Chapter 10

Potato Cytogenetics

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10.1 INTRODUCTION

Common potato, *Solanum tuberosum*, belongs to the section *Petota*, which is subdivided into 21 series with 228 wild and 7 cultivated species (Hawkes, 1994). According to the latest view, the section contains 199 wild and 1 cultivated species (Spooner and Hijmans, 2001; Huamán and Spooner, 2002; Chapter 4, van den Berg and Jacobs, this volume). Cytogenetic research helped to create the genome concept of wild and cultivated potato species (reviewed by Matsubayashi, 1991), to study haploid production and to use haploids in genetics and breeding (reviewed by Peloquin et al., 1991), to monitor the chromosome status of hybrid material (reviewed by Hermsen, 1994) and to investigate chromosome instability (reviewed by Wilkinson, 1994). This chapter surveys the application of cytogenetic methods for the investigation of genomic, evolutionary and species relationships, the integration of genetic and cytological maps, the analysis of genome structure and the detection of introgressions of alien chromatin. Besides traditional cytogenetic methods, the potential of new molecular techniques is considered.

10.2 BASIC CHROMOSOME NUMBER AND POLYPLOID COMPLEXES

Determination of chromosome number for *S. tuberosum* was the beginning of cytogenetic studies of potato. The haploid chromosome number ($n = 24$) was established for the first time by Kihara (1924). Later, the somatic chromosome number ($2n = 48$) was provided by Stow (1926) for varieties of the common potato. Approximately at the same time, the first indications of the existence of different ploidy levels in the wild potatoes were provided by investigators studying meiosis in pollen mother cells of *Solanum chacoense, Solanum jamesii, Solanum fendleri, Solanum* × *edinense* and *Solanum demissum* (Salaman, 1926; Smith, 1927; Vilmorin and Simonet, 1927). Rybin (1929, 1933) first described the whole polyploid series in wild potatoes (2x-3x-4x-5x-6x) and established an entire polyploid series in cultivated species (2x-3x-4x-5x). Rybin (1929) proposed to use differences in ploidy level for taxonomic classification of cultivated potatoes. All species of the section *Petota* have the same basic chromosome number (x = 12). Of the potato species with known chromosome number, 73% are classified as diploid ($2n = 2x = 24$), 4% triploid ($2n = 3x = 36$), 15% tetraploid ($2n = 4x = 48$), 2% pentaploid ($2n = 5x = 60$) and 6% hexaploid ($2n = 6x = 72$) (Hawkes, 1990).

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Two major mechanisms have been proposed to explain the origin of polyploidy: chromosome doubling of somatic cells and formation of unreduced gametes (sexual polyploidization). Harlan and De Wet (1975) argued that almost all polyploids in nature have originated through sexual polyploidization. This is particularly true for the species of the section *Petota*, many of which often form both 2*n* pollen and 2*n* eggs (Watanabe and Peloquin, 1991). 2*n* gametes provide opportunities for gene flow between species with different ploidy levels and/or different endosperm balance numbers (EBNs) (Den Nijs and Peloquin, 1977). Thus, in addition to causing polyploidization, the ability to form 2*n* gametes also facilitated interspecific hybridization, which has played an important role in the evolution of wild and cultivated potatoes and in the formation of polyploid complexes in the section *Petota*. There are two major types of polyploids: autopolyploids, which received their homologous set of chromosomes from one species, and allopolyploids, which received their homologous set of chromosomes from different species. Determination of the type of polyploidy for species in the section *Petota* has been based mainly on the analysis of chromosome pairing in species and their hybrids. In general, strict allotetraploid and allohexaploid species show regular meiosis with bivalent chromosome pairing and extremely low frequency of multivalents. Triploid, pentaploid and autotetraploid species show high frequency of multivalents at metaphase I (MI), irregular meiosis and sterility or very low level of fertility. These species are maintained mainly by vegetative propagation. Some of the polyploids are classified as segmental allopolyploids; they are characterized by 'intermediate' frequencies of multivalents – lower than in autopolyploids and higher than in strict allopolyploids of corresponding ploidy levels.

10.3 GENOME AND SPECIES RELATIONSHIPS

The genome concept has been developed for potato species based on the crossability rate in interspecific combinations, hybrid viability, pollen fertility and the degree of chromosomal homology (Marks, 1955, 1965; Hawkes, 1958; Irikura, 1976; Ramanna and Hermsen, 1981; Hawkes, 1990; Lopez and Hawkes, 1991; Matsubayashi, 1991). Chromosome-pairing relationships in interspecific hybrids and in polyploid species have been interpreted by genome formulas, although authors gave them different symbols. Today, most authors agree on the genome hypothesis of Matsubayashi (1991). According to this hypothesis, five genomes (A, B, C, D and P) are recognized in tuber-bearing species of the section *Petota*. A genome E (Ramanna and Hermsen, 1981) is recognized in non-tuber-bearing species of the closely related section *Etuberosum*.

10.3.1 Genomic designation and relationships of diploid potato species

According to Matsubayashi (1991), all diploid tuber-bearing species growing under extremely diverse climatic conditions and exhibiting a wide range of morphological differences comprise one major genomic group A. No diploid species have ever been identified with B, C, D and P genomes. The basic genome A was proposed for diploid species of the four series, *Tuberosa, Commersoniana, Cuneoalata* and *Megistacroloba*, which all have identical (or very similar) genome(s). As reviewed by Matsubayashi (1991),

hybrids between diploid species with the AA genome show 12 bivalents at MI, regular meiosis and fertile pollen. Diploid hybrids between species having the A genome and the other diploid potatoes show more or less reduced pollen fertility, and their amphidiploids are characterized by preferential pairing (reviewed by Matsubayashi, 1991). It was hypothesized that genomic variants of diploid potatoes of the *Bulbocastana, Ingifolia, Conicibaccata, Morelliformia, Pinnatisecta, Piurana* and *Polyadenia* series differ from the basic A genome by cryptic structural differences and that genomic variants of diploid species of the *Olmosiana* series and *Solanum rachialatum (Ingifolia* series) differ from other variants of the A genome by definite structural differences (Matsubayashi, 1991). The genomic variants of diploid species belonging to the above-mentioned eight series were designated by Matsubayashi (1991) as genome formula A with superscripts corresponding to each taxonomical series. Dvořák (1983) gave another explanation of differential affinity between the genomic variants of diploid potato species. He suggested that rapid evolution of non-coding sequences caused the differentiation of genomes of diploid tuber-bearing species.

10.3.2 Genomic nature and relationships in polyploid potato species

Relatively few polyploid members of the section *Petota* have been identified that appear to be autopolyploids. Multiple cytotypes ('cytotype' – any variety of a species whose chromosome complement differs quantitatively or qualitatively from the standard complement of the species; Rieger et al., 1991) of diploid species may be of autopolyploid origin. Triploid and tetraploid cytotypes are known for many typically diploid potato species (Hawkes, 1990). Triploid cytotypes derive from the union of unreduced ($2n$) and normal (n) gametes of the same diploid species, and tetraploid cytotypes can be produced by the fertilization of $2n$ egg cells with $2n$ pollen of a diploid species. Autotriploids should have a high frequency of trivalents at MI. Indeed, Sanuda Palazuelos (1962) observed up to eight trivalents in a triploid cytotype ($2n = 36$) of *Solanum cardiophyllum*, which is similar to the 8.4–10.3 trivalents per cell formed at MI in synthetic autotriploids (Irikura, 1976).

Among even-level polyploid potato species, multivalents occur very rarely. The frequency of multivalents at MI in *S. tuberosum* ($2n = 4x = 48$) ranging from 1.5 to 5.2 (Matsubayashi, 1991) is much higher than in other tetraploid species but lower than in synthetic autotetraploids. Chromosomes of *S. tuberosum* pair, recombine and segregate randomly as common potato displays tetrasomic inheritance ratios (Bradshaw and Mackay, 1994). Thus, *S. tuberosum* is one of the exceptional examples of a polysomic polyploid (autotetraploid – AAAA genome) in the section *Petota*. Both regular bivalent pairing and univalents at MI were quite frequently observed in dihaploids ('dihaploid' – an individual produced from a tetraploid form, which possesses half the tetraploid number of chromosomes; Rieger et al., 1991) of common potato. Unpaired segments in bivalents of some dihaploids have been reported (Matsubayashi, 1991). Therefore, segmental allotetraploidy and the genome formula AAA^tA^t were proposed by Matsubayashi (1991) for common potato. One possible explanation for the disagreements about the polyploid nature of *S. tuberosum* is the introgression of germplasm of wild and cultivated species into Andigena and Chilean landraces and into varieties of common potato.

Hawkes (1990) hypothesized that about 12% of potato species have a hybrid origin. Allopolyploids can originate from spontaneous interploid crosses between species possessing the same EBN or spontaneous crosses between species with functional 2*n* gametes and different EBNs or crosses between diploid species with the same EBN and mitotic polyploidization following the hybridization event or fertilization between 2*n* male and female gametes of two diploid species. For instance, the triploid species *S.*× *vallis-mexici* is a natural hybrid between *Solanum stoloniferum* ($2n = 48$, EBN = 2) and *Solanum verrucosum* ($2n = 24$, EBN = 2) (Marks, 1958). The pentaploid species *Solanum curtilobum* derived from the fusion of an unreduced (3x) gamete of *Solanum juzepczukii* and a normal (2x) gamete of *Solanum andigenum* ssp. *andigena* (Hawkes, 1962).

Segmental allopolyploidy has been proposed for polyploids of the series *Tuberosa,* *S. chaucha* (AAA^t), *S. juzepczukii* (AAA^a), *S. curtilobum* ($AAAA^aA^t$) and *S. sucrense* (AAA^sA^s), and for the wild species *Solanum acaule* of the *Acaulia* series (AAA^aA^a) by comparing the frequency of multivalent formation at MI in the species and their haploids or hybrids (Matsubayashi, 1991). We also suppose segmental polyploidy for the tetraploid species *Solanum tuguerrense* of the *Piurana* series, although Matsubayashi (1991) considered it as a strict allotetraploid (A^pA^pPP). However, the observation of a high frequency of trivalents at MI (4.5 trivalents +7.5 bivalents +7.5 univalents per cell) in triploid hybrids (AA^pP) of *S. tuguerrense* with *S. verrucosum* (AA) (Marks, 1965) indicates partial homology of the A^p and P genomes. For comparison, in triploid hybrids (AAA^a) between the segmental allotetraploid *S. acaule* and several diploid A-genome species, the frequency of trivalents at MI ranged from 3.0 to 6.5 (Propach, 1937; Swaminathan and Howard, 1953; Irikura, 1976).

Wild polyploid species of the series *Longipedicellata, Conicibaccata* and *Demissa* are considered as strict allopolyploids (disomic polyploids) based on the results of meiotic studies that showed regular bivalent pairing (Marks, 1955, 1965; Hawkes, 1958; Irikura, 1976; Lopez and Hawkes, 1991; Matsubayashi, 1991). According to Dvořák (1983), bivalent chromosomal pairing in allopolyploid potato species can be explained by genetically controlled regulatory mechanisms preventing intergenomic pairing. However, no convincing data confirming this hypothesis have ever been obtained.

All authors agree that strict allopolyploids share one common component genome, which is highly homologous to the A genome of diploid potato species (Marks, 1965; Irikura, 1976; Matsubayashi, 1991). Based on the analysis of chromosome pairing in hybrids, the diploid species *S. verrucosum* (AA) was suggested as the putative contributor of the common A genome of natural allopolyploids (Marks, 1965). A common origin of *S. verrucosum* and Mexican polyploid species was supported by the similarity of their cpDNA (Spooner and Sytsma, 1992) and by geographical and morphological data. Amplified fragment-length polymorphism (AFLP) results also support a close relationship between *S. verrucosum* and members of the *Longipedicellata, Demissa* and *Acaulia* series (Kardolus, 1998).

All authors also agree that strict allopolyploids differ from one another by their second component genome (Marks, 1965; Irikura, 1976; Matsubayashi, 1991). According to Irikura (1976), allopolyploid species differ from one another by the genomic variants of a merged B genome. Thus, genome designation AAB^sB^s was proposed for allotetraploid species of the *Longipedicellata* series, $AAB^sB^sB^dB^d$ for allohexaploid species of the

Demissa series and AAB^aB^a for segmental allotetraploid species of the *S. acaule* series (Irikura, 1976). According to the genome hypothesis of Matsubayashi (1991), strict allopolyploid species differ from one another by their second specific distinct component genomes B, C, P and D. The B component genome has been recognized in the allopolyploid species of the *Longipedicellata* series (AABB). Genome C has been recognized in the allotetraploid species of the *Conicibaccata* series ($A^cA^cC\text{`}C\text{`}$), genome P in the allotetraploid species of the *Piurana* series and D genomes in the allohexaploid species of the *Demissa* series ($AADDD\text{`}D\text{`}$) (Matsubayashi, 1991). A more complex genome composition has been proposed for allohexaploid species of the *Conicibaccata* and *Acaulia* series. It was suggested that *Solanum moscopanum* ($2n = 6x$) contains a genome of *Solanum colombianum* ($A^cA^cC\text{`}C\text{`}$) and an additional, distinct MM genome of unknown diploid species origin (Lopez and Hawkes, 1991). *Solanum albicans* contains a genome of *S. acaule* and an additional, distinct XX genome of unknown origin (Hawkes, 1963; Matsubayashi, 1991). Nuclear restriction fragment-length polymorphism (RFLP) data confirm that *S. acaule* (AAA^aA^a) is an ancestor of *S. albicans* (Nakagawa and Hosaka, 2002).

Hawkes (1990) hypothesized that the B genome was a 'primitive' indigenous genome from Mexico. Irikura (1976) considered *S. cardiophyllum* as a possible donor of the second merged B genome in natural allopolyploids (Irikura, 1976). However, no experimental evidence was provided. Today, most authors agree that the origin of the second component genomes of natural allopolyploids is still unknown. It is unlikely that all diploid progenitors of the A^a, B, C and D genomes disappeared. It is possible that the A^a, B and D genomes were derived from a common ancestor and were then modified during the speciation of allopolyploids. This assumption is supported by molecular data that cluster the A^a, B and D genome-containing species (Kardolus, 1998; Nakagawa and Hosaka, 2002). The meiotic behaviour in hybrids also indicates similarity between the A^a, one of the D genomes and the B genomes. For instance, the high frequency of bivalents (5.3 univalents + 24.4 bivalents + 0.7 trivalents + 0.9 quadrivalents; Bains, 1951) in a pentaploid hybrid (AAA^aDD^d) of *S. demissum* ($AADDD^dD^d$) with *S. acaule* (AAA^aA^a) indicates that parental species share two common genomes. Meiotic configurations (15–17 univalents + 20–21 bivalents + 1 trivalent) in pentaploid hybrids ($AABDD^d$) of *S. demissum* ($AADDD^dD^d$) and *S. stoloniferum* (AABB) mean that bivalents are formed between the two A genomes and that most chromosomes of the B genome and one of the D genomes are paired. To reflect the close relationships between *S. demissum* and members of the *Acaulia* and *Longipedicellata* series, Kardolus (1998) proposed the new genome formula $AAA^aA^aB^dB^d$ for *S. demissum*.

During the evolution of natural allopolyploids, the second component genome could be significantly modified compared with the original ancestral genome donor. The hypothesis of Zohary and Feldman (1962) suggested different rates of parental genome modification in allopolyploid species. According to this hypothesis, one subgenome of natural allopolyploids remains stable and very close to the ancestral genome, whereas the second subgenome is modified relative to its progenitor because of introgressive hybridization. It might be suggested that in potato allopolyploids the A subgenome is stable and the second component genome was significantly modified. For instance, hybrids (genome AAA^aB) between *S. acaule* and species of the *Longipedicellata* series are characterized by a high multivalent frequency (0.8–1.3 quadrivalents + 2.2–3.4 trivalents + 14.2–15.8

bivalents + 6.1–4.5 univalents; Matsubayashi, 1991) that probably could reflect structural chromosomal changes accumulated in the A^a and B subgenomes.

It should be mentioned that some cytogenetic studies lack important information either due to limitations associated with the use of single genotype crosses, a single hybrid clone and a single accession of a polyploid species or due to an insufficient number of meiotic cells analysed. Chromosomal configurations were analysed at MI, whereas a true reflection of pairing has to be observed at the pachytene or zygotene stages. Meiotic studies have been performed by conventional methods with limited power to definitely interpret genome affinity in allopolyploids due to the inability to distinguish intergenomic and intragenomic pairing. Besides, the type of meiotic configurations (bivalents, trivalents or quadrivalents) alone is not a sufficient indicator for determining the nature of polyploidy. Predominantly, bivalent chromosome pairing has been described for several autopolyploid species with tetrasomic inheritance (Crawford and Smith, 1984; Samuel et al., 1990). In such cases, natural pressure for high fertility could select mutations in pairing control genes and result in change from random to preferential pairing in autopolyploids. Studies of inheritance patterns of molecular markers would provide more information about the polysomic or disomic inheritance type of polyploids. Obviously, the existing genome concepts of polyploid species of the section *Petota* need to be developed by further studies.

10.3.3 Genomic designation and relationships of potato and non-tuber-bearing species from closely related sections *Etuberosum, Lycopersicum* and *Juglandifolium*

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All species of the section *Petota* and the closest non-tuber-bearing relatives from sections *Etuberosum, Juglandifolium* and *Lycopersicum* (Spooner et al., 1993) have the same basic chromosome number (x = 12) and similar karyotype morphology. Genome symbol E was given to the species of the section *Etuberosum* based on the specificity of meiotic behaviour and sterility of their diploid hybrids with A-genome tuber-bearing potato species (Ramanna and Hermsen, 1979, 1981). The distinct genome symbol S has been postulated for *Solanum sitiens* and *Solanum lycopersicoides* of the section *Juglandifolium* based on the differences detected among genetic maps of these species and tomato (Pertuze et al., 2002). Symbol L was proposed for tomato (section *Lycopersicum*) on the basis of preferential chromosome pairing and clear-cut parental genome discrimination by using genomic in situ hybridization (GISH) in amphidiploids of the LLEE type between tomato and *Solanum etuberosum* (Gavrilenko et al., 2001).

The results of comparative mapping studies revealed a high level of conservation of most linkage groups of the A, L, S and E genomes as well as genetically detected inversions, translocations and transpositions (Tanksley et al., 1992; Perez et al., 1999; Pertuze et al., 2002).

These results indicate that S- and L-genome species are most closely related and characterized by the lowest genome differentiation. Differentiation between L and A genomes is more profound, and the E genome is the most divergent within these taxa indicating distinctiveness of the section *Etuberosum*.

10.4 KARYOTYPING OF POTATO SPECIES

Potato is not an ideal species for cytogenetic research. Small somatic metaphase chromosomes of *S. tuberosum* ranging in length from 1.0 to 3.5 μm (Dong et al., 2000) are critical for identification. Low level of karyotype divergence among potato species as well as of those from the closely related sections complicates the application of traditional cytogenetic approaches to the analysis of introgression. Another disadvantage of cytogenetic research in potato is the absence of aneuploid stocks such as monosomic and nullisomic lines and lack of well-characterized structural chromosome mutants with translocations, inversions or deletions, which are routinely employed in other species for assigning linkage groups to individual chromosomes or for locating genes on specific chromosomes.

The first attempts to identify specific somatic chromosomes of potato stained with DNA-binding dyes such as aceto-carmine were based on the analysis of chromosome length, centromere position and the presence of secondary constrictions (Shepeleva, 1937; Lamm, 1945; Swaminathan, 1954). However, the small size and slight differences in morphology did not allow to distinguish precisely specific metaphase chromosomes. The distribution of highly repetitive DNA sequence on potato chromosomes was studied using Giemsa C-banding techniques with the aim to distinguish specific chromosomes (Mok et al., 1974; Lee and Hanneman, 1976; Pijnacker and Ferwerda, 1984). Even though significant progress has been made in the identification of Giemsa-stained chromosomes, difficulties persisted in the discrimination among chromosomes with similar morphology and similar C-banding patterns.

The pachytene chromosome complement was described for several diploid species and dihaploid clones of common potato (Haynes, 1964; Yeh and Peloquin, 1965; Marks, 1969; Ramanna and Wagenvoort, 1976; Wagenvoort, 1988). Potato chromosomes at pachytene show dark staining heterochromatin in pericentromeric regions and light staining euchromatin in terminal regions. These staining patterns together with other chromosomal landmarks such as position of centromeres, heterochromatin knobs and the size of telomeres allow to distinguish each of the 12 potato chromosomes. However, wide application of pachytene karyotyping was limited in cytogenetic research of potato because this method is elaborate and time consuming, and it can be applied only to diploid clones with excellent quality of chromosomal preparations.

10.4.1 Fluorescent in situ hybridization-based cytogenetic mapping

Development of fluorescent in situ hybridization (FISH) techniques for plant species provided new opportunities for the characterization of the potato genome, including chromosome identification and analysis of genome structure. The use of FISH with genomic DNA cloned in large-insert vectors such as bacterial artificial chromosomes (BACs), called BAC-FISH, has been an effective approach in mapping small probes containing only a few kilobases of DNA to physical chromosomes (Jiang et al., 1995). This approach has been used by Jiang and colleagues for correlating specific chromosomes with molecular linkage groups of potato. BACs with large genomic DNA insertions of the wild diploid species *Solanum bulbocastanum* were screened using mapped RFLP

markers (Song et al., 2000). RFLP marker-specific BAC clones were labelled as FISH probes that were successfully applied to identify each of the 12 somatic metaphase chromosomes of potato (Dong et al., 2000; Fig. 10.1A). As a result, a larger set of new, chromosome-specific cytogenetic DNA markers (CSCDMs) was established for potato karyotyping to integrate the genetic and cytological maps of potato. This system has the following methodical advantages: CSCDMs clearly discriminate between different chromosomes with similar morphology, CSCDMs can be applied to polyploids with larger

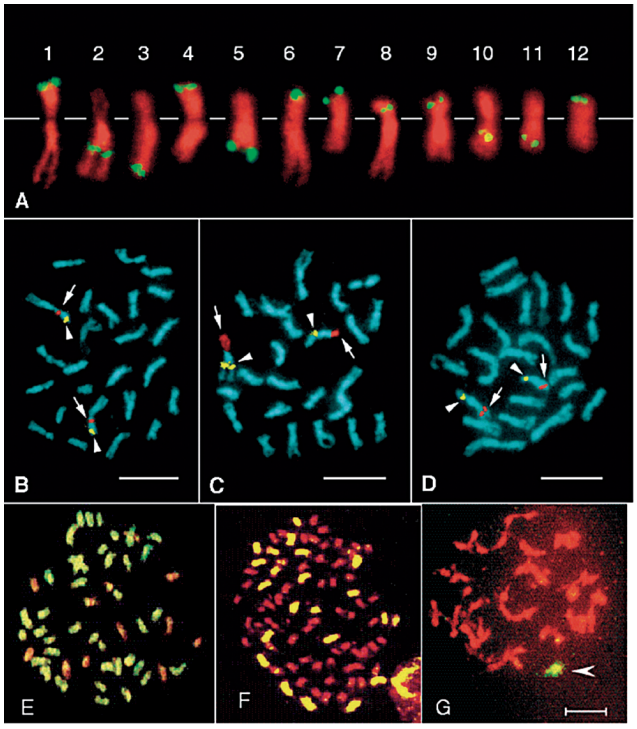

Fig. 10.1. (A) Twelve individual potato chromosomes with fluorescent in situ hybridization (FISH) signals derived from the chromosome-specific cytogenetic DNA markers (CSCDMs). (B) The 5S rRNA genes (red colour and arrows) are located near the centromeres at the same chromosome as chromosome 1-specific DNA marker (yellow colour and arrowheads). (C) The 45S rRNA genes (red colour and arrows) were mapped to the distal region on the short arm of the same chromosome where chromosome 2-specific DNA marker (yellow colour and arrowheads) was located. (D) Bacterial artificial chromosome (BAC) clone, 32A07, which is linked to a potato late blight resistance gene (red colour and arrows), was mapped to the long arm of the same chromosome where the chromosome 8-specific marker (yellow colour and arrowheads) was located. (A–D: from Dong et al., 2000, with kind permission of Springer Science and Business Media.) (E) Genomic in situ hybridization (GISH) of mitotic cells of BC_2 hybrid with 39 chromosomes of potato (yellow colour) and 12 chromosomes of *Solanum etuberosum* (red colour) (Gavrilenko et al., 2003). (F) Hybrid derived from *Solanum nigrum* (+) potato backcross programme with 22 chromosomes of *S. nigrum* (yellow colour) and 36 chromosomes of potato (red colour) (Horsman et al., 2001). (G) Diakinesis stage in the monosomic addition for chromosome 8 of tomato into the potato genome, showing the alien chromosome as a univalent (arrowhead) (Garriga-Calderé et al., 1999). All bars are 10 μm.

chromosome numbers and the quality of chromosome preparations is not so important (Dong et al., 2000).

Visser et al. (1988) were the first to apply in situ hybridization techniques using radioactively labelled repetitive DNA sequences to study genome organization of potato. In further studies, FISH has been used to characterize the distribution of different types of repetitive sequences. Simultaneous hybridization of ribosomal DNA (rDNA) probes with CSCDMs, each labelled with a different fluorochrome, has resulted in mapping two large functionally important families of rDNA sequences of potato (Dong et al., 2000). 5S rDNA genes were located at a single locus near the centromere on the short arm of chromosome 1 (Dong et al., 2000; Fig. 10.1B). A similar location of a single 5S rDNA locus has been detected in tomato using FISH and pachytene analysis (Xu and Earle, 1996a). Only one 5S rDNA locus was found in the S-genome species of the section *Juglandifolium* (Ji et al., 2004). Therefore, no polymorphisms were detected in the number of 5S rDNA loci among the A, L and S genomes.

One major 45S rDNA locus containing 18S, 5, 8S and 26S rRNA genes was found in the nucleolus organizer region (NOR) on the short arm of chromosome 2 in the A, L and S genomes (Fig. 10.1C). Variation in a genome-specific manner was only detected in the number and distribution patterns of minor 45S rDNA loci. Pachytene karyotyping of tomato in combination with FISH revealed four minor 45S rDNA loci that were located in the heterochromatic regions on four chromosomes of the L genome (2L, 6L, 9S and 11S arms) (Xu and Earle, 1996b). In the chromosome complements of the S-genome species, only one minor 45S rDNA locus was detected on chromosomes other than the nucleolar chromosome (Ji et al., 2004). No minor 45S rDNA loci have been reported for the A genome of potato (Dong et al., 2000).

Using FISH, tandemly repeated DNA elements that are highly homologous to the intergenic spacer (IGS) of the 18S–25S rDNA sequence of potato were located at distinct loci in a pericentromeric heterochromatic region on a single (not nucleolar) chromosome of *S. tuberosum* (Stupar et al., 2002). In *S. bulbocastanum*, the same repeated DNA elements were located close to centromeres and distributed on four different chromosomes (Stupar et al., 2002). The other classes of tandem repeats – interstitial telomeric repeats (ITRs) – have been located using FISH in highly condensed centromeric regions of two to seven different chromosomes in several *Solanum* species, and the number of the FISH signals did not correspond to species ploidy level (Tek and Jiang, 2004). The results of FISH on extended DNA fibres revealed that these ITRs are organized in long tandem clusters, suggesting extensive amplification of the ITRs during divergence of potato species (Tek and Jiang, 2004). Both IGS-related repeats and ITRs are highly diverged among a wide range of *Solanum* species indicating their dynamic nature (Stupar et al., 2002; Tek and Jiang, 2004). These results indicate that genome differentiation of the structurally similar, A-genome diploid potatoes might be due to divergence in nucleotide sequences and amplification of different classes of highly repetitive DNA.

Fluorescent in situ hybridization with tandemly repeated, species-specific DNA sequences can be used for comparative karyotyping and for studying introgression. For instance, the pSB1 and pSB7 repeats specific to the E-genome species of the *Etuberosum* section were located mostly in the telomeric and in some centromeric and interstitial areas of the *Solanum brevidens* chromosomes, but not in the *S. tuberosum* chromosomal

complement. Whereas the potato clone pST3 showed signals in telomeric regions of a few chromosomes of *S. tuberosum*, this signal was not detected in *S. brevidens* (Rokka et al., 1998a). Moreover, FISH with *S. brevidens*-specific sequences helped to clarify the genomic composition of hybrids between potato and *S. brevidens* (Rokka et al., 1998b).

Genomic in situ hybridization, based on the use of total genomic DNA as probe, has been developed by Schwarzacher et al. (1989) to identify chromosomes and chromosomal segments of different origin. The ability to discriminate chromatin of different genomes depends on the degree of sequence homology and stringency conditions in the GISH experiments. The standard GISH protocol allows to distinguish genomes sharing 80–85% or less sequence homology (Schwarzacher et al., 1989). Using standard GISH protocols, parental chromosomes were discriminated in wide hybrids between distantly related *Solanum* species belonging to different sections, such as *Petota* (potato) and *Lycopersicum* (tomato) (Garriga-Calderé et al., 1997), *Petota* (potato) and *Etuberosum* (*S. etuberosum* and *S. brevidens*) (Dong et al., 1999, 2001; Gavrilenko et al., 2002, 2003), *Petota* (potato) and *Solanum (Solanum nigrum)* (Horsman et al., 2001), *Etuberosum (S. etuberosum)* and *Lycopersicum* (tomato) (Gavrilenko et al., 2001), *Juglandifolium (S. lycopersicoides* and *S. sitiens*) and *Lycopersicum* (tomato) (Ji et al., 2004). Because the A, E, L and S genomes in wide hybrids can be easily discriminated using standard GISH protocols, these genomes are supposed to have a high level of divergence in their dispersed repetitive DNA sequences. Chromosomes of closely related genomes sharing up to 90–95% sequence homology can be discriminated under higher stringency conditions in combination with an excess of unlabelled blocking DNA in the hybridization mixture (Parokonny et al., 1997). Application of such modified GISH protocols allowed to discriminate chromosomes of closely related parental species belonging to the same section – *Lycopersicum* (Parokonny et al., 1997) or *Juglandifolium* (Ji et al., 2004).

Genomic in situ hybridization was successfully used to establish genome composition of wide hybrids and their derivatives (Fig. 10.1E and F), to discriminate between intergenomic and intragenomic pairing in the genomes of wide hybrids (Garriga-Calderé et al., 1999; Gavrilenko et al., 2001; Ji et al., 2004), to study the specificity of genome interactions such as preferential elimination of chromosomes of one parental genome (Garriga-Calderé et al., 1997; Gavrilenko et al., 2001) and to determine intergenomic translocations (Garriga-Calderé et al., 1997; Dong et al., 2001).

Despite the effectiveness of GISH in detecting chromatin of different origin, GISH alone cannot determine genetic identity of alien chromosomes. Sequential GISH and FISH with CSCDMs performed on the same chromosome preparations made it possible to identify precisely specific homologous chromosomes of the E and A genomes in breeding lines derived from potato (+) *S. brevidens* hybrids (Dong et al., 2001, 2005; Tek et al., 2004). Combination of GISH and FISH with CSCDMs also allowed to determine the specificity of chromosomal re-arrangements (Dong et al., 2001).

10.5 CYTOGENETICS IN POTATO IMPROVEMENT

Wild potato species have been recognized as an important source of useful genes for resistance to pathogens and abiotic stresses (Hawkes, 1994). These gene pools are useful

for the improvement of common potato that has a narrow genetic basis as many other crop species (Ross, 1986). Wild germplasm has been actively utilized in potato breeding for at least 70 years (Bukasov, 1937). Following interspecific crosses and backcrossing, all 11 known *R* genes conferring race-specific resistance to late blight have been introduced into potato varieties from *S. demissum* ($AADDD^dD^d$) (Umareus and Umareus, 1994). The virus resistance genes *Ry, Ra, Na* and *Rx2* have been introgressed into potato from *S. stoloniferum* (AABB) and *S. acaule* (AAA^aA^a), respectively (Solomon-Blackburn and Barker, 2001). Methods used for ploidy manipulation (Hougas and Peloquin, 1958) make most of the potato species with different EBNs cross-compatible with *S. tuberosum*. However, some potentially useful species, e.g. A'A' genome-containing diploid Mexican species or E'E' genome-containing species, cannot be hybridized easily because of the crossing barriers (Hermsen, 1994). The range of hybridization has been broadened using biotechnological methods that allowed to bring into breeding programmes new species such as *S. bulbocastanum, Solanum tarnii, S. etuberosum, S. brevidens* and *S. nigrum*. Following protoplast fusion, backcrossing and embryo or ovule rescue, fertile progenies derived from crosses of wide somatic hybrids with common potato have been produced. Some of these derivatives showed high levels of resistance to diseases. The list includes broad-spectrum resistance to late blight from *S. bulbocastanum* (A^bA^b) (Helgeson et al., 1998; Naess et al., 2000), resistance to tuber soft rot from *S. brevidens* (E^bE^b) (Tek et al., 2004) and resistance to viruses and aphids from *S. etuberosum* (E^eE^e) (Novy et al., 2002; Gavrilenko et al., 2003).

The most recent achievements in detecting introgression are discussed here briefly. Molecular markers and in situ hybridization techniques have been essential for detecting genetic material of wild species at the level of whole chromosomes, chromosomal segments and individual genes. These methods were useful for the development and characterization of heteromorphic aneuploid lines derived from crosses between distantly related taxa. For instance, an entire series of monosomic alien addition lines (MAALs) and two disomic addition lines for tomato chromosomes 10 and 11 ($AAAA + L^{10}$ and $AAAA + L^{11}$) into potato have been established using RFLP and GISH (Garriga-Calderé et al., 1998; Haider Ali et al., 2001; Fig. 10.1G). The application of sequential GISH and FISH with CSCDMs allowed to distinguish addition and substitution lines (Dong et al., 2005). Seven of 12 possible MAALs ($AAAA + E^b$) and one monosomic substitution for chromosome 6 of the E^b genome of *S. brevidens* have been extracted from BC_2 to BC_3 progenies derived from potato (+) *S. brevidens* hybrids (Dong et al., 2005). Importantly, the experiments provided the first evidence for the ability of chromosomes of the two distinct genomes (A and E) to substitute for each other. For practical purposes, these cytogenetic stocks can be useful for assigning unmapped gene(s) to chromosomes. Intergenomic translocations have been identified by using in situ hybridization methods in breeding lines originated from fusion hybrids of potato with tomato (Garriga-Calderé et al., 1997, 1999) and potato with *S. brevidens* (Dong et al., 2001). It must be pointed out that alien chromosome(s) or large alien translocated segments may not be stable when transmitted through backcrossing. Stable introgression can be achieved through crossing over. Following crossing of MAALs or substitution lines with common potato, it might be possible to select genotypes carrying chromosomes that originated because of homologous recombination. However, selection of genotypes with recombinant chromosomes

can be very laborious because of extremely low level of chromosome pairing between the parental genomes A and L (Garriga-Calderé et al., 1999) and limited level of crossing over between A and E genomes (McGrath et al., 1996).

In recent years, new approaches based on molecular markers and genomics have been developed to overcome such limitations. Cloned resistance genes of wild species can be transferred through genetic engineering in susceptible varieties by passing the crossing barriers. Already durable and broad-spectrum resistance against all known races of the late blight pathogen *Phytophthora infestans* has been introgressed from *S. bulbocastanum* into potato by somatic hybridization and subsequent backcrossing (Helgeson et al., 1998; Naess et al., 2001). The major late blight resistance gene *RB* of *S. bulbocastanum* was physically mapped by FISH on potato chromosome VIII (Dong et al., 2000; Fig. 10.1D). *RB* was then cloned using a map-based approach and transformed into susceptible potato varieties (Song et al., 2003).

In conclusion, the introduction of in situ hybridization methods has promoted a significant progress in potato cytogenetics, which has led to the integration of genetic and cytological maps, getting new information about genome structure and detecting introgressions with higher precision. Furthermore, the development and use of molecular techniques will be of great help in better understanding genome evolution and polyploid formation, further development of genetic and physical mapping of genes controlling economically important traits in potato and providing new knowledge about their genetic basis.

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