THE WILD GRAPEVINE IN TUSCANY

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Introduction
The cultivated vine, *Vitis vinifera* L. subsp. *Sativa* belongs from the Vitaceae family (palmate-lobed leaves, berry fruit), subfamily Ampelideoae (plants with climbed stem thanks to cauline tendrils), genre *Vitis*. The latter includes the subgenre *Muscadinia* (with three spontaneous American species, also known as "muscadinia grapes") and the subgenre *Vitis* which consists of 108 species (of which 28 fossils) distributed in four groups:
- American suitable to temperate climates;
- American suitable to warm, tropical and equatorial climates;
- Eastern-Asian;
- European-Asian suitable to temperate climates.

The *Vitis vinifera* includes 2 subspecies, *sylvestris* (wild vines) and *sativa* (cultivated vines).

Wild and cultivated vine
The wild grape (Fig. 1), *Vitis vinifera* L. subsp. *sylvestris* (Gmelin, Hegi) is spontaneous in Europe. It mainly grows in woods or in the thicket where is still possible to identify it, specially in the wetlands of the Mediterranean area and in the Danubian Europe. It is easily recognizable because it is similar to the cultivated vines (according to the most reliable opinions it represents the ancestor) (Fregoni *et al.*, 2005). It differs from these due to substantial ampelographyc differences, the most important of which are:
- the presence of inflorescences only male or female (dioecious plant);
- the bunches and the berries (frequently black berry) are smaller;
- the seeds (Fig. 2) are more rounded with shorter beak (this last character is most important for the classification).

These vines are vulgarly know as "Lambruschi" although doesn’t always exists a true likeness to the homonymous typical grapevine from the Emilia-Romagna’s lowland (Schneider, 2006).

The investigations so far carried out have shown a considerable variety of forms among of which, might be present biotypes of botanical or farming interests. The use of molecular analysis’s methods, now allow to highlight the degree of relationship with the currently cultivated varieties or with uncommon cultivars, in danger of extinction (Costacurta, 2003; Muñoz-Organero *et al.*, 2001). This possibility, however, would require that these botanical entities had had a common pathway or the same area of distribution, in order to enable pollination. Given the richness of grapevine germplasm in Tuscany (Anzani *et al.*, 1993), this eventuality, even if with a low level of probability, could take shape, knowing better both the genetic profile of wild vines and of autochthonous grape varieties that are present in the area.

Researches carried out on archaeological sites (Ciacci *et al.*, Vinum project, Ci.Vin, 2005) have already brought to the identification of molecular profiles of great interest Vignani *et al.*, 2007). The many activities of genotyping of cultivated grapevines through the use of DNA markers, mainly microsatellites (SSR: Simple Sequence Repeat), also allow to carry out a comparative genomics analysis with *Vitis vinifera* subsp. *Sylvestris* (Cunha *et al.*, 2007). Today the idea that the vines are exclusively the result of the domestication of wild vines from the near Middle-East seems to falter, while it advances the hypothesis that some of these are derived from the secondary centers of the Western Europe (Arroyo-Garcìa *et al.*, 2006; Forni, 2012).

It can not finally to be overlooked as some varieties of *V. v. sylvestris* are able to ripe grapes of acceptable quality, with a reduced use of pesticides. In some cases giving a wine fairly respectable (Lovicu, 2007) that can be improvable (if not used directly, however, it could help to identify varieties with different levels of diseases’s susceptibility).
Save the germplasm
The wild grapevine often grows along the rivers by clinging on trees, that regularly are cut, or worse, along ditches continuously cleaned, seriously endangering its natural survival. Our Department is aware about the scientific importance of this plant, for this reason a research project has been undertaken for the safeguard and the ampelographic, molecular and phylogenetic study of wild vines present in the Tuscan Maremma. We have identified and cataloged some wild vines discovered in different habitats in the provinces of Siena and Grosseto. We have found more than 150 accessions of which about seventy were placed in a collection using a specific training system, the Totem (Fig. 3), on which ampelographic, qualitative and molecular (nuclear and chloroplast microsatellites) observations are carried out.

Morphological characters
Within of the collected material (not everything classified yet) the accessions with male flowers are the 19.5%, while those with female flowers represent more than 49% among of which prevailing the black berry (77%) (Tab. 1). It can be noticed, moreover, as the seeds of *V. vinifera* subsp. *sylvestris* are more rounded, with a beak shorter than the *V. vinifera* subsp. *sativa*. The shape of the seeds grape was considered particularly important for the classification and to highlight also the phylogenetic relationship (Terral et al., 2010; Rivera et al., 2007).

The morphological characteristics of the leaves vary widely, from entire to three-lobed or five-lobed, with petiole sinus open or just open and blistering light or moderate (Fig. 4). Quite different even the pilosity of the lower page, ranging from absent to high, characterized by erect hairs or prostrate hairs. Variable is also the color of the veins and the petiole, from purplish red to green.
Analysis of microsatellites loci

This study has revealed very similar genotypes from the same population, but also quite different genotypes regardless the area of origin. We have also found some accessions becoming from wild sativa or natural hybrids between *V. v. subsp. sativa* and *V. v. subsp. sylvestris*. The chloroplast microsatellites analysis have shown that the accessions identify themselves exclusively in chlorotypes A and D: the first is very common in the supposed secondary centers of origin of the Western Europe, while the second predominates in the centers of origin of the Eastern Europe. From our data, the majority of the accessions recovered as *V. v. sylvestris* have the chlorotype A, while the cultivated vines have in prevalence the chlorotype D (Tab. 2). Moreover, the chlorotype D of certain supposed *V. v. sylvestris* confirms that these may result from become wild sativa or intraspecific crossbreedings between *sylvestris* and *sativa* present in the area of identification.

<table>
<thead>
<tr>
<th>Accessions</th>
<th>V. v. sativa</th>
<th>V. v. sylvestris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorotype A</td>
<td>33.33</td>
<td>73.68</td>
</tr>
<tr>
<td>Chlorotype D</td>
<td>66.67</td>
<td>26.32</td>
</tr>
</tbody>
</table>

Table 2 - Chlorotype distribution of the material studied.

The anthocyanin profile

In the first group there are the cv. Sangiovese, cv. Ciliegiolo and four accessions of *sylvestris* which have in common the prevalence of malvin respect to peonin and other anthocyanins, with low ratio of anthocyanins trisubstituted/disubstituted and low presence of acetate and para-coumarylated anthocyanins. In the second group together with the cv. Buonamico there are two accessions with a prevalence of malvin, medium ratio of trisubstituted/disubstituted and medium presence of para-coumarylated malvin. The third cluster contains five accessions of *sylvestris* characterized by prevalence of malvin and greater ratio of trisubstituted/disubstituted with acetate and para-coumarylated anthocyanins. The fourth group includes the cv. Colorino and two *sylvestris* less homogeneous. However, prevails malvin and above all, is higher the ratio of trisubstituted/disubstituted. Finally, cv. Barbera, Tempranillo (accession n° 19, Fig. 5) and the accession n° 64 are markedly diversified in several aspects. The latter accession is different for the prevalence of peonin and the very low ratio of trisubstituted/disubstituted (Fig. 5).
Figure 5 - Dendrogram obtained from the anthocyanin profile in some Tuscan grapes, in accessions of *V. v.* *sylvestris* (Sil_Mar_and number) and other samples recovered in Maremma (operational numerical symbols). (Accession n° 19 Malv_n. Br represent the cv. Tempranillo).

Figure 6 - Profile of anthocyanins in a series of *Vitis sylvestris* and in some Tuscan's vines.

**Conclusions**

The material retrieved, has large morphological and genetic variability, in addition presents very particular anthocyanins profiles of black grapes. The nuclear microsatellites analysis has showed that certain supposed accessions of *Vitis vinifera* subsp. *sylvestris* retrieved in Tuscany seem to derive from the vines already cultivated that become wild, while others accessions would be intraspecific crossbreedings *sativa-sylvestris*. 
The retrieval of *V. v. sylvestris* continues on increasing the number of sites, subjecting the accessions to ampelographic anthocyanin profile of black grapes observations. Further genotyping through other types of molecular DNA markers are in progress.

**Acknowledgements**

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