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Research article

Multi-parameter characterization of water stress tolerance in *Vitis* hybrids for new rootstock selection

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ABSTRACT

Drought in grapevine could be faced using tolerant rootstocks. The present work aims at the evaluation of 25 new genotypes potentially tolerant to drought by using recent methods of phenotypical screening (thermography and on-solid reaction spectroscopy). Plants were grown in well-watered and stressed field conditions. Proxi for transpiration, wood hydrophobicity and starch content were used to characterize and classify the genotypes. The predominant role of the environment was highlighted, nevertheless genotype and genotype × environment interaction showed significant variations as well. Hybrids were classified based on their steady, susceptible or adaptable behavior. The 14 most promising genotypes were identified, 5 of them showing two tolerance mechanisms. In the future, results from this experiment will support viticulture in water limited areas releasing new drought-tolerant interspecific hybrids to be tested after grafting with different scions.

1. Introduction

During last decades, globally averaged temperature gradually increased, and rainfalls became more exceptional (Stocker, 2014). Many vineyard sites, including Mediterranean area, shifted into arid and semi-arid lands (Gago et al., 2014). Major issues are noticed in sandy and gravelly soils, characterized by low water retention (Tramontini et al., 2013) In the current scenario, water becomes a primary resource in grapevine management, affecting both quantity and quality of grape. Dai et al. (2011), identified in the use of drought tolerant rootstocks one of the most promising strategies to overcome the increasing problem of water stress, besides regulated deficit irrigation and the use of cover crops. Rootstocks have been adopted in the modern viticulture to counteract the widespread of phylloxera (*Daktulosphaira vitifoliae*) in the end of the 19th century. However, besides pathogen resistances, the use of rootstocks also affects the interactions between vines and the environment in terms of nutrients uptake, salinity tolerance and water stress resistance. Commercial rootstocks have been based on American species, such as *Vitis riparia* and *Vitis rupestris,* usually bred with *Vitis berlandieri*. However, nowadays around 90% of grown vines in the world are grafted to less than ten different rootstock genotypes, which have substantially unchanged since the begin of the last century (Keller, 2015).

Among American species, *V. champinii* and *V. doaniana* reported the best drought tolerance score, obtained by a multi-parameter analysis in irrigated/not irrigated experiment. Furthermore, low tolerance was shown in un-grafted plants of *V. riparia* and *V. berlandieri* (Padgett-Johnson et al., 2003; Rustioni et al., 2016). A large variability for drought tolerance has also been demonstrated for interspecific hybrids used as rootstocks and *V. berlandieri* x *V. rupestris* hybrids have been shown to be among the most resistant rootstocks to water stress (Ollat et al., 2016; Serra et al., 2014).

Since the end of last century, a new range of interspecific hydrides has been obtained by the breeding of traditional rootstocks, using the plant material of the Università degli Studi di Milano. The main objective was to improve the use efficiency of nutrients and water. *Vitis berlandieri* was used as recurrent genotype in the breeding program (Brancadoro et al., 2014). Most of these genotypes have not been characterized yet and they could represent an interesting source of variability for new rootstock selections. Nevertheless, the value of this collection has been already proved by the identification of interesting rootstocks belonging to the M series (M1; M2; M3; M4). High resistance to limestone was found in M1 and high uptake efficiency of potassium was noticed in M3 (Brancadoro et al., 2014). Rootstock M4 [(*V. vinifera* × *V. berlandieri*) × *V. berlandieri*] was instead proposed as tolerant to abiotic stresses, such as salinity and drought (Brancadoro et al., 2014; Meggio et al., 2014; Corso et al., 2015). According to Meggio et al. (2014) and Corso et al. (2015) M4 demonstrated better acclimatizing

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attitude to water stress than 101.14 (*V. riparia* × *V. rupestris*), a very sensitive rootstock to drought, maintaining a more performant photosynthetic activity during a drought experiment. Among the differentially expressed genes between M4 and 101–14, some of them were related to degradation of hormone abscisic acid (ABA) in the leaves and detoxification of Reactive Oxygen Species (ROS) and, thus, these two physiological processes were suggested to be involved in the stress tolerance (Corso et al., 2015). Other acclimation mechanisms, as the tendency to maintain more opened stomata once plants have been stressed in the past, have been reported (Tombesi et al., 2018).

Thermography is proposed as an innovative technique to estimate the transpiration. Temperature of leaf is strongly related to the stomatal opening and it can be easily recorded by a thermal camera (García-Tejero et al., 2016; Pou et al., 2014; Jones et al., 2002). The advantage of proximal sensing using thermography is the rapid performance, allowing high number of repetitions. Thus, this method is particularly indicated for screening a large number of genotypes within selection programs. Among thermal indexes, the best robustness is achieved with normalized ones, such as CWSI (García-Tejero et al., 2016).

Stems play a crucial role in water transport and drought management. For example, high water transport capacity and low sensitivity to embolism (Lovisolo et al., 2008; Carrier et al., 2016; Charrier et al., 2018) have been found in *Vitis berlandieri* x *Vitis rupestris* hybrids, generally considered tolerant to the water stress, among typical rootstocks (Koundouras et al., 2008; Tramontini et al., 2013). Lovisolo and Schubert (1998) proposed that the susceptibility of grapevines to embolism is linked to the size of their vessels. In particular, smaller vessels are usually less susceptible to embolism. In general, stem architecture and composition strongly affect drought tolerance (Tyree and Ewers, 1991). Hydrophobic barriers can be set up in vine vessels to prevent the water losses (Keller, 2015). Measurement of hydrophobic compounds allocated in the wood is a representative index of the water stress adaptation of vines (Rustioni et al., 2016). Indeed, the variance of the drought tolerance score of ten *Vitis* species, proposed by Padgett-Johnson et al. (2003), was strongly correlated (R^2 = 0.74) to the hydrophobicity of wood (Rustioni et al., 2016).

Moreover, embolism of trunks or shoots can be repaired by release of sugar and potassium ions or through starch remobilization (Keller, 2015; Brodersen et al., 2010; Salleo et al., 2009). Plants normally allocate starch in woody tissues to support the spring resumption. The amount of starch stored as reserve is affected by the water status of plants (Dayer et al., 2013; Herrera et al., 2015) and it is related to the balance between photosynthetic activity and carbon use to support growth. Under drought conditions, closure of stomata reduces the photosynthetic activity and consequently the amount of carbon allocated to starch. In addition, plants tap into starch storage to support the physiological activity. Indeed, a proteomic analysis of vine rootstocks found increasing number of enzymes involved in starch breakdown and sucrose synthesis under water stress, in both susceptible (101.14) and tolerant (M4) genotypes (Prinsi et al., 2018).

Like stomata regulation in leaves, the analyses of woody tissues require a huge number of replicates to provide a representative description of the phenotypic variability. On-solid reactions coupled by reflectance spectroscopy could represent a valid solution for rootstock screening purposes. Recently, two methods based on this approach have been proposed for the quantification of wood hydrophobicity (Rustioni et al., 2016) and starch storage (Rustioni et al., 2017).

The main objectives of this work are: i) to validate the analytical approaches and the multi-parameter assessment for drought studies; ii) to confirm the selection of parameters for rapid screening through proximal sensing; iii) to discriminate the physiological mechanisms for tolerance (adaptive VS constitutive; number of traits involved in the stress responses); iv) to rank and cluster genotypes, according to their drought-tolerance, as a first screening to direct further studies.

2. Materials and methods

2.1. Experimental sites

The study was conducted in 2017 in two different experimental vineyards located in Lombardy (Italy): 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.). The distance between the two experimental sites is 46 km. Six un-grafted vines per genotype were planted in 2014 and 2015 in Riccagioia and Arcagna, respectively. In Riccagioia, plants were spaced 2.40 m inter-row and 1.10 m intra-row, whereas in Arcagna the layout was 3.10 m inter-row and 2.00 m intra-row. Soils are silty-loam and clayey in Arcagna and Riccagioia respectively. The two sites have been characterized by agro-meteorological description. Daily values of temperature and precipitation were monitored in both sites. ARPA Lombardia provided the weather data for Riccagioia (reference station: Voghera), while in Arcagna a meteorological station was located close to the experimental vineyard. Moisture of soils was monitored at two different soil layers (40 cm and 80 cm depth) by WATERMARK Soil Moisture Sensor 200SS, produced by IRROMETER Company, INC., Riverside, California (USA). Drought stress intensity in each site was quantified by stem water potential (Ψ_{stem}) (Choné et al., 2001). Twenty-five replications (one per genotype) in each site were measured by using a Scholander pressure chamber (Scholander et al., 1965), produced by PMS Instrument Company, Corvallis, Oregon (USA). Around midday, leaves were covered for 1 h before analysis. Days of detections were on July 14th (DOY 195) and August 8th (DOY 220).

2.2. Plant material and experimental design

The 25 interspecific hybrids were grown in both sites. They all 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 hybrids of *Vitis* species and it is reported in Table 1. Drought response was measured by leaf thermography and wood reflectance spectroscopy.

2.3. Leaf thermography

Leaf thermography was recorded by using a 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. Data were recorded at two different sampling dates: July 14th, 2017 and August 8th, 2017, during the time laps 11.00 h/14.00 h, as suggested by García-Tejero et al. (2016). In each site, one representative plant was selected for each genotype. Three leaves per plant were selected and data were recorded by thermography in two different points per leaf. Temperatures were obtained by comparing leaves to the dry and wet references (green cardboard) present in each photo. Analyzed leaves were chosen in shade position, as suggested by Jones et al. (2002). Crop Water Stress Index (CWSI) was calculated following the formula proposed by Idso et al. (1981):

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

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

Thermal images were elaborated using the software “InfReC Analyzer NS9500 Lite”.

2.4. Wood reflectance spectroscopy

Wood reflectance spectroscopy was measured on stems (8–10 mm diameter) collected in the middle of December 2017, during vine dormancy. Shoots were stored in a cold room (4 °C) until analyses