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Research Note

Detection of somaclonal variation in grapevine regenerants from protoplasts by RAPD-PCR

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Introduction: Recently regeneration of plants from grapevine protoplasts (*Vitis* sp. "Seyval blanc") has been reported for the first time (REUSTLE *et al.* 1995). This way of regeneration involves prolonged tissue culture with the intrinsic potential of provoking somaclonal variation in other plant species (LARKIN and SCOWCRAFT 1981). We wanted to address the question, if somaclonal variation may be observed in these grapevine regenerants. Hence RAPD-PCR (WILLIAMS *et al.* 1993) was chosen as a method permitting easy and rapid screening for genetic differences. This technique was applied using 60 different 10mer primers in PCR amplifications on 47 grapevine plants, each one regenerated from a single protoplast (protoclone).

Materials and methods: 47 randomly selected shoots of 150 *in vitro* plants representing individual protoclones were frozen in liquid nitrogen, ground to powder and used for DNA extraction according to THOMAS *et al.* 1993. As controls we used *in vitro* grape regenerants from leaf disc-derived somatic embryos and green cuttings of Seyval blanc. For quantification the plant DNA was visually compared to standard DNA fragments on agarose gels (Serva, Heidelberg, Germany; 0.6 % in 89 mM Tris-borate buffer, pH 8.3) and adjusted to 20 ng/µl with TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0). RAPD-PCR essentially followed the procedure of BÜSCHER *et al.* (1993). Primer kits M, O and U from Operon Technologies Inc. (Alameda, CA, USA) were used. *Taq* DNA polymerase was obtained from Eurogentec, Seraing, Belgium, and used at 0.5 U/50 µl reaction. Amplification products were resolved on 1.5 % agarose gels made and run in 0.5 TBE at 7 V/cm, stained for 10 min in 0.5 µg ethidium bromide/ml and documented on Polaroid 665 film.

Hybridization of amplification products was performed after their gelelectrophoretic resolution. For genomic hybridization grapevine DNA was cut with an excess of restriction enzyme *Eco*RI (Boehringer Mannheim, Germany) and the fragments separated on 0.8 % agarose gels. In both cases, DNA was blotted onto Hybond N (Amersham Buchler, Braunschweig, Germany) according to standard protocols (SOUTHERN 1975; SAMBROOK *et al.* 1989). As probe we used a specific amplification product, purified by electroelution from an agarose gel into TE buffer in dialysis bags and labeled with Digoxigenin using the DIG-Labeling Kit (Boehringer Mannheim). Hybridization and detection were carried out according to the specifications of Boehringer. Colorimetric detection with NBT/X-Phosphate or chemiluminescence (recorded on AGFA "Curix" film) were applied using the Boehringer DIG-system.

Results and discussion: Almost all 10mer primers (59 out of 60) yielded RAPD products depending on the individual primer. Between 5 and 20 bands in a size range of 50 to 8500 bp could be resolved on agarose. Only primer OP-O17 did not yield any product. Control and protoclone DNAs produced identical band patterns with 58 primers. One primer however, OP-O11 (5'-GACAGGAGGT-3'), indicated somaclonal variation as an amplification product of about 1680 bp was found to be missing in three distinct protoclones (Fig. 1). The absence of this product

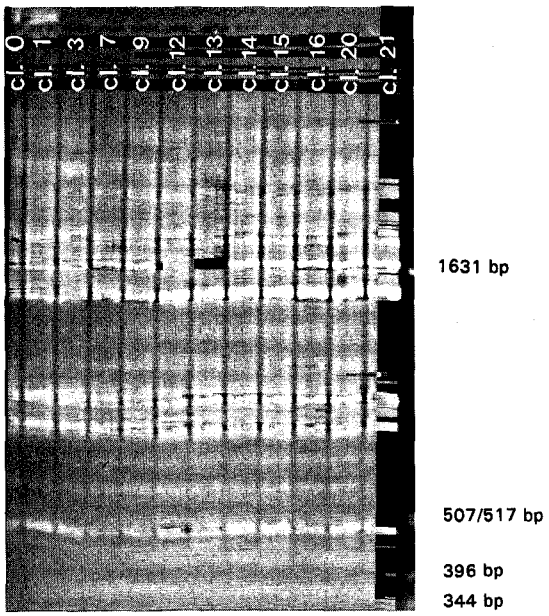

Fig. 1: Amplification products of primer OP-O11 resolved on 1.5 % agarose. Amplified DNA products are from 12 different protoclones. Molecular weight markers are indicated on the right hand side. Clones 12 and 13 showed absence of one DNA amplification product (1680 bp).

was verified by blotting total OP-O11 amplification products and hybridization with the 1680 bp product purified from control amplifications as probe (Fig. 2). In genomic hybridizations, this probe hybridized to the same *Eco*RI fragment in controls and in the protoclones which did not yield this amplification product in RAPD-PCR (Fig. 3). This result indicates, that only a very small genetic change on the primer binding site is responsible for the missing amplification product. Most likely one of the OP-O11 primer binding sites flanking the 1680 bp amplified region was hit by this mutation. This is the first time that molecular evidence is provided for somaclonal variation in grapevine. Moreover, the protoclones affected by this mutation also possess an altered phenotype, as they show strongly increased formation of axillary shoots compared to the control plants and to other protoclones (Fig. 4).

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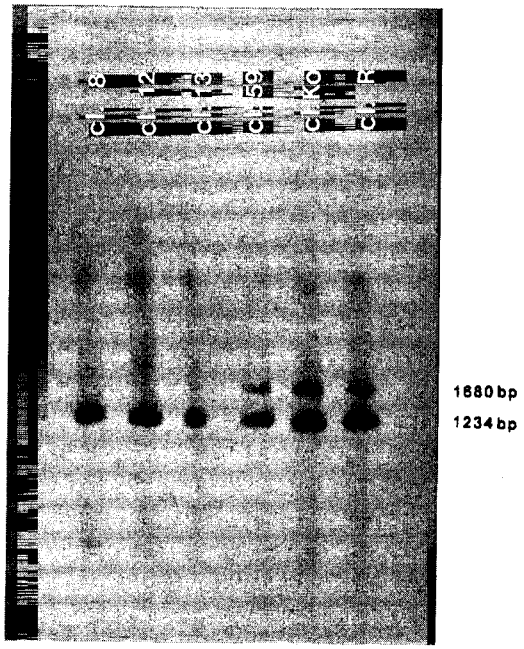

Fig.2: Hybridization of the 1680 bp amplification product missing in the somaclonal mutants (8, 12, 13) to their total OP-O11 RAPD-PCR bands. Clone 59 is an unchanged protoclone, Ko and R are control plants.

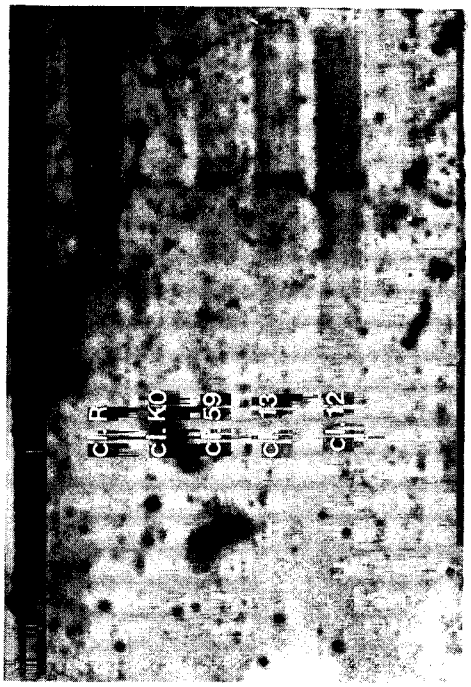

Fig.3: Hybridization of the 1680 bp amplification product missing in the somaclonal mutants to the genomic DNA (*Eco*RI digest).

The three somaclonal mutants all arose from individual protoplasts. However, their common origin from tissue mutated before its use for protoplast preparation is likely. A putative correlation between the altered phenotype and the mutation therefore remains to be investigated.

Fig.4: Phenotype of Seyval blanc regenerants from protoplasts: unchanged (right), one of the somaclonal mutants (left).

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