Wild Grapevine (*Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi) in Italy: Distribution and Preliminary Genetic Analysis

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Abstract

***Vitis vinifera* L. ssp. *sylvestris* grows principally in well preserved natural habitats. Its survival is threatened mainly by human land use practices. In spite of its importance as a progenitor of cultivated forms, very little research has been devoted to the study of its distribution and genetic structure. During the period 2007-2009 a census was carried out in 9 of the 20 Italian regions with major intensification in 4 of them. Eight hundred and twenty plants corresponding to 165 sites were found. Over 50% of these were localized in two regions only. After collection of the wooden material for greenhouse propagation and after rooting of the cuttings, samples were taken to perform genetic analysis using 10 nuclear SSR markers. Some genetic parameters (N_e, H_o, H_e, F_{is}, F_{st} and I) were calculated on the allelic size, grouping plants once for locus and once for region of origin. The results showed Italian wild grapevine expressed altogether high biodiversity and low rate of isolation. In particular plants from Sicilia, in spite of their low number, stood out for their high heterozigosity and low inbreeding and isolation level. This particularity brought the same plants to have the high genetic distance in the NJ phylogenetic tree. PCA analysis separated in 2 well-defined groups along the first component without correspondence with geographical grouping. AMOVA analysis confirmed that the highest variance was placed within populations (only 5% of the overall variance was placed among populations). SSR marker analysis is still in progress to verify the existence of introgression among wild and cultivated compartments and to comprehend the extent of factors driving the genetic structure and the possible pattern of dissemination of wild grapevine in Italy.**

INTRODUCTION

Vitis vinifera L. ssp. *sylvestris* (Gmelin) Hegi is the only ancestral European *Vitis* species, wild progenitor of the cultivated grapevine *Vitis vinifera* L. var. *sativa* (Beck). Past evidences prove that it was present in areas where now is absent (Arnold et al., 1998). So in the last century because of direct and indirect human impact on the environment its spread is drastically decreased in all Europe. Currently it grows in characteristic habitats, like riparian woods, lagoon borders and gullies in hill or mountain areas, where some moisture remains and the natural habitat is better preserved. In spite of its importance as progenitor of the cultivated forms, very little research work has been devoted to the study of its distribution and genetic structure.

Previous research was conducted on distribution of Italian wild populations (Failla et al., 1992; Anzani et al., 1993) from 1985 to 1995. This led to find many sites where wild grapevine grew, but these were not geo-referenced and after 1995 no other census was carried out to deepen the knowledge about wild grapevine spread and to verify the survival of the wild populations previously recorded. Some studies dealt with the genetic analysis of the Italian wild grapevine (Grassi et al., 2003, 2006), but they involved only

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few representative populations.

During the period 2007-2009 a census was carried out in some Italian regions. This work aimed: 1) to improve the knowledge about spread and distribution of the plants on the Italian territory; 2) to collect wooden material for propagation and conservation of germplasm; 3) to fingerprint each collected accession for a population genetic analysis.

MATERIALS AND METHODS

Census and Material Collection

During 2007-2009 period, surveys were carried out in 9 of the 20 Italian Regions to census the wild grapevine populations. Extensive surveys were conducted in 4 regions- Toscana, Lazio, Basilicata and Calabria - and limited surveys were conducted in- Lombardia, Emilia-Romagna, Marche and Sicilia.

During the growing season each individual find was geo-referenced. When possible, during the winter season some wooden material for green-house propagation was collected from each georeferenced find. Rooted cuttings were selected and transferred into pots to allow to growth. At the right moment, a few leaflets were collected from each original plant to conduct genetic analysis.

Genetic Analysis

DNA was extracted with the Qiagen's DNeasy® Plant Mini Kit. The fingerprinting was carried out through the analysis of 10 nuclear SSR marker: VrZag62, VrZag79 (Sefc et al., 1999), VVMD5, VVMD7 (Bowers et al., 1996), VVMD21, VVMD24, VVMD25, VVMD27, VVMD28 (Bowers et al., 1999) and VVS2 (Thomas and Scott, 1993). The PCR reactions followed two different protocols according to the primers' annealing temperature of 50°C (VVMD5, VVMD28 and VVS2 primers) or 56°C (other SSR primers) and number of cycles of 37 and 35 respectively. The allelic size for each locus was read by AB Prism 310 Genetic Analyzer (Applied Biosystems by Life Technology).

Data Analysis

The GenAIEx 6.4 software (Peakall and Smouse, 2006) was used to calculate the number of effective alleles (N_e), the observed and expected heterozigosity (H_o and H_e), the Wright's F-statistics (F_{is} and F_{st}) and the Shannon's Information index (I) for each locus and for each plant's group defined according to region of origin. The same software was used to perform Principal Coordinates Analysis (PCA) and Analysis of Molecular Variance (AMOVA).

The evaluation of the linkages between regional populations was carried out with POPULATIONS 1.2.28 program (Langella, 1999) using Nei's index (Nei, 1973) and 1000 bootstraps, then on this result a neighbor-joining (NJ) tree with PHYLIP 3.69 program (Felsenstein, 1989) was constructed.

RESULTS AND DISCUSSION

Census and Material Collection

During two-year census 820 plants were found corresponding to 165 populations subdivided into 9 regions as shown in Figure 1. Over 50% of these (98 populations) were localized in two regions only: Toscana and Calabria. In the first region they were assembled in the southern part, while in the latter they were scattered on the whole regional territory. Then only 712 plants were green house propagated for conservation and genetic characterization. Thirty-three of the visited populations, reported during a previous census of 1985-1995 period (Failla et al., 1992; Anzani et al., 1993), were found again. For only few plants recently censured it was possible to establish that they were the same already found.

Genetic Analysis

Genetic characterisation was performed to fingerprint each collected accession, to study the genetic structure of the populations collected and to verify the degree of genetic diversity among populations and accessions. All loci were polymorphic, VVS2 and VVMD28 performed the highest number of effective alleles, the highest observed and expected heterozigosity and the highest homogeneity in the allele frequencies distribution through Shannon's Information index (Table 1). Among all alleles and loci analysed, only VVMD21 showed inbreeding depression (F_{is} = 0,445) and genetic differentiation (F_{st} = 0,103).

Altogether plants displayed high genetic diversity (N_e, H_o, H_e and I mean values), little inbreeding (F_{is} mean value) and no differentiation (F_{st} mean value) were detected (Table 2). In spite of their small number (29 individuals), plants from Sicilia stood out for the highest heterozigosity and the lowest levels of inbreeding and differentiation resulting in the best genetically diversified population. In contrast Emilia-Romagna and Marche had the populations most genetically uniform.

The PCA analysis based on the SSR markers explained 42,6% of the total genetic variance. It was not able to separate the regional groups, although scattered points were separated in 2 well-defined groups along the first component (Fig. 2). Currently this result cannot be explained. The AMOVA analysis confirmed that the highest variance was placed within populations (only 5% of the overall variance was placed among populations).

Nevertheless cluster analysis picked out genetic links that reflect the geographic gradient of the regions (Fig. 3). In this phylogenetic tree the branch of the three south regions, Basilicata, Calabria and Sicilia, was well supported by 85 bootstrap's value (data not shown). The weakest branch was the Piemonte plants one (36 bootstrap's value). Probably to evaluate the effective genetic linkage among the regional groups further populations should be found.

CONCLUSIONS

In summary, Italian wild grapevine seems to express high biodiversity and low rate of isolation despite the threats against its survival. This does not allow us to determine clear grouping when plants are considered individually in PCA and AMOVA analyses. The plants from Sicilia are the best genetically diversified and the most distant in the cluster analysis.

SSR marker analysis is still in progress to verify the existence of possible introgression among wild and cultivated compartments and to comprehend the extent of factors driving the genetic structure and the possible pattern of dissemination of wild grapevine in Italy.

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Tables

Table 1. Mean values of number of effective alleles (N_e), observed and expected heterozigosity (H_o and H_e), Wright's F-statistics (F_{is} and F_{st}) and Shannon's Information index (I) calculated for each locus across all plants.

Locus	N_e	H_e	H_o	I	F_{is}	F_{st}
VrZag62	4,5	0,765	0,598	1,829	0,218	0,041
VrZag79	4,3	0,762	0,590	1,853	0,226	0,022
VVMD27	5,4	0,777	0,603	1,949	0,223	0,043
VVMD7	6,3	0,825	0,789	2,169	0,043	0,038
VVS2	8,3	0,867	0,736	2,368	0,151	0,056
VVMD28	8,9	0,884	0,722	2,476	0,183	0,037
VVMD5	4,8	0,765	0,685	1,798	0,104	0,065
VVMD21	2,2	0,507	0,281	1,038	0,445	0,103
VVMD24	3,4	0,691	0,610	1,586	0,118	0,029
VVMD25	4,3	0,760	0,713	1,730	0,061	0,054

Table 2. Mean values of number of effective alleles (N_e), observed and expected heterozigosity (H_o and H_e), Wright's F-statistics (F_{is} and F_{st}) and Shannon's Information index (I) calculated for each plants' group defined according to Region of origin.

Regional group	Ne	He	Ho	I	Fis	Fst
Piemonte	4,5	0,728	0,562	1,714	0,229	0,087
Emilia-Romagna	4,5	0,690	0,544	1,633	0,212	0,135
Toscana	6,5	0,797	0,655	2,110	0,178	0,001
Marche	4,1	0,700	0,538	1,656	0,232	0,123
Lazio	5,5	0,792	0,647	2,007	0,183	0,007
Basilicata	5,1	0,767	0,660	1,928	0,139	0,039
Calabria	5,5	0,795	0,680	2,018	0,145	0,003
Sicilia	6,1	0,812	0,776	1,971	0,045	-0,017
Mean	5,2	0,8	0,633	1,880	0,170	0,047
SE*	0,3	0,0	0,026	0,058	0,019	0,019

*SE = standard error.

Figures

Fig. 1. Map of the wild grapevine distribution as resulted by the 2007-2009 census. On the map Regions where wild populations were found are mentioned.

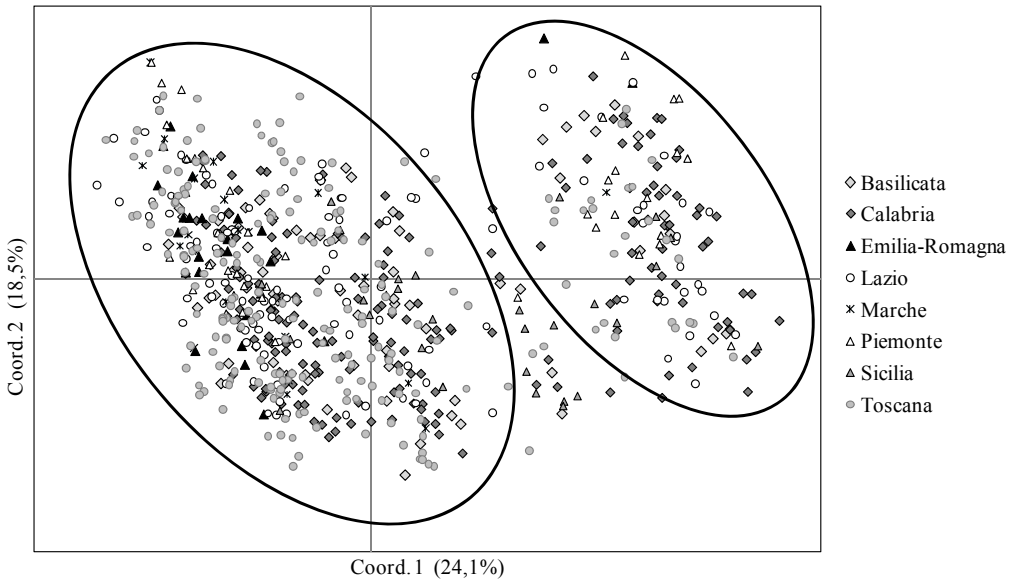

Fig. 2. Scatter plot of the Principal Coordinates Analysis (PCA). The points represent the individuals divided according to the Region of origin. Two ellipses highlight the 2 well-defined groups separated along the first coordinate.

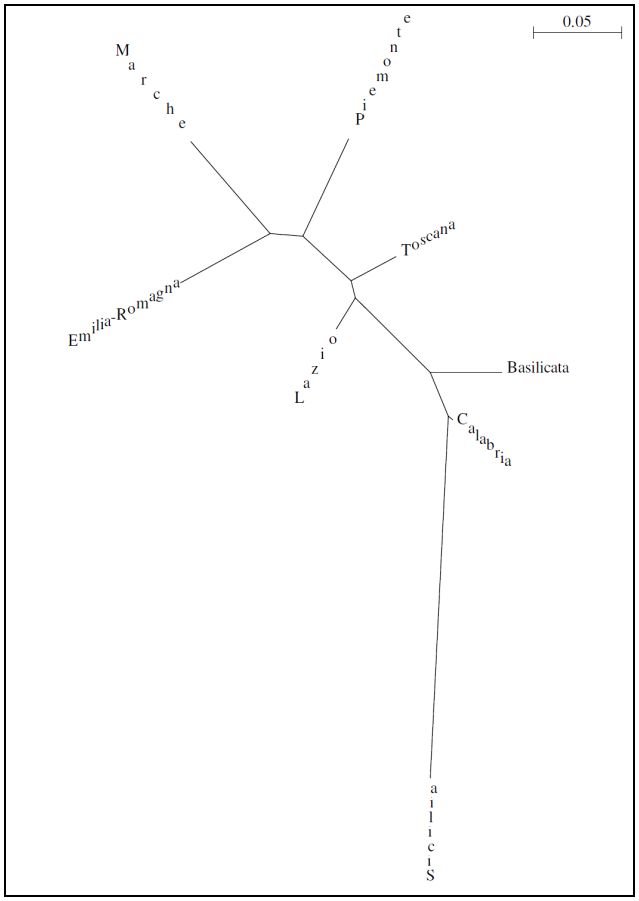

Fig. 3. Unrooted 1000 bootstrapped NJ tree based on Nei's index calculated for all pair-wise comparisons among the regional groups.