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Identification and characterization of *Vitis vinifera* subsp. *sylvestris* populations in north-western Italy

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Summary

The Italian peninsula, for its favorable environmental and geo-morphological conditions, can be considered an ultimate area for survival and development of *Vitis vinifera* subsp. *sylvestris* (Gmelin) Hegi, even though severely affected by human impact. Large surveys started in early 1990 throughout the country. At the time few regions, like Piedmont located in the north-west of the country, were considered lacking of wild *vinifera*. More recent prospection started several years ago, leading to the discovering of five *vinifera sylvestris* populations plus other sites with few individuals. The sites of discovery were described for their ecological features and the identified plants were referenced and characterized by morphology (18 descriptors from the OIV list) and genetics (14 n-SSR loci). The esteemed consistency of each population ranged from 20 to 150 individuals. Morphological and biological traits (dioecious plants, females producing very small roundish black berries), as well as genetic profiles, indicated the observed plants are true *vinifera sylvestris*. As to the ecological requirements, plants were confirmed to be highly dependent on water availability into the soil. The neighbor-joining (NJ) dendrogram resulting from SSR allelic pattern of the individuals belonging to the five populations and to one location with isolated plants, indicated population's genetic similarity broadly reflects site's geographic distance. Considering the numerous reports in the past, spreading and consistency of wild grape germplasm from the region of Piedmont severely decreased over a period of 100–150 years. The relative short distance from wild population's sites and vineyards must also be regarded as a worrying condition because of contamination risks. All means to avoid the loss of this native *Vitis* germplasm must be undertaken by protection policy and proper land management.

Key words: genetic resources; wild grapevine; microsatellite markers; n-SSR; virus.

Introduction

Vitis vinifera subsp. *sylvestris* (Gmelin) Hegi, the wild ancestor of the cultivated grape, is distributed in a large area comprehending Southern and Central Europe, Northern Africa and part of Middle East (ZOHARY and HOPF 1988).

Mainly due to its habitat destruction, often caused by human activities, and to the spreading of overseas pathogens, its presence had severely shrunk since the early twentieth century. Consistency and state of populations inventoried in Europe showed this subspecies is now in most regions an endangered plant (ARNOLD *et al.* 1998). In the last few decades, programs aimed to identify and study wild *vinifera*, and to eventually protect their populations from decline or destruction, were carried out in many European countries, in the Caucasus regions, as well as in northern Africa. True *vinifera sylvestris* individuals and/or populations were inventoried and/or studied in the Iberian peninsula (OCETE *et al.* 2002, CUNHA *et al.* 2007), in France (LACOMBE *et al.* 2003), in central Europe (REGNER *et al.* 2004), in the Balcan regions (DZHAMBAZOVA *et al.* 2009), in the Mediterranean side of Africa (SNOUSSI *et al.* 2004), and in the Middle East. Besides being a source of genetic variability and of genes of potential interest, studies on *sylvestris* grapevines offer a mean of understanding domestication process, cultivated grape evolution, and gene flow through time and regions (for a review see ARROYO-GARCÍA and REVILLA 2013).

The Italian peninsula, for its favorable environmental and geo-morphological conditions, can be considered an ultimate area for survival and development of wild *vinifera*, even though severely affected by human impact. Large surveys started in early 1990 throughout the country (FAILLA *et al.* 1992). Few regions, however, were considered lacking of *vinifera sylvestris* because there were no reports from the State Forestry Service on which those surveys were mainly based. Piedmont, the region located in the north-west of the country at the foot of the Alps, was at that time poorly explored. More recent prospection in the region started several years ago, leading to the discovering of true *vinifera sylvestris* (SCHNEIDER *et al.* 2009). The aims of these studies are: a) to recognize wild grapevine individuals or populations, b) to describe their features and understand their ecology, c) to protect them from damages and extinction, c) to study their genetic structure, and eventually their relationships with the local pool of cultivated varieties. In the present work, wild *vinifera* germplasm still existing in Piedmont (north-western Italy) is presented.

Material and Methods

The research started referring to local literature (when existing) and to other useful records on the occurrence of

subspecies *sylvestris* in the region. The *Herbarium Pedemontanum Taurinensis* (held by the University of Turin), reporting several specimens and their recovery sites dating the second half of 1800, offered useful trails for addressing the prospection. Other clues arose from botanical publications reporting lists of plant species recorded in local surveys (MUSSA 1938, CARREGA and SILLA 1995, ROTTI 1991).

The sites of discovery were described for their ecological features and the main accompanying botanical species. The identified plants were referenced, morphologically characterized by the 18 descriptors from the OIV list (OIV 2007) most significant for wild grapevine characterization (OIV004, 051, 053, 067, 068, 070, 074, 075, 079, 081-1, 083-2, 084, 087, 151, 202, 223, 225, 241). DNA extraction was performed on young leaves sampled from several individuals for each population (51 genotypes), except the Borbera population prospected later. The following n-SSR loci were analyzed on an ABI 3130 sequencer (Applied Biosystems, Foster City, Calif., USA): the 9 loci developed as common markers for international use (GrapeGen06 project: <http://www1.montpellier.inra.fr/grapegen06/technical/index.html>), VvMD21, VvMD26, VvMD36 BOWERS *et al.* 1999), VrZAG67, VrZAG64 (SEFC *et al.* 1999); the allele sizes were detected by GeneMapper software ver. 4.0 (Applied Biosystems). The genetic relationships among the different populations were investigated constructing a neighbor-joining (NJ) dendrogram from a genetic similarity matrix using MEGA v. 5.05 software (TAMURA *et al.* 2011). Genetic distances (1,000 bootstraps) were computed as: $D = [1 - (\text{proportion of shared alleles})]$, using Microsat software (MINCH 1997). The occurrence of GFLV, GVA, GLRaV-1, GLRaV-3 and GFkV viruses was determined through ELISA tests from mature cane samples (GAMBINO *et al.* 2006).

Results and Discussion

Five populations were discovered in the region, besides several plants more or less isolated from two different locations (Table). Two rather close populations were located in the south-east (Gorzente and Piota). Three other sites were found at the foot of the Alps: in the north (Fenera) and western part of the region (Rio corto and Randolera). The esteemed consistency of each population ranged from 20 to 150 individuals. Morphological and biological traits (dioecious individuals, female plants producing very small roundish black berries), as well as genetic profiles, indicated the observed plants are true *vinifera sylvestris*. Leaf morphology was typical of wild *vinifera* and nearly the same in all the sites, except for few characters that seem to mark some populations, like teeth in the lateral sinuses frequent only in Gorzente and Piota.

As to the ecological requirements, plants were confirmed to be highly dependent on water availability into the soil (BIAGINI *et al.* 2014). "Western *Quercus pubescens* woods and related communities" was the habitat prevailing along the Gorzente valley and on Mount Musiné (Rio corto and Randolera) sites, locally mixing up with "*Castanea sativa* woodland". On Fenera site, the "Inner-Alpine *Ononis* steppe forests" were mainly composed by Scots Pine. Both "Meso- and eutrophic *Quercus*, *Carpinus*, *Fraxinus*, *Acer*, *Tilia*, *Ulmus* and related woodland" and "Ravine and slope woodland" were the broadleaved forests detected along Mount Fenera's southern foothills and in the Piota valley, where the dominant species was *Quercus petraea*.

The neighbor-joining (NJ) dendrogram resulting from SSR allelic pattern of the individuals belonging to five populations and to Scrivia location (Figure), indicated population's genetic similarity broadly reflects site's geo-

Table

Main features of the *Vitis vinifera* subsp. *sylvestris* populations discovered in Piedmont (north-western Italy)

| Population/ Location name | Locality | Number of individuals | | Site ecological features | Presence of other <i>Vitis</i> species | | Observed ♂/♀ ratio | Inside protected areas |
|---------------------------------|---|--------------------------|------------------|--|--|---|-----------------------|------------------------------|
| | | esti- mated | inven- toried | | <i>Vitis</i> species | Distance | | |
| Gorzente | Gorzente river valley, Alessandria province | 100 | 21 | Main and tributary river banks, alluvial sediments and mining deposits | <i>V. v. sativa</i> , <i>V. labrusca</i> hybrids | remains of ancient cultivation inside the site | 0,6 | no |
| Piota | Piota river valley, Alessandria province | 150 | 16 | Main and tributary river banks, alluvial sediments and mining deposits | <i>V. v. sativa</i> | 5 km | 2 | no |
| Fenera | Mount Fenera, Vercelli province | 30 | 9 | Screes at the foot of cliffs | <i>V. v. sativa</i> | 2 km | 1 | yes |
| Randolera | Mount Musiné (western slope), Turin province | 20 | 5 | Glacial sediments | <i>V. v. sativa</i> | 2 km | - | no |
| Rio corto | Mount Musiné (southern slope), Turin province | 30 | 10 | Alluvial/glacial sediments on mountain slopes | <i>V. v. sativa</i> | 3 km | 1,7 | no |
| Scrivia | Villalvernia, Alessandria province | - | 3 | River gravel bed | <i>V. v. sativa</i> | 1 km | - | no |
| Borbera | Borghetto di Borbera, Alessandria province | - | 3 | Alluvial rocks (conglomerate) on river banks | <i>V. v. sativa</i> | 1 km | - | no |

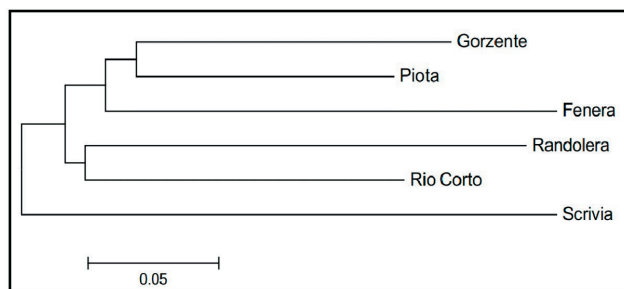


Figure: Neighbor-joining dendrogram based on n-SSR allele sizes (14 loci) of 6 wild *vitifera* populations/locations discovered in Piedmont (north-western Italy).

graphic distance: Gorzente and Piota are located in nearby valleys, Rio Corto and Randolera are likewise close.

ELISA assays were negative for all the harmful viruses on the 30 tested plants. In spite of the observed attacks of fungal pathogens, vines never die because of diseases. The major cause of their disappearing was direct elimination due to forest cuts cleaning riverbanks or building new roads.

Conclusions

Considering the numerous reports in different part of the region in the past, spreading and consistency of wild grape germplasm from the region of Piedmont severely decreased over a period of 100-150 years. Besides direct elimination of plants due to the destruction of their natural habitat, another risk for the survival of this native germplasm is the contamination from cultivated grapevine due to pollen flow, demonstrated by Di Vecchi *et al.* (2008). The relative short distance from wild population's sites and vineyards (Table) must be regarded as a worrying condition.

Indeed, all the means to avoid the loss of this native source of diversity must be undertaken by protection policy and proper land management.

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