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GRAPEVINE ROOTSTOCK CHARACTERIZATION

FOR DROUGHT TOLERANCE

PhD thesis

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Abstract.

Grapevine is worldwide grafted on rootstocks to create a biological barrier to the phylloxera (*Daktulosphaira vitifoliae*). Despite the key role of rootstock in the adaptation to environmental conditions, a limited number of genotypes is available for winegrowers, showing a narrow genetic background. The gap between the importance of rootstocks in abiotic stress tolerance and their low genetic variability leads to consider rootstock breeding as a promising strategy to face climate change. In the last decades, new breeding programs were developed with the aim to provide new rootstocks able to cope with drought and other abiotic stresses. Nowadays, the continuous progress in genetic techniques can assist and accelerate the selection process of new tolerant genotypes.

In the present PhD project, several genotypes at different stages in rootstock selection process were analyzed for drought tolerance. The first part of the thesis focused on 3 genotypes belonging to the recent M-series, the second part was about a new selection of 30 genotypes, coming from different breeding programs, and in the last part a breeding population of 141 genotypes was used for a genome wide association study (GWAS).

The new M-rootstocks (M1, M3 and M4), recently placed on the market, were compared to traditional rootstocks, in order to better understand their behavior under drought. In a pot experiment under controlled conditions, M1, M3 and M4 were compared to nine rootstocks with different genetic background at decreasing levels of water availability. M-rootstock performance under water deficit was similar to the tolerant rootstocks 1103P and 110R, in both phenotypic and genetic responses to water stress. These rootstocks adopted a strategy of tolerance to face water stress, increasing the water use efficiency (WUE) under deficit conditions. To deeply investigate the behavior of tolerant rootstocks under drought, a second experiment in semi-controlled conditions was set up, comparing M4 to 1103P under progressive water deficit, in grafting combination with *V. vinifera* cv Pinot Blanc. Similar performances

were reported by the two grafting combinations under mild to moderate water deficit, but a different response occurred under sever conditions: 1103P reduced stomatal conductance, transpiration, and carbon assimilation more than M4, which was able to preserve water use efficiency and operating efficiency of photosystem II.

In the second part of the thesis 30 new selected genotypes were compared to rootstock M2 for water stress tolerance and nutritional status, in order to characterize the rootstock material before the marketing process and to identify new pre-breeding material. The experiment was carried out in un-grafted conditions for two years and in two experimental fields, characterized by different water availability. Several parameters were analyzed, such as transpiration, WUE, vigor, macronutrients and micronutrients in the leaves. Genotypes ranked for both abiotic stresses and the differences between the two sites allowed to estimate their plasticity for each trait.

Finally, a GWA approach was applied on a breeding population, counting 141 genotypes, in order to identify the genomic regions involved in drought tolerance. The population was genotyped with a 18k SNP array, after the validation on non-vinifera germplasm, belonging to a rootstock core-collection of 70 genotypes. Three phenotyping cycles under increasing water deficit were performed on the breeding population under greenhouse-controlled conditions. Vigor, shoot growth rate, transpiration, stomatal conductance and leaf turgor were estimated for each genotype at different water deficit levels. A group of tolerant genotypes with high performance under water deficit condition was identified and used in GWAS approach to detect the loci associated to drought tolerance of rootstocks.

In conclusion, this work enhanced the knowledge about rootstock response to water deficit, characterized the water tolerance of a large panel of rootstocks and identified potential target genes for future breeding programs.

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STATE OF THE ART

GRAPEVINE ROOTSTOCK SELECTION FOR DROUGHT ADAPTATION: A REVIEW

Abstract.

Adaptation of grapevine to climate change is modulated by rootstocks, as the interface between soil and scion. Rootstock controls the response of the plant to drought and the use of tolerant rootstocks can improve the adaptation to water deficit, preserving the production and the quality of grape. Among available rootstocks, hybrids *Vitis Berlandieri* x *Vitis rupestris* generally reported the best tolerance to water stress, but higher adaptation to drought could be found in other *Vitis* species, never involved in rootstock breeding programs. In contrast to the low diversity of rootstocks, high genetic variability was found in *Vitis* spp, which can be partially related to a more efficient use of water. This diversity should be exploited in future breeding programs to obtain a series of new rootstocks able to cope with climate change. The recent development of innovative methods for genotyping and phenotyping can assist the selection process in future breeding programs. New drought tolerant rootstocks have been recently released and the first studies reported high performance under water deficit conditions. Further efforts will be necessary to select new tolerant rootstocks to guarantee the affinity with the *Vitis vinifera* varieties and the adaptation to all the environments of viticulture.

1. Introduction

During the last century, viticulture has undergone deep changes. The development of technologies and chemistry has led to new viticultural systems, new techniques of agricultural management and new oenological styles. Nowadays, great attention is paid on the quality of grape and wine, besides to the quantity of the production. The quality of wine is strongly affected by environmental factors, such as the climate conditions, the soil characteristics and the biotic and abiotic stresses (Jackson and Lombard, 1993). Nevertheless, the environmental factors changed during the last decades, and they are expected to evolve in the near future. For examples, many areas under vine are expected to experience longer drought period

during the hottest months of the next years (Van Leeuwen et al., 2019). Rainfalls will be more exceptional and intense, causing waterlogging events and higher soil erosion. The use of tolerant rootstock genotype has been identified as a promising strategy of adaptation to climate change (Quénol et al., 2014; Van Leeuwen et al., 2019), but tiny progress was focused on the selection of new rootstocks during the last century. In fact, rootstocks have been introduced in viticulture to create a biological barrier to the phylloxera (*Daktulosphaira vitifoliae*) and the largest part of commercial genotypes has been selected in the end of XIX century. The lack of innovation on rootstocks contrasts with the high technological level achieved for management practices in modern viticultural, and new efforts are required in breeding programs for rootstock selection. In the present work were reviewed: i) the effect of rootstock on adaptation to drought; ii) genetic variability of rootstocks and the possibility to enhance it introducing new diversity from *Vitis* spp; iii) first studies on new rootstocks selected for drought tolerance; iv) innovative phenotyping and genetic methods to assist future breeding for drought tolerant rootstocks.

2. The role of rootstocks in drought adaptation

Plants respond to drought in several ways, according to the entity and the length of the water deficit. Grapevine is able to regulate the water potential under water stress through the control of stomatal conductance, this behavior is defined isohydric. Nevertheless, a large variability of stomatal control within *Vitis* has been reported, ranging from near-isohydric to near-anisohydric responses (Pou and Medrano, 2012). Beside stomatal control, differences among the two behaviors may be explained by the ability of near-isohydric genotypes to inhibit the aquaporins activity in the leaves through the abscisic acid (ABA), reducing the leaf hydraulic conductivity (Coupel-ledru et al., 2017). Near-isohydric genotypes adopt an avoiding strategy to face drought, reducing the physiological activity until the water return available (Delzon, 2015; Ollat et al., 2018). This strategy allows to face long period of water deficit, but it can involve in a delay of vegetative growth and ripening of grape, due to the reduction of the photosynthetic rate. During short to medium drought period, near-anisohydric genotypes are able to maintain a high

physiological activity, preserving the stomatal conductance and coping with the low water potential, but this resistance strategy can be critical for long drought periods. An intermediate behavior can be adopted by some genotypes, reducing the stomatal conductance and maintaining the physiological activity by increasing the water use efficiency.

Rootstock plays a key role in the adaptation of vine to the environmental conditions, being the interface between grapevine variety and soil. A meta-analysis performed by Lavoie-Lamoureux et al (2017) identified in rootstock genotype the main contribution to the total variability of water status, followed by the scion genotype. Minor contribution to the total variance was explained by methodologies for water stress detection and environmental factors. Water absorption is regulated by the rootstock in several ways: deep-rooted genotypes are able to explore deep soil layers and easily find water. In fact, the depth and the architecture of the root system depends on the rootstock genotype, beside to soil characteristics (Alsina et al., 2011; Yıldırım et al., 2018); the amount of aquaporins on the absorption roots is different among rootstock genotypes (Sabir et al., 2021); the transpiration from leaves is regulated by rootstocks, controlling the stomatal conductance through both hormonal and hydraulic signals (Zhang et al., 2016); under water stress conditions, embolisms in roots depend on the vessel size, which in turn depends on the genotype and some rootstocks are more efficient in embolism repair through higher remobilization of osmolytes (Knipfer et al., 2015). Several studies evidenced the differences among commercial rootstocks in response to drought. Lovisolo et al (2008), found that the fraction of root water transport controlled by cellular metabolism in grapevine rootstocks was higher for hybryds *Vitis Berlandieri* x *Vitis rupestris* (140Ru, 775P and 1103P) than *Vitis Berlandieri* x *Vitis riparia* (SO4, 157.11, 420A and K5BB) under water stress condition. They also speculated that rather hybrids with *V. rupestris* embolized less than hybrids with *V. riparia* or they repaired more efficiently from embolisms. A tolerant strategy was shown by 140Ru in grafting combination with Cabernet Sauvignon, Grenache, Merlot and Syrah and 1103P in grafting combination with Cabernet Sauvignon (Koundouras et al., 2008; Tramontini

et al., 2013). In both studies, rootstock SO4 showed an avoiding strategy, reducing the physiologic activity more than 110R and 1103P. Satisha et al (2014), compared the water use efficiency of several rootstocks: Dogridge and Salt Creek (*V. champinii*); St. George (*V. rupestris*); 110 R, 99 R and 1103P (*V. Berlandieri x V. rupestris*); B2-56 (*V. Berlandieri x V. rupestris x V. longii*) and Teleki 5A (*V. Berlandieri x V. riparia*). Under stressed conditions 110R, 1103P, 99R, Dogridge and B2-56 increased the water use efficiency, showing a tolerance strategy.

Table 1. Adaptation strategies to water deficit adopted by the main rootstock genotypes. Be = *Vitis Berlandieri*; Ru = *Vitis rupestris*; Ri = *Vitis riparia*; Ch = *Vitis champinii*; Vi = *Vitis vinifera*

Rootstock	Genetic background	Adaptation strategy	Reference
1103P	Be x Ru	Tolerance	Koundouras et al, 2008 Satisha et al, 2014 Faralli et al, 2021 Bianchi et al, 2020
779P	Be x Ru	Tolerance	Gullo et al, 2018
140Ru	Be x Ru	Tolerance/Resistance	Tramontini et al, 2013 Bianchi et al, 2020
110R	Be x Ru	Tolerance	Satisha et al, 2014 Bianchi et al, 2020
99R	Be x Ru	Tolerance	Satisha et al, 2014
B2-56	Be x Ru	Tolerance	Satisha et al, 2014
St. George	Ru	Avoidance	Satisha et al, 2014
SO4	Be x Ri	Avoidance	Lucini et al, 2020 Koundouras et al, 2008 Tramontini et al, 2013 Bianchi et al, 2020 Faralli et al, 2021 Galbignani et al, 2016
K5BB	Be x Ri	Avoidance	Bianchi et al, 2020
420A	Be x Ri	Avoidance	Bianchi et al, 2020 Gullo et al, 2018
Teleki 5A	Be x Ri	Avoidance	Satisha et al, 2014
1613C	Be x Ri	Avoidance	Satisha et al, 2014
161-49C	Be x Ri	Avoidance	Bianchi et al, 2020
Dogridge	Ch	Tolerance	Satisha et al, 2014
Salt Creek	Ch	Avoidance	Satisha et al, 2014
Schwarzmann	Ri x Ru	Avoidance	Bianchi et al, 2020
101-14	Ri x Ru	Avoidance	Meggio et al, 2014 Corso et al, 2015
41B	Be x Vi	Resistance	Bianchi et al, 2020

Ibacache et al (2016), investigated the yields of nine rootstocks (1613 Couderc, Freedom, Harmony, 1103P, 110R, 99R, 140Ru, Saint George and Salt Creek) grafted with three different varieties of

table grape under semi-arid conditions. The best performance was obtained by Salt Creek (*Vitis champinii*) across all three evaluated cultivars. Adaptation strategies adopted by rootstocks to face drought are summarized in Table 1.

3. Exploitation of genetic variability in *Vitis* spp.

Despite the importance of rootstock in environmental stress adaptation, a limited number of genotypes has been involved in breeding programs. The largest part of rootstocks has been obtained by the crossing of few genotypes, mainly belonging to three species: *Vitis Berlandieri*, *Vitis riparia* and *Vitis rupestris*. In a recent study, Riaz et al (2019) found that only three genotypes belonging to these three species (Rességuier 2, du Lot and Gloire de Montpellier) contributed to a total of 39% of the genetic background of 47 Vitis hybrids, representing the worldwide available rootstocks. Migliaro et al (2019), described the whole genetic variability of a large rootstock collection of 232 unique accessions by only 70 genotypes. According to Ollat et al (2016), the narrow genetic background of rootstocks is a limit for pest resistance of grapevine and adaptation to climate change. Using a multi-criteria approach, Padgett-Johnson et al (2003) compared the water stress tolerance of 17 American *Vitis* species. In the study, *V. riparia*, *V. Berlandieri* and even *V. rupestris* showed low tolerance to water deficit compared to other species, whereas the best performance was reported by *V. champinii* and *V. doaniana*. These results suggested that rootstock variability could be increased involving other American species in breeding programs, which are potentially better acclimatized to drought.

Recent studies revealed the large genetic diversity in the genus *Vitis*, which is potentially available for new rootstock breeding programs (Liang et al., 2019; Wan et al., 2013). Across the temperate area all over the world has been identified approximately 60 species belonging to the genus *Vitis*. Two species belong to the subgenera *Muscadinia* (2n = 40), whereas the others belong to the subgenera *Vitis* (2n = 38). Has been estimated that the divergence between the two subgenera occurred at about 18 Ma (Wan et al., 2013). Within the subgenera *Vitis*, two main clades were described: the first clade included North

American species and the second one the species from Europe and Asia. The Eurasian clade diverged in minor clades, among which the European one was represented by *V. vinifera* in both *sylvestris* and *sativa* subspecies (Liu et al., 2016). According to Wen et al (2018), a rapid radiation of *Vitis* in North America began in the Neogene. American clade was further investigated by Klein et al (2018), identifying two main groups: clade I comprised *V. riparia* and *V. rupestris* together with *V. acerifolia*, *V. arizonica*, and *V. monticola*; clade II consisted of *V. aestivalis*, *V. cinerea*, *V. labrusca*, and *V. mustangensis*. Some genotypes selected among American or Asian clades might be included in future breeding programs to increase the genetic diversity of rootstocks and improve the adaptation to climate change.

4. New rootstock selected for drought tolerance

In the last decades, new breeding programs were developed with the specific purpose to obtain new rootstocks with high tolerance to abiotic stresses. The Georgikon series was recently selected for limestone and water stress tolerance by the Georgikon Faculty of Pannon University, including *V. vinifera* in the parental material. In the same programs, Zamor 17 was selected for drought tolerance, including *V. rupestris* in the genetic background. In a recent study, rootstocks Georgikon 28, Georgikon 121 and Zamor 17 were compared to the sensitive SO4 and the tolerant 1103P in terms of response to water deficit in grafting combination with *Pinot gris* (Faralli et al., 2021). Different responses were induced by rootstocks to the scion. During the experiment, Georgikon 28, Georgikon 121 and SO4 reported higher leaf water potential than Zamor 17 and 1103P, suggesting different behaviors in response to water deficit.

Other four new rootstocks have been recently selected for abiotic stress tolerance by the Department of Agricultural and Environmental Sciences (DiSAA) of the University of Milano. In the breeding program, *Vitis Berlandieri* was used as recurrent genotype. In particular, rootstock M1 was selected for the tolerance to limestone, M2 to cope with several abiotic stresses, M3 for the efficiency in potassium uptake and M4 for the tolerance to drought and salt. Meggio et al (2014), compared M4 to the commercial rootstock 101.14 in terms of biochemical and physiological responses to water stress and

NaCl exposure under controlled environmental conditions. During the experiment, leaf water potential and stomatal conductance (gs) were monitored, whereas sugars, proteins, and nutrients (in particular K, Mg, Ca) were measured in both leaves and roots. The two genotypes reported a similar response under well-watered conditions, but different physiological responses occurred at decreasing water availability. M4 demonstrated better acclimatizing attitude to water stress than 101.14, maintaining a more performant photosynthetic activity during the whole experiment. The same genotypes (M4 and 101.14) were further studied by Corso et al (2015) under progressive drought conditions, using a transcriptomic approach. M4 reported higher expression than 101.14 of genes associated to two different physiological processes: degradation of ABA in the leaves (genes CYP706 and CYP707) and detoxification of reactive oxygen species (genes VvSTS). Thus, M4 was able to keep the stomata open under water deficit and to limit the oxidative stress caused by drought, allowing the root growth, the transpiration from leaves and consequentially the water uptake. The ability of M4 to limit the ROS under water deficit was also suggested by Lucini et al (2020), reporting an increment of gibberellins and cytokinin's in response to the stress using a metabolomic approach. In other studies, M4 was compared to the sensitive SO4 in grafting combination with Sangiovese and to the tolerant 1103P in grafting combination to Grechetto Gentile. In both studies, M4 reported higher photosynthetic activity, transpiration rate, water use efficiency (WUE) and water potential than the control rootstocks (Frioni et al., 2020; Galbignani et al., 2016). Under controlled conditions, rootstocks M1, M3 and M4 adopted a tolerance strategy in response to water deficit, showing a similar WUE to 1103P and 110R (Bianchi et al., 2020). Strategies adopted by the new rootstocks are summarized in Table 2. A new selection of about 30 genotypes has been recently obtained by several crosses, using in the breeding program *V. Berlandieri* as a recurrent genotype. These genotypes are in process of characterization before being released as rootstocks. A first screening for water stress tolerance in un-grafted conditions identified 14 promising genotypes for water stress tolerance, but further studies will be necessary to assess their response to water deficit (Bianchi et al., 2018).

Table 2. Adaptation strategies to water deficit adopted by new rootstock genotypes. Be = *Vitis Berlandieri*; Ru = *Vitis rupestris*; Ri = *Vitis riparia*; Ch = *Vitis champinii*; Vi = *Vitis vinifera*

Rootstock	Genetic background	Adaptation strategy	Reference
Georgikon 28	Be x Vi	Avoidance	Faralli et al, 2021
Georgikon 121	Be x Vi	Avoidance	Faralli et al, 2021
Zamor 17	Be x Ru	Tolerance	Faralli et al, 2021
M1	K5BB x Teleki 5C	Tolerance	Bianchi et al, 2020
M3	K5BB x Teleki 5C	Tolerance	Bianchi et al, 2020
M4	Unknown x 1103P	Tolerance	Lucini et al, 2020 Frioni et al 2020 Bianchi et al, 2020 Galbignani et al, 2016 Corso et al, 2015 Meggio et al, 2014

5. Assisted selection methods for rootstock breeding

Only a limited number of new tolerant rootstocks has been released in the last decades. One of the reasons is the long time required in the breading programs, especially for perennial crops as grapevine. In fact, several years are needed before the breeding population can be characterized for abiotic stress tolerance and to identify the performing genotypes. Nowadays, early selection can be carried out using molecular markers related to the interested phenotypic traits, thanks to the development of genetic techniques in the last years and the availability of high throughput genotyping tools. In grapevine, quantitative trait loci (QTLs) have been detected for several traits, such as the resistance to pest and the quality of grapes (Martinez-Zapater et al., 2010). QTLs related to drought tolerance have been studied in *V. vinifera* by Coupel-Ledru et al (2014) on a pseudo-F1 population obtained by the cross between Syrah and Grenache. In the study, a large number of QTLs were found for transpiration rate and hydraulic conductance, suggesting that drought-tolerance of grapevine is regulated by several genes. The genetic architecture of rootstock control of transpiration and the adaptation to water stress has been investigated by Marguerit et al (2012) on a breeding population of *V. vinifera × V. riparia* hybrids. Also in this study, several QTLs for different traits were identified. In particular, 7 QTLs were related to the water extraction capacity, 2 QTLs were detected for the transpiration rate, 3 QTLs for the acclimatation of transpiration

rate linked to water deficit and 1 QTL was found for the water use efficiency, estimated by carbon isotopes. The confidence intervals of the detected QTLs included the genes involved in the ABA pathway. Further investigation on rootstock control of transpiration was performed by Trenti et al (2021) on a genetic core collection composed by 100 *Vitis* spp. accessions, using a Genome-wide association study (GWAS) approach. In the study, transpiration rate was estimated by thermography and 13 candidate genes were identified in the association analysis. Three of these genes (VIT_13s0019g03040, VIT_17s0000g08960, VIT_18s0001g15390) responded to water deficit in a gene expression analysis on reference rootstocks. These genes codify for Glycosyltransferase, Raffinose synthase and Peroxidase, respectively. The loci related to the rootstock control of water stress are summarized in Table 3.

Table 3. Association between phenotypic traits related to rootstock control of grapevine water status and genetic data through QTL and GWAS analyses. Tr = transpiration rate; TE = transpiration efficiency; TTSW = total transpirable soil water; Ig = stomatal conductance index

Trait	Type	LG	Position	Reference
Tr	QTL	Chr1 (CS)	56.8 ± 10.4 cM	Marguerit et al, 2012
Tr	QTL	Chr17 (CS)	19.2 ± 11.1 cM	Marguerit et al, 2012
TE	QTL	Chr6 (CS)	29.7 ± 9.8 cM	Marguerit et al, 2012
TE	QTL	Chr11 (RGM)	7.0 ± 16.1 cM	Marguerit et al, 2012
TTSW	QTL	Chr3 (RGM)	5.8 ± 4.1 cM	Marguerit et al, 2012
TTSW	QTL	Chr3 (RGM)	22.5 ± 1.0 cM	Marguerit et al, 2012
TTSW	QTL	Chr5 (RGM)	67.5 ± 8.2 cM	Marguerit et al, 2012
TTSW	QTL	Chr11 (RGM)	54.6 ± 11.3 cM	Marguerit et al, 2012
Ig	SNP	Chr18	13,519,938	Trenti et al, 2021
Ig	SNP	Chr17	10,497,222	Trenti et al, 2021
Ig	SNP	Chr13	4,177,522	Trenti et al, 2021

Except the control of transpiration, other physiological mechanisms are involved in adaptation of rootstocks to drought, thus, further studies will be necessary to identify the loci associated to the response to water deficit. Specific assays to detect these loci in breeding populations will allow to assist the selection process and accelerate the long time required for breeding programs of new rootstocks. Another innovative approach that can be used to assist the selection processes is the genomic selection (GS). GS allows to predict the phenotype traits based on genetic data. A GS study was performed in grapevine to predict berry weight, pruning weight, cluster weight and cluster length (Fodor et al., 2020). A diversity

panel of 279 genotypes was used as training population for the phenotype prediction, obtaining the best prediction of each trait with determination coefficient higher than 0.3. As far as this, no studies of GS have been performed on grapevine to predict the tolerance to abiotic stresses.

6. Phenotyping methods for drought tolerance

Rather in traditional or genetic assisted breeding programs, the phenotyping methods play a key role in the selection of genotypes among the breeding populations. Compared to other traits, phenotyping for drought tolerance is challenging, due to the complexity of water deficit adaptation and the high numbers of traits required for the characterization. Direct measurement of water potential, transpiration or water use efficiency are limited by the time required for each measurement, and the consequent difficulties to analyze several genotypes under homogeneous environmental conditions. Thus, indirect, rapid and non-destructive methods are necessary for water stress phenotyping. Water balance has been used to assess the transpiration rate under controlled conditions as the difference between input and output of water. Transpiration can also be estimated by thermography, monitoring the temperature of the leaves. In fact, the reduction of transpiration due to stomatal closure leads to a rise of leaf temperature. Thermal indexes have been developed normalizing the leaf temperature on environmental conditions, such as the stomatal conductance index (Ig) (Jones et al., 2002) and the Crop Water Stress Index (CWSI) (Idso et al., 1981). These indexes reported a strong relation to stomatal conductance, especially when they were measured during the middle of the day (García-tejero et al., 2016; Pou et al., 2014). Beside the thermal infrared, other regions of the spectra are related to the water status of the leaf, and they can be investigated through spectroscopy. In particular, Rapaport et al (2015) identified the spectra regions of visible (530 – 550 nm and 700 – 750 nm) and near-infrared (1380 – 1590 nm) as affected by water deficit. Several optical indexes have been developed using specific wavelengths in these regions to estimate the water potential and the stomatal conductance. Among them, one of the most applied was the water index (WI), which reported a strict relation to the stomatal conductance under controlled and field conditions (Serrano et

al., 2010), whereas a lower but significant regression was observed between WI and the predawn water potential (González-Flor et al., 2019). The new frontier of spectroscopy is represented by the development of chemometric models using hyperspectral data. Chemometric models were designed by Tardaguila et al (2017) to predict the stem water potential and the relative leaf water content (RWC), acquiring the spectra from several varieties of *V. vinifera*.

Intrinsic water use efficiency (iWUE) can be estimated analyzing the isotope carbon patterns. In fact, a reduction of stomatal conductance affects the internal CO_2 and water concentration, reducing preferable $^{12}CO_2$ than the isotope $^{13}CO_2$ (Leavitt and Danzer, 1993). Thus, the ratio of carbon isotopies ($\delta^{13}C$) is related to the ratio of carbon assimilation and the stomatal conductance, noted as intrinsic WUE. Using $\delta^{13}C$, Bota et al (2016) were able to discriminate the genotypes with high iWUE among a panel of 23 varieties of *V. vinifera*. Leaf water potential (Ψ_L) can be estimated by the leaf angle occurring between petiole and leaf blade, which is affected by cell turgor (Smart, 1974). In a recent study, high relation between Ψ_L and leaf angle was found using both manual recording and 3D imaging (Briglia et al., 2020). This method (3D imaging) can also be used to assess the leaf area and consequently the vegetative growth of genotypes (Milella et al., 2019). Active growth of shoots can be also evaluated using the "apex method", developed from Rodriguez Lovelle et al (2009). Major difficulties may be found to phenotype the root system architecture. Destructive analyses after a period of water deficit allow to measure the root biomass produced and the ratio with the epigeal biomass. A periodic observation of root development under controlled conditions is possible using rhizotrons through a transparent material (Dumont et al., 2016; Fort et al., 2017). This method allows to measure the geotropic angle of the root system, which depends on genotype beside to the environmental characteristics (Smart et al., 2006). The use of electrical imaging to investigate the root development for pot and field conditions has been revised by Zhao et al (2019). Innovative methods for water stress detection and their relationship with the relative direct measurements are reported in Table 4. Another critical issue in phenotyping for water stress is the

difficulty to obtain homogeneous conditions among genotypes, even under controlled conditions. Usually, genotypes are compared at the same level of soil water content (SWC), but an approach based on the fraction of transpirable soil water (FTSW) has been proposed (Bindi et al., 2005; Marguerit et al., 2012; Sinclair and Ludlow, 1986). In this approach, the fraction of soil water able to support the transpiration is calculated as the ratio between the water extraction capacity and SWC. In these studies, FTSW were used to fix the response curves of the transpiration rate to water deficit, in order to assess the acclimatation and the plasticity of the trait.

Table 4. Relationship among rapid phenotyping methods and direct measurements of some traits related to the grapevine water status. R^2 = coefficient of determination; r = Pearson Index

Method/Index	Reference method	Goodness	Reference
Thermography			
Ig	gs	$R^2 = 0.78$	Pou et al., 2014
Ig	gs	$R^2 = 0.76$	García-tejero et al., 2016
CWSI	gs	$R^2 = 0.61$	García-tejero et al., 2016
Spectroscopy			
WI	gs	$R^2 = 0.95$	Serrano et al, 2010
WI	Ψ_{PD}	$R^2 = 0.41$	González-Flor et al., 2019
Hyperspectral	Ψ_S	$r_c = 0.82$; $r_{cv} = 0.77$	Tardaguila et al., 2017
Hyperspectral	RWC	$r_c = 0.83$; $r_{cv} = 0.77$	Tardaguila et al., 2017
Carbon isotopes			
$\delta^{13}C$	iWUE	$R^2 = 0.64$	Tomás et al, 2012
Turgor			
Leaf angle	Ψ_L	$R^2 = 0.86$	Briglia et al, 2020
Leaf angle (3D imaging)	Ψ_L	$R^2 = 0.73$	Briglia et al, 2020

7. Conclusions

This work focused on the adaptation to climate change of viticulture through the selection of new drought tolerant rootstock. Several studies reported an important and consistent effect of rootstocks on many parameters related to drought tolerance. Nowadays the genetic diversity of rootstocks is narrow, but it can be enhanced by introducing new genotypes from *Vitis* spp. in future breeding programs. Few genotypes have been recently selected for drought adaptation, showing high tolerance to water deficit in the first characterization studies, nevertheless more efforts are required in this direction. Critical issues in selection process are represented by the difficulties for water stress phenotyping, despite innovative

methods have been recently developed. New phenotyping methods (i.e., thermography, hyperspectral models or 3D imaging) allow to analyze a large number of genotypes maintaining high level of precision. Furthermore, the development of genetic techniques (i.e., QTL, GWAS and GS) can assist the early selection of promising genotypes among the breeding populations, but few studies are available on rootstock control of grapevine water status so far. New efforts are required in rootstock breeding, exploiting the genetic diversity of genus *Vitis* and applying innovative methods in the selection process. Grapevine is grown in several countries under different environmental conditions, which are rapidly evolving along with climate change. Thus, a larger number of rootstocks would allow to adapt to the abiotic stresses of each area, to face the changing climate conditions and to guarantee higher affinity with all the varieties grown all over the world.

8. Relevance of the present PhD project in the state of the art

In this context fitted the present PhD project, which aimed to i) identify new promising rootstocks for drought tolerance, among a large panel of analyzed genotypes; ii) to investigate the physiological response of tolerant rootstocks to water deficit, at both physiological and genetic levels; iii) to identify the genetic regions involved in drought tolerance to assist the selection process of future breeding programs for drought tolerant rootstocks. The project is structured in three main parts.

Specific aim of Part I was to assess the drought tolerance of M-rootstocks in comparison to other commercial genotypes largely used in viticulture, and it included two experiments: the first one is titled "How do novel M-rootstock (Vitis spp.) genotypes cope with drought?" and has been published on Plants (doi:10.3390/plants9101385), the authorship of the study includes Davide Bianchi, Leila Caramanico, Daniele Grossi, Lucio Brancadoro and Gabriella De Lorenzis; the second experiment is unpublished and the title is "The New Grapevine Rootstock M4 Improves Water Use Efficiency of Pinot Blanc Under Severe Water Stress", the contributors are Davide Bianchi, Andrea Caputo, Carola Pozzoli and Lucio Brancadoro.

The main objective of Part II was to characterize a new selection of rootstocks obtained by a recent breeding program for abiotic stress tolerance, and it also consisted in two experiments: the first one was published on Scientia Horticulturae (doi:10.1016/j.scienta.2019.109155) with the title "Phenotyping of the "G series" Vitis hybrids: First screening of the mineral composition" and the authorship includes Davide Bianchi, Daniele Grossi, Giovambattista Simone Di Lorenzo, Yang Zi Ying, Laura Rustioni and Lucio Brancadoro; the second experiment is unpublished and it is titled "Water use efficiency and nutritional status of a new grapevine rootstock selection", written by Davide Bianchi and Lucio Brancadoro.

Part III aimed to characterize the genetic structure and the drought tolerance of a breeding population, to identify genetic regions involved in water deficit adaptation. In the first study, a promising tool for the genetic characterization was validated on grapevine rootstocks and it was published on Diversity (doi:10.3390/d12030103) with the title "Genetic Diversity and Population Structure in a *Vitis* spp. Core Collection Investigated by SNP Markers", the authorship was Davide Bianchi, Lucio Brancadoro and Gabriella De Lorenzis; the second experiment of Part III is unpublished and the title is "A new genomic locus associated to drought tolerance in grapevine rootstocks", with the contribution of Davide Bianchi, Martino Bolognini, Carola Pozzoli, Gabriella De Lorenzis and Lucio Brancadoro.

References

Alsina, M.M., Smart, D.R., Bauerle, T., Herralde, F. De, Biel, C., Stockert, C., Negron, C., Save, R., 2011. Seasonal changes of whole root system conductance by a drought-tolerant grape root system 62, 99–109. https://doi.org/10.1093/jxb/erq247

Bianchi, D., Caramanico, L., Grossi, D., Brancadoro, L., Lorenzis, G. De, 2020. How Do Novel M-Rootstock (Vitis Spp .) Genotypes Cope with Drought ? https://doi.org/10.3390/plants9101385

Bianchi, D., Grossi, D., Tincani, D.T.G., Simone Di Lorenzo, G., Brancadoro, L., Rustioni, L., 2018. Multi-parameter characterization of water stress tolerance in Vitis hybrids for new rootstock selection. Plant Physiol. Biochem. 132, 333–340. https://doi.org/10.1016/j.plaphy.2018.09.018

Bindi, M., Bellesi, S., Orlandini, S., Fibbi, L., Moriondo, M., Sinclair, T., 2005. Influence of Water Deficit Stress on Leaf Area Development and

Transpiration of Sangiovese Grapevines Grown in Pots 3, 68–72.

Bota, J., Tomás, M., Flexas, J., Medrano, H., Escalona, J.M., 2016. Differences among grapevine cultivars in their stomatal behavior and water use efficiency under progressive water stress. Agric. Water Manag. 164, 91–99. https://doi.org/10.1016/j.agwat.2015.07.016

Briglia, N., Williams, K., Wu, D., Li, Y., Tao, S., Corke, F., Montanaro, G., Petrozza, A., Amato, D., Cellini, F., Doonan, J.H., Yang, W., Nuzzo, V., 2020. Image-Based Assessment of Drought Response in Grapevines 11, 1–12. https://doi.org/10.3389/fpls.2020.00595

Corso, M., Vannozzi, A., Maza, E., Vitulo, N., Meggio, F., Pitacco, A., Telatin, A., D'Angelo, M., Feltrin, E., Negri, A.S., Prinsi, B., Valle, G., Ramina, A., Bouzayen, M., Bonghi, C., Lucchin, M., 2015. Comprehensive transcript profiling of two grapevine rootstock genotypes contrasting in drought susceptibility links the phenylpropanoid pathway to enhanced tolerance. J. Exp. Bot. 66, 5739–5752. https://doi.org/10.1093/jxb/erv274

Coupel-ledru, A., Lebon, É., Christophe, A., Doligez, A., Cabrera-bosquet, L., 2014. Genetic variation in a grapevine progeny (Vitis vinifera L . cvs Grenache × Syrah) reveals inconsistencies between maintenance of daytime leaf water potential and response of transpiration rate under drought 65, 6205–6218. https://doi.org/10.1093/jxb/eru228

Coupel-ledru, A., Tyerman, S.D., Masclef, D., Lebon, E., Christophe, A., Edwards, E.J., Simonneau, T., 2017. Abscisic Acid Down-Regulates Hydraulic Conductance of Grapevine Leaves in Isohydric Genotypes Only 1 [OPEN] 175, 1121–1134. https://doi.org/10.1104/pp.17.00698

Delzon, S., 2015. New insight into leaf drought tolerance. Funct. Ecol. 29, 1247–1249. https://doi.org/10.1111/1365-2435.12500

Dumont, C., Cochetel, N., Lauvergeat, V., Cookson, S., Ollat, N., Vivin, P., 2016. Screening root morphology in grafted grapevine using 2D digital images from rhizotrons. Acta Hortic. https://doi.org/10.17660/ActaHortic.2016.1136.29

Faralli, M., Bianchedi, P.L., Bertamini, M., Varotto, C., 2021. Rootstock Genotypes Shape the Response of cv . Pinot gris to Water Deficit. https://doi.org/10.3390/agronomy11010075

Fodor, A., Peros, J., Launay, A., Doligez, A., Bertrand, Y., Roques, M., Beccavin, I., Paslier, L., Romieu, C., Fodor, A., Peros, J., Launay, A., Doligez, A., Berger, G., 2020. Genome-Wide Association Studies (GWAS) and Genomic Selection (GS) in grape : Evaluation of phenotypic prediction methods using a large panel of diversity To cite this version : HAL Id : hal-02801050.

Fort, K., Fraga, J., Grossi, D., Walker, M.A., 2017. Early measures of drought tolerance in four grape rootstocks. J. Am. Soc. Hortic. Sci. 142, 36–46. https://doi.org/10.21273/JASHS03919-16

Frioni, T., Biagioni, A., Squeri, C., Tombesi, S., Gatti, M., Poni, S., 2020. Grafting cv. grechetto gentile vines to new m4 rootstock improves leaf gas exchange and water status as compared to commercial 1103p rootstock. Agronomy 10, 1–14. https://doi.org/10.3390/agronomy10050708

Galbignani, M., Merli, M., Magnanini, E., Bernizzoni, F., Talaverano, I., Gatti, M., Tombesi, S., Palliotti, A., Poni, S., 2016. Gas exchange and water

- use efficiency of cv . Sangiovese grafted to rootstocks of varying water - deficit tolerance. Irrig. Sci. 34, 105–116. https://doi.org/10.1007/s00271-016-0490-z

García-tejero, I.F., Costa, J.M., Egipto, R., Durán-zuazo, V.H., Lima, R.S.N., Lopes, C.M., Chaves, M.M., 2016. Thermal data to monitor crop-water status in irrigated Mediterranean viticulture. Agric. Water Manag. 176, 80–90. https://doi.org/10.1016/j.agwat.2016.05.008

González-Flor, C., Serrano, L., Gorchs, G., 2019. Use of reflectance indices to assess vine water status under mild to moderate water deficits. Agronomy 9, 1–16. https://doi.org/10.3390/agronomy9070346

Ibacache, A., Albornoz, F., Zurita-silva, A., 2016. Scientia Horticulturae Yield responses in Flame seedless , Thompson seedless and Red Globe table grape cultivars are differentially modified by rootstocks under semi arid conditions. Sci. Hortic. (Amsterdam). 204, 25–32. https://doi.org/10.1016/j.scienta.2016.03.040

Idso, S.B., Jackson, R.D., Pinter Jr., P.J., Reginato, R.J., Hatfield, J.L., 1981. Normalizing the stress-degree-day parameter for environmental variability. Agric. Meteorol. 24, 45–55. https://doi.org/https://doi.org/10.1016/0002-1571(81)90032-7

Jackson, D.I., Lombard, P.B., 1993. Environmental and Management Practices Affecting Grape Composition and Wine Quality - A Review. Am. J. Enol. Vitic. 44, 409–430.

Jones, H.G., Stoll, M., Santos, T., Sousa, C. De, Chaves, M.M., Grant, O.M., 2002. Use of infrared thermography for monitoring stomatal closure in the ® eld : application to grapevine 53, 2249–2260. https://doi.org/10.1093/jxb/erf083

Klein, L.L., Miller, A.J., Ciotir, C., Hyma, K., Uribe-Convers, S., Londo, J., 2018. High-throughput sequencing data clarify evolutionary relationships among North American Vitis species and improve identification in USDA Vitis germplasm collections. Am. J. Bot. 105, 215–226. https://doi.org/10.1002/ajb2.1033

Knipfer, T., Eustis, A., Brodersen, C., Walker, A.M., Mcelrone, A.J., 2015. Grapevine species from varied native habitats exhibit differences in embolism formation / repair associated with leaf gas exchange and root pressure 1503–1513. https://doi.org/10.1111/pce.12497

Koundouras, S., Tsialtas, I.T., Zioziou, E., Nikolaou, N., 2008. Agriculture , Ecosystems and Environment Rootstock effects on the adaptive strategies of grapevine (Vitis vinifera L . cv . Cabernet – Sauvignon) under contrasting water status : Leaf physiological and structural responses 128, 86–96. https://doi.org/10.1016/j.agee.2008.05.006

Lavoie-lamoureux, A., Sacco, D., Risse, P., Lovisolo, C., 2017. Factors influencing stomatal conductance in response to water availability in grapevine : a meta-analysis 468–482. https://doi.org/10.1111/ppl.12530

Leavitt, S.W., Danzer, S.R., 1993. Method for Batch Processing Small Wood Samples to Holocellulose for Stable-Carbon Isotope Analysis 5, 87–89.

Liang, Z., Duan, S., Sheng, J., Zhu, S., Ni, X., Shao, J., Liu, C., Nick, P., Du, F., Fan, P., Mao, R., Zhu, Yifan, Deng, W., Yang, M., Huang, H., Liu, Y., Ding, Y., Liu, X., Jiang, J., Zhu, Youyong, Li, S., 2019. Whole-genome resequencing of 472 Vitis accessions for grapevine diversity and demographic

history analyses. Nat. Commun. 1–12. https://doi.org/10.1038/s41467-019-09135-8

Liu, X., Ickert-bond, S.M., Nie, Z., Zhou, Z., Chen, L., Wen, J., 2016. Molecular Phylogenetics and Evolution Phylogeny of the Ampelocissus – Vitis clade in Vitaceae supports the New World origin of the grape genus q. Mol. Phylogenet. Evol. 95, 217–228. https://doi.org/10.1016/j.ympev.2015.10.013

Lovisolo, C., Tramontini, S., Flexas, J., Schubert, A., 2008. Mercurial inhibition of root hydraulic conductance in Vitis spp . rootstocks under water stress 63, 178–182. https://doi.org/10.1016/j.envexpbot.2007.11.005

Lucini, L., Miras-moreno, B., Busconi, M., Marocco, A., Gatti, M., Poni, S., 2020. Plant Science Molecular basis of rootstock-related tolerance to water de fi cit in Vitis vinifera L . cv . Sangiovese : A physiological and metabolomic combined approach. Plant Sci. 299, 110600. https://doi.org/10.1016/j.plantsci.2020.110600

Marguerit, E., Brendel, O., Lebon, E., Leeuwen, C. Van, Ollat, N., 2012. Rootstock control of scion transpiration and its acclimation to water deficit are controlled by different genes.

Martinez-Zapater, J.M., Carmona, M.J., Diaz-riquelme, J., Fernandez, L., Lijavetzky, D., 2010. Grapevine genetics after the genome sequence : https://doi.org/10.1111/j.1755-0238.2009.00073.x

Meggio, F., Prinsi, B., Negri, A.S., Simone Di Lorenzo, G., Lucchini, G., Pitacco, A., Failla, O., Scienza, A., Cocucci, M., Espen, L., 2014. Biochemical and physiological responses of two grapevine rootstock genotypes to drought and salt treatments. Aust. J. Grape Wine Res. 20, 310–323. https://doi.org/10.1111/ajgw.12071

Migliaro, D., De Lorenzis, G., Di Lorenzo, G.S., De Nardi, B., Gardiman, M., Failla, O., Brancadoro, L., Crespan, M., 2019. Grapevine Non- Vinifera Genetic Diversity Assessed by SSR Markers as a Starting-Point for New Rootstock Breeding Programs . Am. J. Enol. Vitic. ajev.2019.18054. https://doi.org/10.5344/ajev.2019.18054

Milella, A., Marani, R., Petitti, A., Reina, G., 2019. In- fi eld high throughput grapevine phenotyping with a consumer-grade depth camera. Comput. Electron. Agric. 156, 293–306. https://doi.org/10.1016/j.compag.2018.11.026

Ollat, N., Cookson, S.J., Destrac-Irvine, A., Lauvergeat, V., Ouaked-Lecourieux, F., Marguerit, E., Barrieu, F., Dai, Z., Duchêne, E., Gambetta, G.A., Gomès, E., Lecourieux, D., Van Leeuwen, C., Simonneau, T., Torregrosa, L., Vivin, P., Delrot, S., 2018. Grapevine adaptation to abiotic stress: An overview. Acta Hortic. 1248, 497–512. https://doi.org/10.17660/ActaHortic.2019.1248.68

Ollat, N., Peccoux, A., Papura, D., Esmenjaud, D., Marguerit, E., Tandonnet, J.P., Bordenave, L., Cookson, S.J., Barrieu, F., Rossdeutsch, L., Lecourt, J., Lauvergeat, V., Vivin, P., Bert, P.F., Delrot, S., 2016. Rootstocks as a component of adaptation to environment, Grapevine in a Changing Environment: A Molecular and Ecophysiological Perspective. https://doi.org/10.1002/9781118735985.ch4

Padgett-Johnson, M., Williams, L.E., Walker, M.A., 2003. Vine water relations, gas exchange, and vegetative growth of seventeen Vitis species grown under irrigated and nonirrigated conditions in California. J. Am. Soc. Hortic. Sci. 128, 269–276.

https://doi.org/10.21273/jashs.128.2.0269

Pou, A., Diago, M.P., Medrano, H., Baluja, J., Tardaguila, J., 2014. Validation of thermal indices for water status identification in grapevine. Agric. Water Manag. 134, 60–72. https://doi.org/10.1016/j.agwat.2013.11.010

Pou, A., Medrano, H., 2012. Anisohydric behaviour in grapevines results in better performance under moderate water stress and recovery than isohydric behaviour 335–349. https://doi.org/10.1007/s11104-012-1206-7

Quénol, H., Marie, G., Barbeau, G., Leeuwen, C. Van, Foss, C., Irimia, L., Rochard, J., Boulanger, J., Tissot, C., Quénol, H., Marie, G., Barbeau, G., Leeuwen, C. Van, Hofmann, M., 2014. Adaptation of viticulture to climate change : high resolution observations of adaptation scenario for viticulture : the adviclim european project To cite this version : HAL Id : hal-01150259 Adapatation of vIticulture to climate change : high resolution o.

Rapaport, T., Hochberg, U., Shoshany, M., Karnieli, A., Rachmilevitch, S., 2015. ISPRS Journal of Photogrammetry and Remote Sensing Combining leaf physiology , hyperspectral imaging and partial least squares-regression (PLS-R) for grapevine water status assessment 109, 88–97. https://doi.org/10.1016/j.isprsjprs.2015.09.003

Riaz, S., Pap, D., Uretsky, J., Laucou, V., Boursiquot, J.M., Kocsis, L., Andrew Walker, M., 2019. Genetic diversity and parentage analysis of grape rootstocks. Theor. Appl. Genet. https://doi.org/10.1007/s00122-019-03320-5

RODRIGUEZ LOVELLE, B., TRAMBOUZE, W., JACQUET, O., 2009. EVALUATION OF THE VINE GROWTH STATUS BY THE “SHOOT TIP METHOD.”

Sabir, F., Zarrouk, O., Noronha, H., Loureiro-dias, M.C., 2021. Biochimie Grapevine aquaporins : Diversity , cellular functions , and ecophysiological perspectives. https://doi.org/10.1016/j.biochi.2021.06.004

Satisha, J., Sahadeo, R.D., Jagdev, S., Ajay Kumar, U., 2014. Moisture and Salinity Stress Induced Changes in Grape Rootstock Cultivars 2014.

Serrano, L., González-flor, C., Gorchs, G., 2010. Agriculture , Ecosystems and Environment Assessing vineyard water status using the reflectance based Water Index. "Agriculture, Ecosyst. Environ. 139, 490–499. https://doi.org/10.1016/j.agee.2010.09.007

Sinclair, T., Ludlow, M., 1986. Influence of Soil Water Supply on the Plant Water Balance of Four Tropical Grain Legumes.

Smart, D.R., Schwass, E., Lakso, A., Morano, L., 2006. From Proceedings of the Soil Environment and Vine Mineral Nutrition Symposium 1.

Smart, R.E., 1974. ASPECTS OF WATER RELATIONS OF THE GRAPEVINE (VITIS VINIFERA) 25.

Tardaguila, J., Fernández-novales, J., Gutiérrez, S., Diago, M.P., 2017. Non-destructive assessment of grapevine water status in the field using a portable NIR spectrophotometer. https://doi.org/10.1002/jsfa.8241

Tramontini, S., Vitali, M., Centioni, L., Schubert, A., Lovisolo, C., 2013. Rootstock control of scion response to water stress in grapevine. Environ. Exp. Bot. 93, 20–26. https://doi.org/10.1016/j.envexpbot.2013.04.001

Trenti, M., Lorenzi, S., Bianchedi, P.L., Grossi, D., Failla, O., Grando, M.S., Emanuelli, F., 2021. Candidate genes and SNPs associated with stomatal conductance under drought stress in Vitis 1–22.

Van Leeuwen, C., Agro, B.S., Irvine, A.D., 2019. An Update on the Impact of Climate Change in Viticulture and Potential Adaptations. https://doi.org/10.3390/agronomy9090514

Wan, Y., Schwaninger, H.R., Baldo, A.M., Labate, J.A., Zhong, G., Simon, C.J., 2013. A phylogenetic analysis of the grape genus (Vitis L .) reveals broad reticulation and concurrent diversification during neogene and quaternary climate change.

Wen, J., Harris, A.J., Kalburgi, Y., Zhang, N., Xu, Y., Zheng, W., Ickert-bond, S.M., Johnson, G., Zimmer, E.A., 2018. Chloroplast phylogenomics of the New World grape species (Vitis , Vitaceae). https://doi.org/10.1111/jse.12447

Yıldırım, K., Yağcı, A., Sucu, S., Tunç, S., 2018. Plant Physiology and Biochemistry Responses of grapevine rootstocks to drought through altered root system architecture and root transcriptomic regulations 127, 256–268. https://doi.org/10.1016/j.plaphy.2018.03.034

Zhang, L., Marguerit, E., Rossdeutsch, L., Ollat, N., Gambetta, G.A., 2016. The influence of grapevine rootstocks on scion growth and drought resistance. Theor. Exp. Plant Physiol. 28, 143–157. https://doi.org/10.1007/s40626-016-0070-x

Zhao, P., Wang, Y., Yan, S., Fan, L., Wang, Z., 2019. Electrical imaging of plant root zone : A review. Comput. Electron. Agric. 167, 105058. https://doi.org/10.1016/j.compag.2019.105058

PART I

HOW DO NOVEL M-ROOTSTOCK (*Vitis* spp.) GENOTYPES COPE WITH DROUGHT?

Abstract

Most of the vineyards around the world are in areas characterized by seasonal drought, where water deficits and high temperatures represent severe constraints on the regular grapevine growth cycle. Although grapevines are well adapted to arid and semi-arid environments, water stress can cause physiological changes, from mild to irreversible. Screening of available *Vitis* spp. genetic diversity for new rootstock breeding programs has been proposed as a way from which new viticulture challenges may be developed. In 2014, novel genotypes (M-rootstocks) were released from the University of Milan. In this work, the behavior of M1, M3 and M4 in response to decreasing water availabilities (80, 50 and 20% soil water content, SWC) was investigated at the physiological and gene expression levels, evaluating gas exchange, stem water potential and transcript abundances of key genes related to ABA biosynthesis (*VvZEP*, *VvNCED1* and *VvNCED2*) and signaling (*VvPP2C4*, *VvSnRK2.6* and *VvABF2*), and comparing them to those of cuttings of nine commercial rootstocks widely used in viticulture. M-rootstocks showed a change at physiological levels at severe water-stressed conditions (20% soil water content, SWC), reducing the stomatal conductance and stem water potential, but maintaining high photosynthetic activity. Water use efficiency was high at water-limiting conditions. The transcriptional changes were observed at 50% SWC, with an increment of transcripts of *VvNCED1* and *VvNCED2* genes. M-rootstocks showed similar behavior to 1103P and 110R rootstocks, two highly tolerant commercial genotypes. These rootstocks adopted a *tolerant* strategy to face water-stressed conditions.

1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the most widely cultivated and prized fruit crops around the world. In arid and semi-arid environments the vines undergo a slow decrease in water availability during the growing season (Chaves et al., 2003). Traditionally, grapevine is a non-irrigated crop due to the adaptation

to water limited conditions, though severe water stress displays from minor to irreversible physiological and biochemical changes (Jackson and Lombard, 1993; Pellegrino et al., 2005).

World viticulture is characterized by the use of *V. vinifera* varieties grafted onto a rootstock (*Vitis* spp.) due to the arrival of phylloxera (*Daktulosphaira vitifoliae* Fitch), a severe threat for grapevine survival, which was accidentally imported into Europe from North America (Gale, 2002). North American *Vitis* species are able to resist to phylloxera due to the co-evolved with the pathogen, therefore they are utilized as rootstocks, as single or inter-specific hybrids. Rootstocks also contribute to control other soil-borne pests such as nematodes, as well as various abiotic constraints, such as drought, salinity, lime-rich soils and deficient mineral nutrition (Bianchi et al., 2020; Bianchi et al., 2018; Cochetel et al., 2017; Vannozzi et al., 2017). They also modify whole plant development, biomass accumulation and phenology (Ollat et al., 2016).

The Mediterranean basin is considered one of the most vulnerable regions of the world to climate change and will potentially have to deal with water scarcity and soil erosion in the next few years (Giorgi and Lionello, 2008; IPCC, 2018). Its climate is characterized by infrequent rainfall (less than 100 days per year) that is unevenly distributed over time (long periods of summer drought) and sometimes quite sparse (about 300 to 500 mm per year in some semi-arid regions). Most climate change scenarios for this area predict a decrease in rainfall and higher temperatures. IPCC forecasts indicate a yearly temperature increase between 2 and 4°C and a decrease in rainfall between 4 and 30% by 2050 (IPCC, 2013). Due to their perennial status, grapevines will be highly vulnerable to environmental changes, representing a substantial risk for viticulture (Schultz, 2000).

Water flows into the plant in a soil-plant-atmosphere continuum (Lazar, 2003). The whole water transport system in the plant is influenced by the anatomical structure of xylem vessels (Shao et al., 2008), hydraulic constraints (Steudle, 2000) and chemical signals (Schachtman and Goodger, 2008; Tombesi et al., 2015). When soil water availability decreases, one of the earliest responses is stomatal closure, in

order to maintain a favorable water balance, buffering the drop of xylem water potential and avoiding embolisms (Hochberg et al., 2016; Jones and Sutherland, 1991). The closure of guard cells leads to a reduction of CO_2 assimilation and H_2O transpiration from leaves, consequently the photosynthetic activity decreases sharply (Medrano et al., 2015).

One of the factors inducing stomatal closure is abscisic acid (ABA), an hormone produced by roots and leaves (Audran et al., 1998; De Smet and Zhang, 2013; Farquhar and Sharkey, 1982; Ikegami et al., 2009; Lovisolo et al., 2016; Manzi et al., 2016, 2015; McAdam et al., 2016; Ren et al., 2007). ABA accumulates in the plant when soil dries out and water potential drops (Farquhar and Sharkey, 1982), the synthesis of which is entrusted to a minor branch of the carotenoid pathway. The early steps of ABA biosynthesis are catalyzed by zeaxanthin epoxidase (ZEP) and 9-*cis*-epoxycarotenoid dioxygenase (NCED) enzymes (Rock et al., 1991). *VvZEP* and *VvNCED* gene expressions are strongly induced by water stress (Qin and Zeevaart, 1999; Rossdeutsch et al., 2016; Speirs et al., 2013) and salt stress (Iuchi et al., 2001). This hormone, through the xylem sap, reaches guard cells, enhancing the content of reactive oxygen species (ROS, especially H_2O_2). Stopping the influx and promoting the efflux of potassium ions (K+) results in a rise of calcium ions (Ca^{2+}) in the cytosol and, consequently, cells lose their turgor. The ABA signaling pathway is mediated by three main components: i) pyrabactin resistance1/pyr1-like/regulatory components of ABA receptors (PYR/PYL/RCAR family of ABA receptors); ii) ABA-regulated Protein Phosphatase 2Cs (PP2CA); iii) ABA-regulated SNRK2 Protein Kinase (SnPK2) (Santiago et al., 2009; Yoshida et al., 2002). Without stimuli, the ABA receptor is an unliganded form and the protein kinase is bound to the protein phosphatase. Specific receptors (PYR/PYL/RCARs) bind to ABA when its concentration increases and the hormone-receptor complex becomes an active site for the protein PP2C. The activated receptor binds to PP2C, frees SnPK2 which in turn is phosphorylated by another protein kinase. Multiple step phosphorylation of SnRK2 activates ABRB (ABRE-binding protein)/ABF (ABRE-binding factor) which induce many ABA-responsive genes' expression (Raghavendra et al., 2010).

In grapevine, the expression of *VvNCED1*, *VvNCED2* and *VvZEP* genes have been directly correlated with ABA accumulation in response to water stress (Rossdeutsch et al., 2016; Soar et al., 2006; Speirs et al., 2013) and their expression was suggested as marker of ABA biosynthesis (Boneh et al., 2012). The expression of genes involved in the ABA signaling pathway revealed that the genes coding for RCAR, SnRK and ABF are downregulated in drought conditions, while *VvPP2C* genes are generally up-regulated (Boneh et al., 2012; Haider et al., 2017).

In the context of global warming, the exploitation of grapevine genetic diversity and the better understanding of plant response to environmental stresses represents the way from which new viticultural challenges may be developed (Bianchi et al., 2020; Migliaro et al., 2019; Vivier and Pretorius, 2002). Although a significant number of efforts in grapevine rootstock selection was carried out so far, less than 10 rootstocks are widely used in viticulture, with negative impact on the grapevine response to biotic and abiotic stresses (Keller, 2015; Ollat et al., 2016). Since 1985, the Department of Agricultural and Environmental Sciences (DiSAA) research group operating at the University of Milan has been working on the selection of new rootstocks able to cope with abiotic stresses. Some genotypes (M-series: M1, M2, M3 and M4) were selected and released in 2014 and registered in the National Register of Vine Varieties (RNVV). M1 and M3 exhibit tolerance to iron-limited conditions (M1 > M3) (Porro et al., 2013; Vannozzi et al., 2017), M2 and M4 display moderate resistance to salinity (Porro et al., 2013; Meggio et al., 2014) and M4 shows high tolerance to drought (Porro et al., 2013; Meggio et al., 2014; Corso et al., 2015).

To assess the drought-tolerance of M-rootstocks in comparison to other commercial genotypes largely used in viticulture, physiological (gas exchange and stem water potential) and transcriptomic performances (genes involved in ABA-synthesis and ABA-mediated responses to drought) were evaluated under well-watered and water-stressed conditions.

2. Results

2.1. The physiological response of grapevine rootstocks to drought

The physiological parameters photosynthesis (Pn), stomatal conductance (Gs), transpiration (E) and stem water potential (Ψ_S) were evaluated on 12 own-rooted grapevine rootstocks under decreasing water availability (from 80 to 20% SWC) (Table 1).

Table 1. List of 12 grapevine rootstocks subjected to water limitation and information about their pedigree (based on Migliaro et al (2019))

Rootstock	Pedigree
1103P	*V. Berlandieri* cv. Resseguier nr. 2 x *V. rupestris* cv. Du Lot
110R	*V. Berlandieri* cv. Boutin x *V. rupestris* cv. Du Lot
140Ru	*V. Berlandieri* cv. Boutin x *V. rupestris* cv. Du Lot
161-49C	*V. Berlandieri x V. riparia*
41B	*V. vinifera* cv. Chasselas x *V. Berlandieri* cv. Planchon
420A	*V. Berlandieri* x *V. riparia*
K5BB	*V. Berlandieri* Resseguier nr. 2 x *V. riparia* cv. Gloire de Montpellier
M1	Kober 5BB x Teleki 5C (*V. Berlandieri* cv. Planchon x *V. riparia*)
M3	Kober 5BB x Teleki 5C
M4	unknown x 1103 P
Schwarzmann	*V. riparia* x *V. rupestris*
SO4	*V. Berlandieri* cv. Resseguier nr. 2 x *V. riparia* cv. Gloire de Montpellier

The physiological activity reported in well-watered conditions (80% SWC) was maintained at 50% SWC and decreased at 20% SWC. The water condition showing the most significant differences (20% SWC) was used to investigate the behavior of each grapevine rootstock under water deficit conditions, in terms of photosynthetic activity and intrinsic water use efficiency (iWUE) (Figure 1). The *V. Berlandieri x V.*

rupestris hybrids (1103P, 110R and 140Ru rootstocks), 41B, M4 and M3 rootstocks carried out high photosynthetic activity under both water conditions, exceeding average levels. The *V. riparia* x *V. Berlandieri* hybrids (161-49C, 420A, K5BB) showed Pn values lower than average values at both water availabilities. The M1 rootstock showed similar Pn values at both conditions, exceeding the average value at 20% SWC (Figure 1a). Differences between genotypes also occurred when iWUE, calculated as the ratio between Pn and stomatal conductance values, was taken into account: 110R, 140Ru and M1 rootstocks maintained high efficiency when SWC decreased; iWUE values of the 161-49C reduced at 20% SWC; the 41B, K5BB and SO4 rootstocks reported iWUE values under average levels at 80% SWC maintaining the same efficiency at 20% ; 1103P, M3 and M4 rootstocks increased their efficiency under the water-stressed condition (Figure 1b).

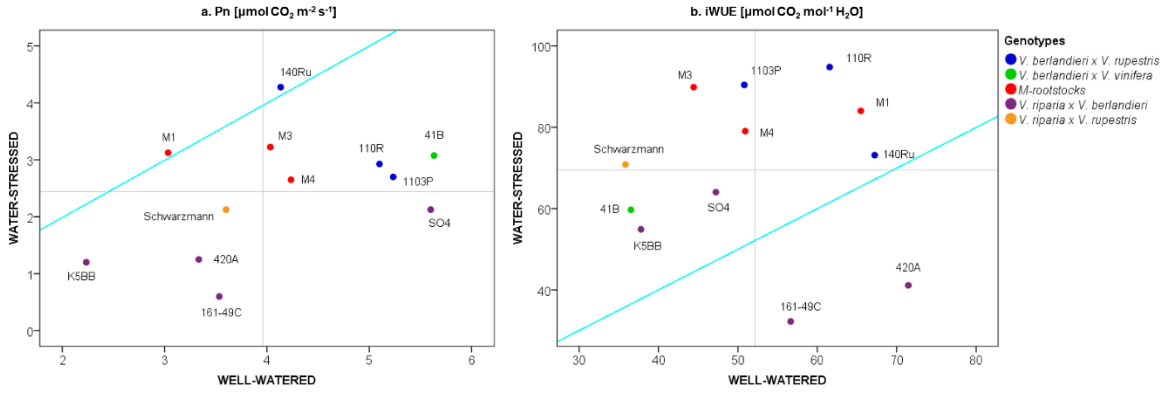


Figure 1. Comparison of performances in 12 own-rooted grapevine rootstocks under both well-watered (80% SWC; soil water content) and water-stressed (20% SWC) in terms of net photosynthesis (Pn) (a) and intrinsic water use efficiency (iWUE) (b). Colors were attributed according to the breeding materials (Table 1). M-rootstock pedigree: M1 – K5BB x Teleki 5C; M3 – K5BB x Teleki 5C; M4 – unknown x 1103P. Lines are set to the mean values of Pn (a) and iWUE (b) for each water condition. Lines 1:1 are reported in cyan

To investigate rootstock WUE in depth, Pn was analyzed as function of Gs under the water-stressed condition (20% SWC). Clear differences emerged in the behaviour of 12 genotypes, resulting in three different groups (Figure 2): i) Group A, genotypes reporting Gs values lower than the water-stressed

threshold (50 mmol H_2O m^{-2} s^{-1}, based on Cifre et al (2005)) and Pn values higher than the general average value (2.5 µmol CO_2 m^{-2} s^{-1}) (1103P, 110R, M1, M3 and M4 rootstocks); ii) Group B, genotypes reporting Gs values lower than the water-stressed threshold and Pn values lower than the general average value (161-49C, 420A, K5BB, Schwarzmann, and SO4 rootstocks); iii) Group C, genotypes reporting Gs values higher than the water-stressed threshold and Pn values higher than the general average value (140Ru and 41B rootstocks).

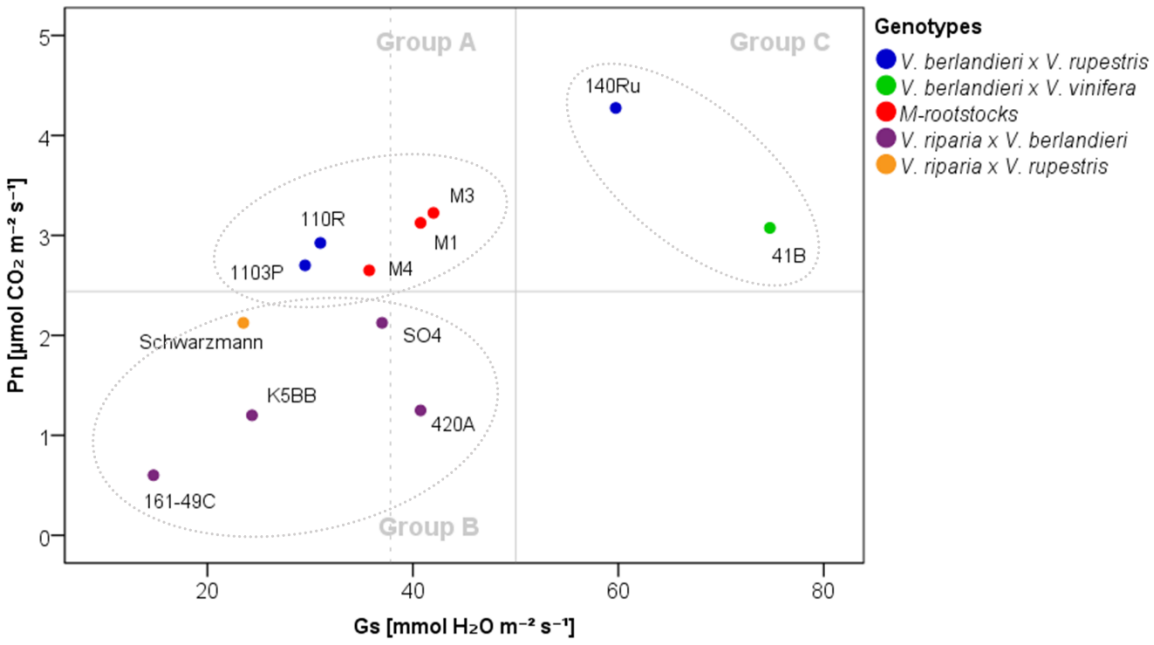


Figure 2. Stomatal conductance (Gs) as function of net photosynthesis (Pn) in 12 own-rooted grapevine rootstocks at and 20% SWC (soil water content). Colors were attributed according to the breeding materials (Table 1). M-rootstock pedigree: M1 – K5BB x Teleki 5C; M3 – K5BB x Teleki 5C; M4 – unknown x 1103P. Thresholds for group identification were set to 50 mmol H_2O m^{-2} s^{-1} (Cifre et al., 2005) for Gs and to average for Pn at 20% SWC. The dotted line shows the average Gs value at 20% SWC

The three rootstock groups (A, B and C) were compared in term of Ψ_S at the decreasing levels of SWC (80, 50 and 20%). Stem water potential settled at -0.4 MPa at 80 and 50% SWC without showing significant differences among groups. At 20% SWC, Group A and C rootstocks decreased Ψ_S value, whereas

Group B rootstocks maintained higher Ψ_S values, without a significant reduction of Ψ_S values with respect to 50% SWC. At 20% SWC, Group A rootstocks reported Ψ_S values lower than Group B rootstocks (Figure 3a). Moreover, the Ψ_S was analyzed as function of stomatal conductance and differences among groups were identified as well (Figure 3b): Group A rootstocks showed, mainly, Gs and Ψ_S levels below stress threshold (50 mmol H_2O m^{-2} s^{-1}, based on Cifre et al (2005)) and average value, respectively; Group B rootstocks showed Gs values below threshold and, mainly, Ψ_S values above the average value (except for K5BB rootstocks); Group C rootstocks showed Gs values exceeding stress threshold, whereas Ψ_S value was higher than the average for 140Ru rootstock and lower than the average for 41B rootstock.

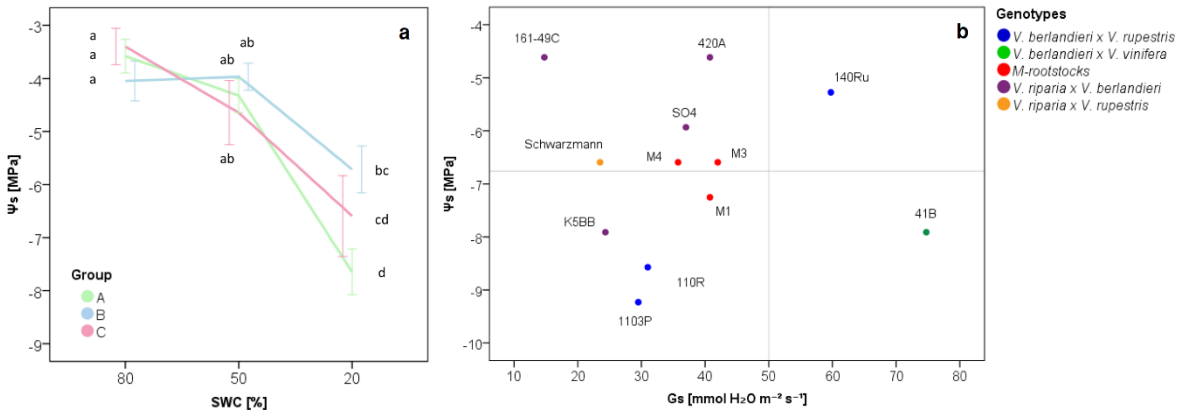


Figure 3. Stem water potential (Ψ_S) as function of decreasing soil water content (SWC) (a) and stomatal conductance (b). Groups are defined in Figure 2, according to the gas exchange values. Group A: 1103P, 110R, M1, M3 and M4 rootstocks; Group B: 161-49C, 420A, K5BB, Schwarzmann and SO4 rootstocks; Group C: 140Ru and 41B rootstocks. Letters show the statistical differences defined according to Tukey post-hoc test at *p*-value 0.05. In plot b, thresholds were set to 50 mmol H_2O m^{-2} s^{-1} (Cifre et al., 2005) for Gs and to average for Ψ_S at 20% SWC

At 20% SWC, groups were compared for all physiological parameters and results are summarized in Table 2. Group B rootstocks significantly differed from Group A and C rootstocks for Pn and Ψ_S values, while Group C rootstocks significantly differed from Group A and B rootstocks for Gs and E values.

Table 2. Average value and standard deviation of physiological parameters for grapevine rootstock genotypes of group A (1103P, 110R, M1, M3 and M4), group B (161-49C, 420A, K5BB, Schwarzmann and

SO4) and group C (140Ru and 41B) at 20% SWC (soil water content). Groups are defined in Figure 2, according to the intrinsic water use efficiency. Pn = net photosynthesis (µmol CO_2 $m^{-2}s^{-1}$); Gs = stomatal conductance (mmol H_2O m^{-2} s^{-1}); E = transpiration (mmol H_2O $m^{-2}s^{-1}$); Ψ_S = stem water potential (MPa). Statistical differences among groups for each parameter are defined according to Tukey post-hoc test at *p*-value 0.05

Parameter	Group A				Group B				Group C			
Pn	2.93	±	0.66	a	1.47	±	1.15	b	3.68	±	1.62	a
Gs	35.80	±	13.40	a	28.26	±	16.16	a	67.25	±	36.23	b
E	0.77	±	0.26	a	0.66	±	0.36	a	1.38	±	0.67	b
Ψ_S	-0.76	±	0.13	a	-0.59	±	0.14	b	-0.66	±	0.14	ab

2.2. The transcriptional response of grapevine rootstocks to drought

Based on the physiological behavior presented in Figure 2, the gene expression values (*VvNCED1*, *VvNCED2*, *VvZEP* in roots and *VvPP2C4, VvSnRK2.6, VvABF2 in* leaves) were compared among the three groups (A, B and C) by discriminant analysis (Figure 4). At 50% SWC, the three groups showed a different transcriptional behavior: Group A and C rootstocks were discriminated along the first function (*p* = 0.000), while Group A and B rootstocks were discriminated along both the first (*p* = 0.034) and the second (*p* = 0.000) functions (Figure 4a). Functions 1 and 2 accounted for 81.0% and 19.0% of total variability, respectively. The most discriminating variables among the groups were *VvABF2* gene for function 1 and *VvNCED1* and *VvNCED2* genes for function 2. Function 1 was significantly correlated to *VvABF2* (0.350) and *VvNCED2* (-0.105) gene expression values and function 2 to *VvZEP* (-0.346), *VvNCED1* (-0.644), *VvSnRK2.6* (0.443) and *VvPP2C4* (0.314) gene expression values. At 20% SWC, Group A and C rootstocks showed a similar behavior for the first function (0.881), different from the one shown by Group B rootstocks. Group B rootstocks were discriminated along the first function from Group A (*p* = 0.000) and C (*p* = 0.000) rootstocks (Figure 4b). The second function discriminated Groups A and C (0.021). Functions

1 and 2 accounted for 88.6% and 11.4% of total variability, respectively. The most discriminating variables among groups were *VvNCED1* and *VvNCED2* genes for function 1 and *VvSnRK2.6* and *VvZEP* genes for function 2. Function 1 reported significant and positive correlations to *VvPP2C4* (0.394) and *VvABF* (0.234) gene expression values, whereas function 2 reported significant and positive correlations to *VvZEP* (0.801), *VvNCED1* (0.872), *VvNCED2* (0.499) and *VvSnRK2.6* (0.156) gene expression values.

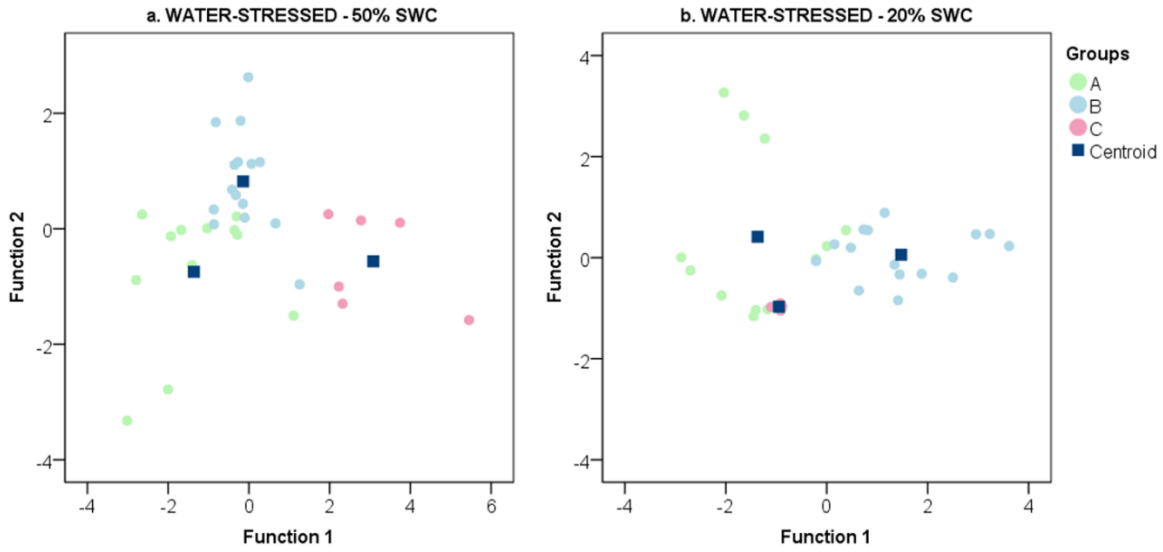


Figure 4. Discriminant analysis of transcript (*VvZEP, VvNCED1, VvNCED2, VvPP2C4, VvSnRK2.6* and *VvABF2* genes) abundance data detected for 12 own-rooted grapevine rootstocks grown under limited water conditions. a: data collected at 50% SWC (soil water content). b: data collected at 20% SWC. The genotypes are classified in three groups (A, B and C), as defined in Figure 2, according to the intrinsic water use efficiency. Group A: 1103P, 110R, M1, M3 and M4 rootstocks; Group B: 161-49C, 420A, K5BB, Schwarzmann and SO4 rootstocks; Group C: 140Ru and 41B rootstocks

In Figure 5, the trend of gene expression for each gene and each group is shown. The *VvZEP* gene showed a significant increment of transcripts only for Group C rootstocks (Figure 5a). For *VvNCED1* gene, the gene expression levels increased significantly at 50% SWC and reached the highest value at 20% SWC for Group A rootstocks (Figure 5b). For *VvPP2C4*, *VvSnK2.6* and *VvABF2* genes, Group B rootstocks showed a significant increment of transcripts at 20% SWC (Figure 5d-f).

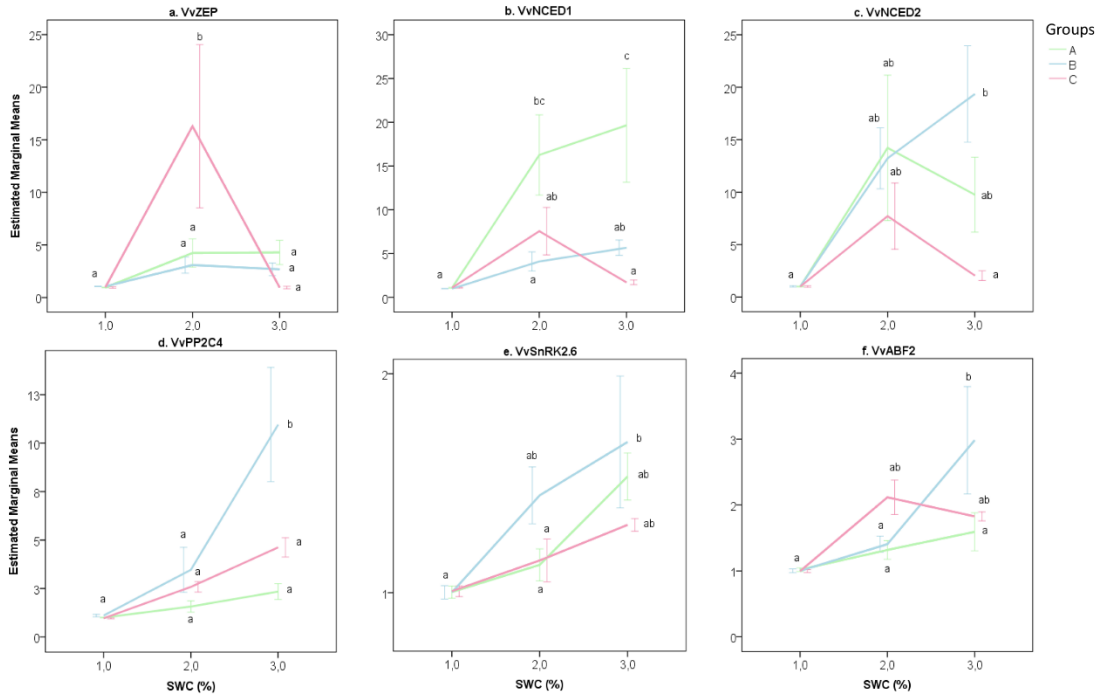


Figure 5. Graphical representation of gene expression data of six genes related to the ABA metabolism in roots (a-c: *VvZEP, VvNCED1* and *VvNCED2*) and leaves (d-f: *VvPP2C4, VvSnRK2.6* and *VvABF2*) of 12 own-rooted grapevine rootstocks grown under limited water conditions (from 80 to 20% of soil water content, SWC). The genotypes are classified in three groups (A, B and C), as defined in Figure 2, according to the intrinsic water use efficiency. Average values and standard error are shown. Statistical differences are defined according to Tukey post-hoc test at *p*-value 0.05. Group A: 1103P, 110R, M1, M3 and M4; Group B: 161-49C, 420A, K5BB, Schwarzmann and SO4; Group C: 140Ru and 41B

In figure 6, gas exchange (Pn, Gs, E) detected at 20% SWC reported a significant negative correlation to the expression of the gene *VvNCED1* obtained at 20% and 50% SWC. Transpiration and stomatal conductance also reported a negative correlation to *VvZEP* at 50% SWC. Vpd at 20% SWC correlated to *VvNCED1* and *VvZEP* expressed at 50% SWC and to *VvNCED1* expressed at 20% SWC. Ψs detected at 20% SWC reported a positive correlation to *VvZEP* at 80% SWC, whereas a negative correlation to *VvPP2C4* and *VvSnRK2.6* at both 80% and 50% SWC.

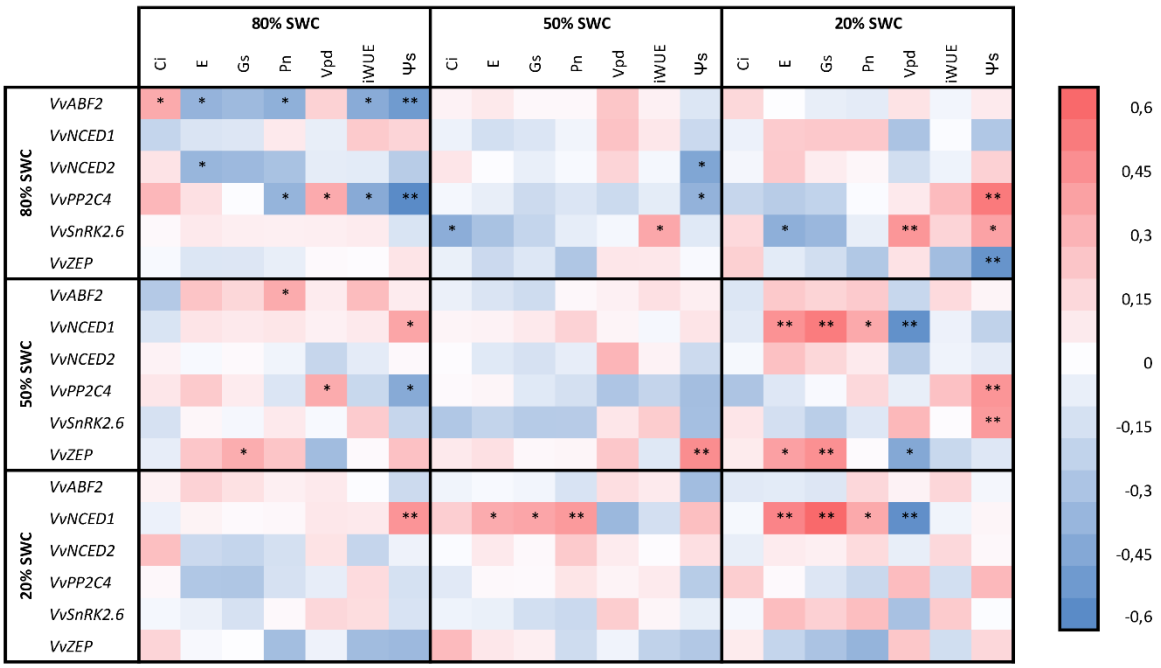


Figure 6. Heatmap of correlation matrix (Pearson Index) among physiological (E, Gs, Pn and Ψ_S) and transcriptional (*VvZEP, VvNCED1, VvNCED2, VvPP2C4, VvSnRK2.6* and *VvABF2* genes) data detected for 12 own-rooted grapevine rootstocks grown under limited water conditions (from 80 to 20% of soil water content). E: Transpiration; Gs: Stomatal conductance; Pn: Photosynthetic activity; Ψ_S: Stem water potential. Statistically significant differences are reported at 0.05 (*) and 0.01 (**) levels

3. Discussion

3.1. Water-limiting conditions for grapevine rootstocks

Grapevine can easily face conditions of mild water stress without their physiological activity being affected, allowing these plants to grow in many marginal areas, usually characterized by limited soil water availability. Roots are the major interface between plants and soil and the first organ to perceive water availability. They are involved in activating key steps for triggering a drought reaction to water stress: signal perception, signal transduction to shoot and leaves and water stress-inducible gene expression (Lovisolo et al., 2016). Therefore, rootstocks play an essential role in the water stress response in

grapevines. In this study, the short-term response to drought of three new-generation (M1, M3 and M4) and nine commercial rootstocks was evaluated. At the physiological level, soil water capacity at 50% was not a limiting condition for M-rootstocks and the nine commercial rootstocks analyzed, with no statistically significant changes occurring in terms of Pn, Gs, E and Ψ_S in comparison with the well-watered condition (80% SWC). Photosynthetic activity reached by all plants under well-watered conditions was lower than regular field activity due to the adaptation of leaves to moderate light [∼PPFD of 600 μmol of photons/(m^2 s)], with values between high and low light conditions obtained by Schubert et al (1996) under field conditions.

3.2. The effect of water stress on grapevine rootstock genotypes

Under water deficit conditions (20% SWC), the *V. Berlandieri x V. rupestris* hybrids (140Ru, 1103P and 110R) and the M-rootstocks and 41B rootstocks maintained high photosynthetic activity in comparison with the *V. riparia* x *V. Berlandieri* hybrids and Schwarzmann rootstocks (Figure 1a). Besides photosynthesis, M-rootstocks and *V. Berlandieri x V. rupestris* hybrids were more efficient in the use of water under limited conditions, reporting higher iWUE values than *V. riparia* x *V. Berlandieri* hybrids and 41B rootstocks (Figure 1b). On reducing the water availability, M3 and M4 rootstocks and most of the commercial rootstocks closed stomata, showing significant differences in Gs values compared to the well-watered condition. M4 and other rootstocks (110R, 161-49C, and SO4) significantly reduced both Gs and Pn values in response to water-stressed conditions (Figure 1). These genotypes are considered “plastic”, due to their ability to modify their performances under different environmental conditions (Bianchi et al., 2018; Silvia Dal Santo et al., 2018; Pinto et al., 2016). However, M1 and 140Ru showed an “elastic” behavior, as they maintain unchanged Pn and iWUE levels under both well-watered and water-stressed conditions.

The genetic background of M-rootstocks and nine commercial grapevine rootstocks became discernible in their performances under water-deficit conditions (Figure 1, Figure 2). In agreement with the literature (Carbonneau, 1985; Lovisolo et al., 2016; Serra et al., 2014), the *V. riparia* x *V. Berlandieri* hybrids (161-49C, 420A, K5BB and SO4 rootstocks) showed lower tolerance to water stress than *V. Berlandieri* x *V. rupestris* hybrids (1103P, 110R and 140Ru rootstocks), with lower Pn values. In Padgett-Johnson et al (2003), *V. rupestris* showed higher drought tolerance than *V. riparia* and *V. Berlandieri.* Schwarzmann (*V. riparia* x *V. rupestris*) showed a performance similar to *V. riparia x V. Berlandieri* hybrids, whereas 41B rootstock (*V. Berlandieri* x *V. vinifera*), typically classified as a tolerant genotype, showed a behaviour similar to *V. Berlandieri* x *V. rupestris* hybrids.

In our study, the performances of M-rootstocks (M1, M3 and M4), characterized by different genetic backgrounds, were similar to that shown by the *V. Berlandieri* x *V. rupestris* hybrids 1103P and 110R. M4 (unknown x 1103 P) and 1103P rootstocks, both considered highly tolerant to water stress (Corso et al., 2015; Lovisolo et al., 2016; Meggio et al., 2014), showed similar performances (Figure 2).

A recent study compared M4 to 1103P in grafting combination with Grechetto Gentile. The two combinations maintained similar water potential under water stress, though M4 showed higher photosynthesis and water use efficiency (Frioni et al., 2020). Galbignani et al (2016) found higher Pn values and higher instantaneous WUE values in Sangiovese grafted on M4 than grafted on SO4 under moderate water-stressed conditions.

Vitis species possess the ability to show different strategic behaviors in response to drought (Padgett-Johnson et al., 2003). In this study, three different strategies based on gas exchange and iWUE were identified in response to severe water-deficit conditions: i) M-rootstocks (M1, M3 and M4), 1103P and 110R rootstocks showed high Pn at limiting Gs values (Group A); ii) Schwarzmann rootstock and *V. riparia x V. Berlandieri* hybrids showed low Pn values at low Gs values (Group B); iii) 140Ru and 41B rootstocks showed high Pn values without reduction of Gs values (Group C) (Figure 2). The three groups

reported differences in stem water potential under low SWC (Figure 3a), as well as in the expression of genes related to ABA biosynthesis and signaling (Figure 4).

3.3. Delineation of group strategies to face drought

Based on physiological performance under water-limiting conditions, the rootstocks were classified into three groups (A, B and C) (Figure 2). The same three clusters were clearly discriminated according to the expression of six genes related to the ABA pathway at both mild (50% SWC) and limiting (20% SWC) water-stressed conditions (Figure 4). ABA mediates many physiological responses of plants to drought: avoidance, as well as tolerance responses. It is synthesized in both roots and leaves (De Smet and Zhang, 2013). In both organs, its levels increase upon exposure to drought and they are accompanied by major changes in gene expression and physiological responses, such as stomatal closure (Tombesi et al., 2015). Differences among groups in physiological activity were only detected under water-limiting conditions (20% SWC), nevertheless the three groups were clearly discriminated at mildly water level (50% SWC) according to their gene expression (Figure 4). At 20% SWC, the discriminant function 1 correlated with *VvPP2C4* and *VvABF2* gene expression in leaves, involved in the ABA signal transduction (Boneh et al., 2012; Chan, 2012; Rattanakon et al., 2016). The discriminant function 2 was mainly correlated with *VvZEP*, *VvNCED1* and *VvNCED2* gene expression in roots, involved in ABA biosynthesis (Rattanakon et al., 2016; Rossdeutsch et al., 2016).

Vines can use several strategies for drought adaptation, including avoidance, tolerance and resistance (Delzon, 2015; Ollat et al., 2018). The expression of genes related to the ABA biosynthetic pathway helped to investigate the strategies adopted by groups to deal with the water deficiency. Group A rootstocks (M1, M3, M4, 1103P and 110R) experienced the stress at 20% SWC (Figure 2), increasing the transcripts of genes related to ABA biosynthesis, especially *VvNCED1* and *VvNCED2* (Figure 5b-c). However, they showed low expression of genes linked to ABA signal transduction, reporting negative

values of discriminant function 1 (Figure 4b). The evidence that genes related to ABA signal transduction (*VvPP2C4*, *VvSnRK2.6*, *VvABF2*) showed low levels of gene expression at low Gs levels allows us to suppose that the stomatal closure in response to ABA rise might be associated with a different mechanism. An alternative way to achieve a fast increment in ABA content is *via* hydrolysis of the ABA-glucosyl ester (ABA-GE), an inactive glucose-conjugated form of ABA (Rattanakon et al., 2016). Nevertheless, Group A rootstocks reduced the stomatal conductance, despite which they retained high Pn activity, proving high water use efficiency (Figure 2, Figure 1b). Photosynthetic activity and stomatal closure involved reductions of both sub-stomatal CO_2 concentration (Ci) and vapor pressure deficit (Vpd). This performance could be ensured by an efficient ROS detoxification system, for which gene expression was noticed for M4 rootstock under water-stressed conditions by Corso et al (2015) (Figure 3). The rootstocks clustered in Group A, including the M-rootstocks (M1, M3 and M4), adopted a *tolerant* strategy (Delzon, 2015), preserving their physiological activity under water stress.

Rootstocks belonging to Group B (161-49C, 420A, K5BB, Schwarzmann and SO4) also reduced the physiological activity at 20% SWC (Figure 1b). Among genes related to ABA biosynthesis, they mainly increased transcripts of *VvAPF2, VvNCED2* and *VvPP2C4* genes (Figure 5c-d). According to the literature, the enhanced activity of VvPP2C genes during drought stress suggests that its primary role is in regulating ABA response (Boneh et al., 2012; Chan, 2012; Haider et al., 2017). As reported by Boneh et al (2012) and Rattanakon et al (2016), transcripts of PP2C genes increase to slow down the activation of the ABA signaling pathway that occurs from a rapid rise of the hormone itself. For Group B rootstocks, stomatal conductance decreased, as well as photosynthetic activity, showing low efficiency in water use (Figure 2, Figure 1b). For this group, low stomatal conductance seemed to buffer the drop of Ψ_S values at decreasing SWC levels (Figure 3a-b) (Figure 6). The strategy adopted by Group B genotypes under water-stressed conditions can be defined as *avoidance* (Delzon, 2015), preserving Ψ_S by reducing the physiological activity

through stomatal closure. The high water potential indicated that they could be adopted in long-term drought conditions.

Unlike other rootstocks, Group C rootstocks (140Ru and 41B) maintained the stomatal conductance under 20% SWC, allowing leaves to continue high photosynthetic activity (Figure 2), regardless of Ψ_S (Figure 3b). The physiological activity performed at 20% SWC could be related to the adaptation of genotype architecture to drought conditions, such as the vessel size (Bianchi et al., 2018; Lovisolo et al., 2018; Tyree and Ewers, 1991) (Figure 6). However, the expression of genes linked to ABA biosynthesis, especially *VvZEP*, rose at 50% SWC before decreasing at lower water availability (Figure 5a). Group C rootstocks showed a *resistant* strategy to water stress under water-limited conditions.

4. Material and Methods

4.1. Plant materials and growth conditions

The experiment was conducted in June 2017, under environmentally controlled conditions in a greenhouse at DiSAA (University of Milan). The greenhouse was equipped with supplementary light and a cooling system, with a 16-hr light [~PPFD of 600 µmol of photons/(m^2 s)] and 8 hr dark photoperiod and a range of temperatures from 23 to 28°C. A total of twelve grapevine rootstocks were analyzed: the worldwide used 1103P, 110R, 114Ru, 161-49C, 41B, 420A, K5BB, Schwarzmann, SO4 and the newly released M1, M3, M4. Nine biological repetitions per genotype were monitored. Two years old cuttings were used. The vines were grown in 4-L plastic pots, trained on 1 m stakes and placed in a randomized complete block design. The growth substrate was composed of 70% sand and 30% peat, supplemented with a layer of expanded clay aggregate on the bottom of the pot to avoid water flooding. During the phenological phase of budding, the plants were maintained in well-watered conditions in order to develop a well-expanded canopy.

4.2. Irrigation management and treatments

Three treatments were performed: 80, 50 and 20% SWC (soil water content). Three plants were collected per treatments, considered as biological replications. The SWC was calculated using the gravimetric method, according to the formula suggested by Gardner et al (2001):

$$SWC = \frac{(\text{fresh weight} - \text{dry weight})}{\text{dry weight}} 100 \quad (1)$$

where fresh weight refers to the soil weight at field capacity and dry weight to the soil dried in an oven at 105°C for 48 hours. Each pot containing one plant was weighed daily for a period of 10 days. When SWC reached the values of 80, 50 and 20%, plants were selected for measurement of physiological parameters and gene expression analysis.

4.3. Plant phenotyping

At each measurement time (80, 50 and 20% SWC), gas exchange parameters and stem water potential (Ψ_S) were evaluated on three different plants (replications) per each rootstock. Both measurements were carried out between 11:00 am and 2:00 pm solar time. Two fully expanded leaves (8th and 9th leaf) per plant were selected to measure gas exchange indicators: photosynthetic activity (Pn; µmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (Gs; mmol H_2O m^{-2} s^{-1}) and transpiration (E; mmol H_2O $m^{-2}s^{-1}$). Gas exchange was measured with a leaf portable photosynthesis system (CIRAS-2, PP Systems, Amesbury, MA, USA) equipped with PLC6 (U) cuvette 18 mm circular (2.5 cm^2 head plate), under constant saturating PPFD of 1500 µmol photons $m^{-2}s^{-1}$, CO_2 concentration of 300 µmol mol^{-1}, block temperature of 25°C and relative humidity between 60% and 70% allowing ~1.5 kPa of VPD inside the leaf chamber. Intrinsic water use efficiency (iWUE) was calculated as Pn/Gs ratio and expressed as µmol CO_2 mol^{-1} H_2O. As suggested by Scholander et al (1965), Ψ_S (MPa) was calculated using the Scholander-pressure chamber (Soil Moisture Equipment Corporation, Santa Barbara, CA, USA). The same leaves used to evaluate gas exchange were

placed in a plastic bag wrapped with an aluminum foil for 1 hr. Subsequently, they were excised with a razor blade and put in the Scholander chamber for the analysis. The Ψ_S value was recorded within 30 sec from the cutting of the leaf by slowly pressurizing the chamber until sap came out from the cut end of the petiole.

4.4. Gene expression analysis

After the *in vivo* measurements of physiological parameters at 80, 50 and 20% SWC, the whole root system and fully expanded leaves (i.e. from the fifth to the eighth node of primary shoot) of the same plants per each rootstock were immediately sampled, frozen in liquid nitrogen and stored at −80°C until RNA extraction. The total RNA was extracted from 100 mg of liquid nitrogen-ground tissue with Spectrum™ Plant Total RNA (Sigma-Aldrich, Germany) commercial kit, according to the manufacturer's instructions. To evaluate RNA quality, 260/230 and 260/280 ratios were checked *via* NanoDrop Spectrophotometer (Thermo Scientific, MA, USA). For those samples showing a 260/230 absorbance ratio lower than 1.8, a lithium-chloride treatment was carried out (as reported in De Lorenzis et al., 2016). RNA integrity was checked by electrophoresis on 1.5% agarose gel. RNA quantification was performed using Qubit® RNA HS Assay Kit by Qubit® 3.0 Fluorometer (Life Technologies, CA, USA). Total RNA (200 ng) was used to synthetize cDNA using 200 U of SuperScript® III Reverse Transcriptase (Thermo Fisher) and 50 µM oligo$(dT)_{20}$ primers in accordance with the manufacturer's instructions. Six genes (Table 3) involved in the response to drought were evaluated *via* real-time RT-PCR. *VvZEP*, *VvNCED1* and *VVNCED2* genes were evaluated in roots and *VvPP2C4*, *VVSnRK2.6* and *VvABF2* genes were evaluated in leaves, based on previous evidence reporting that genes related to ABA biosynthesis are mainly associated with ABA increases in water-stressed roots (Rossdeutsch et al., 2016; Speirs et al., 2013), while genes related to the ABA signal transduction better discriminate the genotypes at leaf level (Rossdeutsch et al., 2016). Ubiquitin (UBI; Fujita et al., 2007) and actin (ACT; Reid et al., 2006) were used as reference genes. RT-PCR

was carried out in a 7300 Real Time PCR System (Applied Biosystems, CA, USA). For each reaction (20 µL), 200 nM of each primer, 2 µL of cDNA (1:100 dilution of the synthesis reaction), 1X SYBR Green Real-Time PCR Master Mix (Thermo Fisher) and water up to 20 µL were added. Thermal cycling was pre-incubation at 95°C for 3 min, followed by 40 cycles of 94°C for 15 sec, 58°C for 20 sec and 72°C for 30 sec. For detecting non-specific amplifications in cDNA samples, a melting cycle with temperature ranging from 65 to 95°C was performed. Each real-time RT-PCR reaction was completed in triplicate. After testing the suitability of ubiquitin and actin as reference genes, ubiquitin was selected to normalize the Ct (cycle threshold) values of all analysed samples, due to a PCR efficiency value more similar to the ones observed for target genes (ranging from 93 to 97%). The expression of each gene in different genotypes and water conditions was calculated by comparing their $2^{-\Delta\Delta Ct}$ values (Livak and Schmittgen, 2001).

Table 3. List of genes evaluated *via* real-time RT-PCR in roots and leaves of 12 own-rooted grapevine rootstocks grown under water deprivation

Genes	Primer sequence (5'→ 3')	Reference	Tissue
VvZEP	F: GGTAAGAAGGAAAGGTTGC R: CAATAGGAGTCCCTGATTTGATGC	(Hayes et al., 2010)	roots
VvNCED1	F: TGCAGAGGACGAGAGTGTAA R: AGCTACACCAAAAGCTACGA	(Hayes et al., 2010)	
VvNCED2	F: ATGCTCAAACCGCCTCTGAT R: TCCCAAGCATTCCAGAGGTG	(Lund et al., 2008)	
VvPP2C4	F: TGGGCTTTGGGATGTTATGT R: TGTGCAGGAGTCTCATCAGC	(Boneh et al., 2012)	leaves
VvSnRK2.6	F: CACCAACCCACCTTGCTATT R: AAACGTGCCTCATCCTCACT	(Boneh et al., 2012)	
VvABF2	F: GGCACCCAGGCTAGTTAA R: GCAGAGTACACGCTAGATTG	(Rossdeutsch et al., 2016)	

4.5. Statistical analysis

Data were analyzed using Microsoft Office Excel and SPSS statistical software (IBM SPSS Statistics 24). A univariate ANOVA model was performed on phenotypical parameters (Pn, Gs, E and Ψ_S) at $p \leq 0.05$ after checking for the assumption of normality and homogeneity of variance. Post-hoc comparisons were performed on phenotypical parameters (Pn, Gs, E and Ψ_S) with Tukey test at $p \leq 0.05$. Gene expression data were used to perform a discriminant analysis, using the values as independent variables and with equal prior probabilities. Groups were identified by bi-plot of Pn and Gs using the available water-stressed threshold for Gs (50 mmol H_2O m^{-2} s^{-1}, based on Cifre et al (2005)) and the mean value for Pn. Differences among groups in terms of discriminant function scores and gene expression were detected by a univariate ANOVA model and a Tukey post-hoc test at $p \leq 0.05$. Correlation among phenotypical parameters and gene expression was determined by Pearson's index at $p = 0.05$ (*) and $p = 0.01$ (**) and viewed as heatmap.

5. Conclusions

In this study, the new M-rootstocks showed a response to water-stressed conditions similar to that of the 1103P and 110R rootstocks, two commercial genotypes typically classified as being highly tolerant. They adopted a tolerant strategy, increasing the transcripts of genes related to ABA biosynthesis, especially *VvNCED1* and *VvNCED2*, maintaining high water use efficiency under water-stressed conditions and preserving physiological activity even with low levels of stomatal conductance. Further studies will be necessary to confirm the performance of M-rootstocks under water stress in field conditions, evaluating rootstock/scion interactions. Nevertheless, a few new grapevine rootstock genotypes are not enough to face the challenges that modern viticulture will have to cope with in the future, therefore, new breeding programs should be planned.

References

Audran, C., Borel, C., Frey, A., Sotta, B., Meyer, C., Simonneau, T., Marion-Poll, A., 1998. Expression studies of the zeaxanthin epoxidase gene in Nicotiana plumbaginifolia. Plant Physiol. 118, 1021–1028. https://doi.org/10.1104/pp.118.3.1021

Bianchi, D., Brancadoro, L., Lorenzis, G. De, 2020a. Genetic Diversity and Population Structure in a Vitis spp . Core Collection Investigated by SNP Markers. https://doi.org/10.3390/d12030103

Bianchi, D., Grossi, D., Simone Di Lorenzo, G., Zi Ying, Y., Rustioni, L., Brancadoro, L., 2020b. Phenotyping of the "G series" Vitis hybrids: First screening of the mineral composition. Sci. Hortic. (Amsterdam). 264. https://doi.org/10.1016/j.scienta.2019.109155

Bianchi, D., Grossi, D., Tincani, D.T.G., Simone Di Lorenzo, G., Brancadoro, L., Rustioni, L., 2018. Multi-parameter characterization of water stress tolerance in Vitis hybrids for new rootstock selection. Plant Physiol. Biochem. 132, 333–340. https://doi.org/10.1016/j.plaphy.2018.09.018

Boneh, U., Biton, I., Zheng, C., Schwartz, A., Ben-Ari, G., 2012. Characterization of potential ABA receptors in Vitis vinifera. Plant Cell Rep. 31, 311–321. https://doi.org/10.1007/s00299-011-1166-z

Carbonneau, A., 1985. The Early Selection of Grapevine Rootstocks for Resistance to Drought Conditions 36, 195–198.

Chan, Z., 2012. Expression profiling of ABA pathway transcripts indicates crosstalk between abiotic and biotic stress responses in Arabidopsis. Genomics 100, 110–115.

Chaves, M.M., Maroco, J.P., Pereira, J.S., 2003. Understanding plant responses to drought — from genes to the whole plant. Funct. Plant Biol. 30, 239. https://doi.org/10.1071/fp02076

Cifre, J., Bota, J., Escalona, J.M., Medrano, H., Flexas, J., 2005. Physiological tools for irrigation scheduling in grapevine (Vitis vinifera L .) An open gate to improve water-use efficiency ? 106, 159–170. https://doi.org/10.1016/j.agee.2004.10.005

Cochetel, N., Escudié, F., Cookson, S.J., Dai, Z., Vivin, P., Bert, P., Muñoz, M.S., Delrot, S., Klopp, C., Ollat, N., Lauvergeat, V., 2017. Root transcriptomic responses of grafted grapevines to heterogeneous nitrogen availability depend on rootstock genotype 68, 4339–4355. https://doi.org/10.1093/jxb/erx224

Corso, M., Vannozzi, A., Maza, E., Vitulo, N., Meggio, F., Pitacco, A., Telatin, A., D'Angelo, M., Feltrin, E., Negri, A.S., Prinsi, B., Valle, G., Ramina, A., Bouzayen, M., Bonghi, C., Lucchin, M., 2015. Comprehensive transcript profiling of two grapevine rootstock genotypes contrasting in drought susceptibility links the phenylpropanoid pathway to enhanced tolerance. J. Exp. Bot. 66, 5739–5752. https://doi.org/10.1093/jxb/erv274

Dal Santo, S., Zenoni, S., Sandri, M., De Lorenzis, G., Magris, G., De Paoli, E., Di Gaspero, G., Del Fabbro, C., Morgante, M., Brancadoro, L., Grossi, D., Fasoli, M., Zuccolotto, P., Tornielli, G.B., Pezzotti, M., 2018. Grapevine field experiments reveal the contribution of genotype, the influence of environment and the effect of their interaction (G×E) on the berry transcriptome. Plant J. 93, 1143–1159. https://doi.org/10.1111/tpj.13834

De Lorenzis, G., Rustioni, L., Parisi, S.G., Zoli, F., Brancadoro, L., 2016. Anthocyanin biosynthesis during berry development in corvina grape. Sci. Hortic. (Amsterdam). 212, 74–80. https://doi.org/10.1016/j.scienta.2016.09.039

De Smet, I., Zhang, H., 2013. Abscisic acid in root growth and development., in: Eshel A. & Beeckman T. (Ed.), Plant Roots : The Hidden Half. CRC Press., Baton Rouge, FL, USA, pp. 16.1–16.13.

Delzon, S., 2015. New insight into leaf drought tolerance. Funct. Ecol. 29, 1247–1249. https://doi.org/10.1111/1365-2435.12500

Farquhar, G.D., Sharkey, T.D., 1982. Stomatal conductance and photosynthesis. Ann. Rev. Plant Biol. 61, 561–591.

Frioni, T., Biagioni, A., Squeri, C., Tombesi, S., Gatti, M., Poni, S., 2020. Grafting cv. grechetto gentile vines to new m4 rootstock improves leaf gas exchange and water status as compared to commercial 1103p rootstock. Agronomy 10, 1–14. https://doi.org/10.3390/agronomy10050708

Fujita, A., Soma, N., Goto-yamamoto, N., Mizuno, A., Kiso, K., Hashizume, K., 2007. Effect of Shading on Proanthocyanidin Biosynthesis in the Grape Berry 76, 112–119.

Galbignani, M., Merli, M., Magnanini, E., Bernizzoni, F., Talaverano, I., Gatti, M., Tombesi, S., Palliotti, A., Poni, S., 2016. Gas exchange and water - use efficiency of cv . Sangiovese grafted to rootstocks of varying water - deficit tolerance. Irrig. Sci. 34, 105–116. https://doi.org/10.1007/s00271-016-0490-z

Gale, G., 2002. Saving the vine from Phylloxera : a never-ending battle, in: Sandler and R. Pindler (Ed.), Wine: A Scientifc Exploration. Taylor and Francis, London, pp. 70–91. https://doi.org/10.1201/9780203361382.ch4

Gardner, C.M.K., Robinson, D., Blyth, K., Cooper, J.D., 2001. Soil Water Content, in: Smith, K.A., Mullins, C.E. (Eds.), Soil and Environmental Analysis: Physical Methods. Marcel Dekker Inc., New York, NY, pp. 13–20.

Giorgi, F., Lionello, P., 2008. Climate change projections for the Mediterranean region. Glob. Planet. Change 63, 90–104.

Haider, M.S., Zhang, Cheng, Kurjogi, M.M., Pervaiz, T., Zheng, T., Zhang, Chaobo, Lide, C., Shangguan, L., Fang, J., 2017. Insights into grapevine defense response against drought as revealed by biochemical, physiological and RNA-Seq analysis. Sci. Rep. 7, 13134. https://doi.org/10.1038/s41598-017-13464-3

Hayes, M.A., Feechan, A., Dry, I.B., 2010. Involvement of Abscisic Acid in the Coordinated Regulation of a Stress-Inducible Hexose Transporter (VvHT5) and a Cell Wall Invertase in Grapevine in Response to Biotrophic Fungal Infection. Plant Physiol. 153, 211–221. https://doi.org/10.1104/pp.110.154765

Hochberg, U., Herrera, J.C., Cochard, H., Badel, E., 2016. Short-time xylem relaxation results in reliable quantification of embolism in grapevine petioles and sheds new light on their hydraulic strategy. Tree Physiol. 36, 748–755. https://doi.org/10.1093/treephys/tpv145

Ikegami, K., Okamoto, M., Seo, M., Koshiba, T., 2009. Activation of abscisic acid biosynthesis in the leaves of Arabidopsis thaliana in response to water deficit. J. Plant Res. 122, 235–243. https://doi.org/10.1007/s10265-008-0201-9

IPCC, 2018. Global warming of 1.5°C, in: Summary for Policymarkerskers. pp. 1–26.

IPCC, 2013. Climate Change 2013: The Physical Science Basis – Summary for Policymakers.

Iuchi, S., Kobayashi, M., Taji, T., Naramoto, M., Seki, M., Kato, T., 2001. Regulation of drought tolerance by gene manipulation of 9- cis - epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in Arabidopsis 27, 325–333.

Jackson, D.I., Lombard, P.B., 1993. Environmental and Management Practices Affecting Grape Composition and Wine Quality - A Review. Am. J. Enol. Vitic. 44, 409–430.

Jones, H., Sutherland, R., 1991. Stomatal control of xylem embolism. Plant, Cell Environ. 14, 607–612.

Keller, M., 2015. The Science of Grapevines. Anatomy and Physiology. Elsevier. https://doi.org/10.1016/C2013-0-06797-7

Lazar, T., 2003. Taiz, L. and Zeiger, E. Plant physiology. 3rd edn. Ann. Bot. 91, 750–751. https://doi.org/10.1093/aob/mcg079

Livak, K.J., Schmittgen, T.D., 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method 25, 402–408. https://doi.org/10.1006/meth.2001.1262

Lovisolo, C., Lavoie-lamoureux, A., Tramontini, S., Ferrandino, A., 2016. Grapevine adaptations to water stress : new perspectives about soil/plant interactions. Theor. Exp. Plant Physiol. 28, 53–66. https://doi.org/10.1007/s40626-016-0057-7

Lovisolo, C., Schubert, A., Leonardo, V., Grugliasco, I.-, Arboree, C., Miglioramento, C., 2018. Effects of water stress on vessel size and xylem hydraulic conductivity in Vitis vinifera L . 49, 693–700.

Lund, S.T., Peng, F.Y., Nayar, T., Reid, K.E., Schlosser, J., 2008. Gene expression analyses in individual grape (Vitis vinifera L.) berries during ripening initiation reveal that pigmentation intensity is a valid indicator of developmental staging within the cluster. Plant Mol. Biol. 68, 301–315. https://doi.org/10.1007/s11103-008-9371-z

Manzi, M., Lado, J., Rodrigo, M.J., Arbona, V., Gómez-Cadenas, A., 2016. ABA accumulation in water-stressed Citrus roots does not rely on carotenoid content in this organ. Plant Sci. 252, 151–161. https://doi.org/10.1016/j.plantsci.2016.07.017

Manzi, M., Lado, J., Rodrigo, M.J., Zacariás, L., Arbona, V., Gómez-Cadenas, A., 2015. Root ABA Accumulation in Long-Term Water-Stressed Plants is Sustained by Hormone Transport from Aerial Organs. Plant Cell Physiol. 56, 2457–2466. https://doi.org/10.1093/pcp/pcv161

McAdam, S.A.M., Brodribb, T.J., Ross, J.J., 2016. Shoot-derived abscisic acid promotes root growth. Plant Cell Environ. 39, 652–659. https://doi.org/10.1111/pce.12669

Medrano, H., Tomás, M., Martorell, S., Flexas, J., Hernández, E., Rosselló, J., Pou, A., Escalona, J.M., Bota, J., 2015. From leaf to whole-plant water use efficiency (WUE) in complex canopies: Limitations of leaf WUE as a selection target. Crop J. 3, 220–228. https://doi.org/10.1016/j.cj.2015.04.002

Meggio, F., Prinsi, B., Negri, A.S., Simone Di Lorenzo, G., Lucchini, G., Pitacco, A., Failla, O., Scienza, A., Cocucci, M., Espen, L., 2014. Biochemical and physiological responses of two grapevine rootstock genotypes to drought and salt treatments. Aust. J. Grape Wine Res. 20, 310–323. https://doi.org/10.1111/ajgw.12071

Migliaro, D., De Lorenzis, G., Di Lorenzo, G.S., Nardi, B. De, Gardiman, M., Failla, O., Brancadoro, L., Crespan, M., 2019. Grapevine non-vinifera genetic diversity assessed by simple sequence repeat markers as a starting point for new rootstock breeding programs. Am. J. Enol. Vitic. 70, 390–397. https://doi.org/10.5344/ajev.2019.18054

Ollat, N., Cookson, S.J., Destrac-Irvine, A., Lauvergeat, V., Ouaked-Lecourieux, F., Marguerit, E., Barrieu, F., Dai, Z., Duchêne, E., Gambetta, G.A., Gomès, E., Lecourieux, D., Van Leeuwen, C., Simonneau, T., Torregrosa, L., Vivin, P., Delrot, S., 2018. Grapevine adaptation to abiotic stress: An overview. Acta Hortic. 1248, 497–512. https://doi.org/10.17660/ActaHortic.2019.1248.68

Ollat, N., Peccoux, A., Papura, D., Esmenjaud, D., Marguerit, E., Tandonnet, J.P., Bordenave, L., Cookson, S.J., Barrieu, F., Rossdeutsch, L., Lecourt, J., Lauvergeat, V., Vivin, P., Bert, P.F., Delrot, S., 2016. Rootstocks as a component of adaptation to environment, Grapevine in a Changing Environment: A Molecular and Ecophysiological Perspective. https://doi.org/10.1002/9781118735985.ch4

Padgett-Johnson, M., Williams, L.E., Walker, M.A., 2003. Vine water relations, gas exchange, and vegetative growth of seventeen Vitis species grown under irrigated and nonirrigated conditions in California. J. Am. Soc. Hortic. Sci. 128, 269–276.

https://doi.org/10.21273/jashs.128.2.0269

Pellegrino, A., Lebon, E., Simonneau, T., Wery, J., 2005. Towards a simple indicator of water stress in grapevine (Vitis vinifera L .) based on the differential sensitivities 306–315.

Pinto, D.L.P., Brancadoro, L., Dal Santo, S., De Lorenzis, G., Pezzotti, M., Meyers, B.C., Pè, M.E., Mica, E., 2016. The influence of genotype and environment on small RNA profiles in grapevine berry. Front. Plant Sci. 7. https://doi.org/10.3389/fpls.2016.01459

Porro, D., Pedò, S., Bertoldi, D., Bortolotti, L., Failla, O., Zamboni, M., 2013. Evaluation of new rootstocks for grapevine: Nutritional aspects, Acta Horticulturae.

Qin, X., Zeevaart, J.A., 1999. The 9-cis-epoxycarotenoid cleavage reaction is the key regulatory step of abscisic acid biosynthesis in water-stressed bean. Proc. Natl. Acad. Sci. U. S. A. 96, 15354–15361. https://doi.org/10.1073/pnas.96.26.15354

Raghavendra, A.S., Gonugunta, V.K., Christmann, A., Grill, E., 2010. ABA perception and signalling. Trends Plant Sci. 15, 395–401. https://doi.org/10.1016/j.tplants.2010.04.006

Rattanakon, S., Ghan, R., Gambetta, G.A., Deluc, L.G., Schlauch, K.A., Cramer, G.R., 2016. Abscisic acid transcriptomic signaling varies with grapevine organ. BMC Plant Biol. 16, 1–16. https://doi.org/10.1186/s12870-016-0763-y

Reid, K.E., Olsson, N., Schlosser, J., Peng, F., Lund, S.T., 2006. An optimized grapevine RNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development. BMC Plant Biol. 6, 27. https://doi.org/10.1186/1471-2229-6-27

Ren, H., Gao, Z., Chen, L., Wei, K., Liu, J., Fan, Y., Davies, W.J., Jia, W., Zhang, J., 2007. Dynamic analysis of ABA accumulation in relation to the rate of ABA catabolism in maize tissues under water deficit. J. Exp. Bot. 58, 211–219. https://doi.org/10.1093/jxb/erl117

Rock, C.D., Heath, T.G., Gage, D.A., Zeevaart, J.A., 1991. Abscisic alcohol is an intermediate in abscisic Acid biosynthesis in a shunt pathway from abscisic aldehyde. Plant Physiol. 97, 670–676. https://doi.org/10.1104/pp.97.2.670

Rossdeutsch, L., Edwards, E., Cookson, S.J., Barrieu, F., Gambetta, G.A., Delrot, S., Ollat, N., 2016. ABA-mediated responses to water deficit separate grapevine genotypes by their genetic background. BMC Plant Biol. 16, 91. https://doi.org/10.1186/s12870-016-0778-4

Santiago, J., Rodrigues, A., Saez, A., Rubio, S., Antoni, R., Dupeux, F., Park, S., Marquez, J., Cutler, S., Rodriguez, P., 2009. Modulation of drought resistance by the abscisic acid receptor PYL5 through inhibition of clade A PP2Cs. Plant J. 60, 575–588. https://doi.org/10.1111/j.1365-313X.2009.03981.x.

Schachtman, D.P., Goodger, J.Q.D., 2008. Chemical root to shoot signaling under drought. Trends Plant Sci. 13, 281–287. https://doi.org/10.1016/j.tplants.2008.04.003

Scholander, P.F., Bradstreet, E.D., Hemmingsen, E.A., Hammel, H.T., 1965. Sap Pressure in Vascular Plants: Negative hydrostatic pressure can be measured in plants. Science (80-.). 148, 339–346. https://doi.org/10.1126/science.148.3668.339

Schubert, A., Restagno, M., Lovisolo, C., 1996. PHOTOSYNTHESIS OF GRAPEVINE LEAVES OF DIFFERENT AGE AT HIGH AND. https://doi.org/10.17660/ActaHortic.1996.427.21

Schultz, H.R., 2000. Climate change and viticulture: a European perspective on climatology, carbon dioxide and UV-B effects. Aust. J. Grape Wine Res. 6, 2–12.

Serra, I., Strever, A., Myburgh, P.A., Deloire, A., 2014. Review: The interaction between rootstocks and cultivars (Vitis vinifera L.) to enhance

drought tolerance in grapevine. Aust. J. Grape Wine Res. 20, 1–14. https://doi.org/10.1111/ajgw.12054

Shao, H.B., Chu, L.Y., Shao, M.A., Abdul Jaleel, C., Hong-Mei, M., 2008. Higher plant antioxidants and redox signaling under environmental stresses. Comp. Rend. Biol. 331, 433–441.

Soar, C.J., Speirs, J., Maffei, S.M., Penrose, A.B., Mc Carthy, M.G., 2006. Grape vine varieties Shiraz and Grenache differ in their stomatal response to VPD: apparent links with ABA physiology and gene expression in leaf tissue. Aust. J. Grape Wine Res. 12, 2–12.

Speirs, J., Binney, A., Collins, M., Edwards, E., Loveys, B., 2013. Expression of ABA synthesis and metabolism genes under different irrigation strategies and atmospheric VPDs is associated with stomatal conductance in grapevine (Vitis vinifera L. cv Cabernet Sauvignon). J. Exp. Bot. 64, 1907–1916. https://doi.org/10.1093/jxb/ert052

Steudle, E., 2000. Water uptake by roots: effects of water deficit. J. Exp. Bot. 51, 1531–1542. https://doi.org/10.1093/jexbot/51.350.1531

Tombesi, S., Nardini, A., Frioni, T., Soccolini, M., Zadra, C., Farinelli, D., Poni, S., Palliotti, A., 2015. Stomatal closure is induced by hydraulic signals and maintained by ABA in drought-stressed grapevine. Sci. Rep. 5, 1–12. https://doi.org/10.1038/srep12449

Tyree, M.T., Ewers, F.W., 1991. The hydraulic architecture of trees and other woody plants 345–360.

Vannozzi, A., Donnini, S., Vigani, G., Corso, M., Valle, G., Vitulo, N., Bonghi, C., Zocchi, G., Lucchin, M., 2017. Transcriptional Characterization of a Widely-Used Grapevine Rootstock Genotype under Different Iron-Limited Conditions. Front. Plant Sci. 7, 1–17. https://doi.org/10.3389/fpls.2016.01994

Vivier, M. a, Pretorius, I.S., 2002. Genetically tailored grapevines for the wine industry. Trends Biotechnol. 20, 472–8.

Yoshida, R., Hobo, T., Ichimura, K., Mizoguchi, T., Takahashi, F., Aronso, J., Ecker, J.R., Shinozaki, K., 2002. ABA-Activated SnRK2 Protein Kinase is Required for Dehydration Stress Signaling in Arabidopsis. Plant Cell Physiol. 43, 1473–1483. https://doi.org/10.1093/pcp/pcf188

THE NEW GRAPEVINE ROOTSTOCK M4 IMPROVES WATER USE EFFICIENCY OF PINOT BLANC UNDER SEVERE WATER STRESS

Abstract

Rootstocks were introduced in viticulture to obtain resistance to a specific biotic stress, but nowadays they represent a promising adaptation strategy to face climate change. A new grapevine rootstock series was recently selected for tolerance to abiotic stresses, such as limestone, nutrient deficit, salt and drought. In particular, tolerance to water deficit was shown by rootstock M4 in both grafted and un-grafted conditions. The aim of this study was to investigate the response of Pinot Blanc grafted onto M4 under progressive water stress, in comparison to the traditional tolerant rootstock 1103P. Water status of the two grafting combination was assessed as predawn water potential, and gas exchange were monitored at midday under the different water stress levels. The two rootstocks showed a similar behavior at mild to moderate water stress. Under sever water stress, M4 maintained higher water potential, transpiration, photosynthetic rate and water use efficiency than 1103P. Chlorophyll fluorescence confirmed that M4 was able to preserve the activity of the photosynthetic apparatus under sever water deficit. In line with previous studies, these results suggested that rootstock M4 can be use with different grafting combinations under arid and semi-arid conditions to face drought.

1. Introduction

In the next years, many areas under vines across the world are expected to experience drought events due to climate change. Innovation in viticulture is required to face drought, in terms of new agricultural techniques and new genetic material (Van Leeuwen et al., 2019). Genetic improvement of scion varieties is considered a long-term adaptation strategy, because it inevitably changes the characteristics of the final product, whereas genetic improvement of rootstocks allows medium-term adaptation to drought (Quénol et al., 2014). Thus, current challenges of viticulture renewed the interest in rootstock selection and some

new genotypes has been recently released. In a recent breeding program where *Vitis Berlandieri* was used as recurrent genotype, four rootstocks (M1, M2, M3, M4) were selected for their tolerance to abiotic stresses. The tolerance to water deficit of rootstocks M1, M3 and M4 was tested under controlled condition, in comparison with 9 commercial rootstocks with various genetic background: the hybrids *Vitis riparia x Vitis Berlandieri* (K5BB, SO4, 420A, 161-49C); *Vitis riparia x Vitis rupestris* (Schwarzmann); *Vitis Berlandieri x Vitis rupestris* (1103P, 110R, 140Ru) and *Vitis Berlandieri x Vitis vinifera* (41B). Under limiting water deficit, the three M-rootstocks showed similar water use efficiency (WUE) to rootstocks 1103P and 110R, considered tolerant to drought (Bianchi et al., 2020). In further studies under controlled conditions, rootstock M4 reported higher tolerance to drought than rootstock 101.14, maintaining the photosynthetic activity under sever water deficit (Corso et al., 2015; Meggio et al., 2014). Both transcriptomic and metabolomic approaches suggested that drought tolerance of M4 could be related to efficient detoxification of ROS (Corso et al., 2015; Lucini et al., 2020). The performance of M4 under water deficit was also tested in grafting combination with Sangiovese, in comparison to rootstock SO4. Results showed that M4 retained higher transpiration and photosynthetic rates under moderate water deficit, and higher WUE than SO4 under sever water stress (Galbignani et al., 2016). In a recent study, M4 was also compared to the tolerant rootstock 1103P, in grafting combination with Grechetto Gentile. In the study, the two rootstocks reported similar leaf water status, but M4 showed a lower reduction of photosyntesis under water deficit and higher WUE than 1103P (Frioni et al., 2020). The aim of this study was to confirm the drought tolerance of rootstock M4 in grafting combination with Pinot Blanc, in comparison to the tolerant rootstock 1103P under long-term progressive water deficit.

2. Material and methods

2.1 Experimental design

The experiment was set up under semi-controlled conditions in 2020, from May (DOY 153) to July (DOY 227). A total of 22 five years old plants of *Vitis vinifera* cv Pinot Blanc were tested in two different grafting combinations, with rootstocks 1103P and M4 (11 biological repetitions per rootstock). Vines were grown in 60 L plastic pots and the substrate was composed of 70% sand and 30% peat, supplemented with a layer of expanded clay aggregate on the bottom of the pot to reduce water flooding. The training system was Guyot. During the phenological phase of budding, vines were maintained in well-watered conditions in order to develop a well-expanded canopy. Soil was maintained at field capacity level till the beginning of the experiment, after that the irrigation was interrupted for the whole period to achieve a progressive water deficit. To avoid water infiltration by rainfall, the tested pots were covered using a plastic film during the whole experimental period. Physiological analysis of each vine was performed in 7 days across the experiment (DOY 153 – 174 – 181 – 190 – 202 – 213 – 227).

2.2. Physiological analysis

The analysis of water potential wwas performed in predawn (Ψ_{pd}), from 3 am to 5:30 am, using a Scholander pressure chamber (Scholander et al., 1965), produced by PMS Instrument Company, Corvallis, Oregon (USA). For the analysis of Ψ_{pd}, one median leaf per plant of about 30 days old was chosen from a primary shoot at each experimental day. Gas exchange was measured in one sunny leaf per each biological repetition using a leaf portable photosynthesis system (CIRAS-3, PP Systems, Amesbury, MA, USA) equipped with PLC6 (U) cuvette 18 mm circular (2.5 cm^2 head plate), under constant saturating PPFD of 1500 µmol photons m^{-2} s^{-1}, CO_2 concentration of 300 µmol mol^{-1}, block temperature of 25°C and relative humidity between 60% and 70% allowing ~1.5 kPa of VPD inside the leaf chamber. Intrinsic water use efficiency (iWUE) was determined as the ratio between carbon assimilation (A) and stomatal conductance (gs). In correspondence to gas exchange measurements on sunny leaves, chlorophyll fluorescence was measured using the CFM-3 Chlorophyll Fluorescence Module connected to CIRAS-3.

2.3. Data analysis

Data were analyzed using Microsoft Office Excel and SPSS statistical software (IBM SPSS Statistics 24). A univariate ANOVA model was performed on physiological parameters (Ψ_{pd}, A, gs, E, iWUE, ϕPSII, Fv'/Fm' and J) at $0.01 < p \leq 0.05$ (*), $0.001 < p \leq 0.01$ (**) and $p \leq 0.001$ (***), after checking for the assumption of normality and homogeneity of variance.

3. Results

The environmental conditions were relatively stable throughout the period when the experiment was conducted. From DOY 153 to DOY 181, photosynthetically active radiation (PAR) measured at midday, remained quite stable, largely above 1000 µmol m^{-2} s^{-1}. Thereafter, on DOY 227 PAR decreased to a minimum of about 700 µmol m^{-2} s^{-1} (Figure 1 a). Air temperature (T) ranged between 34°C and 37°C, except for DOY 213, when temperature increased significantly, recording a maximum of about 39°C (Figure 1 b).

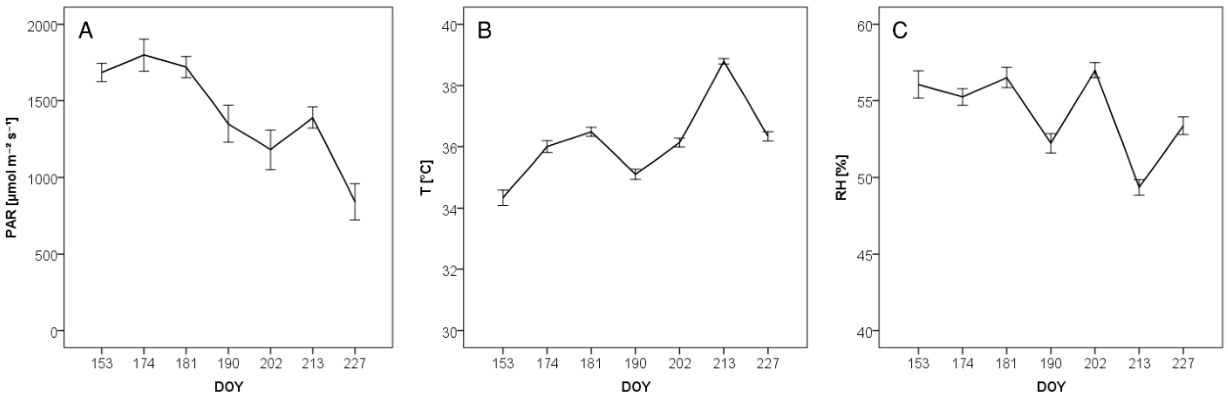


Figure 1. Trends of environmental conditions during the experimental period. A) Average photosynthetically active radiation (PAR). B) Average air temperature (T). C) Average relative humidity (RH). DOY = day of the year

Average daily relative humidity (RH) ranged between 50% and 60% for the whole experimental period, recording a minimum level of 49% on DOY 213. During the experiment, pre-dawn water potential (Ψ_{pd}) gradually decreased and a similar trend of was reported by the two grafting combinations (Figure 2). Significant differences between genotypes only occurred on DOY 227, resulting in a higher Ψ_{pd} for M4 (-0,5 MPa) in comparison to 1103P (-0,9 MPa).

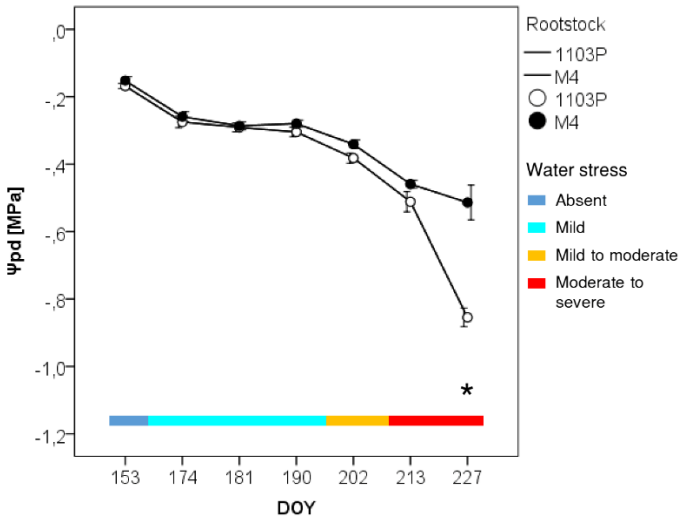


Figure 2. Trend of predawn water potential (Ψ_{pd}) during the experimental period. Significant differences between gentypes are considered for $0.01 < p \leq 0.05$ (*), $0.001 < p \leq 0.01$ (**) and $p \leq 0.001$ (***). DOY = day of the year

The two rootstocks also reported a similar trend in terms of gas exchange. In particular, they maintained a performant photosynthetic activity (about 15 µmol CO_2 m^{-2} s^{-1}), stomatal conductance (about 200 mmol H_2O m^{-2} s^{-1}) and transpiration (about 5 mmol H_2O m^{-2} s^{-1}) until DOY 202 and gas exchange rapidly dropped to minimum levels on DOY 213 and DOY 227 (Figure 3 a, b, c). Significant differences between rootstocks occurred at low level of gas exchange (DOY 213 and DOY 227), with higher A, gs and E for rootstock M4, whereas gas exchange of rootstock 1103P was close to zero (Figure 3 a, b, c). Under

progressive water deficit, 1103P maintained the intrinsic water use efficiency (iWUE) around 80 µmol CO_2 mol^{-1} H_2O until DOY 202, but it was considerably reduced to about 15 µmol CO_2 mol^{-1} H_2O in the last two days, following a similar trend to gas exchange. On the other hand, M4 was able to maintain stable iWUE for the whole experiment, with values around 80 µmol CO_2 mol^{-1} H_2O (Figure 3 d).

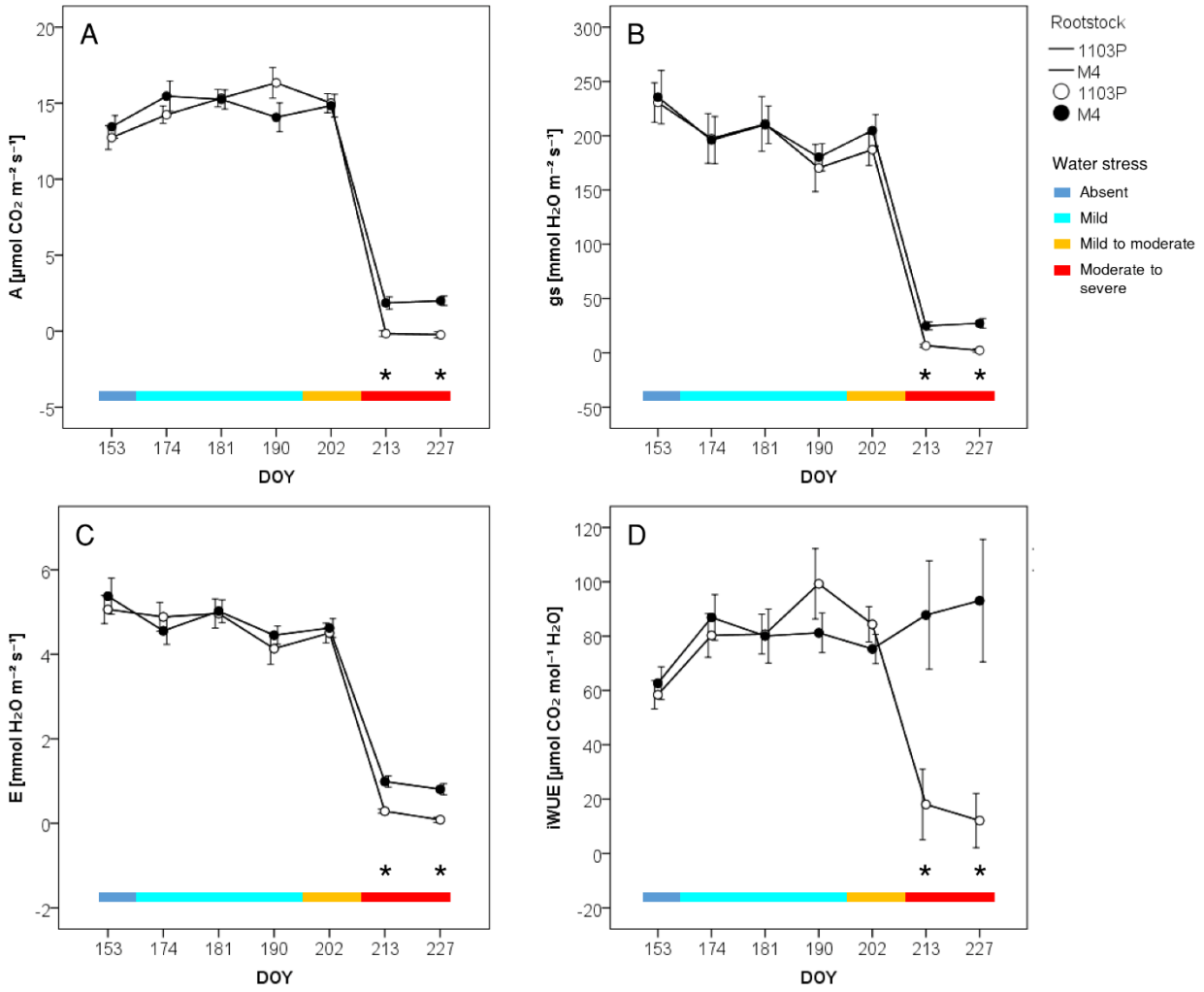


Figure 3. Trends of: A) photosynthetic rate (A); B) stomatal conductance (gs); C) transpiration rate (E); and intrinsic water use efficiency (iWUE) during the experimental period. Significant differences between genotypes are considered for $0.01 < p \leq 0.05$ (*), $0.001 < p \leq 0.01$ (**) and $p \leq 0.001$ (***). DOY = day of the year

Similarly, M4 performed stable operating efficiency of photosystem II during the experiment, with an average value of about 0.50 for ϕPSII (Figure 4 a) and about 0.65 for Fv'/Fm' (Figure 4 b). Thylakoid electron transport rate (J) was also stable at about 250 µmol e^- m^{-2} s^{-1} for M4 under progressive water deficit (Figure 4 c). As for iWUE, rootstock 1103P maintained similar ϕPSII, Fv'/Fm' and J to rootstock M4 until DOY 202, with slightly lower ϕPSII and J on DOY 174. In the last two dates, ϕPSII of 1103P dropped to 0.3, Fv'/Fm' decreased at 0.4 and J reduced to 150 µmol e^- m^{-2} s^{-1} (Figure 4 a, b, c).

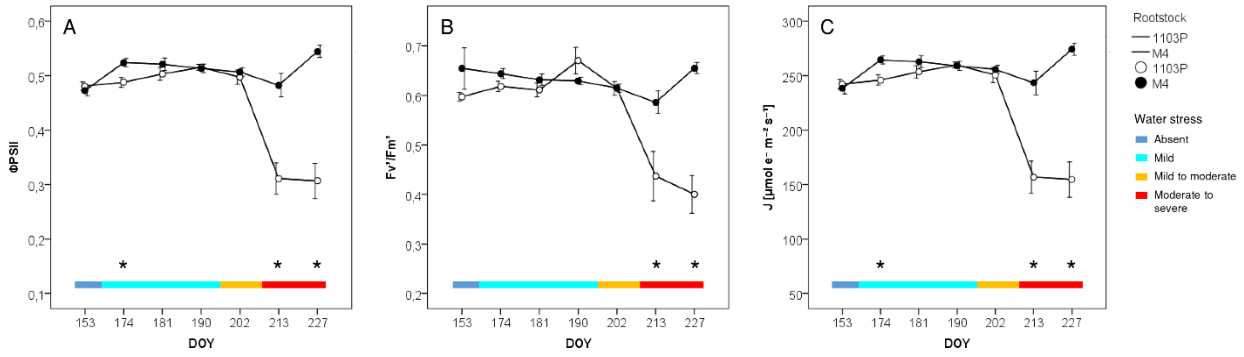


Figure 4. Trends of: A) operating efficiency of photosystem II (ϕPSII); B) operating efficiency of photosystem II, far-red (Fv'/Fm'); C) Thylakoid electron transport rate (J) during the experimental period. Significant differences between genotypes are considered for $0.01 < p \leq 0.05$ (*), $0.001 < p \leq 0.01$ (**) and $p \leq 0.001$ (***). DOY = day of the year

4. Discussion

According to the thresholds defined by Deloire et al. (2004), water stress was absent on DOY 153 ($\Psi_{pd} <$ -2MPa) and became mild from DOY 174 to DOY 190 (-4MPa < Ψ_{pd} < -2MPa). Mild to moderate water stress occurred on DOY 202 and moderate to severe on DOY 213 and DOY 227. Stomatal conductance below the threshold of 50 mmol H_2O m^{-2} s^{-1} confirmed the severe water stress in the last two experimental days (Cifre et al., 2005). The condition of severe water stress can be attributed to the low water availability but also to other limiting environmental conditions, such as the exceptional air temperature of 39°C on

DOY 213, matched by low RH, as well as non-saturating PAR on DOY 227. From mild to moderate water stress, physiological activity was stable for both grafting combinations, in terms of A, gs and E. At mild water stress, iWUE slightly increased from 60 to 80 µmol CO_2 mol^{-1} H_2O and it was maintained until moderate water deficit. Both rootstocks guaranteed high performance to the scion from mild to moderate water stress, whereas under severe conditions significant differences occurred in response to water deficit. Reduction of gas exchange was more evident for 1103P than M4, which showed higher levels of A, gs and E. Photosynthetic activity of M4 under severe stress level can be explained by the good performance of the photosynthetic apparatus, with stable efficiency of PSII and stable electron transport rate in thylakoids. These factors allowed M4 to maintain high iWUE for the whole experiment. These results confirmed the tolerance to water deficit of rootstock M4, already proven in previous studies under different grafting combinations, in comparison to both susceptible (Galbignani et al., 2016; Meggio et al., 2014) and tolerant rootstocks (Frioni et al., 2020). Thus, M4 seemed to be able to improve drought tolerance of different varieties, but the physiological mechanism of response was not fully described. Future studies could further investigate the physiological response to water deficit of rootstock M4 in different grafting combinations, also considering the effect on the production and quality of grape.

References

Bianchi, D., Caramanico, L., Grossi, D., Brancadoro, L., Lorenzis, G. De, 2020. How Do Novel M-Rootstock (Vitis Spp .) Genotypes Cope with Drought ? https://doi.org/10.3390/plants9101385

Cifre, J., Bota, J., Escalona, J.M., Medrano, H., Flexas, J., 2005. Physiological tools for irrigation scheduling in grapevine (Vitis vinifera L .) An open gate to improve water-use efficiency ? 106, 159–170. https://doi.org/10.1016/j.agee.2004.10.005

Corso, M., Vannozzi, A., Maza, E., Vitulo, N., Meggio, F., Pitacco, A., Telatin, A., D'Angelo, M., Feltrin, E., Negri, A.S., Prinsi, B., Valle, G., Ramina, A., Bouzayen, M., Bonghi, C., Lucchin, M., 2015. Comprehensive transcript profiling of two grapevine rootstock genotypes contrasting in drought susceptibility links the phenylpropanoid pathway to enhanced tolerance. J. Exp. Bot. 66, 5739–5752. https://doi.org/10.1093/jxb/erv274

Frioni, T., Biagioni, A., Squeri, C., Tombesi, S., Gatti, M., Poni, S., 2020. Grafting cv. grechetto gentile vines to new m4 rootstock improves leaf gas exchange and water status as compared to commercial 1103p rootstock. Agronomy 10, 1–14. https://doi.org/10.3390/agronomy10050708

Galbignani, M., Merli, M., Magnanini, E., Bernizzoni, F., Talaverano, I., Gatti, M., Tombesi, S., Palliotti, A., Poni, S., 2016. Gas exchange and water - use efficiency of cv . Sangiovese grafted to rootstocks of varying water - deficit tolerance. Irrig. Sci. 34, 105–116. https://doi.org/10.1007/s00271-016-0490-z

Lucini, L., Miras-moreno, B., Busconi, M., Marocco, A., Gatti, M., Poni, S., 2020. Plant Science Molecular basis of rootstock-related tolerance to water de fi cit in Vitis vinifera L . cv . Sangiovese : A physiological and metabolomic combined approach. Plant Sci. 299, 110600. https://doi.org/10.1016/j.plantsci.2020.110600

Meggio, F., Prinsi, B., Negri, A.S., Simone Di Lorenzo, G., Lucchini, G., Pitacco, A., Failla, O., Scienza, A., Cocucci, M., Espen, L., 2014. Biochemical and physiological responses of two grapevine rootstock genotypes to drought and salt treatments. Aust. J. Grape Wine Res. 20, 310–323. https://doi.org/10.1111/ajgw.12071

Quénol, H., Marie, G., Barbeau, G., Leeuwen, C. Van, Foss, C., Irimia, L., Rochard, J., Boulanger, J., Tissot, C., Quénol, H., Marie, G., Barbeau, G., Leeuwen, C. Van, Hofmann, M., 2014. Adaptation of viticulture to climate change : high resolution observations of adaptation scenario for viticulture : the adviclim european project To cite this version : HAL Id : hal-01150259 Adapatation of vIticulture to climate change : high resolution o.

Scholander, P.F., Bradstreet, E.D., Hemmingsen, E.A., Hammel, H.T., 1965. Sap Pressure in Vascular Plants: Negative hydrostatic pressure can be measured in plants. Science (80-.). 148, 339–346. https://doi.org/10.1126/science.148.3668.339

Van Leeuwen, C., Agro, B.S., Irvine, A.D., 2019. An Update on the Impact of Climate Change in Viticulture and Potential Adaptations. https://doi.org/10.3390/agronomy9090514

Vigne, L.A., Eau, E.T.L., Deloire, A., Carbonneau, A., Wang, Z., Ojeda, H., 2004. VINE AND WATER A SHORT REVIEW 1–13.

PART II

PHENOTYPING OF THE "G SERIES" *Vitis* HYBRIDS: FIRST SCREENING OF THE MINERAL COMPOSITION

Abstract

Grapevine rootstocks affect the nutritional status of plants and thus the production and the quality of grape. In this study, a screening of mineral level in vine leaves is performed to a series of 35 *Vitis* hybrids for rootstock selection, in two different growing conditions at two sampling times. Mineral levels were determined by elemental analyzer (N) and ICP-MS (P; K; Mg; Ca; Na; Fe; Cu; Fe) in leave samples. Generally, the effect of growing conditions was predominant, whereas genotype effect and their interaction were significant for N, K, Ca, Mg, Na, Mn and Cu. A cluster analysis was used to identify the affinity of each genotype to K, Mg and Ca. Furthermore, response of genotypes to the different environments was assessed by a plasticity index. An elastic behavior was shown by 14 genotypes. Within this group, genotypes G.05, G.21, G.71, G.76 and G.77 reported high potassium level, beside the already demonstrated tolerance to water stress.

1. Introduction

Mineral deficiencies result in physiological dysfunctions characterized by specific symptoms (Caramanico et al., 2017; Rustioni et al., 2018). Besides the vegetative growth, nutritional disequilibrium affects the production and the quality of grape. For example, nitrogen may affect the Yeast Assimilable Nitrogen (YAN) in the must, involving in microbial instability during fermentation (Bell and Henschke, 2005) and potassium excess may increase the must pH, due to the salification of tartaric acid (Brancadoro et al., 1994; Kodur, 2011). Nutrients are mainly uptaken by the soil, translocated through different plant tissues, and stocked in the wood or moved to leaves and berries. Thus, besides the environmental conditions (including mineral content in the soil, water availability, and, in general, climatic and edaphic conditions), also agronomical practices, affecting plant physiology, influence the nutritional status of vines. In this framework, one of the major effect could be ascribed to the cultivated genotypes of varieties and

rootstocks (Fisarakis et al., 2004; Ibacache and Sierra, 2009; Tomasi et al., 2015). The effect of rootstocks on the nutritional status can be explained by different attitude of each genotype in terms of nutrient uptake, translocation in the shoot and assimilation (Ozdemir et al., 2011). Both root density and distribution are affected by rootstocks (Swanepoel and Southey, 1989) and the differences in the root morphology between genotypes may produce differential nutrient uptakes (Williams and Smith, 1991). Mineral translocation can also be affected by rootstock genotype, for example, by the synthesis of different levels of cytokinins (Skene and Antcliff, 1972).

Nutritional status of genotypes can be largely affected by the environmental conditions, and, thus, the stability of the performances of a rootstock acquire a major importance. Genotypes called 'plastic', respond to each environment strongly modifying their phenotype, while genotypes called 'elastic' maintain similar characteristics in different environments. Recently, different studies were focused on this property (Bianchi et al., 2018; Dal Santo et al., 2018; Pinto et al., 2016; Rustioni et al., n.d.). Rootstock selection should consider the plasticity of genotypes besides their average performance, in order to find elastic genotypes to ensure a good nutritional status under a wide range of environments and plastic genotypes able to adapt to critical conditions. Among commercial rootstocks, SO4 (*V. Berlandieri* × *V. riparia*) and 44-53 M (*V. riparia* × *V. cordifolia* × *V. rupestris*) show high levels of potassium (Brancadoro et al., 1994; Wooldridge et al., 2010). During last decades, a new set of rootstock genotypes was obtained by Università degli Studi di Milano, through a breeding program. Four of them have been recently registered and commercialized with the name of "M" series. Rootstocks M1 and M2 preforms high efficiency in K uptake (Brancadoro et al., 2014; Carnevali et al., 2014). Other genotypes of the collection ("G" series) have been genetically characterized (Migliaro et al., 2019) and they have been tested by Bianchi *et al* (2018) in terms of water stress tolerance, nevertheless their efficiency in nutrient uptakes is still unknown. The aims of the present study are to: i) identify the main synergies and antagonisms in nutrient uptakes among several *Vitis* spp hybrids; ii) evaluate the relative effect of sampling time, growing

condition, genotypes and their interactions on the total variability of nutrient levels in the leaves; iii) provide a first screening of the nutritional status of the 35 different genotypes, including their plasticity.

2. Material and methods

2.1. Experimental plan

The study was conducted in 2017. Nutritional status was assessed as the leaf concentration of the macronutrients N, P, K, Ca, Mg and the micronutrients Na, Mn, Fe, Cu. Experimental sites, sampling times and plant genotypes were considered as variability factors. The experimental sites are Arcagna and Riccagioia, characterized by different environmental conditions. Samplings were carried out on July 14th (DOY 195) and August 8th (DOY 220). The 35 genotypes belong to the collection of Dipartimento di Scienze Agrarie e Ambientali of Università degli Studi di Milano. The breeding material composing the analyzed genotypes includes several interspecific hybrids of *Vitis* species. The analyzed genotypes are reported in Table 1 with indicated the genetic characterization, studied by Migliaro *et al* (2019), and the relative sex of flowers. Genotype M2, belonging to the M series, was used as reference.

2.2. Characterization of growing conditions

The two experimental vineyards are located in Lombardy (Italy): Arcagna (45.340276N, 9.449786E, 83m a.s.l.) and Riccagioia (44.984783N, 9.089038E, 133m a.s.l.). The distance between the two experimental sites is 46km. Six un-grafted vines per genotype were planted in 2014 and 2015 in Riccagioia and Arcagna, respectively. In Riccagioia, plants were spaced 2.40m inter-row and 1.10m intra-row, whereas in Arcagna the layout was 3.10m inter-row and 2.00m intra-row. In both sites, plants were trained in creeping system and spur pruned during winter. Soil characteristics of the two sites are shown in Table 2. According to USDA (United States Department of Agriculture), the soil in Riccagioia is classified as sandy clay loam, whereas the soil in Arcagna is sandy loam. The organic matter (OM) content is higher in Arcagna, but the total cation exchangeable capacity (CEC) is major in Riccagioia. However, due to the different amount of

precipitations during the vegetative period and the different vineyard management (e.g. plant density), plants grown in Arcagna had a sufficient water availability, whereas in Riccagioia vines underwent drought stress, as discussed by Bianchi *et al* (2018) and reported in Table 2.

2.3. Mineral quantification

For each genotype, in each growing condition and in both the considered dates, 3 leaves were collected in the middle of the shoots, then they were dried and grinded. The amount of 0.3g of each sample was mineralized in 10mL of HNO_3 (>60%) at 210°C for 20 mins + 20 mins of cooling and filtered on a 0.45 µm nylon membrane. Concentration of P, K, Ca, Mg, Na, Mn, Fe, Cu were determined in the leaf samples by Inductively Coupled Plasma Mass spectrometry (ICP-MS) using a quadrupole spectrometer (Aurora M90, Bruker). Leaf N concentrations were estimated with an elemental analyzer (NA 1500 series 2 NC, Carlo Erba, Italy), starting by 7mg of each grinded sample.

2.4. Data elaboration

Data were processed using Microsoft Office Excel Professional Plus 2016 and SPSS statistical software (IBM SPSS Statistics 24). Correlations among nutrients were determined by Pearson index and they were significant at the 0.05 (*) and 0.01 (**) levels (2-tailed). Single effects of factors and their interactions were assessed by analysis of variance (ANOVA), performed at the 0.05 (*) and 0.01 (**) levels (2-tailed). To classify the rootstock behaviors based on the absorption of K, Mg and Ca, residues from the averages were normalized at the maximum. Normalized residues were submitted to hierarchical cluster analysis, using the UPGMA method (Unweighted Pair Group Method with Arithmetic Mean). The Plasticity Index (PI) was calculated by the following steps: 1) differences between Riccagioia and Arcagna levels of each nutrient (Δ); 2) residues from the averages of Δ; 3) normalization of the absolute value of residues (PI_X); 4) sum of the PI_N of each nutrient ($PI = PI_P + PI_K + PI_{Mg} + PI_{Ca} + PI_{Mn} + PI_{Na} + PI_{Cu}$).

Table 1. Genetic characterization of the hybrids collection (Migliaro *et al*, 2019) and flower sex

Genotype	Involved species	Breeding material	Flowers*
G.03	*V. Berlandieri - V. riparia*	5 A Gosek x Teleki 5 C	4
G.05	*V. Berlandieri - V. riparia*	K 5 BB x Teleki Stieler	1
G.07	*V. Berlandieri - V. riparia*	161-49 x Resseguierr n. 1	1
G.09	*V. Berlandieri - V. riparia*	K 5 BB x SO 4	1
G.12	*V. Berlandieri - V. riparia - V. rupestris*	Teleki 8 B x 1103 P	1
G.13	*V. Berlandieri - V. riparia - V. rupestris*	Teleki 8 B x 110 R	4
G.16	*V. Berlandieri - V. riparia*	K 5 BB x SO 4	1
G.17	*V. Berlandieri - V. riparia*	225 Ru x 420 A	1
G.19	*V. Berlandieri - V. riparia*	K 5 BB x SO 4	N
G.21	*V. Berlandieri - V. riparia - V. rupestris*	K 5 BB x 110 R	1
G.23	*V. Berlandieri - V. riparia*	K 5 BB x Teleki Stieler	1
G.24	*V. Berlandieri - V. rupestris*	1447P x 110R	1
G.25	*V. Berlandieri - V. riparia*	(Borrisquou x Rupestris Scheele) x 779 P	1
G.26	*V. Berlandieri - V. rupestris - V. vinifera*	(Borrisquou x Rupestris Scheele) x 779 P	3
G.27	*V. Berlandieri - V. rupestris - V. vinifera*	5 A Gosek x SO 4	3
G.28	*V. Berlandieri - V. riparia - V. rupestris*	K 5 BB x 140 Ru	3
G.29	*V. Berlandieri - V. riparia*	225 Ru x 420 A	4
G.30	*V. Berlandieri - V. riparia*	K 5 BB x 420 A	4
G.30 B	*V. Berlandieri - V. riparia - V. rupestris*	K 5 BB x 1103 P	1
G.34	*V. Berlandieri - V. riparia*	K 5 BB x Resseguierr n. 1	4
G.37	*V. Berlandieri - V. riparia*	141-69 x SO 8	1
G.69	*V. Berlandieri - V. riparia - V. rupestris - V. vinifera*	Teleki 8 B x 1045 P	4
G.70	*V. Berlandieri - V. rupestris - V. vinifera*	41 B x Resseguier n. 1	1
G.71	*V. Berlandieri - V. riparia*	Reckendorferr 27 x Teleki 5 C	4
G.72	*V. Berlandieri - V. riparia - V. rupestris*	K 5 BB x 140 Ru	N
G.73	*V. Berlandieri - V. vinifera*	41 B x Resseguier n. 1	1
G.74	*V. Berlandieri - V. cordifolia - V. riparia - V. rupestris*	106-8 x Resseguier n. 1	N
G.74 B	*V. Berlandieri - V. cordifolia - V. riparia - V. rupestris*	106-8 x Resseguier n. 1	N
G.75	*V. Berlandieri - V. vinifera*	41 B x Resseguier n. 1	1
G.76	/	/	N
G.77	*V. Berlandieri - V. riparia*	K 5 BB x 420 A	4
G.81	/	/	1
G.82	*V. Berlandieri - V. rupestris - V. vinifera*	(Borrisquou x Rupestris Scheele) x Resseguierr n. 1	4
G.83	*V. Berlandieri - V. riparia*	Reckendorferr 27 x Teleki 5 C	4
M2	*V. Berlandieri - V. riparia - V. vinifera*	Teleki 8 B x 333 EM	4

* OIV character (151): 1= fully developed stamens and no gynoecium; 2= fully developed stamens and reduced gynoecium; 3= fully developed stamens and fully developed gynoecium; 4= reflexed stamens and fully developed gynoecium. N= no flowers

Table 2. Characterization of the two experimental sites (Riccagioia and Arcagna) in terms of soil proprieties, soil nutrient contents, soil water availability and water stress of plants

	Parameter	U.M.	Riccagioia	Arcagna
Soil proprieties	Sand	*%*	58	75
	Silt	*%*	19	14
	Clay	*%*	23	11
	OM	*g/kg*	8.95	11.3
	CEC	*me 100g^{-1}*	14.5	8
	pH H_2O	*pH*	7.3	6.2
	pH KCl	*pH*	6.9	5.9
Soil nutrient contents	N	*ppm*	5500	4000
	P_2O_5	*ppm*	32.0	74.5
	K	*ppm*	114.0	177.5
	Ca	*ppm*	2770.0	1010.5
	Mg	*ppm*	389.0	170.5
	Fe	*ppm*	41.7	135.2
	Mn	*ppm*	17.2	14.9
	Cu	*ppm*	4.5	11.2
	Na	*ppm*	17.0	8.5
Water characterization	Rainfalls[1]	*mm*	169.4	246.6
	Ψ_{SOIL} [2]	*MPa*	-0.19	-0.13
	Ψ_{STEM} [3]	*MPa*	-1.16	-0.68
	CWSI [4]	-	0.82	0.52

[1] Total precipitation during the vegetative period, ranging from DOY 91 to DOY 243, 2017
[2] Soil water potential, analyzed on DOY 195 and DOY 220 (Bianchi *et al*, 2018)
[3] Stem water potential at midday, analyzed on DOY 195 and DOY 220 (Bianchi *et al*, 2018)

3. Results and discussion

3.1. Mineral compounds in Vitis spp. – synergies and antagonisms

Correlation between nutrient concentration in the leaves is reported in Table 3. The main correlations involve alkali metals; alkaline-earth metals and Mn among transition elements. Non-metals (especially N) were less correlated with other minerals, as well as Cu and Fe among transition elements. High positive

correlations are K–Mn (0.631), Mg–Na (0.623) and Mg–Ca (0.582), whereas main negative correlations are noticed for K–Mg (-0.748), K–Ca (-0.566), Mg–Mn (-0.582) and K–Na (-0.502). Other relevant correlations are shown by P with both K (0.426) and Mn (0.453). Low correlations are found between N and other nutrients, although some of them are significant (P, Mg, Fe, Cu). Finally, positive correlation is shown by Fe and Ca.

Table 3. Pearson correlation between nutrients in leaves. Significant correlations are reported for 0.01<p<0.05 (*) and p<0.01 (**). Synergies between nutrients are shown in green, whereas antagonisms in red. The color intensity is proportioned to the Pearson Index

	N	P	K	Mg	Ca	Mn	Na	Fe	Cu
N	1	-0.241**	0.100	-0.179*	-0.132	-0.050	-0.144	0.201*	0.302**
P	-0.241**	1	0.426**	-0.136	-0.174*	0.453**	-0.141	-0.043	-0.058
K	0.100	0.426**	1	-0.748**	-0.566**	0.631**	-0.502**	0.087	-0.296**
Mg	-0.179*	-0.136	-0.748**	1	0.582**	-0.582**	0.623**	-0.146	0.253**
Ca	-0.132	-0.174*	-0.566**	0.582**	1	-0.349**	0.334**	0.291**	0.265**
Mn	-0.050	0.453**	0.631**	-0.582**	-0.349**	1	-0.586**	0.229**	-0.390**
Na	-0.144	-0.141	-0.502**	0.623**	0.334**	-0.586**	1	-0.193*	0.264**
Fe	0.201*	-0.043	0.087	-0.146	0.291**	0.229**	-0.193*	1	-0.031
Cu	0.302**	-0.058	-0.296**	0.253**	0.265**	-0.390**	0.264**	-0.031	1

Correlation analysis highlights synergies and antagonisms among nutrients (Table 3). Relation between K, Mg and Ca in plants is reported in several studies (Casanova-Gascón et al., 2018; Fisarakis et al., 2004; Garcia et al., 1999; Jakobsen, 1993; Toumi et al., 2016). In particular, K competes for the root uptake against Ca an Mg (Brataševec et al., 2013; Jakobsen, 1993). Antagonistic effect between Na–K and synergies between Na–Ca–Mg highlighted by our results are well known as well (Downton, 1985; Fisarakis

et al., 2004), representing important factors to consider in salinity conditions. Results from this study also highlight a strong relation between Mn and all the other minerals considered, except for N. Significant synergy between Mn and Fe in *Vitis* leaves is also reported by Alagić *et al* (2018). Synergy between N and Cu is also reported by Yruela (2018), whereas the synergy between Fe and Ca is confirmed by Amorós *et al* (2018). Cu and Fe appeared not correlated in our results, and interactions among these two elements are still not clearly defined neither in literature. In fact, copper accumulation in soil may diminish iron uptake (Keller, 2015), however Fe–Cu antagonism is not confirmed in leaves, showing positive correlation (Alagić et al., 2018).

3.2. Mineral content variability among samples

Table 4 shows the percentage of variability explained by factors and their interactions for each nutrient. The main source of variability is the growing condition, except for N and Fe. Nitrogen variance mainly depends on DOY (85% of total variability), whereas the Fe variance is mainly affected by the interaction growing condition × DOY (53%). The influence of genotype factor on the total variability and its interaction with growing condition and DOY is generally lower than other factors, but always higher than the error variability. Significant differences are reported in Table 4: DOY differenced for N, P, Ca, Mg and Cu; the growing condition differenced for P, K, Ca, Mg, Na, Mn and Cu; their interaction (growing condition × DOY) differenced for N, P, K, Ca, Fe and Cu; genotype and its interaction with growing condition differenced for N, K, Mg, Ca, Na, Mn and Cu; significant differenced were not found in DOY × genotype interaction.

Table 4. Variance percentage of nutrient leaf levels explained by each factor and their interactions. Differences are significant for 0.01<p<0.05 (*) and p<0.01 (**). G.C.=growing conditions

NUTRIENT	**G.C.** [%]	**DOY** [%]	**GENOTYPE** [%]	**GENOTYPE×DOY** [%]	**G.C.×DOY** [%]	**GENOTYPE×G.C.** [%]	**ERROR** [%]
N	0.80	85.41**	3.48**	0.69	5.87**	3.08**	0.68
P	51.17**	10.83**	1.84	1.00	32.7**	1.22	1.25
K	95.11**	0.11	0.84**	0.09	2.91**	0.77**	0.17
Mg	91.45**	5.49**	0.96**	0.25	0.82	0.77**	0.26
Ca	67.93**	12.58**	1.81**	0.66	14.88**	1.55**	0.59
Na	98.78**	0.20	0.42*	0.08	0.01	0.43*	0.08
Mn	94.43**	0.70	1.26**	0.56	1.17	1.28**	0.6
Fe	15.76	1.58	7.93	4.67	52.77*	10.25	7.03
Cu	60.51**	21.23**	2.54**	0.58	12.76**	1.77**	0.61

3.3. The role of the growing condition

Nutrient level in leaves is affected by the different growing conditions, except for N and Fe, which did not report significant differences between Arcagna and Riccagioia (Figure 1). Phosphorus level was higher in Arcagna (0.23±0.05%) than in Riccagioia (0.19±0.04%). Potassium had the same trend with larger differences, amounting to 0.92±0.26% in Arcagna and 0.44±0.09% in Riccagioia. Contrarily, calcium and magnesium levels were higher in Riccagioia than Arcagna, amounting respectively to 2.01±0.42% and 1.58±0.28% for Ca, 0.51±0.12% and 0.29±0.09% for Mg. About micronutrients, Mn level was largely higher in Arcagna, whereas Cu and Na concentrations were higher in Riccagioia (Figure 1).

The effect of the environment on the mineral status of grapevines is reported in several studies. Soil characteristics affect the availability of minerals, the presence of calcium carbonate induces Fe deficiency (Bavaresco et al., 2003; Brancadoro et al., 2001), the salinity reduces the nutrients uptake (Ahmad, 2016; Fisarakis et al., 2004; Stevens et al., 1996), the waterlogging reduces N-P uptake and it increases the Na uptake (Gliński and Stępniewski, 1985; Stevens and Prior, 1994), whereas the drought reduces the K uptake and it increases the absorption of Mg and Ca. Synergies and antagonisms among these elements is already described in paragraph 3.1. In this study, the two analyzed sites show different

environmental characteristics, in terms of soil proprieties and water availability (Table 2). Regarding nutrient contents in soils, Arcagna reported higher level of K and lower levels of Mg and Ca than Riccagioia (Table 2). The same trend found in the soil is shown in the vine leaves (Figure 1). In Arcagna, Mn level in leaves is higher than Riccagioia, though the Mn content in the soil is lower. The difference in Mn uptake may be involved by the different soil proprieties in the two sites or the different water availability.

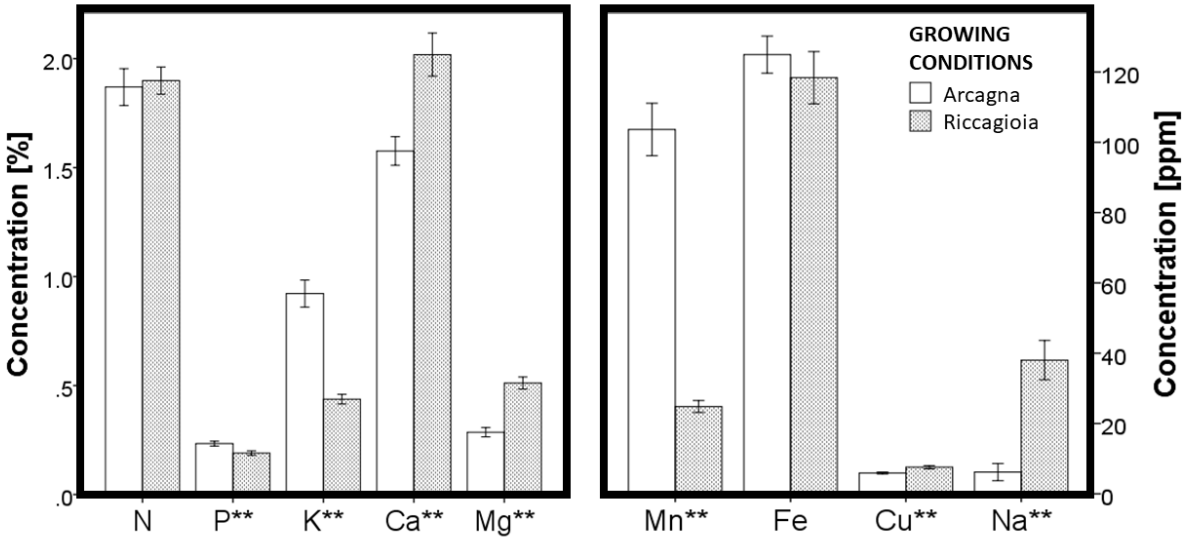


Figure 1 Nutrient leaf levels in the two experimental sites (Arcagna and Riccagioia). Significant differencies are reported for 0.01<p<0.05 (*) and p<0.01 (**)

3.4. The role of the sampling time and its interactions

Only few elements significantly changed from DOY 195 to DOY 220 (Figure 2). During this period nitrogen decreased from 2.04±0.29% to 1.73±0.25%, phosphorous increased from 0.20±0.03% to 0.22±0.06%, calcium level rose from 1.69±0.33% to 1.89±0.46%, magnesium increased from 0.37±0.14% to 0.43±0.16% and copper decreased from 7.20±1.93ppm to 6.28±1.50ppm, whereas other nutrients has not reported significant differences (Figure 2). Interaction of the sampling time with the growing condition were noticed for N, P, K, Ca, Fe and Cu. In Arcagna only N level changed from DOY 195 to DOY 220, reducing of

about 20%. In Riccagioia, Ca level increased during the analyzed period, whereas N, K and Cu levels decreased. Interaction of the sampling time with genotypes was not noticed in nutrients levels.

At DOY 195, nutrient concentration settles around the standard levels reported in literature for different *Vitis* species (*V. vinifera* and *V. lambrusca*) during veraison (Failla et al., 1993; Zengin, 2012). At DOY 220, nitrogen level may decrease because of the remobilization from the analyzed leaves to the younger leaves , through the phloem (Lawlor, 2002; Schreiner, 2016). Contrarily, calcium level widely increased because it is phloem-immobile and it cannot be remobilized (Pradubsuk and Davenport, 2010; Vázquez Vázquez et al., 2016). Concentration of P, K, Mg, Fe Na and Mn didn't change or lightly increased probably because the uptake was balanced by their remobilization in young leaves and fruits (Figure 2). Similar trends between veraison and harvest are reported by Köse *et al* (2016) and Vázquez Vázquez *et al* (2016) on grafted plants. Assimilation of nutrients during the season are also affected by environmental conditions, as shown by DOY × growing condition interaction. In fact, Ca level was stable in Arcagna but it increased in Riccagioia, where water availability was limited. Contrarily, all the genotypes reported the same trend of nutrient levels between the two sampling times, showing a lack of DOY × genotype interaction.

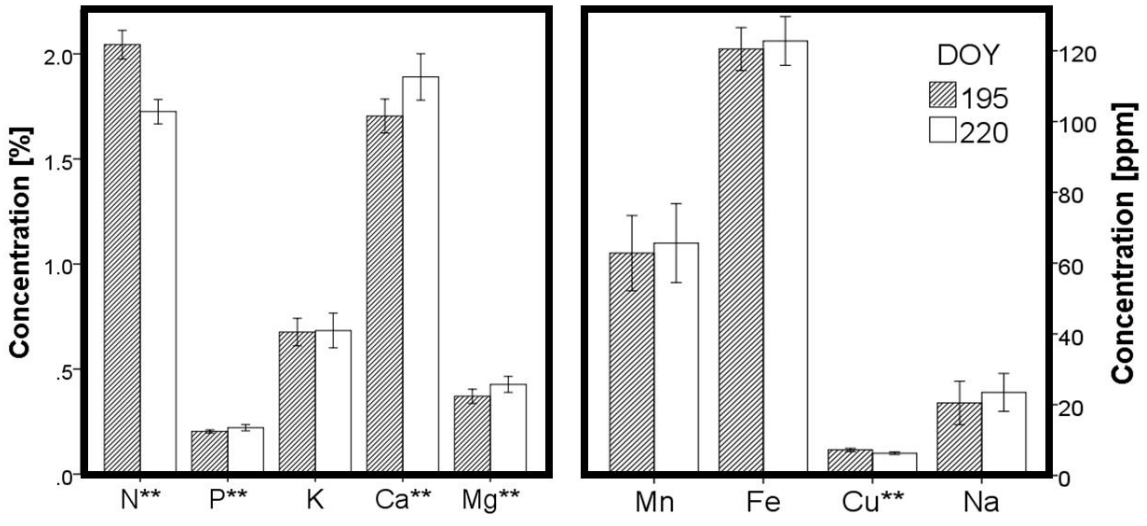


Figure 2 Nutrient leaf levels in the two sampling times (DOY 195 and DOY 220). Significant differencies are reported for 0.01<p<0.05 (*) and p<0.01 (**)

3.5. The role of the genotype

Genotype factor significantly affected the level of all the analyzed nutrients, except for iron and phosphorus. Average levels of both micronutrients and macronutrients in the leaves of each genotype are shown in Table 5: genotype M2 reported the highest N level; the major P uptake was performed by G.30 and G.83; the highest K level was in G.07 and the lowest in G.34; G.19 was the most efficient genotype in Fe uptake. G.28 reported good amount of all the nutrients, except for Mn, whereas G.73 was poor in both macro and micronutrients. The cluster analysis collected the genotypes with similar levels of K, Mg and Ca (Figure 3): group A is composed by nine genotypes with high bent for K; the three genotypes of group B are characterized by low levels of these nutrients; group C includes ten genotypes with high level of Ca and the other genotypes are collected in group D, showing high bent for Mg.

The effect of rootstock genotypes on N status is reported in several studies (Fisarakis et al., 2004; Köse et al., 2016; Somkuwar et al., 2015; Williams and Smith, 1991; Wooldridge et al., 2010), however it is not always confirmed (Angyal et al., 2002; Bavaresco et al., 1993). The effect of grapevine rootstocks on micronutrients have been found (Köse et al., 2016; Ozdemir et al., 2011) and the effect on Fe level is also reported by Bavaresco *et al* (2003), contrarily to this study. Köse *et al* (2016) has not found significant differences in P rootstock levels, as well. Significant rootstock effect on K, Mg and Ca is known in literature (Brancadoro et al., 1994; Fisarakis et al., 2004; Kidman et al., 2014; Köse et al., 2016; Somkuwar et al., 2015; Stevens et al., 1995; Wooldridge et al., 2010). Genotypes can be grouped according to their affinity to K, Mg or Ca, due to the antagonism between these macronutrients (Figure 3). Genotypes in group A may further the K uptake, against Mg or Ca, whereas genotypes in group D may be interesting to avoid Mg deficiencies.

Table 5. Average leaf levels of nutrients for each genotype and their standard deviation. The heatmap shows in blue the nutrient levels over the total average and, in red the levels lower than the average. Least Significant Differences (LSD) are reported for each nutrient ($p<0.05$)

Genotype	Micronutrients				Macronutrients				
	Na [ppm]	Mn [ppm]	Fe [ppm]	Cu [ppm]	N [%]	P [%]	K [%]	Mg [%]	Ca [%]
G.03	32.39 ± 24.70	63.24 ± 37.91	116.37 ± 5.28	5.00 ± 1.31	1.73 ± 0.15	0.17 ± 0.03	0.72 ± 0.42	0.46 ± 0.28	1.64 ± 0.45
G.05	20.37 ± 18.59	61.58 ± 36.40	133.32 ± 11.50	6.75 ± 1.33	2.09 ± 0.34	0.20 ± 0.03	0.77 ± 0.3	0.35 ± 0.14	1.96 ± 0.41
G.07	21.19 ± 13.24	51.12 ± 33.58	129.96 ± 38.72	6.25 ± 1.59	1.78 ± 0.18	0.22 ± 0.08	0.99 ± 0.61	0.32 ± 0.11	1.88 ± 0.73
G.09	18.83 ± 16.70	79.88 ± 67.55	112.86 ± 15.25	6.34 ± 3.18	1.59 ± 0.25	0.21 ± 0.07	0.60 ± 0.30	0.41 ± 0.17	2.23 ± 0.72
G.12	31.52 ± 17.56	76.51 ± 68.14	113.89 ± 8.25	5.81 ± 0.82	1.73 ± 0.21	0.20 ± 0.04	0.63 ± 0.25	0.43 ± 0.11	1.47 ± 0.13
G.13	13.22 ± 9.83	65.26 ± 36.85	126.56 ± 25.11	6.76 ± 0.39	2.23 ± 0.46	0.19 ± 0.02	0.94 ± 0.55	0.34 ± 0.16	1.57 ± 0.50
G.16	15.27 ± 13.83	93.67 ± 79.19	140.67 ± 29.86	8.02 ± 2.27	2.21 ± 0.35	0.22 ± 0.06	0.60 ± 0.15	0.38 ± 0.06	2.08 ± 0.20
G.17	39.97 ± 41.86	62.34 ± 49.49	113.67 ± 15.39	5.47 ± 0.39	1.69 ± 0.29	0.20 ± 0.04	0.49 ± 0.13	0.54 ± 0.11	1.74 ± 0.18
G.19	10.54 ± 7.61	57.43 ± 46.26	154.95 ± 88.21	7.64 ± 1.66	1.87 ± 0.20	0.21 ± 0.06	0.64 ± 0.19	0.25 ± 0.04	1.78 ± 0.36
G.21	19.71 ± 21.33	57.19 ± 31.15	133.06 ± 26.56	5.83 ± 0.51	1.88 ± 0.30	0.22 ± 0.05	0.79 ± 0.44	0.40 ± 0.12	1.65 ± 0.45
G.23	19.12 ± 12.19	89.57 ± 69.51	143.08 ± 23.00	6.32 ± 1.54	2.16 ± 0.37	0.20 ± 0.02	0.79 ± 0.43	0.34 ± 0.13	2.10 ± 0.20
G.24	14.95 ± 20.36	68.68 ± 67.82	128.42 ± 47.28	5.80 ± 1.24	2.04 ± 0.26	0.22 ± 0.07	0.55 ± 0.24	0.47 ± 0.22	1.55 ± 0.57
G.25	20.21 ± 22.56	72.06 ± 67.37	110.59 ± 22.65	6.56 ± 1.17	1.92 ± 0.21	0.17 ± 0.04	0.80 ± 0.38	0.34 ± 0.15	1.75 ± 0.42
G.26	12.46 ± 15.32	62.47 ± 53.64	112.12 ± 30.72	5.78 ± 1.39	1.55 ± 0.50	0.21 ± 0.08	0.50 ± 0.12	0.42 ± 0.06	1.55 ± 0.12
G.27	31.82 ± 37.92	45.73 ± 28.48	113.20 ± 11.92	6.75 ± 1.07	1.78 ± 0.20	0.19 ± 0.04	0.65 ± 0.42	0.33 ± 0.25	1.96 ± 0.51
G.28	31.41 ± 20.24	40.49 ± 11.63	118.69 ± 15.63	7.99 ± 2.44	1.89 ± 0.23	0.23 ± 0.04	0.78 ± 0.45	0.47 ± 0.23	2.09 ± 0.53
G.29	13.89 ± 16.58	36.57 ± 15.52	100.51 ± 9.51	6.23 ± 0.54	1.79 ± 0.19	0.24 ± 0.06	0.72 ± 0.36	0.47 ± 0.21	1.86 ± 0.43
G.30	45.71 ± 49.56	72.92 ± 50.38	110.18 ± 27.75	5.77 ± 1.40	1.76 ± 0.07	0.26 ± 0.05	0.77 ± 0.43	0.47 ± 0.28	1.60 ± 0.27
G.30B	14.95 ± 14.81	85.96 ± 76.26	139.02 ± 37.38	7.34 ± 0.75	1.78 ± 0.39	0.22 ± 0.05	0.70 ± 0.16	0.32 ± 0.14	1.91 ± 0.48
G.34	14.17 ± 12.53	85.41 ± 74.86	106.45 ± 34.62	6.71 ± 1.71	1.86 ± 0.23	0.19 ± 0.04	0.46 ± 0.18	0.40 ± 0.10	1.68 ± 0.36
G.37	21.34 ± 28.31	57.63 ± 43.32	112.82 ± 9.36	7.07 ± 1.32	2.02 ± 0.13	0.25 ± 0.10	0.58 ± 0.22	0.34 ± 0.12	1.80 ± 0.53
G.69	15.73 ± 13.50	61.19 ± 38.72	108.18 ± 11.07	4.72 ± 0.90	1.86 ± 0.37	0.20 ± 0.06	0.78 ± 0.39	0.38 ± 0.17	1.68 ± 0.26
G.70	47.01 ± 62.23	51.79 ± 42.60	114.06 ± 24.22	8.95 ± 2.62	1.87 ± 0.16	0.24 ± 0.04	0.60 ± 0.18	0.54 ± 0.12	1.67 ± 0.10
G.71	16.03 ± 17.70	67.06 ± 36.02	116.09 ± 9.54	7.56 ± 1.34	2.06 ± 0.28	0.21 ± 0.04	0.68 ± 0.16	0.42 ± 0.16	1.76 ± 0.38
G.72	15.01 ± 18.75	55.56 ± 42.14	114.84 ± 7.14	8.22 ± 3.54	1.67 ± 0.32	0.22 ± 0.02	0.51 ± 0.17	0.38 ± 0.11	2.02 ± 0.42
G.73	11.80 ± 12.00	58.08 ± 35.29	102.68 ± 5.18	7.11 ± 3.27	1.89 ± 0.37	0.19 ± 0.03	0.54 ± 0.03	0.35 ± 0.03	1.62 ± 0.17
G.74	12.23 ± 15.04	53.76 ± 28.89	148.85 ± 34.45	6.15 ± 1.01	2.11 ± 0.36	0.20 ± 0.03	0.64 ± 0.26	0.38 ± 0.11	2.05 ± 0.62
G.74B	24.72 ± 23.31	48.97 ± 31.59	124.11 ± 20.91	6.10 ± 1.21	1.90 ± 0.27	0.23 ± 0.07	0.53 ± 0.26	0.50 ± 0.14	1.91 ± 0.23
G.75	9.46 ± 9.48	73.68 ± 57.77	111.77 ± 13.54	7.63 ± 1.16	1.88 ± 0.29	0.20 ± 0.05	0.64 ± 0.22	0.42 ± 0.12	1.72 ± 0.34
G.76	21.79 ± 22.86	40.28 ± 23.10	121.87 ± 24.48	6.77 ± 1.69	1.87 ± 0.19	0.18 ± 0.02	0.72 ± 0.37	0.32 ± 0.22	1.68 ± 0.49
G.77	46.44 ± 42.19	54.47 ± 34.39	106.43 ± 13.47	7.00 ± 1.23	1.73 ± 0.19	0.21 ± 0.04	0.70 ± 0.36	0.33 ± 0.15	1.68 ± 0.35
G.81	30.02 ± 35.38	80.61 ± 43.59	118.62 ± 13.10	6.44 ± 1.86	1.97 ± 0.42	0.22 ± 0.06	0.89 ± 0.43	0.34 ± 0.15	1.80 ± 0.36
G.82	24.02 ± 24.92	64.13 ± 51.33	125.24 ± 13.61	7.98 ± 1.43	1.80 ± 0.46	0.25 ± 0.06	0.63 ± 0.29	0.45 ± 0.17	2.24 ± 0.49
G.83	17.00 ± 10.03	92.18 ± 63.89	143.48 ± 25.34	6.54 ± 1.86	1.71 ± 0.15	0.26 ± 0.03	0.62 ± 0.14	0.44 ± 0.11	1.80 ± 0.33
M2	15.52 ± 18.96	61.08 ± 54.57	133.59 ± 18.15	8.53 ± 2.50	2.29 ± 0.33	0.19 ± 0.02	0.84 ± 0.45	0.42 ± 0.20	1.44 ± 0.36
LSD	*34.86*	*69.61*	*n.s.*	*2.37*	*0.41*	*n.s.*	*0.46*	*0.22*	*0.58*

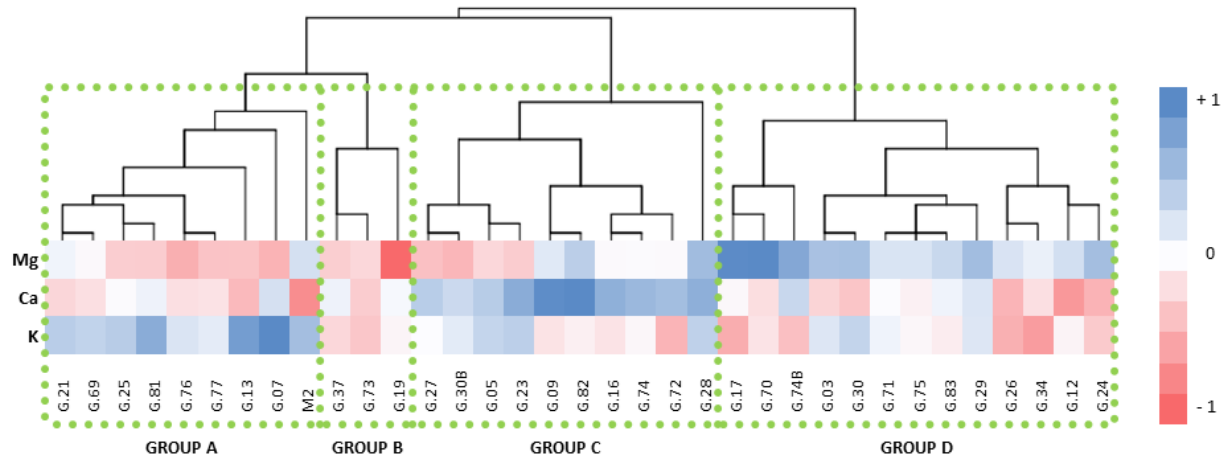


Figure 3 Cluster analysis of genotypes according to their level of potassium, magnesium, and calcium in leaves. Normalized levels over the nutrient average are shown in blue, whereas the levels lower than the average are reported in red. The color intensity is proportioned to the residues from the average levels

3.6. Genotype × growing condition interaction: the rootstock plasticity

Interaction between sites and genotypes is significant for all the nutrients, except for N and Fe (Table 4). Where the interactions were significant, differences of the nutrient levels in the two growing conditions have been used to assess the plasticity of genotypes, through the Plasticity Index (PI), as shown in Figure 4. According to the variability of each element between sites, genotypes have been clustered and classified in group A ($PI < 2$), group B ($2 \leq PI < 3$) and group C ($PI \geq 3$). Genotype M2 is classified in group A with the genotypes G.05, G.71, G.25, G.81, G.75, G.82, G21, G.69, G.24, G.74, G.77, G.37 and G.76.

Besides the average performance, it is important to consider the susceptibility of each genotype to different environmental conditions. Some studies investigated the interaction of rootstock genotypes with salinity (Fisarakis et al., 2004; Stevens et al., 1996) and limestone (Bavaresco et al., 2003) on the mineral uptake. In this study, the interaction has been evaluated through the plasticity of genotypes among the two growing conditions (Figure 4). Group A collects the elastic genotypes, which are stable among environments being less affected by the growing condition, whereas group C includes the plastic genotypes. Genotypes of group B shows a plastic behavior for a few nutrients. Among the elastic genotypes, G.05, G.21, G.71, G.76 and G.77 reported high level of K (Figure 3) and showed at least one mechanism of tolerance to the water stress in a recent study (Bianchi et al., 2018). It is possible to speculate that drought-tolerant genotypes are able to uptake potassium because they maintain a better water flow in water-limited conditions. Moreover, potassium with sugar and starch may repair embolism of trunks and shoots (Brodersen et al., 2010; Keller, 2015; Salleo et al., 2009), providing a better adaptation to drought conditions.

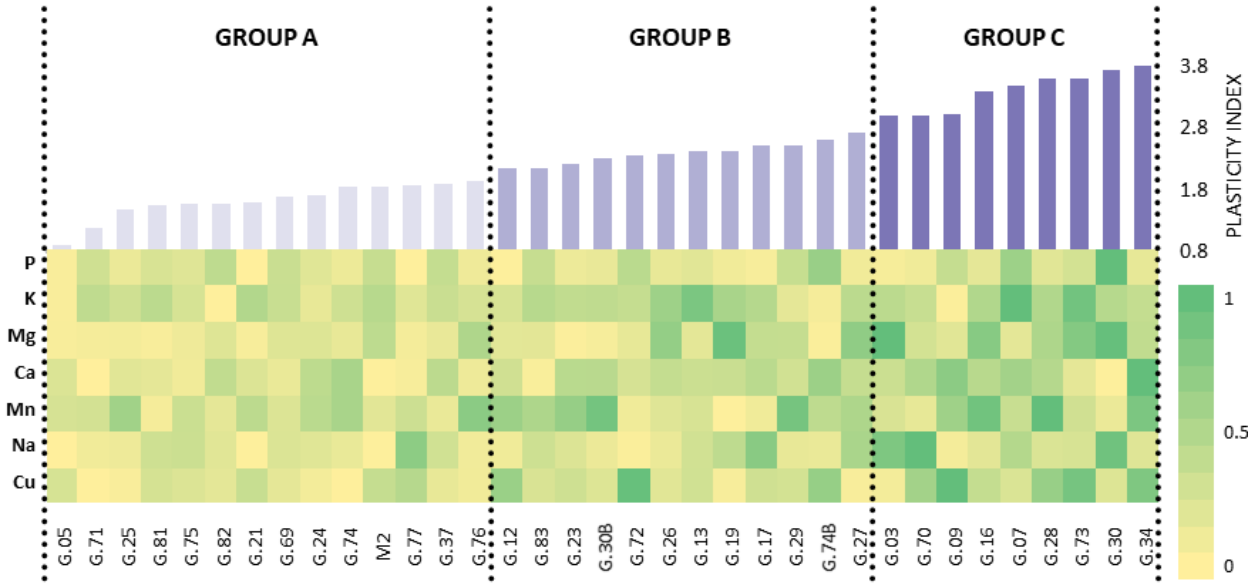


Figure 4 Plasticity Index of genotype (in purple) and the residues between the leaf levels in the two growing conditions for each nutrient. The color intensity is proportioned to the differences between growing conditions

4. Conclusions

In *Vitis* species, the leaf levels of K, Mg, Ca, Na and Mn are strongly correlated. In particular, the synergy K–Mn is opposed to the synergy Mg–Ca–Na. The effect of the sampling time, the growing condition, the genotype and their interactions on the nutritional status is confirmed. Nitrogen level is mainly affected by the sampling time, due to the remobilization in berries, whereas Iron level depends on the interaction between the sampling time and the growing condition. For the other nutrients, growing condition factor represents the main source of variability. Differences among genotypes were used as a first screening of the nutritional status of the 35 un-grafted hybrids, identifying different affinities of genotypes to each nutrient. Hybrids were classified according to their affinity to K, Mg and Ca. Furthermore, plastic or elastic behaviors of genotypes were tested in response to the different growing conditions. This first screening provided an overview on the nutrient uptake efficiency of the hybrid series and their responses to different environments. In further studies, the most promising genotypes of this series should be tested

in grafted condition, considering the interaction with different varieties of *V. vinifera* and the effect of the selected rootstocks on the production and quality of grape.

References

Ahmad, O.A., 2016. Salinity Soil Effects on Yeild, Fruit Quality and Mineral Composition of Superior Seedless Grapevines Grafted on Some Rootstocks. J. Appl. Sci. 16, 359–371. https://doi.org/10.3923/jas.2016.359.371

Alagić, S., Tošić, S.B., Dimitrijević, M.D., Nujkić, M.M., Papludis, A.D., Fogl, V.Z., 2018. The content of the potentially toxic elements, iron and manganese, in the grapevine cv Tamjanika growing near the biggest copper mining/metallurgical complex on the Balkan peninsula: phytoremediation, biomonitoring, and some toxicological aspects. Environ. Sci. Pollut. Res. 25, 34139–34154. https://doi.org/10.1007/s11356-018-3362-7

Amorós, J.A., Bravo, S., Pérez-de-los-Reyes, C., García-Navarro, F.J., Campos, J.A., Sánchez-Ormeño, M., Jiménez-Ballesta, R., Higueras, P., 2018. Iron uptake in vineyard soils and relationships with other elements (Zn, Mn and Ca). The case of Castilla-La Mancha, Central Spain. Appl. Geochemistry 88, 17–22. https://doi.org/10.1016/j.apgeochem.2017.02.009

Angyal, M., Lehoczky, É., Kocsis, L., 2002. Examination of the nutrient uptake by the view of grapevine rootstock-scion interaction. Acta Biol. Szeged. 46, 189–190.

Bavaresco, L., Fraschini, P., Perino, A., 1993. Effect of the rootstock on the occurrence of lime-induced chlorosis of potted *Vitis vinifera* L . cv . ' Pinot blanc ' 305–306.

Bavaresco, L., Giachino, E., Pezzutto, S., 2003. Grapevine Rootstock Effects on Lime-Induced Chlorosis, Nutrient Uptake, and Source–Sink Relationships. J. Plant Nutr. 26, 1451–1465.

Bell, S.J., Henschke, P.A., 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. Aust. J. Grape Wine Res. 11, 242–295. https://doi.org/10.1111/j.1755-0238.2005.tb00028.x

Bianchi, D., Grossi, D., Tincani, D.T.G., Simone Di Lorenzo, G., Brancadoro, L., Rustioni, L., 2018. Multi-parameter characterization of water stress tolerance in *Vitis* hybrids for new rootstock selection. Plant Physiol. Biochem. 132, 333–340. https://doi.org/10.1016/j.plaphy.2018.09.018

Brancadoro, L., Failla, O., Gardiman, M., Rossoni, M., Scienza, A., Zamboni, M., 2014. I nuovi portainnesti della serie M a confronto con altri in commercio, in: Scienza, A., Failla, O., Espen, L. (Eds.), Progetto AGER-SERRES. Strategie Innovative per La Selezione Di Nuovi Portinnesti Di Vite. L'Informatore Agrario, Verona. pp. 25–58.

Brancadoro, L., Reina, L.V.A., Scienza, A., 1994. Potassium content of grapevine during the vegetative period: The role of the rootstock. J. Plant Nutr. 17, 2165–2175. https://doi.org/10.1080/01904169409364872

Brancadoro, L., Tamai, G., Zocchi, G., Failla, O., 2001. Adaptive responses of *Vitis* spp. and *Prunus* spp. to Fe-deficiency induced by HCO<inf>3</inf>-, Acta Horticulturae. https://doi.org/10.17660/ActaHortic.2001.564.44

Brataševec, K., Sivilotti, P., Vodopivec, B.M., 2013. Soil and foliar fertilization affects mineral contents in *Vitis vinifera* L. cv. "rebula" leaves, Journal of Soil Science and Plant Nutrition.

Brodersen, C.R., Mcelrone, A.J., Choat, B., Matthews, M.A., Shackel, K.A., 2010. The Dynamics of Embolism Repair in Xylem : In Vivo Visualizations Using High-Resolution Computed Tomography. Plant Physiol. 154, 1088–1095. https://doi.org/10.1104/pp.110.162396

Caramanico, L., Rustioni, L., De Lorenzis, G., 2017. Iron deficiency stimulates anthocyanin accumulation in grapevine apical leaves. Plant Physiol. Biochem. 119, 286–293. https://doi.org/10.1016/j.plaphy.2017.09.004

Carnevali, P., Brancadoro, L., Failla, O., 2014. Evaluation of four new rootstock genotypes obtained by back cross, Acta Horticulturae. https://doi.org/10.17660/ActaHortic.2014.1046.40

Casanova-Gascón, J., Martín-Ramos, P., Martí-Dalmau, C., Badía-Villas, D., 2018. Nutrients Assimilation and Chlorophyll Contents for Different Grapevine Varieties in Calcareous Soils in the Somontano DO (Spain). Beverages 4, 90. https://doi.org/10.3390/beverages4040090

Dal Santo, S., Zenoni, S., Sandri, M., De Lorenzis, G., Magris, G., De Paoli, E., Di Gaspero, G., Del Fabbro, C., Morgante, M., Brancadoro, L., Grossi, D., Fasoli, M., Zuccolotto, P., Tornielli, G.B., Pezzotti, M., 2018. Grapevine field experiments reveal the contribution of genotype, the influence of environment and the effect of their interaction (G×E) on the berry transcriptome. Plant J. 93, 1143–1159. https://doi.org/10.1111/tpj.13834

Downton, W., 1985. Growth and mineral composition of the sultana grapevine as influenced by salinity and rootstock. Aust. J. Agric. Res. 36, 425–434.

Failla, O., Stringari, G., Porro, D., Scienza, A., 1993. Determination of leaf standards for apple trees and grapevines in northern Italy.

Fisarakis, I., Nikolaou, N., Tsikalas, P., Therios, I., Stavrakas, D., 2004. Effect of salinity and rootstock on concentration of potassium, calcium, magnesium, phosphorus, and nitrate-nitrogen in Thompson seedless grapevine. J. Plant Nutr. 27, 2117–2134. https://doi.org/10.1081/LPLA-200034662

Garcia, M., Daverede, C., Gallego, P., Toumi, M., 1999. Effect of various potassium-calcium ratios on cation nutrition of grape grown hydroponically. J. Plant Nutr. 22, 417–425.

Gliński, J., Stępniewski, W., 1985. Soil aeration and its role for plants. CRC Press, Inc.

Ibacache, A.G., Sierra, C.B., 2009. Influence of rootstocks on nitrogen, phosphorus and potassium content in petioles of four table grape varieties Freedom. Chil. J. Agric. Res. https://doi.org/10.4067/S0718-58392009000400004

Jakobsen, S.T., 1993. Interaction between Plant Nutrients: III. Antagonism between Potassium, Magnesium and Calcium. Acta Agric. Scand. Sect. B — Soil Plant Sci. 43, 1–5.

Keller, M., 2015. The Science of Grapevines: Anatomy and Physiology, Second. ed.

Kidman, C.M., Dry, P.R., McCarthy, M.G., Collins, C., 2014. Effect of rootstock on nutrition, pollination and fertilisation in “Shiraz” (*Vitis vinifera* L.). Vitis. 53 (3), 139-145.

Kodur, S., 2011. Effects of juice pH and potassium on juice and wine quality, and regulation of potassium in grapevines through rootstocks (*Vitis*): A short review. Vitis. 50 (1), 1–6.

Köse, B., Horuz, A., Akınoğlu, G., 2016. Phenological Changes of Leaf Nutrients in *Vitis labrusca* L. Grape Grafted on Different Rootstocks in Heavy Clay Soil Conditions. Erwerbs-Obstbau 58, 211–217. https://doi.org/10.1007/s10341-016-0279-5

Lawlor, D.W., 2002. Carbon and nitrogen assimilation in relation to yield : mechanisms are the key to understanding production systems. J. Exp.

Bot. 53, 773–787.

Migliaro, D., De Lorenzis, G., Di Lorenzo, G.S., De Nardi, B., Gardiman, M., Failla, O., Brancadoro, L., Crespan, M., 2019. Grapevine Non- Vinifera Genetic Diversity Assessed by SSR Markers as a Starting-Point for New Rootstock Breeding Programs . Am. J. Enol. Vitic. ajev.2019.18054. https://doi.org/10.5344/ajev.2019.18054

Ozdemir, G., Ekbic, H.B., Erdem, H., Torun, B., Tangolar, S., 2011. Effect of different zinc application methods on leaf Zn, Fe, Cu and Mn concentrations of ungrafted and grafted Flame Seedless grapevine cultivar and rootstocks (*Vitis* sp.). J. Food, Agric. Environ. 9, 217–222.

Pinto, D.L.P., Brancadoro, L., Dal Santo, S., De Lorenzis, G., Pezzotti, M., Meyers, B.C., Pè, M.E., Mica, E., 2016. The influence of genotype and environment on small RNA profiles in grapevine berry. Front. Plant Sci. 7. https://doi.org/10.3389/fpls.2016.01459

Pradubsuk, S., Davenport, J.R., 2010. Seasonal Uptake and Partitioning of Macronutrients in Mature ' Concord ' Grape 135, 474–483.

Rustioni, L., Bianchi, D., Brancadoro, L., n.d. Drought increases chlorophyll content in stems: a new parameter for *Vitis* rootstock selection.

Rustioni, L., Grossi, D., Brancadoro, L., Failla, O., 2018. Iron, magnesium, nitrogen and potassium deficiency symptom discrimination by reflectance spectroscopy in grapevine leaves. Sci. Hortic. (Amsterdam). 241, 152–159. https://doi.org/10.1016/j.scienta.2018.06.097

Salleo, S., Trifilò, P., Esposito, S., Nardini, A., Lo Gullo, M.A., 2009. Starch-to-sugar conversion in wood parenchyma of field-growing *Laurus nobilis* plants: a component of the signal pathway for embolism repair? Funct. Plant Biol. 36, 815–825.

Schreiner, R.P., 2016. Nutrient uptake and distribution in young pinot noir grapevines over two seasons. Am. J. Enol. Vitic. https://doi.org/10.5344/ajev.2016.16019

Skene, K.G.M., Antcliff, A.J., 1972. A Comparative Study of Cytokinin Levels in Bleeding Sap of *Vitis vinifera* (L.) and the Two Grapevine Rootstocks, Salt Creek and 1613. J. Exp. Bot. 23, 283–293.

Somkuwar, R.G., Taware, P.B., Bhange, M.A., Sharma, J., Khan, I., 2015. Influence of Different Rootstocks on Growth, Photosynthesis, Biochemical Composition, and Nutrient Contents in 'Fantasy Seedless' Grapes. Int. J. Fruit Sci. https://doi.org/10.1080/15538362.2015.1031564

Stevens, R., G, H., Davies G, 1996. Separating the effects of foliar and root salt uptake on growth and mineral composition of four grapevine cultivars on their own roots and on "Ramsey" rootstock. J. Am. Soc. Hortic. Sci. 121, 569–575.

Stevens, R.M., Harvey, G., Aspinall, D., 1995. Grapevine growth of shoots and fruit linearly correlate with water stress indices based on root-weighted soil matric potential. Aust. J. Grape Wine Res. 1, 1322–7130.

Stevens, R.M., Prior, L.D., 1994. The Effect of Transient Waterlogging on the Growth, Leaf Gas Exchange, and Mineral Composition of Potted Sultana Grapevines. Am. J. Enol. Vitic. 45, 285 LP-290.

Swanepoel, J.J., Southey, J.M., 1989. The Influence of Rootstock on the Rooting Pattern of the Grapevine. South African J. Enol. Vitic. 10.

Tomasi, N., Monte, R., Varanini, Z., Cesco, S., Pinton, R., 2015. Induction of nitrate uptake in Sauvignon Blanc and Chardonnay grapevines depends on the scion and is affected by the rootstock. Aust. J. Grape Wine Res. https://doi.org/10.1111/ajgw.12137

Toumi, M., Nedjimi, B., Halitim, A., Garcia, M., 2016. Effects of K-Mg ratio on growth and cation nutrition of *Vitis vinifera* L. cv. "Dattier de Beiruth" grafted on SO4 rootstock. J. Plant Nutr. 39, 904–911.

Vázquez Vázquez, F.A., Pérez Cid, B., Río Segade, S., 2016. Assessment of metal bioavailability in the vineyard soil-grapevine system using different extraction methods. Food Chem. 208, 199–208. https://doi.org/10.1016/j.foodchem.2016.04.005

Williams, L.E., Smith, R.J., 1991. The Effect of Rootstock on the Partitioning of Dry Weight, Nitrogen and Potassium, and Root Distribution of Cabernet Sauvignon Grapevines. Am. J. Enol. Vitic. 42, 118–122.

Wooldridge, J., Louw, P.J.E., Conradie, W.J., 2010. Effects of rootstock on grapevine performance, petiole and must composition, and overall wine score of *Vitis vinifera* cv. Chardonnay and pinot noir. South African J. Enol. Vitic. 31, 45–48.

Zengin, M., 2012. No TitleToprak ve Bitki Analiz Sonuçlarının Yorumlanmasında Temel İlkeler In: Bitki Besleme. Gübretaş Rehb. Kitaplar Dizisi 2, 837–961.

WATER USE EFFICIENCY AND NUTRITIONAL STATUS OF A NEW GRAPEVINE ROOTSTOCK SELECTION

Abstract

Production and quality of grape are determined by hydric and nutritional status of vine. In modern viticulture, the interface between grapevine and soil is represented by the rootstock, which modulates the uptake of water and nutrients. Thus, selection of new rootstocks for abiotic stress tolerance represents an adaptation strategy for viticulture to the new environmental condition imposed by climate change. The aim of this work is to evaluate the water use efficiency and the nutritional status of a selection of 30 new rootstock genotypes coming from several breeding programs. Water use efficiency and gas exchange were measured for each genotype in two environments characterized by different water availability and the levels of N, P, K, Mg, Ca, Na, Fe, Mn and Zn in the leaf blades were determined during the phenological stages of flowering and ripening. Genotypes were classified according to their drought tolerance, vigor and affinity to macronutrients and micronutrients. A total of 14 genotypes showed tolerance to water stress and 10 of them reported high vigor and carbon assimilation. Furthermore, the largest part of drought tolerant genotypes was more affined to Mg than K. In further studies, the most promising rootstocks will be evaluated in grafting combination with *Vitis vinifera*.

1. Introduction

In the new environmental scenario affected by climate change, adaptation of viticulture to abiotic stresses is becoming a crucial issue to preserve the production and the quality of grape and wines. Since the end of XIX century, grapevine is worldwide grafted onto rootstocks to avoid the attack of phylloxera (*Daktulosphaira vitifoliae*). Thus, tolerance of grapevine to abiotic stresses is not only depending on the cultivar, but it is mediated by the rootstock genotype. In a recent study, the effect of rootstock genotype and its interactions on grape yield has been assessed to 13.29 % and the contribute to sugar content has been evaluated in 14.80 % (Migicovsky et al., 2021). The effect of rootstocks on yield and quality of grape

can be explained by the different uptake of water and nutrients from the soil and the vigor induced to the scion (Keller, 2005; Zombardo et al., 2020).

Rootstocks control the water status of vines involving different physiological mechanisms. A main mechanism has been identified in the control of stomatal conductance and transpiration by hydraulic and hormonal signaling (Zhang et al., 2016). Drought tolerant rootstocks showed different levels of stomatal control in response to water deficit. For example, the tolerant rootstock 1103P reduced the water loss by closing the stomata, whereas another tolerant rootstock, M4, maintained higher stomatal conductance under water deficit and increased the water use efficiency (Frioni et al., 2020). This strategy can face short to medium water stress periods supporting the vegetative growth and the ripening of grape, and it may involve physiological systems to detox the reactive oxygen species (ROS) caused by water stress (Corso et al., 2015).

The vigor of the shoot is induced by rootstocks, causing an effect on the vegetative-productive balance (Leão and Chaves, 2020; Migicovsky et al., 2021). In fact, rootstock genotype affected the assimilation of carbon from the air and the uptake of macronutrients from the soil, such as nitrogen and phosphorous, directly related to the development of biomass (Ibacache et al., 2019; Rossdeutsch et al., 2021; Verdugo-Vásquez et al., 2021). Excess or deficit of nitrogen can affect the yeast assimilable nitrogen (YAN) in the must, causing microbial instability during fermentation (Bell and Henschke, 2005).

The uptake of other macronutrients like potassium, magnesium and calcium depends on rootstock genotypes, beside to environmental conditions. In a recent study, rootstocks with *Vitis riparia* in their genetic background reported lower levels of Mg than rootstocks without *V. riparia* (Gautier et al., 2020). Antagonisms in the uptake of K and Mg has been reported in literature, as well as the synergies between Mg and Ca (Casanova-Gascón et al., 2018; Toumi et al., 2016). Rootstocks ranked according to the level of K in leaves, grape and must (Brancadoro et al., 1994). High levels of K in the must can involve

in a reduction of the total acidity, due to the salinification of tartaric acid (Kodur, 2011). The choice of a rootstock affined to Mg rather than K can be made as an agronomical practice to maintain the acidity of wines under unfavorable environmental conditions and to avoid Mg deficit. Rootstocks also affect the uptake of micronutrients, such as iron, manganese, zinc and sodium (Gautier et al., 2020). The ability to uptake iron is relevant with high concentration of limestone in the soil. Some rootstocks can cope with limestone in the soil, and they can be chosen to avoid Fe deficit, inducing specific uptake strategies (Marastoni et al., 2020).

Hydric and nutritional status of vines are driven by environmental conditions; thus, the plasticity of rootstocks play a key role in the interaction of genotypes with the environment: rootstocks that respond to changes of external conditions by modifying their performance are defined “plastic”, whereas rootstocks that maintain a stable performance under different environments are defined “elastic” (Dal Santo et al., 2018; Pinto et al., 2016). The plasticity of specific traits related to hydric and nutritional status should be considered in the selection process of new rootstocks able to cope with abiotic stresses.

The Department of Agricultural and Environmental Sciences (DiSAA) of the University of Milano is currently working on the selection of new grapevine rootstocks for the tolerance to abiotic stresses. The four genotypes of the M-series were recently released to cope with different environmental conditions. In particular, M1 was selected for limestone tolerance, M2 reported high efficiency in K and Mg uptake, M3 showed affinity to K and M4 reported high tolerance to water deficit and salt stress. A new selection of 30 genotypes coming from the same breeding programs is currently under investigation. A first screening on water stress tolerance has been performed in Bianchi et al (2018), whereas a first screening on mineral nutrition was reported in Bianchi et al (2020). The aims of this work are: i) to assess the water use efficiency and the transpiration control of the new selection of genotypes; ii) to evaluate their vigor and carbon assimilation; iii) to analyze their nutritional status in terms of macro and micronutrients and their affinity to potassium or magnesium; iv) to investigate the plasticity of key traits in response to

different environments and v) to identify among them the most promising rootstocks for abiotic stress tolerance.

2. Material and methods

2.1. Experimental design

The experiment was carried out in 2019. New 30 grapevine rootstock genotypes were studied in un-grafted conditions and the commercial rootstock M2 was used as control. The genetic background of all genotypes is reported in Migliaro et al (2019). Genotypes were analyzed under field conditions in two different environments, located in Arcagna (45.340276 N, 9.449786 E, 83 m a.s.l.) and Riccagioia (44.984783 N, 9.089038 E, 133 m a.s.l.). In each site, three biological repetitions per genotype were considered, represented by three different plants. Estimation of water status using thermography was performed in three days per site, during the phenological stages of flowering (DOY 164), veraison (DOY 200) and ripening (DOY 220). During ripening also gas exchange was measured. Concurrently to the phenological stages of flowering and ripening, leaf samples were collected for mineral analysis. To assess the vigor of vines, pruning weight was measured in the two sites during winter.

2.2. Characterization of the experimental sites

The two experimental fields were located in Lombardia, Italy, and the distance between them was about 46 km. The experimental field in Riccagioia was set up in 2014 with inter-row distance among plants of 2.40 m and intra-row distance of 1.10 m. The soil in Riccagioia is classified as sandy clay loam (USDA Textural Soil Classification), reporting 58% of sand, 19% of silt and 23% of clay. Total organic matter amounted to 8.95 g/kg and the CEC was 14.5 me $100g^{-1}$. The values of pH in Riccagioia were 7.3 and 6.9 in H_2O and KCl, respectively. Nitrogen content in the soil was 5500 ppm and the ratio K/Mg was 0.29. Daily meteorological data for Riccagioia were provided by the station network of ARPA Lombardia (reference station of Voghera). The experimental field in Arcagna was set up in 2015 with inter-row distance among

plants of 3.10 m and intra-row distance of 2.00 m. According to the USDA Textural Soil Classification the soil type was sandy loam, with 75% of sand, 14% of silt and 11% of clay. Total organic matter amounted to 11.30 g/kg and the CEC was 8.0 me $100g^{-1}$. The content of N was 4000 ppm and the ratio K/Mg amounted to 1.04. Temperature and precipitations in Arcagna were monitored in situ by a meteorological station. In both sites, vines were trained in creeping system.

2.3. Leaf temperature and gas exchange

On DOY 164, 200 and 220 the water status of plants was monitored by thermography. Thermal images were recorded by the thermal camera Thermo Gear Model G100EX/G120EX (Detector Uncooled focal plane array; Number of pixels 320 H × 240 V; Spectral range 8–14 µm; dynamic resolution at 14 bit), produced by InfReC, NEC Avio Infrared Technologies CO., Ltd. Emittance was set to 0.96, as suggested for grapevine leaves by Grant et al (2006). One image was taken for each biological repetition and three shaded leaves (Jones et al., 2002) were chosen in each image. Temperature was recorded in three points per leaf using the software "InfReC Analyzer NS9500 Lite". Leaf temperature was normalized on a dry reference (T_{dry}) and wet reference (T_{wet}) to determinate the thermal index Crop Water Stress Index (CWSI), proposed by Idso et al (1981) and modified by Jones (1999) as follow:

$$CWSI = \frac{T_C - T_{wet}}{T_{dry} - T_{wet}}$$

T_C = canopy temperature; T_{wet} = reference temperature for fully closed stomata; T_{dry} = reference temperature for fully transpiring leaves.

On DOY 220, gas exchange was measured in one sunny leaf per each biological repetition using a leaf portable photosynthesis system (CIRAS-3, PP Systems, Amesbury, MA, USA) equipped with PLC6 (U) cuvette 18 mm circular (2.5 cm^2 head plate), under constant saturating PPFD of 1500 µmol photons m^{-2} s^{-1}, CO_2 concentration of 300 µmol mol^{-1}, block temperature of 25 °C and relative humidity between 60%

and 70% allowing ~1.5 kPa of VPD inside the leaf chamber. Instantaneous water use efficiency (WUE) was determined as the ratio between carbon assimilation (A) and the transpiration rate (E), whereas intrinsic water use efficiency (iWUE) was calculated as the ratio between A and stomatal conductance (gs). Plasticity of iWUE (diWUE) and A (dA) were calculated as the absolute value of the difference of each trait between the two environments (Arcagna and Riccagioia).

2.4. Mineral analysis

Nutritional status of vines was assessed by the mineral composition of leaves. During flowering (DOY 164) and ripening (DOY 220), 3 samples per biological repetition were collected and each sample was composed by 3 leaves close to bunches. Samples were dried in an oven at 60 °C until a constant mass was achieved and grinded. An amount of 0.3 g for each sample was mineralized in 10 mL of HNO_3 (> 60%) at 210 °C for 20 min + 20 min of cooling and filtered on a 0.45 µm nylon membrane. Levels of P, K, Ca, Mg, Na, Mn, Fe, Zn were determined in the leaf samples by Inductively Coupled Plasma Mass spectrometry (ICP-MS) using a quadrupole spectrometer (Aurora M90, Bruker). An amount of 7 mg for each grinded sample was processed by an elemental analyzer (NA 1500 series 2 NC, Carlo Erba, Italy) to detect the levels of C and N. Photosynthetic nitrogen use efficiency (PNUE) was calculated as the ratio between A and the level of N. The ratios potassium magnesium (KMg) and potassium magnesium calcium (KMgCa) were also determined. Plasticity of KMgCa (dKMgCa) and Fe (dFe) were calculated as the absolute value of the difference of each trait between Arcagna and Riccagioia.

2.5. Statistical analysis

All data were processed using Microsoft Office Excel Professional Plus 2016 and R statistic environment (R Core Team, 2021). Single effect of factors and their interactions were tested by univariate ANOVA model, after accounting for normality of distribution and homogeneity of variance. Level of significance was considered at $p = 0.05$. Data were standardized for multivariate analyses according to z distribution.

Cluster analyses were performed using the Euclidean distance and a complete linkage method. Principal component analyses (PCA) were carried out using the factoextra R package. Venn diagram was obtained using the open-source component for web environment jvenn (Bardou et al., 2014).

3. Results and discussion

3.1. Plasticity of traits in response to the environment

The water and nutritional status of rootstocks was affected by environmental conditions, beside by the genotype. During the vegetative period, the site of Riccagioia reported lower cumulative precipitations than Arcagna, amounting to 202.6 mm and 277,8 mm, respectively. Water deficit in Riccagioia and well-watered conditions in Arcagna were confirmed by the soil water potential, measured along the vegetative period during the phenological stages of flowering, veraison and ripening. On DOY 164, 200 and 220, soil water potential in Riccagioia was -0.04 MPa, -0.17 MPa and -0.20 MPa, respectively, whereas in Arcagna amounted to -0.01 MPa, -0.09 MPa and -0.14 MPa. The effect of the environment and its interaction with the genotype were significant for all the physiological parameters related to water stress, except for WUE (Table 1). Riccagioia reported higher CWSI than Arcagna during all the vegetative period and the larger differences between the two sites occurred in the stages of veraison and ripening. During ripening, average stomatal conduction in Arcagna amounted to 201.71 ± 10.47 mmol H_2O m^{-2} s^{-1}, whereas in Riccagioia was 46.18 ± 3.49 mmol H_2O m^{-2} s^{-1}. The same trend was shown for transpiration and photosynthesis. The photosynthetic rate in Arcagna was higher than in Riccagioia, amounting to 10.43 ± 0.32 and 3.84 ± 0.34 µmol CO_2 m^{-2} s^{-1}, respectively, whereas the iWUE was higher in Riccagioia, amounting to 93.24 ± 8.47 µmol CO_2 mol^{-1} H_2O.

Table 1. Mean values and standard error for each analyzed trait in the two environments (Arcagna and Riccagioia) at each phenological stage: f = flowering; v = veraison; r = ripening; d = dormancy. Significant effect of environment (E), genotype (G) and their interaction (G x E) is reported for $p<0.001$ (***), $0.001<p<0.01$ (**), $0.01<p<0.05$ (*) and $p>0.05$ (n.s.)

Trait	Stage	u.m.	Arcagna	Riccagioia	E	G	G x E
gs	r	mmol H_2O m^{-2} s^{-1}	201.71 ± 10.47	46.18 ± 3.49	***	***	***
E	r	mmol H_2O m^{-2} s^{-1}	3.22 ± 0.12	1.2 ± 0.07	***	***	***
WUE	r	mmol CO_2 mol^{-1} H_2O	3.48 ± 0.13	3.17 ± 0.26	n.s.	***	***
iWUE	r	µmol CO_2 mol^{-1} H_2O	61.68 ± 3.11	93.24 ± 8.47	***	***	***
VPD	r	kPa	1.94 ± 0.06	2.95 ± 0.09	***	***	***
CWSI	f	-	0.39 ± 0.01	0.52 ± 0.01	***	**	***
CWSI	v	-	0.2 ± 0.01	0.71 ± 0.01	***	***	***
CWSI	r	-	0.23 ± 0.01	0.63 ± 0.01	***	***	***
Vigor	d	kg	1.2 ± 0.06	0.48 ± 0.03	***	***	***
A	r	µmol CO_2 m^{-2} s^{-1}	10.43 ± 0.32	3.84 ± 0.34	***	***	***
C	f	%	44.72 ± 0.13	43.48 ± 0.13	***	n.s.	*
C	r	%	43.68 ± 0.38	45.08 ± 0.17	**	n.s.	n.s.
N	f	%	2.99 ± 0.06	2.63 ± 0.04	***	***	***
N	r	%	1.62 ± 0.03	1.86 ± 0.02	***	***	***
PNUE	r	µmol CO_2 $\%N^{-1}$ m^{-2} s^{-1}	4.64 ± 0.14	1.98 ± 0.16	***	***	***
P	f	%	0.32 ± 0.01	0.37 ± 0.02	**	***	**
P	r	%	0.22 ± 0.01	0.22 ± 0.01	n.s.	***	n.s.
K	f	%	0.98 ± 0.02	0.76 ± 0.03	***	***	**
K	r	%	0.95 ± 0.04	0.44 ± 0.01	***	***	***
Mg	f	%	0.18 ± 0.01	0.27 ± 0.01	***	***	***
Mg	r	%	0.31 ± 0.01	0.52 ± 0.01	***	***	***
Ca	f	%	1.54 ± 0.05	1.38 ± 0.05	**	***	**
Ca	r	%	2.57 ± 0.07	2.51 ± 0.05	n.s.	***	***
KMg	f	-	6.03 ± 0.34	2.99 ± 0.14	***	***	***
KMg	r	-	3.75 ± 0.33	0.93 ± 0.05	***	***	***
KMgCa	f	-	0.62 ± 0.03	0.51 ± 0.03	***	***	***
KMgCa	r	-	0.36 ± 0.02	0.15 ± 0.01	***	**	**
Na	f	ppm	37.68 ± 2.86	46.96 ± 3.92	n.s.	n.s.	**
Na	r	ppm	59.36 ± 7.33	34.56 ± 2.27	**	n.s.	n.s.
Fe	f	ppm	235.51 ± 7.44	120.5 ± 5.94	***	***	***
Fe	r	ppm	313.79 ± 8.95	276.03 ± 6.53	***	***	**
Mn	f	ppm	179.16 ± 7.34	144.02 ± 7.43	***	***	***
Mn	r	ppm	156.26 ± 6.94	62.21 ± 2.61	***	***	***
Zn	f	ppm	21.75 ± 1.02	40.06 ± 3.22	***	***	***
Zn	r	ppm	24.91 ± 1.49	25.15 ± 1.46	n.s.	n.s.	n.s.

Differences between the two sites also occurred in the nutritional status of rootstocks. Significant effect of the environment was found for all the nutrients analyzed in the leaves, except for P, Ca and Zn during ripening and Na during flowering (Table 1). Significant interaction of the environment and genotypes was also reported for all nutrients, except for C, P, Na and Zn during ripening. The site of Arcagna reported higher levels of K, Fe and Mn than Riccagioia in both physiological stages, as well as the ratios K/Mg and K/(Mg+Ca). In both stages, the level of Mg was higher in Riccagioia, whereas the level of N was higher in Arcagna during flowering and in Riccagioia during ripening. PNUE in Arcagna during ripening was higher than in Riccagioia, amounting to 4.64 ± 0.14 µmol CO_2 $\%N^{-1}$ m^{-2} s^{-1} and 1.98 ± 0.16 µmol CO_2 $\%N^{-1}$ m^{-2} s^{-1}, respectively. In the end of the season, the produced biomass estimated by pruning weight amounted to 1.2 ± 0.06 kg in Arcagna and 0.48 ± 0.03 kg in Riccagioia.

The measured parameters concurred to establish different water conditions in the two environments. The lack of precipitations in Riccagioia led to low water potential of the soil in the phenological stages of veraison and ripening, and to physiological response of vines in terms of stomatal closure and the consequent reduction of photosynthesis. In fact, according to Cifre et al (2005), the stomatal conductance in Riccagioia was below the threshold of severe water stress amounting to 50 mmol H_2O m^{-2} s^{-1}. Due to stomatal closure, the temperature of the leaves in Riccagioia generally increased, as detected by the thermal index CWSI. According to Bellvert et al (2014), the level of CWSI in Riccagioia showed a condition of severe water stress during veraison and moderate water stress during ripening, whereas the level in Arcagna was below the threshold of mild water stress. The different water conditions in the two environments led to differences in terms of nutrient levels, with higher uptake of K in Arcagna, probably promoted by the wider water availability (Brancadoro et al., 1994). Nonetheless, different levels of water stress and different affinity to nutrients were shown from individual genotypes.

3.2. Water use efficiency and transpiration control

Rootstock genotypes were classified according to gs, E, CWSI, iWUE and their plasticity of iWUE in response to water deficit (diWUE). Based on Euclidean distance, four groups were identified: group 1 counted 9 genotypes; group 2 amounted to 8 genotypes including rootstock M2; 4 genotypes belonged to group 3; and 10 genotypes were collected in group 4 (Figure 1a). The different behaviors among groups were analyzed by PCA including all the parameters related to water stress. Two principal components were identified, representing about the 65% of the total variance. The first component explained the 41.8% of variance and it was positively affected by gs and E, and negatively affected by VPD, CWSI, WUE, iWUE and diWUE. The second principal component explained the 23.6% of variance and it increased along with gs, E, WUE, iWUE, diWUE, and decreased with VPD and CWSI. The biplot of the two first principal components is reported in figure 1b: genotypes belonging to groups 1 and 2 reported high levels of VPD and CWSI; the 4 genotypes in group 3 reported high WUE, iWUE and diWUE; whereas genotypes collected in group 4 reported high gs and E.

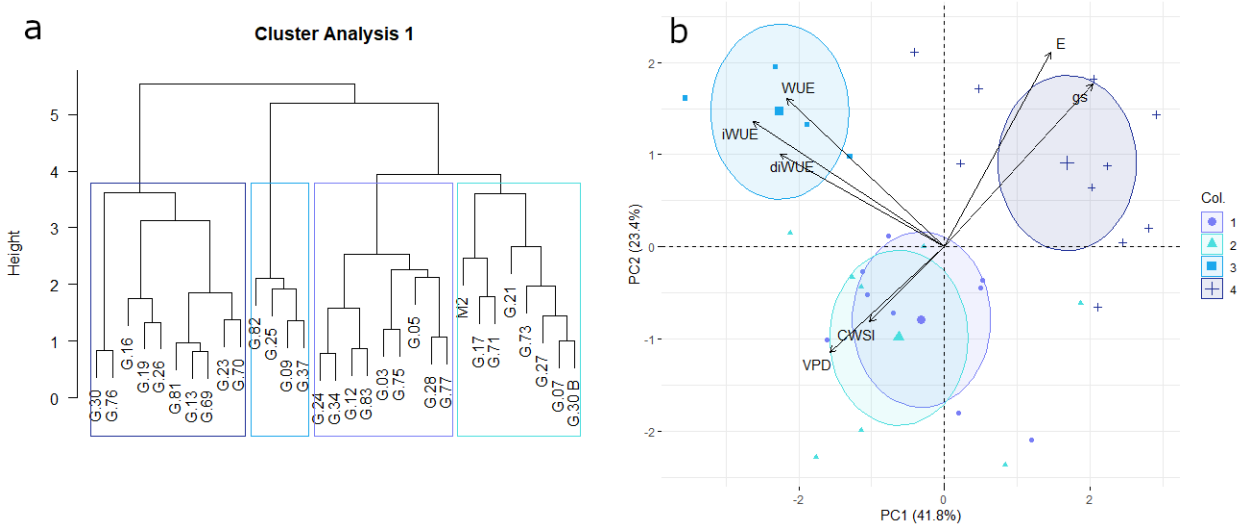


Figure 1. Classification of rootstock genotypes for water use efficiency and transpiration control based on cluster analysis (a) and PCA (b). WUE = instantaneous water use efficiency; iWUE = intrinsic water use efficiency; diWUE = plasticity of water use efficiency; E = transpiration rate; gs = stomatal conductance; VPD = vapor pressure deficit; CWSI = crop water stress index

The groups of genotypes detected by the cluster analysis were confirmed by PCA. The physiological meaning of the first principal component can be identified in the regulation of stomatal conductance. The second principal component was related to the tolerance to water stress, regardless the adopted strategy. The best performance was shown by groups 3 and 4, but different strategies were adopted: group 4 reported the higher transpiration rate, maintaining similar iWUE in the two environments without altering the gas exchange; group 3 reported the higher iWUE, reducing the stomatal conductance but preserving the photosynthetic activity in the environment under water deficit, as shown by diWUE. Groups 1 and 2 were more susceptible to water stress, reporting low iWUE and stomatal conductance, which involved in low transpiration rate and high VPD in the sub-stomatal chamber. Thus, temperature of leaves increased as detected by the thermal index CWSI. Genotypes belonging to groups 3 and 4 reported a better water use efficiency and stomatal conductance than rootstock M2, which is considered tolerant to water deficit conditions. In a preliminary study, some genotypes belonging to group 4 (i.e. G.13, G.19, G.23, G.26, G.30 and G.76) showed a mechanism of tolerance to water stress, rather increasing the wood hydrophobicity to reduce embolisms or repairing them by starch remobilization (Bianchi et al., 2018). In a recent study (Rustioni and Bianchi, 2021), some genotypes of group 4 (G.13, G.16, G.19 and G.81) and G.25, classified in group 3, increased the concentration of chlorophyll in woody tissues under water deficit, as a possible strategy of detoxification related to stem photosynthetic activity.

3.3. Vegetative growth, carbon assimilation and nitrogen use efficiency

A second cluster analysis classified the genotypes according to their vigor (represented by the pruning weight), carbon assimilation, levels of N and P and photosynthetic nitrogen use efficiency. Results of the analysis identified four groups: group 1 was composed by 2 genotypes; group 2 comprised 18 genotypes, including M2; group 3 collected 7 genotypes and group 4 included 4 genotypes (Figure 2 a). Groups were characterized by PCA, considering two principal components able to explain about the 60% of the total

variance. The first principal component represented the 34.3% of the variance and it was negatively affected by vigor and N, whereas it was positively affected by A and PNUE. The second component was mainly related to A, PNUE, C and P and it explained the 25.7% of variance. The highest vigor was reported by genotypes belonging to groups 1 and 3, whereas the highest carbon assimilation and nitrogen use efficiency was performed by groups 3 and 4. Both low vigor and photosynthesis were shown by genotypes belonging to group 2, which reported high level of C and the largest differences in carbon assimilation between the two environments (Figure 2 b).

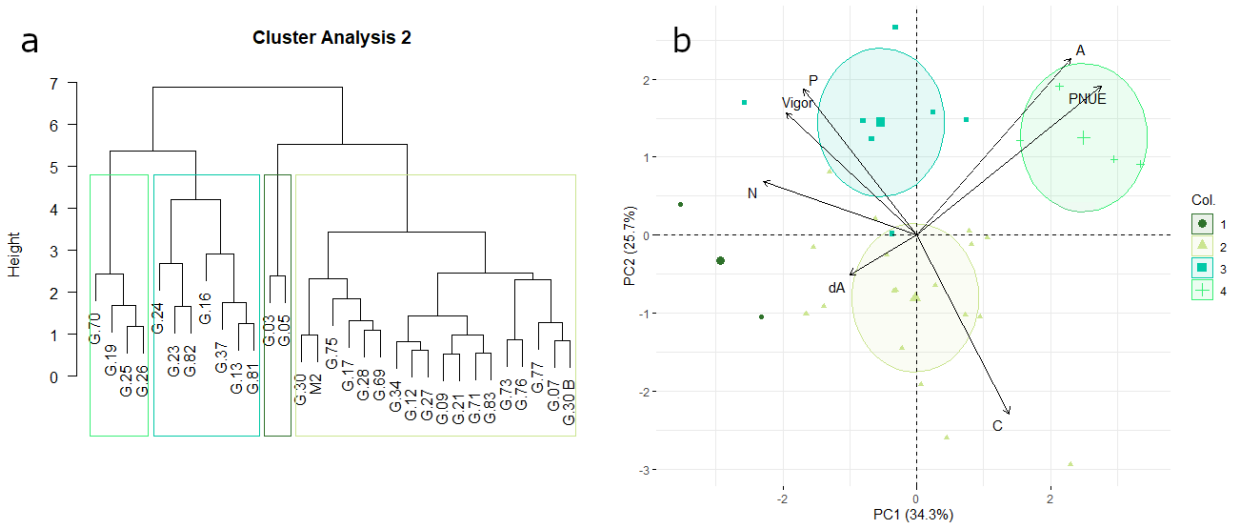


Figure 2. Classification of rootstock genotypes for vigor, carbon assimilation and nitrogen uptake based on cluster analysis (a) and PCA (b). A = carbon assimilation; dA = plasticity of carbon assimilation; PNUE = photosynthetic nitrogen use efficiency; N = nitrogen; P = phosphorous; C = carbon

Wide differences in terms of vigor were found among genotypes in the end of the season. The high vigor of genotypes of group 3 was also supported by high photosynthetic activity during the season and high levels of N in the leaves. Differently, genotypes belonging to group 4 reported low level of N and vigor, but the highest photosynthetic rate during the season. This involved in high photosynthetic nitrogen use efficiency. Group 4 also reported an elastic behavior in terms of photosynthesis, reporting similar performance in the two environments. These results suggested that genotypes of group 4 can be use in

several environments to maintain the photosynthetic activity and to support the vegetative growth and the ripening of grape. On the other hand, genotypes of group 3 can confer vigor to the scion under limiting environments, ensuring high nitrogen and phosphorus uptake. The high N levels of genotypes belonging to group 3 (i.e. G.24, G.37, G.82, G.13, G.16 and G.23) confirmed the results obtained in a first screening on the mineral nutrition of the same genotypes (Bianchi et al., 2020).

3.4. Affinity to potassium or magnesium uptake

Genotypes were further classified according to their affinity to K or Mg. Three groups outcome from cluster analysis. The first group included 5 genotypes, the second group counted 13 genotypes and the third one included 13 genotypes, comprising rootstock M2 (Figure 3 a). Two principal components were found by PCA to explain the 76% of the total variance. The first component represented the 56% of variance and it increased along with the level of Mg and it decreased with the level of K, the ratios k/Mg and K/(Mg+Ca), as well as with the plasticity of K/(Mg+Ca) (i.e. dKMgCa). The second principal component explained the 20% of variance and it is mainly affected by the level of Ca. The three groups of genotypes were well discriminated according to the first principal component (Figure 3 b). Group 1 reported the higher level of K and high values of the ratios K/Mg and K/(Mg+Ca). Group 3 reported high level of Mg and low level of K, whereas group 2 reported intermediate levels of both K and Mg. Group 1 also reported the widest differences between the two environments in terms of K/(Mg+Ca). Two sub-groups can be identified in group 3: the first one included genotypes G.16, G.24, G.25, G.37, G.71 and G.76 which reported high levels of Ca, whereas the second sub-group included genotypes G.17, G.26, G.30, G.30B, G.70 and G.75 with low levels of Ca.

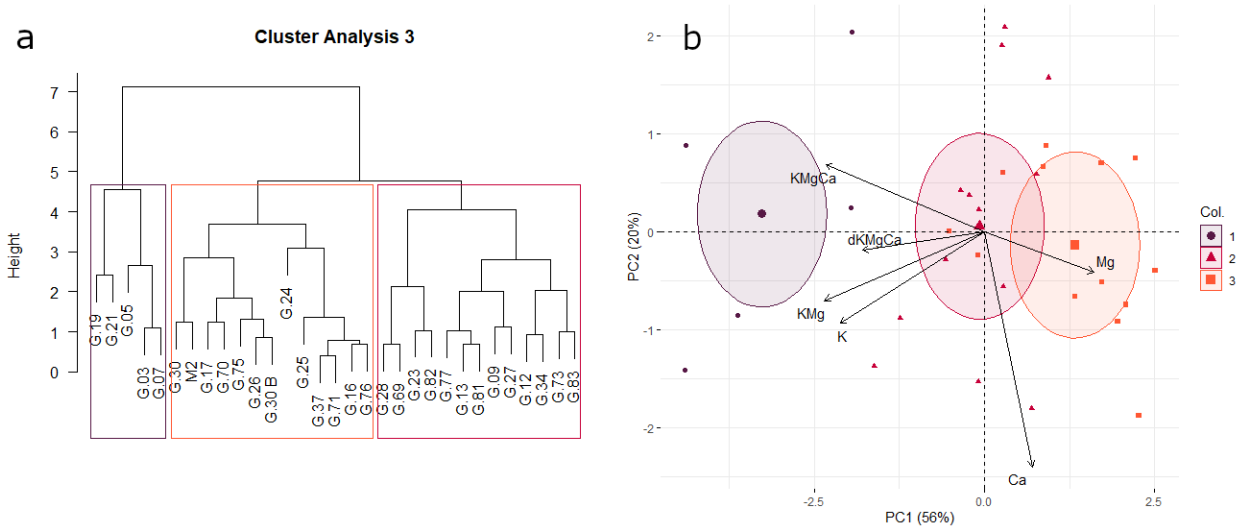


Figure 3. Classification of rootstock genotypes for potassium and magnesium based on cluster analysis (a) and PCA (b). K = potassium; Mg = magnesium; Ca = calcium; KMg = ratio potassium magnesium; KMgCa = ratio potassium magnesium calcium; dKMgCa = plasticity of the ratio potassium magnesium calcium

The three groups identified by cluster analysis showed different affinity to K or Mg. Genotypes with high level of K generally reported low level of Mg, confirming the antagonism in the uptake of these nutrients (Casanova-Gascón et al., 2018; Toumi et al., 2016). Affinity to K and Mg was described by the first principal component of PCA, whereas the second component was related to Ca uptake. Genotypes collected in group 1 were affined to K and excluded Mg. These genotypes have already reported high levels of K in a preliminary study, except for G.19 which reported an average K level (Bianchi et al., 2020). Group 1 also reported the largest differences of the affinity to K, Mg and Ca in response to the environment, showing a plastic behavior. On the other hand, group 3 was affined to Mg, with lower levels of K. In group 3, genotypes G.17, G.24, G.26, G.30, G.70, G.71 and G.75 confirmed the affinity to Mg proven in the first screening (Bianchi et al., 2020). Genotypes in group 3 showed an elastic behavior, maintaining similar levels of Mg and K in the two environments. Avoiding magnesium deficit, they could reduce the risk of desiccation of the rachis. Furthermore, for specific oenological goals these genotypes can be use

as rootstocks to limit the uptake of K, reducing the salinification of tartaric acid and maintaining acidity and freshness of wines (Kodur, 2011).

3.5. Uptake affinity to micronutrients

Differences among genotypes also occurred in terms of micronutrient levels. A cluster analysis was used to classify the genotypes according to their levels of Fe, Mg, Na and Zn. The plasticity of Fe in response to the environment was also considered (dFe). In the analysis four groups were identified (Figure 4 a): group 1 comprised 9 genotypes; group 2 and group 4 counted 4 genotypes each one; and group 3 collected 14 genotypes. Rootstock M2 was included in group 4. PCA approach was used to analyze the differences among groups (Figure 4 b). The first two principal components explained the 40.6% and 22.9% of total variance, respectively. The first principal component was positively affected by the levels of Fe, Mg and by dFe, whereas it was negatively affected by the level of Na. The second principal component was mainly related to the levels of Zn and Na. Group 1 was discriminated by others according to the first principal component, reporting high levels of Mn and Fe, as well as high dFe. The other three groups were discriminated by the second principal component. Levels of Zn and Na were high in group 2 and low in group 4. Group 3 reported average levels of the analyzed micronutrients.

Genotypes showed different affinities to micronutrients. Genotypes in group 1 were able to uptake high levels of Fe and Mn. They also showed a plastic behavior in Fe uptake in response to the environment, with higher levels of Fe in Arcagna. On the other hand, genotypes belonging to group 2 were affined to Zn and Na uptake. Genotypes collected in group 4 reported low levels of all the analyzed micronutrients, and they can be the most susceptible to a deficit of these elements. Genotypes in group 3 resulted particularly interesting for their balance in the levels of micronutrients, reporting no deficit for specific elements. Genotypes in this group have already shown affinity to Na (G.12, G.28, G.30, G.70, G.77, G.81, G.82), Mn (G.09, G.12, G.13, G.23, G.24, G.30, G.75, G.81) and Fe (G.07, G.13, G.19, G.23, G.24, G.82)

in the preliminary study on their nutritional status (Bianchi et al., 2020). In the same study, genotypes of group 1 confirmed high levels of Mn (G.16, G.25, G.30B, G.71, G.83) and Fe (G.16, G.30B, G76, G.83).

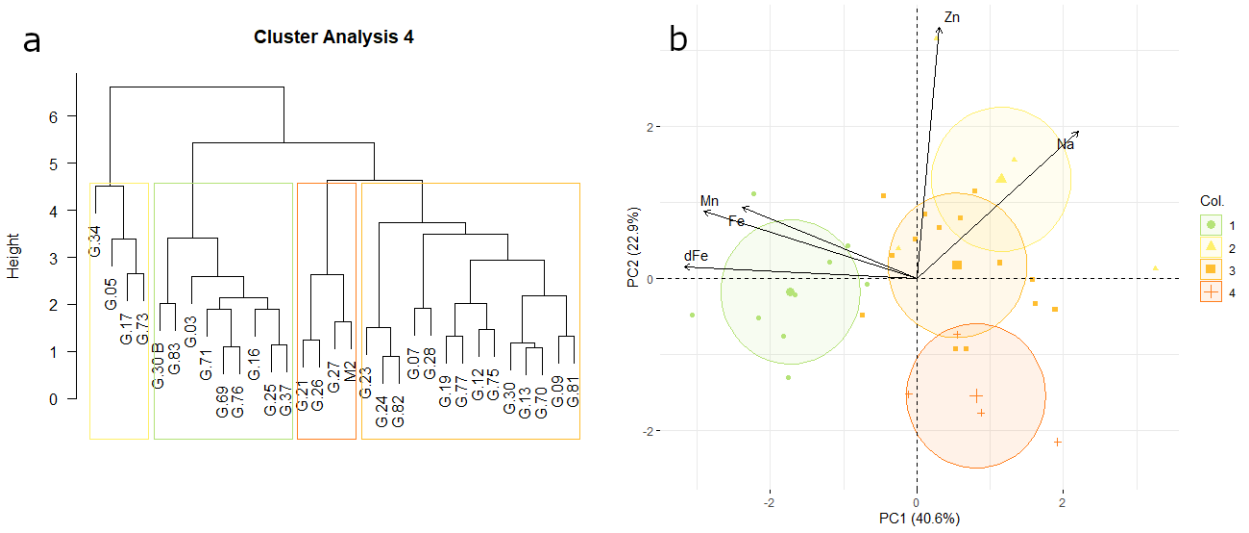


Figure 4. Classification of rootstock genotypes for micronutrients based on cluster analysis (a) and PCA (b). Na = sodium; Zn = zinc; Mn = magnesium; Fe = iron; dFe = plasticity of iron level

3.6. Promising rootstocks for adaptation to abiotic stresses

The groups identified in the four cluster analyses were compared using a Venn diagram (Figure 5). In the diagram, the set "water stress tolerance" considered the genotypes belonging to group 3 and 4 from cluster analysis 1. Similarly, the set "vigor and carbon assimilation" included groups 3 and 4 from cluster analysis 2. The sets "affinity to Mg" and "balance in micronutrients" considered groups 3 from cluster analysis 3 and cluster analysis 4, respectively. Overall, the genotypes in the diagram amounted to 23. A number of 10 genotypes belonged to both the sets "water stress tolerance" and "vigor and carbon assimilation". Among them, 5 genotypes (G.16, G.25, G.26, G.37 and G.70) were also collected in "affinity to Mg", whereas 6 genotypes (G.13, G.19, G.23, G.70, G.81 and G.82) were also included to "balance in

micronutrients". Only G.70 belonged to all the four sets and the control rootstock M2 was only included in the set "affinity to Mg".

Venn diagram was used to summarize the results from the four cluster analyses, in order to characterize the promising genotypes for all the analyzed traits. Except to G.24, all the genotypes with high photosynthetic activity (groups 3 and 4 in cluster analysis 2) also reported high tolerance to water stress, in terms of transpiration or water use efficiency (groups 3 and 4 in cluster analysis 1). The largest part of tolerant genotypes to water stress also reported affinity to Mg rather than K. Among the genotypes affined to Mg, only four of them (G.24, G.30, G.70 and G.75) reported a balance in micronutrients. In future studies, the promising genotypes identified in this work can be further investigated for drought tolerance and mineral nutrition in grafting combination with some cultivars of *Vitis vinifera*, under different environmental conditions. Furthermore, their tolerance to other abiotic stresses such as limestone in the soil could be tested. All the collected data can be used to characterize the new rootstocks and their fundamental role in the adaptation of viticulture to the new environmental scenario imposed by climate change.

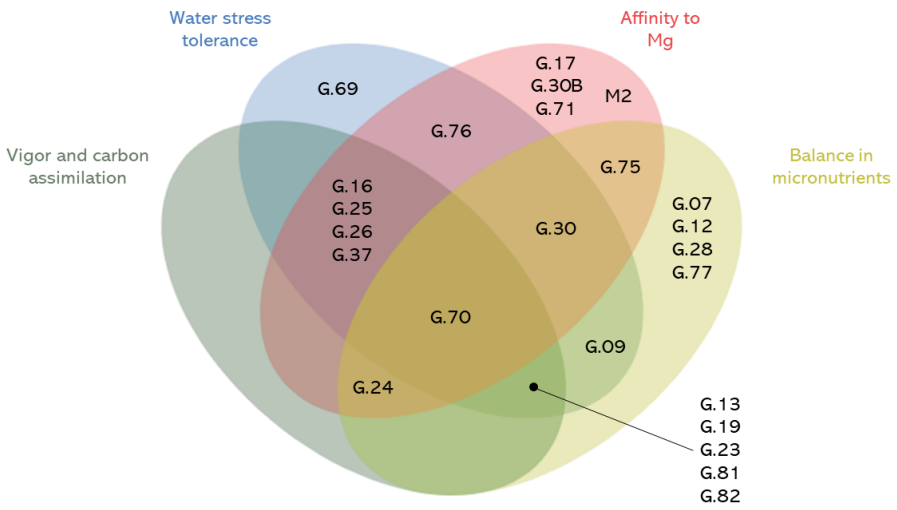
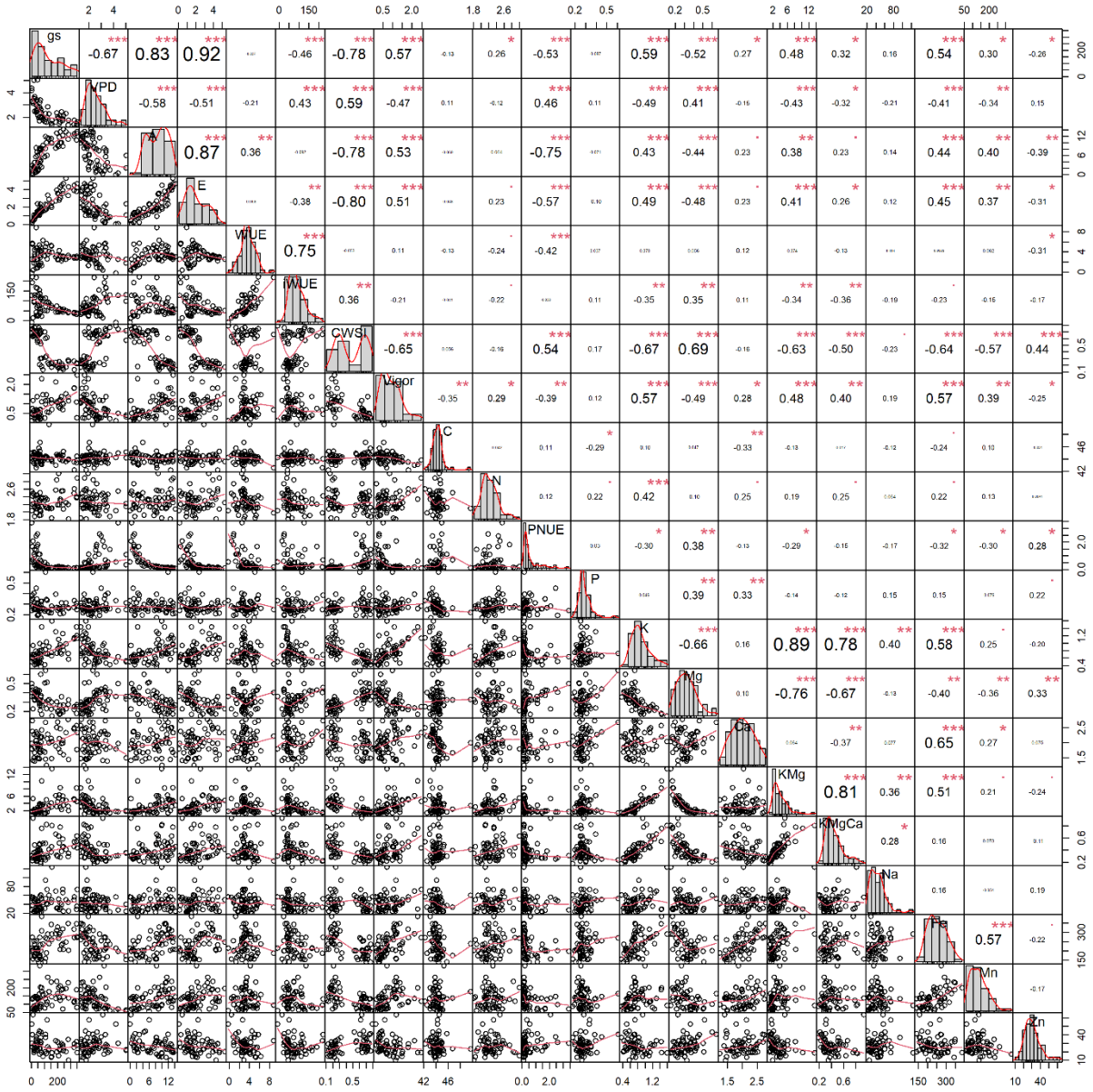


Figure 5. Venn diagram of promising rootstocks for abiotic stress tolerance

Figure S1. Pearson correlation among all the traits analyzed in the study. Significant correlations are considered for 0.01 < p ≤ 0.05 (*), 0.001 < p ≤ 0.01 (**) and p ≤ 0.001 (***)



References

Bardou, P., Mariette, J., Escudié, F., Djemiel, C., Klopp, C., 2014. SOFTWARE Open Access jvenn: an interactive Venn diagram viewer. BMC Bioinformatics 15, 1–7.

Bell, S.J., Henschke, P.A., 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. Aust. J. Grape Wine Res. 11, 242–295. https://doi.org/10.1111/j.1755-0238.2005.tb00028.x

Bellvert, J., Zarco-Tejada, P.J., Girona, J., Fereres, E., 2014. Mapping crop water stress index in a 'Pinot-noir' vineyard: Comparing ground measurements with thermal remote sensing imagery from an unmanned aerial vehicle. Precis. Agric. 15, 361–376. https://doi.org/10.1007/s11119-013-9334-5

Bianchi, D., Grossi, D., Simone Di Lorenzo, G., Zi Ying, Y., Rustioni, L., Brancadoro, L., 2020. Phenotyping of the "G series" Vitis hybrids: First screening of the mineral composition. Sci. Hortic. (Amsterdam). 264. https://doi.org/10.1016/j.scienta.2019.109155

Bianchi, D., Grossi, D., Tincani, D.T.G., Simone Di Lorenzo, G., Brancadoro, L., Rustioni, L., 2018. Multi-parameter characterization of water stress tolerance in Vitis hybrids for new rootstock selection. Plant Physiol. Biochem. 132, 333–340. https://doi.org/10.1016/j.plaphy.2018.09.018

Brancadoro, L., Reina, L.V.A., Scienza, A., 1994. Potassium content of grapevine during the vegetative period: The role of the rootstock. J. Plant Nutr. 17, 2165–2175. https://doi.org/10.1080/01904169409364872

Casanova-Gascón, J., Martín-Ramos, P., Martí-Dalmau, C., Badía-Villas, D., 2018. Nutrients Assimilation and Chlorophyll Contents for Different Grapevine Varieties in Calcareous Soils in the Somontano DO (Spain). Beverages 4, 90. https://doi.org/10.3390/beverages4040090

Cifre, J., Bota, J., Escalona, J.M., Medrano, H., Flexas, J., 2005. Physiological tools for irrigation scheduling in grapevine (Vitis vinifera L .) An open gate to improve water-use efficiency ? 106, 159–170. https://doi.org/10.1016/j.agee.2004.10.005

Corso, M., Vannozzi, A., Maza, E., Vitulo, N., Meggio, F., Pitacco, A., Telatin, A., D'Angelo, M., Feltrin, E., Negri, A.S., Prinsi, B., Valle, G., Ramina, A., Bouzayen, M., Bonghi, C., Lucchin, M., 2015. Comprehensive transcript profiling of two grapevine rootstock genotypes contrasting in drought susceptibility links the phenylpropanoid pathway to enhanced tolerance. J. Exp. Bot. 66, 5739–5752. https://doi.org/10.1093/jxb/erv274

Dal Santo, S., Zenoni, S., Sandri, M., De Lorenzis, G., Magris, G., De Paoli, E., Di Gaspero, G., Del Fabbro, C., Morgante, M., Brancadoro, L., Grossi, D., Fasoli, M., Zuccolotto, P., Tornielli, G.B., Pezzotti, M., 2018. Grapevine field experiments reveal the contribution of genotype, the influence of environment and the effect of their interaction (G×E) on the berry transcriptome. Plant J. 93, 1143–1159. https://doi.org/10.1111/tpj.13834

Frioni, T., Biagioni, A., Squeri, C., Tombesi, S., Gatti, M., Poni, S., 2020. Grafting cv. grechetto gentile vines to new m4 rootstock improves leaf gas exchange and water status as compared to commercial 1103p rootstock. Agronomy 10, 1–14. https://doi.org/10.3390/agronomy10050708

Gautier, A.T., Cookson, S.J., Lagalle, L., Ollat, N., Marguerit, E., 2020. Influence of the three main genetic backgrounds of grapevine rootstocks on petiolar nutrient concentrations of the scion, with a focus on phosphorus. Oeno One 54, 1–13. https://doi.org/10.20870/oeno-one.2020.54.1.2458

Grant, O.M., Chaves, M.M., Jones, H.G., 2006. Optimizing thermal imaging as a technique for detecting stomatal closure induced by drought stress under greenhouse conditions. Physiol. Plant. 127, 507–518. https://doi.org/10.1111/j.1399-3054.2006.00686.x

Ibacache, A., Verdugo-Vásquez, N., Zurita-Silva, A., 2019. Rootstock: Scion combinations and nutrient uptake in grapevines. Fruit Crop. Diagnosis Manag. Nutr. Constraints 297–316. https://doi.org/10.1016/B978-0-12-818732-6.00021-6

Idso, S.B., Jackson, R.D., Pinter Jr., P.J., Reginato, R.J., Hatfield, J.L., 1981. Normalizing the stress-degree-day parameter for environmental variability. Agric. Meteorol. 24, 45–55. https://doi.org/https://doi.org/10.1016/0002-1571(81)90032-7

Jones, H.G., 1999. Use of thermography for quantitative studies of spatial and temporal variation of stomatal conductance over leaf surfaces. Plant, Cell Environ. 22, 1043–1055. https://doi.org/10.1046/j.1365-3040.1999.00468.x

Jones, H.G., Stoll, M., Santos, T., Sousa, C. De, Chaves, M.M., Grant, O.M., 2002. Use of infrared thermography for monitoring stomatal closure in the ® eld : application to grapevine 53, 2249–2260. https://doi.org/10.1093/jxb/erf083

Keller, M., 2005. From the ASEV Soil Environment and Vine Mineral Nutrition Symposium: Deficit irrigation and vine mineral nutrition. Am. J. Enol. Vitic. 56, 267–283.

Kodur, S., 2011. Effects of juice pH and potassium on juice and wine quality, and regulation of potassium in grapevines through rootstocks (Vitis): A short review. Vitis - J. Grapevine Res. https://doi.org/10.1097/MPH.0000000000000505

Leão, P.C. de S., Chaves, A.R. de M., 2020. Agronomic responses of grapevine 'Chenin Blanc' as a function of training systems and rootstocks. Sci. Agric. 78, 1–7. https://doi.org/10.1590/1678-992x-2018-0413

Marastoni, L., Lucini, L., Miras-Moreno, B., Trevisan, M., Sega, D., Zamboni, A., Varanini, Z., 2020. Changes in physiological activities and root exudation profile of two grapevine rootstocks reveal common and specific strategies for Fe acquisition. Sci. Rep. 10, 1–12. https://doi.org/10.1038/s41598-020-75317-w

Migicovsky, Z., Cousins, P., Jordan, L.M., Myles, S., Striegler, R.K., Verdegaal, P., Chitwood, D.H., 2021. Grapevine rootstocks affect growth-related scion phenotypes. Plant Direct 5, 1–11. https://doi.org/10.1002/pld3.324

Migliaro, D., De Lorenzis, G., Simone Di Lorenzo, G., De Nardi, B., Gardiman, M., Failla, O., Brancadoro, L., Crespan, M., 2019. Grapevine non-vinifera genetic diversity assessed by SSR markers as a starting-point for new rootstock breeding programs. Am. J. Enol. Vitic. in press.

Pinto, D.L.P., Brancadoro, L., Dal Santo, S., De Lorenzis, G., Pezzotti, M., Meyers, B.C., Pè, M.E., Mica, E., 2016. The influence of genotype and environment on small RNA profiles in grapevine berry. Front. Plant Sci. 7. https://doi.org/10.3389/fpls.2016.01459

R Core Team, 2021. R: A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria.

Rossdeutsch, L., Schreiner, R.P., Skinkis, P.A., Deluc, L., 2021. Nitrate Uptake and Transport Properties of Two Grapevine Rootstocks With Varying Vigor. Front. Plant Sci. 11, 1–15. https://doi.org/10.3389/fpls.2020.608813

Rustioni, L., Bianchi, D., 2021. Drought increases chlorophyll content in stems of Vitis interspecific hybrids. Theor. Exp. Plant Physiol. 33, 69–78. https://doi.org/10.1007/s40626-021-00195-0

Toumi, M., Nedjimi, B., Halitim, A., Garcia, M., 2016. Effects of K-Mg ratio on growth and cation nutrition of Vitis vinifera L. cv. “Dattier de Beiruth” grafted on SO4 rootstock. J. Plant Nutr. 39, 904–911.

Verdugo-Vásquez, N., Gutiérrez-Gamboa, G., Díaz-Gálvez, I., Ibacache, A., Zurita-Silva, A., 2021. Modifications induced by rootstocks on yield, vigor and nutritional status on vitis vinifera cv syrah under hyper-arid conditions in Northern Chile. Agronomy 11. https://doi.org/10.3390/agronomy11050979

Zhang, L., Marguerit, E., Rossdeutsch, L., Ollat, N., Gambetta, G.A., 2016. The influence of grapevine rootstocks on scion growth and drought resistance. Theor. Exp. Plant Physiol. 28, 143–157. https://doi.org/10.1007/s40626-016-0070-x

Zombardo, A., Crosatti, C., Bagnaresi, P., Bassolino, L., Reshef, N., Puccioni, S., Faccioli, P., Tafuri, A., Delledonne, M., Fait, A., Storchi, P., Cattivelli, L., Mica, E., 2020. Transcriptomic and biochemical investigations support the role of rootstock-scion interaction in grapevine berry quality. BMC Genomics 21, 1–20. https://doi.org/10.1186/s12864-020-06795-5

PART III

GENETIC DIVERSITY AND POPULATION STRUCTURE IN A *VITIS* SPP. CORE COLLECTION INVESTIGATED BY SNP MARKERS

Abstract

Single nucleotide polymorphism (SNP) genotyping arrays are powerful tools to measure the level of genetic polymorphism within a population. The coming of next-generation sequencing technologies led to identify thousands and millions of SNP loci useful to assess the genetic diversity. A *Vitis* genotyping array, containing 18k SNP loci, has been developed and used to detect genetic diversity of *Vitis vinifera* germplasm. So far, this array was not validated on non-*vinifera* genotypes used as grapevine rootstocks. In this work, a core collection of 70 grapevine rootstocks, composed by individuals belonging to *Vitis* species not commonly used in the breeding programs, was genotyped using the 18k SNP genotyping array. SNPs results were compared to the established SSR (Simple Sequence Repeat) markers, in terms of heterozygosity and genetic structure of the core collection. Genotyping array has proved to be a valuable tool for genotyping of grapevine rootstocks, with more than 90% of SNPs successfully amplified. Structure analysis detected a high degree of admixed genotypes, supported by the complex genetic background of non-*vinifera* germplasm. Moreover, SNPs clearly differentiated non-*vinifera* and *vinifera* germplasm. These results represent a first step in studying the genetic diversity of non-conventional breeding material that will be used to select rootstocks with high tolerance to limiting environmental conditions.

1. Introduction

Vitis vinifera, the most important economic fruit species in the modern world, is usually grown on rootstocks (a mixture of non-*vinifera* grapevine species and hybrids) due to its susceptibility to phylloxera attack, a homopteran insect (*Daktulosphaira vitifoliae* Fitch) that feeds on the *V. vinifera* roots (Granett et al., 2001). Nevertheless, rootstocks play a key role in the adaptation of vines to the environmental conditions, affecting the production and the quality of grape and wines. Several studies report an effect

of rootstocks on limestone tolerance (Brancadoro et al., 1995; Ollat et al., 2016), nutrients uptake (Köse et al., 2016) and water stress tolerance (Corso and Bonghi, 2014). Although relevant efforts in grapevine rootstock selection were made on the turn of the 20th century, only few genotypes found a large spread in vineyards and nowadays more than the 90% of *V. vinifera* varieties are grown grafted onto less than 10 rootstocks (Keller, 2015), with negative consequences on the tolerance to biotic and abiotic stresses (Ollat et al., 2016) and on genetic diversity. It was already demonstrated that the genetic background of rootstock germplasm is narrow, traceable in a limited number of species. Based on Riaz et al (2019) results, three genotypes of three *Vitis* species contributed to the 39% of rootstock genetic diversity. In this context, rootstock collections represent a relevant starting-point for new breeding programs, aimed to select new promising genotypes able to face the environmental challenges of modern viticulture.

Whilst much work has been performed to study the genetic diversity of large *V. vinifera* germplasm collections (Bacilieri et al., 2013; Cipriani et al., 2010; Emanuelli et al., 2013; Laucou et al., 2011), few information is available on genetic identification of non-*vinifera* germplasm (Crespan et al., 2009; Cseh et al., 2006; De Andrés et al., 2007; Dzhambazova et al., 2007; Jahnke et al., 2014; Riaz et al., 2019; Sefc et al., 1998; Upadhyay et al., 2007). Recently, the grapevine rootstock collection of University of Milan (Italy), composed of 379 accessions and including the largest part of the rootstock germplasm currently available worldwide, has been genotyped by SSR (Simple Sequence Repeats) to investigate genetic diversity, infer population structure, analyze pedigrees and design a core collection (Migliaro et al., 2019). Molecular analysis identified 232 unique genotypes with a high level of admixture and a narrow genetic background. Among the 232 unique genotypes, 70 genotypes were selected to be included in a core collection designed to capture the entire allelic richness of the non-*vinifera* collection. Some of these genotypes are *Berlandieri × rupestris* and *Berlandieri* × *riparia* varieties (7%), some other have *labrusca* and *vinifera* parentage (30%), but most of them are individuals not still genetically identified or poorly

characterized by the ampelographic and agronomic point of view, making this core collection even more interesting as new materials for the further breeding programs.

So far, SSR markers were one of the most reliable and robust tool used for the genetic characterization of *vinifera* and non-*vinifera* germplasm, widely adopted for their high degree of information provided by the large number of detected alleles per locus (Crespan et al., 2009; Cseh et al., 2006; De Andrés et al., 2007; Dzhambazova et al., 2007; Jahnke et al., 2014; Lin and Walker, 1998; Migliaro et al., 2019; Riaz et al., 2019; Sefc et al., 1998; Upadhyay et al., 2007). Recently, SNP (Single Nucleotide Polymorphism) markers have rapidly gained high popularity in the scene of *V. vinifera* molecular genetics (Cabezas et al., 2011; De Lorenzis et al., 2019; Emanuelli et al., 2013; Laucou et al., 2018; Ruffa et al., 2016). The number of SNP loci used to study the genetic diversity increased as changed the technologies to detect them in the genome. Prior to the emergence of next-generation sequencing (NGS) technologies, SNP sets included tens (Cabezas et al., 2011) or hundreds (Emanuelli et al., 2013) loci. With the coming of NGS technologies, the number of SNP loci rapidly increased up to thousands: 10k (Myles et al., 2010), 18k (Laucou et al., 2018) and 37k (Marrano et al., 2017) SNPs. Their popularity is mainly due to the abundance in the genome (they are the most abundant polymorphisms among the individuals of the same species), amenability to high-throughput detection and high reproducibility, since normalization with reference varieties is not required (De Lorenzis et al., 2019). These molecular markers are widely used to study genetic diversity and to dissect complex traits *via* QTLs (Quantitative Traits Loci) or GWASs (Genome-Wide Association Studies) for breeding program (Laucou et al., 2018).

The most used SNP set is the Vitis18kSNP array, which was set up by the GrapeReSeq Consortium, re-sequencing the genome of 47 *V. vinifera* genotypes and 18 American genotypes, belonging to the species *Vitis aestivalis, Vitis Berlandieri, Vitis cinerea, Vitis labrusca, Vitis lincecumii* and *Muscadinia rotundifolia*. In this project, a total of 18,071 SNPs were selected, a third of which (4,510 SNPs) identified in Northern American species genome (Laucou et al., 2018). Several studies validated the 18k SNP set for

the evaluation of genetic diversity in *V. vinifera* (De Lorenzis et al., 2019, 2017; Degu et al., 2015; Mercati et al., 2016; Ruffa et al., 2016; Sunseri et al., 2018), but Vitis18kSNP array could also represent a potential effective tool for rootstock characterization, due to the consistent number of SNPs detected in Northern American species genome. The aim of this study was to evaluate the goodness of the non-*vinifera* germplasm core collection (Migliaro et al., 2019), representing the whole genetic diversity of grapevine rootstock collection housed at the University of Milan, by Vitis18kSNP array. SNP and SSR profiles have been compared for their usefulness to detect genetic diversity and population structure.

2. Material and methods

2.1. Plant material

Seventy *Vitis* ssp. genotypes, belonging to a core collection identified in Migliaro et al (2019) were genotyped using 18k SNP. The pedigree of 31 genotypes is unknown and the others are 31 hybrids genotypes and 8 traced to pure *Vitis* species (Table 1). The core collection is located in Torrazza Coste, Pavia, Italy (44.984783N, 9.089038E, 133m a.s.l.).

2.2. DNA extraction and SNP genotyping

One hundred milligrams of freeze-fresh young leaf tissue were ground with liquid nitrogen and genomic DNA was extracted using NucleoSpin® Plant II (MACHEREY-NAGEL – Düren, Germany), according to manufacturer's protocol. Concentration of DNA and its quality were checked by electrophoresis on agarose gel, by spectroscopy (260/230 and 260/280 ratios) using NanoDrop Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and the Quant-iT dsDNA HS assay kit for Qubit 3.0 Fluorometer (Thermo Fisher Scientific). SNP genotyping was performed on 200 ng of genomic DNA per sample using the Vitis18kSNP array (Illumina Inc., San Diego, California), containing 18,071 SNPs, by the laboratory of Fondazione Edmund Much (San Michele all'Adige, Trento, Italy).

Table 1. List of the vine rootstock core collection. For each accession is reported the genotype name and the breeding material (Migliaro et al., 2019)

Accession ID	Genotype	Pedigree
1	101.14 Millardet et de Grasset	*V. riparia x V. rupestris*
4	110 Richter	*V. rupestris x V. Berlandieri*
6	1202 C	*V. vinifera x V. rupestris*
10	161.49 Couderc	*V. Berlandieri x V. riparia*
16	3309 Couderc	*V. riparia x V. rupestris*
17	333 Ecole de Montpellier or Tisserand	*V. vinifera x V. Berlandieri*
19	41 B Millardet et de Grasset	*V. vinifera x V. Berlandieri*
28	Cosmo 10	*V. Berlandieri x V. riparia*
29	Dog Ridge	*V. rupestris x V. candicans*
38	Isabella	*V. labrusca x V. vinifera*
39	Jacquez	*V. aestivalis x V. vinifera*
40	Geilweilerhof V.348	*V. vinifera*
41	Kober 5BB	*V. Berlandieri x V. riparia*
42	LN 33 or LLYOD'S NUMBER 33	*V. riparia x V. longii x V. vinifera*
43	Malegue 44.53	*V. riparia x V. cordifolia x V. rupestris*
46	Salt Creek	Unknown
55	*Vitis riparia* Fabre	*V. riparia*
56	*Vitis riparia* Gloire de Montpellier	*V. riparia*
68	Genotype 01	Unknown
69	Genotype 02	*V. Berlandieri x V. riparia x V. cinerea*
70	Genotype 03	*V. Berlandieri x V. riparia x V. cordifolia x V. rupestris*
71	Genotype 04	Unknown
81	Genotype 15	*V. Berlandieri x V. riparia x V. rupestris*
83	Genotype 17	*V. Berlandieri x V. riparia*
84	Genotype 18	Unknown
96	Genotype 29	*V. riparia x V. vinifera x ?*
99	Genotype 33	*V. riparia x V. longii x ?*
112	Genotype 46	Unknown
114	Genotype 48	Unknown
116	Vitis riparia Lombard	*V. riparia*
118	Genotype 52	Unknown
120	Genotype 54	Unknown
121	Genotype 55	Unknown
125	Vitis labrusca Muncy	*V. labrusca*
126	Genotype 60	Unknown

127	Genotype 61	Unknown
129	Genotype 63	*V. Berlandieri x V. riparia x ?*
132	Genotype 66	Unknown
134	Genotype 68	Unknown
136	Genotype 70	*V. vinifera x V. Berlandieri x V. riparia x V. candicans*
140	Genotype 74	*V. Berlandieri x V. riparia*
150	Kober 125 AA	*V. Berlandieri x V. riparia*
152	Genotype 86	Unknown
153	Genotype 87	Unknown
154	Genotype 88	Unknown
155	Genotype 89	Unknown
161	Genotype 95	Unknown
162	Genotype 96	Unknown
163	Genotype 97	Unknown
164	Genotype 98	Unknown
166	Genotype 100	Unknown
169	Genotype 103	Unknown
171	Genotype 105	Unknown
172	Genotype 106	*V. riparia x V. rupestris x ?*
173	Genotype 107	Unknown
176	Genotype 110	Unknown
177	Genotype 111	Unknown
184	Genotype 118	Unknown
187	143 B Millardet et De Grasset	*V. vinifera x ?*
192	202-4 Millardet et De Grasset	*V. riparia x V. longii x ?*
198	33 Ecole de Montpellier	*V. Berlandieri x V. riparia*
199	420 B Millardet et De Grasset	*V. Berlandieri x V. riparia*
206	Dufour 11 F	*V. riparia x V. rupestris*
214	Genotype 148	Unknown
215	*Vitis riparia* Sericea	*V. riparia*
216	*Vitis riparia* Sombre	*V. riparia*
217	*Vitis riparia* Tomenteux	*V. riparia*
223	Genotype 157	*V. labrusca x V. riparia x V. rupestris*
224	Genotype 158	Unknown
231	Genotype 165	Unknown

2.3. Data analysis

For SNP data, samples with call quality value (p50GC) lower than 0.54 and loci with GenTrain (GT) score value lower than 0.6 (De Lorenzis et al., 2015) were filtered from the dataset, as well as those with more than 20% of missing data and monomorphic loci. Number of alleles and their frequency, observed heterozygosity (H_o), expected heterozygosity (H_e) and minor allele frequency (MAF) were assessed using PEAS V1.0 software (Xu et al., 2010). In order to identify the minimum number of SNP loci able to explain the observed diversity in our data set, the accumulation curve approach implemented in the package *poppr* and AMaCAID for R software (R Core Team, 2019) were used. The results were viewed as a barplot.

The genetic structure of the core collection was analyzed using LEA package (Frichot and Franc, 2015) of R software by varying the number of ancestral genetic groups (K) from 1 to 10 in ten repetition runs for each K value. The Principal Components Analysis (PCA) was run by using *adegenet* package of R software (Jombart, 2008) and the first two components values were plotted on a 2-D scatterplot. The genetic distance among genotypes was set up on Nei's distance (Nei, 1972), performed in PEAS, and the clustering was performed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Circular dendrogram was plotted using MEGA 7.0 software (Kumar et al., 2016). The validation of clustering results was performed considering the pairwise Nei's genetic distance. The values were calculated using nei.dist function of R software.

In order to compare SNP and SSR results, the Migliaro et al (2019) core collection SSR profiles were used to detect the number of alleles, H_o and H_e, using the GenAlEx 6.5 software (Peakall and Smouse, 2006). Structure analysis was performed using STRUCTURE 2.0 software (Pritchard et al., 2000). Burn-in and MCMC (Markov Chain Monte Carlo) values were set on 100,000 replicate runs, the number of clusters (K) varied from 1 to 10, and 10 replicate runs were carried out to quantify the variation of the likelihood for each K. The most likely K value was chosen according to Evanno et al. (2005) method. PCA was performed using *adegenet* package and the UPGMA circular dendrogram was drawn using MEGA 7.0

software on the Nei's distance matrix assessed by GenAlEx 6.5. Clustering results were validated by pairwise Nei's genetic distance (GenAlEx 6.5).

To investigate the genetic relationship between non-*vinifera* and *vinifera* germplasm, our dataset was merged with those reported in De Lorenzis et al (2019), Laucou et al (2018) and De Lorenzis et al (2015). The final dataset resulted in 1044 genotypes. PCA and parentage analysis were performed on the new dataset. Parentage analysis was performed to account for first-degree (parent-offspring) relationships among core collection genotypes and *vinifera* genotypes. The analysis was carried out by PLINK 1.07 software, calculating the identity-by-descent (IBD). The following parameters were set: MAF = 0.1 and r^2 of linkage disequilibrium = 0.05. The parent-offspring (PO) relationships among genotypes were assigned based on Z0 (probability of sharing 0 IBD allele identical-by-descent), Z1 (probability to share 1 IBD allele), Z2 (probability to share 2 IBD alleles), and PI-HAT (the relatedness measure measured as PI-HAT = P (IBD = 2) + 0.5 × P (IBD = 1)) parameters. To assign the PO relationships, the experimental values were compared to the theoretical ones: Z0 and Z2 values similar to 0, Z1 similar to 1 and PI-HAT to 0.5. Only relationships with core collection genotypes will be discussed.

3. Results

Seventy *Vitis* ssp. genotypes of a grapevine rootstock core collection (Migliaro et al., 2019) were genotyped using the Vitis18kSNP array. Filtering the genetic profiles for a call quality value (p50GC) higher than 0.54, 66 out of 70 genotypes were retained, probably due to low quality of DNA. Accessions 152, 192, 216, and 231 were not considered for further analysis. The number of SNP loci with GT score value higher than 0.6 was 16,495 (91.3% of the total) and the loci showing a percentage of missing data lower than 20% amounted to 15,688 (86.8%). Finally, 1,508 monomorphic SNPs were removed, obtaining a final dataset of 14,180 SNPs (78.5%) suitable for genetic characterization of the analyzed genotypes. The final dataset accounted for 11,717 *vinifera* SNPs (around 86% of SNPs identified in the *V. vinifera* genome) and

2,463 non-*vinifera* SNPs (around 55% of SNPs identified in the genome of other species). Among the non-*vinifera* SNPs, the *M. rotundifolia* SNPs showed the lowest percentage of loci successfully amplified (19%), while *V. Berlandieri* SNPs showed the highest (66%). Two R packages (*poppr* and AMaCAID) were used to identify the minimum number of loci able to distinguish the 66 genotypes (100% of genetic diversity). The genotype accumulation curves reported in Figure 1 indicated that randomly sampling 64 or 49 SNPs, respectively based on the simulation performed with *poppr* package (Figure 1a) and AMaCAID package (Figure 1b), the 100% of core collection genetic diversity is detected.

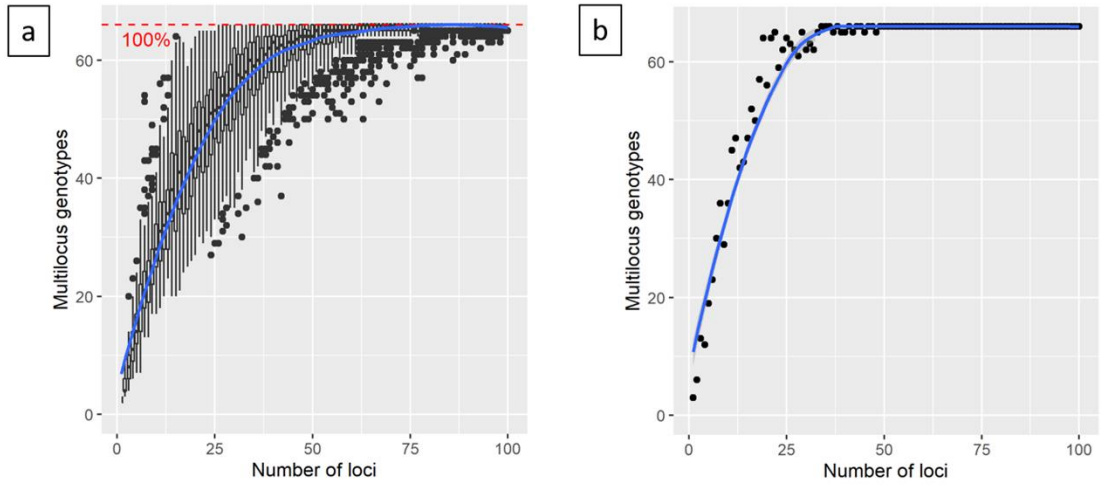


Figure 1. Genotype accumulation curve of 66 grapevine rootstock accessions, genotyped over 14180 SNP loci, obtained with *poppr* package (a) and AMaCAID package (b). Value on Number of loci axis was limited to 100. The red dashed line represents 100% of the total observed genotypes

Genetic diversity of core collection was evaluated using both SNP and SSR molecular markers. The average number of alleles for SNPs is 1.80 and the minor allele frequencies (MAF) is equal to 0.10. The percentage of SNPs reporting MAF higher than 0.05 was about 57%. For the largest part of SNPs (10,162), no difference ($p \leq 0.05$) was found between H_o and H_e values. In the other loci, H_o was lower than H_e in 3,584 SNPs and higher in the remaining 434 SNPs. Both molecular markers showed similar H_o and H_e values (0.143 *vs* 0.157 and 0.823 *vs* 0.879, respectively for SNP and SSR loci) as reported in Table 2.

Table 2. Observed (H_o) and expected (H_e) heterozygosity of the vine rootstock core collection and the ancestral groups identified by the structure analysis, based on SNP and SSR profiles. N = number of genotypes

Marker	Plant material	N	H_o	H_e
SNP	Core collection	66	0.143	0.157
	Group 1	8	0.100	0.095
	Group 2	16	0.303	0.280
	Group 3	15	0.125	0.099
	Group 4	27	0.071	0.067
SSR	Core collection	70	0.823	0.879
	Group 1	10	0.882	0.730
	Group 2	24	0.779	0.771
	Group 3	36	0.836	0.891

In order to identify ancestral population in the analyzed core collection a structure analysis was performed with both SNP and SSR profiles. The analyses estimated the most likely number of ancestral populations at K = 4 for SNPs and K = 3 for SSRs. A bar plot representation of the two structures is shown in Figure 2. Based on SNP profiles, the percentage of admixed genotypes (reporting the predominant K values lower than 0.8) was about 53% (Figure 2a). The SNP-group 1 was the smallest group, where only 12% of genotypes were included, whereas SNP-group 4 was the biggest (41%). *V. Berlandieri* x *V. riparia* genotypes and those having an unknown pedigree were grouped in all the four SNP-groups. The majority of unknown genotypes belonged to SNP-group 4 (11 out of 29). In the SNP-group 1, only three known genotypes were clustered, a *V. Berlandieri* x *V. riparia* (ID 83), a *V. vinifera* x *V. rupestris* (ID 6) and a *V. labrusca* (ID 125). In the SNP-group 2, we had genotypes with other species in their genetic background (such as *V. cordifolia, V. labrusca, V. rupestris* and *V. vinifera*), as well as in the SNP-group 3, where

genotypes with *V. candicans, V. labrusca, V. longii* and *V. rupestris* in their pedigree were clustered. Pure *V. riparia* genotypes were assigned to the SNP-group 4 together with *V. candicans, V. cinerea, V. longii, V. rupestris* and *V. vinifera* genotypes. Based on SSR profiles, 31% of genotypes were admixed and 69% of genotypes were grouped in three ancestral groups (10% of genotypes in SSR-group 1, 23% in SSR-group 2 and 36% in SSR-group 3) (Figure 2b). The *V. Berlandieri* x *V. riparia* genotypes were mainly grouped in ancestral SSR-group 1, whereas *V. rupestris* genotypes were assigned to SSR-group 2 and *V. vinifera* to SSR-group 3.

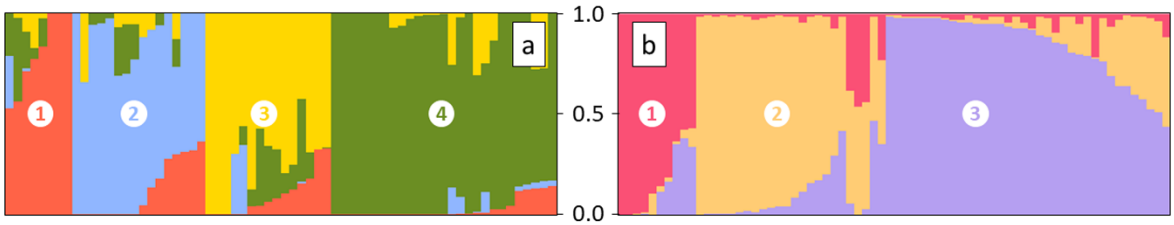


Figure 2. Genetic structure of the grapevine rootstock core collection (66 genotypes) defined using 14k SNP (**a**) and 17 SSR (**b**) molecular markers. Ancestral groups are reported in different colors

PCA was performed to identify correlations among structure groups (Figure 3). Regarding SNPs, the first two principal components (PCs) explained 37% of the total variability. PCA was able to discriminate among the ancestral groups identified by the structure analysis. PC1 separated SNP-group 2 from the others, whereas PC2 highlighted the differences among SNP-group 1, 3 and 4. SNP-group 2 was the group showing the highest diversity, with four out of five not-admixed genotypes (ID 39, 40, 71 and 120) clustered aside from the other genotypes (Figure 3a). Performing PCA on SSR profiles, the first two PCs described the 49% of the total variability. As for SNPs, ancestral groups were discriminated by PCA. SSR-group 2 and 3 were separated along the PC1 and SSR-group 1 along the PC2 (Figure 3b). Admixed genotypes were generally placed in between the genotypes of each ancestral group, independently from the used molecular markers.

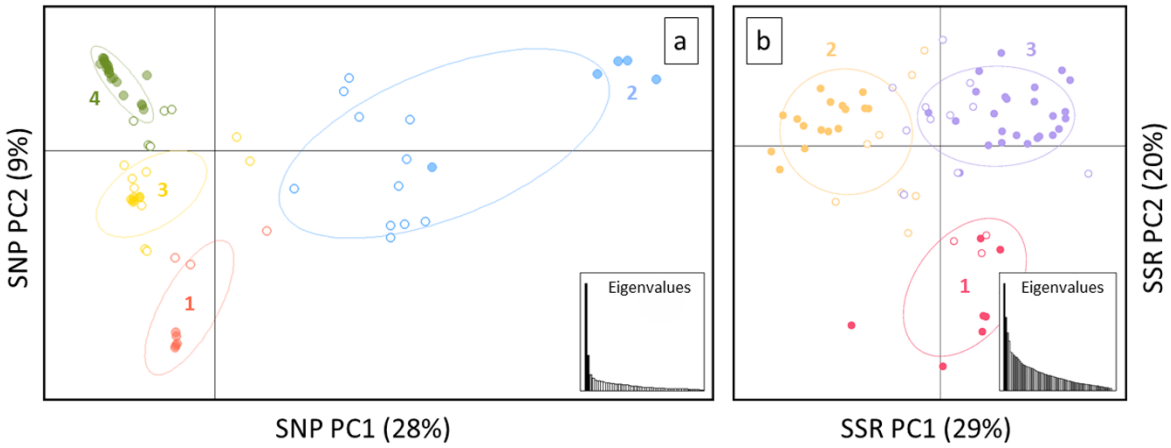


Figure 3. Principal component analysis (PCA) of the grapevine rootstock core collection (66 genotypes), defined using 14k SNP (**a**) and 17 SSR (**b**) molecular markers. Genotypes are classified according to the ancestral groups identified in the structure analysis. White filled dots are admixed genotypes. Colors are according to ancestral groups reported in Figure 2

Genetic distance among genotypes of the grapevine rootstock core collection is reported in Figure 4. Based on the SNP analysis, the genotypes showed different levels of similarity, ranging from 85 to 98%. Using the threshold value of 95% for similarity index, two main groups were identified, one grouping genotypes belonging to the structure SNP-group 1, 3 and 4 and the other genotypes of SNP-group 2. In each cluster, genotypes were clustered according to the ancestral group they belong to. Similarly to the PCA analysis, genotypes of the SNP-group 2 were the most different, with samples 39, 40, 71 and 120 clustering as outgroups. SSR dendrogram showed similarity values ranging from 75 to 95%. Two main clusters were identified (threshold value = 83%), one grouping mainly genotypes of the ancestral SSR-groups 1 and 2 and the other the genotypes of SSR-group 3. Each genotype was clustered according to their ancestral group. Genotypes of SSR-group 3 were the most different (Figure 4b).

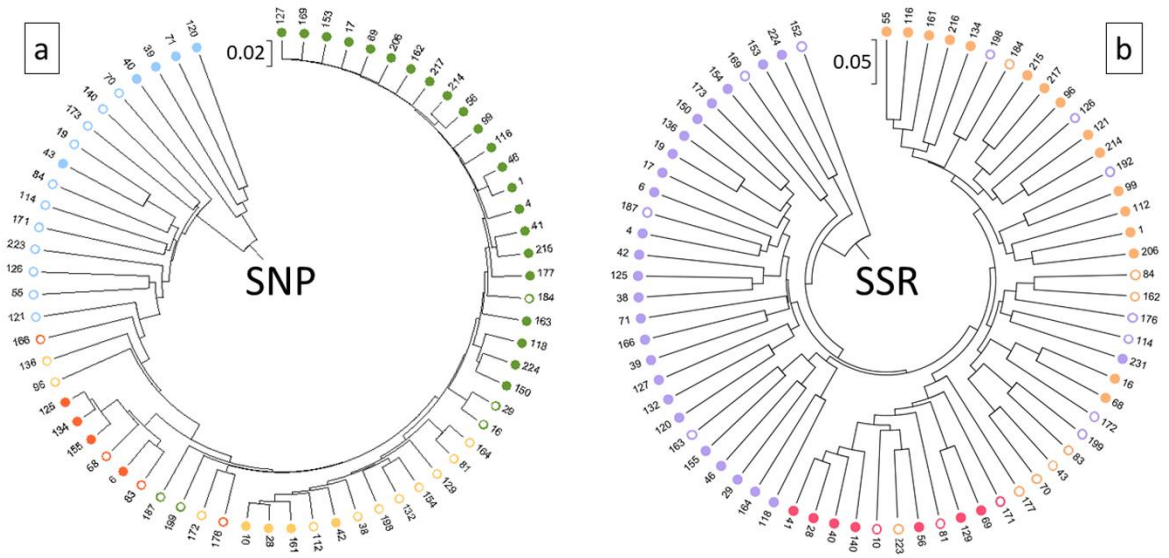


Figure 4. Clustering of the grapevine rootstock core collection (66 genotypes), according to Nei's distance, defined using 14k SNP (**a**) and 17 SSR (**b**) molecular markers. White filled dots are admixed genotypes. Colors are according to ancestral groups reported in Figure 2

H_o and H_e values per each ancestral group were similar each other for both SNP and SSR groups, with H_o, generally, higher than H_e (Table 2). About SNPs, SNP-group 2 and SNP-group 4 were the groups showing the highest (0.303 *vs* 0.280) and lowest (0.071 *vs* 0.067) H_o and H_e values, respectively. The highest pairwise Nei's genetic distance was detected for the combination SNP-group 2 – SNP-group 3 (0.197) and the lowest for the combination SNP-group 3 – SNP-group 4 (0.037). About SSRs, H_o values ranged from 0.779 (SSR-group 2) to 0.882 (SSR-group 1) and H_e values from 0.730 (SSR-group 1) to 0.891 (SSR-group 3). Pairwise Nei's genetic distance showed the highest value for the combination SSR-group 2 – SSR-group 3 (0.674), and the lowest for combination SSR-group 1 – SSR-group 2 (0.484).

In order to highlight the genetic relationship between non-*vinifera* and *vinifera* germplasm (De Lorenzis et al., 2015, 2019; Laucou et al., 2018), a second dataset was built, accounting for 1044 genotypes and 6375 SNPs. PCA results were plotted on a scatter plot (Figure 5). The first two principal components (PCs) explained the 23% of total genetic variability (19 and 4% for PC1 and PC2, respectively). The genotypes were discriminated along the PC1 in two well distinct groups: i) vinifera group; ii) non-vinifera

group. Moreover, some genotypes belonging to the non-*vinifera* dataset overlapped with the *vinifera* genotypes (ID 39, 40, 71, and 120, two genotypes with a *vinifera* background and two genotypes with an unknown pedigree), and some other were strongly differentiated from the rest of non-*vinifera* genotypes. The last group of genotypes were mainly genotypes with a *riparia* background. Some *vinifera*-backgrounded genotypes and some unknown genotypes were included as well.

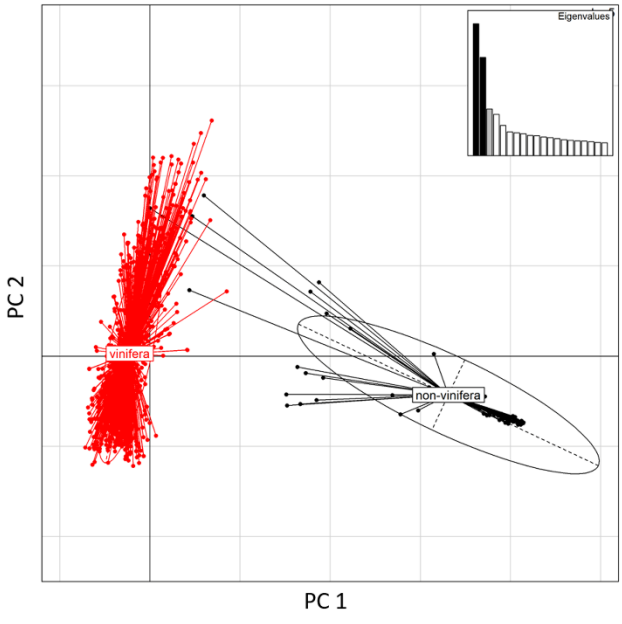


Figure 5. Principal component analysis (PCA) of non-vinifera (66 genotypes) and vinifera genotypes (978 genotypes (De Lorenzis et al., 2015, 2019; Laucou et al., 2018)), defined using 6k SNP molecular markers

On the same dataset, parentage analysis was performed to account for first- and second-degree relationships among core collection genotypes and *vinifera* germplasm (De Lorenzis et al., 2015, 2019; Laucou et al., 2018). Only one PO relationship was observed in the new dataset: ID 40 (Geilweilerhof V.348) = Pinot noir x Riesling. The experimental values for relationship parameters were as follow: i) ID 150 – Pinot noir, Z0 = 0.012, Z1 = 0.923, Z2 = 0.092, PI_HAT = 0.553; ii) ID 150 – Riesling, Z0 = 0.035, Z1 = 0.898, Z2 = 0.058, PI_HAT = 0.507.

4. Discussion

4.1. The 18k SNP genotyping array is a suitable tool to characterize non-vinifera germplasm

Increasing efforts in new rootstock selection require effective tools able to investigate the diversity in the genus *Vitis*. Recently, a 18k SNP genotyping array has been developed, containing 13,561 SNPs isolated from *V. vinifera* and 4,510 SNPs from other *Vitis* species (Laucou et al., 2018). So far, the Vitis18kSNP array was used in several studies on *V. vinifera* germplasm characterization, but its effectiveness on grapevine rootstocks has not been tested yet. In this work, the array was validated on 70 genotypes of a grapevine rootstock core collection, obtaining a final dataset of 14,180 SNP loci. This number of SNP loci was in line with the ones reported for *V. vinifera* germplasm, ranging from 10,041 to 16,501 SNPs (De Lorenzis et al., 2019, 2017; Degu et al., 2015; Mercati et al., 2016; Ruffa et al., 2016; Sunseri et al., 2018), resulting to be an informative tool for grapevine rootstock genetic characterization. Among the core collection genotypes with a known pedigree (Migliaro et al., 2019), the species mostly represented are *V. riparia* (n. 29 genotypes), *V. Berlandieri* (n. 16), *V. rupestris* (n. 11) and *V. vinifera* (n. 10). Although, *V. riparia* and *V. ruprestris*, two species worldwide used in the breeding programs of grapevine rootstocks for their resistance trait to phylloxera (Riaz et al., 2019), were not included in the panel of species used to identify and selected the SNPs (Laucou et al., 2018), genotypes having in their pedigree the genetic background of these two species were successfully analyzed. On the other hand, about the 56 and 59% of SNPs identified in the genome of *V. aestivalis* and *V. cinerea*, respectively, were amplified, even though in the core collection these two species appeared less represented (only two genotypes among the ones with a known pedigree). Nevertheless, it is not possible to exclude that among the unknown genotypes there are some having *aestivalis* and *cinerea* background. These results confirm that molecular markers identified in *V. vinifera* are appropriate to genotype different *Vitis* species and *viceversa*, as already verified for other molecular markers, such as SSR (Sefc et al., 1999), REMAP (D'Onofrio et al., 2010) and

iPBS (Guo et al., 2014). Only the SNPs loci detected in the genome of *M. rotundifolia* were not useful for *Vitis* non-*vinifera* genotyping (only the 19% of *M. rotundifolia* SNPs were successfully amplified). *Muscadinia* (2n = 40) and *Vitis* (2n = 38) are the two subgenera of *Vitis* genus. The two subgenera are distinguishable based on morphological traits and are nearly reproductively isolated, exhibiting significant divergence each other (Wan et al., 2013).

To genotype the *vinifera* germplasm, a set of nine SSRs has been established as reference tools to distinguish among the grapevine cultivars. Seven out of these nine SSR loci were found to be suitable to distinguish among the non-*vinifera* genotypes (Migliaro et al., 2019). Regarding the Visit18kSNP genotyping array, Mercati et al. (2016) suggested a minimal set of 12 SNP loci to discriminate among Sicilian cultivars and Laucou et al. (2018) found 14 as minimal number of SNP loci to distinguish among 783 grapevine cultivars. In this work, a minimum number of SNP loci has been proposed for the non-*vinifera* germplasm as well, using two different R packages. Both packages detected a number of loci (64 and 49 SNPs; Figure 1) higher than the one detected by Laucou et al (2018) and Mercati et al (2016). Because the minimal set of loci can change depending on the genetic diversity of genotypes analyzed, the higher size of minimal SNP set detected for non-*vinifera* germplasm reflects the low genetic distance detected by SNPs in comparison to the one detected by SSR markers (Figure 4).

4.2. SNP profiles reveal a high level of admixture

Genetic characterization of grapevine rootstocks can be performed by different marker types, which results do not always overlap (Emanuelli et al., 2013). In this study, SNP and SSR profiles were compared to assess genetic diversity of the grapevine rootstock core collection. Differences between SNPs and SSRs were observed with respect to heterozygosity (Table 2). As expected due to their multiallelic nature and high level of polymorphism, SSR loci exhibited a significantly higher heterozygosity than bi-allelic SNP loci. The same trend was observed by Emanuelli et al (2013) comparing a set of 384 SNPs to 22 SSRs on 122

rootstock genotypes. In particular, they observed rootstock heterozygosity values of H_o = 0.099 and H_o = 0.734 for SNPs and SSRs, respectively, slightly lower than the heterozygosity observed in this work (Table 2), suggesting that 384 SNPs related to phenotypical traits have the same power than 14k SNPs unrelated to phenotypical traits to detect the heterozygosity. SSR H_e value of core collection (Table 2) was larger than the values detected in other studies about different rootstock material (Dzhambazova et al., 2007; Emanuelli et al., 2013; Sefc et al., 1998; Upadhyay et al., 2007), confirming the uniqueness and preciousness of the analyzed germplasm collection. H_o value of core collection was lower than the H_e for both molecular markers. This result can be addressed to a Wahlund effect, due to population substructure. Indeed, although the high percentage of admixed genotypes (Figure 2), structure groups were detected with both molecular markers. The average minor allele frequency among the 14,180 SNPs (MAF = 0.10) was slightly higher than rootstock germplasm studied by Emanuelli et al (2013) (MAF = 0.08), but lower than the *sativa* compartment (MAF = 0.26).

A different genetic structure was defined according to the marker type: using SSRs, three ancestral groups were identified (Figure 2b), whereas SNPs defined a more complex structure, consisting of four ancestral populations (Figure 2a). Same trend was reported by Laucou et al (2018) on *V. vinifera* cultivars genotyped with the same set of SNP and SSRs. A different result was described by Emanuelli et al (2013), where K = 6 and K = 5 were identified for SSRs and SNPs, respectively, probably due to the lower number of SNP loci used to genotype the individuals. As a result of the higher number of SNP ancestral groups, the percentage of admixed genotypes was lower for SSRs (most of them resulted admixed also for SNP analysis). According to Klein et al (2018), two main clades can be discerned among North American *Vitis* species: clade I comprised *V. riparia* and *V. rupestris* together with *Vitis acerifolia, Vitis arizonica* and *Vitis monticola*; clade II consisted of *Vitis aestivalis, V. cinerea, V. labrusca* and *Vitis mustangensis*. SNP and SSR profiles were not able to capture this division between *V. riparia* and *V. rupestris* and the other species such as *V. labrusca*, probably due to the low number of genotypes having one species in their pedigree.

The core collection was designed to maximize the genetic variation of our *non*-vinifera germplasm collection and a high number of genotypes having a genetic background derived from three or four species have been included. The complex pedigree of selected genotypes supports the high level of admixture, with any strong evidence of differentiation among species.

PCA (Figure 3) and cluster analysis (Figure 4) produced consistent results, which clearly discriminated the structure ancestral groups for both marker types. Nei's genetic distances reflected structure, PCA and cluster distribution, confirming the SNP-group 2 and SSR-group 3 as the most different, although SSR Nei's genetic distance values among groups were higher than SNP ones. Both groups clustered individuals having in their genetic background species different from *V. Berlandieri*, *V. riparia* and *V. rupestris* (the three species mostly used in the rootstock breeding programs (Riaz et al., 2019)), such as *V. aestivalis*, *V. candicans*, *V. cordifolia* and *V. longii*. Some of these genotypes were clustered as much different in comparison to the individuals belonging to the same group (such as ID 39, 40, 71 and 120 for SNP analysis). Based on the comparison between non-*vinifera* and *vinifera* germplasm (Figure 5), these genotypes were assigned to the *vinifera* germplasm. Although *V. vinifera* was not used to breed rootstock material so far, due to their susceptibility of phylloxera (Granett et al., 2001), rootstock (non-*vinifera*) and scion (*vinifera*) do not always make up a successful graft. Indeed, the higher the inter-specificity between rootstock and scion, the higher the incompatibility. For this reason, it could be interesting to investigate these genotypes by the phenotypical point of view for further breeding programs.

In contrast to the trend of the whole core collection, H_o values within the SNP and SSR structure groups were slightly higher than the expected ones (except for the SSR-group 3) (Table 2). This result is due to the absence of clear discrimination among species based on the structure analysis (Figure 2) and low genetic variation (Figure 4) due to inbreeding among species (Riaz et al., 2019). The difficulty in finding a clear differentiation among genotypes with different genetic background can be traced back to

classification of *Vitis* genus. Indeed, the *Vitis* species are interfertile with most of their distribution areas overlapping, where natural hybridization can occur. This hybridization can mix the morphological traits and make difficult the identification of a true species (Wan et al., 2013).

4.3. SNPs performed well in dicriminating non-vinifera and vinifera germplasm

The Vitis18kSNP genotyping array was mainly developed to analyze *V. vinifera* germplasm, but in this work it was demonstrated working well also with non-vinifera germplasm, amplifying a high number of loci and discriminating well among non-vinifera and vinifera germplasm (Figure 5). It was already demonstrated that the two germplasms are clearly differentiated when analyzed with both SSR and SNP molecular markers (Emanuelli et al., 2013; Laucou et al., 2011). The genotyping array strongly discriminated the two germplasms, even though some (four) core collection genotypes overlapped with the vinifera-genotypes. These four genotypes are two (ID 39 and 40) vinifera-backgrounded genotypes (ID 40 has a *V. vinifera* x *V. vinifera* pedigree) and two (ID 71 and 120) unknown genotypes, suggesting a likely vinifera background also for the latter genotypes. In the non-vinifera group, part of riparia genotypes were placed in between non-vinifera and vinifera genotypes, appearing as the less homogeneous genotypes. Because together with riparia genotypes, some vinifera-backgrounded and unknown genotypes were also placed, it can be suggested a “riparia x vinifera” background for those genotypes with an unknown pedigree. The strong differentiation among non-vinifera and vinifera germplasm was also confirmed by the lack of PO relationships between the two groups of genotypes.

4.4. From SSR to SNP genotyping

SNPs are widely used to genotype crops and are markers of choice for QTL and GWAS (Bérard et al., 2009; Ha et al., 2007; Tian et al., 2011) due to their number, distribution and density along the genome. In *V. vinifera*, the genotyping SNP array has been used successfully to investigate the genetic diversity of grapevine, to discriminate among the wild and cultivated compartments, to infer population structure

and to reconstruct the pedigree of cultivars (De Lorenzis et al., 2019, 2017; Laucou et al., 2018; Mercati et al., 2016; Ruffa et al., 2016; Sunseri et al., 2018). Its attractiveness is due to a number of advantages, such as their high reproducibility among the laboratories, transferability, throughput, automatization and inexpensiveness. Nevertheless, the success of this tool will be established once laboratories will fully adopt SNPs as genotyping method, instead of SSRs, and the number of individuals analyzed with SNP array raises, as well as the reference databases. If this shift appears difficult to be applied due to the great efforts made in genotyping the *vinifera* germplasm using a universal panel of 9 SSR loci, for the non-*vinifera* germplasm the genotyping is still at the beginning, making this shift a more feasible change.

5. Conclusions

The genetic base of available *Vitis* rootstocks derived from a restricted number of genotypes, selected among North American *Vitis* species at the end of the XIX century. Considering the relevant role of rootstocks on environmental stress tolerance, the low genetic diversity reduces the ability of grapevine cultivars to adapt to several environmental constraints. This issue can be faced increasing the genetic and phenotypic diversity of breeding material, including non-conventional material in the further breeding programs. Living germplasm collections are valuable resources for exploring the genetic and phenotypic diversity and providing new genetic resources to support plant breeding efforts. The non-*vinifera* collection housed at the University of Milan has been established with the purpose of collecting as much as possible the diversity of non-*vinifera* germplasm and to design a core collection, where the putatively novel breeding material are included. Because the SNP genotyping is becoming even more popular for a number of advantages (rapid processing of large populations and data harmonization), 70 individuals of the non-*vinifera* germplasm core collection have been genotyped by *Vitis* SNP genotyping array. The SNP genotyping array has proved adequate to study the genetic diversity of non-*vinifera* germplasm. The genetic characterization provided the uniqueness and preciousness of the core collection as source of

plant breeding material not commonly used so far. The 18k SNP genotyping array will be a valid tool to assist the selection on the most promising individuals.

References

Bacilieri, R., Lacombe, T., Cunff, L. Le, Vecchi-staraz, M. Di, Laucou, V., Genna, B., Péros, J., This, P., Boursiquot, J., 2013. Genetic structure in cultivated grapevines is linked to geography and human selection. BMC Plant Biol.

Bérard, A., Le Paslier, M.C., Dardevet, M., Exbrayat-Vinson, F., Bonnin, I., Cenci, A., Haudry, A., Brunel, D., Ravel, C., 2009. High-throughput single nucleotide polymorphism genotyping in wheat (Triticum spp.). Plant Biotechnol. J. 7, 364–374. https://doi.org/10.1111/j.1467-7652.2009.00404.x

Brancadoro, L., Rabotti, G., Scienza, A., Zocchi, G., 1995. Mechanisms of Fe-efficiency in roots of Vitis spp. in response to iron deficiency stress. Plant Soil 171, 229–234. https://doi.org/10.1007/BF00010276

Cabezas, J.A., Ibáñez, J., Lijavetzky, D., Vélez, D., Bravo, G., Rodríguez, V., Carreño, I., Jermakow, A.M., Carreño, J., Ruiz-García, L., Thomas, M.R., Martinez-Zapater, J.M., 2011. A 48 SNP set for grapevine cultivar identification. BMC Plant Biol. 11. https://doi.org/10.1186/1471-2229-11-153

Cipriani, G., Spadotto, A., Jurman, I., Di, G., Crespan, M., Meneghetti, S., Frare, E., Vignani, R., Cresti, M., Morgante, M., Pezzotti, M., Pe, E., Policriti, A., Ra, V., 2010. The SSR-based molecular pro W le of 1005 grapevine (Vitis vinifera L .) accessions uncovers new synonymy and parentages , and reveals a large admixture amongst varieties of di V erent geographic origin. Theor Appl Genet 1569–1585. https://doi.org/10.1007/s00122-010-1411-9

Corso, M., Bonghi, C., 2014. Grapevine rootstock effects on abiotic stress tolerance. Plant Sci. Today 1 (3), 108–113. https://doi.org/http://dx.doi.org/10.14719/pst.2014.1.3.64

Crespan, M., Meneghetti, S., Cancellier, S., 2009. Identification and genetic relationship of the principal rootstocks cultivated in Italy. Am. J. Enol. Vitic. 60, 349–356.

Cseh, A., Taller, J., Podmaniczky, P., Kocsis, L., 2006. Comparative analysys of the most widespread grapevine rootstock lines in the world, the teleki lines, with microsatellite markers. Cereal Res Commun 34, 773–776.

D'Onofrio, C., De Lorenzis, G., Giordani, T., Natali, L., Cavallini, A., 2010. Retrotransposon-based molecular markers for grapevine species and cultivars identification 451–466. https://doi.org/10.1007/s11295-009-0263-4

De Andrés, M.T., Cabezas, J.A., Cervera, M.T., Borrego, J., Martínez-Zapater, J.M., Jouve, N., 2007. Molecular characterization of grapevine rootstocks maintained in germplasm collections. Am. J. Enol. Vitic. 58, 75–86.

De Lorenzis, G., Chipashvili, R., Failla, O., Maghradze, D., 2015. Study of genetic variability in Vitis vinifera L. germplasm by high-throughput Vitis18kSNP array: The case of Georgian genetic resources. BMC Plant Biol. 15. https://doi.org/10.1186/s12870-015-0510-9

De Lorenzis, G., Mercati, F., Bergamini, C., Cardone, M.F., Lupini, A., Mauceri, A., Caputo, A.R., Abbate, L., Barbagallo, M.G., Antonacci, D., Sunseri, F., Brancadoro, L., 2019. SNP genotyping elucidates the genetic diversity of Magna Graecia grapevine germplasm and its historical origin and dissemination. BMC Plant Biol. 19, 1–15. https://doi.org/10.1186/s12870-018-1576-y

De Lorenzis, G., Squadrito, M., Rossoni, M., di Lorenzo, G.S., Brancadoro, L., Scienza, A., 2017. Study of intra-varietal diversity in biotypes of Aglianico and Muscat of Alexandria (Vitis vinifera L.) cultivars. Aust. J. Grape Wine Res. 23, 132–142. https://doi.org/10.1111/ajgw.12247

Degu, A., Morcia, C., Tumino, G., Hochberg, U., Toubiana, D., Mattivi, F., Schneider, A., Bosca, P., Cattivelli, L., Terzi, V., Fait, A., 2015. Metabolite profiling elucidates communalities and differences in the polyphenol biosynthetic pathways of red and white Muscat genotypes. Plant Physiol. Biochem. 86, 24–33. https://doi.org/10.1016/j.plaphy.2014.11.006

Dzhambazova, T., Hvarleva, T., Tsvetkov, I.Y., Atanassov, A., 2007. Characterization of Grapevine Rootstocks using Microsatelite Markers. Biodivers. Ecosyst. 2008–2012. https://doi.org/10.1080/13102818.2007.10817414

Emanuelli, F., Lorenzi, S., Grzeskowiak, L., Catalano, V., Stefanini, M., Troggio, M., Myles, S., Martinez-Zapater, J.M., Zyprian, E., Moreira, F.M., Grando, M.S., 2013. Genetic diversity and population structure assessed by SSR and SNP markers in a large germplasm collection of grape. BMC Plant Biol. 13, 1–17. https://doi.org/10.1186/1471-2229-13-39

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Frichot, E., Franc, O., 2015. APPLICATION LEA : An R package for landscape and ecological association studies. MethodsinEcologyandEvolution. https://doi.org/10.1111/2041-210X.12382

Granett, J., Walker, M.A., Kocsis, L., Omer, A.D., 2001. BIOLOGY AND MANAGEMENT OF GRAPE PHYLLOXERA 387–412.

Guo, D., Guo, M., Hou, X., Zhang, G., 2014. Molecular diversity analysis of grape varieties based on iPBS markers. Biochem. Syst. Ecol. 52, 27–32. https://doi.org/10.1016/j.bse.2013.10.008

Ha, B.K., Hussey, R.S., Boerma, H.R., 2007. Development of SNP assays for marker-assisted selection of two southern root-knot nematode resistance QTL in soybean. Crop Sci. 47, 73–82. https://doi.org/10.2135/cropsci2006.10.0660tpg

Jahnke, G., Májer, J., Szoke, B., Varga, P., Kocsisné Molnár, G., Tarczal, E., Kocsis, L., 2014. Analysis of grape rootstocks by microsatellite markers. Acta Hortic. 1046, 617–626. https://doi.org/10.17660/ActaHortic.2014.1046.85

Jombart, T., 2008. adegenet : a R package for the multivariate analysis of genetic markers 24, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Keller, M., 2015. The Science of Grapevines: Anatomy and Physiology, Second. ed.

Klein, L.L., Miller, A.J., Ciotir, C., Hyma, K., Uribe-Convers, S., Londo, J., 2018. High-throughput sequencing data clarify evolutionary relationships among North American Vitis species and improve identification in USDA Vitis germplasm collections. Am. J. Bot. 105, 215–226. https://doi.org/10.1002/ajb2.1033

Köse, B., Horuz, A., Akınoğlu, G., 2016. Phenological Changes of Leaf Nutrients in Vitis labrusca L. Grape Grafted on Different Rootstocks in Heavy Clay Soil Conditions. Erwerbs-Obstbau 58, 211–217. https://doi.org/10.1007/s10341-016-0279-5

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7 : Molecular Evolutionary Genetics Analysis Version 7 . 0 for Bigger Datasets Brief communication 33, 1870–1874. https://doi.org/10.1093/molbev/msw054

Laucou, V., Lacombe, T., Dechesne, F., Siret, R., Bruno, J.P., Dessup, M., Dessup, T., Ortigosa, P., Parra, P., Roux, C., Santoni, S., Varès, D., Péros, J.P., Boursiquot, J.M., This, P., 2011. High throughput analysis of grape genetic diversity as a tool for germplasm collection management. Theor. Appl. Genet. 122, 1233–1245. https://doi.org/10.1007/s00122-010-1527-y

Laucou, V., Launay, A., Bacilieri, R., Lacombe, T., Adam-Blondon, A.F., Bérard, A., Chauveau, A., De Andrés, M.T., Hausmann, L., Ibáñez, J., Le Paslier, M.C., Maghradze, D., Martinez-Zapater, J., Maul, E., Ponnaiah, M., Töpfer, R., Péros, J.P., Boursiquot, J.M., 2018. Extended diversity analysis of cultivated grapevine Vitis vinifera with 10K genome-wide SNPs. PLoS One 13, 1–27. https://doi.org/10.1371/journal.pone.0192540

Lin, H., Walker, M.A., 1998. Identifying grape rootstocks with simple sequence repeat (SSR) DNA markers. Am. J. Enol. Vitic. 49, 403–407.

Marrano, A., Birolo, G., Prazzoli, M.L., Lorenzi, S., Valle, G., Grando, M.S., 2017. SNP-Discovery by RAD-Sequencing in a Germplasm Collection of Wild and Cultivated Grapevines (V . vinifera L .) 1837661, 1–19. https://doi.org/10.1371/journal.pone.0170655

Mercati, F., De Lorenzis, G., Brancadoro, L., Lupini, A., Abenavoli, M.R., Barbagallo, M.G., Di Lorenzo, R., Scienza, A., Sunseri, F., 2016. High-throughput 18K SNP array to assess genetic variability of the main grapevine cultivars from Sicily. Tree Genet. Genomes 12. https://doi.org/10.1007/s11295-016-1021-z

Migliaro, D., De Lorenzis, G., Di Lorenzo, G.S., Nardi, B. De, Gardiman, M., Failla, O., Brancadoro, L., Crespan, M., 2019. Grapevine non-vinifera

genetic diversity assessed by simple sequence repeat markers as a starting point for new rootstock breeding programs. Am. J. Enol. Vitic. 70, 390–397. https://doi.org/10.5344/ajev.2019.18054

Myles, S., Chia, J., Hurwitz, B., Simon, C., Zhong, G.Y., Ware, D., 2010. Rapid Genomic Characterization of the Genus Vitis 5. https://doi.org/10.1371/journal.pone.0008219

Nei, M., 1972. Genetic Distance between Populations. Am. Nat. 106, 283–292. https://doi.org/http://dx.doi.org/10.1086/282771

Ollat, N., Peccoux, A., Papura, D., Esmenjaud, D., Marguerit, E., Tandonnet, J.P., Bordenave, L., Cookson, S.J., Barrieu, F., Rossdeutsch, L., Lecourt, J., Lauvergeat, V., Vivin, P., Bert, P.F., Delrot, S., 2016. Rootstocks as a component of adaptation to environment, Grapevine in a Changing Environment: A Molecular and Ecophysiological Perspective. https://doi.org/10.1002/9781118735985.ch4

Peakall, R., Smouse, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of Population Structure Using Multilocus Genotype Data. Genetics.

R Core Team, 2019. R: A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Au.

Riaz, S., Pap, D., Uretsky, J., Laucou, V., Boursiquot, J.M., Kocsis, L., Andrew Walker, M., 2019. Genetic diversity and parentage analysis of grape rootstocks. Theor. Appl. Genet. https://doi.org/10.1007/s00122-019-03320-5

Ruffa, P., Raimondi, S., Boccacci, P., Abbà, S., Schneider, A., 2016. The key role of “Moscato bianco” and “Malvasia aromatica di Parma” in the parentage of traditional aromatic grape varieties. Tree Genet. Genomes 12. https://doi.org/10.1007/s11295-016-1006-y

Sefc, K., Regner, F., Turetschek, E., Glössl, J., Steinkeller, H., 1999. Identification of microsatellite sequences in Vitis riparia and their applicability for genotyping of different Vitis species. Genome 42, 367–373.

Sefc, K.M., Regner, F., Glössl, J., Steinkellner, H., 1998. Genotyping of grapevine and rootstock cultivars using microsatellite markers. Vitis 37, 15–20.

Sunseri, F., Lupini, A., Mauceri, A., De Lorenzis, G., Araniti, F., Brancadoro, L., Dattola, A., Gullo, G., Zappia, R., Mercati, F., 2018. Single nucleotide polymorphism profiles reveal an admixture genetic structure of grapevine germplasm from Calabria, Italy, uncovering its key role for the diversification of cultivars in the Mediterranean Basin. Aust. J. Grape Wine Res. 24, 345–359. https://doi.org/10.1111/ajgw.12339

Tian, F., Bradbury, P.J., Brown, P.J., Hung, H., Sun, Q., Flint-Garcia, S., Rocheford, T.R., McMullen, M.D., Holland, J.B., Buckler, E.S., 2011. Genome-wide association study of leaf architecture in the maize nested association mapping population. Nat. Genet. 43, 159–162. https://doi.org/10.1038/ng.746

Upadhyay, A., Saboji, M.D., Reddy, S., Deokar, K., Karibasappa, G.S., 2007. AFLP and SSR marker analysis of grape rootstocks in Indian grape germplasm. Sci. Hortic. (Amsterdam). 112, 176–183. https://doi.org/10.1016/j.scienta.2006.12.011

Wan, Y., Schwaninger, H.R., Baldo, A.M., Labate, J.A., Zhong, G., Simon, C.J., 2013. A phylogenetic analysis of the grape genus (Vitis L .) reveals broad reticulation and concurrent diversification during neogene and quaternary climate change.

Xu, S., Gupta, S., Jin, L., 2010. PEAS V1.0: a package for elementary analysis of SNP data. Mol. Ecol. Resour. 1085–1088. https://doi.org/10.1111/j.1755-0998.2010.02862.x

A NEW GENOMIC LOCUS ASSOCIATED TO DROUGHT TOLERANCE IN GRAPEVINE ROOTSTOCKS

Abstract

Rootstocks are worldwide used in modern viticulture, but their genetic base is narrow, and few genotypes are available for winegrowers. New challenges caused by climate change draw attention on the selection of new rootstocks, in order to improve the adaptation of viticulture to abiotic stresses. In breeding programs, early selection of promising genotypes can be achieved using marker-assisted selection (MAS), which allows to considerably reduce the long time required by traditional selection. Nevertheless, drought tolerance related markers have not been detected in grapevine rootstocks so far. The aim of this study was to apply a genome-wide association (GWA) approach to a rootstock breeding population characterized for drought tolerance and genotyped with a 18k SNP array. Phenotyping for drought tolerance was performed under controlled conditions, with a multi-parameter approach. Vegetative growth and transpiration, estimated by thermography, were used to classify the genotypes in four groups according to their response to increasing water deficit. Groups were used in GWA to identify one significant association with one locus in chromosome 6. The detected locus is included in the U-box domain, which play an important role in abiotic stress adaptation in other plant species. This study provides a potential target gene in assisted breeding programs for drought tolerance.

1. Introduction

In the last decades, viticultural areas underwent to increasing temperature and longer drought periods due to climate change. Production and quality of grape and wine are strongly affected by environmental conditions, such as light and water availability, and their interaction with genotypes and viticultural techniques (Van Leeuwen et al., 2019). Resilience of viticulture to climate change can be improved by selecting new plant material adapted to drought conditions. Drought adaptation of vine involves several physiological mechanisms, such as root growth, transpiration control, water use efficiency and embolism

repair through remobilization of osmolytes (Brodersen et al., 2010; Flexas et al., 2009; Steudle, 2000). Selection of drought-adapted rootstocks has been identified as a promising strategy to face climate change (Delzon, 2015; Ollat et al., 2018). Rootstocks are worldwide used in viticulture since the end of XIX century after the spread of phylloxera (*Daktulosphaira vitifoliae*). Unlike the European *Vitis vinifera*, the roots of American *Vitis* species showed resistance to the aphid due to the coevolution in the same areas, and they were used in breeding programs to obtain rootstocks. Nevertheless, the genetic base of rootstocks is narrow. Riaz et al (2019), found that the 39% of the genetic variability among commercial rootstocks is represented by only three genotypes: *Vitis riparia* Gloire de Montpellier, *Vitis rupestris* du Lot and *Vitis Berlandieri* Rességuier. Thus, new efforts in rootstock selection are required: introducing more diversity in future breeding programs could be possible to obtain rootstocks with higher tolerance to drought. The selection of promising genotypes is a crucial step in breeding programs, which require long time especially for perennial crops as grapevine. The recent development of genetic techniques and the availability of high throughput genotyping tools paves the way on the marker-assisted selection (MAS), which allows an early selection of promising genotypes for the interested phenotypic traits. In grapevine, quantitative trait loci (QTLs) related to drought tolerance have been studied in *V. vinifera* by Coupel-Ledru et al (2014) on a pseudo-F1 population obtained by the breeding of Syrah and Grenache. Results of the study suggested that drought-tolerance of grapevine is regulated by several genes, due to the large number of QTLs related to transpiration rate and hydraulic conductance. The genetic architecture of rootstock control to transpiration and the adaptation to water stress were investigated by Marguerit et al (2012) on a breeding population of *V. vinifera* × *V. riparia* hybrids. Also in this study, several QTLs were identified, related to water extraction capacity, transpiration rate, transpiration efficiency, acclimatation of transpiration rate to water deficit and water use efficiency, estimated by carbon isotopes. The confidence intervals of the detected QTLs included the genes involved in the ABA pathway. A Genome-wide association (GWA) approach was used by Trenti et al (2021) to identify the loci related to

transpiration, estimated by thermography, on a genetic core collection composed by 100 *Vitis* spp. accessions. In the analysis, 13 candidate genes were identified and 3 of them, codifying for Glycosyltransferase, Raffinose synthase and Peroxidase, responded to water deficit in a gene expression analysis on reference rootstocks. Except the control of transpiration, other physiological mechanisms are involved in adaptation of rootstocks to drought. The aims of the present study are: i) to characterize the genetic structure of a new breeding population obtained by rootstock M; ii) to phenotype the breeding population for drought tolerance using a multi-parameters approach and to identify promising genotypes for arid conditions; iii) to identify genetic regions involved in drought tolerance through a GWA approach.

2. Material and methods

2.1. Plant material

The breeding population analyzed in this study was obtained in 2012 by the open pollination of rootstock M1. The population included 141 genotypes and it is maintained under field conditions in the germplasm collection of the Department of Agricultural and Environmental Sciences (DiSAA) of University of Milano, located in Torrazza Coste, Pavia, Italy (44.984783 N, 9.089038 E, 133 m a.s.l.). During the pruning period, cuttings of each genotype were collected for genotyping and phenotyping under controlled conditions.

2.2. SNP genotyping

One hundred milligrams of freeze-fresh young leaf tissue were ground with liquid nitrogen, and genomic DNA was extracted using NucleoSpin® Plant II (MACHEREY-NAGEL—Düren, Germany), according to manufacturer's protocol. Concentration of DNA and its quality were checked by electrophoresis on agarose gel by spectroscopy (260/230 and 260/280 ratios) using NanoDrop Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and the Quant-iT dsDNA HS assay kit for Qubit 3.0 Fluorometer (Thermo Fisher Scientific). SNP genotyping was performed on 200 ng of genomic DNA per sample using

the Vitis18kSNP array (Illumina Inc., San Diego, CA, USA), containing 18,071 SNPs, by the laboratory of Fondazione Edmund Much (San Michele all'Adige, Trento, Italy).

2.3. Phenotyping for drought tolerance

The experiment was carried out in 2020 under controlled conditions in the greenhouse of DiSAA (University of Milano). The greenhouse was equipped with supplementary light and a cooling system, with a 16 hr light [~PPFD of 600 µmol of photons m^{-2} s^{-1})] and 8 hr dark photoperiod and a range of temperatures from 23 to 28 °C. One year-old un-grafted cuttings were used for the experiment and during budding, plants were maintained in well-watered conditions. Vines were grown in 4 L plastic pots, filled with a growth substrate composed by 22% of neutral sphagnum peat and 78% of mix growing medium. One shoot per plant was kept and trained on 1 m graduated stake. Three phenotyping cycles were performed during the experiment, and 1 biological repetition per genotype was used in each cycle (a total of 3 repetition per genotype). The experimental plan was at randomized blocks to avoid the effect of the temperature gradient in the greenhouse, caused by the cooling system. At the beginning of each cycle, pots were maintained four days under well-watered at 70% of soil water content (SWC), in order to avoid waterlogging stress. In the next steps, water availability decreased to 50%, 30% and 15% of SWC and it was maintained at the same level for 4 days at each step. Soil water content was calculated according to Gardner et al (2001). Field capacity weight and dry weight of each pot were recorded before the beginning of the experiment. Phenotypic analyses were performed at the beginning and in the end of each SWC level (70%; 50%; 30%, 15%), consisting in pot weighting and in the acquisition of 4 orthogonal images in the visible and thermal infrared regions of spectra. Images were recorded by the thermal camera Thermo Gear Model G100EX/G120EX (Detector Uncooled focal plane array; Number of pixels 320 H × 240 V; Spectral range 8–14 µm; dynamic resolution at 14 bit), produced by InfReC, NEC Avio Infrared Technologies CO., Ltd. Emittance was set to 0.96, as suggested for grapevine leaves by Grant et al (2002). A total of 4 leaves were chosen per genotype and the temperature was recorded in two points per leaf

using the software “InfReC Analyzer NS9500 Lite”. Leaf temperature was normalized on a dry reference (T_{dry}), representing fully closed stomata, and wet reference (T_{wet}), representing fully transpiring leaves, to determinate Crop Water Stress Index (CWSI) and stomatal conductance index (IG), proposed by Idso et al (1981) and Jones et al (2002), respectively. Images in the visible range were used to measure the shoot length (L), leaf surface (LS) and the leaf angle (LA), subtended between the leaf blade and the petiole, using the software ImageJ. Shoot grow rate (SGR) was calculated as the increment in shoot length per day. Evapotranspiration (ET) was calculated as the difference in pot weight per day, including the water added to the pot to maintain the level of SWC.

2.4. Data analysis

For SNP data, samples with call quality value (p50GC) lower than 0.54 and loci with a GenTrain (GT) score value lower than 0.6 (De Lorenzis et al., 2015) were filtered from the dataset, as well as those with more than 20% of missing data and monomorphic loci. The genetic structure of the breeding population was assessed using LEA package (Frichot and Franc, 2015) of R software, varying the number of ancestral genetic groups (K) from 1 to 10 in 10 repetition runs for each K value. The Principal Components Analysis (PCA) on genetic profiles was run by using the *adegenet* package of R software (Jombart, 2008). Genetic distance among genotypes was set up on Nei’s distance (Nei, 1972), performed in PEAS, using unweighted pair group method with arithmetic mean (UPGMA). A circular dendrogram was plotted using MEGA 7.0 software (Kumar et al., 2016).

Association analysis was performed in R software using GAPIT package (Lipka et al., 2012). MLMM (Multiple Locus Mixed Linear Model), FarmCPU (Fixed and random model Circulating Probability Unification) and Blink algorithms were tested. For fixed effect, Q-matrix (for K = 2), detected by LEA, was used as the covariate for association analysis accounting for population structure. A conservative

threshold for assessing SNP significance was calculated based on Bonferroni correction for a type I error rate of 0.05.

3. Results

3.1. Genetic structure characterization of the breeding population

The breeding population was genotyped using an array of 18k SNPs. The genetic profile of all genotypes reported a call quality value (p50GC) higher than 0.54. The total number of analyzed SNP loci was 18,071 and 15,563 (86.12% of the total amount) of them reported a GT score value higher than 0.6. The loci showing a percentage of missing data lower than 20% amounted to 15,344 (84.91%). The monomorphic loci were also filtered to obtain a final dataset of 11,909 SNPs (65.90%) suitable for genetic characterization of the breeding population. A structure analysis was performed to explore the structure of the breeding population. The most likely number of ancestral populations was identified in K = 2. The first ancestral population was composed by 130 genotypes and the second one was composed 12 genotypes, including the parental rootstock M1. A bar plot representation of the structure analysis is shown in figure 1. The population structure was further investigated using a PCA approach and NEI's distance. The first two components identified by PCA represented the 11.23% of the total variance of data, and the fist principal component (PC1) alone accounted for the 6.63% of variance. The two ancestral population identified by the structure analysis were clearly discriminated by PC1 as shown in figure 2. Furthermore, the population structure was confirmed by the larger Nei's genetic distance between the two ancestral groups. Dendrogram in figure 3 reported the Nei's genetic distance among genotypes.

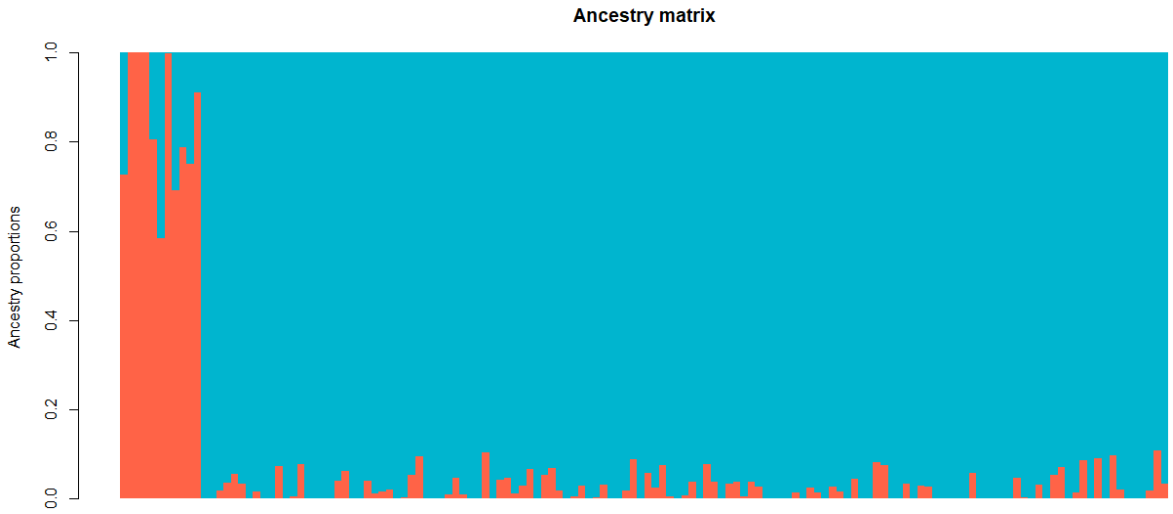


Figure 1. Structure analysis performed by LEA R package at K = 2 of a grapevine breeding population including 141 genotypes and the female parental M1, defined using 12k SNPs molecular markers. In the barplot each genotype is represented by a vertical bar. Ancestral populations are represented with different colors

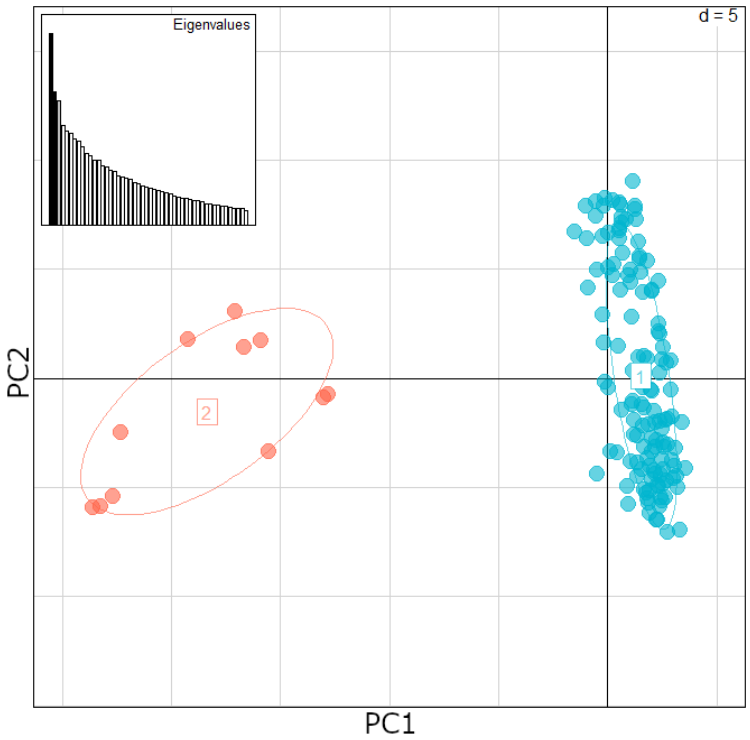


Figure 2. Principal component analysis (PCA) of the grapevine breeding population including 141 genotypes and the female parental M1, defined using 12k SNPs molecular markers. Genotypes are colored according to the ancestral populations identified in the structure analysis in Figure 1

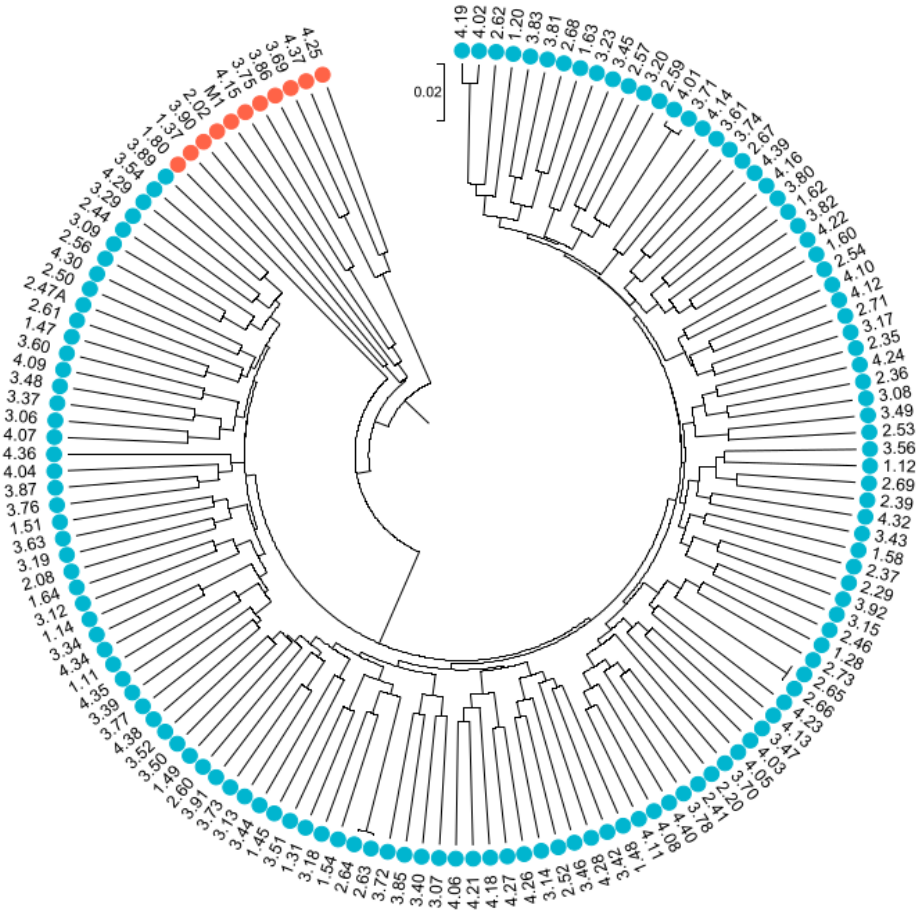


Figure 3. Clustering of the grapevine breeding population including 141 genotypes and the female parental M1, defined using 12k SNPs molecular markers. Genotypes are colored according to the ancestral populations identified in the structure analysis in Figure 1

3.2. Phenotyping for water deficit tolerance

Tolerance to water deficit of the 142 genotypes was evaluated across the three phenotyping cycles by normalizing the data on the mean value of each cycle. The analysis of variance reported a significant effect of SWC on all traits and a significant effect of genotype factor on L, LS, SGR, CWSI and LA. At decreasing levels of water availability, vines gradually reduced SGR, LA, ET and IG with significant differences among all levels of SWC, whereas CWSI gradually increased. L and LS increased from 70% to 30 % of SWC and no

significant difference was found between 30% and 15% of SWC. All traits were significant correlated, as reported in figure 4a. In particular, SGR was strongly correlated to ET (r = 0.76) and ET reported high correlation to the thermal indexes (r = 0.75 with CWSI and r = 0.67 with IG). Correlations were confirmed by a PCA. The first two principal components represented the 74.2% of the total variance of which 59.3% was represented by the only first component. The first component was negatively affected by SGR, IG, ET and LA and positively affected by CWSI, whereas the second principal component was mainly related to L and LS. A trend in SWC was identified according to the first principal component (Figure 4b).

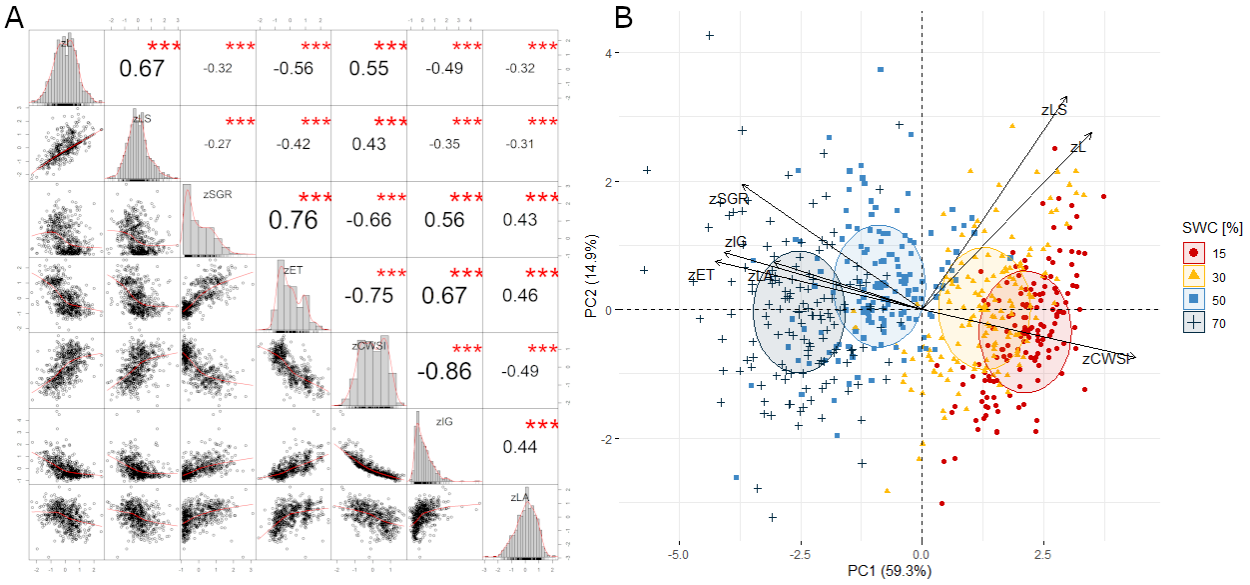


Figure 4. Pearson correlation (A) and principal component analysis (B) on the standardized phenotypic traits: L = shoot length; LS = leaf surface; SGR = shoot growth rate; ET = evapotranspiration; CWSI = crop water stress index; IG = stomatal conductance index; LA = leaf angle; SWC = soil water content. Significant correlations are considered for $0.01 < p \leq 0.05$ (*), $0.001 < p \leq 0.01$ (**) and $p \leq 0.001$ (***)

The SWC of 30% corresponded to the level of stress that stopped the growth in the largest part of vines, so it was chosen to distinguish the behavior of genotypes. Four groups of genotypes were identified at 30% of SWC: groups A1 and A2 reported CWSI below the average level, whereas groups B1

and B2 were above; groups A2 and B2 exceeded the average level of SGR, whereas groups A1 and B1 reported lower SGR. Classification in groups at 30% of SWC according to SGR, CWSI and L is reported in Figure 5. Behavior of the groups identified at 30% of SWC were investigated at the other levels of water availability. A significant effect of groups was found for L during the whole experiment, with longer shoots for groups B1 and B2 (Figure 6a). They also reported higher SGR under well-watered conditions (70% SWC) but under water deficit (30% SWC) the highest SGR was showed by groups A2 and B2 (Figure 6b). Significant effect of the group was found for thermal indexes at 50% and 30% of SWC (Figure 6c; Figure 6d), with lower levels of CWSI reported by groups A1 and A2. Furthermore, a significant effect of the group was found for LA at 30% and 15% of SWC (Figure 6e). In particular, under water deficit group A2 maintained the highest leaf angle.

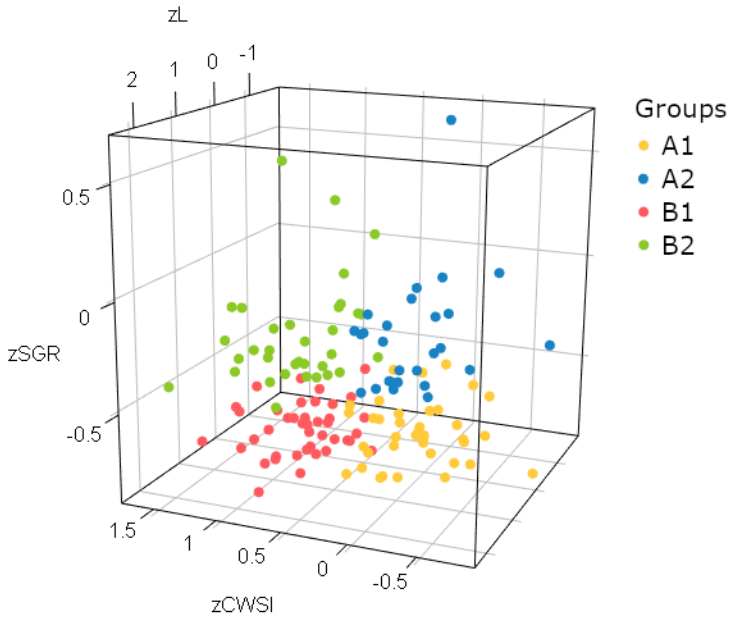


Figure 5. Classification of genotypes according to shoot growth rate (SGR), crop water stress index (CWSI) and shoot length (L) at 30% of soil water content (SWC). Genotypes in groups A2 and B2 reported SGR above the average level. Genotypes in groups A1 and A2 reported CWSI below the average level

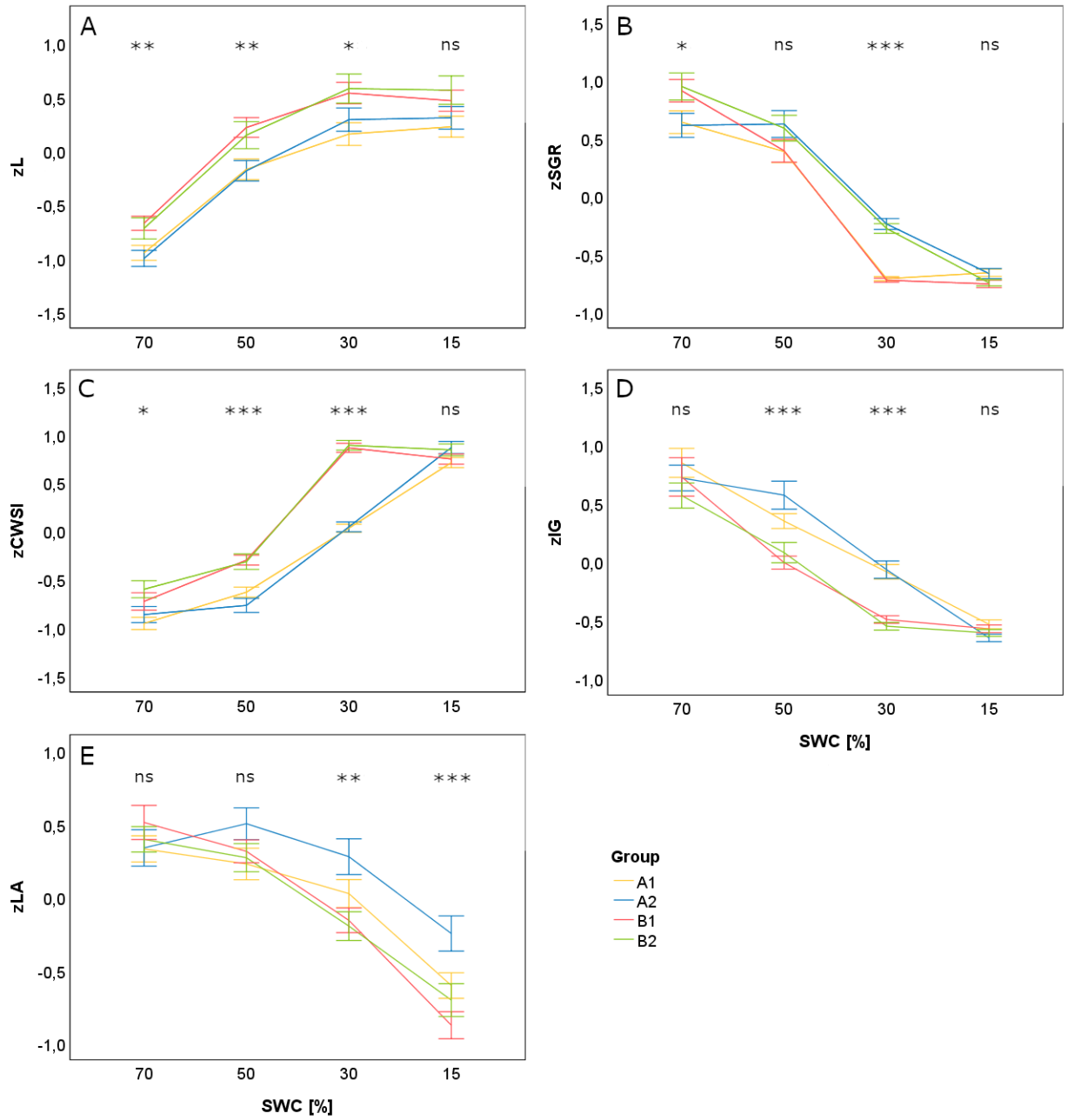


Figure 6. Dynamic of groups performance under decreasing water levels for each standardized phenotypic trait. A) shoot length (L); B) shoot growth rate (SGR); C) crop water stress index (CWSI); D) stomatal conductance index IG); E) leaf angle (LA); SWC = soil water content. Bars represent standard error of means. Significant differences among groups are considered for $0.01 < p \leq 0.05$ (*), $0.001 < p \leq 0.01$ (**) and $p \leq 0.001$ (***)

3.3. GWA analysis and candidate gene prediction

A GWAS approach was used to associate the genetic profiles of the breeding population with phenotypic results. A progressive score was assigned to each phenotypic group according to the tolerance to water stress and used in GWAS. Three different statistical models were used to associate phenotypic and genetic data (MLMM; FarmCPU; Blink). Considering the structure of the breeding population assessed by structure analysis, PCA and Nei's distance, a Q-matrix for K = 2 was used as covariate in the GWA analysis. All the three tested models identified a significant association between an SNP (chr6_5522324_A_G) and water deficit tolerance, reporting a p-values of $6.70e^{-07}$, $1.02e^{-07}$ and $1.12e^{-07}$ for MLMM, FarmCPU and Blink, respectively (Figure 7). The associated SNP was located in chromosome 6 in the position 5,522,324 and it was mapped in the *V. vinifera* reference genome (PN40024 12X) to identify the putative gene related to water deficit tolerance. The predicted gene belongs to the U-Box domain.

4. Discussion

A new frontier in breeding programs is represented by the marker-assisted selection (MAS), which allows to reduce the long time required in the traditional selection process. The selection of new rootstocks has been identified as an adaptation strategy of viticulture to climate change and in particular to face drought (Quénol et al., 2014). In order to apply MAS in the selection of drought tolerant rootstocks, the loci related to the tolerance to water deficit have to be identified. For this purpose, a breeding population belonging of 141 genotypes was characterized in this study at genetic level, as well as at phenotypic level in response to water deficit. The genetic analysis of the population was analyzed using an array of 18k SNPs, developed with 13,561 SNPs isolated from *V. vinifera* and 4510 SNPs from *V. aestivalis, V. Berlandieri, V. labrusca, V. cinerea, V. lincecumï* and *M. rotundifolia* (Laucou et al., 2018). This tool was widely use on *V. vinifera* germplasm (De Lorenzis et al., 2019; Laucou et al., 2018; Mercati et al., 2016) and it was validated on a core collection of 70 genotypes, representing the whole variability of a larger rootstock collection of 232

unique genotypes (Bianchi et al., 2020). Structure analysis, PCA and Nei's distance concurred to define a population structure composed by two different groups of genotypes: a larger group including 130 genotypes and another group including 11 genotypes and rootstock M1. Both groups shared the same female parental (M1), whereas the male parental was different. Phenotyping for drought tolerance was performed under controlled conditions to control the water availability of vines and to reduce the effect of other environmental factors except water deficit. Among the water availability levels, SWC of 30% resulted the most appropriate to identify the different behaviors of genotypes in response to water deficit, because at more sever conditions (15% of SWC) the differences among genotypes were flattened and the vegetative growth was stopped. Interestingly, the groups identified at 30% of SWC showed significant differences also at mild water stress in terms of transpiration (CWSI and IG) and at severe water stress in terms of leaf turgor (LA). Genotypes belonging to the group A2 were able to maintain high growth (SGR) and water status (CWSI, IG and LA) at mild to moderate to severe water deficit. These genotypes can be further studied under field conditions or in grafting combination with *V. vinifera* to become promising rootstocks for arid conditions, or to be used as pre-breeding material. The association of genetic and phenotypic data allowed to identify a locus related to drought tolerance. The association was consistent using different statistical models. The putative gene obtained by the mapping of the locus identified in GWAS belonged to the U-box family genes. Using RNA-seq data from *Medicago truncatula*, Song et al (2017) detected 15 drought-regulated U-box genes, of which 6 of them were also regulated by salt and cold stress. The putative gene was located in chromosome 6, in a genetic region that could be related to the transpiration efficiency, as obtained by QTL approach on an F1 grapevine rootstock population (Marguerit et al., 2012). Nevertheless, any significant association with stomatal conductance index (IG) was identify in chromosome 6 by GWAS approach on a rootstock collection (Trenti et al., 2021). Further studies will be necessary to validate the expression of the putative gene under water deficit, thereafter it can become a target gene in future rootstock breeding programs for drought adaptation.

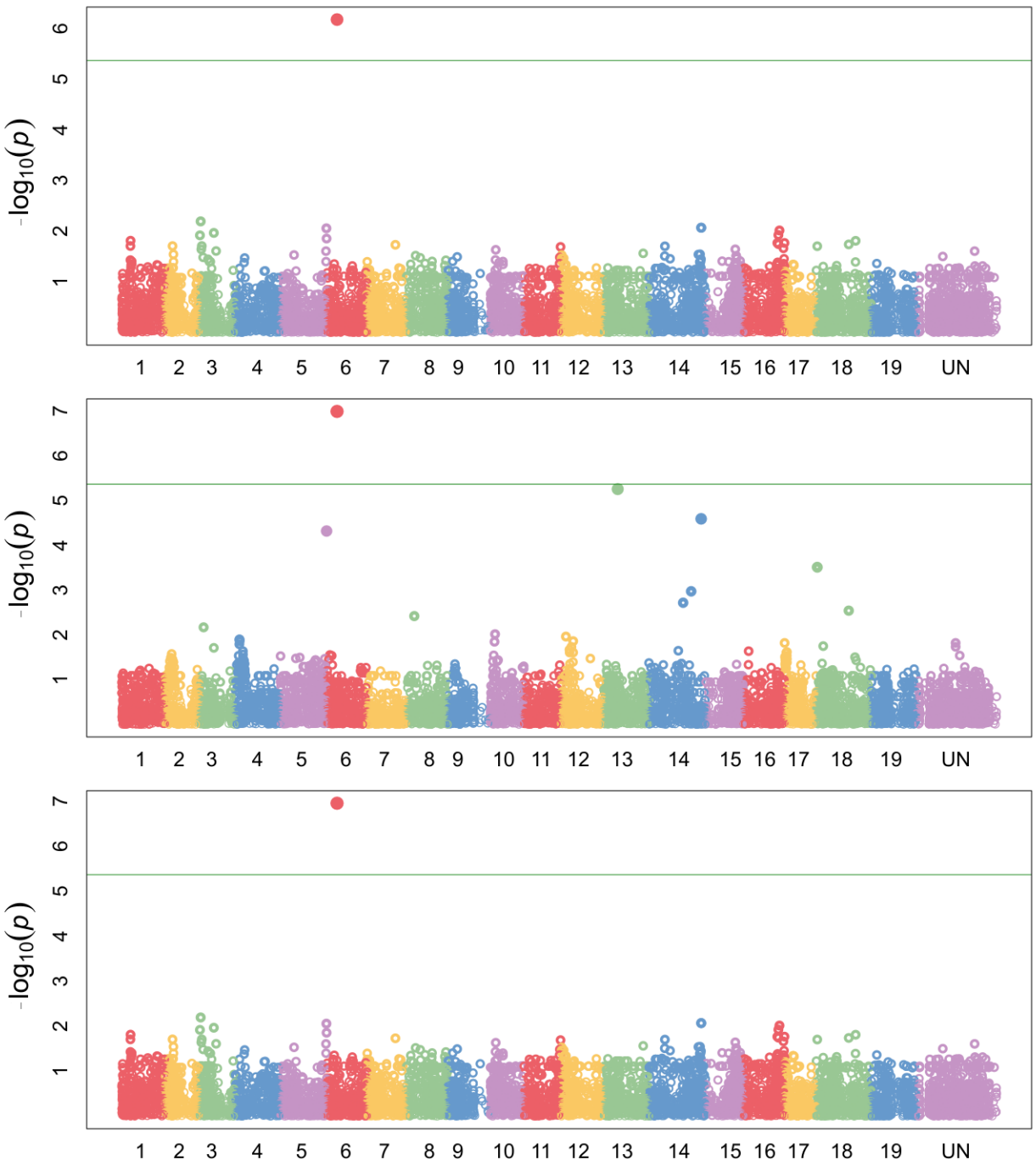


Figure 7. Manhattan plot of -log10 p-values estimated for phenotypic groups in response water deficit in the breeding population genotyped by 18 k SNPs. Significant SNPs are circles above the Bonferroni-adjusted threshold (green horizontal line). Association analysis results of MLMM (A), FarmCPU (B) and Blink (C) algorithms

References

Bianchi, D., Brancadoro, L., Lorenzis, G. De, 2020. Genetic Diversity and Population Structure in a Vitis spp . Core Collection Investigated by SNP Markers. https://doi.org/10.3390/d12030103

Brodersen, C.R., Mcelrone, A.J., Choat, B., Matthews, M.A., Shackel, K.A., 2010. The Dynamics of Embolism Repair in Xylem : In Vivo Visualizations Using High-Resolution Computed Tomography. Plant Physiol. 154, 1088–1095. https://doi.org/10.1104/pp.110.162396

Coupel-ledru, A., Lebon, É., Christophe, A., Doligez, A., Cabrera-bosquet, L., 2014. Genetic variation in a grapevine progeny (Vitis vinifera L . cvs Grenache × Syrah) reveals inconsistencies between maintenance of daytime leaf water potential and response of transpiration rate under drought 65, 6205–6218. https://doi.org/10.1093/jxb/eru228

De Lorenzis, G., Di Lorenzo, G.B.S., Failla, O., Musayev, M.K., Salimov, V., Maghradze, D., Chipashvili, R., 2015. Study of genetic diversity in V. vinifera subsp. sylvestris in Azerbaijan and Georgia and relationship with species of the cultivated compartment, Special Paper of the Geological Society of America.

De Lorenzis, G., Mercati, F., Bergamini, C., Cardone, M.F., Lupini, A., Mauceri, A., Caputo, A.R., Abbate, L., Barbagallo, M.G., Antonacci, D., Sunseri, F., Brancadoro, L., 2019. SNP genotyping elucidates the genetic diversity of Magna Graecia grapevine germplasm and its historical origin and dissemination. BMC Plant Biol. 19, 1–15. https://doi.org/10.1186/s12870-018-1576-y

Delzon, S., 2015. New insight into leaf drought tolerance. Funct. Ecol. 29, 1247–1249. https://doi.org/10.1111/1365-2435.12500

Flexas, J., Barón, M., Bota, J., Ducruet, J.-M., Gallé, A., Galmés, J., Jiménez, M., Pou, A., Ribas-Carbó, M., Sajnani, C., Tomàs, M., Medrano, H., 2009. Photosynthesis limitations during water stress acclimation and recovery in the drought-adapted Vitis hybrid Richter-110 (V. berlandieri×V. rupestris). J. Exp. Bot. 60, 2361–2377. https://doi.org/10.1093/jxb/erp069

Frichot, E., Franc, O., 2015. APPLICATION LEA : An R package for landscape and ecological association studies. MethodsinEcologyandEvolution. https://doi.org/10.1111/2041-210X.12382

Gardner, C.M.K., Robinson, D., Blyth, K., Cooper, J.D., 2001. Soil Water Content, in: Smith, K.A., Mullins, C.E. (Eds.), Soil and Environmental Analysis: Physical Methods. Marcel Dekker Inc., New York, NY, pp. 13–20.

Idso, S.B., Jackson, R.D., Pinter Jr., P.J., Reginato, R.J., Hatfield, J.L., 1981. Normalizing the stress-degree-day parameter for environmental variability. Agric. Meteorol. 24, 45–55. https://doi.org/https://doi.org/10.1016/0002-1571(81)90032-7

Jombart, T., 2008. adegenet : a R package for the multivariate analysis of genetic markers 24, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jones, H.G., Stoll, M., Santos, T., Sousa, C. De, Chaves, M.M., Grant, O.M., 2002. Use of infrared thermography for monitoring stomatal closure in the ® eld : application to grapevine 53, 2249–2260. https://doi.org/10.1093/jxb/erf083

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7 : Molecular Evolutionary Genetics Analysis Version 7 . 0 for Bigger Datasets Brief communication 33, 1870–1874. https://doi.org/10.1093/molbev/msw054

Laucou, V., Launay, A., Bacilieri, R., Lacombe, T., Adam-Blondon, A.F., Bérard, A., Chauveau, A., De Andrés, M.T., Hausmann, L., Ibáñez, J., Le Paslier, M.C., Maghradze, D., Martinez-Zapater, J., Maul, E., Ponnaiah, M., Töpfer, R., Péros, J.P., Boursiquot, J.M., 2018. Extended diversity analysis of cultivated grapevine Vitis vinifera with 10K genome-wide SNPs. PLoS One 13, 1–27. https://doi.org/10.1371/journal.pone.0192540

Lipka, A.E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P.J., Gore, M.A., Buckler, E.S., Zhang, Z., 2012. GAPIT : genome association and prediction integrated tool 28, 2397–2399. https://doi.org/10.1093/bioinformatics/bts444

Marguerit, E., Brendel, O., Lebon, E., Leeuwen, C. Van, Ollat, N., 2012. Rootstock control of scion transpiration and its acclimation to water deficit are controlled by different genes.

Mercati, F., De Lorenzis, G., Brancadoro, L., Lupini, A., Abenavoli, M.R., Barbagallo, M.G., Di Lorenzo, R., Scienza, A., Sunseri, F., 2016. High-throughput 18K SNP array to assess genetic variability of the main grapevine cultivars from Sicily. Tree Genet. Genomes 12. https://doi.org/10.1007/s11295-016-1021-z

Nei, M., 1972. Genetic Distance between Populations. Am. Nat. 106, 283–292. https://doi.org/http://dx.doi.org/10.1086/282771

Ollat, N., Cookson, S.J., Destrac-Irvine, A., Lauvergeat, V., Ouaked-Lecourieux, F., Marguerit, E., Barrieu, F., Dai, Z., Duchêne, E., Gambetta, G.A., Gomès, E., Lecourieux, D., Van Leeuwen, C., Simonneau, T., Torregrosa, L., Vivin, P., Delrot, S., 2018. Grapevine adaptation to abiotic stress: An overview. Acta Hortic. 1248, 497–512. https://doi.org/10.17660/ActaHortic.2019.1248.68

Quénol, H., Marie, G., Barbeau, G., Leeuwen, C. Van, Foss, C., Irimia, L., Rochard, J., Boulanger, J., Tissot, C., Quénol, H., Marie, G., Barbeau, G., Leeuwen, C. Van, Hofmann, M., 2014. Adaptation of viticulture to climate change : high resolution observations of adaptation scenario for viticulture : the adviclim european project To cite this version : HAL Id : hal-01150259 Adapatation of vIticulture to climate change : high resolution o.

Riaz, S., Pap, D., Uretsky, J., Laucou, V., Boursiquot, J.M., Kocsis, L., Andrew Walker, M., 2019. Genetic diversity and parentage analysis of grape rootstocks. Theor. Appl. Genet. https://doi.org/10.1007/s00122-019-03320-5

Song, Jianbo, Mo, X., Yang, H., Yue, L., Song, Jun, Mo, B., 2017. The U-box family genes in Medicago truncatula : Key elements in response to salt , cold , and drought stresses 1–22.

Steudle, E., 2000. Water uptake by roots: effects of water deficit. J. Exp. Bot. 51, 1531–1542. https://doi.org/10.1093/jexbot/51.350.1531

Trenti, M., Lorenzi, S., Bianchedi, P.L., Grossi, D., Failla, O., Grando, M.S., Emanuelli, F., 2021. Candidate genes and SNPs associated with stomatal conductance under drought stress in Vitis 1–22.

Van Leeuwen, C., Agro, B.S., Irvine, A.D., 2019. An Update on the Impact of Climate Change in Viticulture and Potential Adaptations. https://doi.org/10.3390/agronomy9090514

GENERAL CONCLUSIONS

In the present PhD project, a large panel of grapevine rootstock genotypes were analyzed for drought tolerance. All genotypes came from different breeding programs performed by the Department of Agricultural and Environmental Sciences (DiSAA) of the University of Milano, at different steps in the selection process: i) the M-rootstocks, analyzed in the first part of the work (Part I), were recently released and they are available for winegrowers; ii) a new selection of 30 genotypes coming from different breeding programs, in the last steps of characterization before the release (Part II); iii) a breeding population of 141 genotypes obtained by rootstock M1 (Part III). Among these, some promising genotypes could be selected after the characterization process to be used as rootstocks for arid and semi-arid area, increasing the narrow genetic diversity of rootstocks.

In this work, several methods were adopted for water stress phenotyping. Plants were analyzed under controlled condition in greenhouse (Part I, experiment 1; Part III, experiment 2), semi-controlled conditions (Part I, experiment 2) and field conditions (Part II, experiments 1 and 2), depending on the aim of the experiment. Analysis of plant water status were performed with both traditional (e.g., Scholander pressure chamber) and innovative methods (e.g., thermography and imaging). In each experiment, several parameters were considered to characterize the response of vines to water deficit.

Results of the work can be observed at different levels: i) at practical level, with the identification of promising rootstocks able to face drought, such as M-rootstocks (Part I), 14 genotypes belonging to the new selection (Part II), and 25 genotypes belonging to the breeding population (Part III) ; ii) at physiological level, with the investigation of the response of rootstocks to water deficit (Part I), the rootstock control of scion gas exchange (Part I), the interaction between drought tolerance and nutritional status (Part II) and the correlation between plant growth, transpiration and leaf angle (Part III); iii) at genetic level, with the expression analysis of six genes related to the biosynthesis and signaling of ABA (Part I), and the identification of a target locus related to drought tolerance (Part III).

In further studies, promising genotypes can be studied for their tolerance to other abiotic stresses, like limestone or salt, and analyzed in grafting combinations with different varieties of *Vitis vinifera*. For the characterization of the physiological response of tolerant rootstocks, other parameters can be considered, such as root development, aquaporins and vessel size. The target locus related to drought tolerance identified in this work can be validated on reference rootstocks, analyzing the gene expression under water deficit, before to be involved in marker-assisted selection for future breeding programs.