

# Genetic structure and differentiation in cultivated grape, *Vitis vinifera* L.

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## Summary

222 cultivated (*Vitis vinifera*) and 22 wild (*V. vinifera* ssp. *sylvestris*) grape accessions were analysed for genetic diversity and differentiation at eight microsatellite loci. A total of 94 alleles were detected, with extensive polymorphism among the accessions. Multivariate relationships among accessions revealed 16 genetic groups structured into three clusters, supporting the classical eco-geographic grouping of grape cultivars: *occidentalis*, *pontica* and *orientalis*. French cultivars appeared to be distinct and showed close affinity to the wild progenitor, ssp. *sylvestris* from south-western France (Pyrenees) and Tunisia, probably reflecting the origin and domestication history of many of the old wine cultivars from France. There was appreciable level of differentiation between table and wine grape cultivars, and the Muscat types were somewhat distinct within the wine grapes. Contingency  $\chi^2$  analysis indicated significant heterogeneity in allele frequencies among groups at all loci. The observed heterozygosities for different groups ranged from 0.625 to 0.9 with an overall average of 0.771. Genetic relationships among groups suggested hierarchical differentiation within cultivated grape. The gene diversity analysis indicated narrow divergence among groups and that most variation was found within groups (~85%). Partitioning of diversity suggested that the remaining variation is somewhat structured hierarchically at different levels of differentiation. The overall organization of genetic diversity suggests that the germplasm of cultivated grape represents a single complex gene pool and that its structure is determined by strong artificial selection and a vegetative mode of reproduction.

## 1. Introduction

Cultivated grape, *Vitis vinifera* L., is the sole European representative of the genus *Vitis* L., a large member of Vitaceae with ~60 species (Galet, 1988). Two-thirds of these species are native to North America and one-third is distributed over central and east Asia. The cultivated grape is believed to have been domesticated around 4000 BC from a perennial wild grape originally classified as *V. sylvestris* C.C. Gmelin occurring from north-eastern Afghanistan to the southern borders of the Black Sea and the Caspian Sea (Zohary & Spiegel-Roy, 1975; Ketsa & Verheij, 1992). However, based

on a recent archaeological finding in the Zagros mountains of Iran, McGovern *et al.* (1996) suggested 5400–5000 BC as the probable period of domestication of the grape.

Currently, most botanists regard the wild ancestral grape *V. sylvestris* as the primitive form of the cultivated grape because of the close morphological resemblance and free gene flow between them (Heywood & Zohary, 1991) and consequently have reduced its taxonomic status to subspecies level within the *V. vinifera* crop complex (Levadoux, 1956). The wild grapes are predominantly forest climbers and occur in disjunct populations from the Atlantic coast to Tadzhikistan and the western Himalayas (Zohary & Hopf, 1993). They occasionally come into contact with cultivated

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Table 1. *Grape germplasm accessions included in the study (organized into 16 groups as illustrated in Fig. 1). Parentheses list first the grape type (T, table; W, wine; TW, table/wine dual; S, sylvestris), then the country (abbreviated according to the ISO3166 code; ? represents unknown origin) and finally the number of samples assayed*

<b>Group 1</b>	17 <i>Vitis vinifera</i> ssp. <i>sylvestris</i> NT1 (S/TUN/1)
1 Ab jusht (T/AFG/2)	18 Treixadura Blanca (W/ESP/1)
2 Askari (T/IRN/1)	19 Vilana (W/GRC/2)
3 Assirtico (W/GRC/5)	20 Vidiano (W/GRC/1)
4 Azzazy (T/MAR/1)	21 Volitsa B (W/GRC/1)
5 Bez el Anza (W/EGY/1)	<b>Group 4</b>
6 Black Kishmish (T/SUN/3)	1 Affenthaler (W/DEU/1)
7 DVIT715 (T/?/1)	2 Black Damascus (T/?/1)
8 Fahri (T/AFG/1)	3 Cornichon (T/?/1)
9 Hassaine (T/TUR/1)	4 Malvasia (W/ITA/1)
10 Husseine (T/AFG/2)	5 Robola Kokkini (W/GRC/1)
11 Kandahar (T/AFG/1)	6 Sultana (T/TUR/1)
12 Kandahari (T/AFG/1)	7 Sultana Moschata (T/ITA/3)
13 Khalili (T/AFG/1)	8 Tavriz (TW/AZB/1)
14 Kishmish of Vir (T/SUN/1)	9 Tokay (T/ALG/1)
15 Kishmish Sorkh (T/AFG/2)	<b>Group 5</b>
16 Kishmishi (T/AFG/1)	1 Asprouda Mikinon (W/GRC/1)
17 Mavroudi School of Patras (TW/GRC/1)	2 Asprouda Halkidos (W/GRC/2)
18 Monukka (T/AFG/2)	3 Asprouda Zakinthou (W/GRC/1)
19 Takhani (T/AFG/1)	4 Divromos (W/GRC/1)
20 Thompson Seedless (T/TUR/7)	5 DVIT881 (TW/?/1)
<b>Group 2</b>	6 Karvouniariz (W/GRC/1)
1 Black Corinth (T/GRC/2)	7 Lagorthis B (TW/GRC/1)
2 Chaouch (T/GRC/3)	8 Maloukato (W/GRC/2)
3 Dabouki (T/ISR/1)	9 Mammolo Toscano (TW/ITA/1)
4 Daphne (TW/GRC/3)	10 Mavrodaphne (W/GRC/5)
5 Dolcetto (W/ITA/1)	11 Migdali (W/GRC/1)
6 Fraoula Kokkini (T/GRC/1)	12 Prune de Cazouls (T/?/1)
7 Hakiki (T/GRC/2)	13 Skiadopoulo (W/GRC/2)
8 Kislev (T/ISR/1)	14 Tsoupi (TW/GRC/1)
9 Kurutaktas (T/GRC/1)	15 Vertzami (W/GRC/2)
10 Ladikinah (W/GRC/3)	16 Zakinthino (W/GRC/2)
11 Leanoy (W/SUN/1)	<b>Group 6</b>
12 Medaur (T/ISR/1)	1 Sciacarello (W/FRA/1)
13 Migdali (W/GRC/2)	2 <i>V. vinifera</i> ssp. <i>sylvestris</i> NT6 (S/TUN/1)
14 Nava (T/ISR/1)	3 <i>V. vinifera</i> ssp. <i>sylvestris</i> NT10 (S/TUN/1)
15 Nemea (W/GRC/2)	4 <i>V. vinifera</i> ssp. <i>sylvestris</i> NT12 (S/TUN/1)
16 Petinos (T/GRC/1)	<b>Group 7</b>
17 Red Malaga (T/ESP/1)	1 Bequignol (W/FRA/1)
18 Rhasaki Anatolico (T/GRC/1)	2 Cabernet Sauvignon (W/FRA/1)
19 Rodiares (TW/GRC/1)	3 Gewurztraminer (W/FRA/3)
20 Romeiko (W/GRC/3)	4 Mueller-thurgau (W/DEU/1)
21 Shami (T/GRC/1)	5 Perle (W/DEU/1)
22 Sidezitis (T/GRC/1)	6 Petite Manseng (W/FRA/1)
23 Thrapsathiri B (W/GRC/1)	7 Sauvignon Gris (W/FRA/1)
24 Trapanlarin Kara (TW/SUN/1)	8 <i>V. vinifera</i> ssp. <i>sylvestris</i> NT1 (S/TUN/1)
25 Ughetta (W/ITA/1)	9 <i>V. vinifera</i> ssp. <i>sylvestris</i> NT4 (S/TUN/1)
26 White Corinth (T/GRC/1)	10 <i>V. vinifera</i> ssp. <i>sylvestris</i> NT7 (S/TUN/1)
<b>Group 3</b>	11 <i>V. vinifera</i> ssp. <i>sylvestris</i> NT9 (S/TUN/1)
1 A'asemi (T/YEM/1)	12 Touriga Nacional (W/PRT/1)
2 Aglianico (W/ITA/1)	<b>Group 8</b>
3 Boal Dulce (TW/DZA/1)	1 Cabernet Franc (W/FRA/2)
4 Daphnata (TW/GRC/1)	2 Merlot (W/FRA/1)
5 DVIT652 (TW/?/1)	3 Mondeuse noire (W/FRA/1)
6 DVIT789 (TW/?/1)	4 Petite Verdot (W/FRA/1)
7 Goustolidi (W/GRC/3)	5 <i>V. vinifera</i> ssp. <i>sylvestris</i> F1 (S/FRA/1)
8 Kotsifali (W/GRC/3)	6 <i>V. vinifera</i> ssp. <i>sylvestris</i> F2 (S/FRA/1)
9 Lagorthis A (W/GRC/2)	7 <i>V. vinifera</i> ssp. <i>sylvestris</i> F3 (S/FRA/1)
10 Lianoroi (T/GRC/2)	8 <i>V. vinifera</i> ssp. <i>sylvestris</i> F4 (S/FRA/1)
11 Mandilaria (W/GRC/4)	9 <i>V. vinifera</i> ssp. <i>sylvestris</i> F5 (S/FRA/1)
12 Mausac Blanc (W/FRA/1)	10 <i>V. vinifera</i> ssp. <i>sylvestris</i> F6 (S/FRA/2)
13 Monica (W/ITA/3)	11 <i>V. vinifera</i> ssp. <i>sylvestris</i> F7 (S/FRA/1)
14 Pedro Ximenez (W/ESP/1)	12 <i>V. vinifera</i> ssp. <i>sylvestris</i> F8 (S/FRA/1)
15 Plavac Mali (W/HRV/1)	13 <i>V. vinifera</i> ssp. <i>sylvestris</i> F9 (S/FRA/1)
16 Plito (W/GRC/1)	

Table 1. (cont.).

14	<i>V. vinifera</i> ssp. <i>sylvestris</i> F10 (S/FRA/1)	8	Dan ben Chana (T/ISR/1)
15	<i>V. vinifera</i> ssp. <i>sylvestris</i> F11 (S/FRA/1)	9	Dermatas (TW/GRC/2)
16	<i>V. vinifera</i> ssp. <i>sylvestris</i> F12 (S/FRA/1)	10	Doradillo (W/ESP/1)
17	<i>V. vinifera</i> ssp. <i>sylvestris</i> NT3 (S/TUN/1)	11	Emperor (T/USA/2)
18	<i>V. vinifera</i> ssp. <i>sylvestris</i> NT5 (S/TUN/1)	12	Folle Blanche (W/FRA/3)
<b>Group 9</b>		13	Grec Rouge (TW/FRA/1)
1	Aligote (W/FRA/1)	14	Ithani Lefko (W/GRC/2)
2	Chardonnay (W/FRA/2)	15	Ithani Mavro (W/GRC/3)
3	Chasselas Ciotat (TW/FRA/2)	16	Kratosija (W/YUG/1)
4	Lagrain (W/ITA/2)	17	Malaga (T/FRA/1)
5	Madam Matijas (T/HUN/1)	18	Mavroudi (W/GRC/1)
6	Melon (W/FRA/1)	19	Montils (W/FRA/1)
7	Muscat Ottonel (W/FRA/1)	20	Muscat Oliver (TW/HUN/1)
8	Perla di Csaba (TW/ITA/1)	21	Periquita (W/PRT/1)
9	Pinot Nior (W/FRA/7)	22	Perle de Csaba (T/HUN/1)
<b>Group 10</b>		23	Rondinella (W/ITA/1)
1	Durif (W/FRA/1)	24	Ruby Cabernet (W/USA/1)
2	Juracon Noir (W/FRA/1)	25	Sereksiya Rosavi (W/SUN/1)
3	Malbec (W/FRA/1)	26	Terret blanc (W/FRA/1)
4	Marsanne (W/FRA/1)	27	Voivoginovia (TW/CHN/1)
5	Negrette (W/FRA/2)	28	Vranac (W/YUG/2)
6	Peloursin (W/FRA/1)	29	Vuthomato (T/GRC/1)
<b>Group 11</b>		30	Xynomavro (W/GRC/3)
1	Alicante Bouschet (W/FRA/2)	31	Zeni (T/LBN/1)
2	Angulata (T/GRC/1)	32	Zimsko Belo (T/YUG/1)
3	Araklinos (W/GRC/3)	33	Zinfandel (W/YUG/1)
4	Aramon (W/FRA/1)	<b>Group 14</b>	
5	Asprouda Patron (W/GRC/1)	1	Aleatico (W/ITA/1)
6	Aspruda Ariloghi (W/GRC/2)	2	Carolina Blackrose (T/USA/1)
7	Aspruda Mikinon (W/GRC/1)	3	Criolla Mesa (T/ARG/1)
8	Chisostaphylo (W/GRC/1)	4	Kontehgalo (T/GRC/1)
9	Fileri (W/GRC/4)	5	Malagouzia (W/GRC/3)
10	French Colombard (W/FRA/1)	6	Malvasia Bianca (W/ITA/1)
11	Ghlicopati (W/GRC/1)	7	Moschato Mavro (W/GRC/1)
12	Grand Noir (W/FRA/1)	8	Moschato Samou (W/GRC/4)
13	Hunisa (T/TUR/1)	9	Moschardina (W/GRC/1)
14	Jurancon Blanc (W/FRA/1)	10	Muscat of Alexandria (TW/ITA/2)
15	Kontocladi (T/GRC/1)	11	Ohanes (T/ESP/1)
16	Kritiko (W/GRC/1)	12	Opale (T/ITA/1)
17	Liatiko (W/GRC/3)	13	Volitsa A (W/GRC/1)
18	Limberger (W/HUN/3)	<b>Group 15</b>	
19	Petite Bouschet (W/FRA/1)	1	Barbera (W/ITA/2)
20	Sidezitis Proimo (T/GRC/1)	2	Corbeau (W/FRA/1)
21	Thiakon (W/GRC/3)	3	Eftakilo (T/GRC/1)
22	Vernaccia Bianca (W/ITA/1)	4	Frankenthal Blanc (W/DEU/1)
23	Volitzia Lefki (W/GRC/1)	5	Gros Colman (T/SUN/2)
<b>Group 12</b>		6	Kadarka (W/HUN/3)
1	Argant (W/FRA/1)	7	L'arvine (W/CHE/1)
2	Arikaras (TW/GRC/1)	8	Limnio (W/GRC/1)
3	Castelao (W/PRT/1)	9	Muscat Hamburg (T/DEU/2)
4	Folle Noire (W/FRA/1)	10	Queen of the Vineyard (T/HUN/2)
5	Grecanico Dorato (W/ITA/1)	11	Rhazaki (T/LBN/9)
6	Gros Vert (T/LBN/2)	12	Rhazaki Mavro (T/LBN/1)
7	Harslevelu (W/HUN/1)	13	Robin (W/FRA/1)
8	Mission (W/USA/1)	14	Roditis (TW/GRC/7)
9	Negru Virtos (W/ROM/1)	15	Savatiano (W/GRC/5)
10	Palomino (W/ESP/1)	16	Viognier (W/FRA/1)
<b>Group 13</b>		<b>Group 16</b>	
1	Aspiran Noir (W/FRA/1)	1	Athiri (TW/GRC/2)
2	Aspruda Santorinih (W/GRC/3)	2	Mataro (W/ESP/1)
3	Baidh ul Haman (T/AFG/1)	3	Parianoh (W/GRC/4)
4	Black Malvoisie (TW/FRA/2)	4	Somarello Rosso (TW/ITA/1)
5	Burger (W/FRA/1)	5	Sultana Crimson (T/TUR/1)
6	Carignane (W/ESP/1)	6	Thrapsathiri A (W/GRC/1)
7	Casculho (W/PRT/1)	7	Vermentino Favorita (TW/ITA/1)
		8	Zakinthino (W/GRC/1)

forms in nearby vineyards, forming complex introgressive hybrid swarms in transition zones (Zohary & Hopf, 1993). Domestication of grape involved a shift in the mode of reproduction from dioecious to hermaphroditic, ensuring self-pollination without the need for external pollen donors.

The earliest signs of grape cultivation come from Chalcolithic and Early Bronze Age (3500–2300 BC) sites in the Jordan Valley, where wild and cultivated grapes provided fresh fruits, easily stored raisins and juice for fresh consumption and fermentation into wine (Zohary & Hopf, 1993). Over the next 2000 years, grape cultivation spread to the eastern, northern and western parts of Eurasia and to northern Africa, following the trade routes and migration of ancient tribes (De Candolle, 1886; Stager, 1985). Traditional viticulture was based on thousands of distinct cultivars (Einset & Pratt, 1975; Olmo, 1976) exhibiting a wide range of adaptations, growth habits and fruit characteristics. Currently, over 6000 cultivars are documented, including wine, table and raisin types (Alleweldt & Dettweiler, 1992). Nevertheless, cultivar names are often ambiguous owing to transliteration, the substitution of local or regional names for the original cultivar names, the presence of variants within cultivars (clones) and poor documentation of passport data, which includes ecogeographical, climatological and ethnographic information associated with germplasm accessions. Moreover, the wide distribution and long cultivation history of the grape have led to the development of numerous cultivars that have many synonyms, a problem that plagues germplasm collections (Galet, 1990; Ambrosi *et al.*, 1994).

Traditional methods of describing grape vine varieties based on the plant's vegetative and reproductive traits (ampligraphy) have contributed greatly to establishing the identity and relationships among *V. vinifera* cultivars (Krimbas, 1943; Negrul, 1946; Galet, 1979; Boursiquot *et al.*, 1987). Nevertheless, ampelographic traits are often plastic, with a large genotype–environment interaction component rendering them less useful in classifying closely related cultivars. Early efforts to classify the eco-geographic variation within the cultivated grape resulted in the classification of cultivars into groups: *occidentalis*, the small-berried wine grapes of western Europe; *orientalis*, the large-berried table grapes of West Asia; and *pontica*, the intermediate type from the basin of the Black Sea and eastern Europe (Negrul, 1938). Levadoux (1956) summarized the eco-geographic differentiation of wild and cultivated *V. vinifera* populations from western Europe and the Mediterranean region, and discussed the consequences of isolation of eastern and western populations during the glacial period and post-glacial proliferation for population differentiation, climatic adaptations and early

selection and cultivation by man. More recently, Bisson (1995) proposed the eco-regional classification for French cultivars and discussed the implications for grape breeding.

A wide range of biochemical and molecular markers are being used to characterize and classify grape germplasm collections (Calo *et al.*, 1989; Tschammer & Zyprian, 1994; Bowers *et al.*, 1996; Cervera *et al.*, 1998; Tessier *et al.*, 1999). However, the use of molecular markers to study genetic structure and differentiation in *V. vinifera* is limited to a few investigations (Sefc *et al.*, 2000; Dangl *et al.*, 2001). Microsatellite markers, being abundant, multi-allelic and highly polymorphic, provide an efficient and accurate means of detecting genetic polymorphism. Most importantly, their co-dominant nature makes them the markers of choice for population genetic analysis to assess genetic structure and differentiation in germplasm collections and natural populations. The knowledge of the amount and pattern of distribution of genetic variation is central to the development of effective conservation strategies and efficient use of *Vitis* germplasm.

The present study evaluated the genetic diversity, structure and differentiation in a grape germplasm collection using polymorphisms at eight microsatellite loci. In addition, we examined the genetic relationship between cultivated and wild grapes to draw inferences about the history of domestication. Further, we elucidated the possible relationships between geographic origin of cultivars and the pattern of microsatellite polymorphism.

## 2. Materials and methods

### (i) Plant material, DNA extraction and microsatellite analysis

366 *V. vinifera* accessions, including 22 of ssp. *sylvestris*, representing the different grape growing regions of the world were analysed at eight microsatellite loci. 234 of them were from the collection of the National Clonal Germplasm Repository (US Department of Agriculture, Davis, CA). The remaining accessions came from the Foundation Plant Materials Service (University of California, Davis, CA) and from collections and vineyards in Greece (Boutaris, 1999). A list of 244 unique accessions included in the final analyses along with their country of origin is presented in Table 1.

DNA extraction, PCR amplification, electrophoresis and detection of polymorphisms were according to Dangl *et al.* (2001). Eight microsatellite loci (*VVS2* (Thomas & Scott, 1993), *VVMD5*, *VVMD6*, *VVMD7*, *VVMD27*, *VVMD28*, *VVMD31* and *VVMD32* (Bowers *et al.*, 1996; Bowers *et al.*, 1999a)) were successfully amplified. Gels were scored

in two different formats: (1) as binary scores with presence of an allele scored as unity and the absence as zero; and (2) as genotypes based on the allelic composition.

#### (ii) *Data analysis*

The binary data were used to compute the Dice coefficient of association (Dice, 1945) based on the proportion of alleles shared between two accessions for all possible pairwise combinations. The resultant matrix was subjected to a cluster analysis with the neighbour-joining (NJ) algorithm (Saitou & Nei, 1987) to produce an unrooted additive phenetic tree (phenogram). The multilocus microsatellite genotype data were pooled into groups based on the results of NJ analysis and analysed for various within-group genetic variability measures such as the mean number of alleles per locus, polymorphic index and observed and expected levels of heterozygosity. Contingency  $\chi^2$  analysis was performed to determine the heterogeneity among groups. Intergroup relationships were examined by computing unbiased genetic identity and distance coefficients (Nei, 1978) for all possible pairwise comparisons of groups. An unweighted pair group method using arithmetic means (UPGMA) cluster analysis was performed on the genetic identity matrix to visualize intergroup relationships. The principal component analysis (PCA) on the correlation matrix derived from the binary data was used to confirm the results of the two cluster analyses.

An additive tree with the distance Wagner procedure (Swafford, 1981) on the Prevosti distance (Wright, 1978) between all possible pairwise combinations of wine, table and wild grape groups was reconstructed to elucidate the level of genetic divergence among groups.

Gene diversity analysis was performed on the allele frequency data from the 16 groups obtained in the NJ cluster analysis by following the method suggested by Nei (1973). The total gene diversity ( $H_T$ ) was partitioned into gene diversity caused by variation within groups ( $H_G$ ) and the component caused by variation between groups ( $D_{GT}$ ). Differentiation between groups was calculated as  $G_{GT} = D_{GT} \div H_T$ , where  $G_{GT}$  can vary between 0 (when  $H_G = H_T$ ) and 1 (when  $H_G = 0$ ), that is, the groups are fixed for different alleles. Partitioning of gene diversity was according to Nei's (1973) method as extended by Chakraborty (1980), where total gene diversity was apportioned hierarchically according to differentiation observed in the UPGMA tree.

### 3. Results and discussion

The study of genetic structure and differentiation in cultivated grape should include its wild ancestor

*V. vinifera* ssp. *sylvestris* because it forms a continuum with the cultivated grape within the primary gene pool. Accordingly, 22 accessions of ssp. *sylvestris*, originally from southwestern France and northern Tunisia, were included in the study. Among the 366 accessions fingerprinted, many cultivars were represented by more than one accession and a comparison of multilocus allelic profiles confirmed that there were often multiple synonyms for a single cultivar. Thus, 244 unique genotypes, including the 22 *V. vinifera* ssp. *sylvestris* accessions (groups 6, 7 and 8), representing most of the grape-growing regions of the world were selected for further analysis (Table 1).

#### (i) *Levels of polymorphism and genetic relationships among grape cultivars*

Extensive genetic polymorphism and high levels of heterozygosity were observed among the accessions. All eight loci assayed were polymorphic, with the number of alleles per locus ranging from five for *VVMD6* to 19 for *VVMD28*, with a total of 94 alleles among the accessions assayed. Owing to space constraint, only the frequency of the most common allele among the 16 groups obtained in the NJ cluster analysis is presented (complete data on web site – <http://www.ars-grin.gov/dav/>). The pattern of allelic distribution for different loci among accessions and groups, although nonrandom, did not reveal an obvious geographic trend across the collection. This observation is consistent with the historical fact that grape germplasm has been moved around the grape-growing regions of the world, making it difficult to recognize the geographic trends in allele frequencies and genetic identity of many grape cultivars (Sefc *et al.*, 2000).

Because no *a priori* structure or geographic criteria could be assumed, the overall pattern of genetic relationships among accessions was examined using a cluster analysis irrespective of their geographic origin. This established rational groups based on pairwise genetic similarities so that genetic structure and differentiation could be examined. The NJ cluster analysis on the cultivar pairwise Dice coefficient of association matrix (244 × 244) produced an unrooted tree (phenogram) with 16 recognizable groups structured into three major clusters (indicated by arrows in Fig. 1). These clusters were distinct in the cladogram (not shown here), which depicts the inferred historical relationships (cladogenesis) among grape cultivars. Neither the groups nor the clusters exhibited appreciable levels of genetic divergence. The highly dissected branching nature of the phenogram with narrow divergence between groups suggests that most variation is found within groups. However, the branch lengths might not accurately reflect the degree of relationships among cultivars and groups, because



the phenogram is a two-dimensional representation of complex multidimensional variation. The narrow divergence among groups is further substantiated by the fact that the first two orthogonal vectors in the PCA extracted only 26% of the total variation. The lack of significant genetic structure within the cultivated grape gene pool examined does not seem to support the notion that the European cultivars have evolved from indigenous vines representing distinct gene pools with introgression of genes from introduced cultivars playing only a minimum role (Sefc *et al.*, 2000).

With limited historical records on the origin of grape cultivars, it is difficult to infer the overall relationships on the basis of molecular variation alone. Nevertheless, many instances of associations reflecting the close genetic relationships are depicted in the phenogram (Fig. 1, Table 1). For example, the sibling cultivars ‘Chardonnay’, ‘Melon’ and ‘Aligote’ are found in group 9, all sharing the well known ‘Pinot’ pedigree (Bowers *et al.*, 1999b), ‘Sauvignon Gris’, a clone of ‘Sauvignon Blanc’ and ‘Cabernet Sauvignon’ in group 7, and ‘Cabernet Franc’ (Bowers & Meredith, 1997) in group 8, and others such as ‘Peloursin’ and ‘Durif’ (Meredith *et al.*, 1999) in group 9, ‘Müller-thurgau’ and ‘Perle’ (Alleweldt & Dettweiler, 1992) in group 7 and ‘Carignane’ and ‘Ruby Cabernet’ (Olmo, 1948) in group 13 illustrating parent–progeny relationships. The composition of groups within the three clusters in the phenogram, however, allowed for some generalizations about the origin and relationships among many grape cultivars. The three major clusters in the NJ tree were designated as Mediterranean table grape, western European wine grape and central European grape clusters, mainly based on the geographic region to which most cultivars in the clusters belong and their end use as wine, table or dual types (used both as wine and table type).

#### (a) Mediterranean table-grape cluster

This cluster consisted of groups 1 and 2, composing predominantly table grapes from the east Mediterranean region including some of the seedless types along with a number of southern European minor wine varieties. The cultivars of group 1 represent the west Asian region and possess morphological traits typical of the group *orientalis*. These traits, typified by cultivars such as ‘Thompson Seedless’ and ‘Khalili’, include absence or sparsely distributed prostrate hairs on shoot tips, shiny young leaves, mostly glabrous when fully expanded, large and loose clusters with

medium to large, round to elliptical, firm-fleshed fruits (Negrul, 1938; Levadoux, 1956). Group-2 cultivars are mainly table types but also include some dual-use and wine types. These cultivars (e.g. ‘Black Corinth’, ‘White Corinth’ and ‘Chaouch’) display a high density of prostrate to erect hairs on vegetative organs, mainly shoot tips and lower surface of leaves, and juicy small-to-medium round fruits, characteristics of the group *pontica* (Levadoux, 1956) and believed to be intermediate between the groups *orientalis* and *occidentalis* (Negrul, 1938). This assertion is supported by the fact that there are both wine and table types in this group.

#### (b) Western European wine-grape cluster

This cluster is made up of ten groups (3–12), of which five (6–10) contained the French wine grapes and the wild grape *ssp. sylvestris* accessions, with a number of primarily Greek and Italian wine grapes and some table and dual-use types forming five other groups (3, 4, 5, 11 and 12). This cluster reflects the close association between the group *occidentalis*, to which the French wine grapes belong, and the group *pontica*, represented by Greek and Italian wine and table type grapes. The association of French with Greek and Italian wine grape groups in this cluster is probably due to historical exchange of germplasm among these countries. According to Negrul (1938) and Levadoux (1956), cultivars of the *occidentalis* group are closer to the wild grape, *ssp. sylvestris*, and possess many wild characters such as low to medium density of prostrate hairs on leaves, small to medium, round or elliptic juicy berries borne in small to medium-sized compact clusters. Many cultivars that exemplify this morphology are found in groups 6–10: ‘Touriga Nacional’, ‘Gewürztraminer’, ‘Sauvignon Gris’, ‘Cabernet Sauvignon’, ‘Petite Verdot’ and ‘Aligote’. The association of wild and wine grapes in clusters 6, 7 and 8 could be attributed to introgression between the wild and cultivated grapes that occurs spontaneously in transitional habitats, or might represent the autochthonous form of *V. vinifera*. However, further studies with a broader sampling basis are required to develop a full understanding of this association.

#### (c) Central European grape cluster

This cluster involved four groups (13–16) containing the wine types from southern and south-central European regions along with a number of table types

Fig. 1. Neighbour-joining cluster analysis based on the pairwise Dice coefficient of association showing the genetic relationships among grape cultivars. G1–G16 represent the genetic groups recognized for further analyses of genetic structure and differentiation within the cultivated grapes. Arrows at the centre of the phenogram demarcate the three clusters (G1 and G2, Mediterranean table grape cluster; G3–G12, Western European grape cluster; G13–G16, Central European grape cluster).

mostly belonging to the group *pontica*. The degree of differentiation among these groups is marginal and they shared most alleles at all loci, although their frequency varied. However, group 14 appeared to be distinct in containing some of the Muscat types and showed close association with group 13. The intermixing of wine and table types in this cluster was probably due to the infusion of genes from the Near Eastern table types into southern European wine types. This is supported by the fact that the present-day European grape gene pools are made up of cultivars domesticated from the indigenous wild vines, and introduction and introgression of the Near Eastern germplasm (Ambrosi *et al.*, 1994).

In outbreeding perennial species such as grapes, biochemical and nuclear DNA markers often provide weak discrimination between populations because most variation resides within populations (Brown, 1979; Hamrick & Godt, 1990; Brown & Schoen, 1992). The high levels of within-group variation and the simple genetic structure observed in the phenogram probably suggest a complex history of development of grape cultivars. Several mechanisms are thought to have been contributing factor in the development of European grape cultivars (Levadoux, 1956), such as the introduction and spread of wild and semi-domesticated grapes, especially from its native Near Eastern range, domestication of indigenous wild grape, natural hybridization between indigenous and introduced vines, and human selection. Although *in situ* domestication of indigenous wild germplasm is considered to have played an important role in the early cultivar development in Europe and West Asia, continued spread of wild and domesticated germplasm among different grape-growing regions might have acted to homogenize the different regional gene pools over time.

The overall distribution pattern of molecular variation suggests that the cultivated grape represents a single complex gene pool within which historical movement of germplasm, recent introductions, hybridization, and human selection are shaping the genetic structure. Clonal crop germplasm collections are unique in that they are composed of genetically discrete clones representing cultivars, special genotypes, breeding material and somatic mutants, unlike seed crop collections, which represent a dynamic mutation-recombination system. Hawkes (1975) discussed the problems associated with genetic conservation of clonal crops. He aptly pointed out that strong artificial selection and clonal propagation greatly altered the original genetic structure of these crops.

(ii) *Genetic variability within cultivar groups*

The organization of genetic diversity in clonal germplasm collections is reminiscent of historical genetic

Table 2. Frequencies for the most common allele among the 16 groups obtained in the neighbour-joining cluster analysis; - indicates absence of allele. Groupwise observed allele frequencies for different loci are posted in the *Vitis* section at <http://www.ars-grin.gov/dav/>

Locus (alleles per locus)	Most Frequent allele	Group															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Accessions per group	20	25	21	9	16	4	12	18	9	6	24	10	33	13	16	7	
<i>VVS2</i> (15)	133	0.100	0.020	0.143	0.111	0.188	0.250	-	0.028	0.333	0.583	0.400	0.394	0.577	0.344	0.429	
Alleles per group	10	11	7	8	5	3	6	9	5	3	7	6	7	5	9	4	
<i>VVMD5</i> (10)	228	-	0.025	0.100	0.095	0.222	0.063	0.375	0.250	0.111	0.333	0.042	0.200	0.030	0.346	0.125	
Alleles per group	6	8	9	5	6	3	6	7	7	5	7	6	7	8	6	6	
<i>VVMD6</i> (5)	205	-	0.040	-	0.056	0.125	0.750	0.292	0.639	0.556	0.167	0.250	0.061	0.038	0.031	0.071	
Alleles per group	4	5	4	4	3	3	4	4	4	4	5	5	5	4	5	5	
<i>VVMD7</i> (13)	239	0.125	0.020	0.595	0.444	0.750	0.750	0.625	0.333	0.611	0.417	0.350	0.091	0.231	0.063	0.357	
Alleles per group	6	7	8	6	5	3	4	8	8	4	6	5	9	6	8	4	
<i>VVMD27</i> (9)	181	-	0.150	0.060	0.095	0.167	0.250	-	0.208	0.083	0.111	0.417	0.050	0.545	0.038	0.031	
Alleles per group	5	8	8	5	6	3	3	6	6	4	7	5	8	6	6	6	
<i>VVMD28</i> (19)	239	0.100	0.120	0.048	-	0.313	0.500	0.250	0.111	0.167	0.167	0.100	0.061	0.038	0.188	0.143	
Alleles per group	9	11	11	8	7	4	5	8	8	7	8	6	12	8	9	4	
<i>VVMD31</i> (11)	212	0.525	0.2	0.643	0.556	0.406	0.125	0.083	0.583	0.222	0.167	0.1	0.182	0.462	0.563	0.643	
Alleles per group	7	7	6	6	4	3	6	5	5	4	3	6	9	5	5	3	
<i>VVMD32</i> (13)	253	-	0.12	0.214	0.167	0.281	0.375	0.125	0.083	0.056	0.583	0.15	0.258	0.077	0.188	0.071	
Alleles per group	4	10	7	6	7	3	6	4	4	5	2	8	7	7	7	7	



Table 3. Genetic variability measures for the 16 groups obtained in the neighbour-joining cluster analysis. Values in parentheses indicate standard errors

Group	Accessions	Mean alleles per locus	Mean heterozygosity observed	Mean heterozygosity expected
1	20	6.4 (0.8)	0.775 (0.033)	0.742 (0.025)
2	25	8.3 (0.7)	0.770 (0.044)	0.753 (0.022)
3	21	7.5 (0.7)	0.798 (0.037)	0.720 (0.036)
4	9	6.0 (0.5)	0.806 (0.069)	0.750 (0.045)
5	16	5.4 (0.5)	0.727 (0.073)	0.693 (0.046)
6	4	3.0 (0.2)	0.625 (0.116)	0.567 (0.044)
7	12	5.0 (0.4)	0.698 (0.063)	0.672 (0.034)
8	18	6.4 (0.7)	0.646 (0.072)	0.653 (0.034)
9	9	5.1 (0.4)	0.861 (0.041)	0.711 (0.039)
10	6	4.3 (0.6)	0.875 (0.042)	0.729 (0.043)
11	24	6.8 (0.3)	0.771 (0.032)	0.765 (0.020)
12	10	5.9 (0.4)	0.900 (0.033)	0.793 (0.018)
13	33	7.9 (0.6)	0.716 (0.038)	0.752 (0.025)
14	13	6.1 (0.5)	0.798 (0.048)	0.717 (0.031)
15	16	6.9 (0.6)	0.789 (0.033)	0.741 (0.026)
16	7	4.9 (0.5)	0.786 (0.047)	0.698 (0.036)

structure originating from natural evolution, domestication and modern plant breeding. Such a structure could generally be described based on the amount and pattern of distribution of genetic diversity within and between different geographic or genetic groups.

Computation of within-group variability measures on the group-wise pooled genotype data revealed extensive genetic variability in the cultivated grape. All eight loci exhibited three or more abundant alleles and possessed a number of minor alleles in heterozygous combination with major alleles. The contingency  $\chi^2$  analysis revealed significant heterogeneity among the groups for the composition and frequency of alleles at different loci. Because of the large size of the data sets, only the frequency of the most common allele among the groups is presented (Table 2) (complete data on web site). The presence of minor alleles in different groups can be attributed to either rare mutations or infrequent genotypes in the collection. In clonal crops, recombination and spread of alleles are limited by the asexual mode of reproduction and tend to remain rare among a few unique genotypes in which they are present or arise. However, the origin and maintenance of these minor alleles have great significance in the evolution and conservation of diversity in clonally propagated species.

Measures of within-group genetic diversity are summarized in Table 3. The mean number of alleles per locus ranged from 3 for group 6, which is unique in containing three wild grape genotypes from Tunisia along with an accession from Corsica, to 8.3 for group 2, which is composed mostly of table grapes from southern Europe with an average of six alleles per

locus across groups. However, the mean number of alleles per locus, which is an indicator of variability, should be interpreted cautiously because it is sensitive to sample size. All the eight loci assayed were polymorphic in all the 16 groups produced by the NJ cluster analysis. The mean observed heterozygosity levels were slightly higher than the expected panmictic proportions except in two groups, which recorded slightly lower levels. It ranged from 0.625 for group 6 to 0.9 for group 12, with an overall average of 0.771. Such high levels of heterozygosity are commonly observed among clonally propagated, outbreeding, perennial species because it is favoured during selection and is known to confer greater adaptability, vigour and productivity on clonal varieties (Aradhya *et al.*, 1998; Sefc *et al.*, 2000). Grapes, being an outbreeding species, have highly heterozygous cultivars, carry a heavy genetic load and suffer severe inbreeding depression (Olmo, 1976).

### (iii) Genetic structure and hierarchical partitioning of gene diversity

The UPGMA cluster analysis on the pairwise unbiased genetic identities between groups to examine the intergroup relationships produced a slightly different picture of overall relationships (Fig. 2) to the NJ cluster analysis on the Dice coefficient matrix. The identities of the three clusters observed in the NJ cluster analysis were not obvious, but the overall affinities remained mostly unchanged. Examination of various group associations on the UPGMA tree cast more light on the nature and level of organization of genetic variability within the cultivated grape. There are four

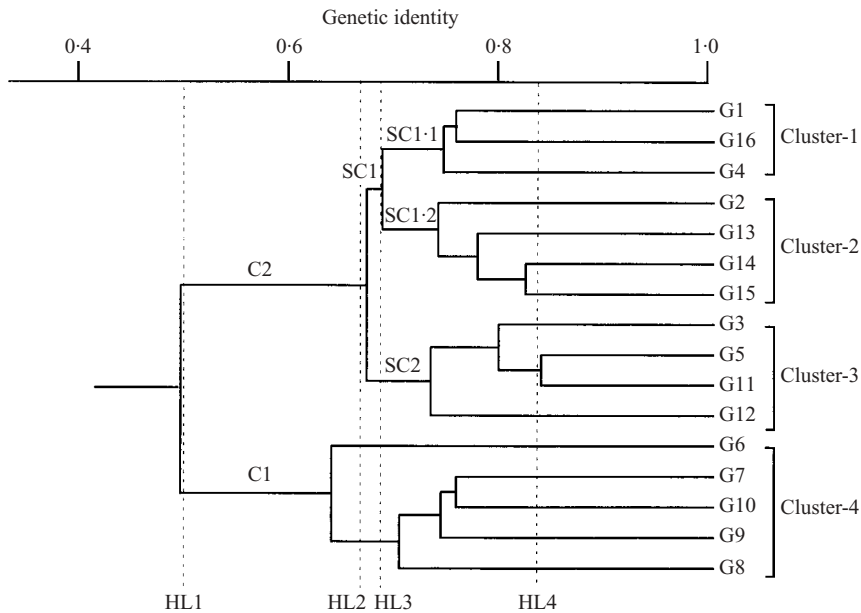


Fig. 2. UPGMA cluster analysis depicting the intergroup relationships at different levels of genetic identity (cophenetic correlation = 0.750). HL1–HL4 refer to differentiation at different hierarchical levels. Cluster 1, predominantly table type belonging to *orientalis* and some to *pontica*; clusters 2 and 3, mostly wine type with some table type belonging to *pontica*; cluster 4, the French wine types representing the group *occidentalis*.

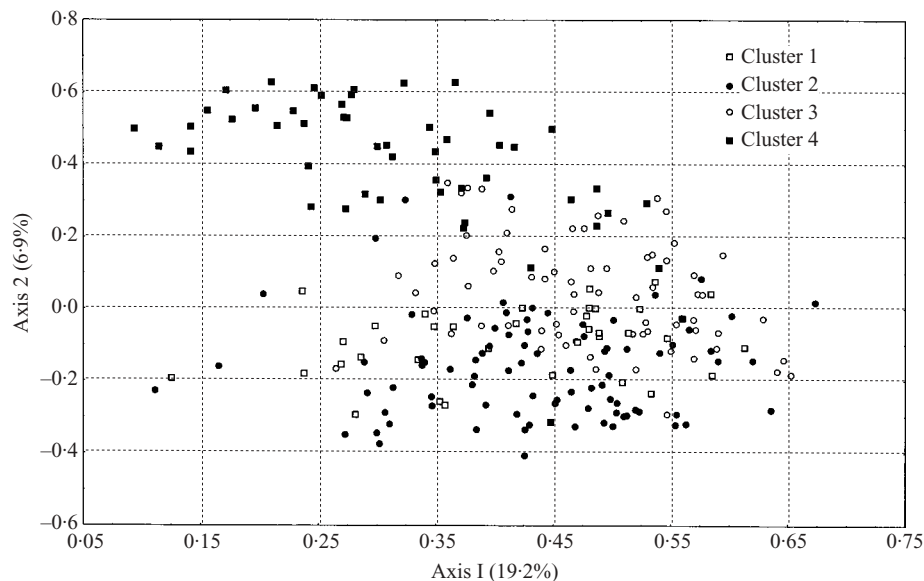


Fig. 3. Two-dimensional projection of grape cultivars along the first two principal axes accounting for ~26% of the total variation. (Clusters correspond to the UPGMA analysis in Fig. 2.)

different hierarchical levels (HL1 to HL4) at which clusters can be recognized on the phenogram. The first split into two major clusters (C1 and C2), occurred at ~50% genetic similarity (HL1), with the French wine grapes (groups 6–10, Fig. 1) forming a cluster (C1). The remaining groups constituted C2, which in turn branched into two sub-clusters (SC1 and SC2) at ~65% genetic similarity (HL2). The sub-cluster SC1 further divided into two clusters (SC1.1 and SC1.2) at ~67% genetic similarity (HL3). Altogether, four clusters, referred as clusters 1 to 4 from

here on, could be visualized (not to be confused with the three clusters observed in the NJ cluster analysis). The French wine types representing the group *occidentalis* formed a distinct cluster (4) and the groups containing mostly wine-type cultivars belonging to the group *pontica* constituted two separate clusters (2 and 3). The groups predominant in table type belonging to *orientalis* and some to *pontica* formed cluster 1. Finally, at ~85% genetic similarity (HL4), the terminal branches represented the 16 groups as seen in the NJ cluster analysis.

Table 4. Frequencies for the most common allele among the four clusters generated in the UPGMA cluster analysis. Clusterwise observed allele frequencies for different loci are posted in the *Vitis* section at <http://www.ars-grin.gov/dav/>. Numbers in parentheses indicate the number of alleles in the group

Locus	Allele	Cluster			
		1	2	3	4
Number of accessions		36	87	71	49
<i>VVS2</i>	133	0.167 (11)	0.305 (12)	0.183 (10)	0.163 (10)
<i>VVMD5</i>	226	0.042 (9)	0.247 (8)	0.296 (9)	0.143 (8)
<i>VVMD6</i>	194	0.139 (5)	0.126 (5)	0.232 (5)	0.01 (5)
<i>VVMD7</i>	239	0.25 (12)	0.086 (11)	0.563 (10)	0.5 (7)
<i>VVMD27</i>	181	0.125 (9)	0.236 (9)	0.232 (9)	0.102 (8)
<i>VVMD28</i>	239	0.083 (13)	0.098 (16)	0.169 (13)	0.194 (14)
<i>VVMD31</i>	210	0.153 (9)	0.207 (11)	0.12 (8)	0.082 (7)
<i>VVMD32</i>	253	0.056 (9)	0.178 (11)	0.148 (11)	0.173 (9)

Table 5. Genetic variability measures for different clusters obtained in the UPGMA cluster analysis. Values in parentheses indicate standard errors

Cluster	Accessions	Mean alleles per locus	Mean heterozygosity observed	Mean heterozygosity expected
1	36	9.1 (0.8)	0.785 (0.031)	0.776 (0.026)
2	87	10.5 (1.2)	0.757 (0.015)	0.789 (0.017)
3	71	9.1 (0.8)	0.787 (0.027)	0.782 (0.027)
4	49	8.9 (0.9)	0.724 (0.032)	0.747 (0.029)

Similar results were obtained with the PCA based on the binary data matrix (244 accessions  $\times$  94 SSR alleles). The first three principal axes accounted for only 31% of the total variation, indicating the complex multidimensional nature of relationships among grape cultivars. Projection of accessions on a two-dimensional plane defined by the first two axes (explaining  $\sim$ 26% of the total variation) revealed four overlapping groups (Fig. 3), similar to the UPGMA cluster analysis. Once again, the French wine grapes occupied a unique non-overlapping zone on the PCA plot (cluster 4), slightly extending into cluster 3, which was a part of the western European wine grapes cluster in the NJ cluster analysis. The accessions from the remaining two clusters (1 and 2) produced overlapping distributions on the PCA plot. However, the extent of overlapping of clusters apparently suggests little differentiation among different groups.

The multivariate approaches used to elucidate the genetic differentiation in cultivated grapes (NJ and UPGMA cluster analyses, and PCA) yielded generally comparable results. Nevertheless, they were chosen to complement each other, because PCA is known to be less sensitive to distances between close neighbours but to represent more precisely distances among

clusters, whereas cluster analysis generally reproduces distances between the close neighbours faithfully but shows distortion among members of large clusters (Sneath & Sokal, 1973).

The frequencies for the most common alleles among the four clusters produced by the UPGMA cluster analysis are presented in Table 4 (complete data on web site). Although clusters did not differ for allelic composition, they differed significantly for the frequency of different alleles, as indicated by the  $\chi^2$  analysis. Further analysis for various within- and between-group diversity parameters indicated appreciable sub-structuring of genetic variability. The mean number of alleles ranged from 8.9 for cluster 4 to 10.5 for cluster 2 (Table 5). The mean observed heterozygosity levels for different clusters conformed to panmictic proportions except for a slight deficiency of heterozygotes in clusters 2 and 4, which might be due to further sub-structuring within clusters.

The gene diversity analysis (Table 6) based on allele frequencies for the 16 different groups obtained by the NJ cluster analysis indicated that the total gene diversity ( $H_T$ ), a measure of mean heterozygosity in the total collection, was quite uniform for all loci, ranging from 0.773 for *VVMD6* to 0.888 for *VVMD28* with an overall average of 0.826, indicating substantial

Table 6. Measures of gene diversity and additive partitioning of total diversity into hierarchical components caused by differentiation in cultivated grapes

Component	VVS2	VVMD5	VVMD6	VVMD7	VVMD27	VVMD28	VVMD31	VVMD32	Mean
Gene diversity ( <i>D</i> )									
$H_T$	0.855	0.861	0.773	0.789	0.834	0.888	0.777	0.834	0.826
$H_G$	0.738	0.752	0.645	0.656	0.668	0.768	0.664	0.712	0.700
$D_{43}$	0.081	0.054	0.062	0.046	0.103	0.080	0.079	0.054	0.070
$D_{32}$	0.009	0.021	0.000	0.012	0.007	0.014	0.012	0.017	0.011
$D_{21}$	0.009	0.014	0.023	0.045	0.005	0.008	0.011	0.002	0.015
$D_{1T}$	0.018	0.020	0.042	0.031	0.051	0.018	0.011	0.049	0.030
Coefficient of gene differentiation ( <i>G</i> )									
$H_G/H_T$	0.863	0.873	0.835	0.830	0.801	0.865	0.855	0.854	0.847
$G_{43/T}$	0.095	0.063	0.080	0.058	0.124	0.090	0.102	0.064	0.084
$G_{32/T}$	0.010	0.024	0.001	0.015	0.008	0.015	0.015	0.020	0.014
$G_{21/T}$	0.010	0.016	0.030	0.057	0.006	0.009	0.014	0.003	0.018
$G_{1T}$	0.021	0.024	0.054	0.040	0.061	0.020	0.014	0.059	0.037

$H_T$ , total gene diversity;  $H_G$ , gene diversity within groups;  $D_{43}$ , gene diversity owing to differentiation at hierarchical level 4 within level 3;  $D_{32}$ , gene diversity owing to differentiation at hierarchical level 3 within level 2;  $D_{21}$ , gene diversity owing to differentiation at hierarchical level 2 within level 1;  $D_{1T}$ , gene diversity owing to differentiation at hierarchical level 1 within total (see Fig. 2 for hierarchical levels of differentiation).

Table 7. Measures of genetic diversity within table, wine and wild grape groups. Value in parentheses indicates standard errors

Group	Accessions	Mean alleles per locus	Mean heterozygosity observed	Mean heterozygosity expected
Wild	22	6.4 (0.8)	0.597 (0.050)	0.682 (0.033)
Table	65	9.8 (1.1)	0.788 (0.030)	0.813 (0.019)
Table/wine	24	8.1 (0.7)	0.823 (0.041)	0.816 (0.018)
Wine	132	10.3 (1.0)	0.768 (0.020)	0.813 (0.016)

amounts of genetic variation in the collection. Earlier studies on *Vitis* have reported similar levels of total gene diversity (heterozygosity) for different SSR loci (Lamboy & Alpha, 1998; Sefc *et al.*, 2000). On average, 84% of the total gene diversity resided within groups with only a marginal level (16%) accounting for genetic differentiation. This situation is common among outcrossing and vegetatively propagated perennial species, which are generally highly heterozygous and maintain high levels of genetic variation within populations (Brown & Schoen, 1992; Hamrick, 1983). Hierarchical partitioning of gene diversity at different levels of differentiation, as seen in the UPGMA cluster analysis has shown that ~4% of the total variation was due to genetic differentiation between French wine types and the rest of the collection. About 8% of the total diversity was due to overall differentiation into 16 groups produced by NJ cluster analysis. However, the eight loci assayed differed in the magnitude of variation accounting for differentiation among groups at different hierarchical levels. Finally, the UPGMA cluster analysis based on the groups obtained in the NJ cluster analysis provided greater support for the classical eco-geographic

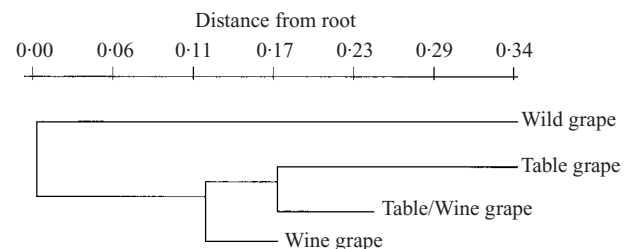


Fig. 4. The distance Wagner tree showing genetic divergence among wine, table and wild grapes.

classification of grape cultivars as compared to the NJ cluster analysis.

#### (iv) Genetic differentiation among wine, table and wild grapes

Grape cultivars are traditionally grouped into wine and table types based on their use. Many of these cultivars are the products of hundreds of years of selection representing the diverse traditional knowledge, preference and culture of grape-growing regions of the world. Therefore, it is interesting to examine the genetic diversity and differentiation within and among

these groups. Allelic composition and frequencies differed significantly among wine, table and wild grape types, with many unique alleles for each type. The mean expected heterozygosity levels ranged from 0.682 for wild grape to 0.816 for the wine and table types (Table 7). The low heterozygosity level of the wild-grape group might be due to inbreeding caused by mating among siblings in the small isolated populations in which they are generally found. Genetic divergence estimated using the distance Wagner procedure indicated that the table grape has highly differentiated from the wine and wild grapes (Fig. 4). This confirms Negrul's observation that the table grapes of the group *orientalis* were uniquely selected under intensive, irrigated agriculture of the ancient oases in the southern parts of the Caucasus for large, branching clusters with medium to large firm-fleshed fruits (Negrul, 1938).

In summary, the gene pool of cultivated grapes surveyed has significant amounts of genetic variation and exhibits narrow differentiation. The French wine grapes appear to be distinct and show close affinity to the ssp. *sylvestris* included in the study. The overall organization of genetic diversity suggests that the germplasm of cultivated grapes represents a single complex gene pool and its genetic structure has been influenced by strong artificial selection. In regard to germplasm management, our results show that the germplasm collection is highly variable and most variation (~85%) is common to all the genetic groups identified. Second, only minimal gains in the variability are possible through extensive collection from diverse eco-geographic sources. Third, unique cultivated genotypes, wild (ssp. *sylvestris*) and spontaneous introgressive hybrids are major sources of new alleles, in addition to the slow process of bud mutation. Finally, diverse wild grape germplasm is a potential source of unique alleles and is important for the improvement of both wine and table grapes.

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