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Ampelometric evaluation of wild grape (*Vitis vinifera L. ssp. sylvestris* (C.C. Gmel.) Hegi) accessions in the germplasm collection of FEM-IASMA, Italy

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Summary

In this paper, 45 wild grapevine accessions collected during two consecutive years were compared for 36 ampelometric traits using digital image analysis. The sample set contained male and female individuals from different geographic regions: Germany, North Italy, Central Italy, South Italy, Sardinia and Turkey. The leaf morphological data from the collected samples suggest that geographic origin, gender and vintage could have an effect on ampelometric traits in this species.

K e y w o r d s : phyllometry; morphological diversity; vintage effect; germplasm collection.

Introduction

The wild grape (*Vitis vinifera* subsp. *sylvestris* C.C. Gmel. Hegi) is an indigenous member of the flora in Eurasian riverbanks and mid-elevation mountains. Because of habitat loss and introgression, this species was drifting towards extinction, and it is important to preserve the remaining populations. Diversity of the wild grape in natural habitats and in germplasm collections is estimated based on molecular, chemotaxonomic and morphological data (Arroyo-García. *et al*. 2006, Cunha *et al.* 2007, Emanuelli *et al.* 2013). However, dioecious nature and a phanerophytic life form of the wild grape, make it difficult to describe its reproductive organs, such as inflorescences, bunches and berries in the indigenous habitats. Therefore, the most accessible sample, a mature leaf and its morphological description (*ampelometry*), supply appropriate information for the characterization of ecotypes and the comparison of genotypes within and between species. For instance, Anzani *et al.* (1990) described 13 phyllometric indices of the wild grape accessions from the grape germplasm collection (ITA362), located in San Michele all'Adige, Italy. They pointed out the sexual trimorphism of the species. Male, female and hermaphrodite samples had unique leaf morphological characteristics. Slimane *et al.* (2010) reported ampelometric data from 23 ecotypes collected in Tunisian wild grape populations, and defined the most dispersed variables. Further phyllometric characteristics, which allowed for the proper discrimination of wild grape populations and genotypes were highlighted by Barth *et al.* (2009) and Ekhvaia and Akhalkatsi (2010).

Although the leaf morphology of *Vitis* sp. is thoroughly known, a year-to-year climatic effect has not yet become a focus of ampelometric investigations. A general observation is that the different climatic conditions, such as radiation and precipitation of certain geographic regions could cause significant alterations in phyllometric traits of plant species, e.g. in the leaf shape and size (Bonan, 2002).

In the present study, digital image analysis of leaf morphology on wild grape accessions from an Italian germplasm collection was carried out to: (1) estimate ampelometric variability among the genotypes originating from different geographic regions, (2) compare leaf morphology of female and male individuals, and (3) evaluate a year-to-year effect on ampelometric traits. We emphasize that the diversity among the ecotypes is intensified by sexual dimorphism observed in this species.

Material and Methods

For this survey, 45 wild grape accessions (Table) in the *ex situ* germplasm collection of the FEM-IASMA was compared for 36 ampelometric traits such as: length of the veins, angle between the veins, depth of sinuses, opening of the petiole sinus, distance between the tip of the lobes etc. (Figure). The growing conditions of the experimental field are detailed in Emanuelli *et al.* (2013). Samples were grouped into 12 ecotypes based on gender and putative geographic origin (Table). Sampling was carried out in July 2012 and 2013 between berry set and *veraison*, according to the OIV (2009) recommendations: consistently 10 leaves per accession. Digitalisation of the leaves was performed with a scanner HP ScanJet at 300 dpi. Ampelometric characterization of the samples was carried out with the GRA.LE.D. software with modifications compared to Bodor *et al.* (2012) to increase the numbers of the investigated characteristics. Statistical analysis was executed in IBM SPSS ver. 20 (IBM Corp.). Morphological differences between male and female individuals and geographic origin were explored by ANOVA, while the between-year effect was evaluated by the t-test.

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Table

List of the wild grape (*Vitis vinifera* subsp. *sylvestris* C.C. Gmel. Hegi) accessions used in this study

No.	Geographic origin	Sex of flowers	Number of accessions
1	Central-Italy	Female	3
2	Central-Italy	Male	5
3	Germany	Female	4
4	Germany	Male	5
5	North-Italy	Female	2
6	North-Italy	Male	4
7	South-Italy	Female	4
8	South-Italy	Male	3
9	Sardinia	Female	6
10	Sardinia	Male	6
11	Turkey	Female	2
12	Turkey	Male	1

Results and Discussion

Ampelometric description of 45 wild grape accessions was carried out in two years. Variability of the leaf characteristics was investigated. The most variable morphological trait was the opening of the petiole sinus in both years, in terms of OIV (2009): wide open, open, closed petiole sinuses were present in the sample set. Overlapping of petiole sinus was observed in a small percent of the samples (< 1 %). The ratio between the main vein and secondary veins (N_1/N_2) was the least variable characteristic in both years. Leaf morphology showed high variability among ecotypes and it was intensified by sexual dimorphism. Our results are in accordance with previous observations and show that both geographic origin and sex of the flowers are related to leaf morphology. We stress that these two factors – gender and origin – are in interaction with each other. This should be taken into consideration when discussing leaf morphology of the ecotypes. Results of ANOVA with ecotypes as factor levels show that almost all morphological traits were significantly different; 5 out of 36 and 6 out of 36 characteristics were not variable among ecotypes in 2012 and 2013, respectively. Among others angle between the veins N_2 and N_3 (OIV 608) and the width of the leaves, the distance between the tips of veins N_1 and N_2, and the angle between N_2 and N_3 were not significantly different among ecotypes. We found leaf morphological differences in both years among male (24 accessions) and female (21 accessions) genotypes. Seven out of the 36 characteristics varied between male and female accession in 2012 while in 2013 variability was higher: 19 out of the 36 characters were differing. The size of the leaf is an important morphological characteristic for discrimination of wild grape accessions; however, in this study the overlapping of the lobes was expected to give false results. Therefore, we refer to the leaf size according to the length of the main veins (N_1; N_2). Average leaf size of female accessions was smaller than that of male genotypes, which result is in contrast with the observations reported by Anzani *et al.* (1990). Our results are in line with previous reports about leaf morphology related to geographic origin of the wild grape samples (Barth *et al.* 2009; Slimane *et al*. 2010).

For example, we found that Turkish samples in the FEM-IASMA collection (3 accessions) had open petiole sinus and small leaves with very short or short veins. The samples of German origin in this sample set (9 accessions) had wide opening of the petiole sinus and leaves with short or medium size veins. Italian accessions were grouped into four sub-groups: North-Italian (6 accessions), Central-Italian (8 accessions), South-Italian (7 accessions) and Sardinian (12 accessions). South-Italian accessions had an open or wide open petiole sinus and relatively small leaves. North-Italian samples had leaves with short or medium size veins, while the accessions from Sardinia and the Central Italian regions had small size of leaves and the open or wide open petiole sinus. Year-to-year effect. The year-to-year or vintage effect has significantly modified leaf morphology. However, a few characteristics were rather stable along the vintages, e.g. the depth of the upper sinuses and the distance between the top of the upper lobes. In general, the leaf size was greater and the petiole sinuses were closed in 2013, compared to 2012.

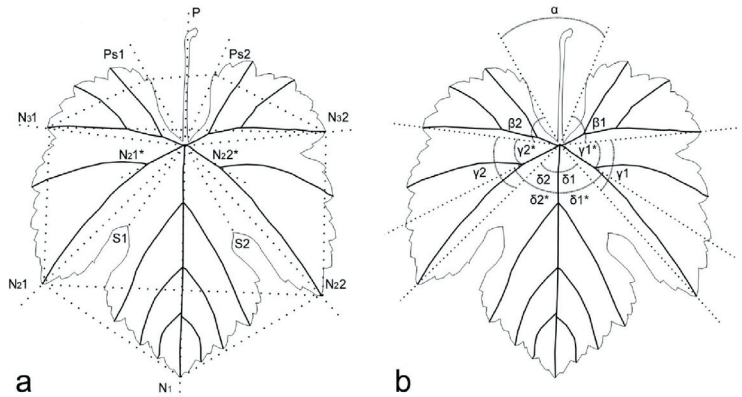

Figure: Ampelometric characteristics of a wild grape (*Vitis vinifera* subsp. *sylvestris* C.C. Gmel. Hegi) leaf investigated in this study: length of the veins (N1, N21, N22, N31, N32), length of the petiole (P), opening of the petiole sinus (Ps1-Ps2), depth of the sinuses (S1; S2), distance between the top of the lobes (N21-N22, N31-N32) (**a**), angles between the veins (β1, β2, γ1, γ1*, γ2, γ2*, δ1, δ1*, δ2, δ2*) opening of the petiole sinus (α) (**b**).

Conclusions

Based on observations in two consecutive years, we concluded that geographic origin, sex of the flowers and vintage had significant effect on leaf morphology of the wild grape. In our study, external factors could be considered as homogenous in this way we did not concerned the effect of bud and shoot load, environmental conditions or the rootstock type. Results of this study show that the year-to-year effect influences morphological characteristics, and therefore this factor should be included in sample comparisons. We highlight that the investigation of leaf morphological characters among the wild grape accessions gives comprehensive results about the diversity in natural habitats as well as in germplasm collections.

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