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Genetic diversity and differentiation within and between cultivated (*Vitis vinifera* L. ssp. *sativa*) and wild (*Vitis vinifera* L. ssp. *sylvestris*) grapes

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Summary

Genetic characterization of 502 diverse grape accessions including 342 cultivated (*V. vinifera* ssp. *sativa*) and 160 wild (*V. vinifera* ssp. *sylvestris*) grapes showed considerable genetic diversity among accessions. A total of 117 alleles were detected across eight SSR loci with the average of 14 alleles per locus. The genetic diversity of wild grapes was slightly lower than that observed in the cultivated grapes probably due to small populations and severe natural selection leading to drift and loss of alleles and heterozygosity in wild grapes. The distance cluster analysis (CA) supported the classical ecogeographic groups with moderate genetic differentiation among them. There was a greater affinity of Occidentalis grape to wild grape from the Caucasus than other groups. However, a number of low to moderate frequency alleles that are present in the cultivated grape are not represented in the wild grape.

K e y w o r d s : genetic diversity, genetic differentiation, domestication, microsatellite.

Introduction

The cultivated grape (*Vitis vinifera* L. ssp. *sativa*) is an important fruit crop of Antiquity. Archaeological records suggest that the cultivation of domesticated grapes began approximately 6000-8000 years ago in the Near East. It is believed to have been domesticated from the wild grape (*Vitis vinifera* L. ssp. *sylvestris*) and later moved to the eastern, northern and western parts of Eurasia and North Africa following trade routes of the ancient civilizations. The Near East origin of cultivated grapes is supported by recent molecular analyses (Aradhya *et al.* 2003, Arroyo-Garcia *et al.* 2006), although there is a possibility that multiple independent domestication events occurred in the Near East and southwestern Europe (Arroyo-Garcia *et al.* 2006).

Although cultivated and wild grapes are easily distinguishable based on berry and cluster characteristics, their taxonomic distinction into two subspecies (*sativa* and *sylvestris*) is still controversial. Genetic evidences have shown that moderate to high gene-flow is possible between wild and cultivated grapes (Di Vecchi-Staraz *et al.* 2009, Arroyo-Garcia *et al.* 2006, Negrul 1938). These findings suggest that further comprehensive studies with broader sampling of germplasm are necessary to develop a full understanding of the genetic relationship between wild and cultivated grapes. Currently, the wild grape is endangered due to several reasons: human pressure on natural resources, out-crossing with cultivated grapes (Di Vecchi-Staraz *et al.* 2009), and lack of conservation efforts to preserve the genetic diversity of wild grapes. Identification and characterization of wild grapes is important to assess the genetic diversity and relationship between wild and cultivated grapes for understanding the domestication of cultivated grapes, as well as for managing genetic diversity in grapevine germplasm collections. In addition, wild grapes could be a valuable source of genes and contribute to future grape breeding programs.

The objectives of this study are to (1) analyze the genetic diversity, and differentiation within and between cultivated and wild grapes, and (2) discuss the results in relation to the domestication history of grape.

Material and Methods

P l a n t m a t e r i a l a n d P C R a s s a y : A total of 502 accessions including 342 cultivated and 160 wild grape accessions were analyzed in this study. Among them, 444 were from the collection of the National Clonal Germplasm Repository (US Department of Agriculture, Davis, CA), and the rest came from a collection of the Institute for Adriatic Crops and Karst Reclamation, Split, Croatia and sites of Albania. Total DNA was isolated from dried leaf tissue using the CTAB method and treated with RNase A to remove RNA contaminants.

E i g h t m i c r o s a t e l l i t e m a r k e r s : *VVS2* (Thomas and Scott 1993), *VVMD5, VVMD7, VVMD27, VVMD31, VVMD34* (Bowers *et al.* 1996, Bowers *et al.* 1999 a), *VrZAG62* and *VrZAG79* (Sefc *et al.* 1999) were PCR amplified in a 4-plex fluorescent dye system (6FAM, VIC, NED, PET) in a 15 µl reaction mixture containing 10 mM Tris–HCl, pH 8.3, and 50 mM KCl (all included in 10X PCR buffer), 2 mM $MgCL_2$, 0.9 pmol of each primer, 0.2 mM of each dNTP, 0.6 U of Taq polymerase (New England BioLabs, Ipswich, MA), and approximately 25 ng of template DNA. The PCR conditions were as follows: 1 cycle of 94 °C for 5 min, 30 cycles of 94 °C for 30 s,

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55 °C for 30 s, and 72 °C for 40 s, and then a final elongation of 72 °C for 7 min. Amplified products were resolved using capillary electrophoresis on a ABI 3130xl Genetic Analyzer with Data Collection software, version 3.0 (Applied Biosystems, Foster City, CA). The data were further analyzed using the Genotyper, Version 2.5 (Applied Biosystems) and data assembled as bi-allelic genotypes and in a binary matrix (1 = presence, 0 = absence) format.

Data analysis: Genetic relationship among accessions was assessed by a cluster analysis (CA) using Neighbor-joining algorithm as implemented in the MEGA 5.0 software (TAMURA *et al.* 2011) using a distance matrix assembled based on the proportion of alleles shared between two accessions for all possible pair-wise combinations. The bootstrap interior branch test (DOPAZO 1994) was used to test the reliability of each interior branch on the tree.

The accessions were grouped according to the results of the CA and the SSR genotype data were subjected to analysis of total and within-group genetic diversity measures such as mean number of alleles per locus (*A*), observed (*Ho*) and expected (*He*) levels of heterozygosity, and fixation index (*F*) for different loci.

Results and Discussion

High levels of genetic polymorphism and heterozygosity (Table) among accessions were observed for the eight microsatellite markers analyzed. The mean number of alleles per locus observed for different loci ranged from 10 for *VVMD34* to 18 for *VVS2* and *VVMD7* with an overall average of 14.0 alleles per locus. The observed heterozygosity ranged from 0.476 for *VVMD34* to 0.837 for *VVS2*. Among the ecogeographic groups, the mean number of alleles per locus ranged from ~10 for *Pontica* and *Orientalis* groups to 8.5 for the *Occidentalis* and 9.9 for the wild grape. The genetic diversity observed among the cultivated grapes was slightly higher than in the wild grapes. The reason for low genetic diversity and heterozygosity for wild grape could be due to the fact that they exist in small isolated populations permanently exposed to severe natural selection pressure, where sibmating leads to inbreeding and loss of alleles and heterozygosity.

Table

Genetic diversity in the grape germplasm used in the study

Locus/ subcluster	N	*A*	H_o	H_e	**F**
VVMD5	502	15.000	0.801	0.877	0.087
VVMD7	497	18.000	0.769	0.857	0.103
VVMD27	502	14.000	0.747	0.848	0.119
VVMD31	501	14.000	0.643	0.769	0.164
VVS2	502	18.000	0.837	0.886	0.056
VrZAG62	479	13.000	0.804	0.837	0.040
VrZAG79	477	15.000	0.767	0.861	0.109
VVMD34	471	10.000	0.476	0.520	0.086
Mean		14.000	0.730	0.807	0.095
Wild grape	127	9.875	0.651	0.749	0.131
Occidentalis-1	52	8.250	0.757	0.737	-0.027
Occidentalis-2	62	10.125	0.721	0.749	0.037
Pontica	105	10.500	0.761	0.746	-0.020
Orientalis	156	11.375	0.770	0.792	0.028
Mean		10.025	0.732	0.755	0.030

N, number of individuals; A, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; F, fixation index

Cluster analysis shows the wild and cultivated grapes are genetically divergent (Figure). A set of Western European wine grape (subcluster 2) predominantly belonging to the *Occidentalis* groups appeared closely related to the wild grape from the Caucasus suggesting direct selection of some of ancient Western European wine grapes. The close affinity of wild grape to the cultivated grape groups as depicted in the CA suggest that the domestication process has produced mild genetic differentiation between the wild and cultivated grape. However, there are many high frequency alleles present in the cultivated grape that are not represented in the wild grape (Data not shown). These alleles were probably low frequency in wild grape and subsequently lost due to range reduction and drift in small isolated populations; these alleles may also have been favored during domestication increasing their frequencies in cultivated grape.

The subclusters and their relationships corroborate the genetic divergence and eco-geographic adaptations as influenced by human selection during early domestication history as hypothesized by NEGRUL (1938). The wide divergence of *Pontica* and *Orientalis* subclusters from the wild grape as compared to *Occidentalis* in CA basically supports NEGRUL's hypothesis (NEGRUL 1938) that these groups have experienced longer history of human selection and naturally show greater genetic divergence compared to *Occidentalis*, which has undergone at most 8,000-10,000 years of human selection for wine production.

ARROYO-GARCIA *et al.* 2006 have reported the possibility of multiple domestication events in different geographic locations in the origin of cultivated grape. Our data, from several geographic sources of wild and cultivated grapes, supports at least two separate domestication events that gave raise to cultivated grape; one derived from the wild grape from Transcaucasia, and another from the wild grape of southern European and North African origin. With wider representation of wild grape, one may be able to demonstrate the multiple domestication events supporting diffused center of domestication of cultivated grape.

The distribution of alleles at the eight SSR locus analyzed revealed no clear geographical trend of clustering among the analyzed accessions of cultivated grapes, but the clear distinction between cultivated and wild grapes is evident. Clear separation of grape cultivars into classical groups as suggested by NEGRUL (1938) is practically difficult considering the wide-spread movement of germplasm along the early trade routes, subsequent gene flow among introduced cultivars from different ecogeographic group, and with native wild grape during early domestication and propagation by seeds. Since the Middle Ages vegetative propagation is believed to have been the preferred way of transportation of grape cultivars. This observation and

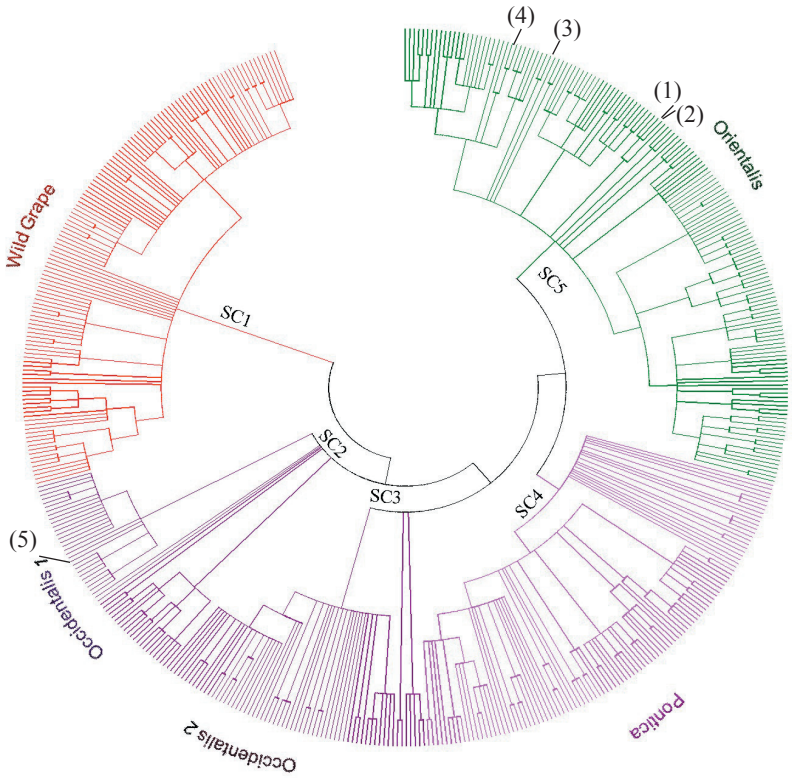

Figure: A condensed minimum evolution tree showing genetic differentiation within and between cultivated and wild grapes - branches are supported at the confidence probability values ≥ 75 % computed using bootstrap interior branch test; SC1: Wild grape, SC2: *Occidentalis1*; SC3: *Occidentalis2*; SC4: *Pontica* and SC5: *Orientalis*. Arrows indicate the position of the cultivars discussed in the text, (1) 'Chardonnay', (2) 'Gamay', (3) 'Olivette Noir', (4) 'Silvaner' and (5) 'Pinot Noir'.

the historical fact that man has moved grape germplasm around the grape-growing regions of the world make it very difficult to identify the geographic trend in the frequency of alleles and to trace the origins of many grape cultivars (Aradhya *et al.* 2003). However, subtle genetic differentiation among different eco-geographic groups is evident in the CA presented here.

There is sufficient evidence from CA that the *Pontica* grape, which is considered an immediate domesticate of wild grape in the Baltic and Pontic regions greatly contributed germplasm for the development of both the Western European wine (*Occidentalis*) and the Near Eastern table (*Orientalis*) grapes during domestication (Negrul 1938).

There are several factors that confound the early development of European grape cultivars, such as the introduction, selection, and spread of genotypes derived from the *Pontica* grape in Transcaucasia, where grape was originally domesticated, and the domestication of native wild grapes and natural hybridization between native wild and introduced vines, and the selection by humans. This has resulted in complex relationship among cultivated wine grape from several wine growing regions of the world. Often in southeastern Europe, grapes from West Asia and the Near East have been bred into wine grape, especially for dessert and sparkling wine and this has brought in genes from the *Orientalis* group into *Occidentalis* grape creating complex relationships among different grape groups.

The uniqueness of table grape cultivars (*Orientalis*), and their divergence from the Western European (*Occidentalis*) wine cultivars seen in CA of this study corroborate earlier reports (Aradhya *et al.* 2003). Several well known Western European cultivars ('Chardonnay' (1), 'Gamay' (2), 'Olivette Noir' (3), and 'Silvaner' (4)) grouped within the *Orientalis* subcluster, indicating their Near Eastern origin. 'Chardonnay' and 'Gamay' are progeny of the single pair of parents 'Pinot Noir' (5) and 'Gouais Blanc' and their contrasting position from other Western European cultivars in the cluster analysis is not surprising considering the Mendelian inheritance of SSR alleles and the Eastern origin of the parent 'Gouais Blanc'. Also, association of several cultivars traditionally grown in Hungary, Croatia, Greece and Italy within *Occidentalis* group could be explained as exchange of germplasm between these countries in the recent past.

In summary, SSR analysis in this paper demonstrated that wild and cultivated grapes are genetically divergent. Within the wild grape there was significant differentiation between the populations from the Caucasus and the Pyrenees and the Atlas Mountain. Our data support the ecogeographic groups proposed by Negrul (1938) and showed that there was differential response of alleles during domestication resulting in marginal differentiation among the groups.

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