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Germplasm conservation of traditional grape varieties in Aosta Valley

Sauvegarde du patrimoine génétique des cépages traditionnels en Vallée d'Aoste

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Abstract

A research project has been started, in order to greatly extend the local germplasm collection of grape varieties traditionally grown in the Aosta Valley viticultural area. While the collection will eventually provide a wide genetic pool serving as a starting point for subsequent selection or breeding projects, the project's main aim is the conservation of the genetic variability still available in the area. This is being done by collecting accessions based on their genetic diversity and geographical origin rather than viticultural potential and sanitary status. The project is underway; nevertheless some interesting results have already been achieved. 770 potential accessions have been identified, coded and tested for virus infections; about 300 of these will eventually be included in the germplasm bank. The identity of each unidentified or uncertain new accession was determined by comparing 9 microsatellite markers to the molecular database developed by CNR-IVV as well as to several public databases and published data; this also allowed the detection of various synonymies. An ancient grape variety, the Gouais blanc, whose presence had never been attested in the studied area, was found in a remote site at 780 m asl. A few accessions were still unidentified; one of these, known to local grape growers as *Puppa de feya*, shows a striking resemblance with a supposedly extinct ancient variety grown in the same area, also called *Puppa de feya*, as accurately described in a XIXth century document.

Keywords: *Vitis vinifera*, autochthonous cultivar, Microsatellites, cultivar identification, virus infection

Introduction

The number of *Vitis vinifera* cultivars present in Italy is rightly believed to be outstanding if compared to other main grape growing European countries. Minor, local, old grape varieties, often autochthonous, sometimes neglected and frequently in danger of disappearing, are estimated as being as many as 2000 in Italy, while they are no more than a few hundred in countries such as France, Germany and Spain. The recovery and the conservation of such a significant heritage regarding grapevine diversity is aimed at the preservation of grape genetic resources within the framework of broader conservation projects having scientific purposes. Due to recent market evolution and trends, the exploitation of local grape varieties and wines represents a valuable opportunity of promoting sustainable economic development, especially in those areas, such as mountain areas which combine hard environmental conditions and tourism potential.
Aosta Valley is a broad alpine valley located in north western Italy, with a long-established viticultural tradition. Its wines, notably those produced with *Malvoisie* (a local Pinot gris biotype), Muscat blanc and *Picotendro* (a local Nebbiolo biotype) were already quite renowned in the Middle Ages. Alongside these allochthonous varieties, a number of native cultivars were ubiquitously grown: a survey dating from 1833 to 1838 mentions more than 20 supposedly autochthonous varieties (Gatta, 1838), which is a considerable number, given the small extent of the area. In the decades following the Phylloxera plague, most vineyards were replanted with Piedmontese and French cultivars, instead of the autochthonous ones. With the exception of three (Petit rouge, Prié blanc, Vien de Nus), most of the latter inexorably became much scarcer (from tens to thousands vines). A few of them probably became extinct.

In order to support the advancement of local viticulture and oenological production through the development of local varieties beside the international ones, over the past decades the Institut Agricole Régional (IAR), often collaborating with other research institutions, has made great efforts in projects which aim to improve and spread autochthonous propagation material. Early work resulted in selections of Nebbiolo, Petit rouge, Vien de Nus, Muscat blanc (Quaglino et al., 1978 , Mannini et al., 1989; Mannini et al., 1992). Several projects followed, on Fumin, Cornalin, Mayolet, Prié rouge, Prié blanc, Vuillerrmin, Bonda (Moriondo et al., 1998; Moriondo, 1999; Moriondo, 2001; Domeneghetti, 2006). Each of these projects involved planting several mother plants in the experimental vineyards. Some of these, namely the most promising for their viticultural and oenological features as well as for their sanitary status, found their permanent place in the vineyards as a starting unit of a germplasm collection. These selections furthered the growth of the regional viticulture, fulfilling the demand for improved planting material. However, for the very reason that these plants were selected with the primary aim of satisfying the demand for varietal improvement, alone, they could not represent the whole genetic diversity accumulated during the past centuries. On the other hand, the regional viticulture is facing a period of major changes and renewal, with most of the old vineyards being replanted; as a consequence, it is becoming more and more difficult to find old, unselected plants. Thus, in order to ensure the conservation of the genetic variability still available in the area, in autumn 2007 a specific research project has been started. The project aims to the germplasm conservation of both autochthonous and traditional grape varieties, where the term "traditional" is referred to varieties cultivated in the area since long time enough to accumulate some degree of genetic variability (i.e. Pinot gris, locally called Malvoisie, Muscat blanc, Nebbiolo, all grown in Aosta Valley since several centuries). Unlike the previous projects, the selection of potential accessions is primarily based on their apparent genetic diversity (as long as it can be inferred by the observed phenotypes) rather than their viticultural and oenological potential. Therefore, besides the best plants (from a production perspective) belonging to the major varieties, the germplasm collection includes accessions representing the minor, currently less exploitable varieties as well as inferior-quality plants belonging to valuable varieties, provided they increase the preserved intra-varietal variability.

1. Methodology

1.1.Mother plant selection

The selection activity was preceded by a thorough documental and bibliographic research, with the aim of elucidating which varieties were grown in the region, their cultivation areas, which viticultural and eonological techniques were adopted. Next, the potentially interesting vineyards and singular vines were located by means of specific inquiries made directly among grape growers, or, often, with the support of the Regional Technical Advisory Service (taking advantage of their extensive knowledge of the territory). During the first *in situ* survey a number of operations were carried out: (1) variety identification (this may require further inspections during different phenological phases, or, in a few cases, DNA analysis); (2) a first visual assessment of the sanitary status, in order to discard the plants patently affected by virus or phytoplasma infections; (3) photographic documentation; (4) assignment of a unique code and GPS georeferentiation (usually with a standard error of about 1 m); (5) sampling of young leaves, when DNA extraction was needed.

Finally from one to a maximum of three accessions per grape variety were selected from each surveyed vineyard, depending on the morphological variability found. Ideally, only virus-free mother plants are retained. However, in a few cases, the rejection of all the infected plants would result in an unacceptable reduction of the intra-varietal genetic variability, or even in complete exclusion from the collection. Consequently, a small fraction of the conservation vineyard was designated to receive virus-infected accessions.

1.2 Sanitary status assessment

All the selected plants were tested for virus infections. A DAS-ELISA assay (Double Antigen Sandwich – Enzyme Linked Immunosorbent Assay) was carried out on one year old cane samples. Samples were

tested for ArMV, GFLV, GLRaV 1-2-3, GVA. An indirect ELISA (DASI-ELISA) method is used for diagnosing GFkV virus. The analyses were carried out by the Regional Phytopathological Service.

While Aosta Valley is still substantially free from Flavescence Dorée disease, recent reports of a few infected plants has given rise to concern about the epidemic potential of this pathogen. Since the gathering of mother plants coming from the entire region, (especially from the most remote and least-attended vineyards) could potentially increase the risks of a rapid spreading of the disease, special care was taken in ensuring that only phytoplasma-free vines would be kept in the collection. Both scions and rootstocks of all the selected mother plants were submitted to thermotherapy treatment (50 °C / 45', following a pre-treatment at 30 °C / 20') prior to grafting. The possible presence of Scaphoideus Titanus was rigorously monitored and an appropriate number of precautionary insecticide treatments were administered in both the nurseries and the collection vineyards. Furthermore, both collection and nurseries, were regularly surveyed by IAR pathologists in order to ensure the detection of any possible symptom as soon as it may appear.

1.3 Varietal identification

The varietal identity of each potential accession was assessed by comparing their plant morphological features with reference cultivars (ampelography). When the plant could not be identified, or the visual observation failed to identify it with certainty and needed further confirmation, microsatellite markers analysis was used. Accessions were analyzed at the 9 loci utilised as common international markers for *Vitis* fingerprinting: VVS2 (Thomas & Scott, 1993), VVMD5, VVMD7 (Bowers et al., 1996), VVMD25, VVMD27, VVMD28, VVMD32 (Bowers et al., 1999), VrZAG62 and VrZAG79 (Sefc et al., 1999). The resulting genetic profiles were then compared to the molecular database developed by CNR-IVV, containing more than 500 genotypes of international and Italian cultivars, specifically including varieties from north western Italy. The comparison with microsatellite profiles available on several public databases and already published data was also carried out. Molecular data were compared using ad hoc developed software as well as Identity 1.0 (Wagner et al., 1999).

2. Results and discussion

Currently 21 distinct autochthonous or traditionally grown grape cultivars are kept in the IAR ampelographic collection, totalling 302 accessions, selected in part during previous research, in part as the result of this project. From the start of the project to the end of the 2009 season, 770 potential accessions have been identified, coded and tested for virus infections; about 300 of these will eventually be included in the collection. Successfully propagating the mother plants and planting the collection plots turned out to be more troublesome than expected. This is because of the poor vegetative status of the scions, often coming from incompletely matured canes of old, badly maintained and unhealthy vines. As a consequence, very low nursery yields are observed. The root taking of the obtained young plants is also very low, mostly due to incomplete soldering of the grafts. Positive ELISA assay results are very common: more than half of the mother plants (56.6%) turned out to be infected by at least one of the tested viruses, GLRaV complex being by far the most widespread. While these infection rates are undoubtedly high (especially considering that all the plants showing clear symptoms of infection are not even tested), they do not differ very much from those observed in similar projects. Since in most cases only healthy mother plants are selected, such a high incidence of virus diseases implies the coding and testing of a much higher number of potential accessions.

Besides selecting a large number of mother plants, during the first years of work, a few findings have occurred which are particularly noteworthy. A single white grape vine recovered from a remote site at 780 m asl called Theilly by the owner was identified, by ampelographic characterization and by DNA analysis, as the ancient variety Gouais blanc. While nowadays Gouais blanc is practically not grown any more, it was extensively cultivated throughout central Europe during the middle ages, playing a major role in the origin of many modern cultivars like Chardonnay, Gamay, Aligoté, Auxerrois, Colombard, Furmint and many other (Boursiquot et al., 2004). Its presence in Aosta Valley, together with several reports from

Piedmontese mountain areas (Schneider et al., 2006), suggests that, in the past, it was probably grown all along the north-western Alpine range, though here it does not seem to have given origin to any relevant progeny. Further *in situ* enquiries confirmed that, until the 1980s, Theilly grapes were used for making a wine of the same name, characterised by a distinct, typical sourness.

A few vines could not be identified. Some of them found in uncultivated plots, could be wild crosses between local varieties that occurred by chance and went neither observed nor named; only one does have a name, as it is known to local growers as *Puppa de feya*. It shows a striking resemblance to the cultivar of the same name accurately described in a XIXth century document. Furthermore, the new finding occurred within the growing area of the ancient variety. The identical name, the close resemblance of morphological traits and the correspondence of growing areas suggest that the new accession may indeed belong to the ancient, supposedly disappeared, cultivar. Some preliminary DNA analyses show a strict relationship with the wide, interrelated family of autochthonous varieties, notably with the local Neyret. Even if the *Puppa de feya* does not have any relevant feature from a production point of view, it remains of some interest as it broadens our regional grapevine genetic heritage, as well as our knowledge of the Aosta Valley viticultural tradition.

3. Conclusions

The aims, methods and selection criteria of an ongoing project on grapevine germplasm conservation in Aosta Valley, have been outlined. While some steps of the work turned out to be more troublesome than expected, notably those relating to the effective propagation of the mother plants and the sanitary status of the accessions, encouraging results have already been achieved, with respect to the amount and the value in terms of genetic diversity of recovered material. When the project will be completed, the collection will provide wide autochthonous genetic pools allowing which could serve as the basis for new selection projects.

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Fig 1: Grape of the native variety *Puppa de feya*
Grappe du cépage autocthone *Puppa feya*