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Genomic DNA fingerprinting of *Vitis vinifera* by the use of multi-loci probes[1])

by

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Charakterisierung genomischer DNA von *Vitis vinifera* mit Hilfe von „Multi-loci-Sonden"

Zusammenfassung: Durch Southern-Transfer von Restriktionsfragmenten genomischer DNA aus *Vitis vinifera* ließen sich Unterschiede zwischen verschiedenen Rebsorten aufzeigen; für die Hybridisierung wurden Sonden aus M 13-DNA und Human-33.6-Minisatelliten-DNA verwendet. Die Restriktionsenzyme *Hinf* I und *Hae* III waren für die Bestimmung sortenspezifischer DNA-Muster gleichermaßen geeignet. Die M 13-Sonde identifizierte in Verbindung mit beiden Enzymen etwa 3mal soviele Banden und zeigte viel mehr Abweichungen zwischen den Sorten auf als die Human-Minisatelliten-Sonde.

Das Differenzierungsverfahren kann bei der Rebe zur Analyse der Abstammung, zur Definition von Merkmalen bei sortengeschützten Reben sowie für Züchtungsprogramme eingesetzt werden, sobald Korrelationen mit wirtschaftlich wichtigen Eigenschaften gesichert sind.

Key words: variety of vine, genome, DNA, analysis, restriction fragment length polymorphism, molecular genetics, taxonomy.

Introduction

Until now isoenzyme polymorphism was used for positive identification of grape cultivars (WEEDEN *et al.* 1988; PARFITT and ARULSEKAR 1989) to provide genetic data to supplement morphological data and to confirm the difference between two closely related varieties (U.S. Plant Pat. No. 3,106, U.S. Plant Pat. No. 5,151).

The introduction of restriction fragment length polymorphism (RFLP) methodologies to plants has significantly improved the capability to discover genetic molecular markers (BECKMANN and SOLLER 1986; PATERSON *et al.* 1988). A major development in this area was the recent discovery of minisatellite DNA sequences (JEFFREYS *et al.* 1985). These sequences are shared between many organisms as hypervariable tandem repeats of a 'core' DNA sequence which are often dispersed throughout the genome and produce polyallelic marker loci. The M 13 DNA (VASSART *et al.* 1987) and the human minisatellite probes (JEFFREYS *et al.* 1985) were found to detect DNA fingerprints in a wide range of organisms. This was demonstrated first in human and animals genomes (JEFFREYS *et al.* 1985; VASSART *et al.* 1987; NAKAMURA *et al.* 1987; GEORGES *et al.* 1988) and in plants (DALLAS 1988; RYSKOV *et al.* 1988, NYBOM *et al.* 1990). In the experiments described by us, the capability of such minisatellite DNA probes to uncover polymorphism in *Vitis vinifera* was tested. Results show that cultivar-specific DNA fingerprints were detected with either of the two probes tested.

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Materials and methods

Grapevine material: Seven genetically unrelated table grape cultivars and selections were chosen for screening. Variation in traits such as seedlessness, colour and origin was considered. These cultivars were: (1) Elul, (SPIEGEL-ROY *et al.* 1983), (2) Sultanina, (3) Dan Ben Hanna, (4) Flame Seedless, (5) Muscat Hamburg, (6) Alphonse Lavallée, (7) Dattier de Beyrouth.

Preparation of Southern blots: DNA was prepared from fresh leaves, collected in the vineyard from several vegetatively propagated plants of each cultivar. Several procedures were tested for efficient extraction of digestible DNA, and the procedure of DELLAPORTA *et al.* (1983) was chosen. Approximately 8—10 µg per lane of total DNA was digested overnight with 40 units of restriction enzyme. DNA was separated on 1 % agarose gel at 3 V/cm (AUSUBEL *et al.* 1987). Southern blotting onto Hybond-N (Amersham UK) nylon membrane was made according to the manufacturer's protocol.

Probe preparation and hybridization: Linearized M 13 DNA and the 33.6 minisatellite probes (JEFFREYS *et al.* 1985) were gel-purified and labelled with ^{32}P[αdCTP] to a specific activity of about 10^9 dpm/µg by a random priming labelling kit (Boehringer) developed by FEINBERG and VOGELSTEIN (1983), and used as recommended by Boehringer. Hybridization was performed for 20 h at 42 °C in hybridization mix containing 5 × SSC, 10 × Denhardt's 0.05 M phosphate buffer, 0.5 % blocking reagent (Boehringer), and 30 % formamide.

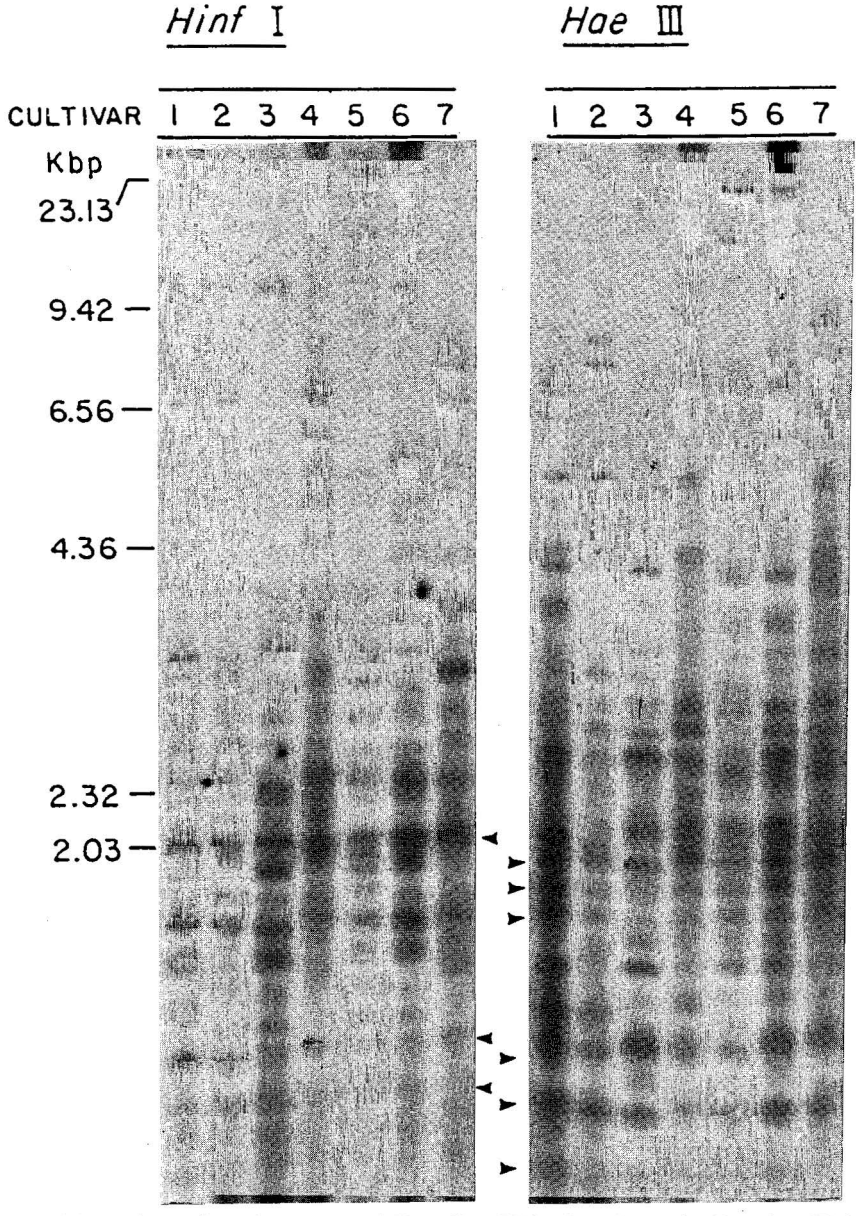
Visualization: Blots were washed twice, with 2 × SSC + 0.1 % SDS at 42 °C for 20 min, and then subjected to X-ray autoradiography for several days, using Agfa RP-2 films. Rehybridization with the 33.6 probe was carried out after strip-wash of the blot according to Amersham's protocol.

Results and discussion

The use of new molecular genetic methodologies to generate markers for a wide range of applications in plants has been discussed (BECKMANN and SOLLER 1986, ALLEWELDT and POSSINGHAM 1988, ROOSE 1988). So far, ongoing breeding programs in grapes utilized mainly morphological (SPIEGEL-ROY 1980), biochemical (BACHMANN and BLAICH 1988), and isozyme (WEEDEN *et al.* 1988; PARFITT and ARULSEKAR 1989) markers.

Seven vegetatively propagated cultivars were subjected to Southern blot analysis with two multi-loci minisatellite probes. As shown in the figure, highly polymorphic patterns were detected when the DNA was hybridized with the M 13 probe. When the *Hinf* I restriction enzyme was used, only 3 bands out of 42 were common to all cultivars. Similarly using the *Hae* III digest only 6 bands out of 37 were common to all cultivars. Bands common to all cultivars were in general smaller than 2 kb in size. The total number of bands per cultivar ranged between 13—27 and 15—21 in the *Hinf* I and *Hae* III digest, respectively. Following strip-washing, the same blot was rehybridized with the 33.6 probe. This resulted in the detection of significantly less bands per lane (not shown due to loss of DNA from the filter by the strip wash). In addition, less variations between cultivars were observed. Polymorphism was uncovered between additional cultivars, using more enzymes, by the procedure described.

We demonstrated the use of multi-loci DNA probes in order to uncover polymorphism between grape cultivars. Some of the bands were detected with both probes.



Hypervariable polymorphism in patterns of *V. vinifera* DNA when digested with either *Hinf* I or *Hae* III endonucleases. Hybridization with M 13 phage DNA probe. Bands common to all cultivars are indicated by arrows. — Grape cultivars: (1) Elul, (2) Sultanina, (3) Dan Ben Hanna, (4) Flame Seedless, (5) Muscat Hamburg, (6) Alphonse Lavallée, (7) Dattier de Beyrouth.

Hypervariabler Polymorphismus in DNA-Bandenmustern von *V. vinifera* nach Behandlung mit den Restriktionsenzymen *Hinf* I oder *Hae* III. Hybridisierung mit M 13-DNA-Sonde. Banden, die in allen Rebsorten auftreten, sind durch Pfeile gekennzeichnet. — Rebsorten s.o.

This suggests that either the same restriction fragment, or coincidental co-migration of two (or more) fragments were detected by these probes. Even so, cultivar identification can be achieved in each probe/enzyme combination. Coincidental co-migration of bands and allelic relationship are still unknown, and will be addressed in F_1 segregating population analysis. At this stage of analysis simple correlation of traits between cultivars with the resulting polymorphism could not be detected.

Our results indicate that the two multi-loci probes, the M 13 phage DNA and the human minisatellite 33.6, detect cultivar specific DNA fingerprints in *V. vinifera* genomic DNA digested by either of the two restriction endonucleases *Hinf* I, *Hae* III.

The demonstration of highly polymorphic patterns within the same genus, using minisatellite probes, will enable cultivar identification analysis, paternity analysis as well as introduction of linkage mapping into ongoing breeding programs.

Summary

Grape cultivars were differentiated using the M 13 phage DNA and a human minisatellite DNA probe for hybridization of Southern blots with *Vitis vinifera* genomic DNA. Cultivar specific DNA fingerprints were detected with either of two restriction enzymes used (*Hinf* I, *Hae* III). The M 13 probe recognized about 3 times more bands and uncovered much more variation between cultivars, with both enzymes, when compared with the human 33.6 minisatellite probe.

This potentially efficient tool to uncover polymorphism can be used in grapes for paternity analysis, to produce markers for patent protection of breeders' rights, and to aid breeding programs, as soon as linkage with economically important traits will be established.

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