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Fluorescence *in situ* hybridization of potato somatohaploids and their somatic hybrid donors using two *Solanum brevidens* specific sequences

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Two *Solanum brevidens* specific repetitive DNA clones (pSB1 and pSB7) were used simultaneously as probes in fluorescence *in situ* hybridization (FISH) for cytological studies of somatohaploids and their somatic hybrid donors. pSB1 was labelled with digoxigenin-11-dUTP and pSB7 was labelled with biotin-14-dATP and they were detected with reporter molecules conjugated to fluorescent dyes using digital imaging. The tandemly repeated sequences hybridized mostly near the telomeres of the chromosomes of *S. brevidens*. Using these two probes, it was possible to identify chromosomes containing repetitive DNA of *S. brevidens* both in the somatic hybrids between *S. brevidens* and *S. tuberosum*, and somatohaploids derived from the somatic hybrids. These cytological analyses showed that for the largest part genomes of the hexaploid somatic hybrids and their anther-derived triploid somatohaploids were composed of the genome of *S. brevidens*.

Key words: chromosome, haploid, repetitive DNA, *Solanum tuberosum*, somatic hybrid, species-specific sequences

Introduction

Fluorescence *in situ* hybridization (FISH) is an important method in chromosome identification and physical mapping. Simultaneous localization of two or more probes (Leitch et al. 1991) or multiple colour FISH (Mukai and Nakahara 1993) and genomic *in situ* hybridization (GISH) (Le et al. 1989, Schwarzacher et al. 1989) can be used in cytological analyses of the organization of genomes in interspecific hybrids.

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We have previously been able to combine the genome of *Solanum brevidens*, which has a broad virus resistance (Valkonen et al. 1992), with *S. tuberosum*, cultivated potato, through protoplast fusion (Rokka et al. 1994). These interspecific somatic hybrids have shown androgenic capacity (Rokka et al. 1995) and few somatohaploids using anther culture (Rokka et al. 1997) have been produced from somatic hybrids. Somatohaploids can be used in potato breeding as a step to combine valuable characters of wild species with good agronomic traits of cultivated potato. Somatohaploids also provide interesting material for genetic studies of interspecific somatic hybrids.

There have been few reports on cytogenetic analysis of tissue culture derived hybrids using *in situ* hybridization. Quantitative changes of repeated sequences, translocations and deletions in tissue culture regenerated sexual hybrids of wheat and rye have been documented (Lapitan et al. 1986, 1988). However, there have been only a few reports using species-specific repetitive DNA probes or total genomic DNA for *in situ* hybridization to analyse somatic hybrids or their derivatives (e.g. Piastuch and Bates 1990, Tempelaar et al. 1991, Itoh et al. 1991, Parokonny et al. 1992, Wolters et al. 1994, Jacobsen et al. 1995).

In this paper we describe the cytological characterization of three somatohaploids of potato and two of their somatic hybrid donor plants with FISH using two species-specific DNA repeats, pSB1 and pSB7, isolated from *S. brevidens* (Pehu et al. 1990) as probes. Both of these DNA clones contain highly repeated sequences and have previously been used as species-specific probes in dot blot analysis for the verification of somatic hybridity (Pehu et al. 1990) and in a survey of DNA sequence similarities between *Solanum* species (Malkamäki et al. 1996). The *S. brevidens* specific probes have also been determined for their chromosomal distribution *in situ*, and localized near the telomeres and in some centromeric and interstitial sites of *S. brevidens* chromosomes, but not in *S. tuberosum* (Rokka et al. 1998).

Material and methods

Two hexaploid somatic hybrids (0502 and 0603) between *S. brevidens* and *S. tuberosum* (Rokka et al. 1994) and three of their triploid anther-derived somatohaploids (0502.1.1.1., 0507.1.2.1. and 0603.1.5.4.) (Rokka et al. 1997) were included in the study.

Roots of the tissue cultured plant material (0502.1.1.1. and 0507.1.2.1.), grown on MS (Murashige and Skoog 1962) with 2% (w/v) sucrose (Merck) and 0.3 µM (0.05 mg/l) NAA (α-naphthaleneacetic acid) (Sigma), were pretreated overnight with 1.25 mM hydroxyurea (Sigma), at 23°C. After the pretreatment, the roots were washed three times with dH_2O and transferred to a Petri dish combining a wet filter paper. After 5–6 hours, the root tips were collected and transferred to an icebath for 16–18 h. The tissue cultured roots of the genotypes 0502, 0603 and 0603.1.5.4. were not treated with hydroxyurea but were directly transferred to an icebath at 4°C for 18–21 h. After the icewater treatment, the root tips were fixed in ice cold methanol:glacial acetic acid (3:1). The metaphase chromosomes were prepared as described by Rokka et al. (1998).

Labelling the plasmids (pUC18) containing the inserts of pSB1 and pSB7 (Pehu et al. 1990) was done by random priming (oligolabelling). The probe pSB1 was labelled with digoxigenin-11-dUTP (Boehringer Mannheim) and the probe pSB7 was labelled with biotin-14-dATP (BRL) (Rokka et al. 1998).

The probe mixture preparation and *in situ* hybridization were carried out as described by Rokka et al. (1998). The slides were incubated before the chromosome denaturation step in a solution of pepsin and rinsed in dH_2O as described by Brown (1995). For hybridization the slides were incubated in a humidity chamber at 37°C for 15–16 hours. After hybridization, the slides were washed in 40% (v/v) formamide in 2xSSC at 42°C for 10 min, 1xSSC at 37°C for 10 min and 0.1xSSC at room temperature for 10 min. The hybridized signals were simultaneous-

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ly detected with PN buffer (0.1 M Na_2HPO_4, 0.1 M NaH_2PO_4, pH 8.0, 0.5% (v/v) Nonidet P-40 (Sigma)) containing 2.5 µg/ml of anti-digoxigenin-rhodamine (Boehringer Mannheim) and 5 µg/ml of fluorescein avidin DN (Vector Laboratories) (Rokka et al. 1998). DAPI stained chromosomes and the hybridized signals were captured with a 100x Zeiss objective using a cooled array CCD (charge-coupled device) collector and digital imaging (Rokka et al. 1998). From one to three chromosome sets were analysed per genotype.

Results and discussion

The chromosome composition of the somatic hybrids of the Solanaceae has not been extensively studied, because the potato chromosomes are small and morphologically similar. In the present work, two somatic hybrids and three anther-derived somatohaploids of *S. brevidens* and *S. tuberosum* were cytologically characterized using two *S. brevidens* specific repetitive DNA sequences simultaneously as probes for *in situ* hybridization.

The two *S. brevidens* specific repeated sequences, pSB1 and pSB7, were previously shown to hybridize *in situ* to *S. brevidens* chromosomes, but not to chromosomes of *S. tuberosum* (Rokka et al. 1998). Using highly stringent washes (40% formamide), which allowed approximately 20% nucleotide mismatches, it was possible to distinguish *S. brevidens* chromosomes from the chromosomes of *S. tuberosum*. Under this stringency, pSB1 and pSB7 were detected in chromosomal regions which are typically known to contain tandemly repeated sequences, such as telomeric areas and some centromeric and interstitial sites. pSB7 hybridized to all 24 chromosomes and pSB1 hybridized to 17–18 chromosomes of *S. brevidens* (Rokka et al. 1998).

The somatohaploids, which were previously described (Rokka et al. 1997), are triploids (2n=3x=32–36) derived from hexaploid (2n=6x=60–71) somatic hybrids between diploid *S. brevidens* (2n=2x=24) and dihaploid *S. tuberosum* (2n=2x=24) Based on counts of chromosomes showing FISH signals, when probed with pSB1 and pSB7, two thirds (≈ 70%) of the genomes of the somatic hybrids (0502 and 0603) were derived from *S. brevidens* and one third (≈ 30%) from *S. tuberosum* (Table 1). This is probably a result of the electrofusion of two proto-

Table 1. Chromosome numbers of the somatic hybrids and somatohaploids between *Solanum brevidens* and *Solanum tuberosum* and the number of chromosomes containing DNA of *S. brevidens* based on FISH (fluorescence *in situ* hybridization) using two *S. brevidens* specific repetitive DNA sequences (pSB1 and pSB7).

Plant	total no. of chromosomes (ploidy level)	no. of chromosomes showing signals of: pSB1	pSB7	total no. of chromosomes containing DNA of *S. brevidens*, both probes simultaneously[a]	
Hybrids:					
Pito dh.45/4(+)*S. brevidens* 0502	68±4 (6x)	45/70	39/70	50/70	~70%
Pito dh.45/4(+)*S. brevidens* 0603	65±5 (6x)	nd.[b]/70	nd.[b]/70	nd.[b]/70	~70%[c]
Somatohaploids:					
Pito dh.45/4(+)*S. brevidens* 0502.1.1.1.	35±1 (3x)	18/36	23/36	29/36	~80%
Pito dh.45/4(+)*S. brevidens* 0507.1.2.1.	33±1 (3x)	21/32	17/32	25/32	~80%
Pito dh.45/4(+)*S. brevidens* 0603.1.5.4.	35±1 (3x)	16/33[d]	26–27/33[d]	26–27/33[d]	~80%

[a] analysed from combined captured images

[b] exact number of chromosomes containing repetitive DNA of *S. brevidens* is not determined

[c] approximative result

[d] one chromosome missing from the set analysed

plasts of *S. brevidens* with one protoplast of dihaploid *S. tuberosum* line (Rokka et al. 1994). This observation is interesting, especially because the hybrids included in this study have shown androgenic capacity, although *S. brevidens* itself is recalcitrant in anther culture (Rokka et al. 1995).

The exact determination of the number of donor species derived chromosomes in somatic hybrids was difficult, because the hybrids showed intragenomic variation in their chromosome numbers (Rokka et al. 1995). Secondly, chromosomal translocations within homoeologous chromosomes and origin of minichromosomes in somatic hybrids are common, as shown between *Nicotiana plumbaginifolia* and *N. tabacum* (Piastuch and Bates 1990), *N. sylvestris* and *N. plumbaginifolia* (Parokonny et al. 1992), *Lycopersicon esculentum* and *S. tuberosum* (Wolters et al. 1994). In the genomes of our somatic hybrids there were fewer than 24 chromosomes which were derived from *S. tuberosum*. Because of the translocations some chromosomes which may only have one arm of a *S. brevidens* chromosome may be distinguished as a chromosome of *S. brevidens*, when these two species-specific sequences are used *in situ*. Pijnacker et al. (1989) reported preferential elimination of *S. phureja* chromosomes in *S. phureja* (+) *S. tuberosum* somatic hybrids whereas Wolters et al. (1994) reported a random elimination of tomato chromosomes in potato (+) tomato somatic hybrids. In our hybrids, elimination of some *S. tuberosum* chromosomes and intergenomic chromosomal rearrangements may have occured, but the exact identification of donor genomes could be more accurately determined using GISH as described for tobacco hybrids by Parokonny et al. (1992) and for potato hybrids by Wolters et al. (1994). In GISH, labelled total DNA from one species is blocked with unlabelled DNA from the other species and used as a probe representing a broader proportion of the genome in *in situ* hybridization than cloned isolated probes of repetitive sequences (Anamthawat-Jónsson et al. 1990, Itoh et al. 1991).

Rokka et al. (1995) found variation in the genome sizes of the somatic hybrids between *S. brevidens* and *S. tuberosum*. These aberrations may not only be due to changes in chromosome numbers, but also due to structural alterations in the karyotypes and differences in the replication of particular (mostly repetitive) DNA families. Quantitative changes in repetitive sequences have been found in protoclones of potato (Landsmann and Uhrig 1985). These changes together with occasional translocations complicate the cytological analysis of the tissue culture derived regenerants.

Solanum brevidens specific DNA was present in 80% of the chromosomes of all the somatohaploids (Table 1). This is possible because some *S. brevidens* chromosomes can pair and recombine with *S. tuberosum* chromosomes as earlier reported by Williams et al. (1993) and McGrath et al. (1996). In meiosis, homoeologous pairing and crossing overs between tomato and potato chromosomes have also been shown in allotetraploid somatic hybrids (Wolters et al. 1994). Examples of *in situ* hybridizations of two somatohaploids (0502.1.1.1. and 0603.1.5.4.) are presented in Figures 1 and 2.

In this work the potential of using species-specific tandemly repeated sequences in cytological characterization of interspecific potato hybrids and their derivatives was shown. Using *in situ* hybridization with *S. brevidens* specific DNA clones, hexaploid somatic hybrids and somatohaploids derived from them via anther culture were shown to have the largest part of their genomes derived from *S. brevidens*, which itself is an androgenically recalcitrant species.

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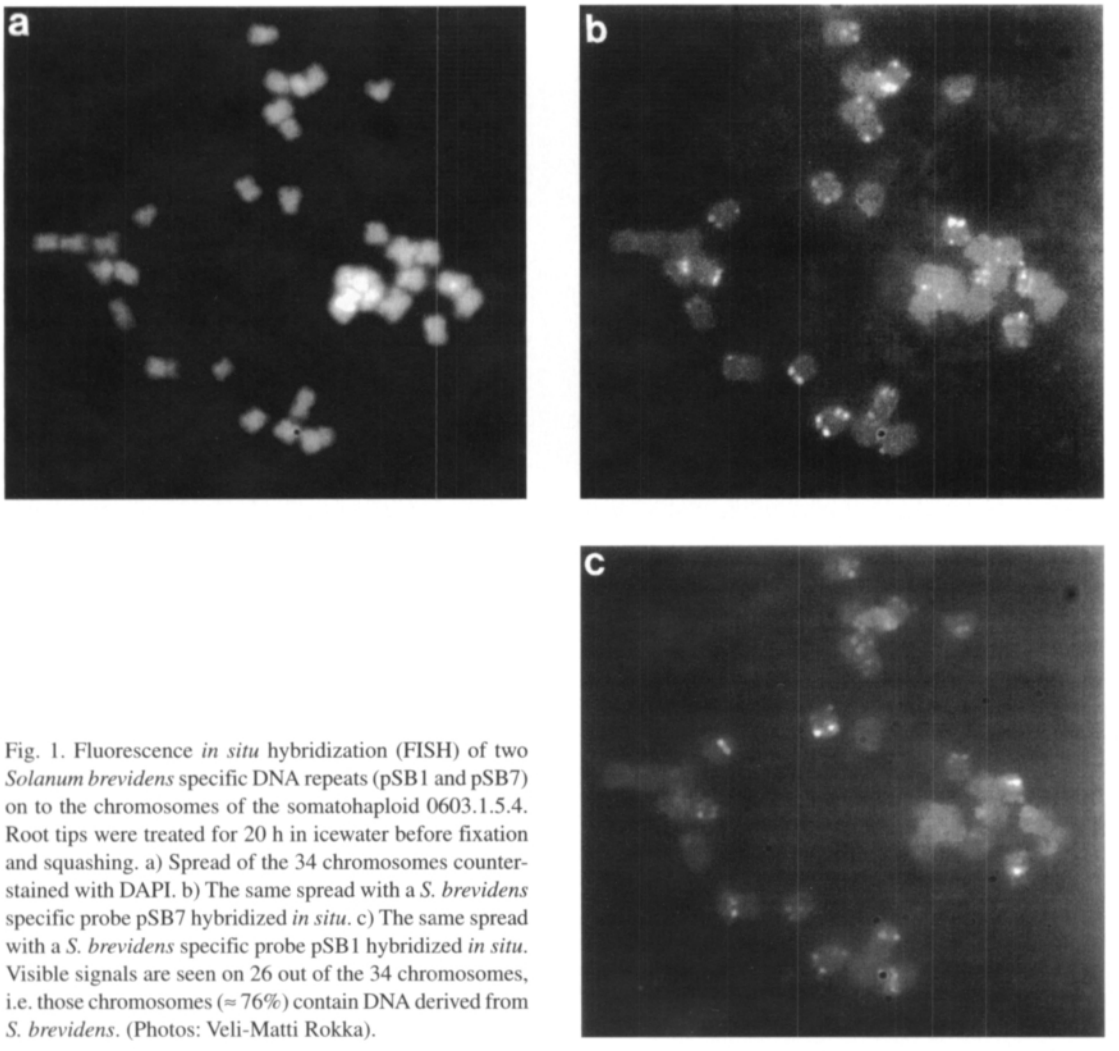

Fig. 1. Fluorescence *in situ* hybridization (FISH) of two *Solanum brevidens* specific DNA repeats (pSB1 and pSB7) on to the chromosomes of the somatohaploid 0603.1.5.4. Root tips were treated for 20 h in icewater before fixation and squashing. a) Spread of the 34 chromosomes counterstained with DAPI. b) The same spread with a *S. brevidens* specific probe pSB7 hybridized *in situ*. c) The same spread with a *S. brevidens* specific probe pSB1 hybridized *in situ*. Visible signals are seen on 26 out of the 34 chromosomes, i.e. those chromosomes (≈ 76%) contain DNA derived from *S. brevidens*. (Photos: Veli-Matti Rokka).

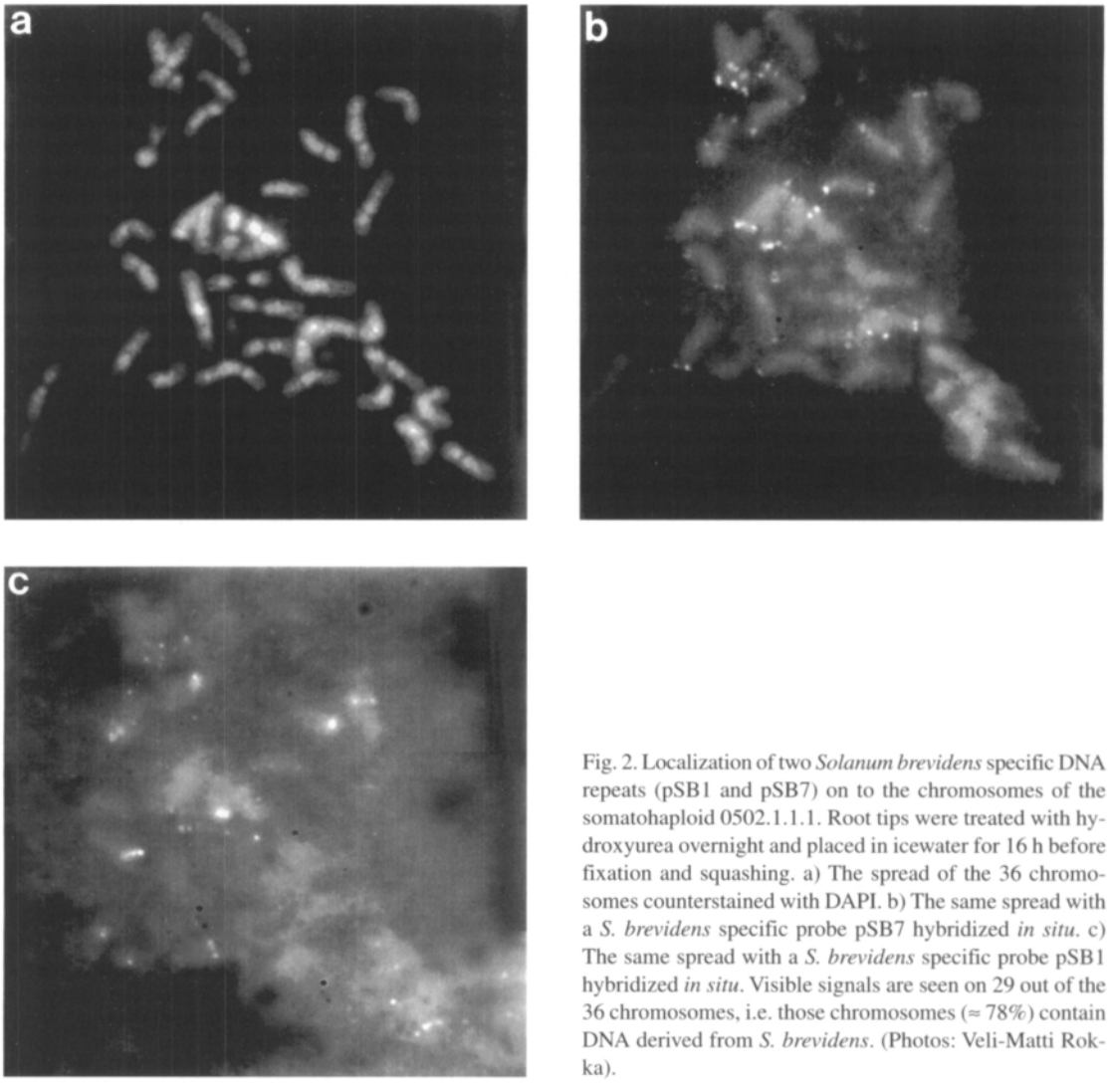

Fig. 2. Localization of two *Solanum brevidens* specific DNA repeats (pSB1 and pSB7) on to the chromosomes of the somatohaploid 0502.1.1.1. Root tips were treated with hydroxyurea overnight and placed in icewater for 16 h before fixation and squashing. a) The spread of the 36 chromosomes counterstained with DAPI. b) The same spread with a *S. brevidens* specific probe pSB7 hybridized *in situ*. c) The same spread with a *S. brevidens* specific probe pSB1 hybridized *in situ*. Visible signals are seen on 29 out of the 36 chromosomes, i.e. those chromosomes (≈ 78%) contain DNA derived from *S. brevidens*. (Photos: Veli-Matti Rokka).

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SELOSTUS

Perunan somaattisten hybridien ja niiden somatohaploidien fluoresenssi *in situ* -hybridisaatio *Solanum brevidens* -lajin spesifisten sekvenssien avulla

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Somaattisia hybridejä, jotka on tuotettu villin perunalajin (*Solanum brevidens*) ja viljellyn perunan (*S. tuberosum*) soluja fuusioimalla, on käytetty ponsiviljelyssä somatohaploidien tuottamiseksi. Somatohaploidit ovat mielenkiintoisia kasvinjalostustutkimuksessa, koska niiden avulla voidaan tutkia hybridien ominaisuuksien periytymistä meioosin jälkeen. Lisäksi somatohaploideja voidaan hyödyntää perunan lajikejalostuksessa risteyttämällä niitä haploidien perunalinjojen kanssa protoplastifuusioiden avulla. Tällöin on kyseessä suvullisen takaisinristeytyksen vaihtoehtoinen jalostusmenetelmä, ja villien perunalajien haluttuja ominaisuuksia voidaan siirtää todennäköisesti tehokkaammin agronomisilta ominaisuuksiltaan hyviin perunalinjoihin.

Tässä työssä tutkittiin kahden *S. brevidens* -lajilta eristetyn toistuvajaksoisen DNA-koettimen (pSB1 ja pSB7) avulla somaattisten hybridien ja niistä tuotettujen somatohaploidien kromosomistoja. Tutkimuksessa käytettiin fluoresenssi *in situ* -hybridisaatiota, jonka avulla yksijuosteinen toistuvajaksoinen DNA voitiin paikallistaa denaturoituihin kromosomeihin, jotka sisälsivät *S. brevidens* -lajin vastaavaa komplementaarista DNA:ta. Fluoresenssi *in situ* -hybridisaatiota varten pSB1-koetin leimattiin digoksigeniini-11-dUTP:lla ja pSB7-koetin biotiini-14-dATP:lla. Kromosomeihin hybridisoituneet leimatut DNA-koettimet paikallistettiin niihin konjugoituneiden fluoresenssiväriaineiden avulla. Molemmat koettimet olivat peräkkäisjaksoista DNA:ta ja hybridisoituivat *S. brevidens* -lajin kromosomien telomeerisiin päihin. Kyseisten koettimien avulla voitiin erottaa somaattisten hybridien ja niiden somatohaploidien ne kromosomit, jotka sisälsivät *S. brevidens* -lajin toistuvajaksoista DNA:ta. Fluoresenssi *in situ* -hybridisaation avulla todettiin, että heksaploidit somaattiset hybridit ja niistä tuotetut triploidit somatohaploidit sisälsivät enemmän *S. brevidens* -lajin genomia kuin *S. tuberosum* -lajilta peräisin olevaa genomia.