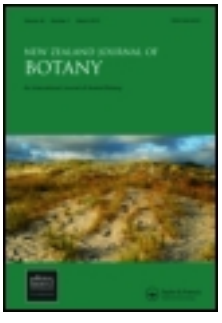

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Variation in rDNA loci of polyploid Solanum elaeagnifolium (Solanaceae)

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RESEARCH ARTICLE

Variation in rDNA loci of polyploid *Solanum elaeagnifolium* (Solanaceae)

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Fluorescence in situ hybridization (FISH) was applied to detect gene rearrangements and genome downsizing in *Solanum elaeagnifolium*, an invasive weed with $2n = 24$, 48 and 72 populations. The probes employed were p*Ta*71 containing the 18S–5.8S–26S gene, and a 5S rDNA probe obtained from *S. stuckertii*. As a result, diploids presented a pair of 18S–5.8S–26S signals and a pair of 5S, whereas tetra- and hexaploids increased their FISH marks proportional to the ploidy level. In all cases, rDNA loci were non-syntenic, and position was conserved along the polyploid series. The cytotypes would have formed recently and drastic rearrangements have not yet occurred. However, in each cell of the 4*x* and 6*x* individuals, signal intensity in one locus of the 18S–5.8S–26S was stronger, suggesting a potential loss of copies in the remaining loci. In addition, one hexaploid showed an extra 5S signal in heterozygosis. The data obtained suggest a recent, recurrent autopolyploidy.

Keywords: *Solanum elaeagnifolium*; polyploidy; rDNA loci; gene loss; genome downsizing

Introduction

Within the large genus *Solanum* L., the subgenus *Leptostemonum* (Dunal) Bitter (the 'spiny solanums'), is remarkable because of its high number of species (± 450), the diversity of habitats its species occupy and its impact on human activity, either as food plants or as weeds (Whalen 1984; Bohs 2005; Levin et al. 2006). *Solanum elaeagnifolium* Cav. is one of these weedy species which deserves special attention: it is a perennial herb, covered with silvery white peltate hairs, and showing a high degree of morphological variation across its distribution area, particularly in leaf shape and number of prickles (Boyd et al. 1984; Stanton et al. 2009, 2012; Zhu et al. 2013). It propagates by means of seeds, creeping rhizomes and root fragments (Fernández & Brevedan 1972; Stanton et al. 2012). There is disagreement about its geographic origin in North or South America, but some authors (Goeden 1971; Boyd et al. 1984) have suggested the southwestern United States and northern Mexico as the most likely centre of origin. The species is an aggressive weed because it competes with crops and produces allelopathic compounds (Mkula 2006); it has spread into many regions of the world beyond its native range, such as Australia, South Africa and the Mediterranean (e.g. Panetta & Mitchell 1991; Mekki 2007; Stanton et al. 2009). Cytologically, a previous work demonstrated a euploid series ($2n = 24$, 48, 72) for populations growing spontaneously in Argentina, and the cytotypes are apparently not randomly distributed (Scaldaferro et al. 2012). Considering geographical distribution, all accessions of *S. elaeagnifolium* from North America (the supposed original area of the species) analysed to date are diploids (Powell & Weedin 2005; Scaldaferro et al. 2012), suggesting that Argentina would be an invaded area where polyploidy may have arisen secondarily.

There are no studies showing morphological differences among the different cytotypes of *S. elaeagnifolium*: apparently the differences are within the continuous range of variability of the species

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(Zhu et al. 2013). Neither are there putative parents nor related species with which *S. elaeagnifolium* could hybridize in the polyploid area, the closest relatives being *S. hindsianum* and *S. tridynamum* from North America (Bohs 2005; Levin et al. 2006). Therefore, in the absence of contrary evidence, it is assumed that polyploids of *S. elaeagnifolium* are autopolyploids.

Polyploidy is important for many aspects described in several review articles (Hegarty & Hiscock 2008; Leitch & Leitch 2008; Van de Peer et al. 2009), and it is regarded as a frequent phenomenon in angiosperms (Soltis & Soltis 1993; Ramsey & Schemske 1998). Polyploids often have a wider geographical range than their diploid parents (Whittemore & Olsen 2011), probably because they would be pre-adapted for resources and habitats out of bounds to their progenitors and are not very well adapted to the parents' habitat (Levin 2004). During the polyploidization process, some phenomena such as gene reordering, genome downsizing and gene silencing may occur (Stebbins 1985; Soltis et al. 2003; Leitch & Bennett 2004; Pires et al. 2004), and their detection is important to recognize chromosomal lineages and cytogeographic patterns (Melo & Guerra 2003; Garcia et al. 2009). For example, studies in *Tragopogon* (Chester et al. 2012) suggest that prolonged chromosomal instability might be common in natural populations after whole-genome duplication, and likewise the nature of *S. elaeagnifolium* in Argentina presents an opportunity to study aspects of autopolyploid evolution. Fluorescence in situ hybridization (FISH) has proved useful for this purpose because it enables the differentiation of chromosome pairs (Moscone et al. 1999; Garcia et al. 2007) and reveals chromosome rearrangements (Heslop-Harrison 2000; Leitch et al. 2008). The procedure also provides information on genome organization and allows chromosomal evolutionary questions to be addressed (Lim et al. 2000; D'Hont 2005; Moscone et al. 2006; Chiarini et al. 2013; Shibata et al. 2013). Variations in the number, signal intensity and position of rDNA loci seem to be common in several plant groups (Frello & Heslop-Harrison 2000; Urdampilleta et al. 2006; Datson & Murray 2006), suggesting their mobility. Within a single species, the number and location of rDNA loci may be conserved or vary considerably among populations with different ploidy levels (Adachi et al. 1997; Vanzela et al. 2002; Tel-Zur et al. 2004), another phenomenon that can be detected using the FISH technique. Thus, in order to determine whether any of these modifications has occurred in the rDNA loci of *S. elaeagnifolium*, the technique was applied with the aim of unravelling whether polyploidy in *S. elaeagnifolium* in Argentina is a case of recent and recurrent autopolyploidy.

Material and methods

The provenance of the plant material studied is shown in Table 1. Mitotic chromosomes were examined in root tips obtained from seeds germinated in Petri dishes. Root tips were pre-treated in saturated *p*-dichlorobenzene in water for 2 h at room temperature, fixed in 3:1 ethanol/acetic acid, washed in distilled water, digested with PECTINEX ® (45 min at 37 °C), and squashed in a drop of 45% acetic acid. After coverslip removal in

Table 1 Provenance of the populations of *S. elaeagnifolium* studied.

Ploidy	Origin of materials	Collection No.
2*x*	USA. Utah State, Washington County, near Zion National Park (UT).	Bohs *w.n.*
2*x*	Argentina. San Juan Prov., Angaco Dept, Quebrada Paso de las Burras (MERL).	Iadiza 1529
2*x*	Argentina. Catamarca Prov., La Paz Dept (CORD).	Cocucci 997
4*x*	Argentina. Buenos Aires Prov., Bahia Blanca Dept, Bahía Blanca City (CORD).	Barboza 2319
6*x*	Argentina. San Luis Prov., Junín Dept, Merlo City (CORD).	Juliani 37
6*x*	Argentina. Buenos Aires Prov., Rojas Dept, Rafael Obligado (CORD).	Scaldaferro 1

liquid nitrogen, the slides were air dried and stored at –20 °C. The location and number of the rDNA sites were determined by using as probes p*Ta*71 containing the 18S–5.8S–26S rDNA (hereafter 35S) labelled with biotin–14-dATP (BioNick, Invitrogen Carlsbad, CA, USA) and a 5S probe created by PCR, using DNA of *Solanum stuckertii* Bitter, and labelled with digoxigenin (Chiarini et al. 2013). The FISH protocol of Schwarzacher & Heslop-Harrison (2000) was followed with minor modifications. The preparations were incubated in 100 µg mL^{-1} RNase, post-fixed in 4% (w/v) paraformaldehyde, dehydrated in a 70–100% graded ethanol series and air-dried. On each slide, 15 µL of hybridization mixture was added (4–6 ng μL^{-1} of probe, 50% formamide, 10% dextran sulfate, 3.3 ng μL^{-1} of salmon DNA, 2× SSC and 0.3% SDS), previously denatured at 70 °C for 10 min. Chromosome denaturation/hybridization was carried out at 90 °C for 10 min, 48 °C for 10 min and 38 °C for 5 min using a thermal cycler (Mastercycler, Eppendorf, Hamburg, Germany), and slides were placed in a humid chamber at 37 °C overnight. The 35S probe was detected with an avidin–FITC conjugate, and the 5S probe was detected with anti-digoxigenin–Rhodamine and then counterstained and mounted with 20 µL antifade (Vectashield Vector Lab., Burlingame, CA, USA), containing 1.5 µL mL^{-1} of DAPI. Photomicrography was carried out with a Zeiss Axiophot microscope equipped with epifluorescence and a digital image capture system. Each of the images taken with different fluorescent filters was merged afterwards using the free software ImageJ (http://rsbweb.nih.gov/ij/).

Results

In all accessions examined, karyotypes are composed mostly of small metacentric chromosomes (mean length: 1.5 µm, Fig. 1). The number of rDNA signals and ploidy level are positively correlated: diploids showed one pair of signals for the 35S and one pair for the 5S, whereas tetra- and hexaploids increased the number of FISH signals proportional to the ploidy level. In all cases, rDNA loci were non-syntenic, and their position was conserved along the polyploid series (Fig. 1). The 35S signals were located in a terminal position on the short arm of submetacentric chromosomes, while the 5S were detected in interstitial/sub-terminal position on metacentric chromosomes. The 4× and 6× individuals showed differences in the intensity of their 35S signals: in each cell, a pair of signals was evidently stronger than the rest (Fig. 1). In hexaploids, differences of the number of 5S were observed: one of the accessions (*Scaldaferro 1*) showed seven 5S signals per cell instead of the expected six (Fig. 1D).

Discussion

Following polyploidization, different types of changes in the genome have been documented. In Solanaceae, for example, an analysis of the 35S rDNA of *Nicotiana* indicated that parental loci were initially maintained in newly formed polyploids, although the sequences within a locus might be subject to concerted evolution, and over periods greater than 1 myr, individual loci would disappear (Kovařík et al. 2008). A different situation occurs in the allotetraploid *N. rustica*, in which the sizes of the 35S rDNA loci showed that the number of rDNA units on one of the parental chromosome sets was amplified and associated with a concomitant reduction in the number of units on the other set (Matyásek et al. 2003). Considering that *S. elaeagnifolium* polyploids increased their FISH marks proportional to the ploidy level, this may suggest that the polyploid series is recently formed and drastic rearrangements have not yet occurred. However, the differences in the intensity of the 35S signals in the 4× and 6× individuals could indicate that some loss of gene copies might be taking place at these loci, as demonstrated in *Nicotiana* (Renny-Byfield et al. 2012). A similar situation was observed in polyploid species of Asteraceae (Adachi et al. 1997; Buggs et al. 2009), in which the number of 35S sites seemed to evolve faster than the 5S sites, as they do not increase with the successive genome additions. This phenomenon could be analogous to the nucleolar dominance or ‘differential amphiplasty’

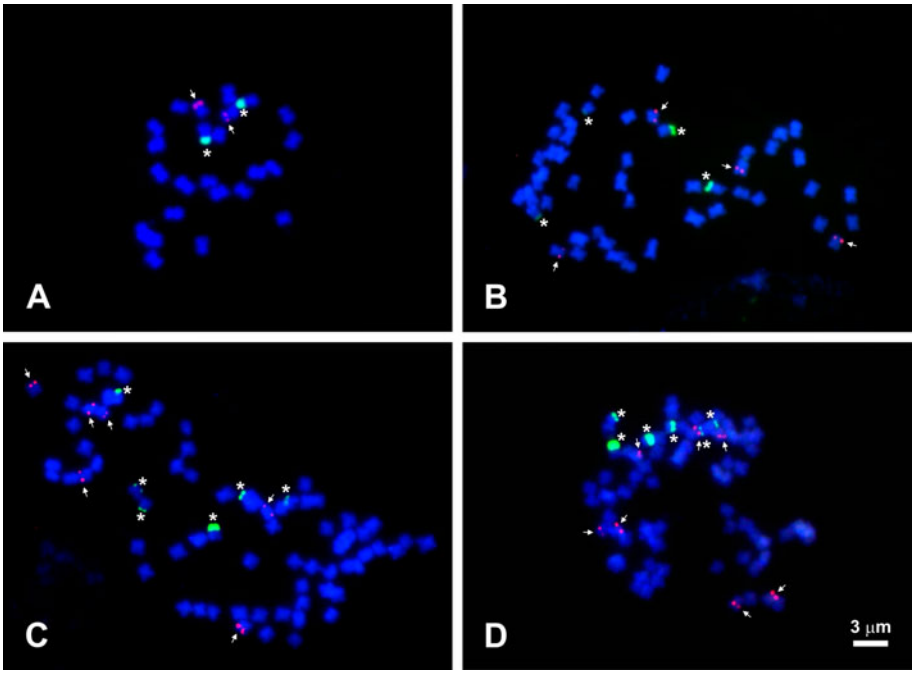

Figure 1 Photomicrographs of metaphases in the three cytotypes of *S. elaeagnifolium* after the FISH technique. **A,** The diploid Iadiza 1529. **B,** The tetraploid Barboza 2319. **C,** The hexaploid Juliani 37. **D,** The hexaploid Scaldaferro 1. Arrows point at the chromosomes with the 18–5.8–26S signal (green, FITC) and asterisks indicate the chromosomes with a 5S signal (red, rhodamine), counterstain in blue (DAPI). All pictures at the same scale. Note the differences in the intensity of 18–5.8–26S signals within each cell in B–D.

that occurs in interspecific hybrids (Pikaard 1999; Volkov et al. 1999) or the competition between B and A chromosomes (Acosta & Moscone 2010).

According to Roa & Guerra (2012) and Garcia et al. (2013), the most common rDNA profiles found in plants consist of two or four 35S sites per diploid karyotype, preferably located in the terminal regions of short chromosome arms. Despite the wide dispersion capacity of these sequences, there would be a tendency for the number of rDNA sites to be restricted. The same authors pointed out that, in polyploids, there is a trend towards a reduction in the number of sites per monoploid complement. In fact, the number of two 35S sites per complement, always associated with nucleolus organizer region satellites, seems to be the rule within the 'spiny solanums' (Rego et al. 2009; Melo et al. 2011; Chiarini et al. 2013), and the results found here for *S. elaeagnifolium* are consistent with this trend. However, the number of 35S sites seems to be variable outside the *Leptostemonum* clade: more than one pair of sites occur in *S. corymbiflorum* (Sendtn.) Bohs from the *Cyphomandropsis* clade, and in other related genera of Solanaceae from the so-called '$x = 12$ clade', such as *Vassobia breviflora* (Sendtn.) Hunz. (Rego et al. 2009).

In diploid species of *Solanum*, more than two pairs of 5S rDNA have been observed in species from different clades, such as *S. trachytrichium* Bitter and *S. gemellum* Mart. ex Sendtn. (Rego et al. 2009), and the same situation, although rare, has been found among some 'spiny solanums' (Chiarini et al. 2013). The usual situation would be one 5S site per haploid complement, *S. elaeagnifolium* being remarkable in this sense, because diploid accessions with one site were found, as well as a polyploid one with an additional site.

Concerning position, in *Solanum* the 5S signals are usually sub-terminal or interstitial, but paracentromeric sites have been detected in *S. lycopersicum* (Lapitan et al. 1991), and in the longest chromosome of the set in potato (Dong et al. 2000). These data suggest that in the genus, the 5S rDNA could be used as a marker of species and/or species groups because its location and number of sites are not conserved.

In flowering plants, evidence has accumulated for both a lack of concerted evolution and variability and rapid rearrangements in 5S rRNA loci (e.g. Raskina et al. 2004; Datson & Murray 2006). In the case of *S. elaeagnifolium*, the emergence of an additional 5S site in one of the accessions can be explained in several ways: subsequent to the polyploidization, chromosome rearrangements and transposition events may occur (Hanson et al. 1996; Krishnan et al. 2001), and minor sites can be added/deleted through non-homologous unequal crossing-over (Seperack et al. 1988). An uncharacterized transpositional process has been suggested to explain these phenomena, and part of the apparent 5S gene dynamism may be explained by activity of small nonautonomous retrotransposons (Kalendar et al. 2008 and citations therein). It has also been suggested that telomeric or sub-telomeric positions of rDNA loci would possibly allow significant chromosome rearrangements/duplications to occur without deleterious effects (Hanson et al. 1996).

Diploid accessions of *S. elaeagnifolium* from both North and South America are similar with regard to rDNA pattern: the FISH technique shows that there were no modifications in number and position of the sites during the hypothesized migration of *S. elaeagnifolium* across the Americas.

The extra 5S signal found in heterozygosis in one of the hexaploids indicates that this individual might have one parent with a duplicated 5S locus, and implies more than one place of origin for the hexaploids. Evidence for recurrent polyploidy was also provided by Scaldaferro et al. (2012), who studied the DNA content in the three cytotypes of *S. elaeagnifolium*. According to their results, 1Cx values differed not only among accessions with different ploidy levels, but also among those of the same ploidy level. This pattern may suggest two possibilities, either some polyploid populations have recently originated and no genome downsizing has yet occurred, and/or there are several places of origin for polyploids in Argentina.

A relationship between genome downsizing and a low number of rDNA loci has been suggested for some species (Garcia et al. 2009). The whole genome downsizing cannot be directly attributed to a decrease in rDNA loci or the number of copies, but it is possible that the rDNA, a highly repetitive gene family, although constituting a small part of the genome itself, may bear some association with overall genome size (Prokopowich et al. 2003). Such an association is not evident in the case of *S. elaeagnifolium*. In fact, DNA content is known for several of the accessions to which the FISH technique was applied (Scaldaferro et al. 2012), and despite some accessions having contents significantly lower than others, all have the same number of 5S sites; except for one accession, which had an extra site.

The proportional increase in rDNA sites with ploidy level could be considered another argument for autopolyploidy, because in many allopolyploids, the 35S sites from one of the parental genomes prevail over the other (Hanson et al. 1996; Matyásek et al. 2003).

In conclusion, the data obtained in this study using the FISH technique support the hypothesis of a recent, recurrent autopolyploidy in *S. elaeagnifolium*. The pattern of variation in rDNA loci number with polyploidy does not fit exactly with the findings from other plant groups (Matyásek et al. 2003; Pires et al. 2004; Garnatje et al. 2006), because it does not always correspond to the exact proportionality (i.e. additivity) or to a relative loss of sites. Further experiments (e.g. haplotypes with microsatellites or genomic in situ hybridization) would be useful in dating precisely each polyploid lineage.

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