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**Research Note**

# Developing molecular genetic markers for grape breeding, using polymerase chain reaction procedures

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K e y w o r d s : Vitis vinifera, random-amplified-polymorphic-DNA, seedlessness, berry color, muscat flavor.

Stenospermocarpic seedlessness in grapes has been discussed in detail by PEARSON (1932) and STOUT (1936), and recently by RAMMING *et al.* (1990), SPIEGEL-ROY *et al.* (1990), and LEDBETTER and BURGOS (1993). A classification of seeded *vs.* seedless individuals is influenced and biased also by several extraneous factors such as berry firmness. Although few genetic factors are assumed to be involved (SPIEGEL-ROY 1979; LEDBETTER and RAMMING 1989), it is hard to overlook the quantitative nature of seed development and its response to environmental effects. We compared several characters of seedlessness in an Early-Muscat x Flame-Seedless progeny. The progeny segregates as to the seedlessness subtraits, as well as to berry colour and muscat flavor. For the present study we analysed seven variables representing traits of seedlessness: mean fresh weight of one seed, total fresh weight of seeds per berry; seed contents evaluated by perceptibility (SPIEGEL-ROY 1979); four seed size categories visually evaluated; degree of hardness of the seed coat; degree of development of the endosperm and degree of development of the embryo (STRIEM *et al.* 1992).

Excluding seeded offsprings at an early stage would be of great value to the breeder concerned with a program aimed at seedlessness. This will be facilitated by establishing a linkage between this trait and reliable markers.

Identification of linkage between DNA markers and loci controlling quantitative traits (QTLs) is rather complicated, requiring a large number of polymorphic markers (BECKMANN and SOLLER 1986). Linear Model Analysis has been applied to quantify the effects of QTL-associated bands.

A new genetic assay to detect nucleotide sequence polymorphisms by polymerase chain reaction (PCR) procedures has been developed (WELSH and MCCLELLAND 1990; WILLIAMS *et al.* 1990). Using the technique for producing molecular genetic markers, more than 160 different 10mer primers (Operon kits A, B, C, E, F, G, H, J, and other primers) were tested by us, some of which were highly polymorphic. Band patterns of two primers, successfully differentiated between 24 different *V. vinifera* grape cultivars examined (STRIEM *et al.*, unpublished, 1992), confirming the suitability of this technique to uncover genetic variability among grape cultivars which was also demonstrated by others (GOGORCENA *et al.* 1993; BÜSCHER *et al.* 1993; JEAN JAQUES *et al.* 1993).

**Results and discussion:** 110 of the primers tested, gave a distinct band pattern. 17 primers distinguished between two closely related cultivars, one is a mutant of the other (Sultanina *vs.* Seeded Sultanina). Bandsharing ratio of 96.1 % was found in this pair, compared to 78.5 % bandsharing ratio between two nonrelated cultivars (Early-Muscat *vs.* Flame-Seedless). 78 primers which uncovered polymorphism between Early-Muscat and Flame-Seedless were tested with 82 individuals of the progeny resulting from the cross between these cultivars. Three traits which segregate in the progeny were investigated: seedlessness, muscat flavor and berry color. More than 400 polymorphic markers were identified. 12 markers were statistically analysed, using SAS procedures.

Significant correlations with several of the seven subtraits of seedlessness were obtained, mainly with the quantitative ones. For example: primer OP-E10, band

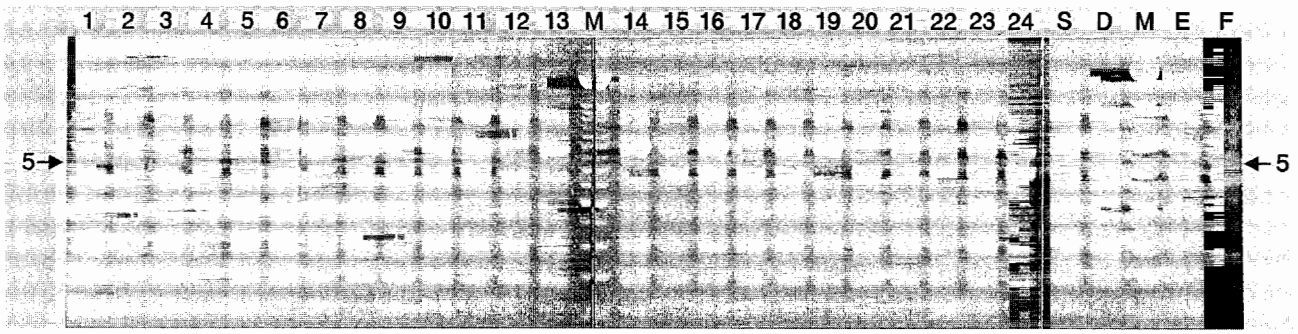


Figure: Electrophoretic separation of RAPD amplification products, using primer OP-E10 (CACCAGGTGA). Lanes 1-24: different individuals of the progeny from the cross of Early-Muscat (E), with Flame-Seedless (F). Marker (M): 100 bp DNA ladder (BRL). (S) DNA mixture of 10 seedless individuals, (D) DNA mixture of 10 seeded individuals. Band #5 (indicated) gave high correlation coeffcients with seedlessness subtraits. DNA was extracted from young grape leaves following the procedure given by LODHI *et al.* (1993). – Conditions for amplification: The PCR mixture contained 10-20 ng template DNA in a 25 µl reaction volume with 2.5 µl 10x buffer (Promega: 10 mM Tris HCl pH 8.0, 50 mM KCl, 0.1 % triton-x100) 2.0 mM $MgCl_2$, 100 µM each dNTP and 1 unit *Taq* polymerase (Promega) and 0.2 µM primer, covered with a drop of mineral oil (Sigma). Amplification was performed in a MJ-Research (PTC-60) thermal cycler for 37 cycles, after initial denaturation for 30 s at 95 °C, one cycle of 1 min at 94 °C, 1 min at 35 °C and 2 min at 72 °C, was followed by 36 cycles consisting of 30 s at 94 °C, 1 min at 35 °C, and 1 min 45 s at 72 °C. Amplification products were resolved by electrophoresis at 10 V/cm in a 2 % NuSieve agarose 3:1 gel, in TAE buffer.

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number 5 (size of about 900 bp, indicated in the Figure), is present in Flame-Seedless, and also in a mixture of DNAs of 10 seedless offsprings, and absent in Early-Muscat band pattern ($r = 0.411$, $P = 0.0017$ with total fresh weight of seeds per berry).

By analysing several markers we found that the quantitative traits (concerning the fresh weight of seeds), had higher correlation coefficients and linear effects than the qualitative traits (evaluation of the seed content by size and percetibility, and the degree of development of the seed components). Multiple linear regression analysis resulted in higher coefficients with the quantitative traits when 7 markers as independent variables were included in the model ($R = 0.779$, with total fresh weight of seeds per berry).

Significant correlations with muscat flavor were found with 11 markers (for example: $r = 0.575$ with marker 62.12), and 16 markers showed significant correlations with berry color (for example: $r = -0.508$ with marker 91.7). In multiple linear regression analysis, when 4 markers were included in the model, correlations were $R = 0.935$ for muscat flavor and $R = 0.898$ for berry color. It could be suggested that these higher correlations with the taste and color are due to fewer genes involved in controlling these traits than that of seedlessness.

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