UNIVERSITY OF VERONA

DEPARTMENT OF

BIOTECHNOLOGY

GRADUATE SCHOOL OF

NATURAL SCIENCES AND ENGINEERING

DOCTORAL PROGRAM IN

BIOTECHNOLOGY

WITH THE FINANCIAL CONTRIBUTION OF

Cavit s.c.

CICLO XXXI

INVESTIGATING THE GENETIC BASIS OF DROUGHT STRESS RESPONSE IN GRAPE ROOTSTOCKS

S.S.D. AGR/03

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Abstract

Consequences of climate change are becoming markedly worrying, since average surface temperatures are constantly going up and extreme climatic events are getting more frequent and intense, posing a considerable threat to worldwide viticulture. Among different abiotic stresses, drought is the factor that has a greater influence on plant physiology with a drastic impact on grape yield and quality. To overcome the deleterious effects of drought, plants adopt a multitude of physiological, biochemical and molecular mechanisms at cellular and systemic levels. Therefore, understanding the complexity of plant's response to water deficit represents a major challenge for sustainable winegrowing. Especially, the development of strategies to reduce water consumption and to improve water-use efficiency (WUE) in vines will be fundamental in future years. Furthermore, the regulation of water use is particularly influenced by rootstocks, on which cultivars are generally grafted to cope with phylloxera infestations. The adaptation to drought indeed seems to be a cooperative action between scions and rootstocks, by means of hydraulic conductivity, chemical signalling and exchange of genetic material. However, a very few number of works were focused on identifying the genetic regions of grape rootstocks responsible for drought tolerance mechanisms.

In this regard, the present research aimed to identify genetic determinism of phenotypic traits associated with drought tolerance. A genome-wide association study (GWAS) approach has been applied on an '*ad hoc*' association mapping panel including different Vitis species, in order to dissect the genomic bases of transpiration-related traits and to identify genetic regions of grape rootstocks involved in drought tolerance, thereby potentially relevant for crop improvement. The panel was first genotyped with the commercial GrapeReSeq Illumina 20K SNP array and infrared thermography has been applied to estimate stomatal conductance values and to assess water status during progressive water stress and re-watering in two years. Some significant marker-trait associations were detected and a good list of candidate genes with a feasible role in drought response were identified.

The physiological responses to drought were further investigate in four commercial rootstocks, 101.14 Millardet et de Grasset (*V. riparia* x *V. rupestris)*, Selection Oppenhiem 4 (*V. riparia* x *V. berlandieri*), 110 Richter (*V. rupestris* x *V. berlandieri*) and Riparia Gloire de Montpellier (*V. riparia*). Differences were observed among genotypes and between water stress experiments that were performed in pots and in hydroponics. Furthermore, the application of osmotic stress in a hydroponic system has proved to be a useful method to

evaluate the short-term stress response, especially for a rapid screening of stomatal sensitivity.

In addition, a pilot study on a reduced subset of *Vitis sylvestris* genotypes exposed to water deficit treatment was carried out to evaluate their drought tolerance, because they represent a source of natural genetic diversity that could be exploited for future breeding programs.

Taken together, a step forward to understand the basis of genetic variability of the response to water deprivation in grape rootstocks has been done in the present research. Moreover, it has been proved that different phenotyping approaches may help to dissect a highly complex trait such as water stress response.

CHAPTER 1

1.1 Plant abiotic stresses

Our climate is changing and strongly influencing human life and natural systems. Consequences of global warming are becoming particularly alarming, since the average surface temperature has increased by about 1 degree Celsius in the past 50 years, according to the National Oceanic and Atmospheric Administration (NOAA) and National Aeronautics and Space Administration (NASA) data (NOAA, 2016; GISTEMP Team, 2018). In addition, the Intergovernmental Panel on Climate Change (IPCC) predicted that atmospheric CO_2 concentration will reach to 720–1000 ppm by the end of this century, which could rise average surface temperatures by 3.5 °C. Besides, it is very likely that extreme climatic events will occur more often and become more intense in many regions with a drastic impact on crop production worldwide (IPCC report, 2014; Tollefson, 2018). Abiotic stresses, including drought, flooding, freezing, radiation (UV-B and UV-A) and heat waves adversely affect plant physiology. At first, they alter plant growth limiting vigour and vegetative development below optimum levels, but if stresses are prolonged, they lead to decline or death of shoots (Bechtold & Field, 2018). It is therefore crucial for plants to employ an efficient acclimatory response to survive in challenging environments. Adaption to abiotic stresses is a dynamic process including multifarious mechanisms, which are mostly determined by genetic plasticity (Bartels & Sunkar, 2005). In fact, plants are generally exposed to a combination of these stresses, such as drought and heat, which causes a very specific response (Suzuki et al., 2014; Zandalinas et al., 2017). To ensure their survival in the natural environment and to maintain growth, plants induce a multiplicity of physiological and biochemical signaling pathways that may interact and inhibit one another. Moreover, they activate many stress-related metabolic and gene regulatory networks (Nelson et al., 2007; Cramer et al., 2011; Yoshida et al., 2014; Zhu, 2016) and different type of molecules act as signal transducers such as reactive oxygen species (ROS) (Mittler et al., 2004), calcium (Bowler & Fluhr, 2000), hormones (Xu et al., 2006; Kazan, 2015; Vishwakarma et al., 2017) and sugars (Jossier, 2009).

1.2 Drought stress in plants

Water supply will pose one of the most relevant threats to the world, both in advanced and in developing countries. Climate models indicate that droughts will become more intense, more frequent, and longer, combined with an increased net irrigation requirement (NIR) (Zhao et al., 2015). The report of the World Resources Institute (WRI) estimates a substantially reduction of water resources across several regions, including the Mediterranean, within 2040 (Luck et al., 2015). Drought is the factor, among the different abiotic stress, which mostly reduces crop productivity worldwide (Boyer et al., 2013). Therefore, understanding the complexity of plant's response to water deficit poses a major challenge for researchers.

To overcome the deleterious effects of water stress and to complete their reproductive cycle, plants have adopted several adaptive strategies. Following deficit recognition they can cope with drought maintaining high tissue water potential (drought avoidance), withstanding at low water potential (drought tolerance) or limiting their life cycle to wet periods (drought escape) (Bohnert et al., 1995). Mechanisms underlying drought avoidance include reduction of stomatal conductance (Martin-StPaul et al., 2017), development of extensive root systems (Bengough et al., 2006), decrease in transpiration area (Blum et al., 1996) and improvement of xylem water capacity (Chaves & Oliveira, 2004). On the other hand drought tolerance is usually characterized by adaptive traits, which consist of accumulation of osmoprotectants (Peters et al, 2007), biosynthesis of epicuticular waxes (Shepherd & Griffiths, 2006) and remobilization of stem water-soluble carbohydrates (WSC) (Gupta et al., 2011). In the initial phase of stress, plant growth is rapidly inhibited ("acute response") whereas the subsequent period ("adaptation response") is characterized by the recovery of growth rate and by the adaptation to the constraints (Skirycz & Inzé, 2010). Basically, the limitation of growth is not only an adverse effect of resource shortages, but also an important process of water deficit adaptation (Levitt et al., 1972).

1.2.1 Physiological, molecular and biochemical mechanisms to tolerate water constraints

Plant drought stress resilience is the result of complex and dynamic physiological, biochemical and molecular processes at cellular and systemic levels (Figure 1). First and foremost, water deficit causes cellular dehydration and growth repression. As a consequence of external water potential reduction, water leaks out from the cells, causing a decrease in cell volume and of turgor pressure. Furthermore, the concentration of solutes inside the plasma membrane determines deformation of the cell wall and can lead to irreversible damages including protein denaturation (Feng et al., 2016). Aquaporins activity plays a prominent role in the regulation of water flow during the stress, reducing water uptake into the expanding cells and, consequently, inhibit plant growth (Javot et al., 2003). Drought stress also affects cell wall composition causing the deposition of phenolics and lignin monomers, which form covalently cross-links with cell wall glycoproteins. These mechanisms are attributed to expansin activity and cause cell wall inextensibility (Moore et al., 2008; Tenhaken, 2014). Inhibition of plant growth is also mediated by DELLA proteins that are in turn regulated by the plant hormones gibberellins (GAs) (Zentella et al., 2007; Navarro et al., 2008).

A fundamental mechanism to avoid plant dehydration is represented by stomatal closure (Martin-StPaul et al., 2017). Stomata constitute the first barrier to limit excessive decreases in water potential maintaining adequate tissue water content, without exceeding the hydraulic system capacity, to prevent wilting and stem embolism (Bartlett et al., 2016). The aperture of stomata is regulated by a pair of specialized epidermal cells called guard cells, which surround stomatal pores. Drought conditions cause a drop in the turgor pressure of the guard cells that release potassium ions and water through osmosis, thereby resulting in stomata closure (Ache et al., 2010). The phytohormone abscisic acid (ABA) plays a pivotal role in the stress signal transduction and transmission to leaf guard cells. ABA is synthetized in mesophyll cells of water stressed leaves (McAdam et al., 2018) and is transported on the epidermal layer, by specific transporters (Kuromori, 2018), to elicit stomatal closure. ABA also modulates the osmotically induced release of water from guard cells by activating plasma membrane-intrinsic proteins (PIPs) (Grondin et al., 2015). Moreover, Takahashi et al. (2018) recently identified a small signalling peptide, CLAVATA3/EMBRYO-SURROUNDING REGION-RELATED 25 (CLE25), which transmits water-deficiency signals from roots to leaves through vascular tissues, and regulates stomatal closure by modulating ABA biosynthesis. These findings elucidated the mechanisms of root-to-shoot signaling under drought. Xylem

tissue is in fact strongly involved in the water stress response and in the regulation of leaf transpiration rate (Comstock, 2002). In addition to improving water uptake from the soil, the xylem system perceives soil water status reducing leaf water potential, and thereby stomatal conductance. Stomata response to changes in hydraulic conductivity in turn allows to maintain water supply and prevent xylem cavitation (Jones & Sutherland, 1991), which may result in leaf and plant mortality.

Stomata also adjust the uptake of carbon dioxide and oxygen to optimize the respiration under water scarcity. Thus, the net photosynthetic rate strongly decreases together with stomatal conductance. The early phases of stress are characterized by changes in the cellular carbon metabolism with a reduction in the contents and activities of photosynthetic enzymes, such as ribulose- 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) (Lawlor et al., 2002) or phosphoenol pyruvate carboxylase (PEP-case) (Boyer et al., 1997). Prolonged drought stress results in the down regulation of photosystem II (PSII) activity which leads to a substantial loss of photosynthetic pigments together with the degradation of thylakoid membrane structure (Reddy et al., 2004). Consequently, photochemical efficiency is reduced, the rate of O_2 photoreduction increases and reactive oxygen species (ROS) (O_2-, H_2O_2, OH) accumulate in chloroplasts. These oxygen free radicals cause several oxidative damages such as protein denaturation, phospholipid peroxidation and oxidation of DNA (Noctor & Foyer, 1998). The anti-oxidative defense system preventing excessive damages caused by ROS accumulation includes non-enzymatic and enzymatic components (Møller et al., 2007). The non-enzymatic antioxidants are divided in ROS-scavengers (ascorbic acid (AsA), reduced glutathione (GSH), α-tocopherol) and pigments (carotenoids, flavonoids and phenolics) (Das & Roychoudhury, 2014). On the other hand, the major antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), peroxidases (APX, GPX) (Mittler et al., 2004). The accumulation of protective compatible solutes represents one of the most valuable mechanisms adopted by plants to reduce ROS production, thereby limiting the deleterious effects of water stress (Hare et al., 1998; Taji et al., 2002; Van den Ende & Valluru, 2009). These compounds, also known as osmoprotectants, are divided in three categories: betaines, sugars/ polyols, and amino acids. The more representative group is composed by trehalose and raffinose family oligosaccharides (RFOs) sugars, such as sucrose, galactinol, raffinose, stachyose, mannitol and myo-inisitol (Bachmann & Keller, 1995; Nishizawa et al., 2008). In addition to their antioxidant function, these compounds are involved in the osmotic adjustment process, which consist in concentrating osmotically active substances inside the cell: in vacuoles, cytosol, and chloroplasts. This leads to a reduction of osmotic potential that

reestablish cell turgor, hence stabilizing the protein structures and maintaining the membrane integrity (Sanders & Arndt, 2012). Moreover, these carbohydrates constitute an alternative energy resource that can be accumulated without affecting primary metabolism and promote the process of dehydration recovery (Peters et al., 2007; Egert et al., 2015).

Finally, when water stress reaches intolerable levels, the last resort practiced by plants is the induction of programmed cell death (PCD) (Petrov, 2015). This senescence mechanism is usually mediated by ROS and involves some proteases, such as cysteine proteases, which are drought-specific and do not take place under natural aging (Khanna-Chopra et al.,1999). Leaf senescence represents an adaptive strategy to save water and to redistribute nutrients stored in older leaves to growing plant tissues (Munné-Bosch & Alegre, 2004).

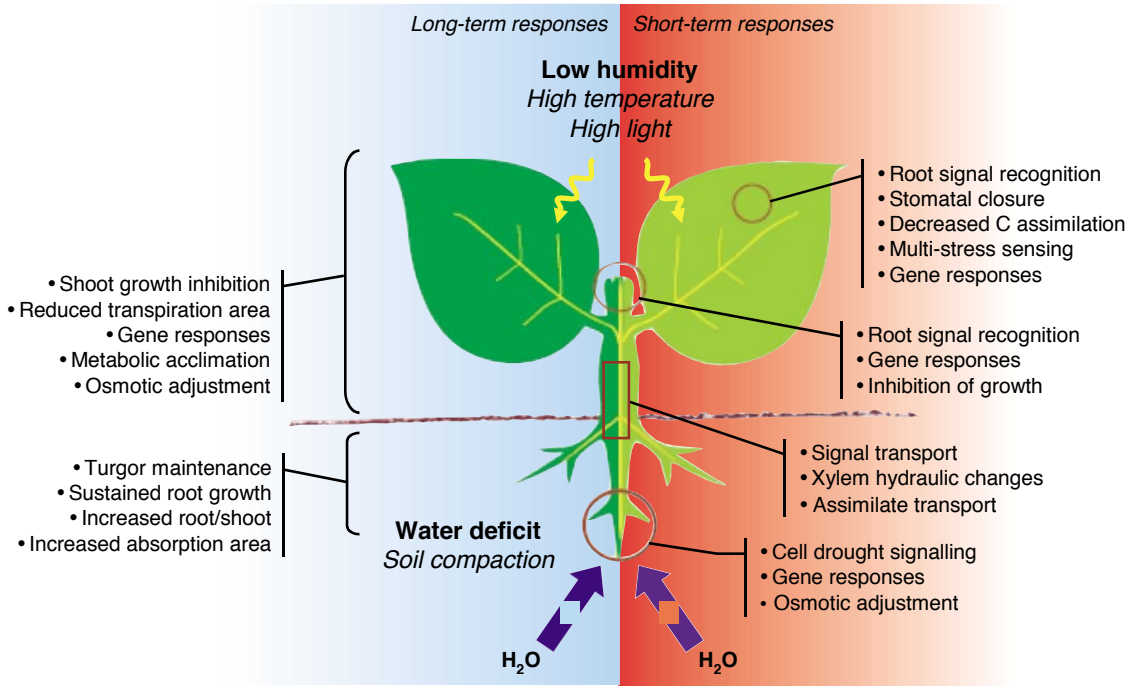

Figure 1: Whole plant physiological, molecular and biochemical responses to drought stress (Source: Chaves et al., 2003)

1.2.2 Gene pathways involved in the drought stress response

A well-structured network of plant's response pathways confers adaptation and tolerance to the water constraints. Genes taking part in these mechanisms can be essentially divided in two categories: functional and regulatory genes. Functional genes are directly involved and their products are proteins and enzymes with predominant roles in protecting cell structures, reducing water loss, reestablishing osmotic homeostasis and repairing damage. On the other hand, regulatory genes contributes to drought signal perception and transduction.

A large part of functional genes codify for primary and secondary metabolites, which participate in osmoprotection by osmotic adjustment and free radical scavenging. Polyamines (PAs) (Cappell et al., 2004), glycine betaine (GB) (Quan et al., 2004) and proline (Vendruscolo et al., 2007) synthases were modulated during drought and confer stress tolerance. Moreover, as already mentioned in the previous chapter, genes involved in the biosynthesis of trehalose (Nuccio et al., 2015), RFOs (Taji et al., 2002), mannitol (Abebe et al., 2003) and antioxidant enzymes (D1az-Vivancos) limit the deleterious effects of water stress and of ROS accumulation. Some functional genes directly reduce transpirational water loss inducing stomatal closure, eg. *SLAC1* that codify for an anion channel (Geiger et al., 2009), NADPH oxidases (*AtrbohD* and *AtrbohF)* (Kwak et al., 2003) or *CaDIL1* that synthesize a late embryogenesis abundant (LEA) protein (Lim et al., 2018). Finally, it is important to emphasise the role of the aquaporin gene family, which regulates the movement of water through plant compartments and could improve the ability to save water (Zhou et al., 2012).

Drought regulatory network involves several families of transcription factors (TFs), *cis*-acting elements, protein kinases, receptor-like kinases and plant hormones. Regulatory genes could be subdivided in a ABA-independent and in a ABA-dependent signal transduction pathway (Yamaguchi-Shinozaki and Shinozaki, 2006) (Figure 2). Indeed, the hormone abscisic acid (ABA) plays a fundamental role in regulating drought-induced gene expression. Furthermore, the limiting step in its biosynthesis is controlled by 9-*cis*-epoxycarotenoid dioxygenase 3 *(NCED3)* (Qin & Zeevaart 1999), whose expression is promptly induced by water stress. Then, the Ser/Thr protein kinases *Casein Kinase 2 (CK2)* and *Sucrose Non Fermenting Kinase 2 (SnRK2)* (Yoshida et al., 2002; Vilela et al., 2015), the phosphatases *PP2C* (Komatsu et al., 2013) and the *pyrabactin resistance 1-like (PYL)* receptor (Park et al., 2009) mediate first phases of ABA signaling after stress perception. This enzymatic complex activate the bZIP-type transcription factors *ABA-responsive element (ABRE) binding protein 1 (AREB1)* and *ABRE-binding factor 2 (ABF2)* by phosphorylation (Fujita et al., 2005; Furihata et al., 2006; Yoshida

et al., 2015), which in turn regulate several downstream genes containing ABRE *cis*-regulatory element (CRE) (PyACGTGG/TC) in their promoter regions. Many of these stress-responsive genes are TFs involved in the regulation of many processes in plants: MYb/MYc (Cominelli et al., 2005; Zhao et al., 2014), NAC (Hu et al., 2006), WRKY (Jiang et al., 2012), and NF-Y (Nelson et al., 2007; Li et al., 2008). An alternative ABA-independent signal transduction pathway regulate the expression of other drought responsive genes, which contain dehydration-responsive element binding protein (DREB) in their promoter regions (Nakashima et al., 2009). The main regulons of this pathway are Dehydration-responsive element binding protein 1 (DREB1)/C-repeat binding factor (CBF) and DREB2 (Liu et al., 1998). They recognize a 9 bp long (TACCGACAT) conserved *cis*-element DRE and activate the expression of several downstream genes (Maruyama et al., 2009; Cheng et al., 2012). However, TFs and stress-responsive genes activated by ABA-independent and ABA-dependent pathways could interact with each other, hence they constitute a highly complex network that has not totally revealed yet.

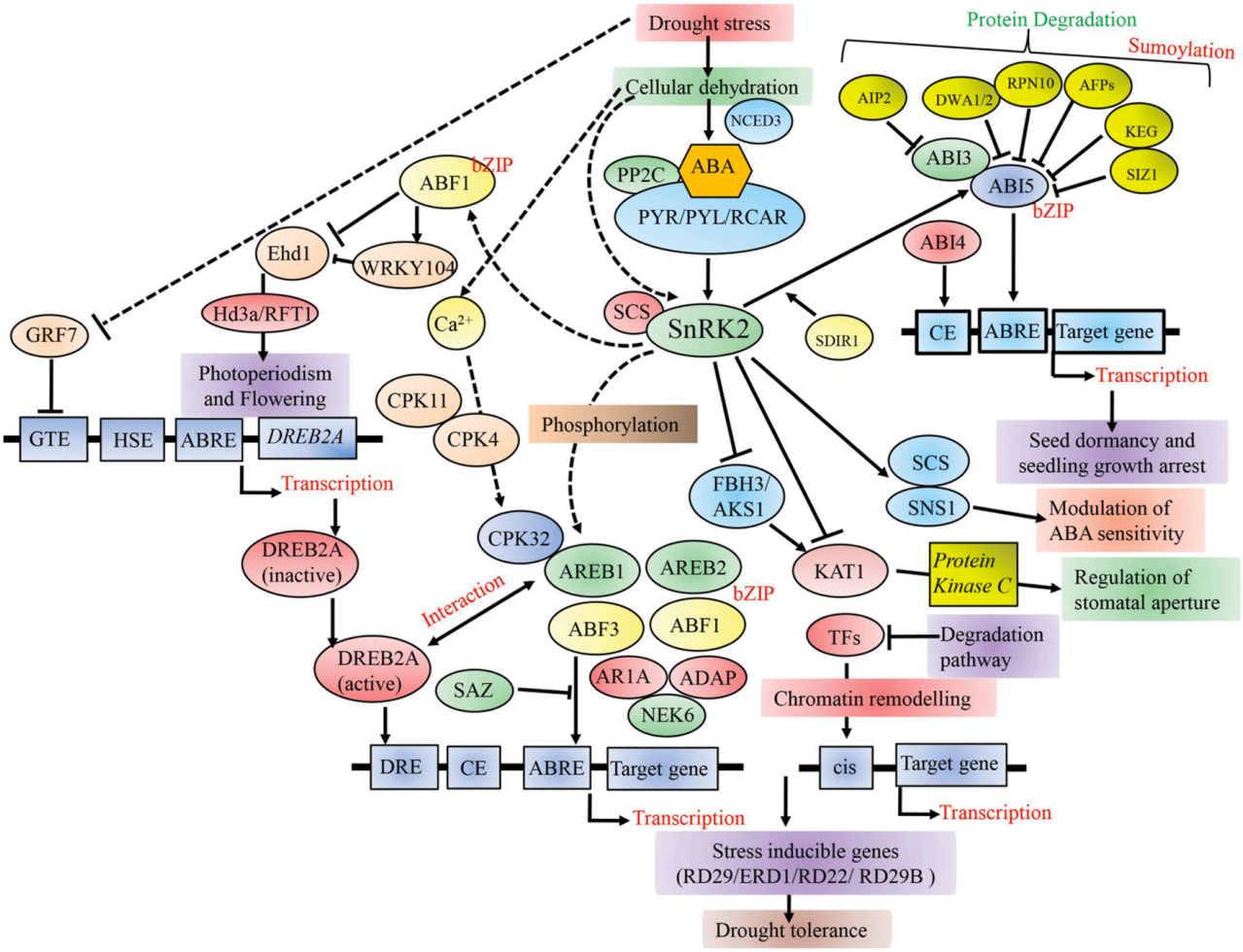

Figure 2: A schematic representation of drought regulatory network and of different TFs involved in dehydration response in plants. Drought signal perception leads to activation of both abscisic acid (ABA)-dependent and ABA-independent pathways. ABA-dependent pathway: accumulation of ABA leads to activation of sub class III SnRK2s through PYR/PYL/RCAR-PP2C receptor complex. SnRK2s phosphorylates four transcription factors ABA-responsive element (ABRE) binding protein 1 (AREB1), AREB2, ABRE binding factor 3 (ABF3), and ABF1 that regulate most of the downstream genes by binding to the ABRE *cis*-element present in their promoter region. ABA-independent pathways: *DREB2A* expression, which is a key TF of this pathway, is regulated by GRF7. Additionally, AREB/ABFs interact with DREB2A indicating a crosstalk between ABA-dependent and ABA-independent pathways under drought stress. (Source: Joshi et al., 2016)

1.3 Grapevine response to water deficit

Grapevine is considered to be a relatively drought tolerant plant, thus the impact of climate change on viticulture sustainability is subject of lively debate (Hannah et al. 2013; Van Leeuwen et al. 2013; Mosedale et al. 2015; Charrier et al. 2018). Global warming could expand the grape production in new areas, and on the contrary, some of the main wine-growing regions may suffer negative economic consequences owing to lower grape yield. However, data collected during several seasons in two of the most important worldwide wine regions, Napa Valley and Bordeaux, showed that grapevines hardly reach drought stress level result in significant embolisms and leaf mortality (Charrier et al. 2018). What is certain is that strategies to reduce water consumption and to improve water-use efficiency (WUE) in vines are fundamental for the viticulture sustainability (Dos Santos et al., 2003).

Grapevines are mostly cultivated in drought prone areas and therefore manifest specific physiological responses and morphological changes under water constraints. Shoot and leaf growth is recessed in the first phases of stress, thereby limiting the vegetative development of the vines (Simonneau et al., 2017). Consequently, there is a decrease of evaporative surfaces and of water demand. Moreover, stressed grapevines exhibit drought avoidance mechanisms such as reduction in the stomatal conductance (Chaves & Oliveira, 2004; Hochberg et al., 2017) and in the xylem hydraulic conductivity (Lovisolo et al., 2002). A quick stomata closure, with the consequent decline of transpiration allow to prevent embolisation together with an increase in WUE and a decrease in carboxylation efficiency. Stomatal movements in response to water deficit is controlled by abscisic acid (ABA), which is accumulated in the stomatal guard cells. A transcription factor of MYB family, VVMYB60, was demonstrated to have a prominent role in this mechanism in grapevine (Galbiati et al., 2010). And several ABA biosynthetic genes, such as *NCED1*, *NCED2* and *ABA 8'-hydroxylase (Hyd1)*, were expressed in different grape tissue (roots, xylem sap, and leaves) in response to water deficit (Speirs et al., 2013). Furthermore, the expression of genes related to ABA metabolism and signalling could differentiate *Vitis* genotypes on the basis of their drought tolerance level (Rossdeutsch et al., 2016). However, as reported in the previous chapter, plant mechanisms of water deficit perception and ABA root-to-shoot signalling are not yet completely understood (Zhu et al., 2016).

These drought-avoidance physiological responses are associated with a decrease of plant water potential, in order to maintain water conductivity and photosynthetic activity, in consequence of soil drying as negative soil water potential arises (Chaves et al., 2010).

Nevertheless, different cultivar apparently exhibit distinct behaviors in response to the water potential decline. Some grapevine genotypes adopt a near-isohydric behaviour, which allow them to maintain a higher water potential under stress condition by means of a promptly stomatal closure. On the other hand near-anisohydric varieties tend to keep their stomata open despite strongly reductions in water potential (Schulz, 2003; Chaves et al., 2010). However, the separation of grape genotypes in these two stress-responsive categories is still controversial, since some cultivars can display contradictory behaviors depending on the environmental conditions (Lovisolo et al., 2010; Rogiers et al., 2012; Tomás et al., 2014; Lavoie-Lamoureux et al. 2017). Although, the genetic variations in the two responsive categories were widely investigated (Coupel-Ledru et al., 2014; Dal Santo et al., 2016), and more recently, Charrier et al. (2018) even belied the different stomatal regulation under moderate drought stress between the more representative anisohydric and isohydric varieties, respectively, Syrah and Grenache.

As drought stress becomes more severe the photosynthesis rate is drastically limited. More specifically, the net CO_2 assimilation (A_n) is inhibited and Rubisco activity is impaired (Flexas et al., 2002), which cause an increase of intracellular reactive oxygen species (ROS) (Carvalho et al., 2015). The accumulation of inorganic ions and compatible solutes counteracts the deleterious effects of ROS in grapevine as osmoprotectants (Pillet et al., 2012) in addition to antioxidant enzymes (Vidigal et al., 2014) and metabolites, such as flavonoids or stilbenoids (Król et al., 2014; Houillé et al., 2015). Furthermore, osmolytes maintain the cell turgor under drought by means of osmotic adjustment mechanisms (Conde et al., 2015). Therefore, the expression of several grapevine sugar transporter genes, e.g. *VvHT5*, *VvSUC11* and *VvGIN2,* was enhanced in leaf tissue during water stress (Medici et al., 2014).

A valuable long-term response to improve the water uptake and WUE under drought conditions is represented by deep rooting, which involved expansins (Lovisolo et al., 2010) and aquaporins (AQPs) activities (Vandeleur et al., 2009). Root development is strongly influenced by soil type, but mostly depend on rootstock genotype (Bauerle et al., 2008). Conversely, AQPs have a prominent role in water transport from roots to leaves and their gene expression and functions was widely studied in response to water deficit (Perrone et al., 2012; Pou et al., 2013).

Water stress, obviously, has also a drastic impact on fruit quality limiting berry size and production yields. Moreover, grape berry composition is significantly affected in both primary and secondary metabolites content (Deluc et al., 2009). Several studies investigated the effect of drought on sugars (Gaudillère et al., 2002), phenolics (Ojeda et al., 2002; Castellarin et al.,

2011; Santesteban et al., 2011) and terpenoids (Song et al., 2012) concentrations. Lastly, Savoi et al. (2017) highlighted, through a multi-omics approach, genes, metabolites and signal transduction pathways involved in berry metabolism under drought. Nevertheless, it should be emphasized that a mild water deficit could be beneficial for wine quality. Regulated deficit irrigation (RDI) promotes the accumulation of sugars in fruit inhibiting lateral shoot growth (Chaves et al., 2010) and enhances the production of flavour compounds (Ripoll et al., 2014). On the contrary, excessive irrigation might affect fruit pigments and sugar contents, thereby decreasing the quality of wines (Medrano et al., 2003). Thus, an accurate and fine modulation of water usage could improve fruit characteristics. Furthermore, the regulation of plant water balance is strongly influenced by rootstocks, which exhibit a large variability in WUE, therefore the selection of an appropriate scion-rootstock combination is crucial.

1.3.1 Role of rootstocks in the regulation of water use

At the end of the 19th century the spread of phylloxera devastated European vineyards. To cope with this plague vines were grafted on phylloxera-resistant American *Vitis* species and almost all vineyards over the world are still grafted (Granett et al., 2002). Therefore, rootstocks influence the vigour of scions, the production yield (Stevens et al., 2010) and their berry composition (Ruhl et al., 1988; Cortell et al., 2007). The effect of rootstock was also investigated in the regulation of water use (Gambetta et al., 2012) and in the adaptability to stress conditions (Keller et al., 2012; Meggio et al., 2014). Regarding drought tolerance, a considerable variability was observed among rootstock genotypes (Serra et al., 2014) and also stomatal control of scions is strongly influenced by rootstock (Marguerit et al., 2012; Tramontini et al., 2013; Peccoux et al., 2017). So the adaptation to drought seems to be a cooperative action between scions and rootstocks, which control hydraulic conductivity and transmit chemical signals to the scions that in turn regulate stomatal conductance. The efficiency in soil water extraction during drought period may differ between rootstock genotypes (Bauerle et al., 2008) and a prominent role of aquaporins (VvPIP2-1, VvPIP2-2) in root hydraulic management was demonstrated (Gambetta et al., 2012). Therefore, drought tolerant rootstocks showed an enhanced hydraulic conductivity in response to water deficit (Barrios-Masias et al., 2015) together with a different production of ABA (Rossdeutsch et al., 2016). On the other hand, as regard scion transpiration, Marguerit et al. (2012) identified some rootstock genomic regions associated with stomatal movements during drought. Furthermore, rootstocks are also involved in the recruitment of bacterial communities associated with the rhizosphere (Marasco et al., 2018), among which plant growth promoting (PGP) bacteria that may protect plants from drought (Rolli et al., 2014).

Transcriptomic studies investigated the effect of rootstocks on root, leaf and berry metabolism in water stressed grapevines (Berdeja et al., 2015; Corso et al., 2015). However, genetic bases of drought response in rootstocks are largely unknown at present and very few works investigated if rootstocks alter the gene expression of scions (Cookson et al., 2013; Chitarra et al., 2017) or if there is an exchange of genetic material between them (Yang et al., 2015; Pagliarani et al., 2017). This is probably due to difficulties in experimental conditions, which must take account of the synergic interaction of soil, rootstock and scion on the overall drought response (Zhang et al., 2016). Finally, from an agronomic point of view, a drought tolerant rootstocks should protect itself from stress damages and at the same time maintaining productivity and yields in scions. Therefore, all the factors that contribute to

water stress resilience in this absolutely complex rootstock-scion interaction have to be deeply investigated and it is going to be a pivotal point in the next years.

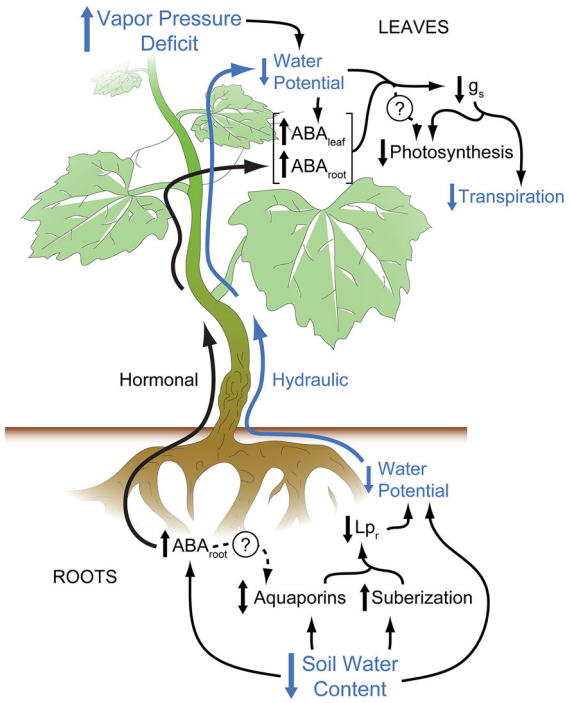

Figure 3 Schematic representation of various mechanisms through which rootstock influences scion behavior under drought. (Source: Zhang et al., 2016)

Objectives

Grapevine responses to water stress are controlled by complex regulatory events mediated by physiological, biochemical and molecular processes as well as environmental factors. Additionally, understanding the genetic determinism of traits valuable for drought stress tolerance in grapevine is quite complicated; especially for its highly heterozygous genome and for the unrevealed mechanisms of interactions between cultivars and rootstocks on which scions are usually grafted in viticulture. Therefore, the present research aims to provide further information on the genetic basis of water stress response in grapevine rootstocks and has been structured as follows:

- in Chapter 2 the aim of the study is to identify genetic regions associated with stomatal regulation involved in drought tolerance through a Genome Wide Association Study on an association mapping panel including different Vitis species.
- in Chapter 3 the aim is to deeply investigate the function of a drought-associated candidate gene, formerly identified in GWAS, and to accurately characterize the physiological responses to drought in four commercial rootstocks differentially adapted to drought.
- Chapter 4 aims to evaluate the exploitation of *V. vinifera* subsp *sylvestris* genotypes for drought tolerance related traits, through a pilot study on a reduced subset of accessions, currently maintained *ex-situ* at the (Edmund Mach Foundation) FEM grape repository.

CHAPTER 2

A genome-wide association study to uncover the genetic basis of drought response in grapevine rootstocks

Introduction

Revealing novel mechanisms for water stress resilience in natural populations could be an efficient strategy for crop improvement to face daunting climate change challenges. Selective breeding deeply influenced the genetic diversity of modern crops over the past years. Natural variations were selected from crop germplasm, wild species or plants adapted to survive in extreme ecological niches (Mickelbart, 2015). Nevertheless, the genetic basis of phenotypic variations associated with drought tolerance mechanisms, such us stomatal regulation (Buckley, 2005), water-use efficiency (Tuberosa, 2012) or osmotic adjustment (Hare et al., 2008), are generally poorly understood. Currently, fundamental advances are made in plants genomics with the availability of reference genomes and high-throughput sequencing technologies (1001 Genomes Consortium, 2016). This prompted plant scientists to deepen the study of plant genetic diversity (Huang & Han, 2014), detecting genetic regions associated with drought tolerance (Harris et al., 2007; Uga et al., 2011; Yu, 2017; Dittberner et al.,2018) and identifying genes and regulatory elements within these regions (Nelson et al, 2007; Mao et al., 2015). Among the various approaches for identifying genes involved in phenotypic traits, quantitative trait loci (QTL) mapping (Takeda & Matsuoka, 2008) and genome-wide association study (GWAS) (Aranzana, 2005) are the most adopted. The main difference between QTL and GWAS strategies is that the former is applied to populations obtained by controlled crosses in order to dissect the co-segregation of phenotypic traits with molecular markers, whereas the latter is based on populations of unrelated individuals and at least ideally aims to genetically explain a large portion of species-wide variation (Bazakos et al., 2017). QTL mapping studies allow the detection of rare variants in comparison with GWAS studies, as long as those alleles are carried at least by one parent and segregate in the progeny. On the other hand, the resolution of QTL intervals is usually low and might include over a hundred genes, due to the scarcity of recombination events occurred (Esch et al., 2007). On the contrary, GWAS exploit a multitude of recombination events happened during the history in natural population. Therefore, this provides higher mapping resolution and smaller

haplotype blocks that could be associated with phenotypic traits of interest in species with a fast linkage disequilibrium (LD) decay, such as grapevine (Platt et al., 2010). Moreover, GWAS is usually preferable to identify significant genotype-phenotype correlations for polygenic traits, even though there are some limitations such as the missing heritability of traits and the population stratification (Korte & Farlow, 2013).

Several studies were conducted on grapevine to identify genetic regions, and major genes within them, involved in fruit quality traits (Battilana et al., 2009; Costantini et al., 2015), in agronomic traits (Houel et al., 2015; Tandonnet et al., 2018), in tolerance to biotic (Teh et al., 2015; Sapkota et al, 2018; Smith et al., 2018) and abiotic stresses (Marguerit et al., 2012; Coupel-Ledru et al., 2014; Henderson et al., 2017). However, GWAS approach was rarely applied (Chitwood et al., 2014; Marrano et al., 2018) on account of limitations in designing an adequate association panel, which should represent much genetic diversity as possible, without complex patterns of population stratification or cryptic relatedness (Nicolas et al., 2016). Moreover, GWAS requires a sufficient number of Single Nucleotide Polymorphisms (SNPs) to ensure the coverage of most genomic regions and a reasonable power to identify variants associated with phenotypic traits, particularly in crop species with low level of LD. Genotyping-by-sequencing (GBS) is a useful and cost-effective method to discover a large number of SNPs, thanks to advances that has been made in Next Generation Sequencing (NGS) technology (Elshire et al., 2011). Although, the low coverage of sequencing and the difficulties in handling high amount of raw sequences may result in several genomic region with missing data. Alternatively, SNP arrays rely on the prior production of sequence information, following by the identification and validation of polymorphisms (Myles et al., 2010; Le Paslier et al., 2013).

Drought tolerance in plants is substantially due to a complex and dynamic response that involves numerous plant tissues and biological pathways. Therefore, correct and accurate phenotyping plays a pivotal role in the dissection of genetic basis of drought response (Tuberosa, 2102). The main difficulties encountered in grape phenotyping are the large sizes of vines, their perennial nature, as well as the long juvenile phases; in addition some methods for evaluating water stress status, such as water potential (Ψ) measurement, are invasive (Hsiao, 1973; Campbell, 1985). Spreading of new high-throughput and high-resolution phenotyping tools, which collect phenotypic data with precise, non-invasive and non-destructive methods, facilitate the record and the repeatability of measurements (Furbank & Tester, 2011). The application of chlorophyll fluorescence, RGB, near infrared (NIR) and hyperspectral imaging to assess grapevine phenotypic traits has become more common in

recent years (Ghozlen et al., 2010; Kicherer et al., 2017). In particular, infrared thermography has been successfully used to monitor stomatal conductance and water status in vineyards (Costa et al., 2012; Gago et al., 2017; Diago et al., 2018; Gutiérrez et al., 2018; Bianchi et al., 2018). Infrared thermography can detects subtle differences in surface temperature by measuring emitted infrared radiation, which is dependent on the rate of transpiration and could be a valuable indicator of stomatal conductance and of water stress severity (Jones, 1999).

In the present research the application of infrared thermography allowed to evaluate the grapevine rootstocks response to water deficit in an ad hoc core-collection, reducing the time for collecting phenotypic traits, and thus allowing the screening of numerous genotypes. A GWAS approach was adopted to dissect the genomic bases of transpiration-related traits and aimed to identify genetic regions involved in drought resilience potentially relevant for crop improvement.

Materials and methods

Definition of a genetic core collection

The association population consisted of 100 grapevine (*Vitis* spp.*)* accessions, including non-*vinifera Vitis* species (Rootstocks) and interspecific hybrids (Hybrids). This genetic core collection was created in order to maximize the genetic diversity within a germplasm collections and was based on a set of 22 SSR and 384 SNPs markers as described by Emanuelli et al. 2013. To construct the genetic core collections the Maximization (M) method (Schoen & Brown, 1993), which is implemented in the MSTRAT software (Gouesnard et al., 2001), was applied. The M strategy selects specific combinations of accessions while maximizing the number of observed alleles at each marker locus and the MSTRAT uses iterative procedures to select samples with the highest allelic diversity. The final number of iterations per MSTRAT run was 200, while the number of repetitions for core sampling was 100. Putative core collections exhibiting the same allelic richness were ranked using Nei's diversity index (Nei, 1987). The accessions that were most often present in the 100 replicates were retained as the final core collection. The genetic structure of the association panel was analyzed with STRUCTURE software v1.0 (Pritchard et al., 2000), which uses a variational Bayesian framework for approximate inference of subpopulations (Falush et al., 2003). A number of ancestral genetic groups (K), ranging from 1 to 10, was tested by 10 independent iterations for each K. The most likely K value was chosen running the algorithm for multiple choices of K and by plotting the marginal likelihood of the data. The software CLUMPP v1.1.2 (Jakobsson & Rosenberg, 2007) was used to find optimal alignments of the independent runs and the output was used directly as input into the program for cluster visualization DISTRUCT v1.1 (Rosenberg, 2004).

SNP genotyping

The commercial GrapeReseq 20K SNPs array, which contains 15022 SNPs from *Vitis vinifera* genotypes and 4978 SNPs from *Vitis* species, was used to genotype the whole population with the Infinium technology following the manufacturer's instructions (Illumina, Inc., San Diego, CA, USA). The genomic DNA of the Pinot Noir cultivar was used as control. The raw SNP data generated were clustered and automatically called using the Genotyping Module V1.9 of the Illumina GenomeStudio Data Analysis software. SNPs with a Call Freq score 0 and a GenTrain score < 0.6 were filtered out. Markers with a Cluster Sep score < 0.4 were visually inspected

for accuracy of the SNP calling. SNPs with R mean score > 0.3 and with clusters not overlapped were retained.

Plant material and drought stress experiment conditions

All plants were multiplied vegetatively to give six replicates and were grown in a greenhouse under partially controlled climate conditions. The vines grew in a 5-L pot filled with a substrate composed of sandy loam soil and peat (4:1 in volume). Soil water content (SWC) was determined by gravimetric method from the difference in weight between the wet and the dry soil (Black, 1965). Two irrigation treatments were established. Three plants were irrigated maintaining the 90% of SWC (control plants) and three plants (water stressed plants) were subjected to a gradual drought stress: a moderate stable water deficit (50% of SWC for 7 days), following by a severe stable water deficit (30% of SWC for 7 days) and lastly a recovery period (90% for 5 days). The experiment was repeated for three successive years: 2012 (1° year), 2013 (2° year) and only on a small subset of population in 2014 (3° year).

Thermal indices and stomatal conductance estimation

The physiological response to drought was evaluated over 30 days. To evaluate the effect of water stress thermal images of the grapevine leaf canopies were elaborated using the software InfReC Analyzer (NS9500LT). Stomatal conductance was estimated from two different thermal indices: crop water stress index (CSWI) (Eq. 1) (Idso et al., 1981) and thermal index (I_g) (Eq. 2) (Jones, 1999).

$$CWSI = \frac{T_{canopy} - T_{wet}}{T_{dry} - T_{wet}} \quad (1) \qquad IG = \frac{T_{dry} - T_{canopy}}{T_{canopy} - T_{wet}} \quad (2)$$

where T_{canopy} (°C) was the temperature deduced from the thermal images of six sun-exposed mature leaves per vine, T_{dry} (°C) and T_{wet} (°C) are the temperature detected on the "reference surfaces" made with cardboard.

Stomatal conductance (g_s) and transpiration were measured with a steady state porometer (Licor Li-1600) in the third experimental year.

Association analysis

Genotype-phenotype associations were tested using the average value of each trait in each year separately. If the phenotype scores were not normally distributed they were transformed using the logarithm function. GWAS was performed applying three models which account for different confounding factors to prevent spurious marker-trait associations. The first model applied was the General Linear Model (GLM), which considers the population structure calculated with STRUCTURE as a cofactor. The GLM can be described by the following matrix notation:

$y_i = \mu + x_i\beta + Q\nu + \varepsilon$ (1)

where y_i is the phenotypic value of i^{th} sample, μ is the model intercept, β is a vector of SNP effects, ν is a vector of population effect and ε is a vector of residual effects, Q is the matrix from STRUCTURE which presents the individual probabilities to belong to a subpopulation. The second model employed was the Mixed Linear model, which extends equation (1) taking also into account a kinship matrix (K) to determine the degree of genetic covariance between pairs of individuals (Yu et al., 2006). Using TASSEL v. 5.2 (Bradbury et al. 2007) a centered identical-by-state K matrix was estimated with the method of Endelman and Jannink. The third model (Q+K model) including both a fixed effect as the population structure matrix (Q) and a random effect as the kinship matrix (K). A quantile-quantile (Q-Q) plot was drawn to determine the model which better accounts for population structure and familial relatedness in the marker-trait association. P-values adjustment for multiple testing was adopted: in addition to the Bonferroni-corrected critical p-values, q-values were also calculated based on their corresponding p-values to identify significant associations between a trait and the SNPs. The q-value is a measure of significance in terms of False Discovery Rate (FDR) (Benjamini & Hochberg, 1995) that limits the false positive results while offering a more liberal criterion than Bonferroni correction factor. A q value of 0.1 was used as significant association threshold (Benjamini & Yekutieli, 2005). GWAS results were visualized with Manhattan plots that were yielded from the qqman and CMplot packages of R software (Turner, 2014).

Results

Construction of a genetic core collection of rootstocks

The study was conducted on 100 *Vitis* spp accessions included in a genetic core collection. A two-steps procedure was applied in order to define a restricted set of genetically highly diverse *Vitis* accession to be used as an ad hoc association panel. Firstly, a core collection was created from *non-vinifera Vitis* species and interspecific hybrids used for fruit production maintained at Fondazione Edmund Mach in grape germplasm collection (Emanuelli et al. 2013), to maximize the allelic diversity among wild *Vitis*, rootstocks and hybrids accessions based on microsatellites. Thus, based on M-method, 98 accessions (out of 231) were sufficient to capture all 412 alleles (Figure 1).

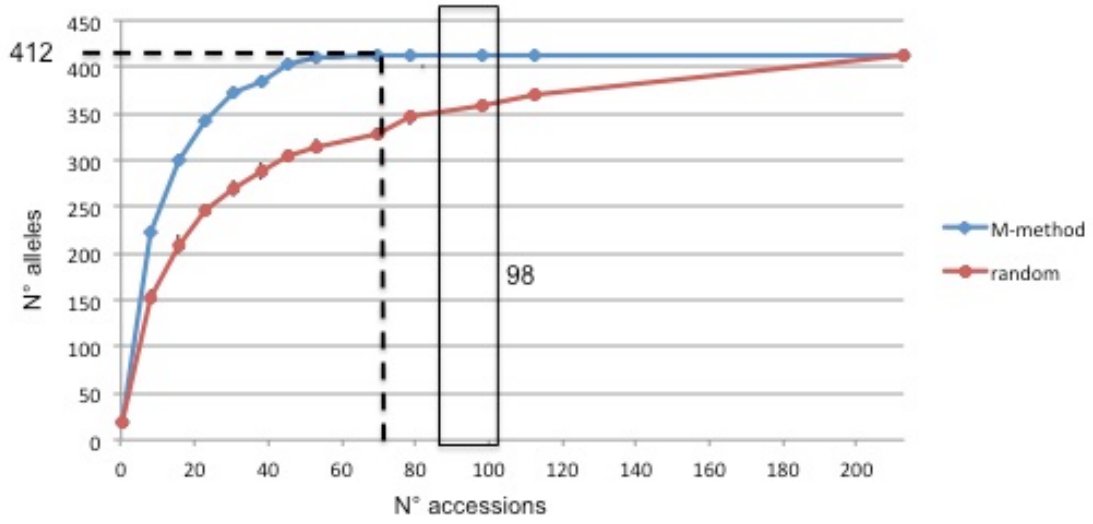

Figure 1: Redundancy curves developed for genetic core collections using the M-method (in blue) and random sampling (in red) with standard deviations, captured in ten independent sampling runs. Plot shows the accumulation of allelic diversity with increasing core size.

Later, 41 rootstock accessions deriving from the breeding program of the University of Milano and six commercial rootstocks (1103 Paulsen, Kober 5BB, Selection Oppenhiem 4, 41 B Millardet et de Grasset, 101.14 Millardet et de Grasset and 140 Ruggeri) were included to obtain a panel of 145 individuals. To better understand the genetic structure of the analyzed population, the clustering algorithm implemented in STRUCTURE software was used by exploring different possible numbers of subpopulations.

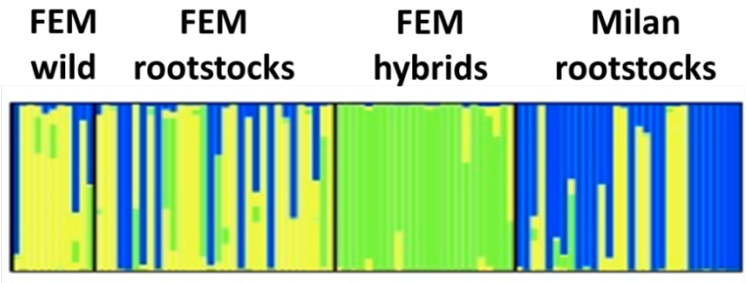

Figure 2: Population structure of the genetic core collection using the model-based program STRUCTURE. Barplot of admixture proportions of FEM wild and rootstocks, FEM hybrids and Milan rootstocks subpopulations, as estimated by STRUCTURE at K = 3. Each individual is represented as a single vertical bar, reflecting assignment probabilities to each of the three groups. K1: yellow bars; K2: green bars; K3: blue bars. Black line separates individuals of four predefined groups.

The ΔK criterion suggested by Evanno et al. (2005) gave the highest value at two groups distinguishing hybrids, which are interspecific crosses with several backcrosses on *V. vinifera*, and *non-vinifera* accessions (rootstocks and wild species). However, an additional peak of ΔK was found also at K=3, resulting in the separation of the rootstocks and wild individuals from the FEM germplasm in a subgroup, whereas rootstocks of the University of Milano clustered together in a third separated group (Figure 2). In order to obtain an association panel easy to handle, which adequately capture much genetic diversity as possible with a minimum of repetitiveness, it was further reduced to 100 samples based again on the M-method. A this step the 6 commercial rootstocks and 4 rootstock selections derived from Milano University's breeding program (M1, M2, M3, M4) were arbitrary forced to be included. In the final association panel, the number of different alleles (A) retained by the SSRs was 422. The observed and expected heterozygosity were 0.77 and 0.87, respectively. While considering the two groups (Rootstocks and Hybrids) separately the diversity parameters were different. Statistics of genetic variation and origin of samples are summarized in Table 1 and in Figure 3.

Table 1: Summary statistics of genetic variation at 22 SSR loci in the genetic core collection. A: number of different alleles; H_E: unbiased expected heterozygosity; H_O: observed heterozygosity.

Group	n° genotypes	A	H_o	H_e
Rootstocks	74	374	0,75	0,86
Hybrids	26	265	0,82	0,84
Total	100	422	0,77	0,87

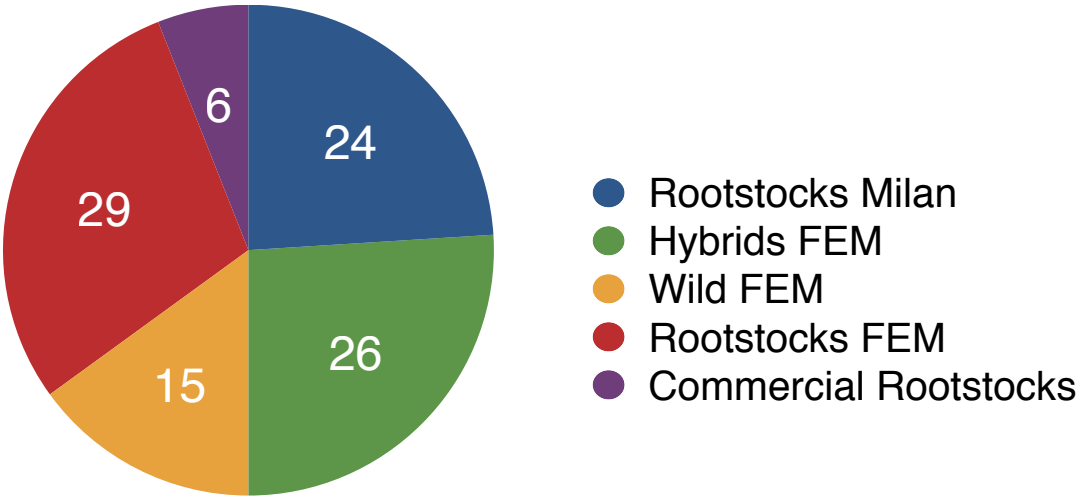

Figure 3: Origin of 100 grapevine accessions included in the final genetic core collection.

Genetic core collection was genotyped using the commercial GrapeReseq 20K SNPs array. After removing low quality loci, the filtered data set was made up of 16.562 SNPs. Moreover, after the identification of missing genotypes, SNPs with a minor allele frequency (MAF) lower than 0.1 were removed constituting a final number of 7132 SNPs. A pairwise distance matrix derived from a modified Euclidean distance for all polymorphic SNPs was calculated to construct neighbor-joining tree using MEGA V5.0 software (Tamura et al., 2011). As a result, the 100 accessions could be clustered into three groups, which contained 44, 28 and 28 accessions, respectively (Figure 4).

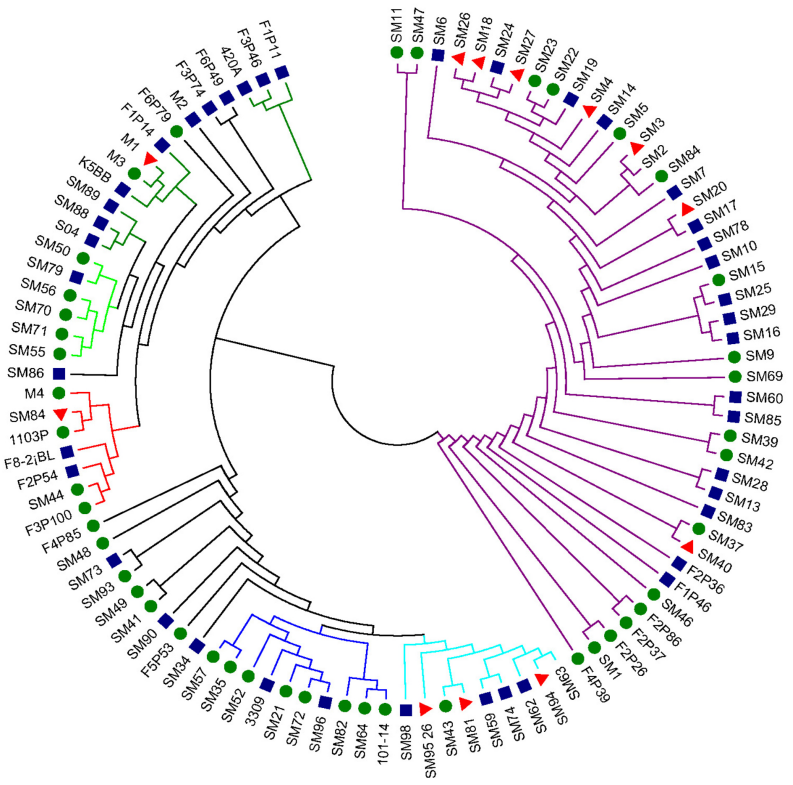

Figure 4: Neighbor-joining tree of the genetic core collection constructed with SNPs of GrapeReseq 20K SNPs array (stringency of 500 bootstrap-replicates). Branches are highlighted in different colors according to the assumed pedigree of individuals: violet (*V. vinifera*), light blue (V. *riparia*), blue (V. *riparia* x V. *rupestris*), red (V. *berlandieri* x V. *rupestris*), green (other American *Vitis spp*) and dark green (V. *berlandieri* x V. *riparia*).

Stomatal conductance of rootstock population under drought stress

To evaluate the transpiration rate under drought stress in the association population, vines of the 100 accessions were subjected to deficit irrigation or were maintained in well-watered conditions for 30 days in two years. Drought stress treatment resulted in a significant decrease of stomatal conductance (I_g), and thereby stressed plants exhibit higher crop water stress index (CSWI) values (Figure 5). Statistically significant differences were observed between control (WW) and water stressed (WS) plants in both years (Table 2). In the first year experiment, WS vines were monitored for or a week at well-water condition before undergoing the water stress (three timepoints of evaluation T1-T3). Unexpectedly, a significant decrease of transpiration compared to WW plants was observed at T2 maybe due to a mistake in daily watering. However, this difference disappears in the next detecting point (T3) with WS and WW plants being fully comparable before the beginning of the stress.

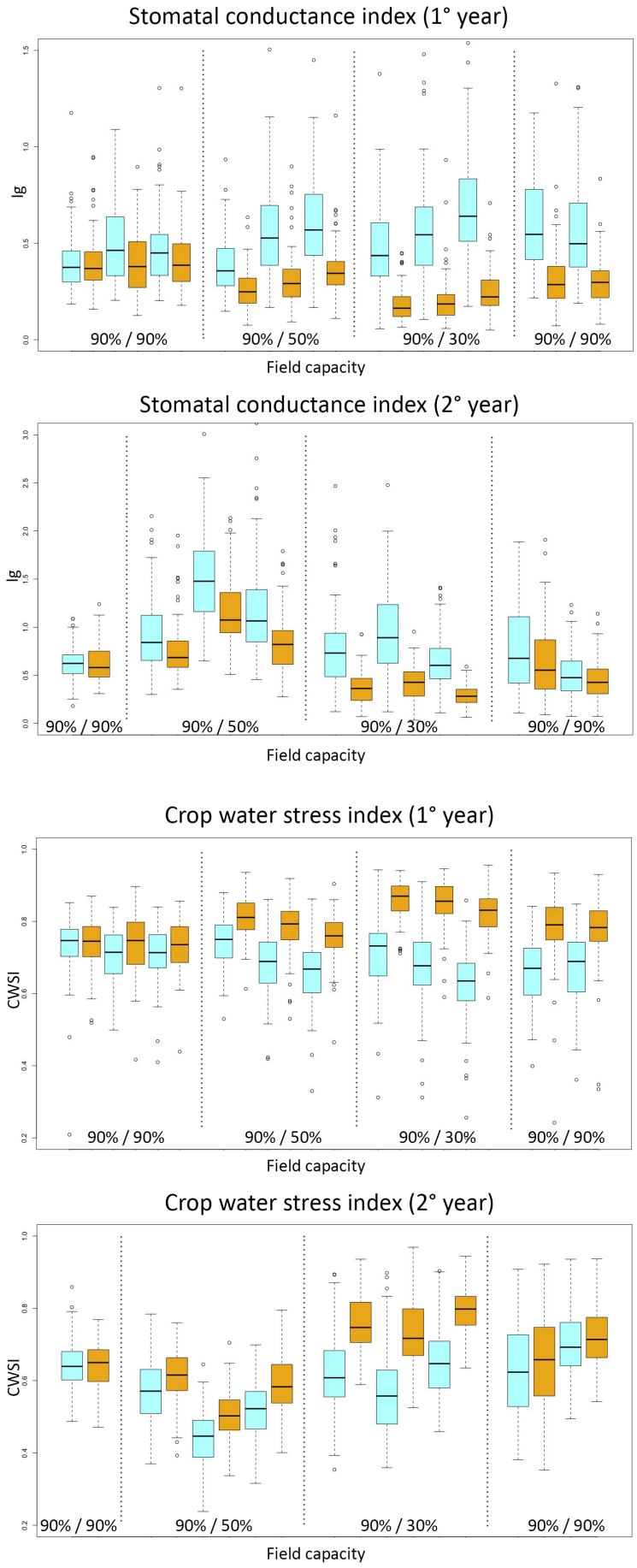

Figure 5: Comparison of stomatal conductance and crop water stress indices between well-watered (WW) (in blue) and water stressed plants (WS) (in yellow) during the water stress experiments in the two years

Table 2: Descriptive statistics of the phenotypic data from control (WW) and water stressed plants (WS) in each year of phenotyping. Asterisks denote significant differences according to Mann-Whitney U test between WS and WW on the same time point. *, **, and *** indicate significantly different values at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

Year	Time points	Treatment	Field capacity (%)	I_g		CWSI	
				Mean	SD	Mean	SD
1°	T1	WW	90	0,410	0,159	0,727	0,088
		WS	90	0,402	0,156	0,738	0,067
	T2	WW	90	0,490	0,206	0,704	0,083
		WS	90	0,424 *	0,234	0,739 ***	0,083
	T3	WW	90	0,487	0,236	0,710	0,080
		WS	90	0,418	0,169	0,733	0,070
	T4	WW	90	0,379	0,140	0,747	0,066
		WS	50	0,264 ***	0,104	0,807 ***	0,059
	T5	WW	90	0,576	0,284	0,681	0,089
		WS	50	0,318 ***	0,153	0,785 ***	0,074
	T6	WW	90	0,623	0,295	0,655	0,088
		WS	50	0,365 ***	0,146	0,755 ***	0,067
	T7	WW	90	0,513	0,307	0,705	0,095
		WS	30	0,184 ***	0,088	0,859 ***	0,054
	T8	WW	90	0,615	0,357	0,667	0,117
		WS	30	0,205 ***	0,132	0,850 ***	0,066
	T9	WW	90	0,765	0,467	0,614	0,111
		WS	30	0,247 ***	0,117	0,821 ***	0,066
	T10	WW	90	0,625	0,317	0,660	0,103
		WS	90	0,352 ***	0,345	0,779 ***	0,097
	T11	WW	90	0,571	0,282	0,675	0,093
		WS	90	0,325 ***	0,222	0,776 ***	0,093
2°	T1	WW	90	0,627	0,183	0,639	0,068
		WS	90	0,623	0,191	0,639	0,067
	T2	WW	90	0,926	0,384	0,566	0,090
		WS	90	0,763 **	0,300	0,612 ***	0,074

	T3	WW	90	1,540	0,510	0,443	0,075
		WS	50	1,172 ***	0,343	0,503 ***	0,069
2°	T4	WW	90	1,220	0,623	0,518	0,089
		WS	50	0,847 ***	0,309	0,589 ***	0,079
	T5	WW	90	0,798	0,442	0,615	0,110
		WS	30	0,372 ***	0,174	0,759 ***	0,079
	T6	WW	90	0,947	0,466	0,570	0,123
		WS	30	0,410 ***	0,185	0,738 ***	0,094
	T7	WW	90	0,651	0,281	0,649	0,094
		WS	30	0,289 ***	0,108	0,795 ***	0,062
	T8	WW	90	0,782	0,438	0,621	0,125
		WS	90	0,659	0,393	0,653	0,130
	T9	WW	90	0,513	0,242	0,699	0,092
		WS	90	0,458	0,201	0,715	0,083

Genome-wide associations

The genome-wide association study (GWAS) was conducted for the transpiration traits related to stomatal conductance using both GLM and MLM methods. The GLM + Q was chosen as the best model based on Quantile-Quantile plots comparisons for associations found for most of the trait under investigation. The MLM + K model was instead preferred at T6 and T10. Marker-trait significant associations were identified for stomatal conductance (Ig) values at time points T5, T6, T7 and T10 in water stressed plants in the first year experiment (Figure 6). In Table 3 are reported SNPs that had significant p-values after Bonferroni or False Discovery Rate (FDR) corrections (suggestive). Among these the most significant markers were the SNP chr17_10497222_C_T that showed a significant association during severe water stress (T7), two SNPs (chr13_11950617_C_T, chr18_13519938_C_T) statistically significant under moderate water stress (T6) and two SNPs (chr3_7009222_A_G and chr16_21122534_A_G) that were significantly associated with transpiration during stress recovery (T10). Other 13 suggestive associations that did not pass Bonferroni correction were detected during moderate stress (T5, T6) and recovery (T10).

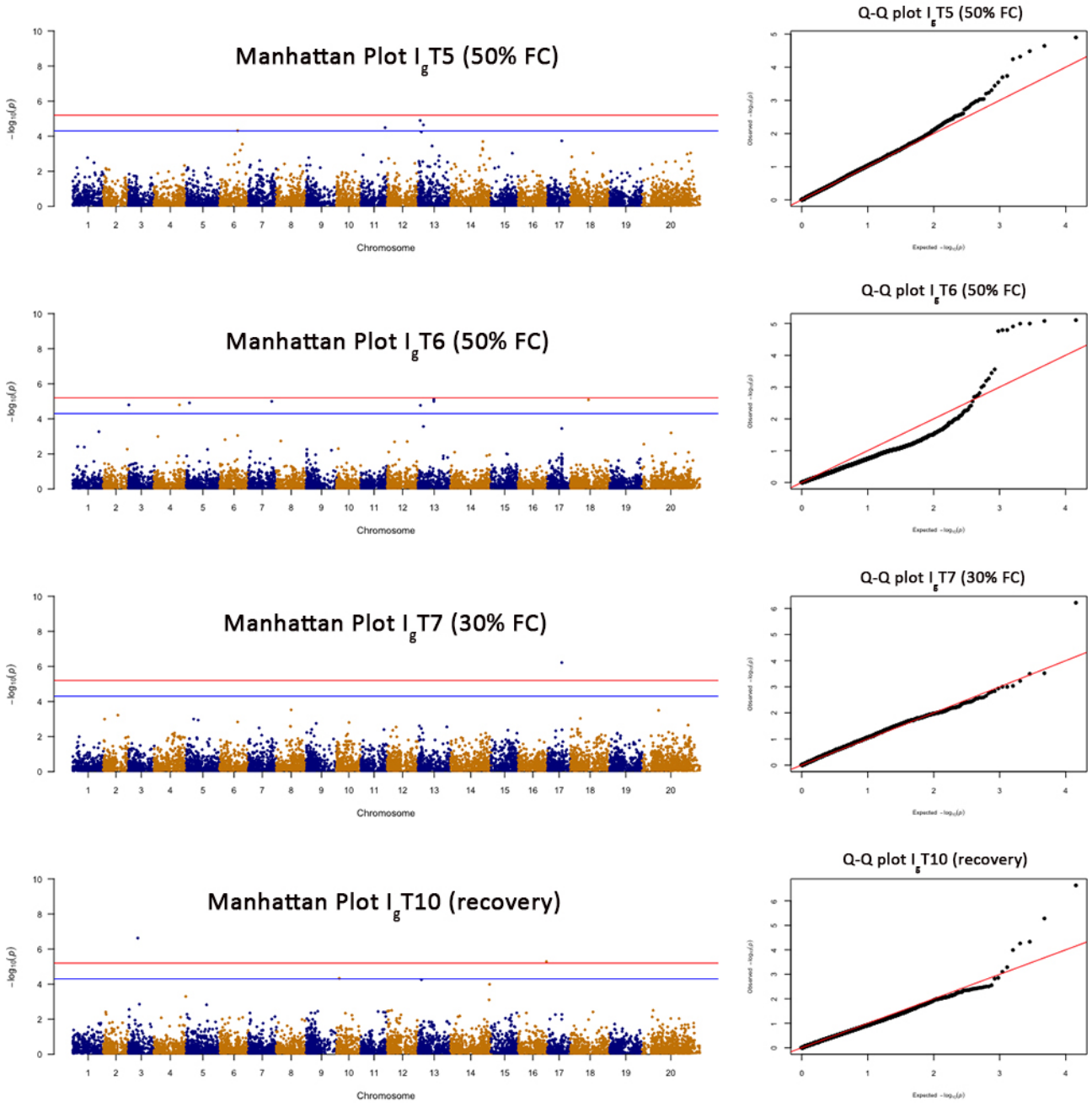

Figure 6: Manhattan plots and respective quantile–quantile (Q-Q) plots of association analysis between stomatal conductance (I_g) values and all SNP sites at time points T5, T6, T7 and T10 of first year experiment. The log10 P-values are plotted against the position on each of the 20 chromosomes. The horizontal blue and red lines indicate respectively the Bonferroni-corrected p-value and False Discovery Rate (FDR) significance threshold.

GWAS with phenotypic data collected during the second year experiment did not identify any significant association after Bonferroni correction (Figure 7). However, suggestive marker-trait association based on significant q-values (FDR corrections) could be found at T1 and T9, respectively before and after water stress (recovery), when plants were at 90% of field capacity.

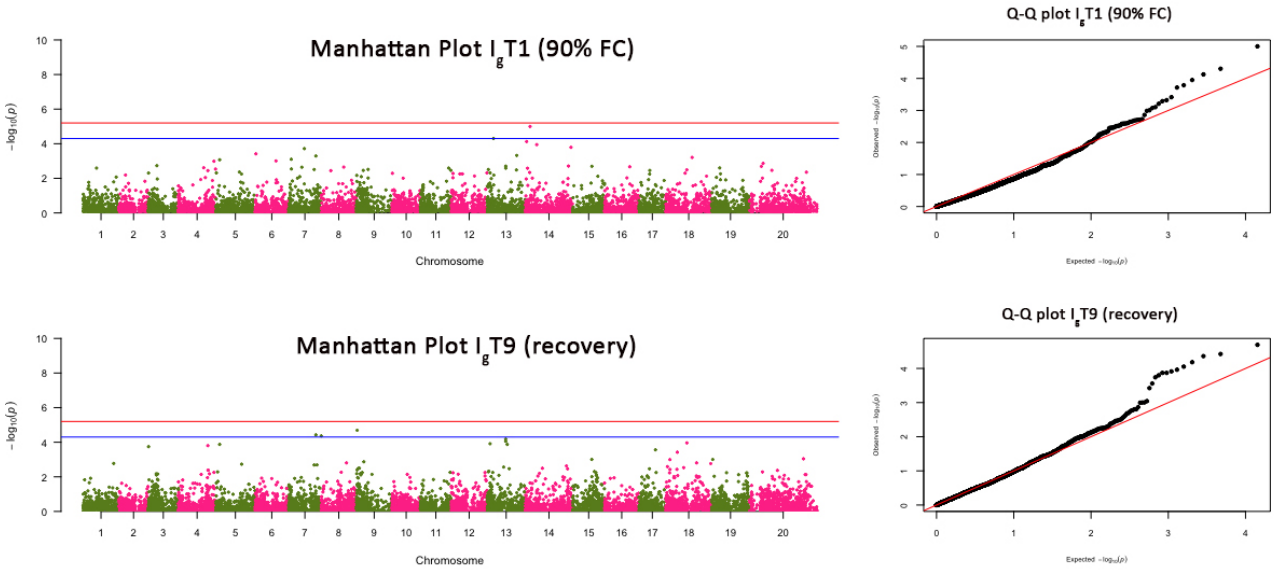

Figure 7: Manhattan plots and respective quantile–quantile (Q-Q) plots of association analysis between stomatal conductance (I_g) values and all SNP sites at time points T1 and T9 of second year experiment. The log10 P-values are plotted against the position on each of the 20 chromosomes. The horizontal blue and red lines indicate respectively the Bonferroni-corrected p-value and False Discovery Rate (FDR) significance threshold.

A circular Manhattan plot (Figure 8) summarizes all the association results of both experiments. For example, on chromosome 13 it is possible to identify shared association signals between timepoints T6 (1° year) and T9 (2° year). Similarly, the statistically significant association signal on chromosome 17, which was detected at T7 during the first year, recur at T5 (1° year) and a T9 (2° year) although they are under the threshold of significance.

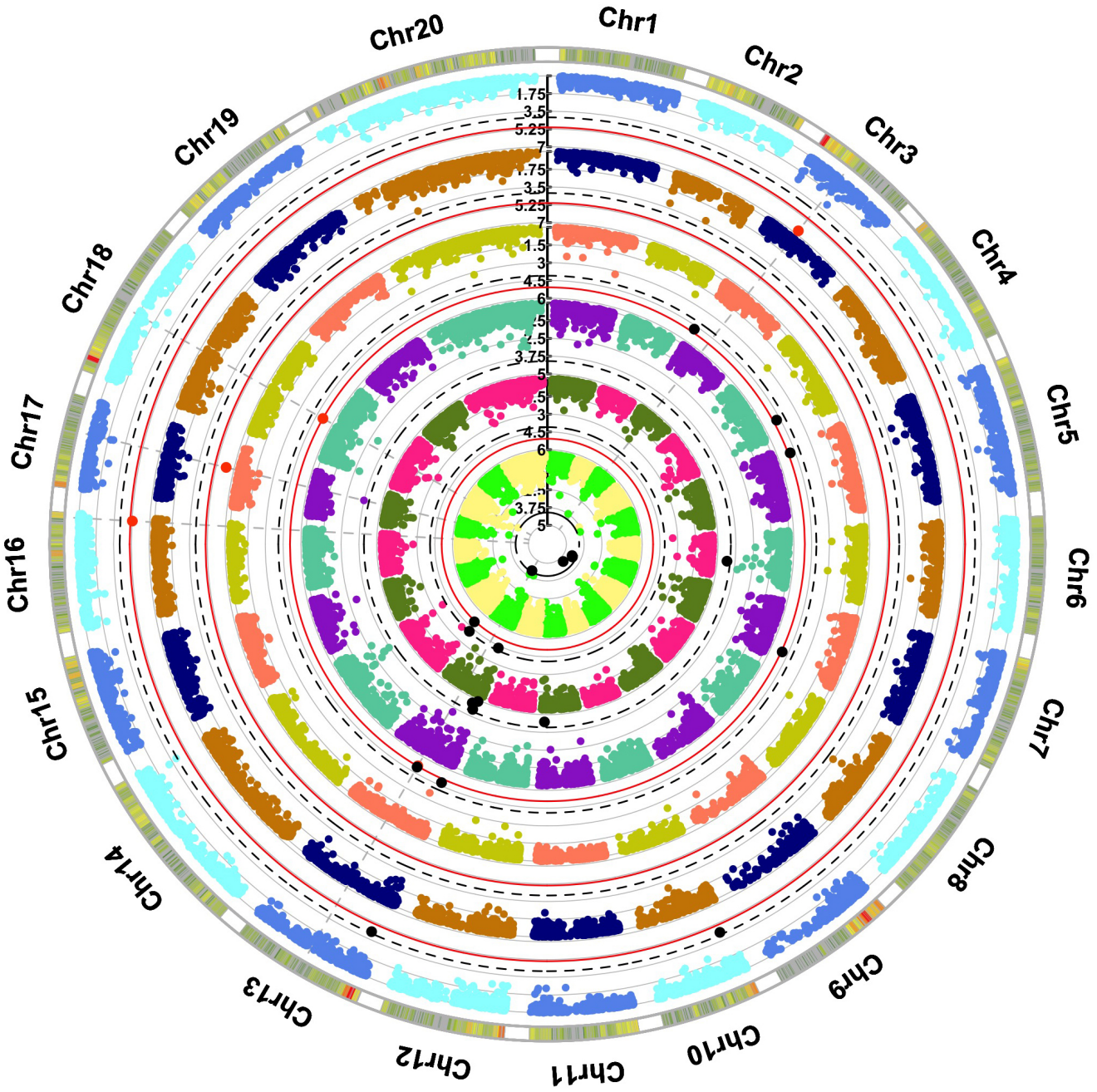

Figure 8 : Circular Manhattan plot of association analysis between stomatal conductance (I_g) values and all SNP sites at time points T5 (aquamarin, violet), T6 (dark yellow, salmon), T7 (blue, orange), and T10 (azure, light blue) of first year experiment and time points T1 (dark green, pink) and T9 (green, yellow) of second year experiment. The red and black dots indicate respectively significant values according to the Bonferroni-corrected *p*-value and False Discovery Rate (FDR).

To identify potential candidate genes, the associated SNPs were examined. Firstly, it was considered whether polymorphisms would be localized in genic regions. Out of the 24 significant SNPs 15 were located within genes, while the remaining SNPs were in intergenic regions. For those markers located outside gene regions or in genes functionally non-annotated, the 20 kilobases surrounding them were scanned, since Linkage Disequilibrium (LD) decay rapidly in grapevine (Nicolas et al., 2016). The 14 candidate genes for their biological functions related to water stress response or for their position are listed in Table 4.

Table 3: SNPs significantly associated to stomatal conductance (Ig) values, with the corresponding p-values. SNPs significantly associated according to the Bonferroni-corrected p-value are reported in the first lines. R^2: the proportion of phenotypic variance explained by the marker. Positions are referred to V1 annotation of the *Vitis vinifera* genome (http://www.genoscope. cns.fr).

Trait	Year	SNP	Chr	Pos	P value	Qtl effect	R^2
I_g T6	1	chr13_11950617_C_T	13	11950617	7.80E-06	A/D	0.39
I_g T6	1	chr18_13519938_C_T	18	13519938	8.30E-06	D	0.38
I_g T7	1	chr17_10497222_C_T	17	10497222	6.07E-07	D	0.25
I_g T10	1	chr3_7009222_A_G	3	7009222	2.34E-07	A/D	0.50
I_g T10	1	chr16_21122534_A_G	16	21122534	5.24E-06	A	0.56
I_g T5	1	chr6_13441720_C_T	6	13441720	4.82E-05	A	0.22
I_g T5	1	chr11_18012075_T_C	11	18012075	3.28E-05	A	0.22
I_g T5	1	chr13_10652062_A_G	13	10652062	3.64E-04	D	0.17
I_g T5	1	chr13_4177522_C_T	13	4177522	2.29E-05	A	0.24
I_g T5	1	chr13_1833944_A_G	13	1833944	1.27E-05	A	0.18
I_g T6	1	chr7_17388970_A_G	7	17388970	9.99E-06	A/D	0.40
I_g T6	1	chr13_11952742_G_T	13	11952742	1.01E-05	A/D	0.40
I_g T6	1	chr5_2431422_C_T	5	2431422	1.24E-05	A/D	0.41
I_g T6	1	chr4_18754964_C_T	4	18754964	1.60E-05	A/D	0.37
I_g T6	1	chr3_235211_C_T	3	235211	1.60E-05	A/D	0.36
I_g T6	1	chr13_2031649_T_C	13	2031649	1.73E-05	A/D	0.36
I_g T10	1	chr10_1989600_G_T	10	1989600	4.71E-05	A/D	0.42
I_g T10	1	chr13_2751641_A_C	13	2751641	5.51E-05	A/D	0.40
I_g T1	2	chr14_3096968_G_T	14	3096968	9.96E-06	A	0.25
I_g T1	2	chr13_4177522_C_T	13	4177522	4.98E-05	A	0.22
I_g T9	2	chr7_17388970_A_G	7	17388970	3.78E-05	A/D	0.23
I_g T9	2	chr7_20777757_C_T	7	20777757	4.36E-05	D	0.27
I_g T9	2	chr9_553031_C_T	9	553031	2.04E-05	A/D	0.27
I_g T9	2	chr13_11950617_C_T	13	11950617	6.57E-05	A/D	0.23

Table 4: List of candidate genes functionally annotated in the genome V1 stored in the GENOSCOPE database.

Trait	Candidate gene	Description	Chr	Start	Stop
I_g T6	*VIT_18s0001g15390*	Peroxidase	18	13521135	13522636
I_g T7	*VIT_17s0000g08960*	Raffinose synthase	17	10494444	10498141
I_g T10	*VIT_03s0091g00570*	Transcription factor	3	6998808	6999512
I_g T10	*VIT_16s0098g00780*	Iaa-amino acid hydrolase	16	21120452	21126524
I_g T10	*VIT_16s0098g00760*	Transcription factor	16	21111871	21115426
I_g T5	*VIT_06s0009g01570*	Serrate rna effector molecule	6	13438002	13465222
I_g T5	*VIT_11s0052g00570*	Auxin-induced protein 5NG4-like	11	18007469	18008509
I_g T5	*VIT_13s0106g00790*	Mevalonate diphosphate decarboxylase	13	10642954	10652636
I_g T5	*VIT_13s0019g03040*	Quercetin glucosyltransferase	13	4177111	4179273
I_g T6	*VIT_05s0020g00540*	β-xylosidase/α -arabinofuranosidase	5	2435691	2438632
I_g T10	*VIT_10s0003g00760*	Glutamate receptor protein	10	1992263	1998191
I_g T1	*VIT_14s0128g00480*	Eukaryotic translation initiation factor 3	14	3092047	3097166
I_g T9	*VIT_09s0002g00810*	peroxisomal (S)-2-hydroxy-acid oxidase	9	547420	552404

Validation of GWAS results

To validate the marker-trait association found for SNP within gene *VIT_17s0000g08960*, 10 rootstock varieties homozygous and 6 heterozygous for that polymorphism were selected for a further water stress study. Transpiration rates of WW and WS plants were measured throughout the experiment (Figure 9) with a steady state porometer. Significant differences between the two groups were found at the beginning of stress (T1) in WW plants and at moderate water stress (T3) in WS plants (Table 5). Furthermore, rootstocks heterozygous for SNP exhibited a reduced transpiration rate compared with other varieties also during severe stress (T4, T5).

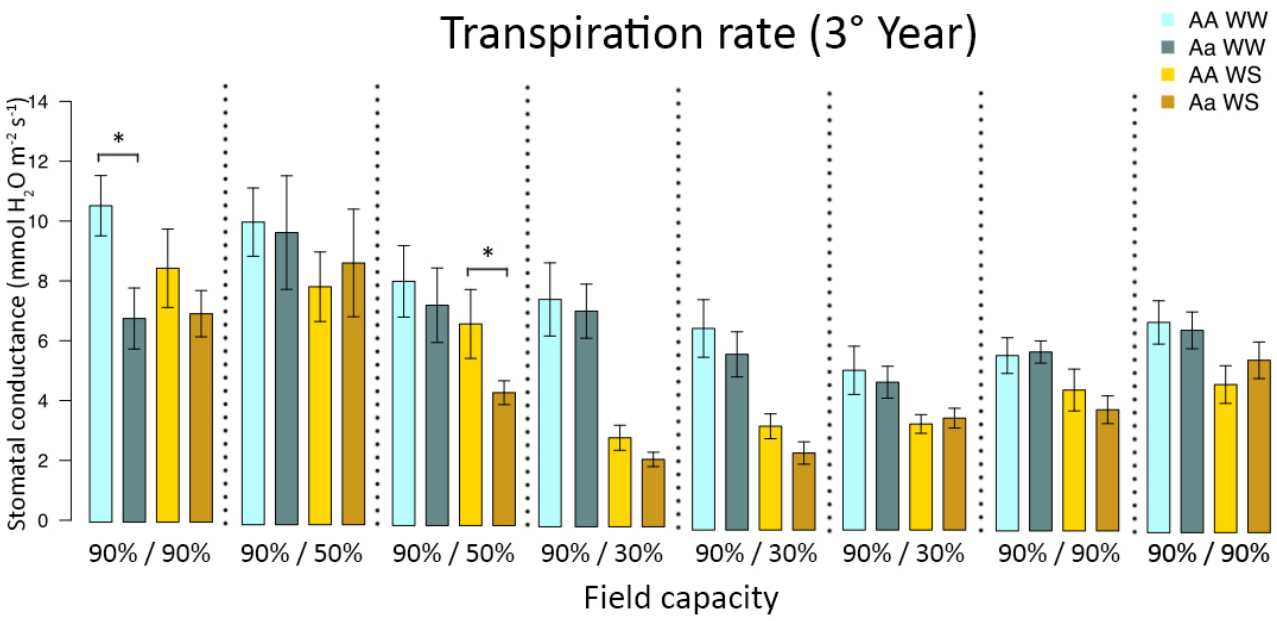

Figure 9: Comparison of transpiration rate of plants homozygous for the chr17_10497222_C_T SNP (WW light blue, WS yellow) and plants heterozygous for the SNP (WW blue, WS dark yellow) during the water stress experiments in the third year.

Table 5: Descriptive statistics of transpiration of plants homozygous and heterozygous for the chr17_10497222_C_T SNP. Asterisks denote significant differences according to Mann-Whitney U test between plants on the same time point and under the same treatment at $p < 0.05$.

Time points	Treatment	Field capacity (%)	SNP chr17_10497222_C_T (CT overdominance effect)	Transpiration rate	
				Mean	SD
T1	WW	90	TT/CC	10,58	3,19
			CT	6,81 *	2,50
	WS	90	TT/CC	8,49	4,15
			CT	6,97	1,90
T2	WW	90	TT/CC	10,11	3,43
			CT	9,76	4,65
	WS	50	TT/CC	7,95	3,68
			CT	8,75	4,40
T3	WW	90	TT/CC	8,16	3,78
			CT	7,36	3,05
	WS	50	TT/CC	7,27	3,42
			CT	4,44 *	0,98
T4	WW	90	TT/CC	7,60	3,86
			CT	7,21	2,21
	WS	30	TT/CC	2,98	1,32
			CT	2,25	0,59
T5	WW	90	TT/CC	6,74	3,04
			CT	5,87	1,85
	WS	30	TT/CC	3,47	1,31
			CT	2,57	0,91
T6	WW	90	TT/CC	5,34	2,55
			CT	4,94	1,31
	WS	30	TT/CC	3,55	0,99
			CT	3,74	0,81
T7	WW	90	TT/CC	5,86	1,89
			CT	5,98	0,91
	WS	90	TT/CC	4,71	2,21
			CT	4,05	1,14
T8	WW	90	TT/CC	7,03	2,29
			CT	6,76	1,51
	WS	90	TT/CC	4,95	1,99
			CT	5,76	1,50

Discussion

Water deficit poses a threat to the sustainable viticulture with serious economic consequences for producers. Therefore, there is a pressing need to extend our understanding of the intricate nature of drought tolerance in grapevine. Breeding grape rootstocks for resilience to water deficit is an achievable strategy to improve water use efficiency (WUE) (Tomás et al., 2014), maintaining the desirable varietal characteristics of scions (Serra et al., 2014). Thus, increasing the availability of genetic determinisms related to tolerance traits could improve the efficiency of this method, in particular using recognizable tags (molecular markers) to target stress-responsive genes (Duchêne, 2016).

The existing grape germplasms are valuable genetic resources that could be examined for seeking phenotypic variations in drought tolerance mechanisms. Constructing a genetic core collection has proved to be an adequate strategy to obtain a optimal number of rootstock genotypes, which represents the whole genetic diversity and captures all the most frequent alleles of a large germplasm, similarly to previous studies of genetic core collection development in grapevine (Le Cunff et al., 2008; Emanuelli et al., 2013; Nicolas et al., 2016). Furthermore, the use of genetic core collection for marker-trait association studies was applied in several plant species with excellent results (McKhann et al., 2004; Zhang et al., 2014; Campoy et al., 2016).

Grapevine WUE under droughts is strongly influenced by plant transpiration rate, which is therefore considered as potential target for its improvement. (Chaves et al., 2010). Thermal infrared imaging was confirmed as very suitable tool for the estimation of stomatal conductance. During all the three experimental years, rootstocks exhibited significant higher canopy temperatures in comparison with their controls when were subjected to water stress, reflecting their water status. Moreover, it was demonstrated that I_g and CWSI parameters deduced from thermal images are significantly correlated with water stress indicators, such as leaf water potential (ΨL), non-photochemical quenching (NPQ) or efficiency of light use by the photosystem II (PSII) (Matese et al., 2018). This approach allowed a fast assessment of the transpiration rate in whole rootstock population (600 vines) on the same day and during a specific time window to limit environmental influence, which would have been impossible with a porometer. In fact since the initial development of the thermography method by Blum et al. (1982), water status of different kind of crops has been widely studied in diverse research works, including grapevine (Bellvert et al., 2014; Sepulveda-Reyes et al., 2016; Gago et al., 2017).

GWAS studies are currently a valuable approach to detect plant genes involved in phenotypic traits, particularly for those with polygenic inheritance, such as drought tolerance. Although, these analyses are not widely carried out in grapevine (Fodor et al., 2014; Chitwood et al., 2014; Tello et al., 2016; Marrano et al., 2018; Laucou et al., 2018). According to Nicolas et al. (2016) the ideal association panel for GWAS in grapevine should combine limited relatedness with minimal structure. The panel designed for this study was composed by hybrids, wild *vinifera* and rootstocks varieties that include the main American Vitis species, such as *V. riparia*, *V. berlandieri* and *V. rupestris*, in their pedigree. Therefore, it ensures a large genetic variability and additionally exhibited unexplored variations for biotic and abiotic stresses resilience (Carbonneau, 1985; Boso et al., 2014).

However, GWAS analysis identified few SNPs associated with studied phenotypic traits, which passed the Bonferroni significance threshold. The decrease of statistical power would be caused by the rapid decay of linkage disequilibrium (LD) in grapevine (Nicolas et al., 2016; Marrano et al., 2017; Laucou et al., 2018) that might require a large number of SNPs to evenly cover the genomic region. Furthermore, drought tolerance is a trait with complex polygenic determinism and with a strong environmental interaction. Hence, its marker-trait association analysis may necessitate highly precise phenotypic data, and an experimental panel including more individuals and replicates, in order to detect minor effect QTLs.

The prominent role of rootstocks in regulating scion stomatal conductance under water deficit has been demonstrated in different studies (Soar et al., 2006; Koundouras et al., 2008; Tramontini et al., 2013; Peccoux et al., 2017), but on the other hand, a very few number of works investigated the genetic determinisms involved in the stomatal regulation. Marguerit et al. (2012) identified, through a QTL analysis, rootstock genetic regions linked to the transpiration control of scions, evaluating drought response of a single scion genotype grafted on 138 individuals from a *V. vinifera* cv. Cabernet-Sauvignon × *V. riparia* cv. Gloire de Montpellier cross. Later, Coupel-Ledru et al. (2014) dissected the genetic basis of stomatal sensitivity between iso- and anisohydric grapevines in a progeny (*Vitis vinifera* L. cvs. Grenache×Syrah) again with a QTL approach. Nevertheless, until now these remain the only ones studies focused on identifying the genetic regions responsible for stomatal control under water stress.

The association mapping approach adopted in this study detected significant genotype-phenotype associations during the various stages of drought stress experiment. The most interesting significantly associated SNP marker was identified under severe water deficit condition in first year experiment (at 30% of field capacity). Moreover, other association

signals for the same marker, which not exhibited significant p-values after multiple testing corrections, were found in the first year (at 50% of FC) and in the second year (at recovery stage). Additionally, the association of SNP chr17_10497222_C_T with a different rate of transpiration under drought was validate in a small group of rootstock varieties in a third year experiment. Indeed, genotypes with heterozygous SNP (CT) exhibited a significant reduction of stomatal conductance compared with genotypes carrying homozygous SNP (CC or TT) at 50% FC. The SNP chr17_10497222_C_T is located in the coding region of *VIT_17s0000g08960*, which codes for a raffinose synthase. The raffinose family of oligosaccharides (RFOs) has a fundamental role in protecting plants against abiotic stresses (Sengupta et al., 2015). These proteins confer tolerance against drought stress acting as signaling compounds through the phloem, and as storage of additional energy resources. Moreover, they have a ROS scavenging function and stabilize cellular membranes and photosynthetic apparatus. Indeed, the accumulation of these carbohydrates improved the water stress tolerance in several plants, such as *Arabidopsis thaliana* (Taji et al., 2002; Nishizawa et al., 2008; Sun et al., 2003), *Medicago sativa* (Kang et al., 2011), *Xerophyta viscosa* (Peters et al., 2007), maize (Gu et al., 2016), coffee (Dos Santos et al., 2015) and apple (Falavigna et al., 2018). Furthermore, it has been reported a role of osmolytes in the regulation of stomata aperture (Daloso et al., 2016). The involvement of *VIT_17s0000g08960* in drought response mechanisms, including ABA-mediated signalling, is confirmed by transcriptomic studies in grapevine. It was differentially modulated in the leaves of isohydric and anisohydric varieties under water deficit (Dal Santo et al., 2016) and it was up-regulated both in transgenic grape cells overexpressing *VvABF2* (Nicolas et al., 2014) than in berries after ABA treatment (Pilati et al., 2017). Among the other significantly associated markers, the SNP chr18_13519938_C_T is positioned within the promoter region of another drought responsive gene, *VIT_18s0001g15390*, which encodes a peroxidase protein. Peroxidases are antioxidant enzymes that prevent excessive damages caused by ROS accumulation and their concentrations are highly modulated under abiotic stresses (Barcelo et al., 2003; Mittler et al., 2004). The other three statistically significant polymorphisms after Bonferroni adjustment, chr3_7009222_A_G, chr16_21122534_A_G and chr13_11950617_C_T, map near a TF involved in transcription initiation, in the intronic region of a iaa-amino acid hydrolase and in a non-annotated gene prediction, respectively. Since these genes could not be considered directly related to water stress response, surrounding genomic regions were scanned without finding credible candidate genes. The Bonferroni correction test is the most applied for assessing the threshold value of associations. However, it is often too conservative and some signals may not pass its stringent criteria. Thus, suggestive SNPs were also considered to detect other marker-trait associations base on False

Discovery Rate (FDR). Among them, all markers identified were found only during one stage of stress, except chr13_4177522_C_T. This SNP, located in the coding region of a quercetin glucosyltransferase protein (VIT_13s0019g03040), was found significant both under moderate water deficit and well-watered condition. This enzyme is responsible for a late step in grapevine anthocyanin biosynthesis, which serve to mitigate the effects of water stress in leaves (Chalker-Scott et al., 1999). Lastly, marker chr13_10652062_A_G was found associated in vines under moderate drought stress and is positioned in the coding region of mevalonate diphosphate decarboxylase (MVD) (VIT_13s0106g00790). This is a limiting enzyme of mevalonate isoprenoid pathway (Bach, 1995) responsible for the formation of sterols, which play an essential role in maintaining membranes structure and in preventing oxidative stress damages (Posé et al., 2009). Make a comparison of these results with published genetic studies of the transpiration under drought (Marguerit et al., 2012; Coupel-Ledru et al., 2014) is quite complex. Even though they performed a comprehensive characterization of the population over the course of the water treatment, the low density of markers limited the resolution of QTL confidence intervals, which included large chromosomic regions. However, the large part of significantly associated SNPs identified in this study colocalized in those QTL regions and indicated more restricted positions. Therefore, valuable approaches to dissect multiple complex traits, such as drought stress response, will consist in the integration of QTL mapping and GWAS for identifying useful candidate genes.

Conclusions

This study used an association mapping panel, including different Vitis species, to identify genomic regions associated with stomatal regulation in response to drought stress. This represent an important tolerance trait, whose genetic determinisms are far from being completely understood. Phenotyping with thermal infrared images has proved to be a useful method to evaluate the drought responses of large population. Significant marker-trait associations were detected, despite the complexity of the trait under investigation and its polygenic inheritance. In particular, the association of an SNP in a gene of the raffinose family of oligosaccharides (RFOs) with reduced transpiration rate during drought, was validated in a further water stress experiment. This and other candidate genes identified with GWAS analysis will be evaluated in more controlled conditions, considering also the interactions between rootstock and scion.

CHAPTER 3

Evaluation and characterization of a candidate gene involved in water stress response

Introduction

Water deficit strongly affects many physiological features of plants. For instance, the excess of light energy, not convertible into biochemical energy, leads to a dramatic accumulation of reactive oxygen species (ROS) (Li et al., 2009). To overcome oxidative damages caused by these chemical compounds, plants have developed a complex and dynamic defense system involving enzymatic and non-enzymatic antioxidants (Noctor & Foyer, 1998; Chaves et al., 2003). Among the latter, compatible solutes or osmolytes are small organic molecules, which stabilize membranes and proteins, maintain cell turgor and reestablish cellular homeostasis (Yancey, 2005). Compatible solutes are divided into three major groups: (1) sugars (e.g. glucose, sucrose, trehalose, and raffinose) (Taji et al., 2002); (2) sugar alcohols or polyols (e.g. mannitol, glycerol, and sorbitol) (Conde et al., 2011); (3) amino acids and ammonium compounds (e.g. proline, methionine and glycine-betaine) (Einset & Connolly, 2009). Osmolytes, in addition to their key role as osmoprotecants, are involved in other regulatory processes of plant adaptation to drought stress, such as osmotic adjustment (Chen & Jiang, 2010), carbon storage, signal transduction (Rolland et al., 2006) and also in the regulation of stomatal conductance (Kelly et al., 2013).

The raffinose family oligosaccharides (RFOs) is one of the most important class of soluble carbohydrates, which are synthesized by specific α-galactosyltransferases with the subsequent addition of galactosyl moieties (Keller & Pharr, 1996). Galactinol (Gol) is formed from UDP-galactose (UDP-Gal) and L-*myo*-inositol by the activity of galactinol synthase (GolS), raffinose synthase (RafS) synthesizes raffinose (Raf) by transferring a galactosyl moiety from Gol to sucrose, and finally stachyose (Sta) is produced starting from Raf using Gol as galactosyl donor in a reaction catalyzed by stachyose synthase (StaS). Increased synthesis of RFOs in response to abiotic stresses are reported in several plant species, such as arabidopsis (Taji et al., 2002; Nishizawa et al., 2008), tomato (Downie et al., 2003), maize (Gu et al. 2016), rice (Maruyama et al., 2014), coffee (Dos Santos et al., 2011), cucumber (Gu et al., 2018) chickpea (Salvi et al., 2018), medicago (Kang et al., 2011; Zhou et al., 2013),

Craterostigma (Egert et al., 2015) and *Ajuga reptans* (Bachmann & Keller, 1995). Little is known about RFOs metabolism in grapevine and their physiological relevance in the plant, but Pillet et al. (2012) reported an accumulation of these carbohydrates in berries after heat stress exposure and high concentrations of RFOs is associated with cold tolerance in *Vitis amurensis* (Sun et al., 2018).

Genetic engineering is a valuable approach for improvement drought tolerance in plants (Parmar et al., 2017). Significant advances have been made in this technology through the application of systems biology approaches to elucidate and characterize gene combinations and genomic regions involved in the complex mechanisms of water stress response (Cramer et al., 2011). Plants that exhibit increased drought resilience were generated by the expression of regulatory genes (Tsai-Hung et al., 2002; Fujita et al., 2005; Oh et al., 2005; Hu et al., 2006; Nelson et al., 2007; Jeong et al., 2013), metabolic genes (Iuchi et al., 2001; Chen & Murata, 2002; Park et al., 2005; Karim et al., 2007; Sato & Yokoya, 2008; Wang et al., 2008; Faize et al., 2011; Yue et al., 2011; Estrada-Melo et al., 2015) and genes associated with post-translational regulation (De Block et al., 2005; Ning et al., 2010; Zou et al., 2010; Hozain et al., 2012). Furthermore, the overexpression of RFOs genes confers enhanced drought tolerance in Arabidopsis (Taji et al., 2002; Sun et al., 2013), and rice (Selvaraj et al; 2017). As regards grapevine, very few genetic transformation studies, which aimed to improve abiotic stress tolerance, are reported (Jin et al., 2009; Zok et al., 2010; Tillet et al., 2012; He et al., 2018). Interestingly, the overexpression of a stress responsive MYB related transcription factor, which regulates RFOs synthesis, enhances cold tolerance in grape transgenic calli (Sun et al., 2018) and Daldoul et al. (2017) demonstrated that *E.coli* bacterial colonies, overexpressing the stress-responsive grapevine gene α-galactosidase *Vv-α-gal/SIP,* showed higher tolerance to abiotic stress.

For this reasons the function of a drought-associated raffinose synthase gene (*VIT_17s0000g08960),* formerly identified by means of a Genome-wide association study (GWAS) for water stress tolerance in grapevine rootstocks, has to be more deeply investigated. Moreover, this and other genes involved in RFOs metabolism could be efficient targets for grapevine water deficit resilience.

Materials and methods

Gene amplification and resequencing

Genomic DNA of 85 rootstock genotypes was extracted from freeze-dried leaf tissue, after grinding with mortar and pestle, using the DNeasy® Plant Mini Kit (QIAGEN, Hilden, Germany), DNA concentration and purity were checked both by agarose gel electrophoresis and by the the spectrophotometer NanoDrop ND-8000 (NanoDrop Technologies, Wilmington, DE, USA). The *VIT_17s0000g08960* gene was directly sequenced. Gene-specific primers were designed using Primer 3 software (Rozen and Skaletsky, 2000) and were synthesized based on the genomic sequence of *V. vinifera* gene annotation v2.1 hosted on http://genomes.cribi.unipd.it/grape (Vitulo et al., 2014). A total of 3678 bp of the *VIT_17s0000g08960* locus, from the initial ATG start codon to the TGA stop codon, was resequenced. Both strands of four partially overlapping amplicons were sequenced and assembled in a contiguous sequence. Primers used to amplify PCR fragments were also employed for the resequencing and are listed in Table 1, Polymerase chain reactions (PCR) were performed in a final volume of 20 µl contained 20 ng of genomic DNA, 2 of µl 10× PCR buffer (Roche, Indianapolis, IN, USA), 0,2 mM of each dNTP, 0,6 µM of each primer, 1,5 mM MgCl2 and 0,4 unit of FastStart Taq DNA Polymerase (Roche). Thermocycling consisted of an initial denaturation of the template DNA at 95°C for 15 min, followed by 11 cycles of 95°C for 45 s, 65°C (touch- down step from 65°C to 60°C) for 45 s and 72°C for 1 min, and another 24 cycles of 95°C for 45 s, 60°C for 45 s and 72°C for 1 min, with a final extension of 10 min at 72°C. Amplified products were analyzed in 1,5% agarose gel. PCR products were purified with ExoSAP-IT™ PCR Product Cleanup Reagent (Amersham Pharmacia Biotech, Uppsala, Sweden) and sequenced in both directions with the Big Dye® Terminator v 3,1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). After precipitation, the sequencing products were mixed with 15 µl of HiDiTM formamide and subjected to capillary electrophoresis in an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems). Sequences were processed with the Sequencing analysis v 3.7 software (Applied Biosystems), finally STADEN package v2.0.0 (Staden, 1996) was used to analyze the DNA sequences.

Nucleotide polymorphisms and diversity

The estimation and frequency of polymorphisms were defined using the DnaSP software (Librado et al., 2003), based on the SNPs and INDELs detected in *VIT_17s0000g08960* cDNA. Nucleotide diversity was evaluated with the parameter π (Nei et al., 1979), which is the

average number of nucleotide differences per site between two sequences. The neutral mutation parameter θ (Watterson et al., 1975) was calculated from the total number of mutations. Tajima's D test and Fu and Li's D test implemented in DnaSP were used to estimate neutrality of the SNP polymorphisms, taking the dataset as a whole and the rootstock and hybrid groups into consideration separately. Prediction of tolerability of amino acid substitution at all positions was calculated with the software tool PROVEAN (Protein Variation Effect Analyzer) (Choi & Chan, 2015). The hypothesis of neutral polymorphisms was tested using Tajima's D (Tajima, 1989) and Fu and Li's D (Fu & Li, 1993) tests.

Cloning SO4 rootstock full-ORF *VIT_17s0000g08960* cDNA

The *VIT_17s0000g08960* cDNA, lacking its stop codon, was amplified using a Phusion® High-Fidelity DNA polymerase (New England Biolabs, Ipswich, MA, USA) with the primer pair (Table 1), and the resulting amplicon was purified from agarose gel using QIAquick Gel Extraction Kit (QIAGEN) and was cloned into an entry vector pENTR™/D-TOPO® (Invitrogen, Carlsbad, CA, USA). The sequence-confirmed entry vector was recombined into pK7WG2 plant binary vector (Karimi et al., 2002), under the control of 35S promoter, using LR ClonaseTM II enzyme mix (Invitrogen).

Embryogenic callus induction of four rootstock genotypes

Plant material of rootstock genotypes: 101.14 Millardet et de Grasset (101.14 Mgt)(*V. riparia* x *V. rupestris)*, Selection Oppenhiem 4 SO_4 (*V. riparia* x *V. berlandieri*), 110 Richter (110R) (*V. rupestris* x *V. berlandieri*) and Riparia Gloire de Montpellier (RgM) (*V. riparia*) was harvested from field germplasm collection of Edmund Mach Foundation (San Michele all'Adige, Italy). Inflorescences of grape flowers were collected at the developmental stage 55 of BBCH-scale (Lorenz et al., 1994), when they are swelling but not completely separated. After a brief rising with tap water, inflorescences were sterilized in a sodium hypochlorite solution (5%) for 20 min. Then five rinses were performed in distilled water and they were stored for 2 days at 4 °C. Under binocular microscope, the anthers were separated from ovaries and were plated with the adaxial face on the culture medium B (Murashige & Skoog-based (MS) medium added with 2,4-dichlorophenoxyacetic acid (2,4D) 1 mg/l, 6-benzylaminopurine (BAP) 1 mg/l and 0,4% phytagel) (Perrin et al., 2004) and were maintained in darkness at 24°C. Subsequently, anther or ovary-derived calli were sub-cultured, according to Perrin et al. (2001), on MPM01 (Nitsch and Nitsch-based (NN) medium modified added with 2,4D 1 mg/l and BAP 0,25 mg/l) and MPM1 (MPM01 added with 0,7 g/l MES) media. When embryogenic calli were obtained

they were maintained and alternately transferred every 40 days to the GS1CA ad PIV media (Franks et al., 1998).

Agrobacterium-mediated transformation of rootstock genotypes

Gene transfer was performed during co-cultures of 101.14 Mgt, SO4, RgM and 110R embryogenic calli with the *Agrobacterium tumefaciens* strain EHA105 (Hood et al., 1993) containing a pK7WG2 plant binary vector (Karimi et al., 2002), with the *VIT_17s0000g08960* cDNA under the control of the CaMV-35S promoter, according to Dalla Costa et al. (2014). After several months of selection on GS1CA medium supplemented with 1 g/L Timentin and 150 mg/L kanamycin in the dark at 25 °C, the more developed embryos were transferred to NN medium supplemented with kanamycin 25 mg/L at 25°C and 16 h-light photoperiod for embryo differentiation and germination. Subsequently they were transferred into WP medium (Lloyd and McCown, 1981) for root and apical growth.

Plant materials and water stress treatment conditions

The experiment was conducted on one-year-old potted (9 L) rooted cuttings of four grapevine rootstock genotypes (101.14 Mgt, SO4, RgM and 110R) in a semi-sealed greenhouse (Figure 2). Twelve vines for each rootstock genotype were subjected to water stress by completely suspending irrigation for 15 days (water stress group, WS), while other six vines were maintained at about 90% of maximum water availability (well watered group, WW). The growing medium was composed of a sand-peat mixture (1:1 in volume) with a field capacity of 35% [(vol water/vol soil) × 100]. The volumetric soil moisture content per pot was monitored with a ML3 ThetaProbe Soil Moisture Sensor (Delta-T Devices, London, UK). The pot surface was covered with a plastic film to avoid soil water evaporation. The experimental plan was completely randomized.

Water stress experiment in hydroponic culture with Polyethylene glycol (PEG)

Grapevine rootstock genotypes, 101.14 Mgt, SO4, RgM and 110R were in vitro propagated on half MS medium, supplemented with indole-3 butyric acid (IBA) 0.1 mg/l, and were grown under a photoperiod (8 h dark, 16 h light) at 25 °C for 1 month. After a period of acclimation 36 *ex vitro* plantlets of each genotype with heights of 4–6 cm were transferred to 1/2 Hoagland nutrient solution modified in hydroponic pots (5 L) with continuous aeration at temperature (25°C day, 23°C night), 50% relative humidity and photoperiod (8 h dark, 16 h light) for two

weeks. A 2% concentration of PEG–6000 was added into the solution to decrease water potential for mimicking drought stress.

Physiological measurements

Physiological measurements were carried out on two healthy adult primary leaves grown between the 6th and the 10th node of the primary shoots. One fully expanded leaf of each water stressed vine was collected and immediately frozen in liquid nitrogen and stored at –80 C° to be used in gene expression experiments. Chlorophyll fluorescence parameters: maximum quantum efficiency of PS II photochemistry (Fv/Fm) and Performance Index (PI) were measured using fluorimeter Handy PEA (Hansatech, Kings Lynn, UK). PI parameter (Strasser et al., 2000) was according to the equation:

$$\mathrm{PI}_{\mathrm{abs}} = \frac{1-(F_0/F_{\mathrm{M}})}{\mathrm{M}_0/\mathrm{V}_{\mathrm{J}}} \times \frac{F_{\mathrm{M}}-F_0}{F_0} \times \frac{1-\mathrm{V}_{\mathrm{J}}}{\mathrm{V}_{\mathrm{J}}}$$

F_o= fluorescence intensity at 50 μs; F_J= fluorescence intensity at 2 ms; F_M= maximal fluorescence intensity; V= relative variable fluorescence at 2 ms calculated as $V_J = (F_J - F_o)/(F_M - F_o)$; M_o= initial slope of fluorescence kinetics, calculated as $M_o = 4^* (F300\ \mu s - Fo)/(F_M - F_o)$. Dark adaption was achieved by covering the sample area with a leafclip for at least 15 minutes. Leaf chlorophyll content was measured with a Chlorophyll Meter SPAD-502 (Konica Minolta Sensing Inc,, Osaka, Japan). Stomatal conductance (g_s) was measured with a portable porometer (SC-1 Leaf porometer, Decagon Devices, Pullman, WA, USA).

Statistical analyses

All statistical analyses were performed using R packages 'stats', 'agricolae' and 'companion' v3.5.1 (R Core Team, 2013). For mean comparisons, several tests were used depending on homoscedasticity pre-tests. Parametric Student's *t*-test (one parameter) or one-way ANOVA were performed to data displaying a normal distribution and equal variance between treatments. Otherwise, non-parametric Mann-Whitney U test (one parameter) and one-way Kruskal–Wallis were performed. For classification tests, a comparison of least-square means at a 0.05 significance level and a Fisher's Least Significant Difference (LSD) or Dunn's tests were performed.

Table 1: List of primers used for cloning and sequencing *VIT_17s0000g08960* coding region.

Primer name	Sequence
SIP1cDNA fw	CACCATGGCTCCCAGCTTGAGCAAAGG
SIP1cDNA rv	GAACAAGTACTCTACAATTGACA
AMP1 fw	CGCGTTCCCATGTCTTAGC
AMP1 rv	GGTTGTCTCCTACGTGCATG
AMP2 fw	AGCCGGGAGAAGACGACAAC
AMP2 rv	ATTGCAGCCGAGAGTGGAG
AMP3 fw	CTATFFAGGATTTGGCCGTG
AMP3 rv	ACATGGGTGGGTGGATTGAA
AMP4 fw	CCGAACGGCACATTTTGG
AMP4 rv	ACTTGAACCCCAACCGTATG

Results

Description and nucleotide diversity of the candidate gene *VIT_17s0000g08960*

Candidate gene and nucleotide diversity observed through analysis of 2343 bp of the *VIT_17s0000g08960* coding sequence among the 85 rootstock genotypes are shown in Table 2. *VIT_17s0000g08960* gene contains 4 exons and this structure corresponded to the gene prediction deposited on Grape Genome Database. A total of 134 SNPs were identified and then named and scored according to their position on *VIT_17s0000g08960* ORF of *V. vinifera* gene annotation v2.1. SNP variation among the 85 rootstock accessions corresponded to an average of one SNP every 17 bp. Only one INDEL was found in exonic regions.

Nucleotide polymorphisms and prediction of tolerability of amino acid exchanges in the candidate gene *VIT_17s0000g08960*

Genetic variation at the nucleotide level was estimated from mean nucleotide diversity (π = 0,007) and the number of segregating sites (θ = 0,011) Nucleotide diversity was not equally distributed among site categories since π value was three times higher for synonymous sites than for non-synonymous sites (Table 3). Besides, nucleotide variation and diversity were separately estimated (Table 4) by grouping the accessions into different phenotypic classes (rootstock and hybrid). The rootstock class has higher frequency of polymorphic sites (one every 21 bp) than the hybrid class (one every 33 bp) but it has also reduced nucleotide diversity (π = 0,006, θ = 0,011) compared with the hybrid class (π = 0,007, θ = 0,007). Neutrality tests were estimated using two values, Tajima's D value and Fu and Li's F value. Both tests indicated that the polymorphisms did not reveal any significant deviation from neutrality in the dataset as a whole and in the subsets of rootstocks and hybrids.

Table 2: Nucleotide diversity of *VIT_17s0000g08960* protein coding region in grape rootstocks population.

Parameters	Overall
Varieties	85
Genomic ATG-TGA	3678 bp
Full-ORF cDNA	2343 bp
Predicted protein	780 aa
Exons	4
Introns	3
Number of polymorphic sites	135
SNPs	134
INDELS	1
Frequency of SNPs	1 per 17 bp
Frequency of INDELS	1 per 2343
Synonymous changes	65
Non-synonymous changes	70
Synonymous vs non-synonymous mutations	0,9:1

Table 3: Overall polymorphisms in the *VIT_17s0000g08960* gene.

All			Synonymous		Non Synonymous	
S	π	θ	S	π	S	π
134	0,007	0,011	65	0,015	70	0,005

The impact of non-synonymous substitutions on biological function of protein was predicted for all 70 mutations detected, hence 17 showed a PROVEAN score below -2,5 that indicates a probable structural alteration of protein (Table 5). Additionally, some of these deleterious mutations occur in a significant proportion of the rootstock population.

Table 4: Comparison of *VIT_17s0000g08960* nucleotide diversity in different phenotypic classes.

Parameters	Overall	Rootstocks	Hybrids
Varieties	85	48	37
Number of polymorphic sites	135	109	71
Frequency of polymorphic sites	1:17	1:21	1:33
Synonymous changes	65	49	36
Non-synonymous changes	70	54	34
Synonymous vs non-synonymous mutations	0,9:1	0,9:1	1,1:1
Mean nucleotide diversity (π/θ)	0,007/0,011	0,006/0,011	0,007/0,007
Mean Tajima D	-1,25 ns	-1,36	-0,06
Fu and Li's D	0,29	-1,21	0,27
Shared mutation	52		
Mutation polymorphic only in one group		64	20

Table 5: Prediction of tolerability of amino acid exchanges.

Site	Amino acid change	Provean score	Prediction	Frequency
57	Q19H	-0,473	Neutral	1/85
67	S23A	-0,345	Neutral	1/85
68	S23L	-0,979	Neutral	1/85
74	T25I	-2,617	Deleterious	2/85
91	F31I	-0,114	Neutral	5/85
136	I46F	-3,167	Deleterious	1/85
139	V47M	-1,000	Neutral	16/85
143	A48V	0,521	Neutral	50/85
161	S54I	-0,581	Neutral	64/85
212	A71V	-1,556	Neutral	4/85
217	E73Q	-1,165	Neutral	16/85
227	S76N	-2,181	Neutral	34/85

230	R77L	-4,161	Deleterious	1/85
238	V80I	-0,095	Neutral	1/85
244	V82I	0,696	Neutral	25/85
266	P89R	5,105	Neutral	18/85
266	P89L	-0,338	Neutral	11/85
320	T107S	0,285	Neutral	3/85
340	H114N	-0,508	Neutral	11/85
347	T116N	-4,558	Deleterious	1/85
358	I120L	0,286	Neutral	2/85
380	G127D	-0,352	Neutral	2/85
451	D151N	-1,896	Neutral	1/85
469	V157L	-0,43	Neutral	2/85
478	G160C	-8,205	Deleterious	10/85
494	R165P	-1,325	Neutral	6/85
500	S167F	-2,615	Deleterious	1/85
547	E183K	-0,821	Neutral	6/85
570	K190N	-2,914	Deleterious	6/85
610	E204Q	-1,791	Neutral	4/85
642	F214L	-5,509	Deleterious	1/85
646	W216L	-11,936	Deleterious	1/85
685	E229K	-1,701	Neutral	45/85
687	E229D	-1,211	Neutral	8/85
709	G237S	0,663	Neutral	40/85
736	G246C	-3,552	Deleterious	4/85
796	D266N	-1,031	Neutral	1/85
798	D266E	-0,423	Neutral	5/85
806	G269D	-1,118	Neutral	8/85
863	E288A	-5,362	Deleterious	2/85
904	Q302E	0,394	Neutral	6/85
907	E303K	-0,573	Neutral	4/85
916	M306V	-1,393	Neutral	4/85
946	E316K	-0,299	Neutral	2/85
947	E316V	-3,985	Deleterious	1/85

967	V323L	-2,569	Deleterious	9/85
1016	N339S	-0,021	Neutral	4/85
1031	P344L	-2,591	Deleterious	5/85
1183	S395P	-3,334	Deleterious	1/85
1231	M411L	-0,961	Neutral	1/85
1378	T460S	-0,744	Neutral	1/85
1382	I461T	-1,472	Neutral	26/85
1402	D468N	-4,847	Deleterious	1/85
1643	T548S	0,325	Neutral	13/85
1643	T548I	-2,445	Neutral	1/85
1687	A563S	-1,429	Neutral	2/85
1759	L587I	-1,155	Neutral	4/85
1768	Y590N	-5,534	Neutral	9/85
1768	Y590H	-2,978	Neutral	3/85
1835	K612R	-0,721	Neutral	4/85
1858	T620A	-1,015	Neutral	16/85
1871	L624S	1,004	Neutral	2/85
1878	N626K	-0,634	Neutral	1/85
1964	E655G	-0,536	Neutral	1/85
1986	K662N	-1,504	Neutral	29/85
2039	T680I	-4,523	Deleterious	8/85
2076	N692K	-0,118	Neutral	12/85
2147	E716A	-1,347	Neutral	1/85
2219	E740A	-1,619	Neutral	1/85

To evaluate the evolutionary relationships of VIT_17s0000g08960, it was conducted a phylogenetic analysis based on full-length protein sequences of Raffinose family oligosaccharides (RFOs) proteins of grapevine and other representative sequenced plant species. Neighbor-joining-tree (Figure 1) showed that the raffinose synthase (RAFs) protein GSVIVT01007681001 codified by *VIT_17s0000g08960* has close evolutionary relationship with RAFs proteins of *Arabidopsis thaliana (*AT5G40390), *Oriza sativa* (Os01g07530.1) and *Cucumis sativus* (Cucsa.098650.1). AT5G40390 protein is the solely responsible for raffinose accumulation in Arabidopsis leaves under drought stress (Egert et al., 2013). Moreover, there

is a distinct separation between the three enzymes of raffinose family oligosaccharide (RFO) pathway.

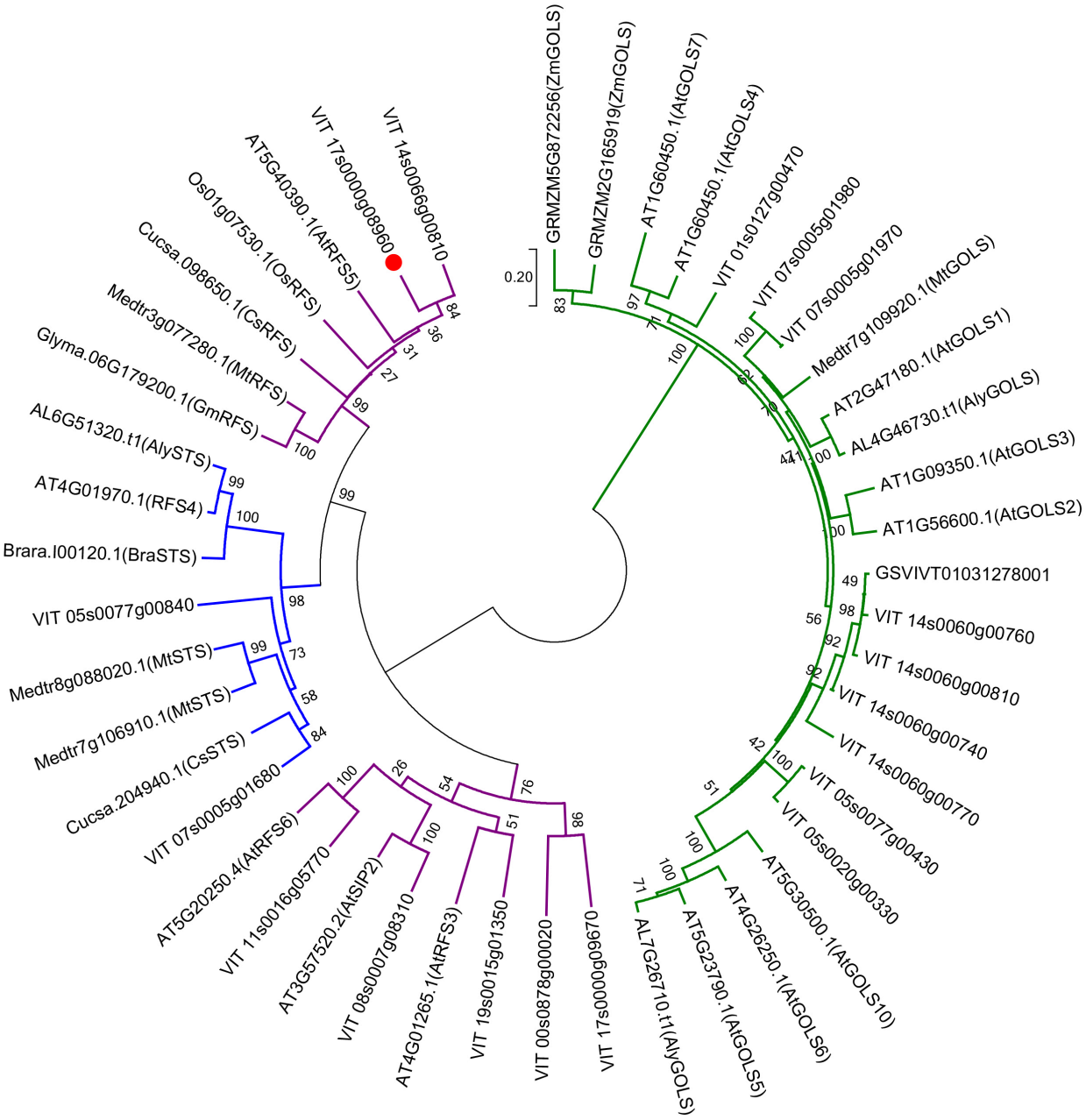

Figure 1: Phylogenetic relation of VIT_17s0000g08960 (marked with with red dot) with Raffinose family oligosaccharides (RFOs) proteins of grapevine and other species (*Arabidopsis thaliana, Arabidopsis lyrata, Brassica rapa, Cucumis sativus, Glycine max, Medicago truncatula, Oriza sativa, Zea mays*). Neighbor-joining tree was constructed using MEGA 6.0 with stringency of 500 bootstrap-replicates. Clustering of RFOs proteins into different subgroups are indicated with different colors: galactinol synthases GOLs (green), raffinose synthases RAFs (violet) and stachyose synthase STs (blue). Proteins are named according V. *vinifera* gene annotation v2.1 hosted on http://genomes.cribi.unipd.it/grape and Phytozome annotation for other species.

Water stress experiment on potted rootstocks

To investigate the effects of drought stress on plant physiology four rootstock genotypes (SO4, 101.14Mgt, 110R and RGM) were subjected to stress by withholding water. These genotypes were selected both to represent putatively different classes of response to WS and based on the SNP chr17_10497222_C_T at the candidate gene (Table 6).

Table 6: Roostock classification based on response to drought in field (1)(Fregoni, 1977) and in greenhouse (2)(Carbonneau, 1985) and on the SNP chr17_10497222_C_T.

Genotype	WS response class (Serra et al., 2014)	SNP (CT overdominance effect)
SO4 (*V. riparia* x *V. berlandieri*)	sensitive (1) / resistant (2)	CT
101.14Mgt (*V. riparia* x *V. rupestris)*	sensitive (1,2)	TT
110R (*V. rupestris* x *V. berlandieri*)	highly resistant (1,2)	TT
RGM (*V. riparia*)	very sensitive (1,2)	CC

Volumetric soil water content was determined throughout the experiment to monitor the stress evolution. Plants of water stress group (WS) showed a a continuous decrease in soil water content (Figure 3a) with a substantial decline at five days from the beginning of the experiment. Interestingly, starting from 9 days after stopping irrigation, water content was significantly higher in SO4 compared to other genotypes. In well watered group (WW) soil moisture was maintained around 30% during the entire experimental period (Figure 3b). After withholding water for two weeks the leaves of SO4 remained almost green and turgid, 110R and 101.14 showed some signs of plant stress and RGM vines were considerably damaged (Figure 4).

Figure 2: Grape rootstock plants that were subjected to drought stress in 2018.

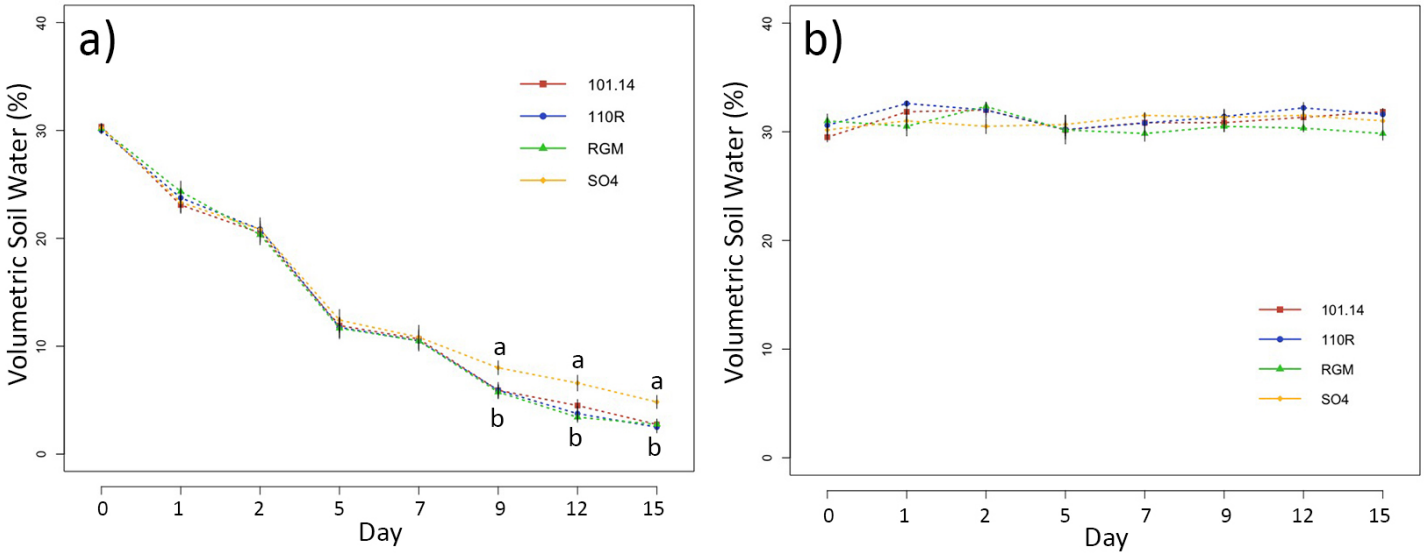

Figure 3: Volumetric soil water content throughout the progression of the drought stress experiment of water stress group (a) and of control group (b). Values represent average measurements ± SE of twelve replicates (WS) and six replicates (WW). Data were analysed using one-way ANOVA with LSD *post-hoc* test, and letters indicate significant differences between genotypes on the same day at $p < 0.05$.

Figure 4: Richter 110, Riparia Gloire de Montpellier, 101.14 Millardet et de Grasset and SO4 Selection Oppenhiem rootstocks of water stress group (WS) and well watered group (WW) at the end of the experiment.

Physiological effects of drought stress on potted rootstocks

Stomatal conductance (g_S) is consider a reference parameter of plant status in response to drought. In plants of water stress group (WS) transpiration was significantly reduced by water deficit in all the rootstocks (Figure 5). A significant genotype effect was observed at the beginning of the experiment and five days after withholding irrigation (Figure 6). Initially 110R showed the highest stomatal conductance values, whereas SO4 had higher transpiration rate than other genotypes during drought progression. At the end of the experiment no significant differences in transpiration were observed among rootstock genotypes with a nearly complete closure of the stomata (Figure 6). Surprisingly, transpiration was significantly reduced after 12 days also in control plants (Figure 7 and Table 7). Chlorophyll fluorescence parameters reflect the maximum efficiency of PSII photochemistry (Fv/Fm) can be taken as indicator of drought stress. Plant subjected to water stress showed different levels of stress tolerance between genotypes (Figure 8). In RGM stressed plants Fv/Fm values significantly decreased in comparison to their control plants after 7 days withholding water, in 110R drought treatment had a relevant impact on photosynthetic efficiency at day 9, whereas 101.14Mgt and SO4 showed a similar patterns with a significant decrease of Fv/Fm after 15 days.

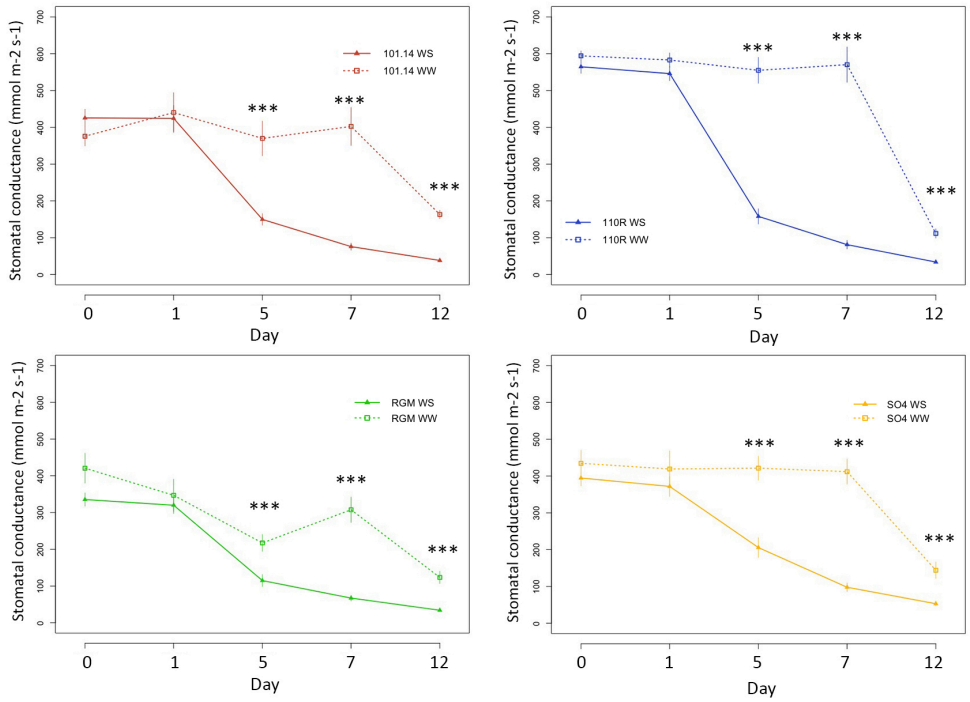

Figure 5: Stomatal conductance of water stressed (WS) and well watered (WW) 101.14Mgt (red), 110R (blue), RGM (green) and SO4 (yellow) throughout the experiment. Values represent average measurements ± SE, *n = 12* (WW) and n=24 (WS). Significant differences between treatments on the same day were tested

with Mann-Whitney U test, and asterisks indicate significantly different values at $p \leq 0.05$ (*), $p \leq 0.01$ (**), and $p \leq 0.001$ (***).

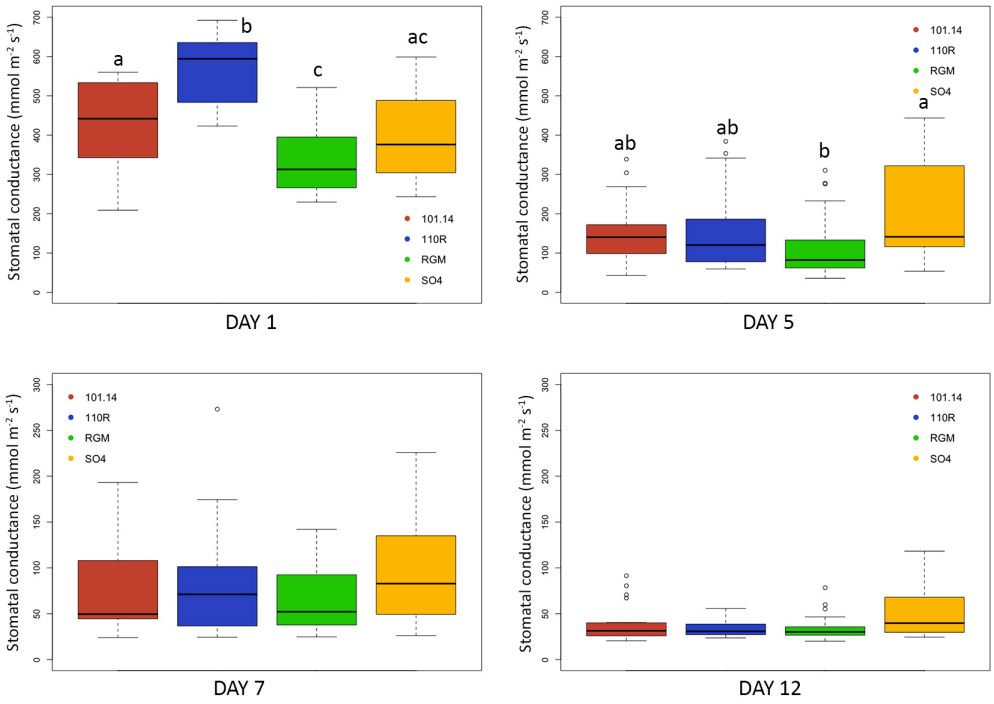

Figure 6: Boxplots of stomatal conductance measured on 101.14Mgt (red), 110R (blue), RGM (green) and SO4 (yellow) water stressed plants at 1, 5, 7 and 12 days from the beginning of experiments (n=24). Significant differences between genotypes on the same day were tested with with Kruskal Wallis Test, and letters indicate significantly different values at $p \leq 0.05$ according to Dunn's test.

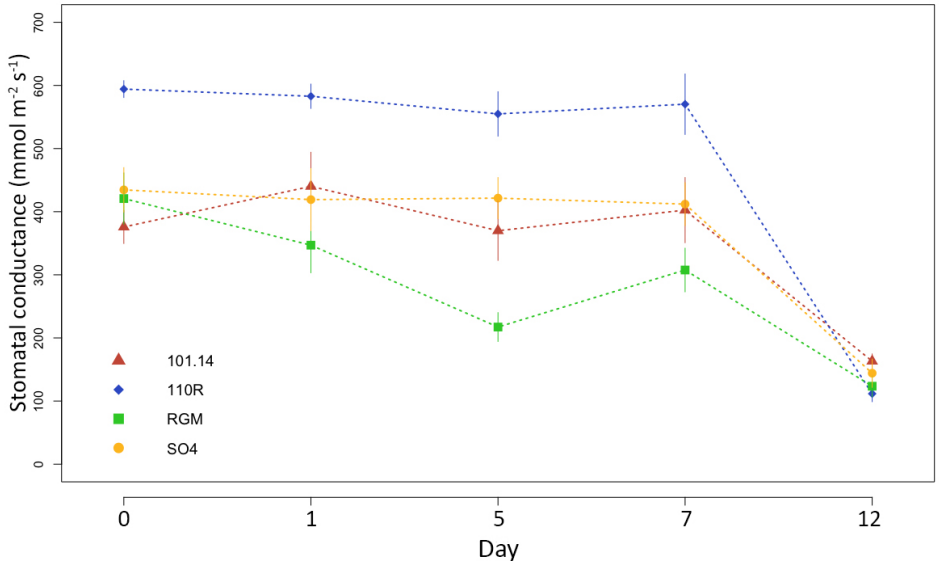

Figure 7: Stomatal conductance of well watered (WW) 101.14Mgt (red), 110R (blue), RGM (green) and SO4 (yellow) throughout the experiment. Values represent average measurements ± SE (*n = 12).*

Table 7: Stomatal conductance of well watered (WW) 101.14Mgt, 110R, RGM and SO4 at 0, 1, 5, 7 and 12 days from the beginning of experiments. Values represent average measurements ± SD (*n = 12).* Significant differences within genotypes throughout the experiment were tested with with Kruskal Wallis Test, and letters indicate significantly different values at $p \leq 0.05$ according to Dunn's test.

DAY	101.14Mgt	110R	RGM	SO4
0	375,8 ± 90,4 **a**	594,2 ± 42,0 **a**	429,8 ± 140,9 **a**	436,6 ± 122,1 **a**
1	440,1 ± 187,1 **a**	582,9 ± 54,5 **a**	346,9 ± 151,5 **ab**	419,1 ± 170,3 **a**
5	369,8 ± 162,8 **a**	554,9 ± 111,1 **a**	217,3 ± 79 **bc**	421,5 ± 112,9 **a**
7	402,5 ± 178,7 **a**	570,3 ± 151,4 **a**	307,6 ± 119,5 **ab**	412,0 ± 117,6 **a**
12	163,2 ± 39,5 **b**	111,6 ± 39,8 **b**	123,5 ± 57,4 **c**	144,2 ± 77,6 **b**

Regarding Performance Index (PI), which is essentially an indicator of sample vitality that considers the concentration of photosynthetic reaction centers and the force of the light and dark reactions, a substantial decrease of these parameter was observed in 101.14Mgt, 110R, RGM after 12 days of water stress treatment. On the other hand PI of SO_4 stressed plants was significantly reduced in comparison to their controls only at the end of the experiment (Figure 9). Chlorophyll content was also checked for both experimental conditions (WW and WS) during the experiment by SPAD measurements (Figure 10). Stressed plants of 101.14Mgt and RGM showed a significant decrease of SPAD values at the last day of stress while SO_4 leaf chlorophyll content differ from controls after 12 days. On the contrary no variation in this parameter was registered for 110R.

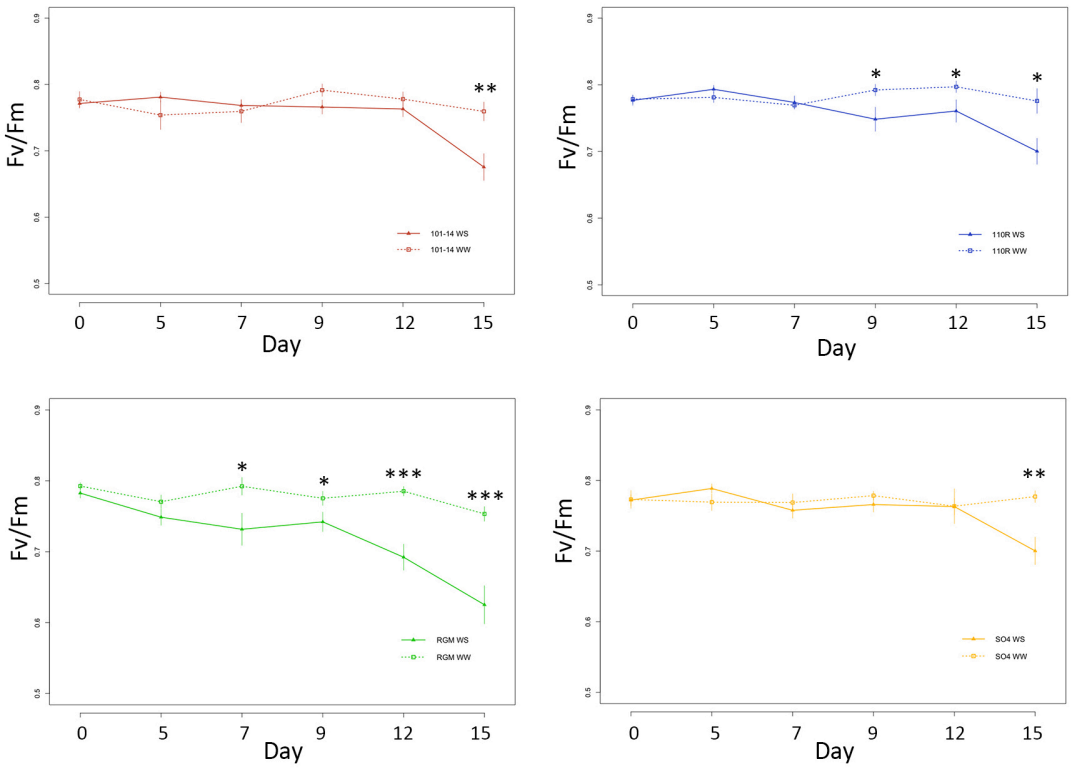

Figure 8: Maximum quantum yield of PSII (Fv/Fm) in water stressed (WS) and well watered (WW) 101.14Mgt (red), 110R (blue), RGM (green) and SO4 (yellow) throughout the experiment. Values represent average measurements± SE, *n = 12* (WW) and n=24 (WS). Significant differences between treatments on the same day were tested with Mann-Whitney U test, and asterisks indicate significantly different values at $p \leq 0.05$ (*), $p \leq 0.01$ (**), and $p \leq 0.001$ (***)

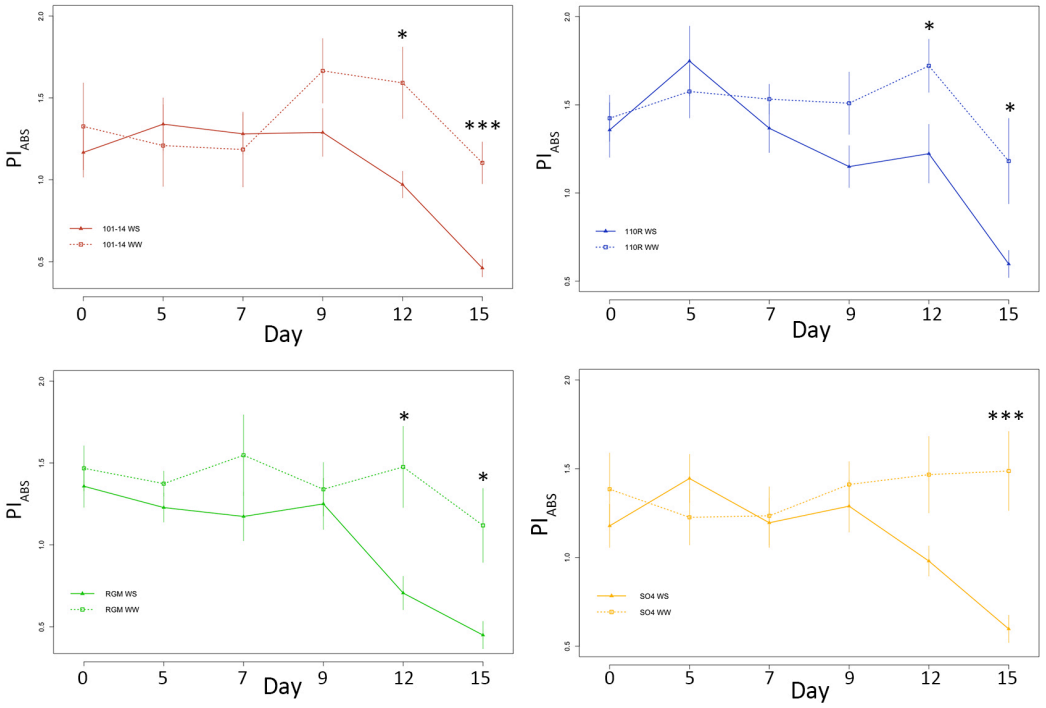

Figure 9: Relative values of the photosynthetic performance index (PI_{ABS}) of water stressed (WS) and well watered (WW) 101.14Mgt (red), 110R (blue), RGM (green) and SO4 (yellow) throughout the experiment. Values represent average measurements ± SE, *n = 12* (WW) and n=24 (WS). Significant differences between treatments on the same day were tested with Mann-Whitney U test, and asterisks indicate significantly different values at $p \leq 0.05$ (*), $p \leq 0.01$ (**), and $p \leq 0.001$ (***)

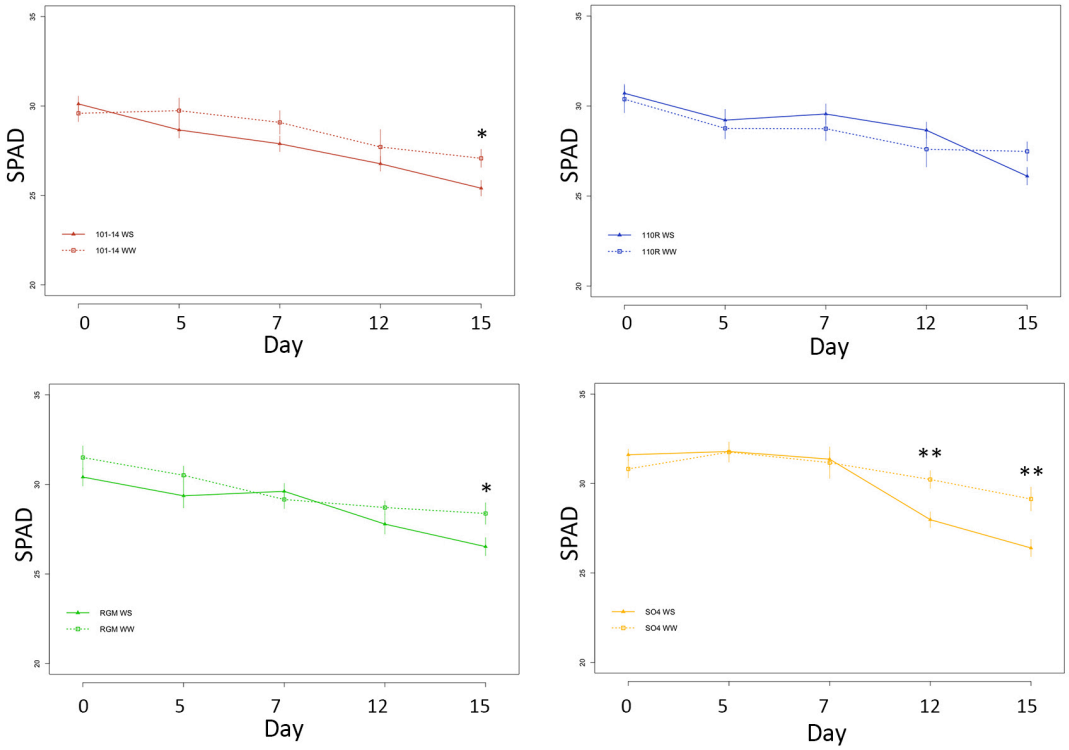

Figure 10: Chlorophyll content (SPAD index) of water stressed (WS) and well watered (WW) leaves of 101.14Mgt (red), 110R (blue), RGM (green) and SO4 (yellow) throughout the experiment. Values represent average measurements ± SE, *n = 12* (WW) and n=24 (WS). Significant differences between treatments on the same day were tested with Student's *t*-test, and asterisks indicate significantly different values at $p \leq 0.05$ (*), $p \leq 0.01$ (**), and $p \leq 0.001$ (***)

Water stress experiment in hydroponics

The physiological responses of the same rootstock varieties RGM, 101.14Mgt, SO4 and 110R were evaluated in a hydroponic culture system (Figure 11). It was developed an effective method to mimic the water stress condition by adding polyethylene glycol (PEG) at the nutrient solution. After the exposure to this simulated water stress for 7 days, 101.14Mgt plants showed the highest survival rate compared to other genotypes (Figure 12). This experiment was repeated two times and proved to be a useful method to ascertain the drought tolerance level of grapevines in a simplified and strictly controlled way.

Figure 11: Grape rootstock plants grown in a hydroponic system.

Physiological effects of drought stress in hydroponics

Stomatal conductance was significantly reduced in SO4, 110R and RGM under PEG stress compared with their controls in both the experiments. More specifically, in SO4 and 110R the treatment resulted in statistically significant stomatal closure after 1 or 2 days, whereas RGM plants diminished their transpiration only ofter 2 or 3 days. On the contrary 101.14Mgt showed a transpiration rate very similar to control also 3 days after stress imposition (Figure 13). No statistically significant effect of PEG treatment on the photosynthetic efficiency and the chlorophyll content was observed in all the four genotypes throughout the experiments (Table 8). But among the varieties, 110R exhibited the highest values of Fv/Fm, PI_{ABS} and SPAD index at all time points measured and in both the experiments. Likewise, 101.14Mgt plants showed always the lowest values in these physiological parameters.

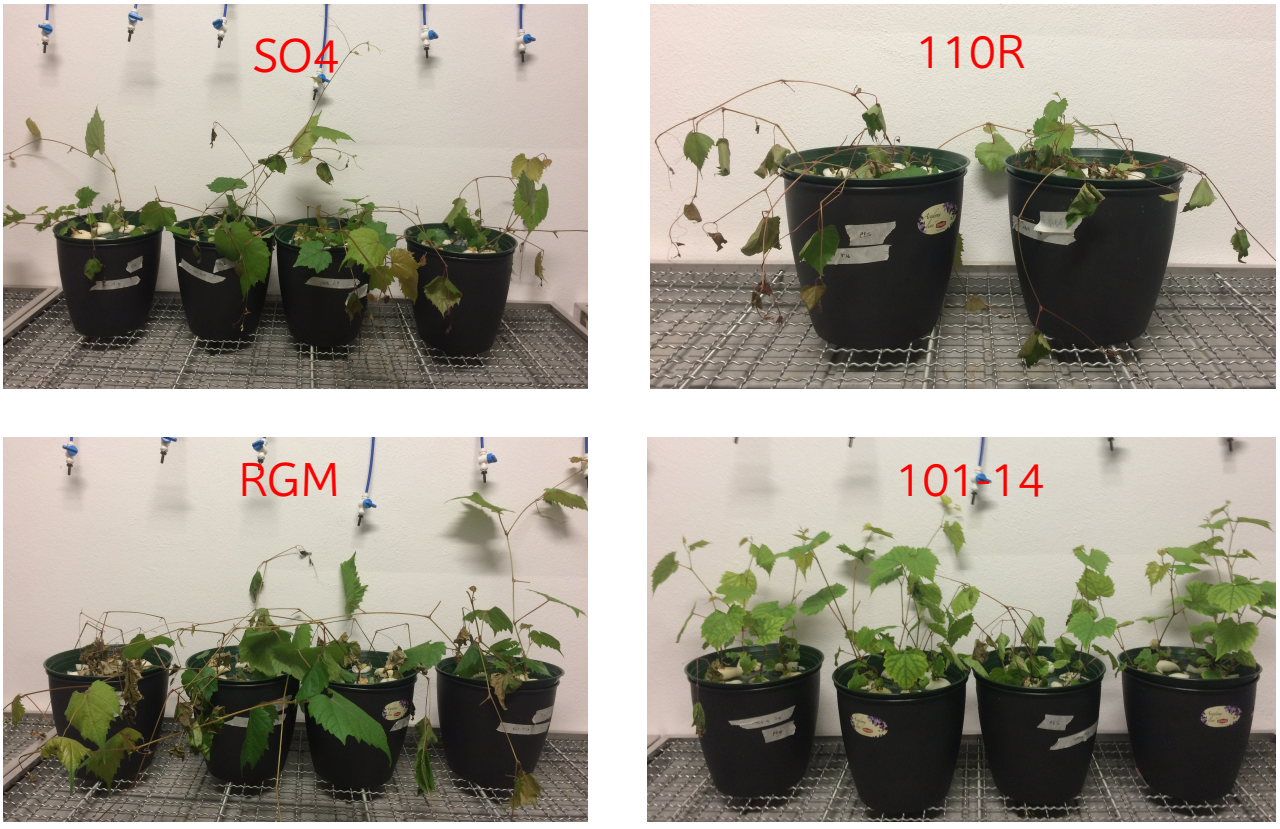

Figure 12: SO4, 110R, RGM and 101.14Mgt rootstocks after exposure to PEG-induced drought stress for 7 days

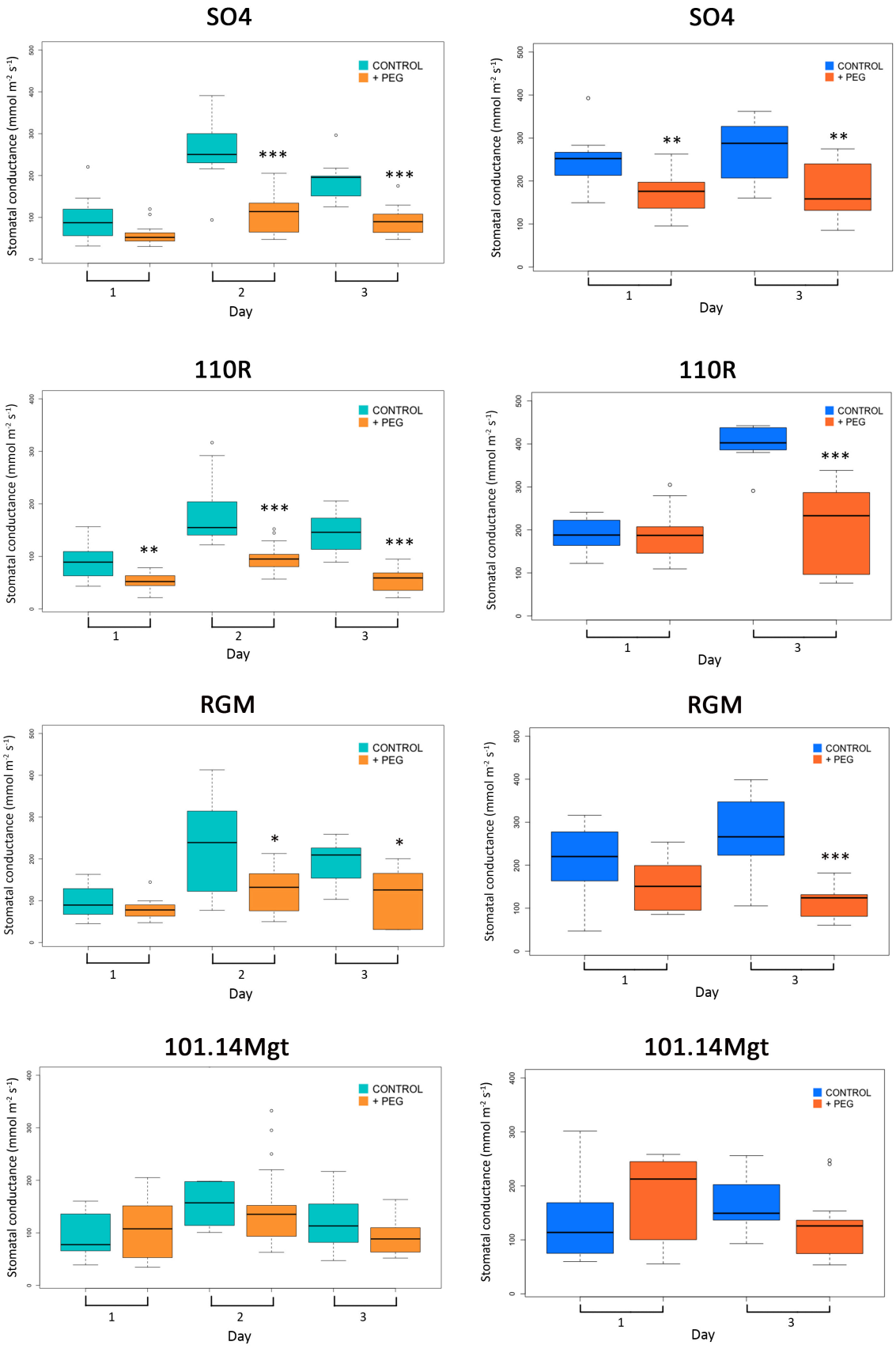

Figure 13: Boxplots of stomatal conductance measured on 101.14Mgt, 110R, RGM, and SO4 throughout the experiments; first experiment (left) and second experiment (right) *n=12* (CONTROL) and n=24 (+PEG). Significant differences between treatments on the same day were tested with Mann-Whitney U test, and asterisks indicate significantly different values at $p \leq 0.05$ (*), $p \leq 0.01$ (**), and $p \leq 0.001$ (***)

Table 8: Maximum quantum yield of PSII (Fv/Fm), relative values of the photosynthetic performance index (PI_{ABS}) and chlorophyll content (SPAD index) measured on 101.14Mgt, 110R, RGM, and SO4 throughout the two experiments in hydroponics, *n=12* (Control) and n=24 (+PEG).

Experiment	Day	Genotype	Treatment	Fv/Fm		PI Index		SPAD	
				Mean	SD	Mean	SD	Mean	SD
1	0	101.14Mgt	Control	0,376	0,187	0,101	0,150	18,8	2,6
		RGM	Control	0,779	0,035	0,864	0,306	29,2	1,5
		SO4	Control	0,714	0,039	0,680	0,577	27,3	3,2
		110R	Control	0,795	0,019	1,548	0,349	32,3	1,8
	1	101.14Mgt	+PEG	0,404	0,231	0,063	0,069	19,1	3,7
			Control	0,415	0,285	0,143	0,260	20,1	4,2
		RGM	+PEG	0,762	0,076	0,973	0,514	30,3	2,0
			Control	0,791	0,020	0,938	0,308	30,5	2,2
		SO4	+PEG	0,699	0,107	0,613	0,350	26,9	3,0
			Control	0,615	0,173	0,440	0,473	26,1	3,1
		110R	+PEG	0,798	0,027	1,481	0,468	32,5	1,9
			Control	0,812	0,005	1,685	0,432	32,2	2,7
	2	101.14Mgt	+PEG	0,438	0,248	0,132	0,244	17,9	3,6
			Control	0,586	0,182	0,146	0,206	21,2	3,0
		RGM	+PEG	0,759	0,044	0,765	0,336	30,6	2,3
			Control	0,798	0,022	1,041	0,332	30,3	2,6
		SO4	+PEG	0,715	0,075	0,554	0,305	27,4	2,8
			Control	0,678	0,124	0,531	0,398	27,2	2,0
		110R	+PEG	0,776	0,031	1,113	0,372	31,6	2,4
			Control	0,787	0,024	1,229	0,500	32,2	2,4
	3	101.14Mgt	+PEG	0,431	0,246	0,042	0,060	19,0	3,3
			Control	0,515	0,225	0,095	0,131	20,9	2,7
		RGM	+PEG	0,748	0,048	0,564	0,327	30,0	2,7
			Control	0,778	0,024	0,869	0,368	29,2	2,6
		SO4	+PEG	0,710	0,081	0,520	0,299	27,9	2,9
			Control	0,743	0,053	0,700	0,394	26,5	1,8
		110R	+PEG	0,750	0,052	0,882	0,479	31,1	2,3
			Control	0,776	0,029	1,136	0,466	30,9	2,5

Experiment	Day	Genotype	Treatment	Fv/Fm		PI Index		SPAD	
				Mean	SD	Mean	SD	Mean	SD
2	0	101.14Mgt	Control	0,682	0,078	0,232	0,218	22,9	1,6
		RGM	Control	0,777	0,020	0,665	0,165	27,4	1,1
		SO4	Control	0,764	0,045	0,625	0,301	23,6	1,7
		110R	Control	0,807	0,016	1,126	0,353	28,0	2,5
	1	101.14Mgt	+PEG	0,606	0,175	0,189	0,218	21,8	2,7
			Control	0,654	0,109	0,193	0,204	22,6	2,0
		RGM	+PEG	0,783	0,035	0,786	0,249	26,9	1,1
			Control	0,787	0,054	1,009	0,388	28,2	1,1
		SO4	+PEG	0,767	0,025	0,584	0,501	23,4	4,6
			Control	0,745	0,068	0,682	0,365	24,3	1,4
		110R	+PEG	0,822	0,004	1,552	0,410	28,4	2,2
			Control	0,820	0,015	1,543	0,447	29,2	2,1
	3	101.14Mgt	+PEG	0,489	0,164	0,096	0,082	21,5	2,8
			Control	0,533	0,202	0,110	0,124	22,2	2,3
		RGM	+PEG	0,735	0,054	0,370	0,211	26,7	1,2
			Control	0,773	0,024	0,520	0,365	27,8	1,4
		SO4	+PEG	0,740	0,050	0,420	0,261	23,1	3,7
			Control	0,703	0,066	0,317	0,214	24,3	2,0
		110R	+PEG	0,788	0,017	0,620	0,489	28,2	2,6
			Control	0,787	0,036	0,941	0,621	28,9	1,8

Embryogenic callus induction in four rootstock genotypes

Starting from explants of about one hundred inflorescences of RGM, 101.14Mgt, SO4 and 110R, embryogenic calli were obtained for all the four varieties (Figure 14). In each plate anthers of five inflorescences were detached from the calyx, taking care not to damage filaments, and were plated together with their ovaries. After about 2 months non embryogenic dry, non embryogenic watery and pre-embryogenic calli were grown from anther cultures. The latter were transferred and embryogenic calli development occurred in 6-8 months.

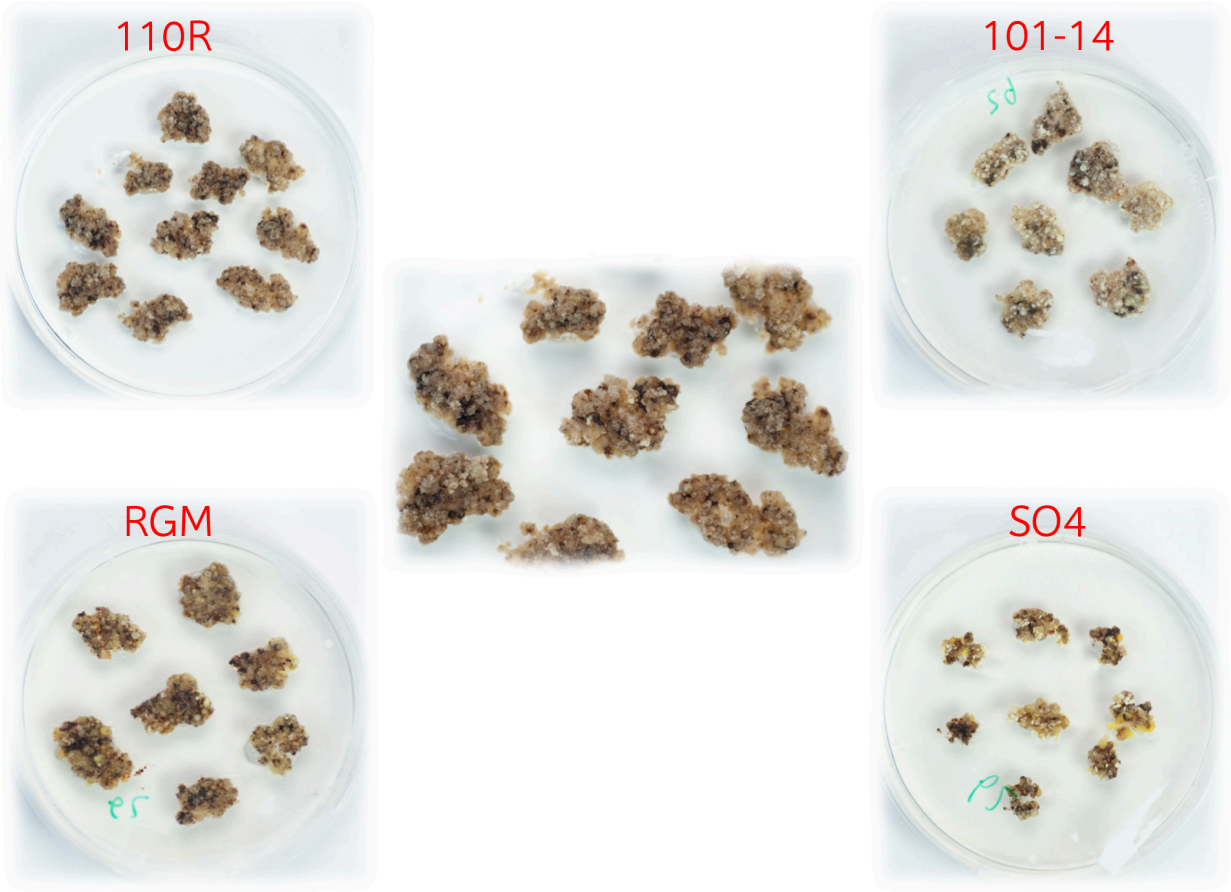

Figure 14: Embryogenic calli derived from anthers cultures of RGM, 101.14Mgt, SO4 and 110R. An enlargement of 110R's callus is depicted in the centre.

Discussion

GWAS identified a statically significant association between stomatal conductance and a SNP in the RFO gene *VIT_17s0000g08960*. This could be a suitable candidate gene for controlling drought functional traits based on its biological significance (Sengupta et al., 2015). Therefore, its entire coding region has been sequenced in 85 rootstock genotypes in order to detect a potential causative variant. The nucleotide diversity of *VIT_17s0000g08960* (π = 0,007) is higher than the average values observed in grapevine gene regions that are reported in literature (π = 0,0040-0,0051) (Owens, 2003; Lijavetsky et al., 2007; Le Cunff et al., 2008; Fernandez et al., 2014), which is consistent to the complex nature of the highly diverse association panel that includes different Vitis species and hybrids and thereby presenting a large genetic variability. Interspecific hybrids, which all include V. *vinifera* in their pedigrees, showed a lower frequency of polymorphic sites compared with other rootstock genotypes. On the other hand, if mutations in the non-coding portions of the genome are considered, the genetic diversity in grapevine is substantially higher both in wild and cultivated varieties ranged from π = 0,015 and π = 0,014, respectively (Zhou et al., 2017). Although a recently published whole-genome resequencing of 472 *Vitis* accessions revised downwards these estimates, reporting nucleotide diversity values of π = 0,0035 for wild and π = 0,0055 for domesticated cultivars (Liang et al., 2019).Unfortunately, none of the non-synonymous changes of *VIT_17s0000g08960* coding region proved to be in LD with the associated variant identified in GWAS. Thus, putative causative mutation in LD with the significant synonymous SNP could be located in genomic regions that have not been investigated and sequenced in the present experiment; cis-regulatory sequences can be localized in intragenic (introns) or intergenic (promoter and enhancer) regions closely surrounding the gene and its associated SNP and need to be further investigated. Nevertheless, the effect of some non-synonymous substitutions, especially those that might have a negative impact on biological function of protein and that are widespread in sequenced varieties (e.g. G160C and Y590N), may be characterized in further functional studies.

Phylogenetic analysis of the protein codified by *VIT_17s0000g08960* has demonstrated that it is closely related to RAFs proteins of other plant species. Among these, stress-inducible protein RS5 (AT5G40390) of Arabidopsis has proved to be the solely responsible for raffinose accumulation in leaves under water stress (Egert et al., 2013). Additionally, the role of *VIT_17s0000g08960* in drought stress response was also supported by the *in silico* analysis of its promoter, which identified several ABA-responsive elements (ABRE) and dehydration-

responsive element binding (DREB) motifs (ACGTG, RYACGTGGYR, YACGTGGC, ACGTGKC, ACCGAC) (Iwasaki et al., 1995; Kang et al., 2002; Dubouzet et al., 2003; Narusaka et al., 2003; Simpson et al., 2003).

Four rootstock genotypes, RGM, 101.14Mgt, SO4 and 110R, which had different variations in the associated SNP chr17_10497222_C_T, were selected to deeply investigated their physiological responses under drought. Differences were observed both among studied genotypes and the two experimental settings applied. As soil water content decreased, SO4 vines exhibited the highest water use efficiency (WUE) in pot stress experiment. However, the drought tolerance degree of this genotype varies greatly depending on experiment conditions. Carbonneau (1985) described SO4 vines, which were subjected to water deficit in small containers, as high tolerant, whereas some water stress experiment studies in vineyards considered them drought-sensitive (Southey, 1992; Dry et al. 2007). Interestingly, Tramontini et al. (2013) reported that different grapevine genotypes grafted on SO4, grown under water-limiting condition in small pot, preserved the soil water in a more efficient way compared with the same varieties grafted on high tolerant rootstock, 140 Ruggeri. In accordance with literature (Carbonneau, 1985) the rootstock genotype that exhibited the lowest drought tolerance in our pot experiment was RGM. It showed an early reduction of transpiration rate, a strongly impairment of photosynthetic efficiency and extensive physical damage. On the other hand, the higher drought tolerance of 110R (Flexas et al., 2009) might not have been completely expressed, because the limited volume of pots does not allow of exploiting its capability to develop extensive root system. Stomatal regulation results are substantially agree with other studies, which report that as drought stress becomes more severe transpiration level is drastically reduced in all the grapevine genotypes, including near-anisohydric varieties, and significant differences in stomatal conductance among them are not detected (Lovisolo et al., 2010; Lavoie-Lamoureux el al., 2017; Charrier et al., 2018). Regarding the unexpected strong decrease of transpiration in well-watered plants on the last day of measurements, possible signals between neighboring plants exposed to abiotic stresses with chemical warnings exchange have been already hypothesized in other studies (Vickers et al., 2009), although the underlying mechanisms are unknown.

The application of osmotic stress in a hydroponic system has proved to be a useful method to evaluate the short-term stress response of different grapevine genotypes in strictly controlled condition as reported by Tattersall et al. (2007) and Su et al. (2015). Particularly, it was possible to classify the genotypes according to their stomatal sensitivity with great precision and reproducibility. Therefore, it should be applied for a rapid screening of genotypes with

different stomatal responses under drought, but on the other hand this method is not completely appropriate to evaluate the actual tolerance to water stress, in fact 101.14Mgt, generally considered to be drought-sensitive under field and pot conditions (Carbonnneau, 1985; Alsina et al., 2011), showed the highest survival rate keeping stomata open.

In the GWAS experiment described in the previous chapter, SNP chr17_10497222_C_T resulted significantly associated with stomatal closure in drought stress conditions with an overdominance effect, in fact heterozygous (CT) genotypes showed lower stomatal conductance in comparison with homozygous genotypes (CC or TT). In this respect, commercial rootstocks that are representative of the three phenotypic classes were deeply characterized. SO4 was the only heterozygous one and its performances were compared with other individuals. Therefore, it has proved to be the genotype more able to preserve soil moisture in the pot experiment and at the same time showed the quicker stomal closure, together with 110R, in the hydroponic experiment. Additionally, Tramontini et al. (2013) observed that scions grafted on SO4 showed an improved WUE in comparison with same varieties grafted on 140 Ruggeri. Although, testing other rootstock varieties carrying the same SNP would definitely enable to evaluate its overall effect, particularly on different genetic backgrounds.

Finally, a functional study of *VIT_17s0000908960* has been planned in the same four well-characterized rootstock varieties. According to Bouquet et al. (1982) and Perrin et al. (2004) a satisfactory rate of callogenesis was obtained from isolated anthers of all the varieties. Gene transfer experiments aimed to test *VIT_17s0000908960* function were performed and plants regenerated from transgenic calli must be verified to ensure the transgene integration.

Conclusions

This study proposes *VIT_17s0000g08960* as candidate gene for drought stress tolerance in grapevine. Its coding sequence has shown to be not so conserved among Vitis species, in addition some non-synonymous mutations were identified and further studies are required to assess their functional effects. Furthermore, phylogenetic analysis of VIT_17s0000g08960 protein sequence and examination of promoter's cis-acting regulatory elements have proved its role in water stress response. The characterization of four rootstock genotypes with different phenotyping approaches helped to dissect the complex physiological regulation of drought response, in particular stomatal closure. Further studies will be required to validate SNP chr17_10497222_C_T as molecular marker associated with stomatal control, thereby it will be implemented into breeding strategies. Indeed, the selection of vines that promptly limit their transpiration in response to water deficit represents an opportunity to increase WUE. Moreover, further insights of *VIT_17s0000g08960* function are expected from ongoing gene expression and genetic transformation studies.

CHAPTER 4

Physiological characterization of *V. vinifera* subsp. s*ylvestris* genotypes under drought stress

Introduction

The wild grapevine, *V. vinifera* subsp *sylvestris,* is considered as the ancestor of the cultivated grapevine (*Vitis vinifera* L.) and represents the only endemic species of the *Vitaceae* in Europe (Arroyo-García et al., 2006). These wild relatives of modern grape cultivars are widely spread in several European countries (Heywood & Zohary, 1991) and some germplasm collections were created for their preservation and characterization (Maul et al., 2012). Currently, scientists show an increasing interest in *V. sylvestris* genotypes, owing to their valuable genetic resources of natural stress tolerance, including abiotic (Askri et al., 2012; Cambrollé et al., 2014; Cambrollé et al., 2015) and biotic (Duan et al., 2016; Guan et al., 2016) resilience factors. Therefore, strategies for sustainable agriculture might consider the re-introduction of these traits in cultivated grapevines. Furthermore, ongoing challenges in viticulture, caused by climate change and by limitations of pesticide use, have made root system as a major target to improve grapevine productivity. Undeniable benefits for the scions, such as better compatibility and higher yield, could be imparted by innovative rootstocks obtained from the wild grapevines. Indeed, compatibility between rootstock and scion is the primary factor that prevents the failing of grafting (Aloni et al. 2010). Additionally, some studies investigated the susceptibility of the different accessions of *V. sylvestris* to phylloxera from this perspective (Ocete et al., 2011; Campus et al., 2014).

This is a pilot study to evaluate the feasibility of exploiting *V. sylvestris* genotypes for some drought tolerance related traits and eventually characterize them in larger scale study.

Materials and methods

Plant materials and water stress treatments conditions

The experiment was conducted on one-year-old potted (9 L) rooted cuttings of eight Vitis *sylvestris* accessions, which were selected from the germplasm collection of Edmund Mach Foundation (San Michele all'Adige, Italy), in a semi-sealed greenhouse. Nine or twelve vines for each rootstock genotype were subjected to water stress by completely suspending irrigation for 21 days. The growing medium was composed of a sand-peat mixture (1:1 in volume) with a field capacity of 35% [(vol water/vol soil) × 100]. The volumetric soil moisture content per pot was monitored with a ML3 ThetaProbe Soil Moisture Sensor (Delta-T Devices, London, UK). The pot surface was covered with a plastic film to avoid soil water evaporation. The experimental plan was completely randomized.

Physiological measurements

Physiological measurements were carried out on two healthy adult primary leaves grown between the 6th and the 10th node of the primary shoots. One fully expanded leaf of each water stressed vine was collected and immediately frozen in liquid nitrogen and stored at –80 C° to be used in gene expression experiments. Chlorophyll fluorescence parameters: maximum quantum efficiency of PSII photochemistry (Fv/Fm) and Performance Index (PI) were measured using fluorimeter Handy PEA (Hansatech, Kings Lynn, UK). PI parameter (Strasser et al., 2000) was according to the equation:

$$\mathrm{PI}_{\mathrm{abs}} = \frac{1-(F_0/F_{\mathrm{M}})}{\mathrm{M}_0/\mathrm{V}_{\mathrm{J}}} \times \frac{F_{\mathrm{M}}-F_0}{F_0} \times \frac{1-\mathrm{V}_{\mathrm{J}}}{\mathrm{V}_{\mathrm{J}}}$$

F_o= fluorescence intensity at 50 μs; F_J= fluorescence intensity at 2 ms; F_M= maximal fluorescence intensity; V= relative variable fluorescence at 2 ms calculated as $V_J = (F_J - F_o)/(F_M - F_o)$; M_o= initial slope of fluorescence kinetics, calculated as $M_o = 4^* (F300\ \mu_s - Fo)/(F_M - F_o)$. Dark adaption was achieved by covering the sample area with a leafclip for at least 15 minutes. Leaf chlorophyll content was measured with a Chlorophyll Meter SPAD-502 (Konica Minolta Sensing Inc,, Osaka, Japan). Stomatal conductance (g_s) was measured with a portable porometer (SC-1 Leaf porometer, Decagon Devices, Pullman, WA, USA).

Statistical analyses

All statistical analyses were performed using R packages 'stats', 'agricolae' and 'companion' v3.5.1 (R Core Team, 2013). For mean comparisons, several tests were used depending on homoscedasticity pre-tests. Parametric Student's *t*-test (one parameter) or one-way ANOVA were performed to data displaying a normal distribution and equal variance between treatments. Otherwise, non-parametric Mann-Whitney U test (one parameter) and one-way Kruskal–Wallis were performed. For classification tests, a comparison of least-square means at a 0,05 significance level and a Fisher's Least Significant Difference (LSD) or Dunn's tests were performed.

Results

Water stress experiment on potted V. *sylvestris* genotypes

Eight V. *sylvestris* genotypes were subjected to stress by withholding water for three weeks aiming to determine the level of drought tolerance in grapevine wild species (Figure 1). Volumetric soil water content (SWC) was determined throughout the experiment to monitor the stress evolution (Figure 2 and Table 1). After three and seven days from the beginning of the experiment, three accessions (F, G, H) showed a significant decrease of SWC in comparison with the other genotypes. However, this difference disappeared as water stress became more severe. At the end of the experiment some genotypes exhibited a great tolerance to the stress imposed and their leaves remained almost green and turgid, whereas other vines showed a widely leaf senescence and additional drought damages (Figure 3).

Figure 1: One of the Vitis *sylvestris* accession that was subjected to water stress

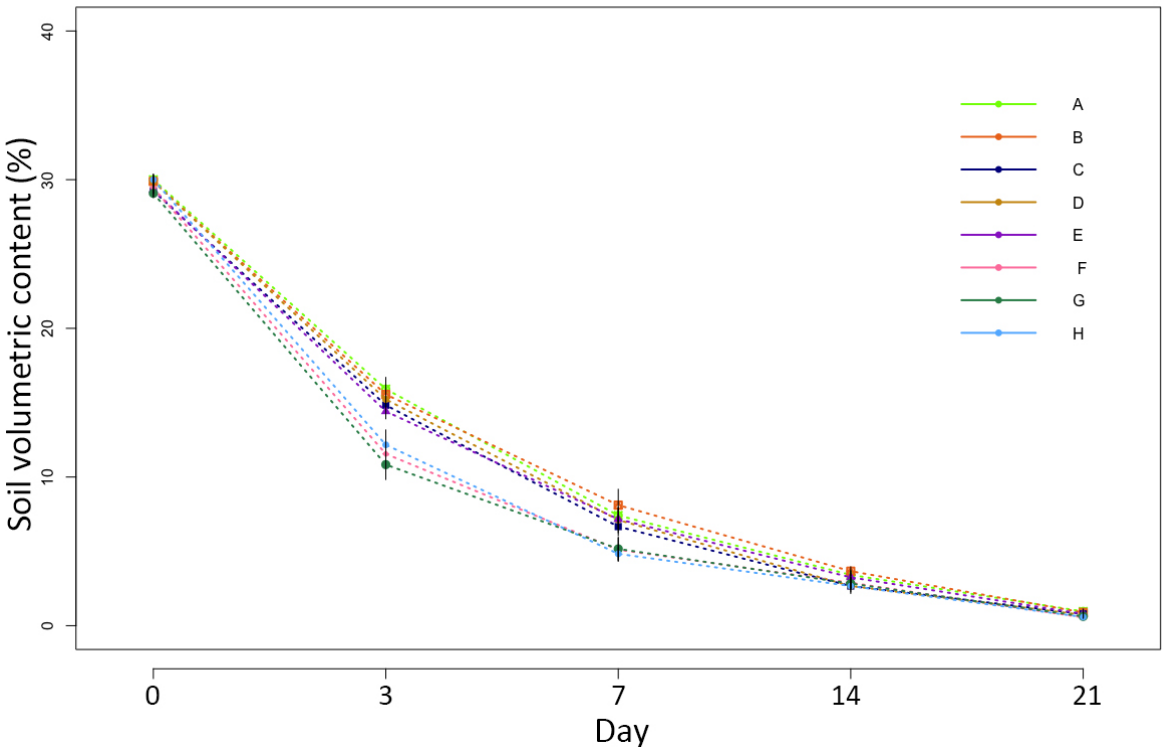

Figure 2: Volumetric soil water content throughout the progression of the drought stress experiment. Values represent average measurements ± SE of twelve replicates (A, C, E, G, H) and nine replicates (B, D, F).

Table 1: Volumetric soil water content of V. *Sylvestris* plants during drought stress experiment. Data were analysed using one-way ANOVA with LSD *post-hoc* test, and letters indicate significant differences between genotypes on the same day at $p < 0.05$.

Day	Genotype	SWC (%)	
		Mean	SD
1	A	30,0	1,3
	B	29,9	1,3
	C	29,3	1,3
	D	29,9	1,1
	E	29,3	0,9
	F	29,4	1,1
	G	29,1	1,0
	H	30,0	1,2
3	A	15,9 a	2,0
	B	15,6 a	3,4
	C	14,8 a	2,0
	D	15,2 a	2,5
	E	14,4 ab	1,7

	F	11,6 c	3,5
	G	10,8 c	3,5
	H	12,2 bc	3,5
7	A	7,4 a	1,6
	B	8,1 a	3,2
	C	6,7 abc	2,1
	D	7,1 ab	2,6
	E	7,2 a	2,0
	F	5,1 bc	2,3
	G	5,2 bc	2,8
	H	4,8 c	1,4
14	A	3,4	1,1
	B	3,7	0,9
	C	2,7	1,4
	D	2,7	1,5
	E	3,3	1,4
	F	2,9	1,1
	G	2,8	1,3
	H	2,7	0,7
21	A	1,0	0,5
	B	0,9	0,5
	C	0,8	0,3
	D	0,7	0,3
	E	0,8	0,5
	F	0,6	0,2
	G	0,6	0,2
	H	0,6	0,2

Figure 3: V. *sylvestris* accession “E” and “G” at the end of the experiment.

Physiological effects of drought stress

Stomatal conductance was measured during the progress of drought experiment. Differences among genotypes were observed after 3 days, when accession H exhibited the highest stomatal conductance values (Figure 4). Conversely, accession E had higher transpiration than other genotypes at day 7 and 14. No difference in stomatal conductance was observed between plants when the soil content was close to 0 after three weeks without irrigation.

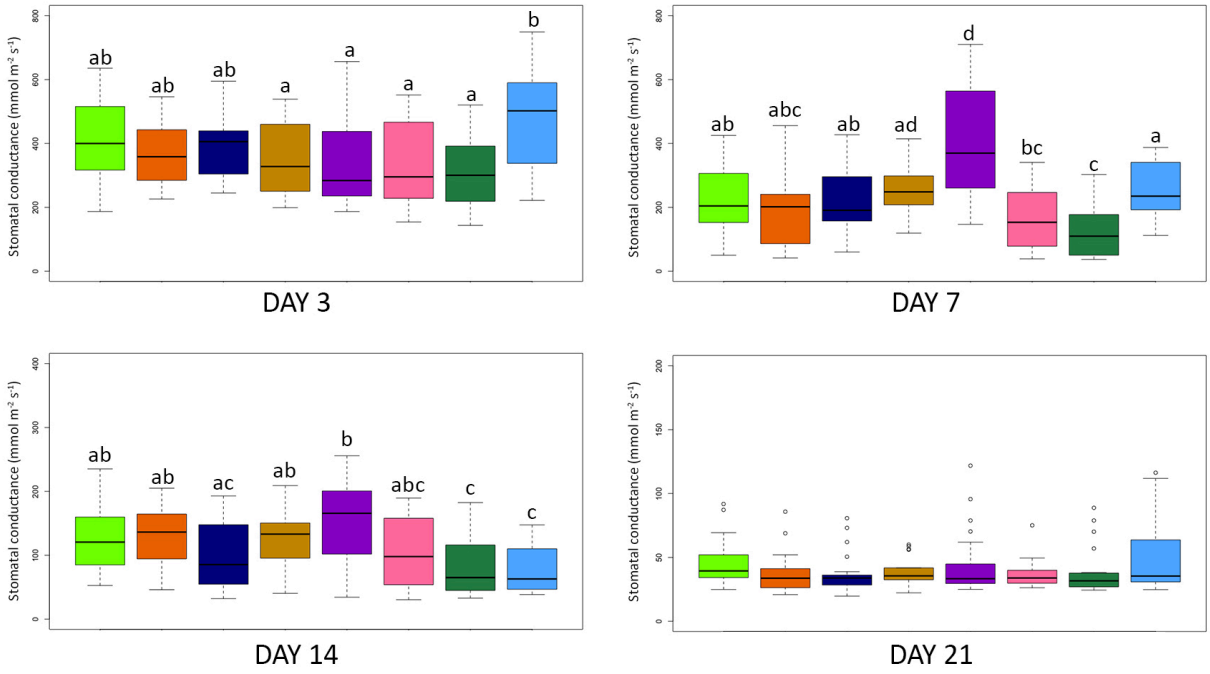

Figure 4: Boxplots of stomatal conductance measured on 8 different V. *Sylvestris* genotypes, A (green), B (orange), C (blue), D (gold), E (violet), F (pink), G (dark green) and H (light blue), 3, 7, 14 and 21 days after withdrawal of irrigation (A, C, E, G, H n=24; B, D, F n= 18) . Significant differences between genotypes on the same day were tested with with Kruskal Wallis Test, and letters indicate significantly different values at $p \leq 0.05$ according to Dunn’s test.

Regarding the photosynthetic efficiency, both the maximum efficiency of PSII photochemistry (Fv/Fm) than PI_{ABS}, which represents the overall PSII photochemical performance, varied a lot among studied V. *sylvestris* accessions throughout the various phases of stress (Figure 5 and Figure 6). In particular, differences in Fv/Fm values between genotypes were statistically significant at at all time points measured (Table 2), whereas PI_{ABS} values at day 14 did not reveal any differences (Table 3). Accessions A and E showed the greatest drought tolerance level with higher values in both parameters, compared to other plants, after three weeks without irrigation. On the other hand accession G resulted the less tolerant genotype and a showed a strongly drop of photochemical performances.

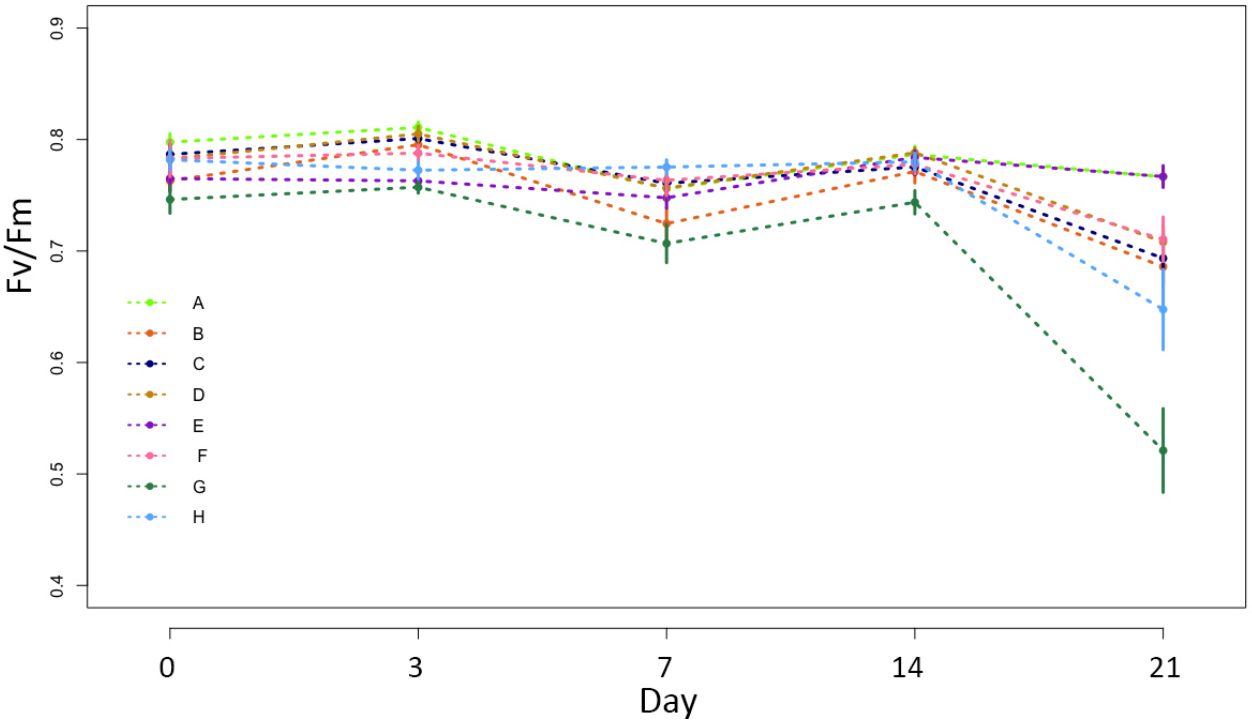

Figure 5: Maximum quantum yield of PSII (Fv/Fm) of V. *Sylvestris* genotypes throughout the experiment. Values represent average measurements± SE, n = 24 (A, C, E, G, H) and n=18 (B, D, F).

Chlorophyll content was highly differentiated among these wild grapevine, but this parameter did not vary much in response to stress (Figure 7). Leaves of accession H had the greatest amount of chlorophyll, while the lowest SPAD values were measured in leaves of accession G (Table 4).

Table 2: Maximum quantum yield of PSII (Fv/Fm) measured on V. *Sylvestris* genotypes throughout the experiment (A, C, E, G, H n=24; B, D, F n= 18). Significant differences between genotypes on the same day were tested with with Kruskal Wallis Test, and letters indicate significantly different values at $p \leq 0.05$ according to Dunn's test.

Day	Genotype	Fv/Fm	
		Mean	SD
1	A	0,798 a	0,037
	B	0,763 bc	0,040
	C	0,787 ab	0,045
	D	0,783 abc	0,039
	E	0,765 bc	0,055
	F	0,783 ab	0,069
	G	0,746 c	0,060
	H	0,782 ab	0,044
3	A	0,811 a	0,023
	B	0,795 abc	0,019
	C	0,801 ab	0,017
	D	0,805 ab	0,029
	E	0,763 d	0,032
	F	0,788 bc	0,032
	G	0,757 d	0,026
	H	0,772 cd	0,037
7	A	0,756 ab	0,063
	B	0,725 ac	0,062
	C	0,761 abc	0,031
	D	0,756 abc	0,045
	E	0,748 abc	0,046
	F	0,763 b	0,058
	G	0,707 c	0,084
	H	0,775 b	0,032
	A	0,787 a	0,036
	B	0,771 ab	0,044
	C	0,775 ab	0,035

14	D	0,788 a	0,019
	E	0,784 a	0,029
	F	0,778 ab	0,037
	G	0,744 b	0,051
	H	0,780 a	0,038
21	A	0,767 a	0,040
	B	0,686 b	0,146
	C	0,693 b	0,121
	D	0,708 b	0,062
	E	0,767 a	0,048
	F	0,710 b	0,085
	G	0,521 c	0,184
	H	0,648 b	0,177

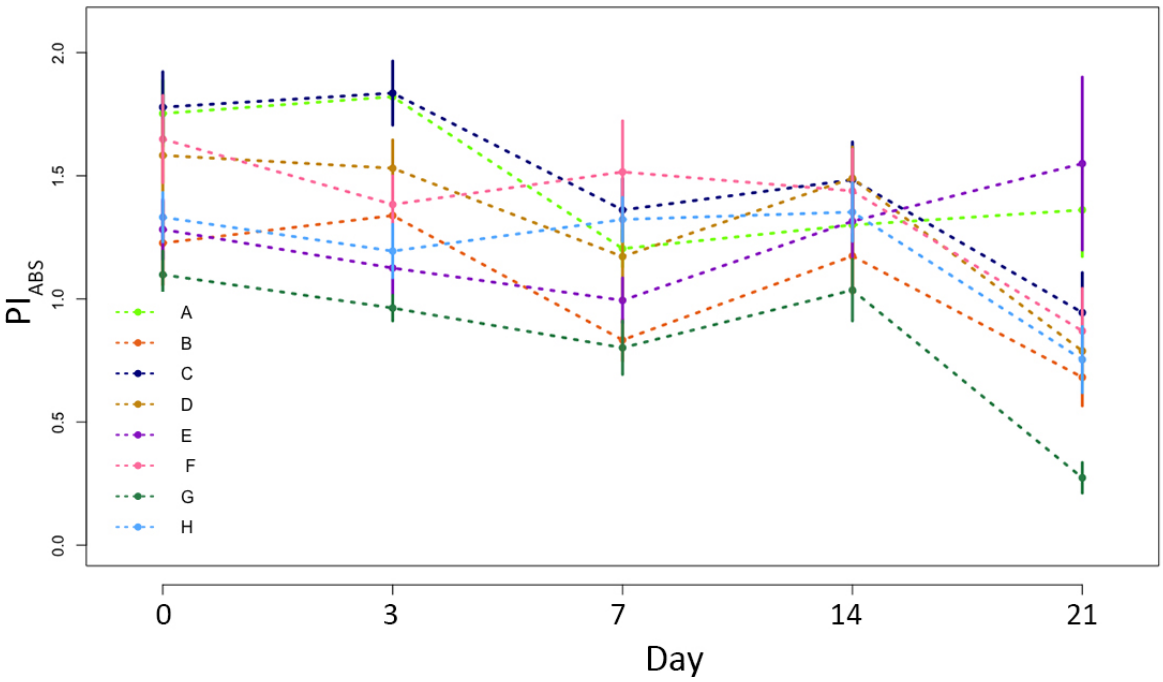

Figure 6: Relative values of the photosynthetic performance index (PI_{ABS}) of V. *Sylvestris* genotypes throughout the experiment. Values represent average measurements± SE, n = 24 (A, C, E, G, H) and n=18 (B, D, F).

Table 3: Relative values of the photosynthetic performance index (PI_{ABS}) measured on V. *Sylvestris* genotypes throughout the experiment (A, C, E, G, H n=24; B, D, F n= 18). Significant differences between genotypes on the same day were tested with with Kruskal Wallis Test, and letters indicate significantly different values at $p \leq 0.05$ according to Dunn's test.

Day	Genotype	Performance Index	
		Mean	SD
1	A	1,753 a	0,656
	B	1,226 bc	0,720
	C	1,778 a	0,705
	D	1,582 abc	0,612
	E	1,282 abc	0,595
	F	1,649 ab	0,750
	G	1,098 c	0,470
	H	1,331 abc	0,492
3	A	1,821 a	0,500
	B	1,338 bc	0,394
	C	1,835 ad	0,637
	D	1,53 abd	0,488
	E	1,125 ce	0,557
	F	1,383 bcd	0,493
	G	0,963 e	0,256
	H	1,194 bce	0,533
7	A	1,204 a	0,368
	B	0,833 b	0,361
	C	1,361 a	0,622
	D	1,171 ab	0,773
	E	0,994 ab	0,447
	F	1,515 a	0,880
	G	0,801 b	0,531
	H	1,322 a	0,438
	A	1,3	0,556
	B	1,174	0,656
	C	1,483	0,752

14	D	1,49	0,537
	E	1,315	0,707
	F	1,438	0,718
	G	1,035	0,612
	H	1,352	0,580
21	A	1,361 a	0,927
	B	0,681 b	0,492
	C	0,944 ab	0,795
	D	0,788 ab	0,553
	E	1,549 a	1,720
	F	0,869 ab	0,735
	G	0,273 c	0,304
	H	0,753 b	0,660

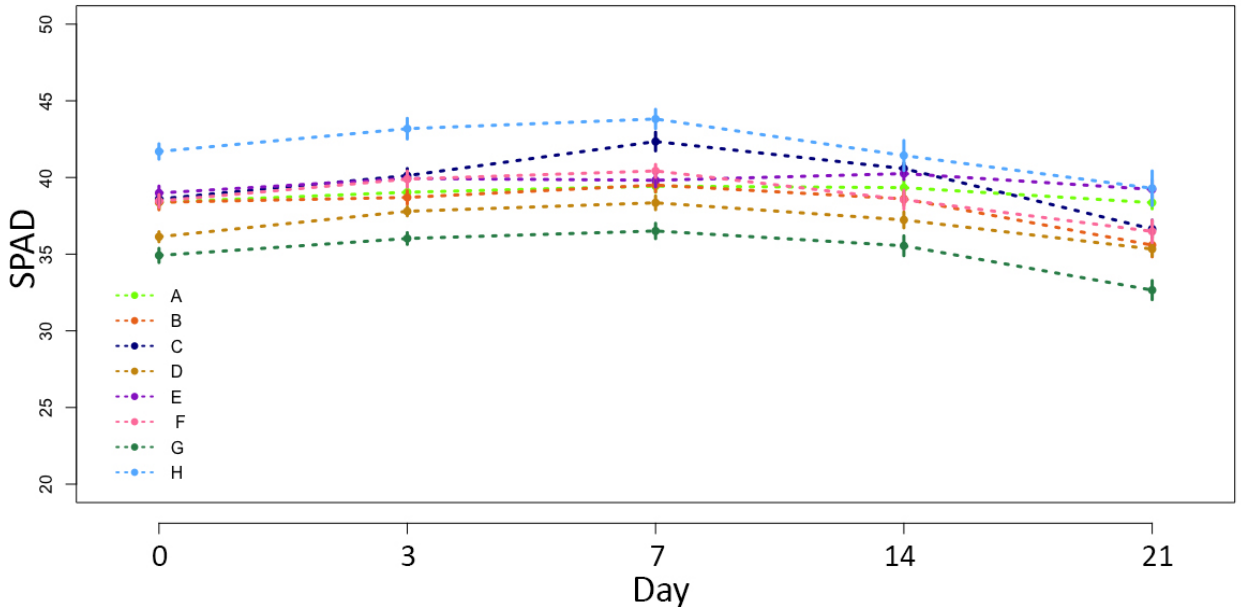

Figure 7: Chlorophyll content (SPAD index) of V. *Sylvestris* genotypes throughout the experiment. Values represent average measurements± SE, n = 24 (A, C, E, G, H) and n=18 (B, D, F).

Table 4: Chlorophyll content (SPAD index) measured on V. *Sylvestris* genotypes throughout the experiment (A, C, E, G, H n=24; B, D, F n= 18). Significant differences between genotypes on the same day were tested with with Kruskal Wallis Test, and letters indicate significantly different values at $p \leq 0.05$ according to Dunn's test.

Day	Genotype	SPAD	
		Mean	SD
1	A	38,4 a	1,9
	B	38,4 a	2,1
	C	38,6 a	1,6
	D	36,1 b	1,4
	E	39 a	2,2
	F	38,5 a	1,8
	G	34,9 b	2,3
	H	41,7 c	2,4
3	A	39,0 ab	2,0
	B	38,7 ab	1,8
	C	40,1 a	2,2
	D	37,8 bc	1,3
	E	39,9 a	2,0
	F	39,9 a	2,2
	G	36 c	1,9
	H	43,2 d	3,3
7	A	39,4 ab	2,0
	B	39,5 ab	1,9
	C	42,3 cd	3,0
	D	38,4 ae	1,9
	E	39,8 ab	2,1
	F	40,4 bc	1,8
	G	36,5 e	2,4
	H	43,8 d	3,1
	A	39,3 ab	2,4
	B	38,6 ac	1,7
	C	40,6 b	1,8

14	D	37,2 cd	2,2
	E	40,3 ab	1,9
	F	38,6 ac	2,6
	G	35,5 d	3,2
	H	41,4 b	4,8
21	A	38,4 ab	2,0
	B	35,6 c	3,2
	C	36,6 ac	2,8
	D	35,3 cd	1,9
	E	39,2 b	3,0
	F	36,5 ac	3,1
	G	32,7 d	3,0
	H	39,3 b	5,5

Discussion

The domestication process of the cultivated *V. vinifera* subsp. *sativa* from the wild ancestor *V. vinifera* subsp. *sylvestris*, which was focused on trait selection for yield improvement and rapid growth, caused an unintentional loss of resilience factors. Therefore, the genetic diversity of natural wild grapevine populations could be exploited for identify genetic factors that confer tolerance to drought stress. Especially since, they have to survive in adverse environmental conditions of their natural habitats without relying on human intervention. Moreover, a recent study (Marrano et al., 2018) identified genomic regions, corresponding to genes involved in abiotic stresses response, with divergent allele frequencies between wild grapevines and cultivated varieties. However, insufficient information on performances of *V. sylvestris* under water stress conditions restricts their use for resilience breeding strategies.

This preliminary investigation on eight different *V. sylvestris* genotypes has proved that there are variations in the physiological responses to drought among wild grapes. Besides a different stomatal regulation during the various phases of stress, some wild grape varieties exhibited a photosynthetic activity substantially unaffected despite the severity of the stress imposed. Genotypic variations of chlorophyll fluorescence, including of the maximum quantum efficiency of PSII photochemistry, provide a valid assessment of plant tolerance to severe water stress (Baker & Rosenqvist, 2004). The more tolerant *V. sylvestris* varieties showed Fv/Fm values, after an extreme water deficit (3 weeks without irrigation), similar to those that are measured in different Vitis genotypes subjected to a less intense drought stress (Guan et al., 2004). Hence, the evaluation of the photosynthetic performance and stomatal conductance of other *V. sylvestris* genotypes under stress conditions, could provide valuable information by which to rank them according to their tolerance and consequently identifying genetic determinism for drought stress tolerance. Furthermore, the most promising individuals might be implemented into breeding strategies targeted to next-generation rootstocks production, which should combine resilience to abiotic stresses (drought, salinity) (Askri et al., 2012), phylloxera tolerance (including new strains that are able to break the resistance of American Vitis species) (Walker et al., 2014) and improved compatibility with the scions (Pina & Errea P, 2005).

Conclusions

This preliminary screening of drought tolerance in *V. vinifera* subsp. *sativa* demonstrated that some water stress resilience factors might be identified in these plants. However, additional studies are needed to evaluate their physiological responses to drought stress in comparison with well watered plants or with other Vitis species subjected to the same treatment.

General conclusion

Grapevine water use efficiency is becoming a key issue for sustainable viticulture to deal with climate change. The present research has provided valuable information on water stress response in several Vitis genotypes, and has identified alternative approaches to investigate this matter.

Regarding the assessment of water deficit effects, different phenotyping methods were employed, among which thermal infrared imaging and osmotic stress treatment in hydroponics. As result, physiological responses were divergent depending on stress imposition system. Infrared thermography has proved to be appropriate to evaluate the drought responses of large population while Polyethylene glycol (PEG)-induced osmotic stress in hydroponics was useful for a rapid screening of the short-term stress response, especially stomatal regulation.

GWAS revealed limits that should be taken into account, such us as the low number of SNPs evaluated and the rapid decay of linkage disequilibrium (LD), but on the other hand some associations were found despite the complexity of the trait, characterized by polygenic inheritance and strongly influenced by environmental conditions. Based on marker-trait association results, a list of candidate genes was assembled which deserve further characterization. Especially, the characterization of a raffinose synthase genes under drought showed encouraging results, even if no causative mutation was found. Therefore, further studies are required to assess its functional effect, including gene expression analysis and investigations on interactions between rootstock and scion.

The evaluation of water stress response with different phenotyping approaches on four rootstock genotypes, differentially classified based on adaptation to drought, helped to reveal this complex physiological regulation, in particular stomatal closure. Commercial rootstocks SO4 notably showed an interesting response to water deficit, which is consistent with observations detected in GWAS for genotypes heterozygous for the SNP (chr17_10497222_C_T). Although, future studies on different rootstock/scion combination are necessary to clarify their role in stomatal regulation during drought stress.

Taken together, these results represent a step forward in the dissection of grape rootstocks mechanisms of drought tolerance. In addition, a preliminary screening of drought stress tolerance in *V. sylvestris* genotypes has proven that some resilience factors from the ancestors

of cultivated grapevines could be identified and exploited in view of a sustainable agriculture.

References

1001 Genomes Consortium (2016) 1,135 Genomes Reveal the Global Pattern of Polymorphism in Arabidopsis thaliana. *Cell* 166, 481-91

Ache P, Bauer H, Kollist H, Al-Rasheid KA, Lautner S, Hartung W, Hedrich R (2010) Stomatal action directly feeds back on leaf turgor: new insights into the regulation of the plant water status from non-invasive pressure probe measurements. *Plant J* 62, 1072–1082

Aloni B, Cohen R, Karni L, Aktas H, Edelstein M (2010) Hormonal signalling in rootstock-scion interaction. Sci Hortic 127, 119– 126

Alsina MM, Smart DR, Bauerle T, de Herralde F, Biel B, Stockert C, Negron C, Save R (2011) Seasonal changes of whole root system conductance by a drought-tolerant grape root system. *J Exp Bot* 62, 99–109

Aranzana MJ, Kim S, Zhao K, Bakker E, Horton M, Jakob K, Lister C, Molitor J, Shindo C, Tang C, Toomajian C, Traw B, Zheng H, Bergelson J, Dean C, Marjoram P, Nordborg M (2005) Genome-wide association mapping in Arabidopsis identifies previously known flowering time and pathogen resistance genes. PLoS Genet 1(5), e60

Arroyo-García R, Ruiz-García L, Bolling L, Ocete R, López MA, Arnold C, Ergul A, Söylemezoğlu G, Uzun HI, Cabello F, Ibáñez J, Aradhya MK, Atanassov A, Atanassov I, Balint S, Cenis JL, Costantini L, Gorislavets S, Grando MS, Klein BY, McGovern PE, Merdinoglu D, Pejic I, Pelsy F, Primikirios N, Risovannaya V, Roubelakis- Angelakis KA, Snoussi H, Sotiri P, Tamhankar S, This P, Troshin L, Malpica JM, Lefort F, Martinez-Zapater JM (2006) Multiple origins of cultivated grapevine (*Vitis vinifera* ssp. *sativa*) based on chloroplast DNA polymorphisms. *Mol Ecol* 15, 3707–3714

Askri H, Daldoul S, Ben Ammar A, Rejeb S, Jardak R, Rejeb MN, Mliki A, Ghorbel A (2012) Short term response of wild grapevines (*Vitis vinifera* ssp. *sylvestris*) to NaCl salinity exposure: changes of some physiological and molecular characteristics. *Acta Physiol Plant* 34, 957–968

Bach TJ (1995) Some new aspects of isoprenoid biosynthesis in plants - A review. *Lipids* 30, 191–202

Bachmann M, Keller F (1995) Metabolism of the raffinose family oligosaccharides in leaves of Ajuga reptans L. Inter and intracellular compartmentation. *Plant Physiol* 109, 991–998

Baker NR, Rosenqvist E (2004) Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *J Exp Bot* 55, 1607–1621

Barceló AR, Pomar F, Lopez-Serano M, Pedreno MA (2003) Peroxidase: a multifunctional enzyme in grapevines. *Funct Plant Biol* 30, 557–591

Barrios-Masias FH, Knipfer T, McElrone AJ (2015) Differential responses of grapevine rootstocks to water stress are associated with adjustments in fine root hydraulic physiology and suberization. *J Exp Bot* 66, 6069-6078

Bartels D, Sunkar R (2005) Drought and Salt Tolerance in Plants. *Crit Rev Plant Sci* 24, 23-58

Bartlett MK , Klein T, Jansen S, Choat B, Sack L. (2016) The correlations and sequence of plant stomatal, hydraulic, and wilting responses to drought. *Proc Natl Acad Sci USA* 113, 13098–13103

Battilana J, Costantini L, Emanuelli F, Sevini F, Segala C, Moser S, Velasco R, Versini G, Grando MS (2009) The 1-deoxy-D: -xylulose 5-phosphate synthase gene co-localizes with a major QTL affecting monoterpene content in grapevine. Theor Appl Genet 118, 653-69

Bauerle TL, Smart DR, Bauerle WL, Stockert C Eissenstat, DM (2008) Root foraging in response to heterogeneous soil moisture in two grapevines that differ in potential growth rate. *New Phytol* 179, 857-866

Bazakos C, Hanemian M, Trontin C, Jimenez-Gomez JM, Loudet O (2017) New strategies and tools in quantitative genetics: how to go from the phenotype to the genotype. *Annu Rev Plant Biol* 68, 435-455

Bechtold U, Field B (2018) Molecular mechanisms controlling plant growth during abiotic stress. *J Exp Bot* 69, 2753–2758

Bellvert J, Zarco-Tejada PJ, Girona J, Fereres E (2014) Mapping crop water stress index in a 'Pinot-noir' vine- yard: comparing ground measurements with thermal remote sensing imagery from an unmanned aerial vehicle. *Precis agric* 15, 361–376

Bengough AG, Bransby MF, Hans J, McKenna SJ, Roberts TJ, Valentine TA (2006) Root responses to soil physical conditions; growth dynamics from field to cell. *J Exp Bot* 57, 437–447

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc 57, 289–300

Benjamini Y, Yekutieli D (2005) Quantitative trait loci analysis using the false discovery rate. *Genetics* 171, 783–790

Berdeja M, Nicolas P, Kappel C, Dai ZW, Hilbert G, Peccoux A, Lafontaine M, Ollat N, Gomès E, Delrot S. Water limitation and rootstock genotype interact to alter grape berry metabolism through transcriptome reprogramming. *Hortic Res* 2, 15012

Bianchi D, Grossi D, Tincani DTG, 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* 15, 333-340

Black CA (1965) Methods of Soil Analysis: Part I, Physical and Mineralogical Properties. American Society of Agronomy, Madison, Wisconsin.

Blum A (1996) Crop responses to drought and the interpretation of adaptation. *Plant Growth Regul* 20, 35–148

Blum A, Mayer J, Gozlan G (1982) Infrared thermal sensing of plant canopies as a screening technique for dehydration avoidance in wheat. *Field Crops Res* 5, 137-146

Bohnert HJ, Nelson DE, Jensen RG (1995) Adaptations to Environmental Stresses. *Plant Cell* 7, 1099–111

Boso S, Alonso-Villaverde V, Gago P, Santiago JL, Martínez MC (2014) Susceptibility to downy mildew (Plasmopara viticola) of different Vitis varieties. *Crop Prot* 63, 26–35

Bowler C, Fluhr R (2000) The role of calcium and activated oxygens as signals for controlling cross-tolerance. *Trends Plant Sci* 5, 241-246

Boyer JS, Wong SC, Farquhar GD (1997) CO_2 and water vapor exchange across leaf cuticle (epidermis) at various water potentials. *Plant Physiol* 114, 185–91

Boyer, JS, Byrne P, Cassman KG, Cooper M, Delmer D, Greene T, Gruis F, Habben J, Hausmann N, Kenny N, Lafitte R, Paszkiewicz S, Porter D, Schlegel A, Schussler J, Setter T, Shanahan J, Sharp RE, Vyn TJ, Warner D, Gaffney J (2013) The U.S. drought of 2012 in perspective: a call to action. *Glob Food Secur* 2, 139–143

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* 19, 2633–2635

Buckley TN (2005) The control of stomata by water balance. *New Phytol* 168, 275–292

Cambrollé J, Garcìa JL, Figueroa ME, Cantos M (2014) Physiological responses to soil lime in wild grapevine (*Vitis vinifera* ssp. *sylvestris*). *Environ Exp Bot* 105, 25–31

Cambrollé J, Garcìa JL, Figueroa ME, Cantos M (2015) Evaluating wild grapevine tolerance to copper toxicity. *Chemosphere* 120, 171-178

Campbell GS (1985) Instruments for measuring plant water potential and its components. *In Instrumentation for Environmental Physiology* (Marshall & Woodward), Cambridge University Press, Cambridge. pp. 193-214

Campoy JA, Lerigoleur-Balsemin E, Christmann H, Beauvieux R, Girollet N, Quero-García J, Dirlewanger E, Barreneche T (2016) Genetic diversity, linkage disequilibrium, population structure and construction of a core collection of Prunus avium L. landraces and bred cultivars. *BMC Plant Biol* 16, 1

Campus D, Farci M, Pili G, Lovicu G (2014) Preliminary Investigations on the Tolerance of European Wild Grape (*Vitis vinifera* subsp. *sylvestris*) against Phylloxera (*Daktulosphaira vitifoliae* Fitch). *Acta Hortic* 1032, 203-205

Capell T, Bassie L, Christou P (2004) Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. *Proc Natl Acad Sci USA* 101, 9909–9914

Carbonneau A (1985) The early selection of grapevine rootstocks for resistance to drought conditions. *Am J Enol Vitic* 36, 195–198

Carvalho LC, Vidigal P, Amâncio S Oxidative stress homeostasis in grapevine (*Vitis vinifera L.*) *Front Environ Sci* 2015, 3:20

Castellarin SD, Gambetta GA, Wada H, Shackel KA, Matthews MA (2011) Fruit ripening in Vitis *vinifera*: spatiotemporal relationships among turgor, sugar accumulation, and anthocyanin biosynthesis. *J Exp Bot* 62, 4345–4354

Chalker-Scott L (1999) Environmental significance of anthocyanins in plant stress responses. *Photochem Photobiol* 70, 1–9

Charrier G, Delzon S, Domec JC, Zhang L, Delmas CEL, Merlin I, Corso D, King A, Ojeda H, Ollat N, Prieto JA, Scholach H, Skinner P, van Leeuwen C, Gambetta GA (2018) Drought will not leave your glass empty: Low risk of hydraulic failure revealed by long-term drought observations in world's top wine regions. *Sci Adv* 4, eaa06969

Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought — from genes to the whole plant. *Func Plant Biol* 30, 239-264

Chaves MM, Oliveira, MM (2004) Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. *J Exp Bot* 55, 2365–2384

Chaves MM, Zarrouk O, Francisco R, Costa JM, Santos T, Regalado AP, Rodrigues ML, Lopes CM (2010) Grapevine under deficit irrigation: hints from physiological and molecular data. *Ann Bot* 105, 661-676

Chen H, Jiang JG (2010) Osmotic adjustment and plant adaptation to environmental changes related to drought and salinity. *Environ Rev* 18, 309–319

Chen THH, Murata N (2002) Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. *Curr Opin Plant Biol* 5, 250–257

Cheng MC, Hsieh EJ, Chen JH, Che HY, Lin TP (2012) Arabidopsis RGLG2, functioning as a RING E3 ligase, interacts with AtERF53 and negatively regulates the plant drought stress response. *Plant Physiol* 158, 363–375

Chitarra W, Perrone I, Avanzato CG, Minio A, Boccacci P, Santini D, Gilardi G, Siciliano I, Gullino ML, Delledonne M, Mannini F, Gambino G (2017) Grapevine Grafting: Scion Transcript Profiling and Defense-Related Metabolites Induced by Rootstocks. *Front Plant Sci* 27, 8:654

Chitwood DH, Ranjan A, Martinez C, Headland L, Thiem T, Kumar R, Covington MF, Hatcher T, Naylor DT, Zimmerman S, Downs N, Raymundo N, Buckler ES, Maloof JN,

Aradhya M, Prins B, Li L, Myles S, Sinha N (2014) A modern ampelography: a genetic basis for leaf shape and venation patterning in grape. *Plant Physiol* 164, 259–272

Choi Y, Chan AP (2015) PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics* 31, 2745-2747

Cominelli E, Galbiati M, Vavasseur A, Conti L, Sala T, Vuylsteke M, Leonhardt N, Dellaporta SL, Tonelli C. (2005) A guard-cell-specific MYB transcription factor regulates stomatal movements and plant drought tolerance. *Curr Biol* 15, 1196–1200

Comstock J (2002) Hydraulic and chemical signalling in the control of stomatal conductance and transpiration. *J Exp Bo*t 53, 195–200

Conde A, Regalado A, Rodrigues D, Costa JM, Blumwald E, Chaves MM, Gerós H (2015) Polyols in grape berry: transport and metabolic adjustments as a physiological strategy for water-deficit stress tolerance in grapevine. *J Exp Bot* 66, 889–906

Conde A, Silva P, Agasse A, Conde C, Gerós H (2011) Mannitol transport and mannitol dehydrogenase activities are coordinated in Olea europaea under salt and osmotic stresses. *Plant Cell Physiol* 52, 1766–1775

Cookson SJ, Clemente-Moreno MJ, Hevin C, Mendome LZ, Delrot S, Trossat-Magnin C, Ollat N (2013) Graft union formation in grapevine induces transcriptional changes related to cell wall modification, wounding, hormone signalling, and secondary metabolism. *J Exp Bot* 64, 2997–3008

Corso M, Vannozzi A, Maza E, Vitulo N, Meggio F, Pitacco A, Telatin A, D'Angelo M, Feltrin E, Negri AS, 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 enhance tolerance. *J Exp Bot* 66, 5739–5752

Cortell JM, Halbleib M, Gallagher AV, Righetti TL, Kennedy JA. Influence of vine vigor on grape (*Vitis vinifera* L. cv. Pinot Noir) anthocyanins. Anthocyanin concentration and composition in fruit. *J Agric Food Chem* 55, 6575–6584

Costa JM, Grant OM, Chaves MM (2012) Thermography to explore plant– environment interactions. *J Exp Bot* 64, 3937-3949

Costantini L, Malacarne G, Lorenzi S, Troggio M, Mattivi F, Moser C, Grando MS (2015) New candidate genes for the fine regulation of the colour of grapes. J Exp Bot 66, 4427–4440

Coupel-Ledru A, Lebon É, Christophe A, Doligez A, Cabrera-Bosquet L, Péchier P, Hamard P, This P, Simonneau T (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. *J Exp Bot* 65, 6205–6218

Cramer GR, Urano K, Delrot S, Pezzotti M, Shinozaki K (2011) Effects of abiotic stress on plants: a systems biology perspective. *BMC Plant Biol* 11, 163

Dal Santo S, Palliotti A, Zenoni S, Tornielli GB, Fasoli M, Paci P, Tombesi S, Frioni T, Silvestroni O, Bellincontro A, d'Onofrio C, Matarese F, Gatti M, Poni S, Pezzotti M (2016) Distinct transcriptome responses to water limitation in isohydric and anisohydric grapevine cultivars. *BMC Genomics* 17, 815

Daldoul S, Amar AB, Gargouri M, Limam H, Mliki A, Wetzel T (2018) A Grapevine-Inducible Gene Vv-α-gal/SIP Confers Salt and Desiccation Tolerance in Escherichia coli and Tobacco at Germinative Stage. *Biochem Genet* 56, 78-92

Dalla Costa L, Pinto-Sintra AL, Campa M, Poletti V, Martinelli L, Malnoy M, (2014) Development of analytical tools for evaluating the effect of T-DNA chimeric integration on transgene expression in vegetatively propagated plants. *Plant Cell Tissue Organ Cul*t 118, 471–484

Daloso M, dos Anjos L, Fernie AR (2016) Roles of sucrose in guard cell regulation. *New Phytol* 211, 809-818

Das K, Roychoudhury R (2014) Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants *Front Plant Sci* 2, 53

De Block M, Verduyn C, De Brouwer D, Cornelissen M (2005) Poly(ADP-ribose) polymerase in plants affects energy homeostasis, cell death and stress tolerance. *Plant J* 41, 95–106

Deluc LG, Quilici DR, Decendit A, Grimplet J, Wheatley MD, Schlauch KA, Mérillon JM, Cushman JC, Cramer GR (2009) Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. *BMC Genomics* 10, 212

Diago MP, Fernández-Novales J, Gutiérrez S, Marañón M, T J (2018) Development and Validation of a New Methodology to Assess the Vineyard Water Status by On-the-Go Near Infrared Spectroscopy. *Front Plant Sci* 9, 59

Dittberner H, Korte A, Mettler-Altmann A, Weber APM, Monroe G, de Meaux J (2018) Natural variation in stomata size contributes to the local adaptation of water-use efficiency in Arabidopsis thaliana. *Mol Ecol* 27, 1-14

D1az-Vivancos P, Faize L, Nicolas E, Clemente-Moreno MJ, Bru-Martinez R, Burgos L, Hernández JA(2016) Transformation of plum plants with a cytosolic ascorbate peroxidase transgene leads to enhanced water stress tolerance. *Ann Bot* 117, 1121–1131

Dos Santos TB, Budzinski IG, Marur CJ, Petkowicz CL, Pereira LF, Vieira LG (2011) Expression of three galactinol synthase isoforms in *Coffea arabica* L. and accumulation of raffinose and stachyose in response to abiotic stresses. *Plant Physiol Biochem* 49, 441-448

Dos Santos TB, de Lima, RB, Nagashima GT, Petkowicz CL, Carpentieri-Pipolo V, Pereira LFP, Domingues DS, Vieira LGE (2015) Galactinol synthase transcriptional profile in two genotypes of Coffea canephora with contrasting tolerance to drought. *Genet Mol Biol* 38, 182-190

Dos Santos TP, Lopes CM, Rodrigues ML, de Souza CR, Maroco JP, Pereira JS, Silva JR, Chaves MM (2003) Partial rootzone drying: effects on growth and fruit quality of field grown grapevines. *Funct Plant Biol* 30, 663–671

Downie B, Gurusinghe S, Dahal P, Thacker RR, Snyder JC, Nonogaki H, Yim K, Fukanaga K, Alvarado V, Bradford KJ (2003) Expression of a Galactinol synthase gene in tomato seeds is up-regulated before maturation desiccation and again after imbibition whenever radicle protrusion is prevented. *Plant Physiol* 131, 1347–59

Dry N (2007) Grapevine Rootstocks: Selection and Management for South Australian Vineyards. Lythrum Press (Adelaide), Stepney, Australia. 85

Duan D, Fischer S, Merz P, Bogs J, Riemann M, and Nick P (2016) An ancestral allele of grapevine transcription factor MYB14 promotes plant defence. *J Exp Bot* 67, 1795-1804

Dubouzet JG, Sakuma Y, Ito Y, Kasuga M, Dubouzet EG, Miura S, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) OsDREB genes in rice, Oryza sativa L., encode transcription activators that function in drought-, high-, salt- and cold-responsive gene expression. *Plant J* 33, 751-763

Duchêne E (2016) How can grapevine genetics contribute to the adaptation to climate change. *OENO One* 50, 113-124

Egert A, Eicher B, Keller F, Peters S (2015). Evidence for water deficit-induced mass increases of raffinose family oligosaccharides (RFOs) in the leaves of three Craterostigma resurrection plant species *Front Physiol* 6, 1–6

Egert A, Keller F and Peters S (2013) Abiotic stress-induced accumulation of raffinose in Arabidopsis leaves is mediated by a single raffinose synthase (RS5, At5g40390). *BMC Plant Biol* 13, e218

Einset J, and Connolly EL (2009) Glycine betaine enhances extracellular processes blocking ROS signaling during stress. *Plant Signal Behav* 4, 197–199

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One* 6, 1–10

Emanuelli F, Lorenzi S, Grzeskowiak L, Catalano V, Stefanini M, Troggio M, Myles M, Martinez-Zapater JM, Zyprian E, Moreira FM and Grando MS (2013) Genetic diversity and population structure assessed by SSR and SNP markers in a large germplasm collection of grape. *BMC Plant Biol* 13, 39

Endelman JB, Jannink J-L (2013) Shrinkage Estimation of the Realized Relationship Matrix. *G3-Genes Genom Genet* 2, 1405–1413

Esch E, Szymaniak JM, Yates H, Pawlowski WP, Buckler ES (2007) Using crossover breakpoints in recombinant inbred lines to identify quantitative trait loci controlling the global recombination frequency. *Genetics* 177, 1851–8

Estrada-Melo AC, Reid MS, Jiang CZ (2015) Overexpression of an ABA biosynthesis gene using a stress-inducible promoter enhances drought resistance in petunia. *Hortic Res* 2, 15013

Evanno G, Regnaut S, Goudet J (2005) Detecting the Number of Clusters of Individuals Using the Software Structure: A Simulation Study. *Mol Ecol* 14, 2611-2620

Faize M, Burgos L, Faize L, Piqueras A, Nicolas E, Barba-Espin G, Clemente-Moreno MJ, Alcobendas R, Artlip T, Hernandez JA (2011) Involvement of cytosolic ascorbate peroxidase and Cu/Zn-superoxide dismutase for improved tolerance against drought stress. *J Exp Bot* 62, 2599–2613

Falavigna VS, Porto DD, Miotto YE, Santos HPD, Oliveira PRD, Margis-Pinheiro, M, Pasquali G, Revers LF (2018) Evolutionary diversification of galactinol synthases in Rosaceae: adaptive roles of galactinol and raffinose during apple bud dormancy. *J Exp Bo*t 69, 1247–1259

Falush D, Stephens M, Pritchard JK (2003) Inference of Population Structure Using Multilocus Genotype Data: Linked Loci and Correlated Allele Frequencies. Genetics 164, 1567–1587

Feng W, Lindner H, Robbins NE, Dinneny JR (2016) Growing out of stress: The role of cell- and organ-scale growth control in plant water-stress responses. *Plant Cell* 28, 1769–1782

Fernandez L, Le Cunff L, Tello J, Lacombe T, Boursiquot JM, Fournier-Level A, Bravo G, Lalet S, Torregrosa L, This P, Mar- tinez-Zapater JM (2014) Haplotype diversity of VvTFL1A gene and association with cluster traits in grapevine (V. vinifera). *BMC Plant Bio*l 14, 209

Flexas J, Barón M, Bota J, Ducruet JM, Galle A, Galmés J, Jimenez M, Pou A, Ribas-Carbó M, Sajnani C, Tomás M, Medrano H (2009) Photosynthesis limitations during wáter stress acclimation and recovery in the drought-adapted Vitis hybrid Richter-110 (V. *berlandieri* x V. *rupestris*). *J Exp Bot* 60, 2361–2377

Flexas J, Bota J, Escalona JM, Sampol B, Medrano H (2002) Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. *Funct Plant Biol* 29, 461–471

Fodor A, Segura V, Denis M, Neuenschwander S, Fournier-Level A, Chatelet P, Homa FAA, Lacombe T, This P, Cunff L Le (2014) Genome-wide prediction methods in highly diverse and heterozygous species: Proof-of-concept through simulation in grapevine. *PLoS One* 9, e11

Franks T, Gang HD, Thomas M (1998) Regeneration of transgenic shape Vitis *vinifera L*, Sultana plants: genotypic and phenotypic analysis. *Mol Breed* 4, 321–333

Fu YX, Li WH (1993) Statistical tests of neutrality of mutations. *Genetics* 133, 693–709

Fujita Y, Fujita M, Sato R, Maruyama K, Parvez MM, Seki M, Hiratsu K, Ohme-Takagi M, Shinozaki K, Yamaguchi-Shinozaki K (2005) AREB1 is a transcription activator of novel ABRE-dependent ABA signaling that enhances drought stress tolerance in Arabidopsis. *Plant Cell* 17, 3470-3488

Furbank RT, Tester M (2011) Phenomics – technologies to relieve the phenotyping bottleneck. Trends Plant Sci 16, 635–644

Furihata T, Maruyama K, Fujita Y, Umezawa T, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K (2006) Abscisic acid-dependent multisite phosphorylation regulates the activity of a transcription activator AREB1. *Proc Natl Acad Sci USA* 103, 1988-1993

Gago J, Fernie AR, Nikoloski Z, Tohge T, Martorell S, Escalona JM, Ribas-Carbó M, Flexas J, Medrano H (2017) Integrative field scale phenotyping for investigating metabolic components of water stress within a vineyard. *Plant Methods* 13, 90

Galbiati M, Matus J, Francia P, Rusconi F, Cañón P, Medina C, Conti L, Cominelli E, Tonelli C, Arce-Johnson P (2011) The grapevine guard cell-related VvMYB60 transcription factor is involved in the regulation of stomatal activity and is differentially expressed in response to ABA and osmotic stress. *BMC Plant Biol* 21, 142

Gambetta GA, Manuck CM, Drucker ST, Shaghasi T, Fort K, Matthews MA, Walker MA, McElrone AJ (2012) The relationship between root hydraulics and scion vigour across Vitis rootstocks: what role do root aquaporins play? *J Exp Bot* 63, 6445– 6455

Gaudillère JP, Van Leeuwen C, Ollat N (2002) Carbon isotope composition of sugars in grapevine, an integrated indicator of vineyard water status. *J Exp Bot* 53, 757–763

Geiger D, Scherzer S, Mumm P, Stange A, Marten I, Bauer H, Ache P, Matschi S, Liese A, Al-Rasheid KA, Romeis T, Hedrich R (2009) Activity of guard cell anion channel SLAC1 is controlled by drought-stress signaling kinase-phosphatase pair. *Proc Natl Acad Sci USA* 106, 21425–21430

Ghozlen NB, Cerovic ZG, Germain C, Toutain S, Latouche G (2010) Non-destructive optical monitoring of grape maturation by proximal sensing. *Sensors* 10, 10040

GISTEMP Team (2018) GISS Surface Temperature Analysis (GISTEMP). NASA Goddard Institute for Space Studies. https://data.giss.nasa.gov/gistemp/

Gouesnard B, Bataillon TM, Decoux G, Rozale C, Schoen DJ, David JL (20019) MSTRAT: An Algorithm for Building Germ Plasm Core Collections by Maximizing Allelic or Phenotypic Richness. *J Hered* 92, 93–94

Granett J, Walker MA, Kocsis L, Omer AD (2001) Biology and management of grape Phylloxera. *Annu Rev Entomol* 46, 387– 412

GrapeReSeq Illumina 20K (http://urgi.versailles.inra.fr/Species/Vitis/GrapeReSeq_Illumina_20K)

Grondin A, Rodrigues O, Verdoucq L, Merlot S, Leonhardt N, Maurel C (2015) Aquaporins contribute to ABA-triggered stomatal closure through OST1-mediated phosphorylation. *Plant Cell* 27, 1945-1954

Gu H, Lu M, Zhang Z, Xu J, Cao W, Miao M (2018) Metabolic process of raffinose family oligosaccharides during cold stress and recovery in cucumber leaves. *J Plant Physiol* 225, 112-120

Gu L, Zhang Y, Zhang M, Li T, Dirk LM, Downie B, Zhao T (2015) ZmGOLS2, a target of transcription factor ZmDREB2A, offers similar protection against abiotic stress as ZmDREB2A. *Plant Mol Biol* 90, 157–170

Guan XQ, Zhao SJ, Li DQ, Shu HR (2004) Photoprotective function of photorespiration in several grapevine cultivars under drought stress. *Photosynthetica* 42, 31–36

Guan X, Essaki H, Laloue H, Nick P, Bertsch C, Chong J (2016) Mining new resources for grape resistance against Botryosphaeriaceae: a focus on Vitis *vinifera* ssp. *sylvestris*. *Plant Pathol* 65, 273-284

Gupta A, Kaur K, Kaur N (2011) Stem Reserve Mobilization and Sink Activity in Wheat under Drought Conditions. *American J Plant Sci* 2:70-77

Gutiérrez S, Diago MP, Fernández-Novales J, Tardaguila J (2018) Vineyard water status assessment using on-the-go thermal imaging and machine learning. *PLoS ONE* 13, e0192037

Hannah L, Roehrdanz PR, Ikegami M, Shepard AV, Shaw MR, Tabor G, Zhi L, Marquet PA, Hijmans RJ (2013) Climate change, wine, and conservation. *Proc Natl Acad Sci USA* 110, 6907–6912

Hare PD, Cress WA, Van Staden J (1998) Dissecting the roles of osmolyte accumulation during stress. *Plant Cell Environ* 21, 535–553

Harris K, Subudhi P, Borrell A, Jordan D, Rosenow D, Nguyen H, Klein P, Muller J (2007) Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. *J Exp Bot* 58, 327–338

He R, Zhuang Y, Cai Y, Agüero CB, Liu S, Wu J, Deng S, Walker MA, Lu J, Zhang Y (2018) Overexpression of 9-cis-Epoxycarotenoid Dioxygenase Cisgene in Grapevine Increases Drought Tolerance and Results in Pleiotropic Effects. *Front Plant Sci* 9, 970

Henderson SW, Dunlevy JD, Wu Y, Blackmore DH, Walker RR, Edwards EJ, Gilliham M, Walker AR (2017) Functional differences in transport properties of natural HKT1; 1 variants influence shoot Na+ exclusion in grapevine rootstocks. *New Phytol* 217, 1113-1127

Heywood V, Zohary D (1991) A catalogue of wild relatives of cultivated plants native to Europe. *Flora Mediterr* 5, 375–415

Hoagland DR, Arnon DI (1938) The water-culture method for growing plants without soil (Circular (California Agricultural Experiment Station). Ed Berkeley, Calif : University of California, College of Agriculture

Hochberg U, Windt CW, Ponomarenko A, Zhang Y-J, Gersony J, Rockwell FE, Holbrook NM (2017) Stomatal closure, basal leaf embolism, and shedding protect the hydraulic integrity of grape stems. *Plant Physiol* 174, 764–775

Hood EE, Gelvin SB, Melchers LS, Hoekema A (1993) New Agrobacterium helper plasmids for gene-transfer to plants. *Transgenic Res* 2, 208–218

Houel C, Chatbanyong R, Doligez A, Rienth M, Foria S, Luchaire N, Roux C, Adivèze A, Lopez G, Farnos M, Pellegrino A, This P, Romieu C, Torregrosa L: (2015) Identification of stable QTLs for vegetative and reproductive traits in the microvine (Vitis vinifera L.) using the 18 K Infinium chip. *BMC Plant Biol* 15, 205

Houillé B, Besseau S, Delanoue G, Oudin A, Papon N, Clastre M, Simkin AJ, Guérin L, Courdavault V, Giglioli-Guivarc'h N, Lanoue A (2015) Composition and tissue-specific distribution of stilbenoids in grape canes are affected by downy mildew pressure in the vineyard. *J Agric Food Chem* 63, 8472–8477

Hozain M, Abdelmageed H, Lee J, Kang M, Fokar M, Allen RD, Holaday AS (2012) Over-expressing AtSAP5 in cotton up-regulates putative stress responsive genes and improves the tolerance to rapidly developing water deficit and moderate heat stress. *Plant Physiol* 169, 1261–1270

Hsiao TC (1973) Plant responses to water stress. *Annu Rev Plant Physiol* 24, 519-70

Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q, (2006) Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *Proc Natl Acad Sci USA* 103, 12987–12992

Huang X, Han B (2014) Natural variations and genome-wide association studies in crop plants. *Annu Rev Plant Biol* 65, 531–51

Idso SB, Jackson RD, Pinter PJ, Reginato RJ, Hat eld JL (1981) Normalizing the stress-degree-day parameter for environmental variability. *Agric Meteorol* 24, 45–55

IPCC report (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Iuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T, Tabata S, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K (2001) Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in Arabidopsis. *Plant J* 27, 325–333

Iwasaki T, Yamaguchi-Shinozaki K, Shinozaki KI (1995) Identification of a cis-regulatory region of a gene in Arabidopsis *thaliana* whose induction by dehydration is mediated by abscisic acid and requires protein synthesis. *Mol Gen Genet* 247, 391-398

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23, 1801–1806

Javot H, Lauvergeat V, Santoni V, Martin-Laurent F, Güçlü J, Vinh J, Heyes J, Franck KI, Schäffner AR, Bouchez D, Maurel C (2003) Role of a single aquaporin isoform in root water uptake. *Plant Cell* 15, 509–522

Jeong JS, Kim YS, Redillas MC, Jang G, Jung H, Bang SW, Choi YD, Ha SH, Reuzeau C, Kim JK (2013) OsNAC5 overexpression enlarges root diameter in rice plants leading to enhanced drought tolerance and increased grain yield in the field. *Plant Biotechnol J* 11, 101–114

Jiang YJ, Liang G, and Yu DQ (2012) Activated expression of WRKY57 confers drought tolerance in Arabidopsis. *Mol Plant* 5, 1375–1388

Jin WM, Dong J, Hu YL, Lin ZP, Xu XF, Han ZH. Improved cold-resistant performance in transgenic grape (*Vitis vinifera L.*) overexpressing cold-inducible transcription factors AtDREB1b. *Hortic Sci* 44, 35–39

Jones HG (1999) Use of infrared thermometry for estimation of stomatal conductance as a possible aid to irrigation scheduling. *Agric For Meteorol* 95, 139–149

Jones HG, Sutherland RA (1991) Stomatal control of xylem embolism. *Plant Cell Environ* 14, 607–612

Joshi R, Wani SH, Singh B, Bohra A, Dar ZA, Lone AA, Pareek A, Singla-Pareek SL (2016) Transcription factors and plants response to drought stress: current understanding and future directions. *Front Plant Sci* 7, 1029

Jossier M, Bouly JP, Meimoun P, Arjmand A, Lessard P, Hawley S, Grahame HD, Thomas M: (2009) SnRK1 (SNF1-related kinase 1) has a central role in sugar and ABA signalling in Arabidopsis thaliana. *Plant J* 59, 316-328

Kang JY, Choi HI, Im MY, Kim SY (2002) Arabidopsis basic leucine zipper proteins that mediate stress-responsive abscisic acid signaling. *Plant Cell* 14, 343-357

Kang Y, Han Y, Torres-Jerez I, Wang M, Tang Y, Monteros M, Udvardi M (2011) System responses to long-term drought and re-watering of two contrasting alfalfa varieties. *Plant J* 68, 871–889

Karim S, Aronsson H, Ericson H, Pirhonen M, Leyman B, Welin B, Mantyla E, Palva ET, Van Dijck P, Holmstrom KO (2007) Improved drought tolerance without undesired side effects in transgenic plants producing trehalose. *Plant Mol Biol* 64, 371–386

Karimi M, Inzé D, Depicker A (2002) GATEWAY™ vectors for Agrobacterium-mediated plant transformation. *Trends Plant Sci* 7, 193–195

Kazan K (2015) Diverse roles of jasmonates and ethylene in abiotic stress tolerance. *Trends Plant Sci* 20, 219–229

Keller F, Pharr DM (1996) "Metabolism of carbohydrates in sinks and sources: galactosyl-sucrose oligosaccharides," in *Photoassimilate Distribution in Plants and Crops: Source–Sink Relationships*, eds E. Zamski and A. A. Schaffer (New York: Marcel Dekker), 157–183

Keller M, Mills LJ, Harbertson JF (2012) Rootstock effects on deficit-irrigated winegrapes in a dry climate: vigor, yield formation, and fruit ripening. *Am J Enol Vitic* 63, 29–39

Kelly G, Moshelion M, David-Schwartz R, Halperin O, Wallach R, Attia Z, Belausov E, Granot D (2013) Hexokinase mediates stomatal closure. *Plant J* 75, 977–988

Khanna-Chopra R, Srivalli B, Ahlawat YS (1999) Drought induces many forms of cysteine proteases not observed during natural senescence. *Biochem Biophys Res Commun* 255, 324–327

Kicherer A, Herzog K, Bendel N, Klück HC, Backhaus A, Wieland M, Rose JC, Klingbeil L, Läbe T, Hohl C, Petry W, Kuhlmann H, Seiffert U, Töpfer R (2017) Phenoliner: A New Field Phenotyping Platform for Grapevine Research. *Sensors* 17(7), 1625

Komatsu K, Suzuki N, Kuwamura M, Nishikawa Y, Nakatani M, Ohtawa H, Takezawa D, Seki M, Tanaka M, Taji T, Hayashi T, & Sakata Y (2013) Group A PP2Cs evolved in land plants as key regulators of intrinsic desiccation tolerance. *Nat Commun* 4, 2219

Korte A, Farlow A (2013) The advantages and limitations of trait analysis with GWAS: a review. *Plant Methods* 9, 29

Koundouras S, Tsialtas IT, Zioziou E, Nikolaou N (2008) Rootstock effects on the adaptive strategies of grapevine (*Vitis vinifera L.* cv. Cabernet–Sauvignon) under con- trasting water status: leaf physiological and structural responses. *Agric Ecosyst Environ* 128,86–96

Król A, Amarowicz R, Weidner S (2014) Changes in the composition of phenolic compounds and antioxidant properties of grapevine roots and leaves (*Vitis vinifera L.*) under continuous of long-term drought stress. *Acta Physiologiae Plantarum* 36, 1491–1499

Kuromori T, Seo M, Shinozaki K (2018) ABA Transport and Plant Water Stress Responses. *Trends Plant Sci* 23, 513-522

Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JD, Schroeder JI (2003) NADPH oxidase AtrbohD and AtrbohF genes function in ROS-dependent ABA signaling in Arabidopsis. *EMBO J* 22, 2623-2633

Laucou V, Launay A, Bacilieri R, Lacombe T, Adam-Blondon AF, Bérard A, Chauveau A, de Andrés MT, Hausmann L, Ibáñez J, Le Paslier MC, Maghradze D, Martinez-Zapater JM, Maul E, Ponnaiah M, Töpfer R, Péros JP, Boursiquot JM (2018) Extended diversity analysis of cultivated grapevine *Vitis vinifera* with 10K genome-wide SNPs. *PLoS ONE* 13, e0192540

Lavoie-Lamoureux A, Sacco D, Risse PA, Lovisolo C (2017) Factors influencing stomatal conductance in response to water availability in grapevine: A meta-analysis. *Physiol Plant* 159, 468–482

Lawlor DW (2002) Limitation to photosynthesis in water stressed leaves: stomata vs. metabolism and the role of ATP. *Ann Bo*t 89, 1–15

Le Cunff L, Fournier-Level A, Laucou V, Vezzulli S, Lacombe T, Adam-Blondon AF, Boursiquot JM, This P (2008) Construction of nested genetic core collections to optimize the exploitation of natural diversity in *Vitis vinifera* L. subsp. *sativa*. *BMC Plant Biol* 8, 31

Le Paslier MC, Choisne N, Bacilieri R, Bounon R, Boursiquot J, Bras M, Brunel D, Di Gaspero G, Hausmann L, Lacombe T, Laucou V, Launay A, Martinez-Zapater JM, Morgante M, Raj P., Ponnaiah M, Quesneville H, Scalabrin S, Torres-Perez R, Adam-Blondon AF (2013) The GrapeReSeq 18 k Vitis genotyping chip. In 9th International symposium grapevine physiology and biotechnology: International Society for Horticultural Science 123

Levitt J (1972) Responses of Plants to Environmental Stresses. New York, NY: Academic Press, 698

Li WX, Oono Y, Zhu JH, He XJ, Wu JM, Iida K, Lu XY, Cui XP, Jin HL, Zhu JK (2008) The Arabidopsis NFYA5 transcription factor is regulated transcriptionally and posttranscriptionally to promote drought resistance. *Plant Cell* 20, 2238-2251

Li Z, Wakao S, Fischer BB, Niyogi KK (2009) Sensing and responding to excess light. *Annu Rev Plant Biol* 60, 239–260

Liang ZC, Duan SC, Sheng J, Zhu SS, Ni XM, Shao JH, Liu CH, Nick P, Du F, Fan PG, Mao RZ, Zhu YF, Deng WP, Yang M, Huang HC, Liu YX, Ding YQ, Liu XJ, Jiang JF, Zhu YY, He XH, Chen W, Li SH, Dong Y (2019) Whole-genome resequencing of 472 Vitis accessions for grapevine diversity and demographic history analyses. Nat Commun 10, 1190

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphims data. *Bioinformatic*s 25, 1451-1452

Lijavetzky D, Cabezas JA, Ibanez A, Rodriguez V, Martinez-Zapater JM (2007) High throughput SNP discovery and genotyping in grapevine (*Vitis vinifera L.*) by combining a re-sequencing approach and SNPlex technology. *BMC Genomics* 8, 424

Lim J, Lim CW, Lee SC (2018) The Pepper Late Embryogenesis Abundant Protein, CaDIL1, Positively Regulates Drought Tolerance and ABA Signaling. *Front Plant Sci* 9, 1301

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain, separate two cellular signal transduction pathways in drought- and low temperature-responsive gene expression, respectively, in Arabidopsis. *Plant Cell* 10, 1391–1406

Lloyd G, McCown B (1981) Commercially feasible micropropagation of Mountain Laurel, Kalmia latifolia, by use of shoot tip culture. *Combined Proceedings - International Plant Propagator's Society* 30, 421-427

Lorenz DH, Eichhorn KW, Bleiholder H, Klose R, Meier U, Weber E (1994) Phänologische Entwicklungsstadien der Weinrebe (*Vitis vinifera L,*). *Vitic Enol Sci* 49, 66–70

Lovisolo C, Hartung W, Schubert A (2002) Whole-plant hydraulic conductance and root-to-shoot flow of abscisic acid are independently affected by water stress in grapevines. *Funct Plant Biol* 29, 1349–1356

Lovisolo C, Perrone I, Carra A, Ferrandino A, Flexas J, Medrano H, Schubert A (2010) Drought-induced changes in development and function of grapevine (Vitis spp.) organs and in their hydraulic and non-hydraulic interactions at the whole-plant level : a physiological and molecular update. *Funct Plant Biol* 37, 98-116

Luck M, Landis M, Gassert F (2015) Aqueduct Water Stress Projections: Decadal Projections of Water Supply and Demand Using CMIP5 GCMs. Technical Note.Washington, D.C.: World Resources Institute

Mao H, Wang H, Liu S, Li Z, Yang X, Yan J, Li J, Tran LP, Qin F (2015) A transposable element in a NAC gene is associated with drought tolerance in maize seedlings. *Nat Commun* 6, 8326

Marasco R, Rolli E, Fusi M, Michoud G, Daffonchio D (2018) Grapevine rootstocks shape underground bacterial microbiome and networking but not potential functionality. *Microbiome* 6, 3

Marguerit E, Brendel O, Lebon E, Van Leeuwen C, Ollat N (2012) Rootstock control of scion transpiration and its acclimation to water deficit are controlled by different genes. *New Phytol* 194, 416–429

Marrano A, Birolo G, Prazzoli ML, Lorenzi S, Valle G, Grando MS (2017) SNP-Discovery by RAD-Sequencing in a germplasm collection of wild and cultivated grapevines (*V. vinifera L.*). *PLoS ONE* 12, e0170655

Marrano A, Micheletti D, Lorenzi S, Neale D, Grando MS (2018) Genomic signatures of different adaptations to environmental stimuli between wild and cultivated *Vitis vinifera L. Hort J* 5, 34

Martin-StPaul N, Delzon S, Cochard H (2017) Plant resistance to drought depends on timely stomatal closure. *Ecol Lett* 20, 1437–1447

Maruyama K, Takeda M, Kidokoro S, Yamada K, Sakuma Y, Urano K, Fujita M, Yoshiwara K, Matsukura S, Morishita Y, Sasaki R, Suzuki H, Saito K, Shibata D, Shinozaki K, Yamaguchi-Shinozaki K (2009) Metabolic pathways involved in cold acclimation identified by integrated analysis of metabolites and transcripts regulated by DREB1A and DREB2A. *Plant Physiol* 150, 1972–1980

Maruyama K, Urano K, Yoshiwara K, Morishita Y, Sakurai N, Suzuki H, Kojima M, Sakakibara H, Shibata D, Saito K, Shinozaki K, Yamaguchi-Shinozaki K (2014) Integrated analysis of the effects of cold and dehydration on rice metabolites, phytohormones, and gene transcripts. *Plant Physiol* 164, 1759–1771

Matese A, Baraldi R, Berton A, Cesaraccio C, Di Gennaro SF, Duce P, Facini O, Mameli MG, Piga A, Zaldei A (2018) Estimation of water stress in grapevines using proximal and remote sensing methods. *Rem Sens* 10, 114

Maul E, Sudharma KN, Kecke S, Marx G, Müller C, Audeguin L, Boselli M, Boursiquot JM, Bucchetti B, Cabello F, Carraro F, Crespan M, De Andrés MT, Dias JE, Ekhvaia J, Gaforio L, Gardiman M, Grando MS, Gyropoulos D, Jandurova O, Kiss E, Kontic J, Kozma P, Lacombe T, Laucou V, Legrand D, Maghradze D, Marinoni D, Maletic E, Moreira Maia F, Muñoz G, Nakhutsrishvili G, Pijic I, Peterlunger E, Pitsoli D, Pospisilova D, Preiner D, Raimondi S, Regner F, Savin G, Savvides S, Schneider A, Sereno C, Simon S, Staraz M, Zulini L, Bacilieri R, This P (2012) The European Vitis Database (wwweu-vitisde): a technical innovation through an online uploading and interactive modification system. *Vitis* 51, 79-85

McAdam SAM, Brodribb TJ (2018) Mesophyll Cells Are the Main Site of Abscisic Acid Biosynthesis in Water-Stressed Leaves. *Plant Physiol* 178, 201-209

McKhann HI, Camilleri C, Berard A, Bataillon T, David JL, Reboud X, Le Corre V, Caloustian C, Gut IG, Brunel D (2004) Nested core collections maximizing genetic diversity in Arabidopsis thaliana. *Plant J* 38, 193-202

Medici A, Laloi M, Atanassova R (2014) Profiling of sugar transporter genes in grapevine coping with water deficit. *FEBS Lett* 588, 3989–3997

Medrano H, Escalona JM, Cifre J, Bota J, Flexas J (2003) A ten-year study on the physiology of two Spanish grapevine cultivars under field conditions: effects of water availability from leaf photosynthesis to grape yield and quality. *Funct Plant Biol* 30, 607-619

Meggio, F., Prinsi, B., Negri, A. S., Di Lorenzo, G. S., Lucchini, G., Pitacco, A., Failla, O., Scienza A, Cocucci C, 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

Mickelbart PV, Hasegawa PM, Bailey-Serres J (2015) Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. *Nat Rev Genet* 16, 237–251

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. *Trends Plant Sci* 9, 490-498

Møller IM, Jensen PE, Hansson A (2007) Oxidative modifications to cellular components in plants. *Annu Rev Plant Biol* 58, 459-481

Moore JP, Vicré-Gibouin M, Farrant JM, Driouich A (2008) Adaptations of higher plant cell walls to water loss: drought versus desiccation. *Physiol Plan*t 134, 237-245

Mosedale JR, Wilson RJ, Maclean IMD (2015) Climate Change and Crop Exposure to Adverse Weather: Changes to Frost Risk and Grapevine Flowering Conditions. *PLoS ONE* 10, e0141218

Munné-Bosch S, Alegre L (2004) Die and let live: leaf senescence contributes to plant survival under drought stress. *Funct Plant Biol* 31, 203–216

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15, 473–497

Myles S, Chia JM, Hurwitz B, Simon C, Zhong GY, Buckler E, Ware D (2010) Rapid genomic characterization of the genus Vitis. *PLoS One* 5, e821

Nakashima K, Ito Y, Yamaguchi-Shinozaki K (2009) Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. *Plant Physiol* 149, 88–95

Narusaka Y, Nakashima K, Shinwari ZK, Sakuma Y, Furihata T, Abe H, Narusaka M, Shinozaki K, Yamaguchi-Shinozaki K (2003) Interaction between two cis-acting elements, ABRE and DRE, in ABA-dependent expression of Arabidopsis rd29A gene in response to dehydration and high-salinity stresses. *Plant J* 34, 137-148

Navarro L, Bari R, Achard P, Lison P, Nemri A, Harberd NP, Jones JDG (2008) DELLAs control plant immune responses by modulating the balance of jasmonic acid and salicylic acid signaling. *Curr Biol* 18, 650-655

Nei L (1987) Molecular evolutionary genetics. New York: Columbia University Press

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Acad Sci USA* 76, 5269-5273

Nelson D, Repetti P, Adams T, Creelman R, Wu J, Warner D, Anstrom D, Bensen R, Castiglioni P, Donnarummo M, Hinchey B, Kumimoto R, Maszle D, Canales R, Krolikowski K, Dotson S, Gutterson N, Ratcliffe O, Heard J (2007) Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. *Proc Natl Acad Sci USA* 104, 16450–16455

Nelson DE, Repetti PP, Adams TR, Creelman RA, Wu J, Warner DC, Anstrom DC, Bensen RJ, Castiglioni PP, Donnarummo MG, Hinchey BS, Kumimoto RW, Maszle DR, Canales RD, Krolikowski KA, Dotson SB, Gutterson N, Ratcliffe OJ, Heard JE (2007) Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. *Proc Natl Acad Sci USA* 104, 16450-16455

Nicolas SD, Péros JP, Lacombe T, Launay A, Le Paslier MC, Bérard A, Mangin B, Valière S, Martins F, Le Cunff L, Laucou V, Bacilieri R, Dereeper A, Chatelet P, This P, Doligez A (2016) Genetic diversity, linkage disequilibrium and power of a large grapevine (*Vitis vinifera L*) diversity panel newly designed for association studies. *BMC Plant Biol* 16, 74

Nicolas, P, Lecourieux D, Kappel C, Cluzet S, Cramer G, Delrot S, Lecourieux F (2014) The basic leucine zipper transcription factor ABSCISIC ACID RESPONSE ELEMENT-BINDING FACTOR2 is an important transcriptional regulator of abscisic acid-dependent grape berry ripening processes. *Plant Physiol* 164, 365–383

Ning J, Li X, Hicks LM, Xiong L (2010) A Raf-like MAPKKK gene DSM1 mediates drought resistance through reactive oxygen species cavenging in rice. *Plant Physiol* 152, 876–890

Nishizawa A, Yabuta Y, Shigeoka S (2008) Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. *Plant Physiol* 147, 1251-63

Nitsch JP, Nitsch C (1969) Haploid plants from pollen grains. *Science* 163, 85–87

NOAA National Centers for Environmental Information (2018) State of the Climate: Global Climate Report for Annual 2016, published online January 2017, retrieved on April 9, 2018 from https://www.ncdc.noaa.gov/sotc/global/201613.

Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. *Annu Rev Plant Biol* 49, 249–279

Nonami H, Boyer JS (1990) Primary events regulating stem growth at low water potentials. *Plant Physiol* 94, 1601–1609

Nuccio ML, Wu J, Mowers R, Zhou HP, Meghji M, Primavesi LF, Paul MJ, Chen X, Gao Y, Haq ue E, Basu SS, Lagrimini LM (2015) Expression of trehalose-6-phosphate phosphatase in maize ears improves yield in well-watered and drought conditions. *Nat Biotechnol* 33, 862–869

Ocete R, Arnold C, Failla O, Lovicu G, Biagini B, Imazio S, Lara M, Maghradze D (2011) Considerations on the European wild grapevine (*Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi) and Phylloxera infestation. *Vitis* 50, 97–98

Oh SJ, Song SI, Kim YS, Jang HJ, Kim SY, Kim M, Kim YK, Nahm BH, Kim JK (2005) Arabidopsis CBF3/DREB1A andABF3 in transgenic rice increased tolerance to abiotic stress without stunting growth. *Plant Physiol* 138, 341–351

Ojeda H, Andary C, Kraeva E, Carbonneau A, Deloire A (2002) Influence of pre- and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *Am J Enol Vitic* 53, 261–267

Owens CL (2003) SNP Detection and Genotyping in Vitis. *Acta Hortic* 603, 139-140

Pagliarani C, Vitali M, Ferrero M, Vitulo N, Incarbone M, Lovisolo C, Valle G, Schubert A (2017) Accumulation of MicroRNAs differentially modulated by drought is affected by grafting in grapevine. *Plant Physiol* 173, 2180–2195

Park BJ, Liu Z, Kanno A, Kameya T (2005) Genetic improvement of Chinese cabbage for salt and drought tolerance by constitutive expression of a B. *napus* LEA gene. *Plant Sci* 169, 553–558

Park SY, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, Lumba S, Santiago J, Rodrigues A, Chow TF, Alfred SE, Bonetta D, Finkelstein R, Provart NJ, Desveaux D, Rodriguez PL, McCourt P, Zhu JK, Schroeder JI, Volkman BF, Cutler SR (2009) Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science* 324, 1068–1071

Parmar N, Singh KH, Sharma D, Singh L, Kumar P, Nanjundan J, Khan YJ, Chauhan DK, Thakur AK (2017) Genetic engineering strategies for biotic and abiotic stress tolerance and quality enhancement in horticultural crops: a comprehensive review. *3 Biotech* 7, 239

Peccoux A, Loveys B, Zhu J, Gambetta GA, Delrot S, Vivin P, Schultz HR, Ollat N, Dai Z (2017) Dissecting the rootstock control of scion transpiration using model-assisted analyses in grapevine. *Tree Physiol* 38, 1026-1040

Perrin M, Gertz C, Masson JE (2004) High efficiency initiation of regenerable embryogenic callus from anther filaments of 19 grapevine genotypes grown worldwide. *Plant Sci* 167, 1343–1349

Perrin M, Martin D, Joly D, Demangeat G, This P, Masson JE (2001) Medium-dependent response of grapevine somatic embryogenic cells. *Plant Sci* 161, 107-116

Perrone I, Gambino G, Chitarra W, Vitali M, Pagliarani C, Riccomagno N, Balestrini R, Kaldenhoff R, Uehlein N, Gribaudo I, Schubert A, Lovisolo C (2012) The grapevine root-specific aquaporin VvPIP2;4N controls root hydraulic conductance and leaf gas exchange under well-watered conditions but not under water stress. *Plant Physio*l 160, 965–977

Peters S, Mundree SG, Thomson JA, Farrant JM, Keller F (2007) Protection mechanisms in the resurrection plant Xerophyta viscosa (Baker): both sucrose and raffinose family oligosaccharides (RFOs) accumulate in leaves in response to water deficit. *J Exp Bot* 58, 1947–1956

Petrov V, Hille J, Mueller-Roeber B, Gechev TS (2015) ROS-mediated abiotic stress-induced programmed cell death in plants. *Front Plant Sci* 6, 69

Pillet J, Egert A, Pieri P, Lecourieux F, Kappel C, Charon J, Gomès E, Keller F, Delrot S, Lecourieux D (2012) VvGOLS1 and VvHsfA2 are involved in the heat stress responses in grapevine berries. *Plant Cell Physiol* 53, 1776–1792

Pina A, Errea P (2005) A review of new advances in mechanism of graft compatibility–incompatibility. *Sci Hortic* 106, 1–11

Platt A, Vilhjálmsson BJ, Nordborg, M (2010) Conditions under which genome-wide association studies will be positively misleading. *Genetics* 186, 1045-1052

Posé D, Castanedo I, Borsani O, Nieto B, Rosado A, Taconnat L, Ferrer A, Dolan L, Valpuesta V, Botella MA (2009) Identification of the Arabidopsis dry2/sqe1-5 mutant reveals a central role for sterols in drought tolerance and regulation of reactive oxygen species. *Plant J* 59, 63–76

Pou A, Medrano H, Flexas J, Tyerman SD (2012) A putative role for TIP and PIP aquaporins in dynamics of leaf hydraulic and stomatal conductances in grapevine under water stress and re-watering. *Plant Cell Environ* 36, 828–843

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959

Qin X, Zeevaart JAD (1999) The 9-cis-epoxycarotenoid cleavage reaction is the key regulatory step of abscisic acid biosynthesis in water-stressed bean. *Proc Natl Acad Sci USA* 96, 15354–15361

Quan R, Shang M, Zhang H, Zhao Y, Zhang J (2004) Engineering of enhanced glycine betaine synthesis improves drought tolerance in maize. *Plant Biotechnol J* 2, 477–486

R Core Team (2013) R: A language and environment for statisticalcomputing, R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Reddy AR, Chaitanya KV, Vivekanandan M (2004) Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J Plant Physiol* 161, 1189–1202

Ripoll J, Urban L, Staudt M, Lopez-Lauri F, Bidel LPR, Bertin N (2014) Water shortage and quality of fleshy fruits— making the most of the unavoidable. *J Exp Bot* 65, 4097–4117

Rogiers SY, Greer DH, Hatfield JM, Hutton RJ, Clarke SJ, Hutchinson PA, Somers A (2012) Stomatal response of an anisohydric grapevine cultivar to evaporative demand, available soil moisture and abscisic acid. *Tree Physiol* 32, 249–261

Rolland F, Baena-Gonzalez E, Sheen J (2006) Sugar sensing and signaling in plants: conserved and novel mechanisms. *Annu Rev Plant Biol* 57, 675–709

Rolli E, Marasco R, Vigani G, Ettoumi B, Mapelli F, Deangelis ML, Gandolfi C, Casati E, Previtali F, Gerbino R, Pierotti Cei F, Borin S, Sorlini C, Zocchi G, Daffonchio D (2015) Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. *Environ Microbiol* 17, 316–331

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. *Mol Ecol Notes* 4, 137–138

Rossdeutsch L, Edwards E, Cookson SJ, Barrieu F, Gambetta GA, Delrot S, Ollat N (2016) ABA-mediated responses to water deficit separate grapevine genotypes by their genetic background. *BMC Plant Biol* 16, 91

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol* 132, 365-386

Ruhl EH, Clingeleffer PR, Nicholas PR, Cirami RM, McCarthy MG, Whiting JR (1988) Effect of rootstocks on berry weight and pH, mineral content and organic acid concentrations of grape juice of some wine varieties. *Aust J Exp Agric* 28, 119-125

Salvi P, Kamble NU, Majee M Stress-Inducible Galactinol Synthase of Chickpea (CaGolS) is Implicated in Heat and Oxidative Stress Tolerance Through Reducing Stress-Induced Excessive Reactive Oxygen Species Accumulation. *Plant Cell Physiol* 59, 155-166

Sanders GJ, Arndt SK (2012) Osmotic Adjustment Under Drought Conditions. In: Aroca R. (eds) Plant Responses to Drought Stress. Springer, Berlin, Heidelberg

Santesteban LG, Miranda C, Royo JB (2011) Regulated deficit irrigation effects on growth, yield, grape quality and individual anthocyanin composition in Vitis *vinifera* L. cv. 'Tempranillo'. *Agric Water Manage* 98, 1171–1179

Sapkota S, Chen LL, Yang S, Hyma KE, Cadle-Davidson L, Hwang C (2018) Construction of a high-density linkage map and QTL detection of downy mildew resistance in *Vitis aestivalis*-derived 'Norton'. *Theor Appl Genet* 1-11

Sato Y, Yokoya S (2008) Enhanced tolerance to drought stress in transgenic rice plants overexpressing a small heat-shock protein, sHSP17. *Plant Cell Rep* 27, 329–334

Savoi S, Wong DCJ, Degu A, Herrera JC, Bucchetti B, Peterlunger E, Fait A, Mattivi F2, Castellarin SD (2017) Multi-omics and integrated network analyses reveal new insights into the systems relationships between metabolites, structural genes, and transcriptional regulators in developing grape berries (*Vitis vinifera L.*) exposed to water deficit. *Front Plant Sci* 8, 1124

Schoen DJ, Brown AH (1993) Conservation of allelic richness in wild crop relatives is aided by assessment of genetic markers. *Proc Natl Acad Sci USA* 90, 10623–10627

Schultz HR (2003) Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field-grown *Vitis vinifera* L. cultivars during drought. *Plant Cell Environ* 26, 1393–405

Selvaraj MG, Ishizaki T, Valencia M, Ogawa S, Dedicova B, Ogata T, Yoshiwara, Maruyama K, Kusano M, Saito K, Takahashi F, Shinozaki K, Nakashima K, Ishitani M(2017) Overexpression of an Arabidopsis thaliana galactinol synthase gene improves drought tolerance in transgenic rice and increased grain yield in the field. *Plant Biotechnol J* 15, 1465–1477

Sengupta S, Mukherjee S, Basak P, Majumder AL (2015) Significance of galactinol and raffinose family oligosaccharide synthesis in plants. *Front Plant Sci* 6, 656

Sepulveda-Reyes D, Ingram B, Bardeen M, Zuñiga M, Ortega-Farıas S, Poblete-Echeverra C (2016) Selecting Canopy Zones and Thresholding Approaches to Assess Grapevine Water Status by Using Aerial and Ground-Based Thermal Imaging. *Remote Sens* 8, 822

Serra I, Strever A, Myburgh PA, 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

Shepherd T, Wynne Griffiths D (2006) The effects of stress on plant cuticular waxes. *New Phytol* 171, 469–499

Simonneau T, Lebon E, Coupel-Ledru A, Marguerit E, Rossdeutsch L, Ollat N (2017) Adapting plant material to face water stress in vineyards: which physiological targets for an optimal control of plant water status ? *OENO One,* 51, 2

Simpson SD, Nakashima K, Narusaka Y, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) Two different novel cis-acting elements of erd1, a clpA homologous Arabidopsis gene function in induction by dehydration stress and dark-induced senescence. *Plant J* 33, 259-270

Skirycz A, Inzé D (2010) More from less: plant growth under limited water. *Curr Opin Biotechnol* 21:197–203

Smith HM, Smith BP, Morales NB, Moskwa S, Clingeleffer PR, Thomas MR (2018) SNP markers tightly linked to root knot nematode resistance in grapevine (Vitis cinerea) identified by a genotyping-by-sequencing approach followed by Sequenom MassARRAY validation. *PL0S ONE* 13, e0193121

Soar C, Dry P, Loveys B (2006) Scion photosynthesis and leaf gas exchange in *Vitis vinifera L.* cv. Shiraz: mediation of rootstock effects via xylem sap ABA. *Aust J Grape Wine Res* 12, 82–96

Song J, Shellie KC, Wang H, Qian MC (2012) Influence of deficit irrigation and kaolin particle film on grape composition and volatile compounds in Merlot grape (*Vitis vinifera L.*). *Food Chem* 134, 841–850

Southey JM (1992) Grapevine rootstock performance under diverse conditions in South Africa. *Proceedings of Rootstock Seminar: A Worldwide Perspective*. American Society for Enology Viticulture: Davis (Eds.). 27–51

Speirs J, Binney A, Collins M, Edwards E, Loveys BR (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, 1907–16

Staden R (1996) The Staden sequence analysis package. *Mol Biotechnol* 5, 233–241

Stevens RM, Pech JM, Gibberd MR, Walker RR, Nicholas PR. Reduced irrigation and rootstock effects on vegetative growth, yield and its components, and leaf physiological responses of Shiraz. *Austral J Grape Wine Res* 16, 413–425

Strasser RJ, Srivastava A, Tsimilli-Michael M (2000): The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Yunus M., Pathre U., Mohanty P. (eds.): *Probing Photosynthesis: Mechanisms, Regulation and Adaptation* Taylor and Francis, London 445–483

Su LY, Dai Z, Li SH, Xin HP (2015) A novel system for evaluating drought-cold tolerance of grapevines using chlorophyll fluorescence. *BMC Plant Biol* 15, 82.

Sun Z, Qi X, Wang Z, Li P, Wu C, Zhang H, Zhao Y (2013) Overexpression of TsGOLS2, a galactinol synthase, in Arabidopsis thaliana enhances tolerance to high salinity and osmotic stresses. *Plant Physiol Biochem* 69, 82–89

Sun X, Matus JT, Wong DCJ, Wang Z, Chai F, Zhang L, Fang T, Zhao L, Wang Y, Han Y, Wang Q, Li S, Liang Z, Xin H (2018) The GARP/MYB-related grape transcription factor AQUILO

improves cold tolerance and promotes the accumulation of raffinose family oligosaccharides. *J Exp Bot* 69, 1749-1764

Suzuki N, Rivero RM, Shulaev V, Blumwald E, Mittler R (2014) Abiotic and biotic stress combinations. *New Phyto*l 203, 32–43

Taji T, Oshumi C, Iuchi S, Seki M, Kasuga M, Kobayashi M, Yamaguchi-Shinozaki K, Shinozaki K (2002) Important roles of drought and cold inducible genes for galactinol synthase in stress tolerance in Arabidopsis thaliana. *Plant J* 29, 417–426

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123, 585–595

Takahashi F, Suzuki T, Osakabe Y, Betsuyaku S, Kondo Y, Dohmae N, Fukuda H,Yamaguchi-Shinozaki K,Shinozaki K (2018) A small peptide modulates stomatal control via abscisic acid in long-distance signalling. *Nature* 556, 235–238

Takeda S, Matsuoka M (2008) Genetic approaches to crop improvement: responding to environmental and population changes. *Nat Rev Genet* 9, 444–57

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol Biol Evol* 28, 2731–9

Tandonnet JP, Marguerit E, Cookson SJ, Ollat N (2018) Genetic architecture of aerial and root traits in field-grown grafted grapevines is largely independent. *Theor Appl Genet* 131, 903-915

Tattersall EA, Grimplet J, Deluc L, Wheatley MD, Vincent D, Osborne C, Ergul A, Lomen E, Blank RR, Schlauch KA, Cushman JC, Cramer GR (2007) Transcript abundance profiles reveal larger and more complex responses of grapevine to chilling compared to osmotic and salinity stress. *Funct Integr Genomics* 7, 317-333

Teh SL, Fresnedo-Ramirez J, Clark MD, Gadoury DM, Sun Q, Cadle-Davidson L, Luby JJ (2017) Genetic dissection of powdery mildew resistance in interspecific half-sib grapevine families using SNP-based maps. *Mol Breeding* 37, 1

Tello J, Torres-Pérez R, Grimplet J, Ibáñez J (2016) Association analysis of grapevine bunch traits using a comprehensive approach. *Theor Appl Genet* 119, 227-242

Tenhaken R (2014) Cell wall remodeling under abiotic stress. *Front Plant Sci* 5, 771

Tillet RL, Wheatley MD, Tattersall EA, Schlauch KA, Cramer GR, Cushman JC (2012) The *Vitis vinifera* C-repeat binding protein (VvCBF4) transcriptional factor enhances freezing tolerance in wine grape. *Plant Biotech J* 10, 105-124

Tollefson J (2018) Clock ticking on climate action. *Nature* 562, 172-173

Tomás M, Medrano H, Escalona JM, Martorell S, Pou A, Ribas-Carbó M, Flexas J (2014) Variability of water use efficiency in grapevines. *Environ Exp Bot* 103, 148–157

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

Tsai-Hung H, Jent-turn L, Yee-yung C, Ming-Tsair C (2002) Heterology expression of the Arabidopsis C-Repeat/Dehydration Response Element Binding Factor 1 gene confers elevated tolerance to chilling and oxidative stresses in transgenic tomato. *Plant Physiol* 130, 618–626

Tuberosa R. (2012) Phenotyping for drought tolerance of crops in the genomics era. *Front Physiol* 3, 347

Turner, SD (2014) qqman: an R package for visualizing GWAS results using QQ and Manhattan plots. bioRxiv.005165

Uga Y, Okuno K, Yano M (2011) Dro1, a major QTL involved in deep rooting of rice under upland field conditions. *J Exp Bot* 62, 2485–2494

Van den Ende W, Valluru R (2009) Sucrose, sucrosyl oligosaccharides, and oxidative stress: scavenging and salvaging. *J Exp Bot* 60, 9–18

van Leeuwen C, Schultz HR, Garcia de Cortazar-Atauri I, Duchene E, Ollat N, Pieri P, Bois B, Goutouly JP, Quenol H, Touzard JM, Malheiro A, Bavarescok L, Delrot S (2013) Why climate change will not dramatically decrease viticultural suitability in main wine-producing areas by. *Proc Natl Acad Sci USA* 110, 3051–3052

Vandeleur RK, Mayo G, Shelden MC, Gilliham M, Kaiser BN, Tyerman SD (2009) The role of plasma membrane intrinsic protein aquaporins in water transport through roots: diurnal and drought stress responses reveal different strategies between isohydric and anisohydric cultivars of grapevine. *Plant Physiol* 149, 445–460

Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. *Trends Plant Sci* 9, 490–98

Vendruscolo EC, Schuster I, Pileggi M, Scapim CA, Molinari HB, Marur CJ, Vieira LG (2007) Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. *J Plant Physiol* 164, 1367-1376

Vickers CE, Gershenzon J, Lerdau MT, Loreto FA (2009) Unified mechanism of action for volatile isoprenoids in plant abiotic stress. *Nat Chem Biol* 5, 283–91

Vidigal P, Martin-Hernandez AM, Guiu-Aragonés V, Amâncio S, Carvalho LC (2014). Selective silencing of 2Cys and type-IIB Peroxiredoxins discloses their roles in cell redox state and stress signaling. *J Integr Plant Biol* 57, 591-601

Vilela B, Najar E, Lumbreras V, Leung J, Pages M (2015). Casein kinase 2 negatively regulates abscisic acid-activated SnRK2s in the core abscisic acid-signaling module. *Mol Plant* 8, 709–721

Vishwakarma, K, Upadhyay, N, Kumar, N, Yadav, G, Singh, J, Mishra, RK, Kumar, V, Verma, R, Upadhyay, R, Pandey, M (2017) Abscisic acid signaling and abiotic stress tolerance in plants: a review on current knowledge and future prospects. *Front Plant Sci* 8, 161

Vitulo N, Forcato C, Carpinelli EC, Telatin A, Campagna D, D'Angelo M, Zimbello R, Corso M, Vannozzi A, Bonghi C, Lucchin M, Valle G (2014) A deep survey of alternative splicing in grape reveals changes in the splicing machinery related to tissue, stress condition and genotype. *BMC Plant Biol* 14, 99

Walker MA, Lund K, Agüero C, Riaz S, Fort K, Heinitz C, Romero N (2014) Breeding grape rootstocks for resistance to phylloxera and nematodes - It's not always easy. *Acta Hortic* 1045, 89-97

Wang CR, Yang AF, Yue GD, Gao Q, Yin HY, Zhang JR (2008) Enhanced expression of phospholipase C 1 (ZmPLC1) improves drought tolerance in transgenic maize. *Planta* 227, 1127–1140

Watterson GA (1975) On the number of segregation sites. *Theor Pop Biol* 7, 256-276

Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, Ismail AM, Bailey-Serres J, Ronald PC, Mackill DJ (2006) Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature* 442, 705–708

Yamaguchi-Shinozaki K, Shinozaki K (2006) Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu Rev Plant Biol* 57, 781–803

Yancey PH (2005) Organic osmolytes as compatible, metabolic and counteracting cryoprotectants in high osmolarity and other stresses. *J Exp Bot* 208, 2819–2830

Yang Y, Mao L, Jittayasothorn Y, Kang Y, Jiao C, Fei Z, Zhong GY (2015) Messenger RNA exchange between scions and rootstocks in grafted grapevines. *BMC Plant Biol* 15, 251

Yoshida R, Hobo T, Ichimura K, Mizoguchi T, Takahashi F, Aronso J, Ecker JR, Shinozaki K (2002) ABA-activated SnRK2 protein kinase is required for dehydration stress signaling in Arabidopsis. *Plant Cell Physiol* 43, 1473-1483

Yoshida T, Fujita Y, Maruyama K, Mogami J, Todaka D, Shinozaki K, Yamaguchi-Shinozaki K (2015) Four Arabidopsis AREB/ABF transcription factors function predominantly in gene expression downstream of SnRK2 kinases in abscisic acid signalling in response to osmotic stress. *Plant Cell Environ* 38, 35–49

Yoshida T, Mogami J, and Yamaguchi-Shinozaki K (2014) ABA-dependent and ABA-independent signaling in response to osmotic stress in plants. *Curr Opin Plant Biol* 21, 133–139

Yu J, Pressoir G, Briggs WH, Vroh Bi I, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nat Genet* 38, 203–8

Yu LX (2017) Identification of single-nucleotide polymorphic loci associated with biomass yield under water deficit in alfalfa (Medicago sativa L.) using genome-wide sequencing and association mapping. *Front Plant Sci* 8, 1152

Yue Y, Zhang M, Zhang J, Duan L, Li Z (2011) Arabidopsis LOS5/ABA3 overexpression in transgenic tobacco (*Nicotiana tabacum* cv Xanthi-nc) results in enhanced drought tolerance. *Plant Sci* 181, 405–411

Zandalinas SI, Mittler R, Balfagon D, Arbona V, Gómez-Cadenas A (2017) Plant adaptations to the combination of drought and high temperatures. *Physiol Plant* 162, 2-12

Zentella R, Zhang Z, Park M, Thomas SG, Endo A, Murase K, Fleet CM, Jikumaru Y, Nambara E, Kamiya Y, Tai-ping S (2007) Global analysis of DELLA direct targets in early gibberellin signaling in *Arabidopsis. Plant Cell* 19, 3037–3057

Zhang L , Marguerit E, Rossdeutsch L, Ollat N, Gambetta GA (2016) The influence of grapevine rootstocks on scion growth and drought resistance. *Theor Exp Plant Phys*, 28, 143-157

Zhang P, Liu X, Tong H, Lu Y, Li J (2014) Association mapping for important agronomic traits in core collection of rice (Oryza sativa L.) with SSR markers. *PLoS ONE* 9, e111508

Zhao G, Webber H, Hoffmann H, Wolf J, Siebert S, Ewert F (2015) The implication of irrigation in climate change impact assessment: a European-wide study. *Glob Chang Biol* 21, 4031-4048

Zhao Y, Xing L, Wang X, Hou YJ, Gao J, Wang P, Duan CG, Zhu X, Zhu JK (2014) The ABA receptor PYL8 promotes lateral root growth by enhancing MYB77-dependent transcription of auxin- responsive genes. *Science Signal* 7, ra53

Zhou S, Hu W, Deng X, Ma Z, Chen L, Huang C, Wang C, Wang J, He Y, Yang G, He G (2012) Overexpression of the wheat aquaporin gene, TaAQP7, enhances drought tolerance in transgenic tobacco. *PLoS ONE* 7, e52439

Zhuo Ch, Wang T, Lu S, Zhao Y, Li X, Guoa Z (2013) A cold responsive galactinol synthase gene from Medicago falcata (MfGolS1) is induced by myo-inositol and confers multiple tolerances to abiotic stresses. *Physiol Plant* 149, 67–78

Zhou Y, Massonnet M, Sanjak JS, Cantu D, Gaut BS (2017) Evolutionary genomics of grape (Vitis vinifera ssp. vinifera) domestication. *Proc Natl Acad Sci USA* 114 (44), 11715-11720

Zhu JK (2016) Abiotic stress signaling and responses in plants. *Cell* 167, 313–324

Zok A, Olah R, Heideg E, Horvath VG, Kos PB, Majer P, Varadi GY, Szegedi E (2010) Effect of Medicago sativa ferritin gene on stress tolerance in transgenic grapevine. *Plant Cell Tiss Org Cult* 100, 339-344

Zou JJ, Wei FJ, Wang C, Wu JJ, Ratnasekera D, Liu WX, Wu WH (2010) Arabidopsis calcium-dependent protein kinase CPK10 functions in abscisic acid- and Ca2+-mediated stomatal regulation in response to drought stress. *Plant Physiol* 154, 1232–1243