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RFLP analysis of asymmetric somatic hybrids between *Solanum tuberosum* and irradiated *S. brevidens*

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Abstract. The nuclear genome composition of five asymmetric somatic hybrids, obtained by fusion of leaf protoplasts from *Solanum tuberosum* and gamma-irradiated leaf protoplasts from *S. brevidens*, have been analyzed at the molecular level. An analysis of 21 loci using linkage group-specific restriction fragment length polymorphism (RFLP) was included in the study. All five hybrids contained a complete set of the loci studied from *S. tuberosum*. The degree of elimination of alleles from the irradiated *S. brevidens* donor genome ranged from 10–65% in the five asymmetric hybrids analyzed. The detection of incomplete chromosomes, as well as non-parental bands in Southern hybridizations with RFLP markers, revealed extensive chromosome rearrangements in the asymmetric hybrids.

Key words: *Solanum brevidens* – Asymmetric somatic hybrids – Protoplast fusion – Restriction fragment length polymorphism – Potato

Introduction

The transfer of desirable traits from wild germplasm into cultivated species is a central method in crop improvement. For this purpose, interspecific sexual hybrids may be constructed. However, the formation of sexual hybrids is often limited to closely related species whereas symmetric somatic hybrids obtained by fusion of protoplasts of two different species can be relatively easily generated in some species combinations (Ferreira and Zelcer 1989; Puite 1991). An obstacle in the utilization of interspecific hybrids, both sexual and somatic, is that they contain many unwanted traits of the wild species and are often sterile. These problems may be overcome by partial genome transfer techniques such as asymmetric somatic hybridization.

Cytological analysis of asymmetric somatic hybrids has been successful in determining the chromosome number, and in a few cases of hybrids composed of species with distinct chromosomal morphology, in identifying the parental origin of the chromosomes. In somatic hybrids of *Arabidopsis thaliana* with *Brassica campestris* (Gleba and Hoffmann 1978), *Atropa belladonna* with *Nicotiana chinensis* (Gleba et al. 1982) and *A. belladonna* with *N. plumbaginifolia* (Gleba et al. 1988), cytological observation detected a possible introgression of genomic sequences between the chromosomes of the two species.

In the genus *Solanum*, chromosome morphologies are not distinct enough to allow for karyotyping individual species. However, a nearly-saturated linkage map of potato and tomato has been produced, which includes more than 1000 RFLP markers on all 12 chromosomes (Gebhardt et al. 1991; Tanksley et al. 1992). Utilizing this map, Williams et al. (1990) used a selection of linkage group-specific RFLP markers to distinguish the chromosomes of *S. brevidens* from those of potato (*S. tuberosum*) in interspecific somatic hybrids. A similar approach was employed to analyze the genomic composition of asymmetric hybrids between *Lycopersicon esculentum* and irradiated *L. peruvianum* or *L. pennellii* (Melzer and O'Connell 1990, 1992; Wijbrandi et al. 1990).

We have produced asymmetric somatic hybrids between *S. tuberosum* and *S. brevidens* by fusing

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gamma-irradiated protoplasts of *S. brevidens* with untreated *S. tuberosum* (Xu et al. 1993). In this paper we describe the use of linkage group-specific RFLP markers for molecular characterization of five such asymmetric somatic hybrids.

Materials and methods

Plant material

Asymmetric hybrid plants were obtained from independent protoplast fusion events (Xu et al. 1993). A dihaploid line (PDH40, 2n = 2x = 24) derived from *S. tuberosum* cv "Pentland Crown" was used as recipient and the diploid, wild species *S. brevidens* Phil (CPC2451, 2n = 2x = 24) was used as the donor in donor-recipient fusion experiments. The *S. brevidens* parent was irradiated before fusion with a dose of either 300 Gray (asymmetric hybrid 10363 and 10732) or 500 Gray (asymmetric hybrids 40111, 50013 and 70021), using a ^{60}Co source (120 Gy/min at the Gray Radiological Institute, Watford, UK).

Probes used in RFLP analysis

The following 21 tomato genomic (TG) clones, representing 12 potato chromosome linkage groups, were used to analyze the nuclear DNA composition of the asymmetric hybrids: chromosome 1, TG53, TG71; chromosome 2, TG31, TG48; chromosome 3, TG94, TG134; chromosome 4, TG22; chromosome 5, TG23; chromosome 6, TG115, TG118; chromosome 7, TG20; chromosome 8, TG16, TG45; chromosome 9, TG35, TG18; chromosome 10, TG43, TG63; chromosome 11, TG30, TG47; chromosome 12, TG28, TG68 (Tanksley et al. 1992). The TG clones were kindly supplied by Dr. S. D. Tanksley, Cornell University, Ithaca, USA. These clones were amplified for labelling by the polymerase chain reaction (PCR) using M-13 forward and reverse primers. With only a few exceptions, most of the clones produced the expected insert size after PCR. In these cases, the PCR products were separated by electrophoresis in a low-melting-temperature gel, and the fragment of the correct size was cut out and used as a probe.

DNA isolation, restriction and southern transfer

Total cellular DNA was isolated from in-vitro-cultured plant materials using the method of Draper et al. (1988).

Ten micrograms of cellular DNA was restricted for 5 h at 37 °C according to the supplier's instructions (New England Biolabs) and separated by agarose-gel (0.8%) electrophoresis in TRIS-borate EDTA (TBE) buffer at 2 v/cm for 18 h. After electrophoresis, the gel was depurinated for 10 min in 0.25 N HCl, denatured for 30 min in 0.5 M NaCl + 1.5 M NaCl solution and neutralized for 20 min in 1 M NH_4OAc solution. The DNA was then transferred onto a DNA-binding filter, Hybond-N (Amersham) in 1 M NH_4OAc, by capillary transfer.

Labelling and hybridization

The TG clones were digoxigenin-labelled according to the instructions of the supplier (Boehringer Mannheim, cat. no. 1093657). Hybridization and immunological detection were also carried out following the procedures given by the supplier. Bound probes were removed by incubating the filters in dimethylformamide (DMF) at 60 °C and in 0.2 N NaOH + 0.1% SDS for 30 min at 37 °C, respectively. The filters were re-used 2 or 3 times.

Results

The five selected asymmetric hybrid plants were less vigorous than the parental species, *S. tuberosum* and *S. brevidens*, or the previously characterized symmetric hybrids between these two species (Pehu et al. 1989). The overall morphology of the asymmetric hybrids was similar to the non-irradiated parent *S. tuberosum* PDH40. Four of the hybrids had abnormal leaf morphology, characterized by fused leaflets and rough leaf surface, probably due to the difference in the growth rate between sectors. No flowers have been observed in the five asymmetric hybrids. One of them, 10732, was characterized by poor rooting.

To identify probe and restriction enzyme combinations which would reveal polymorphisms between the two parents, Southern blots of DNA from *S. tuberosum* and *S. brevidens* were hybridized to each of the RFLP markers selected for this study. Each probe was hybridized to filters of *Hin*dIII-digested genomic DNA of the parental species. Of the 21 probes surveyed (representing all of the 12 chromosomes of potato), 18 showed polymorphism between the fusion parents. Three of the probes (TG45, TG30 and TG18) revealed polymorphism between the fusion parents when DNA

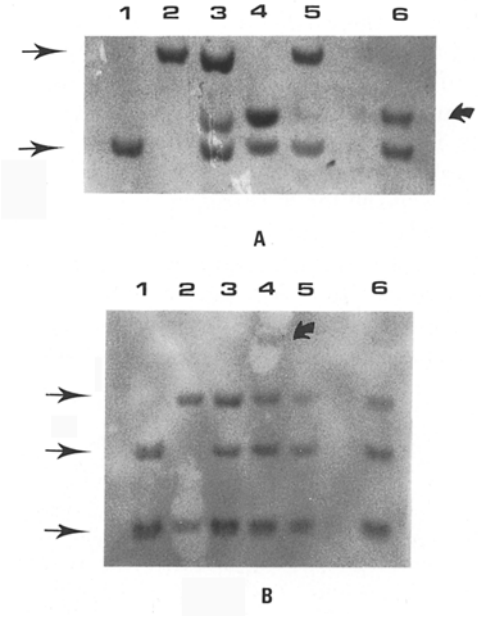

Fig. 1. Analysis of the loci TG43 (*A*) and TG22 (*B*) of the asymmetric hybrids. *Lanes 1 and 2* contain DNA isolated from the fusion parents *S. tuberosum* PDH40 and *S. brevidens*, respectively. *Lanes* 3–6 contain DNA isolated from the asymmetric hybrids 70021, 40111, 10363 and 10732, respectively. The DNA was restricted with *Hin*dIII and probed with the tomato genomic clones TG43 and TG22. Bands of interest are indicated by *arrow heads*. The non-parental bands are indicated by *curved arrows*

was digested with *Eco*RI. Many of the probe/enzyme combinations produced an RFLP with multiple fragments. Often in these patterns, the parental species had one or more bands in common.

The molecular map of the potato genome has enabled us to readily score the extent of nuclear hybridity in the somatic hybrids. Using the linkage group-specific markers, 21 loci were surveyed in this study in five asymmetric hybrids between *S. tuberosum* and irradiated *S. brevidens*. With respect to the number of *S. tuberosum*- and *S. brevidens*-specific fragments hybridizing, two classes of hybridization patterns were distinguished: a single and a multifragment pattern. As an example of the first class, the hybridization pattern for one locus, TG43, located on chromosome 10, is shown in Fig. 1A. *Hin*dIII digestion of potato DNA revealed a species-specific polymorphism for the TG43 sequence; a single fragment is observed in DNA isolated from *S. brevidens*, while another single fragment is observed in DNA isolated from *S. tuberosum* after probing with TG43. All of the five asymmetric hybrids had the *S. tuberosum*-specific bands (data not shown for asymmetric hybrid 50013). Asymmetric hybrids 70021 and 10363 were hybrid at this locus and displayed both parental fragments, whereas asymmetric hybrids 40111, 50013 and 10732 were scored as having the *S. tuberosum* fragment. Asymmetric hybrids 70021, 40111 and 10732 had a new band which did not exist in either parent (lanes 3, 4, 6 in Fig. 1A; indicated by curved arrow). The second class, the multi-fragment pattern, was represented by 11 probes (TG20, TG115, TG94, TG68, TG118, TG22, TG31, TG16, TG53, TG35, TG18) and showed a pattern as exemplified by clone TG22 in Fig. 1B: one band is present in both parental species, one band is specific for *S. tuberosum* and one band is specific for *S. brevidens*. Four of the asymmetric hybrids surveyed for the presence of *S. brevidens* TG22 alleles, were found to contain either one or no band specific to *S. brevidens* in addition to having one band from *S. tuberosum* and one band present in both parental species. Asymmetric hybrid 40111 had a new band which did not exist in either parent (lane 4 in Fig. 1B; indicated by curved arrow). Similar extra bands could be observed on five of the asymmetric hybrids probed with TG16, TG53 and TG31 and individual asymmetric hybrids with certain TG clones (Table 1).

A summary of the number of hybrid and non-hybrid loci for each of the five asymmetric hybrids, and their map location, is presented in Fig. 2.

It is difficult to quantify the intensity of the bands after NBT/BCCP immunological detection. However, the hybridization intensities of signals in dot-blots (probed by pST10, a *S. tuberosum*-specific sequence) of these asymmetric hybrids, as judged by visual inspection (data not shown), were slightly less than the untreated *S. tuberosum* parent. It suggested that the asymmetric hybrids might contain one *S. tuberosum* genome. According to the chromosome counts of metaphase plates from root tip cells and RFLP data, the asymmetric hybrids probably had one diploid *S. tuberosum* genome (i.e., 24 chromosome) and 7–22 chromosomes/chromosome fragments (Table 2).

In general, the chromosome numbers derived from the RFLP mapping data are approximately equal to the numbers determined by counts on metaphase plates of root tips (Table 2). In the asymmetric hybrids, the total number of complete plus incomplete chromosomes was higher than the number of chromosomes counted in root tip cells. In three out of five asymmetric hybrids, the total number of complete chromosomes from *S. tuberosum* and *S. brevidens* as determined by RFLP analysis was lower than the number of chromosomes counted.

Discussion

In this paper we have shown that RFLP analysis is an effective means of characterizing the genomic composition of asymmetric somatic hybrids obtainend after fusion of untreated protoplasts of *S. tuberosum* and

Table 1. The appearance of non-parental bands in the RFLP analysis of the five asymmetric hybrids. Ch., Chromosome. "+", indicating the presence of a non-parental band in the hybrid. "–", indicating the absence of a non-parental band in the hybrid

Genotype	Ch. 1 TG53	Ch. 2 TG31	Ch. 4 TG22	Ch. 8 TG16	Ch. 10		Ch. 12 TG 28
					TG43	TG63	
10363	+	+	–	+	–	–	–
10732	+	+	–	+	+	–	+
40111	+	+	+	+	+	+	–
50013	+	+	–	+	–	–	–
70021	+	+	–	+	+	+	+

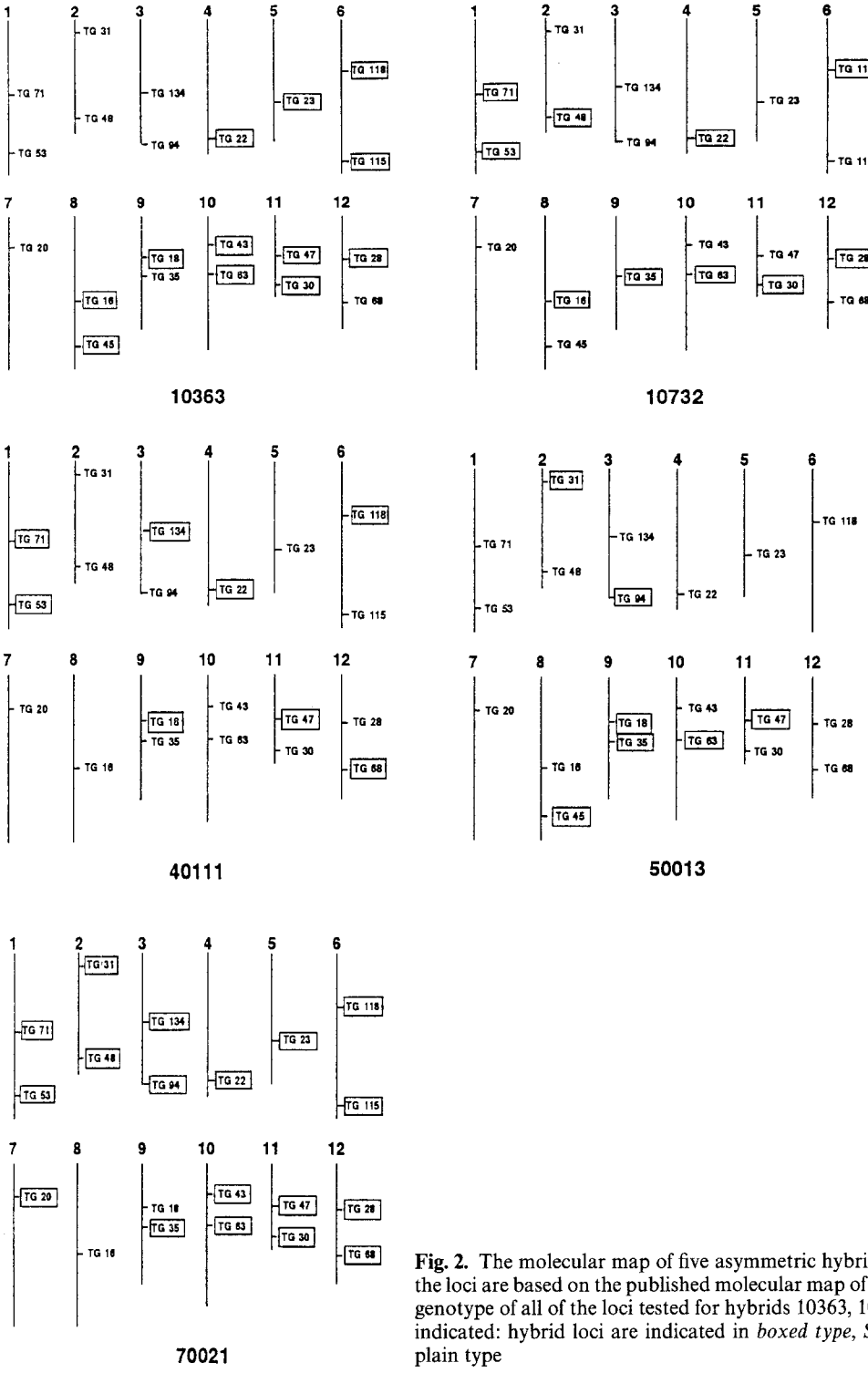

Fig. 2. The molecular map of five asymmetric hybrids. The chromosomal location of the loci are based on the published molecular map of potato (Tanksley et al. 1992). The genotype of all of the loci tested for hybrids 10363, 10732, 40111, 50013 and 70021 are indicated: hybrid loci are indicated in *boxed type*, *S. tuberosum* loci are indicated in plain type

irradiated protoplasts of *S. brevidens*. Using only one restriction enzyme, we could readily establish diagnostic RFLPs for 18 of the 21 molecular markers tested. These RFLPs allow each linkage group of the two different *Solanum* species to be identified in the hybrid by at least two markers. It is thus evident that a high degree of sequence divergence has taken place between the species and resulted in detectable polymorphism at the 21 loci used. Similar results in this species combination have been observed by Williams et al. (1990).

Through the analysis of linkage group-specific RFLPs, we have been able to produce a detailed

Table 2. The number of *S. tuberosum* (T) and *S. brevidens* (B) chromosomes in five asymmetric hybrids, as determined by RFLP analysis with 21 single-copy clones and by chromosome counts in metaphase plates of root tip cells

Genotype	Chromosome number RFLP analysis				Total c (inc.)	Metaphases 2n
	Complete		Incomplete	Lost		
	T	B	B	B		
10363	24	12	4	8	36(+4)	32–36
10732	24	4	12	4	28(+12)	32–36
40111	24	4	10	8	28(+10)	32–36
50013	24	2	10	12	26(+10)	28–32
70021	24	22	2	2	46(+2)	42–46
S. tuberosum	24	0	0	0	24(+0)	24
S. brevidens		24	0	0	24(+0)	24

The total number of chromosomes presumed complete, indicated as "c", and the total number of incomplete chromosomes, "+ inc." in parentheses, of each genotype are given

molecular profile for five asymmetric hybrids, which are independent regenerants from different calli (Xu et al. 1993). The five asymmetric hybrids contained a full complement of chromosomes from *S. tuberosum* and between 35–90% of the donor *S. brevidens* genome, as determined by RFLP analysis. Several authors have reported highly asymmetric somatic hybrids, with only one or a few chromosomes from an irradiated donor, in other fusion combinations, e.g., between *Nicotiana tabacum* and *N. plumbaginifolia* (Bates et al. 1987), *N. plumbaginifolia* and *Petunia hybrida* (Hinnisdaels et al. 1991), and *N. tabacum* and *Daucus carota* (Dudits et al. 1987). The limited chromosome elimination after gamma-ray irradiation observed in our study is consistent with data obtained in most of the interspecific (and intra- or inter-generic) asymmetric hybrids, e.g., between *Solanum* species (Sidorov et al. 1987; Fehér et al. 1992), *L. esculentum* and *L. peruvianum* (Wijbrandi et al. 1990), *N. plumbaginifolia* and *A. belladonna* (Gleba et al. 1988), *N. plumbaginifolia* and *N. sylvestris* (Famelaer et al. 1989). It is also possible that a dose of 300 and 500 Gy of gamma rays is not sufficient to cause extensive chromosome elimination from the *S. brevidens* in the casse of our donor-recipient fusion.

The extra bands which did not exist in either parent were quite common in some asymmetric hybrids with certain TG clones, indicating that rearrangements of the DNA have occurred. Similar results have been observed by Fehér et al. (1992) for the same species combination and by Sjödin and Glimelius (1989) in asymmetric hybrids of *Brassica*. In the present study, quite a few extra bands were found in the asymmetric hybrids, which could be a result of chromosomal recombination. Another possible cause could be somaclonal variation during plant regeneration from protoplasts (Karp et al. 1989).

While the presence of both parental alleles is indicated by a hybrid score in the RFLP analysis, the physical location of the *S. brevidens* allele in the genome is not known. There are two possibilities: either the *S. brevidens* allele has integrated into a *S. tuberosum* chromosome or the *S. brevidens* allele is present in the genome on an *S. brevidens* chromosome or a mini-chromosome. In Fig. 2, asymmetric hybrids 10363 and 70021 contain chromosomes in which two of the loci (in each case the two loci represented the same chromosome but were separated by a relatively large recombination distance) tested scored as hybrid (i.e., chromosome 6 in 10363 and chromosome 2 in 70021). In these cases, the most likely explanation is that the asymmetric hybrids contain at least one intact copy of the *S. brevidens* chromosome in addition to the *S. tuberosum* homologue. If the region near the centromere scores as hybrid for a particular chromosome, with the flanking regions scoring as *S. tuberosum*, it is possible that a minichromosome from *S. brevidens* is present in the hybrid. The total number of complete plus incomplete chromosomes as determined by RFLP analysis was higher than the number counted in metaphase plates in all of the five asymmetric hybrids (Table 2). The most likely explanation is that chromosome fragments generated by irradiation were involved in rearrangements such as translocations, although which chromosome were involved and where on the chromosome the rearrangement occurred is not known. This is in agreement with the previous findings of Wijbrandi et al. (1990) and Melzer and O'Connell (1992). In-situ hybridization with species-specific probes may clarify this hypothesis (Itoh et al. 1991).

The karyotype of the asymmetric hybrids correlated with their morphology. The asymmetric hybrids contained a complete set of the loci from *S. tuberosum*,

plus some loci from the donor *S. brevidens*. The morphology of the asymmetric hybrids was more similar to *S. tuberosum* than to *S. brevidens*. As shown in Tables 1 and 2, the asymmetric hybrid 10732 was found to have more incomplete chromosomes from *S. brevidens* and quite a few non-parental bands, which indicated extensive DNA rearrangements. This hybrid was characterized by very poor roots. Collectively, the asymmetric hybrids were less viable than either the fusion parents or the symmetric hybrids in terms of shoot regeneration, root formation and plant growth. A possible explanation for this is the unbalanced genome of the asymmetric hybrids where irradiation of the donor induced extensive DNA rearrangements. We have previously shown loss of vigor and abnormal morphology to be associated with aneuploidy (Pehu et al. 1989).

The *S. brevidens* parent appeared to be homozygous for all of the loci analyzed by the probes used in this study. This was supported by the finding that the asymmetric hybrids surveyed for the presence of the *S. brevidens* alleles, were found to contain either none or all of the bands from the parent. Similar results have been observed by Williams et al. (1990) and Williams and Helgeson (1991). In their study, they found that *S. brevidens* was homozygous for all but 3 of the 70 RFLP loci.

In conclusion, even with the limited number of fusion products surveyed, our studies showed that asymmetric somatic hybrids containing the recipient *S. tuberosum* genome plus a partial genome from the donor *S. brevidens* could be achieved by "gamma-fusion". However, limited chromosome elimination has been found and the irradiation resulted in extensive chromosome rearrangements and chromosome breaks. Hence, these are drawbacks to the application of this technology for practical breeding purposes. Analysis of a large population of asymmetric hybrids and use of higher doses (above 500 Gy) of donor irradiation will give a more accurate evaluation of the potential of the "gamma-fusion" technique in potato breeding.

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