Modello 1

PROGETTO DI RICERCA / PROJECT

Cognome e nome del candidato **Applicant's Name**	**MODINA DAVIDE**
Corso di dottorato **PhD**	**Dottorato in agricoltura, ambiente e bioenergia**
Codice di riferimento della linea di ricerca	R10/1

1) Titolo del progetto / Project title

INFLUENCE OF THE INTERACTION SCION X ROOTSTOCK ON GRAPEVINE RESPONSE TO DROUGHT STRESS AS A TOOL IN NEW GRAPEVINE ROOTSTOCKS BREEDING PROGRAMS.

2) Sommario

Considering climate change, viticulture will face several challenges. Particularly, an increasing of vine-growth areas under severe drought stress is expected in the next years. This could affect quality and yield of grapes, importance and style of wines and, generally, viticultural potential of many areas. Many studies suggest adaptive strategies to new environmental condition and use of drought-tolerant rootstocks is considered an effective mid-term tool in order to reducing and managing those negative impact. However, the number of usable rootstocks is limited and many of them was selected in late XIX century, when grapevine cultivation was totally different from nowadays. So, there is a general demand in drought tolerant rootstocks, suitable to a modern viticulture. In order to reach this purpose, could be important for breeders have more information about response of grapevine to drought stress and, particularly, on the complex interaction scion x rootstocks. Deepen this topic could give to breeders a key tool for their breeding programs.

The aim of this project is to evaluate scion x rootstock interaction on grapevine drought tolerance strategy, under reduced water availability and combining both scion and rootstock with different drought response strategy.

This information could become a key to guide breeders during selection of new rootstocks customized on specific cultivars or environments. Furthermore, it could be possible help farmers towards the best rootstock x scion choice.

3) Obiettivi e rilevanza dei risultati ottenibili nel contesto dello stato dell'arte / Project aims and their relevance in the context of the state of the art

A large part of the new challenges in viticulture are related to the climate change (Fraga et al., 2012; Malheiro et al., 2010), that can impact on style, personality and importance of wines (Jones, 2008), influencing yield and grape quality (Kenny & Harrison 1992; Jones 2005). Particularly, many vine-growth areas, will be exposed to increased water deficit, due to the weight of evapotranspiration in water balance (Shultz, 2016; Santos et al., 2003; Malheiro et al., 2010), impairing the plant growth, yield and quality of grape (Fregoni., 2013). In light of these, appropriate adaptive and mitigation strategies in order to reducing and managing those impact should be considering (Tubiello & Fischer 2007, Reidsma et al. 2010).

Several authors proposed the use of water-use efficient and drought-tolerant rootstocks as mid-term strategic and anticipatory tool for adapting viticulture to climate change (Fraga et al. 2012, Berdeja et al. 2015, Bianchi et al. 2018, Romero et al. 2018, Sabir and Sahin 2018; Neethling et al. 2017, van Leeuwen et al. 2019). Studies suggest that specific rootstocks–scion interactions and different experimental/soil–climate conditions are also important for rootstock performance and WUE yield in grapevines (Zhang et al. 2016).

Italian vine nursery sector:

The vine nursery sector in Italy plays an important role. According to the Italian Ministry of Agricultural, Food and Forestry Policies, in the 500 Italian nurseries are produced 80.000.000 – 110.000.000 vine grafted per year (about a third of them is exported) with an estimated value of € 100.000.000 - € 140.000.000.

Regarding rootstocks, the fields for mother plants occupy an area of 1950 ha and there is an increasing interest on new genotype. Indeed, the offer of rootstocks' genotypes is scarce: 5 (1103 Paulsen, 110 Richter, 140 Ruggeri, K5BB and SO4) of the 40 authorized to the cultivation in Italy are used for the 80% of the national vine-growth area (Scienza, 2018). Furthermore, most of them was selected in late '800, with the principal aim to fight Phylloxera (*Viteus vitifoliae)*. So, if the cultivation operations and technologies are being adapting to new viticultural models, the rootstocks are instead the same since a century, despite all the new challenge in this important sector.

About this, in 2014 was released 4 new rootstocks (called M-series) result of the breeding program of the University of Milan. These new genotypes have been well appreciated by farmers and in the season 2019/2020 about 1 million of vine plants were grafted on these rootstocks (Scienza, 2018). Particularly, M4 has been recognized as a promising material to establish vineyards in areas that are vulnerable to summer-related droughts (Frioni et al., 2020).

Response of grapevine to drought

In general, drought tolerance is an intricate concept (Tardieu et al.2012) that is linked to a combination of severe stresses (e.g., lack of available water, high temperatures, high evaporative demand).

It is hypothesized that grapevine has two types of stomatal response to water stress: (i) *isohydric cultivars* maintain constant leaf water potential by reducing stomatal conductance. (ii) *anisoidric cultivars* have a low stomatic control and a pronounced fluctuation of leaf water potential.

The effectness of this strategies is related to the enviremental conditions. For example, avoiding water stress through early closing of stomata could be positive under high stress conditions (Tardieau et al., 2012), but conversely could be negative in case of mild stress, involving a reduction in photosynthesis and an increase in the risk of heat stress due limited leaf area cooling (Tardieau et al., 2018).

However, the classification of *Vitis vinifera* cultivar in isohydric and anisohydric is controversial. In fact, It has been demonstrated that the same variety can have an isohydric or anisoidric behavior depending on the intensity of the stress condition (Levin et al., 2020; Hochberg et al., 2018; Schultz, 2003). Moreover several studies suggest that the same cultivar, grafted on different rootstocks, has different responses to water stress (Tandonnet et al., 2010; Faralli et al., 2021). Indeed, the rootstock could induced a different accumulation of ABA and stomatal sensitivy to ABA (Tombesi et al., 2015), hydraulic signals (Schultz et al., 2003), root acquaporine activity (Gambetta et al., 2012) and osmotic adjustment capacity (Prinsi et al., 2018).

Finally, it was well demostrated that the interaction between scion and rootstock is very closely (Serra et al., 2014; Tandonnet et al., 2010) and this more affect drought tolerance (Pou et al., 2008; Gambetta et al., 2012; Serra et al., 2014). Booth scion and rootstock use specific strategy of drought tolerance, so the question to answer is if the scion drought tolerance strategy is dominant on the strategy induced by rootstock or viceversa.

Drought tollerance response of rootstock M4 ((Vitis vinifera × Vitis berlandieri) × Vitis berlandieri cv. Resseguier n.1) was analyze in several study: (i) it was compared to rootstocks conventional classify as poor tolerance to water shortage (Galbignani et al., 2016; Merli et al., 2016; Meggio et al., 2014; Corso et al., 2015; Prinsi et al., 2018) or drought tolerant (Corso et al., 2016; Frioni et al., 2020). (ii) It was tested on near anisohydric (Galbignani et al., 2016; Merli et al., 2016; Corso et al., 2016) or near isohydric scion (Frioni et al., 2020). Neverless it was never compared in the same experiment grafted on isohyidric and anisohydric scion. Furthermore all experiments were conducted in plot.

Objective of the project

With the broad goal on mind to improve resilience of viticulture, able to thrive in the future scenario awaiting agriculture in the context of climate change, the principal aims of the project is:

- Evaluate scion x rootstock interaction on grapevine drought tolerance strategy under reduced water availability combining both scion and rootstock with isohydric or anisohydric strategy.

Relevance of the project

The result of the project could:

- **become a key to guide breeders on selection of new rootstocks customized on specific cultivars or environment.**
- help farmers towards the best rootstock x scion choice.
- Improve knowledge in precision irrigation management

4) Descrizione del progetto / Project description

The project will be divided into four work packages (WPs) distributed over the three years of the PhD program, as reported in Table 1.

Month	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S
Year	1st year									2nd year												3rd year											
WP1																																	
WP2																																	
WP3																																	
WP4																																	

Table 1. Timeline of the project. Activities of WP1 starts on January 2022. WP5 activities end September 30th of the third year.

With its aims, this project will be able to reach different useful accomplishments for research related to breeding programs of the present and the future, through the study of the interaction scion x rootstock in grapevine drought tolerance response in different environmental condition (available water; plot/field).

This information could be used in the rootstock breeding program, during phenotyping and genotyping screening of new genotypes.

WP1 literature review.

During this WP, the literature about grapevine drought tolerance strategies will be deepened and reviewed to collect necessary information for the development of the project.

Why literature review?

The drought tolerance strategies used by the grapevine, in particular the isohydric / anisohydric classification, is a controversial topic. In fact, studies suggest different hypotheses about it, as well as different methods for its evaluation. For this reason, their careful evaluation is necessary

WP2 Plant phenotyping and genotyping (in pots).

Sangiovese it is renowned as a near-anisohydric cultivar, while Montepulciano a near-isohydric (Tombesi et al., 2014). Rootstocks M4 and 1103 P are both considered drought tolerant (Frioni et al., 2020) using different strategies. A recent study compared M4 to 1103P in grafting combination with Grechetto Gentile. The two combinations maintained similar water potential under water stress, though M4 showed higher photosynthesis and water use efficiency (Frioni et al., 2020). This WP analyses 4 grafting combination (Sangiovese x M4; Sangiovese x 1103P; Montepulciano x M4; Montepulciano x 1103P) and 4 own-rooted (Sangiovese, Montepulciano, 1103P, M4).

2.1 Plant material and treatment layout.

The 4 grafting combination (Sangiovese x M4; Sangiovese x 1103P; Montepulciano x M4; Montepulciano x 1103P) and the 4 own-rooted (Sangiovese, Montepulciano, 1103P, M4) are planting in 40 litres pots. For each of that, 6 biological replicas are used. Then the 48 plants are randomly assigned to two treatments: well-watered (WW) and water-stressed (WS). When the plants achieved 18-20 unfolded leaf and the air temperatures are high, irrigation are stopped in all WS plant, until reaching a severe drought stress (around Ψ_{pd} **-0,8 MPa**). After that, WS plant are re-watered. WW plants receive regular irrigation during the entire vegetative season. Since the high volume of the plots, it's not possible using the gravimetric method for estimate the Soil Water Content (SWC). However, it is possible use the pre-dawn water potential (Ψ_{pd}) as a proxy of soil water potential (SWP) and through the estimation of the soil water retention curve, estimate the SWC.

2.2 Assessing response to drought stress.

The following parameters will measure to evaluate the response of different grafting combination and own rooted plants to drought stress. Timing of samples will be as following, for each treatment:

	No water deficit	Mild water deficit	Moderate water deficit	Severe water deficit	No water deficit
Ψ_{pd} (MPa)	From 0 to -0.3	From -0.3 to -0.5	From -0.5 to -0.8	<- 0.8	From 0 to -0.3
Time	1	2	3	4	5

Time interval between each sampling could depend on the seasonal weather condition. Water stress condition is defined using Predawn Water Potential thresholds suggest by Carbonneau (1998).

2.2.1 Gas Exchange Parameters and Chlorophyll Fluorescence.

Through a Leaf Portable Photosynthesis System, gas exchange indicators will be measured: photosynthetic activity (Pn; μmol CO2 m^{-2} s^{-1}), stomatal conductance (Gs; mmol H2O m^{-2} s^{-1}) transpiration (E; mmol H2O m^{-2} s^{-1}) and the water use efficiency (WUE_{leaf}). In order to evaluate the efficiency of photosystem II, Chlorophyl Fluorescence will be measured.

2.2.2 Leaf Water Status.

Seasonal progress of water stress will be monitored using a Scholander Chamber (Scholander et al., 1965) through the measure of the pre-dawn water potential prior to sunrise. At midday, the leaf water potential will be measured to evaluate the leaf water status. Thermal imagines will be taken to estimate transpiration.

2.2.3 Estimation of the canopy development

At each time, using a smart-app, LAI will be estimated. Furthermore, the increasing of the shoot's length will be evaluated. During winter pruning, wood will be weighed in order to evaluate the canopy biomass produce during the season.

2.2.4 Analysis of differential expressed genes under drought conditions via RNA-seq

At each time, differential expressed genes under drought conditions will be evaluated via RNA-seq in order to identify some key genes involved in the response of grapevine rootstocks to water shortage for the future breeding program.

2.3 *Data analyse*

At the end of the experiment, data will analyse and compared to literature. Results could be used as useful phenotypic and genotypic characters in rootstocks breeding programs.

WP3 Plant Phenotyping (in vineyard)

The same WP2 experimental design will be conduct in field (excluding task 2.2.4, due possible high cost of the analysis), using productive grapevine plants. This WP is focused only on the 4 grafting combination (Sangiovese x M4; Sangiovese x 1103P; Montepulciano x M4; Montepulciano x 1103P) and on own rooted rootstock (1103; M4). This WP could take place in commercial vineyards during the period of the phD course planned in the partner company.

Why in vineyard experiment?

Unlike the pot test, the work in the vineyard is more subject to seasonal weather. However, under natural soil, root can express its exploration capacity, while pot constraint has effect on biomass allocation, nutrient uptake and root confinement. Furthermore, pot size has been shown to be a major determinant of biomass growth in crop plants grown in controlled environmental conditions (Porter et al., 2012). In light of this, several authors suggest taking experiment in open field condition (e.g., Faralli et al., 2021; Bianchi et al., 2020).

WP4 Thesis writing and related activity

This WP will be dedicated to publication of scientific papers (a review about the PhD topic will be publish at the end of the 1st year; preliminary results could be published at the end of the 2nd year) and to the thesis writing and discussion (concentrated especially in the last three months of the phD course).

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