Allopolyploid speciation of the Mexican tetraploid potato species *Solanum stoloniferum* and *S. hjertingii* revealed by genomic in situ hybridization

Galina Pendinen, Tatjana Gavrilenko, Jiming Jiang, and David M. Spooner

Abstract: Thirty-six percent of the wild potato (*Solanum* L. section *Petota* Dumort.) species are polyploid, and about half of the polyploids are tetraploid species ($2n = 4x = 48$). Determination of the type of polyploidy and development of the genome concept for members of section *Petota* traditionally has been based on the analysis of chromosome pairing in species and their hybrids and, most recently, DNA sequence phylogenetics. Based on these data, the genome designation AABB was proposed for Mexican tetraploid species of series *Longipedicellata* Buk. We investigated this hypothesis with genomic in situ hybridization (GISH) for both representatives of the series, *S. stoloniferum* Schltdl. and *S. hjertingii* Hawkes. GISH analysis supports an AABB genome constitution for these species, with *S. verrucosum* Schltdl. (or its progenitor) supported as the A genome donor and another North or Central American diploid species (*S. cardiophyllum* Lindl., *S. ehrenbergii* (Bitter) Rydb., or *S. jamesii* Torrey) as the B genome donor. GISH analysis of chromosome pairing of *S. stoloniferum* also confirms the strict allopolyploid nature of this species. In addition, fluorescence in situ hybridization data suggest that 45S rDNA regions of the two genomes of *S. stoloniferum* were changed during coevolution of A and B genomes of this allotetraploid species.

Key words: allopolyploid, FISH, GISH, potato, section *Petota*.

Résumé : Trente-six pour cent des espèces sauvages de pommes de terre (*Solanum* L. section *Petota* Dumort.) sont polyploïdes et environ la moitié de celles-ci sont tétraploïdes ($2n = 4x = 48$). La détermination du niveau de ploïdie et le développement du concept du génome chez les membres de la section *Petota* s'est traditionnellement appuyée sur l'analyse de l'appariement chromosomique chez les espèces et leurs hybrides ainsi que plus récemment sur la phylogénie des séquences d'ADN. Sur la base de ces données, une formule génomique AABB a été proposée pour les espèces tétraploïdes mexicaines de la série *Longipedicellata* Buk. Les auteurs ont examiné cette hypothèse à l'aide de l'hybridation génomique in situ (GISH) pour deux espèces représentatives de la série, *S. stoloniferum* Schltdl. et *S. hjertingii* Hawkes. L'analyse GISH vient appuyer l'hypothèse d'une constitution génomique AABB chez ces espèces et le *S. verrucosum* Schhtdl. (ou son ancêtre) serait la source du génome A tandis que le génome B aurait été contribué par d'autres espèces diploïdes de l'Amérique centrale ou du nord comme le *S. cardiophyllum* Lindl., le *S. ehrenbergii* (Bitter) Rydb. ou le *S. jamesii* Torrey. L'analyse GISH de l'appariement chromosomique chez le *S. stoloniferum* confirme également la nature strictement allopolyploïde de cette espèce. De plus, les résultats d'une analyse par hybridation in situ en fluorescence suggèrent que les régions d'ADNr 45S des deux génomes du *S. stoloniferum* auraient été modifiés au cours de la co-évolution des génomes A et B chez cette espèce allotétraploïde.

Mots-clés : allopolyploïde, FISH, GISH, pomme de terre, section *Petota*.

[Traduit par la Rédaction]

Introduction

Potatoes are members of *Solanum* L. section *Petota* Dumort., which contains approximately 190 wild species (Spooner and Salas 2006) and 4 cultivated species (Spooner et al. 2007). Within the section, 36% of species are polyploids and 64% are diploids ($2n = 2x = 24$) (Hijmans et al. 2007). Determination of the type of polyploidy and develop-

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G. Pendinen and T. Gavrilenko. N.I. Vavilov Institute of Plant Industry, Bolshaya Morskaya Street, 42-44, St. Petersburg, 190000, Russia.
J. Jiang. Department of Horticulture, University of Wisconsin, 1575 Linden Drive, Madison, WI 53706-1590, USA.
D.M. Spooner.[1] USDA, Agricultural Research Service, Department of Horticulture, University of Wisconsin, 1575 Linden Drive, Madison, WI 53706-1590, USA.

[1]Corresponding author (e-mail: david.spooner@ars.usda.gov).

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ment of the genome concept for species of section *Petota* traditionally has been based mainly on the analysis of chromosome pairing in species and their hybrids (Marks 1955, 1965; Hawkes 1958; Irikura 1976; Ramanna and Hermsen 1981; López and Hawkes 1991; Matsubayashi 1991; Gavrilenko 2007). Chromosome pairing relationships in interspecific hybrids and in polyploid species have been interpreted by genome formulae, although authors have used different genome symbols.

Wild polyploid species of series *Longipedicellata* Buk. are considered to be strict allopolyploids (AABB genome) based on meiotic analyses (Wangenheim 1955; Irikura 1976; Matsubayashi 1991). The diploid species *S. verrucosum* Schltdl. was suggested as the putative contributor of the A genome (Marks 1965; Irikura 1976), a result supported by plastid DNA phylogenies (Spooner and Sytsma 1992) and by geographical and morphological data (Spooner et al. 2000).

The B genome donor of the polyploids of series *Longipedicellata* is still unknown. Hawkes (1994) hypothesized that the B genome was an ancestral "primitive" indigenous genome from Mexico, and Irikura (1976) considered *S. cardiophyllum* Lindl. as a possible donor of the B genome in Mexican tetraploids. However, no experimental evidence about the B genome progenitor was provided by classical genome analysis. The DNA sequence results of Spooner et al. (2008) supported an allopolyploid origin of members of series *Longipedicellata*, from the North and Central American diploid species *S. verrucosum* and any one of the 13 remaining North and Central American diploid species.

This is the first study applying GISH analysis to investigate genome origins of natural polyploids within section *Petota*. GISH analysis has been used to investigate somatic hybrids between potato (section *Petota*) and members of other sections of *Solanum* (Dong et al. 1999; Gavrilenko 2007). The objective of our research is to study the polyploid origin of Mexican tetraploid species of series *Longipedicellata* (*S. stoloniferum* Schltdl. and *S. hjertingii* Hawkes) and to test the evolutionary relationships of these taxa relative to representative Mexican or Central American diploids. We chose the diploid species *S. verrucosum* as a putative A genome progenitor and *S. cardiophyllum*, *S. ehrenbergii* (Bitter) Rydb., and *S. jamesii* Torrey as putative B genome progenitors for *S. stoloniferum* and *S. hjertingii*, based on prior hypotheses of classical genome analyses (Marks 1965; Irikura 1976) and the nuclear DNA sequence phylogeny of Spooner et al. (2008), which showed these species to represent a sample of the diversity in these clades.

Materials and methods

Plant materials

Four accessions of *S. stoloniferum* (PI 186544, PI 497998, PI 255546, PI 251740) and one accession of *S. hjertingii* (PI 545713) were used in GISH analysis. *Solanum stoloniferum* is much more widespread than *S. hjertingii*, so we evaluated more accessions of this species. Both species are tetraploid ($2n = 4x = 48$). The diploid species *Solanum verrucosum* ($2n = 2x = 24$) (PI 545745) was used as a putative A genome progenitor species. Putative B genome progenitor species included *S. cardiophyllum* (PI 595465), *S. ehrenbergii* (PI 275216), and *S. jamesii* (PI 458424); these three species are diploid ($2n = 2x = 24$). Spooner et al. (2004) recognized 13 species of Mexican diploids and these were chosen as random representatives of this group. We also included in GISH *S. andreanum* Baker (PI 320345) and *S. piurae* Bitter (PI 310997), which are also diploid ($2n = 2x = 24$). We chose these species because they were shown to be members of the distinctive *Piurana* clade in the phylogenetic study of Spooner et al. (2008).

GISH and FISH

Slide preparation

Roots were collected from greenhouse-grown plants and pretreated in 2 mmol/L 8-hydroxyquinoline at 20 °C for 3 h. Root tips and flower buds were fixed in a solution of 100% ethanol and glacial acetic acid (3:1) and stored in a freezer (–20 °C) until use.

Root tips were digested with 4% cellulase and 1% pectinase at 37 °C for 80 min. The macerated root tips were suspended by forceps in a drop of 45% acetic acid and squashed. Slides were pretreated in pepsin solution (final concentration 0.1 mg/mL) for 45 min at 37 °C and subsequently incubated in RNase A solution (6 μL of 10 μg/μL stock solution + 24 μL of 2× SSC per slide, 40 min at 37 °C) and then formaldehyde solution (4% for 10 min). After each treatment slides were washed 3 times in 2× SSC buffer for 5 min at room temperature. Finally slides were incubated in a 70%, 90%, 100% ethanol series, for 3 min each at room temperature.

DNA extraction and labeling

Genomic DNA was isolated from the putative diploid A and B genome progenitor species using young leaves of greenhouse-grown plants. The GISH technique was performed according to published protocols (Leitch et al. 1994; Schrader et al. 2000; Dong et al. 2001) with minor modifications. DNA was labeled with either digoxigenin-UTP or biotin-UTP by nick translation (DIG- and Biotin-Nick Translation Mix, Roche Applied Science, cat. Nos. 11745816910 and 11745824910).

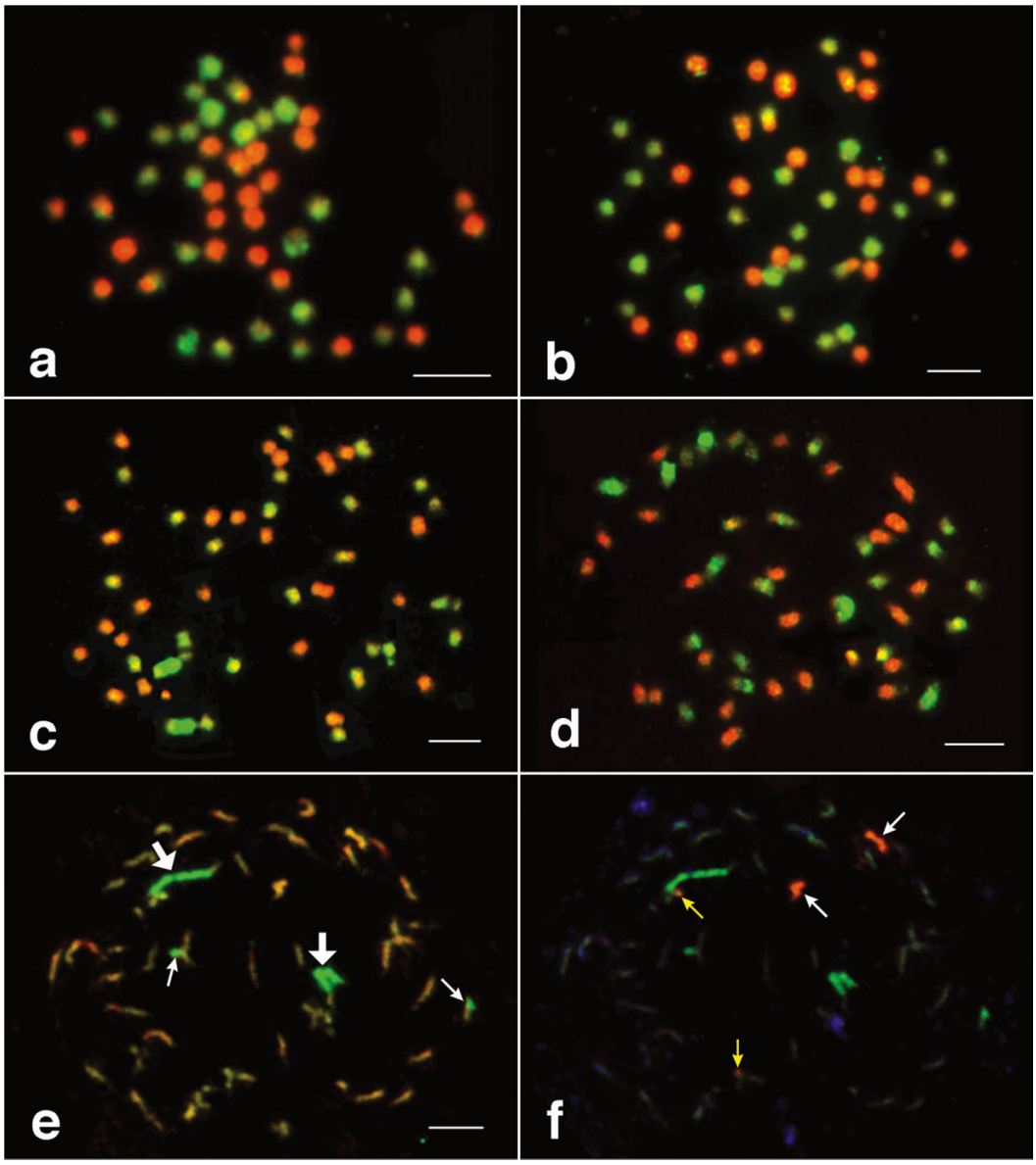
Hybridization

The hybridization mix (40 μL per slide) for GISH was prepared with differentially labeled DNA from the putative parental species (species 1 and 2) and included sheared fish sperm DNA (20 μg), probe DNA of species 1 (100 ng), probe DNA of species 2 (100 ng), 10% dextran sulfate, and deionized formamide (50%). Hybridization was performed overnight at 37 °C. FISH was performed using rDNA probes followed Dong et al. (2000). Denaturation of probes and slides was performed as described by Schrader et al. (2000). Stringent washing was performed as follows: 3 times in 2× SSC for 5 min each at 37 °C, 3 times in 0.1× SSC for 5 min each at 42 °C, and 2 times in 2× SSC for 5 min each at room temperature.

Detection

DIG-labeled DNA was detected with rhodamine anti-DIG conjugate and biotin-labeled probes were detected with FITC-conjugated avidin (Roche Applied Science, cat. Nos. 11207750910 and 11975595910). After addition

Fig. 1. GISH analysis of tetraploid ($2n = 4x = 48$) *Solanum stoloniferum* and *S. hjertingii* using diploid ($2n = 2x = 24$) putative A genome (*S. verrucosum*) and B genome (*S. cardiophyllum*, *S. ehrenbergii*, *S. jamesii*) donor species. (*a*) Somatic chromosomes of *S. stoloniferum* probed with labeled DNA from *S. verrucosum* (red) and *S. jamesii* (green). (*b*) Somatic chromosomes of *S. stoloniferum* probed with labeled DNA from *S. verrucosum* (red) and *S. ehrenbergii* (green). (*c*) Somatic chromosomes of *S. stoloniferum* probed with labeled DNA from *S. verrucosum* (red) and *S. cardiophyllum* (green). (*d*) Somatic chromosomes of *S. hjertingii* probed with labeled DNA from *S. verrucosum* (red) and *S. jamesii* (green). (*e*) Somatic chromosomes of *S. stoloniferum* probed with labeled DNA from *S. verrucosum* (red) and *S. andreanum* (green). A large fragment (big arrows) and a small fragment (small arrows) from two pairs of *S. stoloniferum* chromosomes showed bright green color. Color differentiation was not observed on the rest of the chromosomes. (*f*) The same metaphase cell in (*e*) was hybridized with a 45S ribosomal RNA gene probe (red). The two large (white arrows) and two small (yellow arrows) FISH sites are not located on the chromosomes with color differentiation in GISH analysis. Bars = 5 μm.



of 29 μL of blocking reagent (30 mg BSA in 999 μL 4× SSC), slides were incubated for 30 min at room temperature. The antibody solution, consisting of 1 μL of anti-DIG–rhodamine stock solution + 1 μL of avidin-fluorescein stock solution + 28 μL of detection buffer (0.1 g BSA dissolved in 9.9 mL 4× SSC, pH 7.4), was added to each slide and slides were incubated for 45 min at 37 °C. Slides were washed 3 times in 4× SSC (pH 7.4) for 5 min each at 42 °C. Chromosomes were counterstained by 4′,6-diamidino-2-phenylindole (DAPI) in Vectashield antifade solution (Vector Laboratories).

All images were captured digitally using a SenSys CCD (charge-coupled device) camera (Roper Scientific, Tucson, Arizona) attached to an Olympus BX60 epifluorescence microscope. The CCD camera was controlled using IPLab Spectrum version 3.1 software (Signal Analytics, Vienna, Virginia) on a Macintosh computer.

Results

GISH analysis of the mitotic chromosomes of Mexican tetraploid species

We probed mitotic chromosomes of the Mexican tetraploid species *S. stoloniferum* and *S. hjertingii* with differentially labeled DNA of their putative diploid progenitors in three series of GISH experiments: (1) *S. jamesii* (biotin) and *S. verrucosum* (DIG), (2) *S. cardiophyllum* (biotin) and *S. verrucosum* (DIG), and (3) *S. ehrenbergii* (biotin) and *S. verrucosum* (DIG).

With all three probe combinations, half of the 48 chromosomes from *S. stoloniferum* were clearly labeled red (due to the contribution by *S. verrucosum*) and half were labeled green (due to the contribution by *S. cardiophyllum*, *S. ehrenbergii*, or *S. jamesii*) (Figs. 1*a*–1*c*, Table 1). Similar GISH results were obtained from *S. hjertingii* (Fig. 1*d*). These results confirmed a significant differentiation between the genome from *S. verrucosum* and the genomes from the three putative B genome progenitor species. The GISH results also showed that *S. cardiophyllum*, *S. ehrenbergii*, *S. jamesii*, or a diploid species closely related to these three species contributed the B genome of the Mexican allotetraploid species.

We also used *S. andreanum* and *S. piurae* in GISH analysis. We labeled the *S. verrucosum* DNA in red and the DNA from *S. andreanum* and *S. piurae* in green. Most of the *S. stoloniferum* and *S. hjertingii* chromosomes were preferentially labeled in red in GISH. These results suggest that neither *S. andreanum* nor *S. piurae* is the B genome progenitor and *S. verrucosum* is genomically closer to the B genome chromosomes of the tetraploids than *S. andreanum* or *S. piurae*.

Only two pairs of chromosomes in *S. stoloniferum* (as well as in *S. hjertingii*) had segments that were labeled in green owing to hybridization to the *S. andreanum* probe. The preferential green labeling on the first pair spanned the majority of the chromosome, including the centromere. The green labeling on the second pair included a small section in the proximal region on the short arm (Fig. 1*e*). FISH analysis using a 45S ribosomal RNA gene probe showed that these two pairs of chromosomes were not associated with the 45S rDNA (Fig. 1*f*). These results show that *S. andreanum* and *S. piurae* are more distantly related to the B component genome of *S. stoloniferum* than are *S. cardiophyllum*, *S. ehrenbergii*, and *S. jamesii*.

GISH analysis of chromosome pairing of Mexican tetraploid species

Meiotic analysis showed that the 48 chromosomes of *S. stoloniferum* regularly pair as 24 bivalents at diakinesis (Table 2) without any meiotic irregularities. GISH analysis of meiotic chromosomes revealed only intragenomic chromosome pairing (Fig. 2*a*), i.e., no intergenomic pairing associations, in 29 GISH-analyzed meiotic cells, confirming the allopolyploid nature of this tetraploid species.

FISH analysis of the distribution of 45S and 5S rDNA sites in the genome of potato species

One 45S rDNA locus and one 5S rDNA locus were found in all diploid species (Figs. 2*b*, 2*c*). The 45S rDNA was lo-

Table 1. Summary of diploid and tetraploid species used in GISH analysis.

Species and accessions used for chromosome preparations	Diploid species used for GISH probe combinations	No. of GISH experiments	No. of slides analyzed	No. of cells analyzed[a]	GISH results summary
S. stoloniferum (PI 186544, PI 497998, PI 255546, PI 251740)	ver + jam	4	5	37	Complete color differentiation of the parental chromosomes: 24 red + 24 green
	ver + cph	5	7	44	
	ver + ehr	2	2	8	
S. hjertingii (PI 545713)	ver + jam	2	2	12	Complete color differentiation of the parental chromosomes: 24 red + 24 green
	ver + cph	2	2	12	
	ver + ehr	1	1	6	
S. stoloniferum (PI 255546)	ver + pur	1	1	7	Only 4 chromosome fragments from sto were color-differentiated with pur
S. stoloniferum (PI 251740, PI 255546, PI 497998, PI 186544)	ver + adr	4	5	37	Only 4 chromosome fragments from sto were color-differentiated with adr
S. hjertingii (PI 545713)	ver + adr	1	1	7	Only 4 chromosome fragments from hje were color-differentiated with adr
S. stoloniferum (PI 186544, PI 255546)	jam + adr	2	2	13	Color differentiation between parental chromosomes was not observed

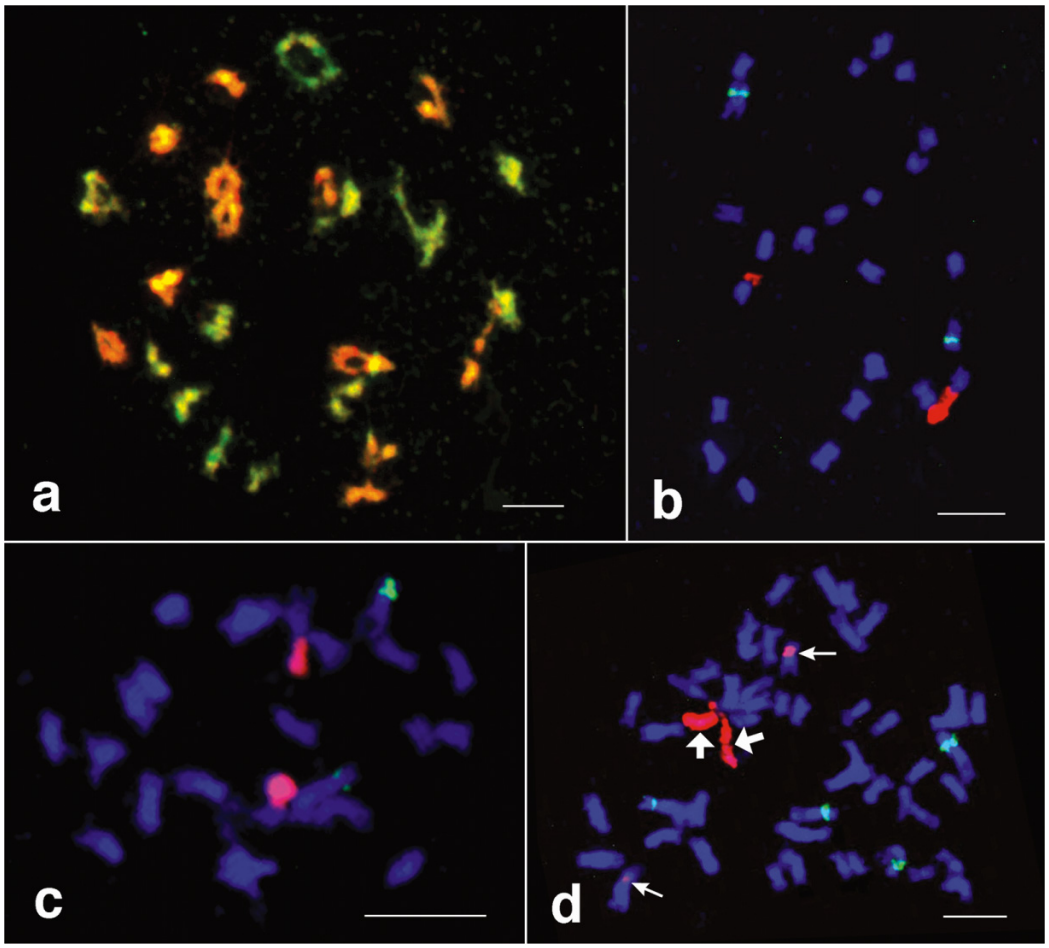
Note: Standard three-letter potato species codes are as follows: adr, *S. andreanum*; cph, *S. cardiophyllum*; ehr, *S. ehrenbergii*; hje, *S. hjertingii*; jam, *S. jamesii*; pur, *S. piurae*, sto, *S. stoloniferum*; ver, *S. verrucosum*.

[a]Cells with complete chromosome numbers, without overlapping, were included in analysis.

Table 2. Mean chromosome pairing per pollen mother cell (PMC) in *Solanum stoloniferum* at diakinesis, analyzed by GISH (hybridization with differentially labeled DNA of *S. verrucosum* and *S. jamesii*).

		No. and type of chromosomal associations per PMC			
Genotype	No. of PMCs analyzed	II, genome A	II, genome B	II, genome A + B	IV, genome A – B
Solanum stoloniferum (PI 497998)	29	12	12	0	0

Fig. 2. Chromosomal pairing and mapping of 45S rDNA in tetraploid ($2n = 4x = 48$) *S. stoloniferum* and its putative diploid ($2n = 2x = 24$) progenitor species, *S. jamesii* and *S. verrucosum*. (*a*) GISH analysis of chromosomal pairing of *S. stoloniferum* at diakinesis. Pairing between A genome chromosomes (red, labeled DNA from *S. verrucosum*) and B genome chromosomes (green, labeled DNA from *S. jamesii*) was not observed. (*b*) Detection of 45S rDNA (red) and 5S rDNA (green) in *S. verrucosum*. (*c*) Detection of 45S rDNA (red) and 5S rDNA (green) in *S. jamesii*. (*d*) Detection of 45S rDNA (red) and 5S rDNA (green) in *S. stoloniferum*. Two large (big arrows) and two small (small arrows) 45S rDNA hybridization sites were observed. Bars = 5 μm.



cated at the terminal region and the 5S rDNA at the proximal region of the short arm of the chromosomes. These locations are similar to those reported in *S. tuberosum* L. (Dong et al. 2000). We observed the expected two 45S rDNA loci and two 5S rDNA loci in the tetraploid species (Fig. 2*d*). The two 45S rDNA loci in *S. stoloniferum* showed dramatic size differences (Fig. 2*d*). In sequential GISH and FISH experiments it was shown that the large 45S rDNA locus was located on a pair of B genome chromosomes and the small locus on a pair of A genome chromosomes (data not shown). These results show that the 45S rDNA locus from the A genome has undergone significant rearrangement since the allopolyploidization event.

Discussion

Hawkes (1990) partitioned species of section *Petota* into 21 taxonomic series, but recent plastid DNA phylogenetic analyses (e.g., Spooner and Castillo 1997) divided section *Petota* into 4 clades. These 4 clades comprise (1) the North and Central American diploid species, exclusive of *S. bulbocastanum* Dunal, *S. cardiophyllum*, and *S. verrucosum*, (2) *S. bulbocastanum* and *S. cardiophyllum*, (3) all examined members of the South American series *Piurana* and some South American species classified to other series, such as *S. andreanum*; and (4) all remaining South American species as well as the North and Central American polyploid species

and *S. verrucosum*. A recent nuclear DNA sequence phylogenetic analysis (Spooner et al. 2008) yielded similar results, except (1) the species of plastid clades 1 and 2 are combined into a single clade, and (2) clades 1+2, 3, and 4 form a polytomy, not a defined sister group structure as with the plastid DNA phylogenies. Our present analysis supports an allopolyploid origin of members of series *Longipedicellata*, with the diploid species *S. verrucosum* in clade 4 as the A genome contributor and one of the remaining North and Central American diploid species in clade 1+2 as the B genome donor.

Here we provide the first demonstration of genome origins of natural potato polyploids (*S. hjertingii* and *S. stoloniferum*) with GISH. Our results are concordant with the results of Spooner et al. (2008) in showing clear discrimination of two divergent parental genomes (genomes A and B) matching those of extant diploids but not other genomes of more distantly related species such as *S. andreanum* and *S. piurae* (see below). Component genome A of tetraploid species is highly homologous to the genome of *S. verrucosum* and genome B is homologous to the genomes of the Mexican and Central American diploid species *S. cardiophyllum*, *S. ehrenbergii*, and *S. jamesii*, or a diploid species closely related to them. These latter three species, members of clade 1+2, are very similar and we can therefore confirm clade-specific progenitors but not species-specific progenitors. In addition, results of GISH analysis of meiotic preparations of *S. stoloniferum* confirm the strict allopolyploid nature of this species.

Matsubayashi (1991) designated diploid ($2n = 2x = 24$) Mexican or Central American species of Hawkes's (1990) series *Pinnatisecta* (*S. cardiophyllum*, *S. ehrenbergii*, *S. jamesii*) as possessing an $A^{pi}A^{pi}$ genome. Taking into account the significant differentiation between the genome of *S. verrucosum* and the genomes of the three Mexican diploid species (*S. cardiophyllum, S. ehrenbergii*, *S. jamesii*), from both our new results and the phylogenetic results of Spooner et al. (2008) we propose to change the genome symbols $A^{pi}A^{pi}$ (Matsubayashi 1991) for the diploid species *S. cardiophyllum*, *S. ehrenbergii*, and *S. jamesii* to BB.

We also used *S. andreanum* and *S. piurae* to test the clade 1 specific nature of *S. cardiophyllum*, *S. ehrenbergii*, and *S. jamesii*. Most of the *S. stoloniferum* and *S. hjertingii* chromosomes were preferentially labeled in red in GISH because of hybridization with DNA of *S. verrucosum*. A and B component genomes of these allotetraploid species are homoeologous because intergenomic pairing at meiosis is inhibited (probably because their chromosomes were structurally altered during evolution of *S. stoloniferum* and *S. hjertingii*). When the hybridization mixture contained the DNA of both diploid progenitors, differential labeling of two component genomes of *S. stoloniferum* (as well as *S. hjertingii*) was observed. In the absence of DNA of the B genome diploid species (*S. cardiophyllum*, *S. ehrenbergii*, *S. jamesii*) in the GISH hybridization mixture, *S. verrucosum* DNA could hybridize to both A and B genome chromosomes of *S. stoloniferum* (the same result occurred with *S. hjertingii*).

Only two pairs of chromosomes in both *S. stoloniferum* and *S. hjertingii* had segments that were labeled in green owing to hybridization to the *S. andreanum* probe. Our results indicate that genomes of the diploid South American species *S. andreanum* and *S. piurae* and genomes of the tetraploid Mexican species *S. stoloniferum* and *S. hjertingii* have homologous segments only on two chromosome pairs. The GISH results indicate that the genome of *S. andreanum* has diverged from both the A genome of *S. verrucosum* and the B genome of Mexican diploid species (*S. cardiophyllum*, *S. ehrenbergii*, *S. jamesii*). Partial homology of the two chromosome pairs of *S. stoloniferum* with the genomes of *S. andreanum* and *S. piurae* (and the two chromosome pairs of *S. hjertingii* with the genome of *S. andreanum*) suggests a possible introgression of genetic material of *S. andreanum* and *S. piurae* (or closely related species) into the genomes of these allotetraploid species. Our results also indicate that *S. andreanum* and *S. piurae* are more distantly related to the B genome of *S. stoloniferum* than are *S. cardiophyllum*, *S. ehrenbergii*, and *S. jamesii*. Further research is needed to show relationships and the origins of these four fragments relative to *S. andreanum*, *S. piurae*, or other species.

Here we also determined the number and location of 45S and 5S rDNA loci among Mexican and Central American tetraploid and diploid species. The number and location of rDNA loci in *S. stoloniferum* was as expected based on our results of FISH of four diploid species. The 5S rDNA pattern of *S. stoloniferum* was similar to those in the parental species. However, the 45S rDNA locus from the B genome chromosome in *S. stoloniferum* appeared to be significantly bigger than those in the parental species. The FISH patterns suggest that a nucleolar dominance event may have occurred after the polyploidization. Thus, only the 45S rRNA genes from the B genome are active in *S. stoloniferum*.

In summary, our results provide experimental evidence of the allopolyploid nature and speciation of the Mexican tetraploid species *S. stoloniferum* and *S. hjertingii*. Our continuing research is using GISH and FISH to test other genome origins outlined in Spooner et al. (2008). From a practical point of view, multiple disease resistances have been reported in germplasm containing *S. stoloniferum* (DeJong et al. 2001). *Solanum stoloniferum* has been extensively used in potato breeding because of viral resistances. For example, desirable genes from *S. stoloniferum* (Ry_{sto} and Na_{sto}, which confer resistance to potato viruses Y and A, respectively) have been transferred through sexual crosses into modern potato cultivars (Solomon-Blackburn and Barker 2001). Knowledge of the genome constitution of Mexican polyploids will clarify understanding of the inheritance of desirable genes in these wild species and help to plan further strategies for potato improvement.

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References

DeJong, H., Proudfoot, K.G., and Murphy, A.M. 2001. The germplasm release of F87084, a fertile, adapted clone with multiple disease resistances. Am. J. Potato Res. **78**: 141–149.

Dong, F., Novy, R.G., Helgeson, J.P., and Jiang, J. 1999. Cytological characterization of potato–*Solanum etuberosum* somatic hybrids and their backcross progenies by genomic in situ hybridization. Genome, **42**: 987–992. doi:10.1139/gen-42-5-987.

Dong, F., Song, J., Naess, S.K., Helgeson, J.P., Gebhardt, C., and Jiang, J. 2000. Development and applications of a set of chromosome-specific cytogenetic DNA markers in potato. Theor. Appl. Genet. **101**: 1001–1007. doi:10.1007/s001220051573.

Dong, F., McGrath, J.M., Helgeson, J.P., and Jiang, J. 2001. The genetic identity of alien chromosomes in potato breeding lines revealed by sequential GISH and FISH analyses using chromosome-specific cytogenetic DNA markers. Genome, **44**: 729–734. doi:10.1139/gen-44-4-729. PMID:11550910.

Gavrilenko, T. 2007. Potato cytogenetics. Ch. 10. *In* Potato biology and biotechnology: advances and perspectives. *Edited by* D. Vreugdenhil, J. Bradshaw, C. Gebhardt, F. Govers, D.K.L. MacKerron, M.A. Taylor, and H.A. Ross. Elsevier, Amsterdam. pp. 203–216.

Hawkes, J.G. 1958. Kartoffel. I. Taxonomy, cytology and crossability. *In* Handbuch Pflanzenzüchtung. Vol. III. *Edited by* H. Kappert and W. Rudorf. Paul Parey, Berlin and Hamburg. pp. 1–43.

Hawkes, J.G. 1990. The potato: evolution, biodiversity and genetic resources. Belhaven Press, Oxford.

Hawkes, J.G. 1994. Origins of cultivated potatoes and species relationships. *In* Potato genetics. *Edited by* J.E. Bradshaw and G.R. Mackay. CAB International, Wallingford, U.K. pp. 3–42.

Hijmans, R., Gavrilenko, T., Stephenson, S., Bamberg, J., Salas, A., and Spooner, D.M. 2007. Geographic and environmental range expansion through polyploidy in wild potatoes (*Solanum* section *Petota*). Glob. Ecol. Biogeogr. **16**: 485–495. doi:10.1111/j.1466-8238.2007.00308.x.

Irikura, Y. 1976. Cytogenetic studies on the haploid plants of tuber-bearing *Solanum* species. II. Cytogenetic investigations on haploid plants and interspecific hybrids by utilizing haploidy. Res. Bull. Hokkaido Natl. Agric. Exp. Stn. **115**: 1–80.

Leitch, A.R., Schwarzacher, T., Jacson, D., and Leitch, I.J. 1994. *In situ* hybridization: a practical guide. Royal Microscopical Society, Microscopy Handbooks. 27. BIOS Scientific Publishers, Oxford.

López, L.E., and Hawkes, J.G. 1991. Cytology and genome constitution of wild tuber-bearing *Solanum* species in the series *Conicibaccata. In* Solanaceae III: Taxonomy, chemistry, evolution. *Edited by* J.G. Hawkes, R.N. Lester, M. Nee, and R. Estrada. Royal Botanic Gardens, Kew, England. pp. 327–346.

Marks, G.E. 1955. Cytogenetic studies in tuberous *Solanum* species. I. Genomic differentiation in the group *Demissa*. J. Genet. **53**: 262–269.

Marks, G.E. 1965. Cytogenetic studies in tuberous *Solanum* species. III. Species relationships in some South and Central American species. New Phytol. **64**: 293–306. doi:10.1111/j.1469-8137.1965.tb05398.x.

Matsubayashi, M. 1991. Phylogenetic relationships in the potato and its related species. *In* Chromosome engineering in plants: genetics, breeding, evolution. Part B. *Edited by* T. Tsuchiya and P.K. Gupta. Elsevier, Amsterdam. pp. 93–118.

Ramanna, M.S., and Hermsen, J.G.Th. 1981. Structural hybridity in the series *Etuberosa* of the genus *Solanum* and its bearing on crossability. Euphytica, **30**: 15–31. doi:10.1007/BF00033655.

Schrader, O., Budahn, H., and Ahne, R. 2000. Detection of 5S and 25S rDNA genes in *Sinapis alba*, *Raphanus sativus* and *Brassica napus* by double fluorescence *in situ* hybridization. Theor. Appl. Genet. **100**: 665–669. doi:10.1007/s001220051337.

Solomon-Blackburn, R., and Barker, H. 2001. A review of host major-gene resistance to potato viruses X, Y, A and V in potato: genes, genetics and mapped locations. Heredity, **86**: 8–16. doi:10.1046/j.1365-2540.2001.00798.x. PMID:11298811.

Spooner, D.M., and Castillo, R. 1997. Reexamination of series relationships of South American wild potatoes (Solanaceae: *Solanum* sect. *Petota*): evidence from chloroplast DNA restriction site variation. Am. J. Bot. **84**: 671–685. doi:10.2307/2445904.

Spooner, D.M., and Salas, A. 2006. Structure, biosystematics, and genetic resources. *In* Handbook of potato production, improvement, and post-harvest management. *Edited by* J. Gopal and S.M. Paul Khurana. Haworth's Press, Inc., Binghampton, N.Y. pp. 1–39.

Spooner, D.M., and Sytsma, K.J. 1992. Reexamination of series relationships of Mexican and Central American wild potatoes (*Solanum* sect. *Petota*): evidence from chloroplast DNA restriction site variation. Syst. Bot. **17**: 432–448. doi:10.2307/2419483.

Spooner, D.M., Van Den Berg, R.G., and Miller, J.T. 2000. Species and series boundaries of *Solanum* series *Longipedicellata* (Solanaceae) and phenetically similar species in ser. *Demissa* and ser. *Tuberosa*: implications for a practical taxonomy of section *Petota*. Am. J. Bot. **87**: 113–130.

Spooner, D.M., van den Berg, R.G., Rodriguez, A., Bamberg, J., Hijmans, R.J., and Lara-Cabrera, S.I. 2004. Wild potatoes (*Solanum* section *Petota*) of North and Central America. Syst. Bot. Monogr. **68**: 1–209 + 9 pl.

Spooner, D.M., Núñez, J., Trujillo, G., del Rosario Herrera, M., Guzmán, F., and Ghislain, M. 2007. Extensive SSR genotyping of potato landraces supports a major reevaluation of their gene pool structure and classification. Proc. Natl. Acad. Sci. U.S.A. **104**: 19398–19403. doi:10.1073/pnas.0709796104. PMID:18042704.

Spooner, D.M., Rodríguez, F., Polgár, Z., Ballard, H.E., Jr., and Jansky, S.H. 2008. Genomic origins of potato polyploids: GBSSI gene sequencing data. Crop Sci. **48**(Suppl.): S27–S36. doi:10.2135/cropsci2007.09.0504tpg.

Wangenheim, K.H.F. 1955. Zur Ursache der Kreuzungsschwierigkeiten zwischen *Solanum tuberosum* L., and *S. acaule* Bitt. bzw. *S. stoloniferum* Schlechtd. et Bouche. Z. Pflanzenzuecht. **34**: 7–48.