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Identification of sixteen grapevine rootstocks by RFLP and RFLP analysis of nuclear DNA extracted from the wood[1])

by

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Identification de 16 porte-greffes de vigne par analyse RFLP, et analyse RFLP d'ADN nucléaire extrait à partir du bois

Résumé : Nous avons identifié 16 porte-greffes du genre *Vitis* par la méthodologie d'analyse du polymorphisme de longueur des fragments de restriction (RFLP) de l'ADN avec l'enzyme de restriction *Hinf* I. Pour cela, nous avons utilisé comme sondes des fragments d'ADN uniques ou peu répétés du génome de la variété Chardonnay de *Vitis vinifera*. L'analyse RFLP de cinq clones de SO 4 (*V. berlandieri* × *V. riparia*) et de trois clones de 41 B Mgt (*V. berlandieri* × *V. vinifera*) avec quatre sondes RFLP et l'enzyme *Hinf* I n'a pas permis de différencier les clones d'un même hybride, dont les génomes sont extrêmement proches puisqu'ils sont obtenus de façon végétative. Nous présentons aussi une méthode simple d'extraction d'ADN nucléaire à partir du bois de vigne. Les analyses RFLP de cet ADN ont donné des résultats identiques à ceux obtenus avec l'ADN des feuilles. Nous sommes maintenant en mesure de proposer que la méthodologie d'analyse RFLP complète ou remplace dans certains cas les méthodes ampélographiques d'identification des porte-greffes du genre *Vitis*. L'élargissement de cette application est envisagé pour les variétés de *Vitis vinifera*.

Key words: grapevine, rootstock, RFLP, identification, nuclear DNA.

Introduction

Varieties, hybrids and species of the genus *Vitis* are identified by their ampelographical characteristics (GALET 1956). The usual identification methods can be extended by molecular analysis methodologies: in several studies, the variability of the isoenzyme or of the pollen wall proteins electrophoresis patterns has been exploited for *Vitis vinifera* (WOLFE 1976; SUBDEN *et al.* 1987; BENIN *et al.* 1988; TEDESCO *et al.* 1989). To circumvent many problems of unreliable identification criteria due to these methodologies, the use of stable genetic markers has been proposed (SOLLER and BECKMANN 1983). A DNA analysis method called DNA restriction fragment length polymorphism (RFLP) analysis allows identification by using highly variable genomic DNA sequences; with this method, varieties of *Solanum tuberosum* ssp. *tuberosum* can be distinguished for example (GEBHARDT *et al.* 1989). The RFLP methodology is independent of the environment and reproducible. It has already been applied to genomic DNA from *V. vinifera* varieties by using heterologous probes derived from phage M13 or the human probe 33.6 (STRIEM *et al.* 1990). Another study described the use of the heterologous cDNA of

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the phenylalanine ammonia-lyase (PAL) gene of *Daucus carota*, which can distinguish between different cultivars of *V. vinifera* and the rootstock variety 3309 Couderc (YAMAMOTO *et al.* 1991).

In an earlier report we have demonstrated the usefulness of unique or lowly repeated sequences of the *V. vinifera* genome (Chardonnay variety) to identify 9 grapevine rootstock varieties by RFLP analysis (BOURQUIN *et al.* 1991). We present here an extension of this study to 16 rootstock varieties, and the analysis of five SO 4 (*V. berlandieri* × *V. riparia*) clones and three 41 B Mgt (*V. berlandieri* × *V. vinifera*) clones. In addition, we describe a technique to isolate nuclear DNA from grapevine wood. This DNA is of sufficient quality to be used in RFLP analysis.

Material and methods

Plant material

Some woody twigs harvested after the vegetative period of the following rootstocks were provided by the Etablissement National Technique pour l'Amélioration de la Viticulture (ENTAV) at le Grau du Roi: 41 B Mgt (*V. berlandieri* × *V. vinifera*), clones 195 and 153; Fercal [(*V. berlandieri* × *V. vinifera*) × (*V. berlandieri* × *V. vinifera*)], clone 242; *V. riparia* Gloire, clone 1; SO 4 (*V. berlandieri* × *V. riparia*), clones 5 and 102; 420 A (*V. berlandieri* × *V. riparia*), clone 11; Vialla (*V. labrusca* × *V. riparia*), clone 116T1; *V. rupestris* du Lot, clone 110; 110 Richter (*V. berlandieri* × *V. rupestris*), clone 118; 140 Ruggeri (*V. berlandieri* × *V. rupestris*), clone 101; 1103 Paulsen (*V. berlandieri* × *V. rupestris*), clone 113; 99 Richter (*V. berlandieri* × *V. rupestris*), clone 96; 196-17 Castel [(*V. rupestris* × *V. vinifera*) × *V. riparia*], clone 99. The SO 4 clones 165, 166 and 157, 41 B Mgt clone 172, Kober 5 BB (*V. berlandieri* × *V. riparia*), clone 259; 161-49 Couderc (*V. berlandieri* × *V. riparia*), clone 171, 3309 Couderc (*V. riparia* × *V. rupestris*), clone 173; LN33 (1613 Couderc × Thompson Seedless), clone D, have been obtained from the Laboratory of Grapevine Pathology of the INRA Institute at Colmar. Each rootstock was cultured in the greenhouse by forcing a part of these twigs in a mixture of 50 % sand and 50 % perlite. Leaves obtained from the rootstocks were stored at −80 °C.

Purification of nuclear DNA

Leaf nuclear DNA was extracted as described (BOURQUIN *et al.* 1991) with one modification: leaves were cut with an electrical vegetable cutter, and subsequently grinded in a mortar in the presence of extraction buffer (50 ml for 10 g of leaves).

To extract nuclear DNA from woody tissue, the epidermis of a woody twig was removed and the cortical parenchyma was scraped with a scalpel. Nuclear DNA was extracted from the shavings in the same way as from the leaves.

Preparation of probes

Pst I restriction fragments of nuclear DNA of the Chardonnay *V. vinifera* variety were used as probes (BOURQUIN *et al.* 1991). Fragments were labelled with ^{32}P-α-dCTP according to FEINBERG and VOGELSTEIN (1983).

DNA restriction, electrophoresis, electrotransfer, hybridization and autoradiography

All analyses were done with the restriction enzyme *Hinf* I. Restriction fragments were separated by vertical electrophoresis on denaturing 4 % Long Ranger gels

(Serva), 140 × 170 × 1 mm. Electrophoresis was stopped when the bromophenol blue marker had reached the bottom of the gel. Additional details and procedures for electrotransfer, hybridization and autoradiography are precised in GEBHARDT *et al.* (1989), and BOURQUIN *et al.* (1991).

Results

Preselection of hybridization probes

To save time and material, interesting probes were preselected with a small representative group of 6 rootstocks: SO 4, Kober 5 BB and 420 A (*V. berlandieri* × *V. riparia*), 110 Richter (*V. berlandieri* × *V. rupestris*), Vialla (*V. labrusca* × *V. riparia*) and 41 B Mgt (*V. berlandieri* × *V. vinifera*). 32 Chardonnay nuclear DNA fragments were tested on *Hinf* I digested nuclear DNAs of this group. 10 DNA fragments (CGMUMM3 to CGMUMM12) which displayed a sufficient level of polymorphism were selected. In addition, the 2 probes CGMUMM1 and CGMUMM2 used earlier (BOURQUIN *et al.* 1991) were added to this group.

Selection of identification probes

CGMUMM1 to CGMUMM12 were tested on *Hinf* I digested nuclear DNAs of the 16 rootstock varieties (Material and methods). Probes were selected in such a way that the 16 rootstocks would be distinguished and would yield easily identified patterns. 7 probes did not fulfill these criteria: 5 probes displayed too little variation or too little resolution under the electrophoresis conditions used; 2 probes revealed too complex hybridization patterns. The remaining 5 probes were useful. The patterns revealed by CGMUMM3 (Figure, left) are different for 11 out of 16 rootstock varieties. A and G on one side, and D, N and F on the other side cannot be distinguished. CGMUMM4 (Figure, right) permits identification of 8 out of 16 rootstocks. A and G, C and O, K and L, M and N are indistinguishable with this probe; D and E show an additional weak band of small size which is cut off in the Figure. CGMUMM5 is somewhat more complex: strong bands permit classification of rootstocks in 6 groups A; B; C, F, G, J; D, E, M, N; H, I, O, P; K, L. Weak polymorphic bands of large size are also observed. The 16 rootstocks can be distinguished with the 3 probes CGMUMM3, 4 and 5. A and G can be identified by CGMUMM5; D, N, F by CGMUMM4. If the weak bands revealed by CGMUMM5 are also used, this probe distinguishes nearly all rootstock varieties. Finally, it is possible to identify the 16 rootstocks when combining differently some selected probes.

Assay of clones identification

The 5 clones 5, 102, 157, 165, and 166 of SO 4 and the 3 clones 153, 172, 195 of 41 B Mgt were investigated with CGMUMM2, 3, 6, and the complex probe CGMUMM13 which revealed 15 to 18 bands in SO 4 and 41 B Mgt. These probes did not reveal any polymorphism between the clones of a same hybrid (results not shown).

RFLP analysis with DNA extracted from woody tissue

Identification of rootstock materials by RFLP would be greatly simplified if analysis could be done directly on the woody tissues of the rootstocks. A method was therefore developed to extract nuclear DNA from woody tissues (Material and methods). The quality of the DNA and the hybridization patterns were compared with those of the DNA obtained from leaf material: CGMUMM2 and 6 were tested on *Hinf* I digested

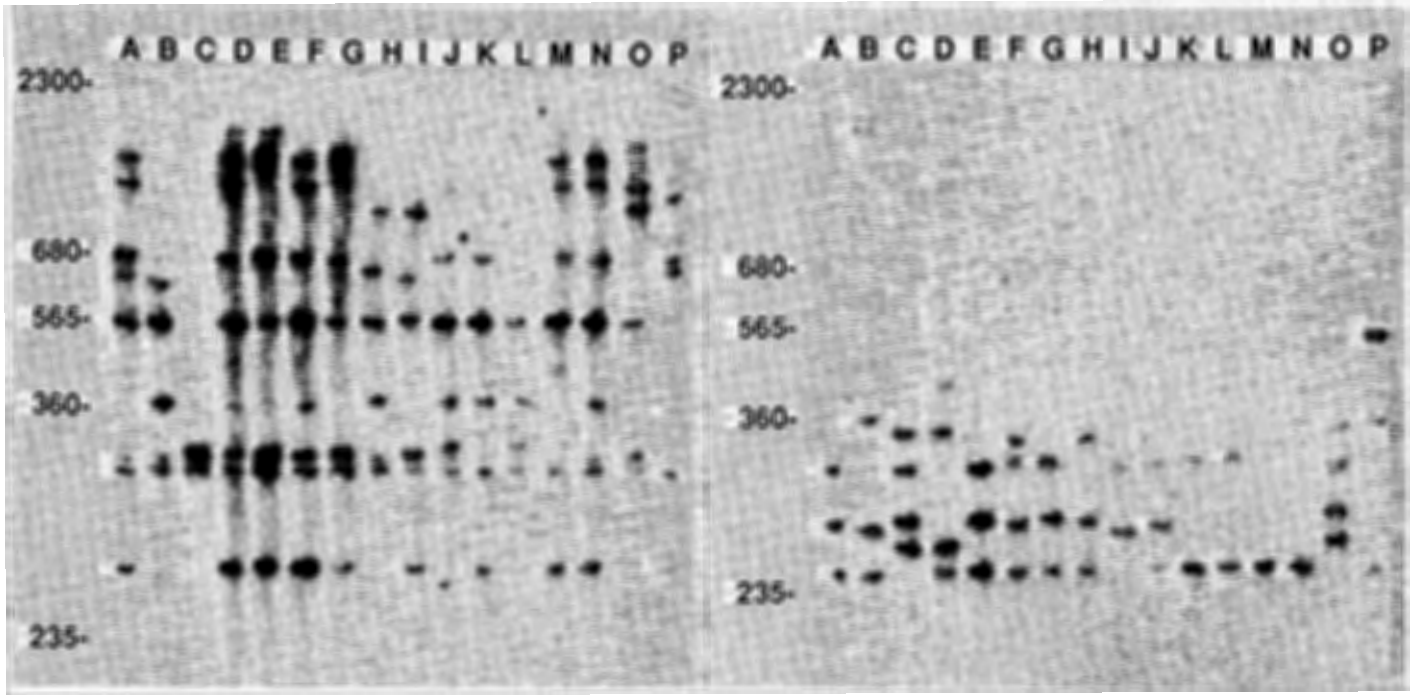

Figure: RFLP analysis of 16 grapevine rootstocks with the restriction enzyme *Hinf* I and the Chardonnay probe CGMUMM3 (left) and CGMUMM4 (right). A: 41 B Mgt (*V. berlandieri* × *V. vinifera*), clone 195; B: Fercal [(*V. berlandieri* × *V. vinifera*) × (*V. berlandieri* × *V. vinifera*)]; C: *V. riparia* Gloire; D: SO 4 (*V. berlandieri* × *V. riparia*), clone 5; E: Kober 5 BB (*V. berlandieri* × *V. riparia*); F: 420 A (*V. berlandieri* × *V. riparia*); G: 161-49 Couderc (*V. berlandieri* × *V. riparia*); H: Vialla (*V. labrusca* × *V. riparia*); I: 3309 Couderc (*V. riparia* × *V. rupestris*); J: *V. rupestris* du Lot; K: 110 Richter (*V. berlandieri* × *V. rupestris*); L: 140 Ruggeri (*V. berlandieri* × *V. rupestris*); M: 1103 Paulsen (*V. berlandieri* × *V. rupestris*); N: 99 Richter (*V. berlandieri* × *V. rupestris*); O: 196-17 Castel [(*V. rupestris* × *V. vinifera*) × *V. riparia*]; P: LN33 (1613 Couderc × Thompson Seedless). The length of the restriction fragments is indicated in bases pairs numbers.

Analyse RFLP de 16 porte-greffes de vigne avec l'enzyme de restriction *Hinf* I et la sonde de Chardonnay CGMUMM3 (à gauche) et CGMUMM4 (à droite). Pour le détail des pistes A à P, *cf.* la liste ci-dessus. La longueur des fragments de restriction est indiquée en nombres de paires de bases.

DNA of the 16 rootstock varieties, and DNA from woody and leaf material were also compared in the 4 experiments with SO 4 and 41 B Mgt clones. No significant differences were observed.

Discussion

The RFLP results presented here confirm the earlier observed high degree of polymorphism in *Vitis* rootstock hybrids, which can be revealed by digestion with *Hinf* I. We anticipate that a similar degree of variation will be obtained for the different *Vitis* species from which the hybrids were derived. With our RFLP analysis methodology, we are now able to distinguish efficiently the majority of the commercialized rootstocks. This method has the advantage of being independent of external factors and to be highly reproducible. Clones of a same variety could so far not be distinguished by RFLP analysis, which is not surprising, since these clones were obtained by vegetative multiplication and therefore genetically extremely similar. But this property of the RFLP identification probes demonstrates interestingly their varietal specificity. It may be possible to distinguish clones with the use of highly repeated sequences.

A simple DNA extraction method for rootstock woody tissues enables analysis of commercially traded rootstock materials without the necessity of inducing the growth of leaves. In our experiments, wood extracted DNA shows the same hybridization patterns as leaf DNA.

We believe that RFLP analysis will be a useful method to complement the commonly used ampelographical identification methods, not only for rootstocks but also for *Vitis vinifera* varieties. In some cases it may replace the traditional methods.

Summary

Sixteen rootstocks of the *Vitis* genus have been identified by the RFLP analysis methodology with the restriction enzyme *Hinf* I. Uniques or moderately repeated DNA sequences of the nuclear genome of the Chardonnay *V. vinifera* variety were used as probes. RFLP analysis of 5 clones of SO 4 (*V. berlandieri* × *V. riparia*) and of 3 clones of 41 B Mgt (*V. berlandieri* × *V. vinifera*) with 4 probes and *Hinf* I did not lead to any polymorphism. This is not surprising because of the vegetative origin of the clones. A simple method of nuclear DNA extraction of wood is described for the grapevine. We propose now the RFLP analysis methodology to complement or to replace in certain cases the ampelographical methods of identification of the rootstocks. The extension of this application to the *V. vinifera* varieties is considered.

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