

Research Note

Grapevine cvs Primitivo, Zinfandel and Crljenak kastelanski: Molecular analysis by AFLP

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Introduction: The cv. Primitivo, grown in Apulia, southern Italy, is often associated with the well known Californian cv. Zinfandel since they are thought to be synonyms (WOLFE 1976, BOWERS *et al.* 1993). Recently the Italian origin of Zinfandel has been questioned (MALETIĆ *et al.* 2004); it was hypothesized to be of Croatian origin after having discovered a grapevine named Crljeak kastelanski in Dalmatia. The fact that in Apulia there are different clones of the cv. Primitivo known under different names, e.g. Primitivo di Gioia, di Turi, di Manduria, may have caused some confusion in the identification of the vegetative material propagated elsewhere.

The AFLP technique, enabling simultaneous analysis of a large number of marker loci throughout the genome and a near perfect replicability, is remarkably powerful as a large number of molecular or morphological variables provides a

more precise estimate of genotype differentiation and reduces the variance caused by over- and under-sampling certain regions of the genome (TIVANG *et al.* 1994). The objective of this research was to evaluate the variability among some Primitivo clones and to study their relationship with cvs Zinfandel and Crljenak kastelanski by AFLP.

Material and Methods: Several clones of cv. Primitivo were collected in different areas of Apulia (southern Italy) by the Department Protezione delle Piante e Microbiologia Applicata, Università di Bari, and preserved at the grapevine germplasm collection CRSA 'B. Caramia' Locorotondo (Bari). Nine clones of these were selected for this study. In addition one representative clone of the cv. Zinfandel (from the University of California, Davis) and one representative clone of the cv. Crljenak kastelanski from Croatia were provided by Prof Pejic, University of Zagreb; they had been analysed in a similar study using microsatellite markers (MALETIĆ *et al.* 2004).

The DNA extraction and AFLP analyses were carried out as described in FANIZZA *et al.* (2003) using 50 EcoRI/MseI primer combinations (Table).

Results and Discussion: The result of the AFLP analysis is reported in the Table. It reveals that 50 primer combinations produced 2,928 scorable AFLP markers. Most of these markers were monomorphic (2,919) and very few were polymorphic (9). The detected polymorphisms were confirmed by repeated analyses (one polymorphism is shown in Figure) since the AFLP reproducibility was about 99 %. The Table shows that most of the Primitivo clones have the same AFLP patterns as both cvs Zinfandel and Crljenak kastelanski. The high similarity among these cultivars has been reported earlier using other markers (WOLFE 1976, BOWERS *et al.* 1993, MALETIĆ *et al.* 2004). All this suggests that cvs

Table

Primer combinations and polymorphisms from AFLP analysis of cvs Primitivo clones (P), Zinfandel and Crljenak kastelanski

EcoRI primers	MseI primers	Polymorphic primers	Monomorphic bands	No. of Polymorphic bands (genotype)
AAG	CAC, CTG, CAT, CCA, CAG, CTC	CAG	370	1 (P-Ma. 7*)
ATT	CAC, CTG, CAT, CCA, CAG, CTC	CAG	385	1 (P-Pi. 1)
ATC	CAC, CTG, CAT, CCA, CAG, CTC	CAG	377	1 (P-Pi. 1)
ACA	CAC, CTG, CAT, CCA, CAG, CTC	CAG	328	1 (P-Ma. 7)
AAC	CAC, CTG, CCA, CAG, CTC	CCA	268	1 (P-Pi. 1)
ACC	CAC, CTG, CCA, CAG, CTC	CTG	275	1 (P-Ve. I 16)
		CCA		1 (P-Ve. I 16)
ACT	CAC, CTG, CCA, CAG, CTC	CAG	241	1 (P-Pi. 1)
ATA	CAC, CTG, CCA, CAG, CTC	CAC	267	1 (P-Ma. 7)
ATG	CAC, CTG, CAT, CCA, CAG, CTC		408	
Total			2919	9

* Code number of Primitivo clones

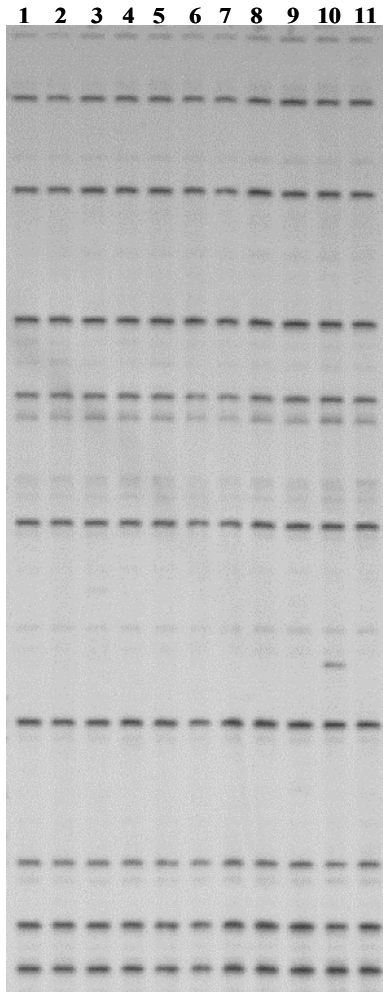


Figure: AFLP profiles for one primer combination *EcoRI* (ACT)-*MseI* (CAG). Genotypes from left to right: (1) Primitivo-Ba.I 21, (2) Primitivo-Ba.II P6 F1, (3) Primitivo-Ma. 7, (4) Primitivo-Ve.I P16, (5) Primitivo-Ba.II P2 H3, (6) Primitivo-Ma.P24 C2, (7) Primitivo-Mu. 13, (8) Zinfandel, (9) Crljenak kastelanski, (10) Primitivo-Pi. 1, (11) Primitivo-Pa. V.

Primitivo, Zinfandel and Crljenak kastelanski are the same variety or clones of the same variety; it should be noted that slight genetic differences might exist among these genotypes since a mutation can be restricted to a very small region of the genome or involve a point mutation in a DNA regulatory sequence which would be difficult to be detected by AFLP or similar techniques.

The Table shows also some variability among the clones of cv. Primitivo (three of them differ from all the other genotypes for a limited number of AFLP bands). Other authors also observed AFLP polymorphisms among clones within old wine grape cultivars (CERVERA *et al.* 2002, FORNECK *et al.* 2003) but not within recent table grape cultivars (CERVERA *et al.* 2000, FANIZZA *et al.* 2003). No deduction can be drawn on the nature of the variability within the Primitivo population as well as on the origin of Primitivo, Zinfandel and Crljenak kastelanski cultivars. Some authors (MALETIĆ *et al.* 2004), using a parentage analysis and 16-25 microsatellite loci, have questioned the Italian origin of cv. Zinfandel and claimed a Croatian origin after having found a genotype named Crljenak kastelanski in Dalmatia; a parentage analy-

sis crucially depends on the number of markers used and the population sample size (none of the Apulian grapevines was included in the group of the Italian cultivars), on the correct evaluation of the rate and model of mutation, microsatellite stability, of which little is known for *Vitis*. Vegetative grapevine material may have spread from one coast of the Adriatic sea to the other; indeed, during the Roman Empire, Apulia (Italy) and Dalmatia (Croatia) had close commercial relations and socio-cultural and religious synergisms are documented since the IV-VI centuries; in addition, migration occurred between the two coasts. Confusion in the variety denomination might have occurred due to the structure and type of viticulture in the past (the Apulian viticulture was characterized by multi-genotype vineyards up to the XVIII century (PACICHELLI 1703) and this might have caused errors in the variety identification). Errors in variety identification might have occurred also in the USA when grape varieties were introduced. AMERINE and WINKLER (1942) reported that the names of grape varieties introduced from Europe to California in the XIX century were confused in many cases. Reliable documents, proving the provenance of these cultivars, are not available and no conclusion can be drawn on their origin. Thus analyses on the basis of molecular resemblance existing now for the material at hand and on the basis of a large number of markers (AFLP or other molecular markers), are a more realistic approach to confirm that the cvs Primitivo, Zinfandel and Crljenak kastelanski are the same variety or clones of the same variety.

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