

Research Note

Chloroplast SSR markers to assess DNA diversity in wild and cultivated grapevines

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Introduction: Simple sequence repeats (SSRs) are abundant and well-distributed throughout the nuclear genomes of *Vitis vinifera* L. Simple sequences length polymorphism (SSLP), caused by variation in the number of repeat units, can be detected easily by PCR using pairs of primers designed from unique sequences bordering the SSR motifs. In the last years SSLP analysis was used for cultivar genotyping and for defining genetic relationships among different varieties. Recently simple sequence repeats of several plant species were found in chloroplast DNA (PROVAN *et al.* 1999; BRYAN *et al.* 1999). CpSSRs result a powerful tool for many aspects of evolutionary population biology (PROVAN *et al.* 2001), including population histories (ECHT *et al.* 1998) and the level of differentiation (McCAULEY 1995) because organelle genomes are typically non-recombinant, uniparentally inherited and effectively haploid. Universal angiosperm chloroplast microsatellite primers were developed by WEISING and GARDNER (1999) on the bordering sequences of 10 cpSSRs. In this study 8 of these primer pairs were tested on wild and cultivated grapevines and the degree of variation was evaluated.

Material and Methods: A total of 12 cultivated and 12 wild Italian grapevines were analysed in this study (Table). Cultivated grapevines were collected from the grapevine col-

lection of C.I.VI.FRU.CE. (Regional Centre for Agriculture), Riccagioia, Pavia, Italy. Wild accessions were obtained from several populations in Italy: 2 plants from Rome, 4 from Grosseto, 1 from Isernia, 1 from Potenza, 2 from Cosenza, and 2 from Nuoro.

Genomic DNA was extracted in 5 ml of "CTAB buffer" (2 % CTAB, 100 mM Tris-HCl, pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl, 1% w/v polyvinylpyrrolidone, 0.1 % v/v (β-mercaptoethanol) as described by LABRA (2001).

C p S S R a n a l y s i s : DNA was analysed at the following 8 microsatellite *loci*: ccmp2, ccmp3, ccmp4, ccmp5, ccmp6, ccmp8, ccmp9, ccmp10 (WEISING and GARDNER 1999). The analysis was performed by adding 10 ng of total DNA to a 20 μl PCR mixture containing, 3 ng of labelled forward primer, 5 ng of reverse primer, 200 ng of each dNTP, 0.5 U Dynazyme II (Celbio, Italy) and 2 μl of Dynazyme buffer. The forward primer was end-labelled with ³³P-ATP (Amersham, Italy). PCR amplification was performed with the following thermal cycles: 3 min at 94 °C; 35 cycles of denaturation (45 s at 94 °C), annealing (30 s at 50 °C) and extension (1 min at 72 °C); then a final step for 7 min at 72 °C. In the case of ccmp2 the annealing temperature was 53 °C. A total of 1.5 μl of the PCR-amplified mixture was added to an equal volume of loading buffer (80 % formamide, 1 mg ml⁻¹ xylene cyanol FF, 1 mg ml⁻¹ bromophenol blue, 10 M EDTA, pH 8.0), denatured for 5 min at 92 °C, loaded onto a 6 % denaturing polyacrylamide gel and electrophoresed in TBE electrophoresis buffer for 3 h at 80 W. The gel was finally fixed in 10 % acetic acid and exposed to an X-ray film for 24 h. Polymorphic bands were scored by visual inspection of the resulting autoradiograms.

Results and Discussion: DNA amplification with the 8 primer pairs used for chloroplast SSR analysis showed that 2 (ccmp3 and ccmp10) out of the 8 analysed *loci* were polymorphic in a total of 24 individuals. Two (106 and 107 bp) and 3 (114, 115, and 116 bp) different size variants were found at *locus* ccmp3 and at *locus* ccmp10, respectively. Size variants of both combined *loci* defines a total of 5 different haplotypes (Table). The most frequent haplotypes, both in cultivated and in wild grapes, were I and IV. Any private

Table

Haplotypes and allele sizes (bp) of ccmp3 and ccmp10 SSR markers, found in *Vitis vinifera* ssp. *sativa* and *silvestris*

Haplotype	cpSSR <i>loci</i>		<i>Vitis vinifera</i> ssp. <i>sativa</i>	<i>Vitis vinifera</i> ssp. <i>silvestris</i> (number of accessions and location of population)
	ccmp3	ccmp10		
I	107	115	Sangiovese, Cesanese, Girò, Ciliegiolo, Lambrusco graspa rossa	2, Rome
II	106	115	Fiudedda	1, Grosseto; 1, Isernia
III	107	114	***	1, Potenza
IV	106	114	Lambrusco foglia frastagliata, Lambrusco Maestri, Pinot Nero, Canaiolo, Aglianico, Grechetto nero Sardo	2, Cosenza; 2 Nuoro; 2 Grosseto
V	106	116	***	1, Matera

allele was detected in wild or cultivated accession. These preliminary results show that all analysed *loci* had a clear reproducible amplification product (Figure). Detected polymorphisms were not sufficient to early distinguish a clear distinction between the two subspecies or to define genetic relationships.

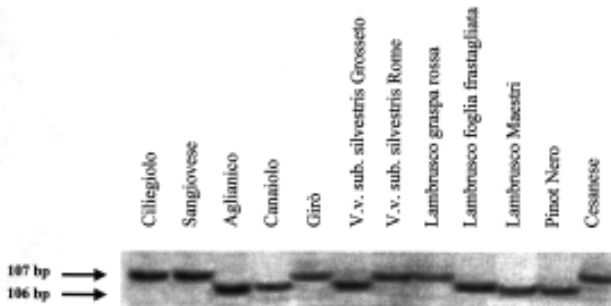


Figure: An example of cpSSR polymorphism (*locus* *ccmp3*) in 12 samples of *Vitis vinifera* ssp. *sativa* and *silvestris*.

The good results obtained in amplification and the high degree of reproducible allele bands allow us to consider cpSSRs as a powerful tool to investigate many aspects of grapevine domestication and diffusion (GRASSI *et al.* 2002).

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