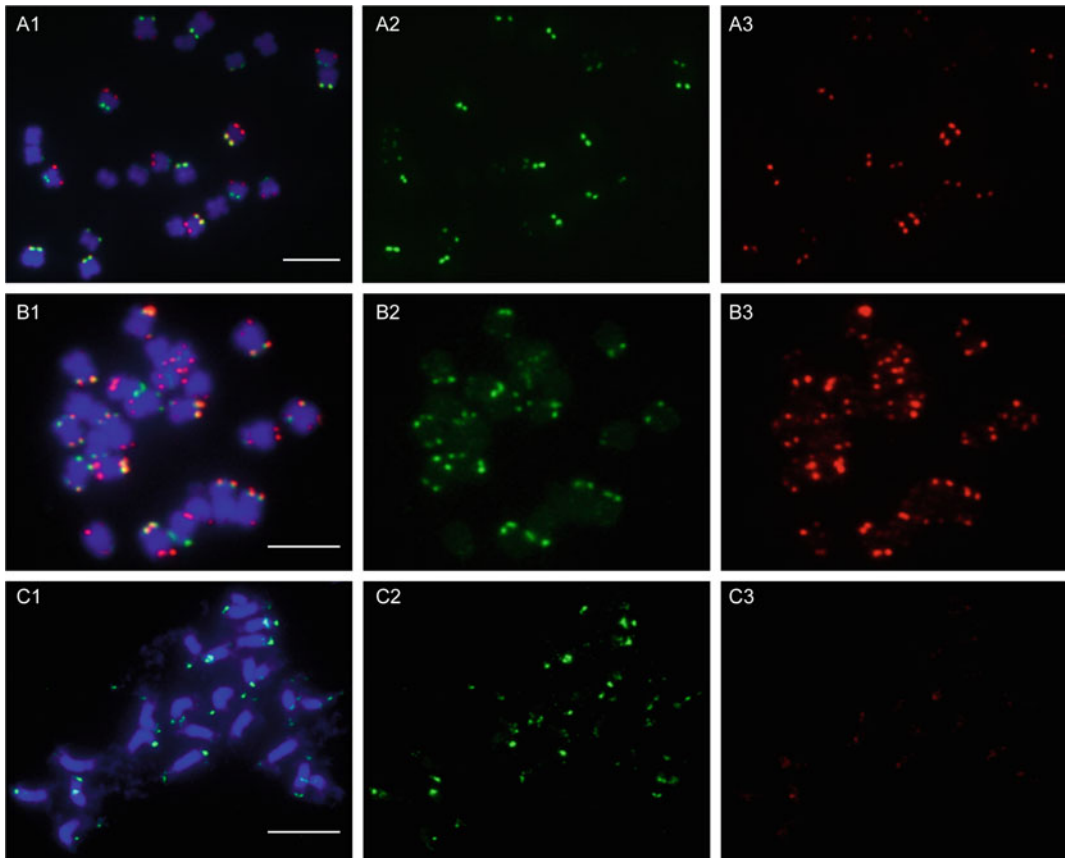

Fig. 2.4 Comparative FISH mapping of the subtelomeric repeats CL14 (green) and CL34 (red), among three different *Solanum* species (Torres et al. 2011). Photographs by Torres G. A. and Jiang J. **a1–a3** DM1-3 potato (A genome); **b1–b3** *S. verrucosum* (A genome); **c1–c3** *S. palustre* (E genome). Bars = 5 μm

2.7.3 Pericentromeric Repeats in *Solanum*

The pericentromeric heterochromatin of most eukaryotic organisms contains large amounts of satellite repeats characterized by different degrees of lineage-specificity. In addition to the IGS-related repeats, telomeric-like sequences are located in the (peri)centromeric regions of several chromosomes of many *Solanum* species. An early genetic linkage mapping study detected short arrays of interstitial telomeric repeats (ITRs) in the centromeric regions of several tomato chromosomes (Presting et al. 1996). In potatoes, pSbTC1, an ITR sequence isolated from *S. bulbocastanum*, generated strong FISH signals in the pericentromeric heterochromatin as well as weak signals in the telomeric regions of several chromosomes of potato and various wild relatives (Table 2.2; Tek and Jiang 2004). The 2.8 kb monomers of pSbTC satellite consisted of exclusively degenerated telomeric DNA sequences, and their long tandem clusters spanned several megabases (Tek and Jiang 2004). This suggested that, differently from tomato (Presting et al. 1996), ITR-like repeats in potatoes have undergone massive local amplification, and therefore, they are not simple footprints of ancient events of chromosome rearrangements (Tek and Jiang 2004). In addition, a FISH survey using a telomeric DNA probe indicated that species with B (*S. bulbocastanum* and *S. pinnatisectum*) and P (*S. paucissectum*) genomes are particularly rich of ITR-like satellites, mainly in

the centromeric/pericentromeric regions of several chromosomes (He et al. 2013). By contrast, tomato did not show any distinct interstitial telomeric signals which corroborated the finding that centromeric and pericentromeric regions of the wild potato relatives, but not tomato, contain megabase-sized arrays of telomeric-like sequences (He et al. 2013). Additional repetitive sequences have been mapped to the pericentromeric heterochromatin of various *Solanum* species (Table 2.2), including a species-specific satellite repeat (Sobo) identified in the *S. bulbocastanum* genome (Tek et al. 2005). Sobo mapped on chromosome 7 of some *S. bulbocastanum* accessions in hemizygous condition, spanning > 350 kb of pericentromeric heterochromatin (Tek et al. 2005). Interestingly, the Sobo repeat was not detected in any other *Solanum* species, which suggested that Sobo repeat is a recently amplified satellite repeat, pointing to the dynamic nature of the satellite DNA (Tek et al. 2005).

2.7.4 Centromeric Repeats in *Solanum*

The centromeres are essential for the faithful segregation of sister chromatids during cell divisions. Centromeric DNA in most eukaryotes consists of long arrays of satellite repeats and/or retrotransposons, and it is among the most rapidly evolving sequences in the genome. Repeat–based centromeres are thought to have evolved from "neocentromeres" arose in novel sites, usually in gene-poor environment, by an accumulation of the specific histone variant CenH3. Given time, these "neocentromers" are believed to evolve into mature centromeres through the "invasion" of satellite DNA (reviewed in Kalitsis and Choo 2012; Jiang 2013; Plohl et al. 2014; Oliveira and Torres 2018).

Potato and its relatives have recently provided a new model system to support the hypothesis of centromere evolution from neocentromere (Gong et al. 2012; Zhang et al. 2014). A genome-wide characterization of the DNA sequences associated to CENH3 nucleosomes has shown that each potato centromeres contains distinct DNA sequences (Gong et al. 2012). Five potato chromosomes did not include any satellite DNA, but consisted primarily of single- or low-copy DNA sequences, including active genes (Gong et al. 2012). Thus, the DNA structure of these five potato centromeres is thought to resemble "immature" neocentromeres (reviewed in Jiang 2013; Gong et al. 2012). In contrast, the centromere of the other six potato chromosomes (1, 2, 3, 5, 7, and 8) are composed of megabase-sized satellite repeat arrays that are specific to individual chromosomes (Table 2.2). The centromere of potato chromosome 9 contains two different satellites, as well as single-copy sequences (Table 2.2). The monomer sizes of these satellite repeats range from ~980 bp to >5.3 kb, and the satellites form long arrays from ~900 kb to > 4 Mb, likely occupying the entire functional cores of the centromeres (Gong et al. 2012). Comparative FISH mapping of the potato centromeric satellites in wild *Solanum* representatives of the genomes A (*S. verrucosum*), B (*S. jamesii*), P (*S. chromatophilum*), and E (*S. etuberosum* and *S. palustre*) indicated that St49 is likely an ancient repeat belonging to an ITR family, and it is present in all species analyzed (Gong et al. 2012). By contrast, the other potato satellite repeats appeared to be amplified recently from retrotransposon-related sequences. These repeats either hybridized only to the closely related *S. verrucosum*, or were absent in all species, suggesting a rapid evolution from repeatless neocentromeres to repeat-based centromeres (Gong et al. 2012). The sequence specificity of the potato centromeres has opened opportunities to comparative analysis of homoeologous centromeres among related species. Isolation of satellite repeats associated with CENH3 in *S. verrucosum* revealed that homoeologous centromeric sequences between *S. verrucosum* and potato were restricted to a single centromere (Cen9). Four *S. verrucosum* centromeres (Cen2, Cen4, Cen7, and Cen10) contained distinct satellite repeats (Table 2.2; Zhang et al. 2014). Strikingly, the same four centromeres in potato contained either different satellite repeats (Cen2 and Cen7) or exclusively

single/low-copy sequences (Cen4 and Cen10). Comparative FISH mapping among *Solanum* species representatives of the genomes A, B, P, and E revealed the absence of the *S. verrucosum* centromeric repeats in most species analyzed, confirming the rapid divergence of the centromeric sequences and suggesting a recent emergence of these centromeric satellites in the *S. verrucosum* genome.

There are no such genome-wide studies in tomato and its relatives. Chang et al. (2008) cytologically mapped a repeat element named TGRIV to the primary constrictions of all tomato pachytene chromosomes. Similar to the sequences of other satellite repeats identified in *Solanum*, TGRIV likely derived from a retrotransposon, the Ty3-Gypsy GYPSODE1 (Chang et al. 2008). However, the association of TGRIV with the tomato centromeric chromatin and its distribution in the tomato relatives remain to be explored.

2.8 Conclusions and Perspectives

Comparative linkage maps and classical cytogenetic studies indicated a conserved genome structure and high collinearity within potato and tomato wild relatives. However, along with high-throughput comparative sequencing, comparative BAC FISH mapping and an increase of resolution in cytogenetic technologies (such as that achieved with comparative genome mapping through nanochannels) are providing evidence for substantial structural rearrangements as well as striking differences in the repeat composition of heterochromatic domains among these species. Many rearrangements found among tomato species involve inversions located in heterochromatic pericentromeric regions, which would be difficult to detect by genetic mapping. Such comparative FISH studies are still sparse among potatoes. However, oligo-based chromosome painting is expected to facilitate these studies by avoiding technical difficulties due to repeat-rich BACs and allowing reciprocal comparative analysis using oligo-probes designed on any sequenced *Solanum* genome. In addition, oligo-based chromosome painting enables the monitoring of pairing of homologous/homeologous chromosomes during meiosis, which provides useful insights into differentiation and recombination between the parental genomes of species and experimental hybrids. Cytogenomic studies have demonstrated the complex structure of the centromeric and pericentromeric regions of several *Solanum* species, associated with impressive variation in the sequence composition of homologous centromeres/pericentromeres within accessions and cultivars as well as homeologous centromeres/pericentromeres among closely related species. In the future, similar studies in tomato and its relatives may reveal whether such sequence diversity at the (peri)centromeres is common across the genus *Solanum,* or it is a phenomenon restricted within potatoes. Cytogenetics will continue to support high-throughput comparative sequencing studies to identify the landscape of structural variants and reveal genome diversity across potato and tomato wild relatives.

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