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Molecular approach to assess the origin of cv. Marzemino

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Summary

DNA marker analysis was used to determine the varietal identity of Marzemino accessions in public collections and private Italian vineyards; relationships among this varietal group and Vertzami, a traditional Greek cultivar, were also investigated through SSR and AFLP approaches. Molecular results strongly support the relationship among Vertzami cultivars growing in Greece, Marzemino and several Italian accessions selected on the basis of etymological similarity. SSR data exclude a direct descent of Marzemino, or other related Italian varieties, from Vertzami; on the other hand the level of similarity among Vertzami, Marzemino and some related varieties indicates a possible common ancestor. None of the accessions is considered as common ancestor but on the basis of genomic variability in the Marzemino group and of the relationships with the other Italian cultivars a probable Italian ancestor is supposed.

K e y w o r d s : AFLP, Marzemino, microsatellite, SSR, *Vitis vinifera* L.

A b b r e v i a t i o n s : SSR = Simple Sequence Repeat; AFLP = Amplified Fragment Length Polymorphism.

Introduction

Viticulture is one of the oldest agricultural activities dating back to 4700 B.C. (Olmo 1976; Jackson 1994; Olmo 1995). About 2000 years later it was introduced to Greece and from there to other parts of Europe (Forni 1996).

European germplasm originates from grapes domesticated and imported from the Near East (Forni 1996), as well as from cultivars derived from direct domestication of wild relatives which have their origin in western Europe. This gave rise to a broad range of grapevine cultivars whose discrimination and correct identification became very difficult (Calo *et al*. 2001).

This study aims to identify the place of origin of the cv. Marzemino. From Friuli, Veneto and Trentino this superior variety is supposed to have spread to Lombardy and Emilia Romagna. Actually the name Marzemino has often been used to define a family of cultivars with a high variability (Calo *et al*. 2001). This makes investigation about its origin difficult; some archeological data (Cattaneo and De Marinis 1993), historical documentations and chemotaxonomical analysis (Scienza and Failla 1996) suggest that Marzemino originates from Asia Minor; from here it was supposed to be brought to Cyprus (Di Rovasenda 1877) and subsequently to Greece (Euboea). Here, the cultivar is called Varsami or Marzavi mavro (Logothetis 1970). Recently, in Lefkas (Greece) the occurrence of a vine called Vertzami was reported; this is the only evidence for grapevines with etymological similarity with the cultivar under examination. On the other hand, in Italy, several varieties (Barzemina, Romagnola, Barzemina Emiliana, Marzemino bianco, Barzeminone) have names that seem to be, more or less, strictly related to Marzemino.

This work used two different molecular marker techniques (AFLP and SSR) to asses the putative origin of Marzemino and to investigate the antique routes and ways of its spread.

Material and Methods

P l a n t m a t e r i a l : The 32 grapevine accessions (*V. vinifera* L.) included 12 accessions of Vertzami from different sites in Lefkas and 11 accessions and/or putative clones or mutations of Marzemino from different localities and germplasm collections: the accessions named Marzemino Rovereto and Marzemino Trento were from IASMA, Agricultural Research Institute, S. Michele all'Adige Trento, Italy, while Marzemino Brescia were collected from the Provincial Centre for Viticulture, Brescia, Italy.

In the analysis we also included 1 accession of Marzemino bianco; 1 accession of Marzemina bianca; 3 accessions and putative clones of Barzemino (a presumed synonym of Marzemino in Lombardy) from germplasm collections or old and marginal vineyards; 2 different Balsamina (another presumed synonym of Marzemino) and Barzeminone accessions from germplasm collections (ERSAL, Pavia, Italy).

D N A a n a l y s i s : DNA was extracted from young leaves (diameter: 1-2 cm) harvested from rooted cuttings. They were frozen in liquid nitrogen and ground to fine powder. Extraction and purification was obtained as described by Labra *et al.* (2001).

S S R a n a l y s i s : DNA was analysed at the following 13 microsatellite *loci*: VVS2, VVS4 (Thomas and Scott 1993), VVMD5, VVMD6, VVMD7 (Bowers *et al*. 1996), VVMD17, VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD31 and VVMD34 (Bowers *et al*. 1999).The analysis was performed by adding 10 ng of genomic DNA to a 25 µl PCR mixture containing 0.25 µM of the DNA primer pair specified for each microsatellite *locus*, 200 µM of each of the 4 dNTPs, 0.5 U Dynazyme and Dynazyme buffer (Finnzyme,

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Finland) as specified by the supplier. PCR amplification was performed with a programmable thermal controller (PTC 100, MJ Research Inc., USA) with the following thermal cycles: 7 min at 94 °C; 35 cycles of denaturation (45 s at 94 °C), annealing (30 s at 52 °C) and extension (1 min at 72 °C), followed by a final step for 7 min at 72 °C.

A total of 10 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 mM EDTA, pH 8.0) and 5 µl of this mixture was analysed by electrophoresis on a 4.5% sequencing polyacrylamide gel in TBE electrophoresis buffer (50 mM boric acid, 1 mM EDTA, pH 8.0) for 3 h at 80 W. The gel was fixed in 10 % acetic acids and stained with silver as described by Echt *et al.* (1999).

AFLP analysis: AFLP was performed as described in Rossoni *et al.* (2003). Primer pairs used in the selective amplification were E31 - M32, E32 - M36, E32 - M38 and E33 - M38 of Tab. 1.

Data analysis: SSR fingerprints were evaluated by visual inspection of silver stained gels. In accordance with numeric taxonomy principles, each microsatellite allele was scored as a binary character for its absence (0) or presence (1). Presence was scored as (1) independently for the heterozygous or homozygous state. Similarity-dissimilarity matrices were computed with the Jaccard's coefficient:

JC = a/(n-d);

where: a = traits present in both compared genotypes; n = total number of polymorphic traits; d = traits absent in both compared genotypes. The final products of data processing were dendrograms constructed by cluster analysis based upon UPGMA (unweighted pair-group method with arithmetical averages).

AFLP fingerprints were evaluated by visual inspection of autoradiograms. DNA bands were scored for their presence (1) or absence (0) and the resulting data matrices were analysed using the SPSS programme (SPSS Inc. 1998). Variation in band intensity was not considered as a criterion for polymorphism. Similarity-dissimilarity matrices were computed with the Jaccard coefficient for qualitative data. A dendrogram was constructed by cluster analysis based upon the UPGMA algorithm.

Table 1

DNA primers for AFLP analysis

Name	DNA sequence	Variable extension
M32	5'-GATGAGTCCTGAGTAA-3'	AAC
M36	5'-GATGAGTCCTGAGTAA-3'	ACC
M38	5'-GATGAGTCCTGAGTAA-3'	ACT
E31	5'-GACTGCGTACCAATTC-3'	AAA
E32	5'-GACTGCGTACCAATTC-3'	AAC
E33	5'-GACTGCGTACCAATTC-3'	AAG

Results

Molecular markers have been used to evaluate the degree of similarity among Marzemino and Vertzami accessions. In addition varieties with etymological similarities have been considered to better define the relationships among Marzemino and Vertzami. Marzemino and Vertzami accessions showed identical SSR profiles for each variety (Tab. 2), consequently only one sample of each cultivar was considered in the resulting dendrogram (Fig. 1). The Jaccard's similarity index between Marzemino and Vertzami was only 0.45 with 11 shared SSR allelles. This value excludes a direct relationship between the two cultivars but suggests a possible common ancestor. Barzemino had the SSR profile of Marzemino. Marzemino bianco (0.95), Balsamina Emiliana and Romagnola (0.85) are very closely related to Marzemino and we can hypothesize that these cultivars may have the same progenitors. An intermediate value of similarity between Marzemino and Vertzami was found for Marzemina bianca and Barzeminone.

To better define the intravarietal variability of the Marzemino and Vertzami cultivars growing in different areas AFLP analysis was performed on a total of 32 samples. A total of 219 bands was detected by using 4 AFLP primer combinations, of these 54 (24.65 %) were polymorphic. Results, summarised in a dendrogram (Fig. 2), show the absence of genomic variability among the Vertzami accessions

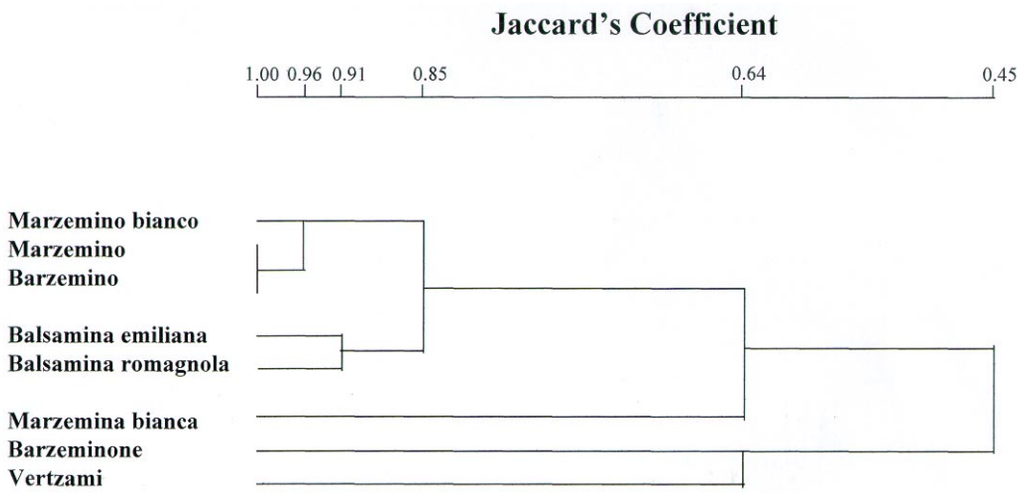

Fig. 1: Dendrogram representing the similarity among the accessions according to SSR markers.

Table 2

Size of microsatellite alleles (bp) observed for all analysed accessions

Accession	VVS2		VVS4		VVMD5		VVMD6		VVMD7		VVMD17		VVMD21		VVMD24		VVMD25		VVMD27		VVMD28		VVMD31		VVMD34	
Marzemino	129	129	166	173	222	228	197	206	237	260	220	222	243	249	210	214	253	253	181	190	231	247	212	216	240	240
Barzemino	129	129	166	173	222	228	197	206	237	260	220	222	243	249	210	214	253	253	181	190	231	247	212	216	240	240
Marzemino bianco	129	129	166	166	222	222	197	206	237	260	220	222	243	249	210	214	253	253	181	190	231	247	212	216	240	240
Marzemina bianca	129	129	166	173	222	222	197	206	237	254	220	222	243	249	210	214	253	253	179	190	233	239	212	216	240	240
Barzeminone	140	129	166	166	222	228	197	206	237	254	220	222	243	256	210	210	253	253	181	190	233	239	204	212	240	240
Vertzami	129	140	166	173	222	228	190	206	237	254	220	222	243	256	208	219	243	253	179	190	233	239	204	212	240	240
Balsamina emiliana	129	129	166	173	222	228	197	206	237	260	220	222	243	249	210	214	243	253	181	190	231	247	204	212	240	240
Balsamina romagnola	129	129	166	173	222	228	197	206	237	260	220	222	249	249	210	214	243	253	181	190	231	247	204	212	240	240

collected from Leukade (Greece), on the contrary a low variability (5-6 % of Jaccard's index) was detected among the Marzemino group. Balsamina Romagnola, Balsamina Emiliana and Barzeminone showed an intermediate value between Marzemino and Vertzami in agreement with the SSR analysis.On the basis of results obtained with both molecular tools we conclude that Vertzami accessions are clones of the same variety, on the other side AFLP has highlighted that several putative cultivars have to be considered clones of Marzemino (Fig. 2), with the exception of Balsamina emiliana, Balsamina romagnola and Barzeminone as previously reported (Calo *et al*. 2001).

Discussion

Information on the origin and relationship among plant cultivars is of great interest both for germplasm preservation and for cultivar improvement, *i.e.* breeding and biotechnology (Karp 1998). Research on direct ancestors of local varieties (local wild vines or cultivated vines from other regions) is still an open issue.

We have now produced molecular evidence that strongly supports the relationship among the Vertzami cultivars growing in Greece, Marzemino and several accessions selected on the basis of etymological similarity. At least for this group, we can conclude that there is a correlation between the origin of cultivar names and the history of varieties. SSR data exclude a direct origin of Marzemino or other related Italian varieties (Barzemino, Balsamina emiliana and Balsamina romagnola) from Vertzami; on the other hand, the level of similarity was in agreement with a possible common ancestor among Vertzami, Marzemino and some varieties closely related (*i.e*. Barzeminone). None of the accessions may be considered as the common ancestor but on the basis of genomic variability in the Marzemino group and of relationships among some Italian cultivars (Marzemino Bianco, Barzemino, Balsamina romagnola and emiliana) we can conclude that the probable ancestor was an old Italian and not a Greek grapevine.

These considerations are also supported by historical reports describing Vertzami as an old Italian variety spread out from Venice populations into Greece during the colonization of Leukades in the 14th century B.C . (Falcetti and Campostrini 1997).

We conclude that the origin of Marzemino-Vertzami and similar investigations for other grapevine varieties are important in order to add details to the model of grapevine spread. The results presented in this paper are consistent with a significant diffusion of grapevine varieties strongly influenced by human movements (Zohary and Hops 1993; Labra 2002).

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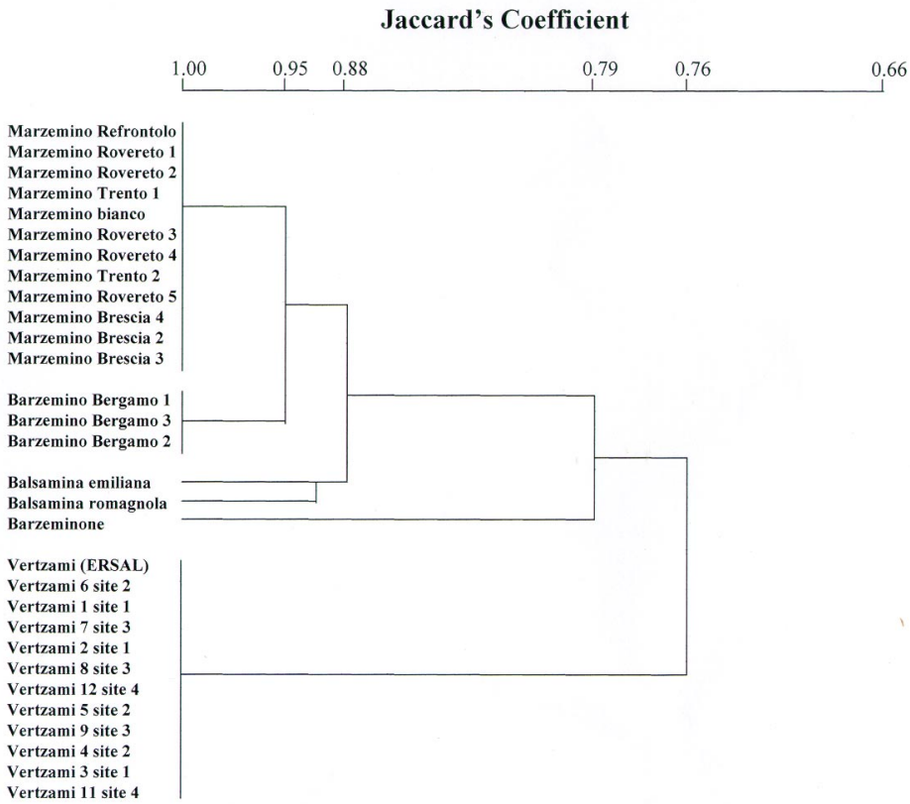

Fig. 2: Dendrogram representing the similarity among the accessions according to AFLP markers.

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