Use of RAPD markers to screen somatic hybrids between *Solanum tuberosum* and *S. brevidens*

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Summary The identification of somatic hybrids between *Solanum tuberosum* and *S. brevidens* can be carried out using polymerase chain reaction (PCR) and arbitrary 10-mer primers to generate random amplified polymorphic DNA (RAPD) markers. Five commercial primers have been tested. Each primer directed the amplification of a genome-specific "fingerprint" for the fusion parents and *S. brevidens*. The size of the amplified DNA fragments ranged from 100 to 1800 base pairs. The somatic hybrids showed a combination of the parental banding profiles with four of the five primers surveyed, whereas regenerants from one of the parents had the same or a similar banding pattern to that of the parent. Thus RAPD markers provide a quick, simple and preliminary screening method for putative somatic hybrids.

Key Words. Somatic hybrids, DNA polymorphism, potato, *Solanum brevidens*, RAPD, PCR

Abbreviations: EDTA - ethylenediaminetetraacetic acid, PCR - polymerase chain reaction, RAPD - random amplified polymorphic DNA, RFLP - restriction fragment length polymorphisms, TBE - Tris-borate-EDTA buffer, Tris - trizma base

Introduction

Somatic hybridization is increasingly used to combine complete or partial genomes of sexually incompatible species as well as the reconstitution of hybrids that can be sexually produced in breeding programs (Fish et al. 1987, 1988; Jones 1988; Chaput et al. 1990; Bates 1992). Putative hybrid plants are generally identified by one or more of the following methods: intermediate morphology (Austin et al 1986; Fish et al. 1987, 1988), isozyme markers (Gieba et al. 1984; Chen et al. 1989), restriction fragment length polymorphisms (RFLPs) (Pehu et al. 1989; Pental et al. 1988) and species-specific probes (Saul and Potrykus 1984; Pehu et al. 1990). However, all of these methods rely on the use of full-size plants either for a comprehensive morphological study or mass extraction of DNA or proteins. These identification methods are time-consuming as is the growth period of the plant to maturity. A quick and simple method which could detect hybridity of fusion products at either the callus level or a juvenile stage of growth would be of great advantage for a preliminary screening in these somatic hybridization programs.

Williams et al. (1990) and Welsh and McClelland (1990) have recently reported the use of a PCR technique by which DNA sequences in total genomic DNA are amplified using arbitrary 10 base pair primers to generate randomly amplified polymorphic DNA (RAPD) markers. The amplification products are visualized by agarose gel electrophoresis and ethidium bromide staining. The bands produced are frequently polymorphic and inherited in a Mendelian fashion. This method is quick, inexpensive and can be used on minute quantities of material. In plants the technique has been successfully used for linkage map studies in tomato (Klein-Lankhorst et al. 1991) and conifers (Carlson et al. 1991) and also cultivar identification in Brassicas (Hu and Quiros 1991).

In this paper we report on the use of RAPD markers to analyze previously characterized somatic hybrids between *Solanum tuberosum* (dihaploid line PDH40) and *S. brevidens*, a non-tuber bearing wild species with multiple virus resistance (Valkonen et al. 1992).

Materials and Methods

Plant material: Five somatic hybrids between *S. tuberosum* and *S. brevidens* produced by Fish et al. (1987, 1988) and the parental species *S. tuberosum* PDH40 and *S. brevidens* were used. Two protoplast regenerants of *S. tuberosum* were also used, for full details see Table 1.

DNA extraction: The DNA extraction was carried out according to Edwards et al. (1991) with the following modifications. A small piece of leaf tissue was macerated in a...
1.5 ml Eppendorf tube, using a glass rod, at room temperature for 15 seconds. 400 μl of extraction buffer (100 mM Tris HCl pH 8.0, 500 mM NaCl, 50 mM EDTA, 0.7 μl/ml 2-mercaptoethanol) was added and the sample vortexed for 5 seconds. The extracts were centrifuged at 13,000 rpm for 1 minute and 350 μl of the supernatant was transferred to a fresh Eppendorf tube. 4μl of RNase (stock concentration 10 mg/ml) was added and the tube was incubated at 37 °C for 30 mins. 350 μl isopropanol was used to precipitate the DNA. After centrifugation, the pellet was dissolved in 100 μl sterile distilled water. 1 μl of this sample was used for a 25μl volume PCR reaction.

DNA amplification: Five commercial 10-mer primers (A02, A05, A10, A12 and A16) from Operon Technologies (Alameda, California) were used for PCR amplification. The sequences of the primers A02, A05, A10, A12 and A16 were 5'-TGCCGAGCTG-3', 5'-AGGGGTCTTG-3', 5'-GTGATCGCAG-3', 5'-TCGGCGATAG-3' and 5'-AGCCAGCGAA-3', respectively. The PCR amplification was following the protocol reported by Williams et al. (1990) with minor modifications. The reaction components were: 1x reaction buffer, 0.2 μM of dNTPs, 1 μM primer, 0.5 unit of Taq polymerase (Promega), 25ng of genomic DNA in sterile distilled water. The final volume for each amplification reaction was 25 μl. The PCR machine (PTC-10, MJ Research, Inc.) was programmed for an initial denaturation step of 95°C for 3 min 30s and then 40 cycles of 95°C for 35s, 35°C for 35s and 72°C for 1 min 20s. On completion, 10μl of each sample was loaded onto a 2.0% agarose gel and run in 1xTBE buffer at 4 V/cm for 5 hr. The DNA molecular marker VIII (Boehringer Mannheim) was used as a molecular weight standard.

Results

Five Operon primers were randomly selected to test the RAPD markers produced in the parental species, S. brevidens and PDH40. All of the primers produced 1-5 bands in both species, with distinct banding profiles for each, although one of the primers (A12) produced one common band in PDH40 and S. brevidens in addition to the species specific bands.

Of the five primers tested, four produced significantly different banding patterns to be of use in identifying the somatic hybrids between PDH40 and S. brevidens. Two examples are given in Fig. 1. The hybrids had a combination of both PDH40 and S. brevidens banding patterns, whereas the PDH40 protoplast regenerants had either the same banding profile as that of the parent, (lane 1 in Fig. 1b) or a very similar pattern (lane 1 in Fig. 1a).

When a 50:50 mixture of PDH40 and S. brevidens DNA was made and used in the same PCR reactions, the banding profiles produced were usually the sum of the bands of each parent.

However some of the parents’ weak bands disappeared in both the parental DNA mixture (lane m in Fig. 1a)

![Fig. 1. RAPD profiles generated by primer A10 (a) and A05 (b). Lane 1-2, regenerants from one fusion parent S. tuberosum PDH 40. Lane 3-7, somatic hybrids. Lane 8-9, fusion parents (see Table 1 for the details). m is a DNA mixture of parents. The size of the molecular standard is indicated.](image-url)