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# Genotype assessment of grape regenerants from floral explants

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## Summary

**A molecular typing of regenerant vines based on co-dominant simple sequence repeat (SSR) markers was applied for checking recombination events during somatic embryogenesis from floral explants. Twenty-one samples of somatic embryos and plantlets from embryogenic callus of both anthers and ovaries of the *V. vinifera* cv. Chardonnay, the rootstock Kober 125 AA, and the accession *V. rupestris* du Lot were randomly chosen from a number of regenerant lines. The genotype at polymorphic VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79 loci was produced and compared with reference patterns. No recombination events were detected in the cells involved in the somatic embryogenesis induction of all the checked samples, since all of them generated the same SSR profile of the grape variety from which explants were isolated.**

K e y w o r d s : grape, recombination, somatic embryogenesis, SSR markers.

## Introduction

Among the various tissues exploited, anthers and ovaries resulted the most valuable source for somatic embryogenesis induction in the *Vitis* genus (Martinelli and Gribaudo 2001). Anthers have been initially attempted for dihaploid plant recovering in genetic improvement programs (Gresshoff and Doy 1974; Hirabayashi *et al.* 1976; Zou and Li 1981; Cao 1990), and are still the most widely applied organs for grapevine somatic embryogenesis initiation (Martinelli and Gribaudo 2001). Even less adopted, ovaries also proved to be suitable material for establishing somatic embryogenesis and, according to our experience, in most cases gave higher efficiencies than anthers (Martinelli *et al.* 2001, 2003).

When applying flower explants, the involvement of sexual cells in the regeneration is possible, thus recombination resulting from likely meiotic events could occur. As a consequence, possibly recombination events require an accurate checking.

In grape, it is generally accepted that anther-derived embryos have a somatic origin (Altamura *et al.* 1992; Faure *et al.* 1996), however participation of haploid pollinic cells has been documented (Zou and Li 1981; Rajasekaran and Mullins 1983; Altamura *et al.* 1992). Besides, callusing has been described as nucellar origin when ovules are adopted (Srinivasan and Mullins 1980); however, there is a lack of detailed studies on ontogeny of grape somatic embryogenesis from ovaries.

Usually, histology and cytology are the adopted tools for assessing the involvement of meiotic cells during morphogenesis (Rajasekaran and Mullins 1983; Newton and Goussard 1990; Altamura *et al.* 1992; Faure *et al.* 1996; Sefc *et al.* 1997; Salunkhe *et al.* 1999). However, the large number (38), the small size (average 1 µm) (Lodhi and Reisch, 1995) and the difficult staining (Rajasekaran and Mullins, 1979) of grape chromosomes are critical constraints. Besides, no information on the regenerant genotypes is achieved.

Molecular typing seems to be the most promising candidate as effective and certain assay for evaluating the floral regeneration products. Among available DNA markers, microsatellites have become the favorite type for identification of grapevine cultivars, pedigree reconstruction and genome mapping, being an almost unlimited source of polymorphic sites. Microsatellites are simple sequence repeated (SSR) motifs present in the plant nuclear DNA that are locus specific, highly polymorphic and co-dominant. This latter property, moreover, allows the discrimination between homozygotes and heterozygotes (Sefc *et al.* 2001).
Genotype assessment at few SSR loci was used in this study to check the occurrence of recombination in samples of somatic embryos and plantlets regenerated form embryogenic callus of both anthers and ovaries of the cv. Chardonnay, the rootstock Kober 125 AA, and the accession *V. rupestris* du Lot.

## Material and Methods

P l a n t m a t e r i a l : Leaves of micropropagated plantlets converted from two anther- and from 5 ovary-embryogenic callus lines, respectively, of *Vitis vinifera* cv. Chardonnay and of the rootstock Kober 125 AA (*V. riparia* x *V. Berlandieri*) were selected. Besides, 7 mature somatic embryos were randomly chosen from each of two lines of both anther- and ovary-embryogenic callus of the accession *Vitis rupestris* du Lot (Tab. 1). Embryogenesis induction, culture maintenance and plant recovery were performed starting from inflorescences collected from the vineyard 10 - 14 d before bloom, when anthers became translucent yellow, according to Martinelli *et al.* (2001, 2003; Figure).

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Table 1

Schematic presentation of the samples selected for the assays

| Sample number | Genotype | Nature of sample | |
|---|---|---|---|
| 1 - 2 | *V. vinifera* cv. Chardonnay | Leaves from a plantlet derived from anther | 2 different lines |
| 3 - 7 | Kober 125 AA | Leaves from a plantlet derived from ovary | 5 different lines |
| 8 - 21 | *V. rupestris* du Lot | 4 somatic embryos derived from anther | 2 different lines |
| | | 3 somatic embryos derived from ovary | 2 different lines |
| 22 - 24 | *V. rupestris* du Lot | Leaves from an *in vivo* plant | 3 accessions |

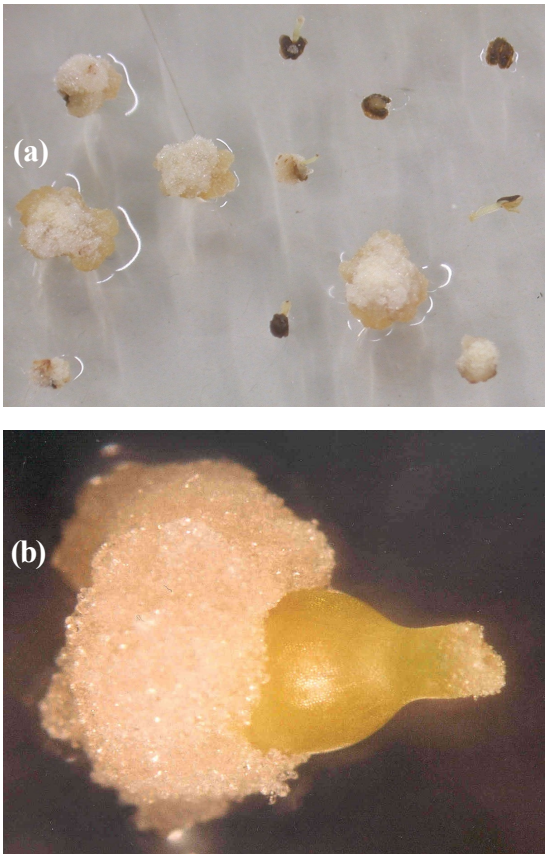


Figure: Callusing from immature *Vitis rupestris* du Lot anther (**a**) and ovary (**b**) explants dissected from *in vivo*-grown inflorescences, 10 - 14 d before bloom. Embryogenic callus induction, somatic embryo development and conversion into plantlets, and long-term embryogenic cultures maintenance are performed according to MARTINELLI *et al.* (2001, 2003).

Molecular assays: Genomic DNA was extracted and PCR amplified at selected SSR loci. Fragment size was determined by capillary electrophoresis and GeneScan software using the ABI310 genetic analyzer. The procedure was described in GRANDO and FRISINGHELLI (1998).

Genotypes at 6 loci VVS2, VVMD5, VVMD7, VVMD27, ZAG62 and VrZAG79 proposed by the GENRES 081 EU Project as universal set of microsatellite markers for grapevine characterisation (THIS *et al.* 2004) were generated and compared with true-to-type Chardonnay, Kober 125 AA and *V. rupestris* du Lot SSR profiles.

## Results and Discussion

Within the several regenerant lines obtained from anthers and ovaries of the cv. Chardonnay, the rootstock Kober 125 AA, and the accession *V. rupestris* du Lot, samples were randomly chosen from clones regenerated from different embryogenic callus lines, thus well representing the different types of tissues participating in the morphogenic processes (Tab.1). The selected plant material resulted a valuable sample for assaying possible recombination events during somatic embryogenesis from grape floral explants. Moreover, the assays conducted on somatic embryos showed the feasibility of discriminating likely recombination events at early stages of regeneration, before plant conversion induction. Although destructive, if embryo sampling is representative of the regenerant population, such analysis would be a useful tool to gain more knowledge of the cultured material. Tab. 2 reports the genotype at each microsatellite locus investigated.

Based on recent mapping experiments (GRANDO *et al.* 2003, RIAZ *et al.* 2004) the 6 loci used in this study are located on 4 different linkage groups, thus representing a quite good subset of the grape chromosomes. Moreover, nearly all the loci were heterozygous thus highly informative with regard to the grape sources of our explants.

Chardonnay and Kober 125 AA regenerant SSR profiles were compared with the genotypes stored in our database and confirmed by data reported in literature at http://www.ismaa.it/areabioav/gmc.html. Besides, three accessions of *V. rupestris* du Lot were genotyped to provide a control for samples 8 - 21.

All the patterns at microsatellite loci obtained from the 21 *in vitro* samples investigated were fully consistent with the molecular genotype of the three grape varieties. These results prove that in all the selected samples no recombination events occurred in the cells involved during the morphogenic process from anthers and ovaries thus excluding the participation of cells of the sexual lines in the morphogenesis.

There might be some questions on whether the regenerants originate from both somatic and germinal cells, since analysis should be unable to distinguish chimerical tissues in these cases. As for anthers, it should be noticed however that the likelihood of the participation of a mixed cell population to morphogenesis resulted negligible, since

Table 2

DNA profiles of Chardonnay (1-2), Kober 125 AA (3-7) and *V. rupestris* du Lot (8-24) samples analysed at 6 microsatellite loci (core set)

| Sample Number | Loci (bp) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | VVS2 | | VVMD5 | | VVMD7 | | VVMD27 | | VrZAG62 | | VrZAG79 | |
| 1 - 2 | 134 | 140 | 231 | 235 | 237 | 241 | 179 | 187 | 188 | 196 | 241 | 243 |
| 3 - 7 | 140 | 145 | 233 | 261 | 231 | 243 | 189 | 204 | 190 | 213 | 249 | 257 |
| 8 - 24 | 134 | 134 | 233 | 265 | 255 | 259 | 202 | 204 | 196 | 196 | 257 | 261 |

accurate histological analysis already proved the single cell origin of grape somatic embryogenesis initiation (Altamura *et al.* 1992; Faure *et al.* 1996). In the case of ovary, on the other hand, no support in literature is available, claiming for further detailed studies towards a better understanding of grape somatic embryogenesis.

Molecular analysis of grape regenerants from floral explants and from ovaries of other species is not reported in literature, and only a few studies on anther-derived populations of herbaceous crops are available (Foisset and Delourme 1996). For these assays, RFLPs and RAPDs have been mostly exploited (Heun *et al.* 1991; Rivard *et al.* 1996; Manninen 2000). However, SSRs have recently been described for potato as a valuable tool for discriminating homozygous doubled monoploid from heterozygous anther-derived diploid plants (Chani *et al.* 2000), and for detecting segregation distortion in androgenic plant populations (Chani *et al.* 2002).

Accordingly, the molecular analysis based on Simple Sequence Repeat markers proposed here produced a valuable approach for an accurate genetic analysis of the regenerated plant material.

As already reported in literature (Martinelli and Gribaudo 2001), ovary has been infrequently adopted for grape somatic embryogenesis induction and detailed studies on regenerant ontogeny are not available. Besides, our results confirm that occurrence of dihaploid lines from anther cultures is extremely difficult in grape, while the histological origin of cells involved in morphogenesis is still controversial (Rajasekaran and Mullins 1983; Altamura *et al.* 1992; Faure *et al.* 1996). For these reasons, we believe that when floral explants are applied, the origin of the cells involved in morphogenesis should always be checked properly.

Finally, we would like to stress that the assay proposed here would be a valuable tool for discriminating possibly off-type plantlets originating from somaclonal variation, from off-type plantlets originating from gametic cells.

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