

PhD School on Agriculture, Environment and Bioenergy

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(XL cycle, 2024-27)

Project draft

1. Field of interest

AGRI-03/A Arboricoltura Generale e Coltivazioni Arboree

2. Project title

Research and Evaluation of Alternative Vitis Species for New Rootstocks' Breeding

3. Tutor: Prof. Lucio Brancadoro

co-tutor/s: Prof. Gabriella De Lorenzis

4. Relevance of the topic and state of the art:

The ongoing climate change is currently leading to new challenges for European viticulturists. Among these challenges, the most complex lies in the need to find new ways to address the abiotic stresses imposed by the progressive rise in temperatures, including water stress and saline stress (Quénol et al., 2014). In this context genetic research in viticulture is increasingly focusing on the role played by rootstocks, considered as one of the most important agronomic tools for vineyard management, as well as one of the most effective mediators of traditional cultivar adaptation to a changing environment (Khan et al., 2020). Moreover, working on the molecular bases of rootstocks allows the development of climate change adaptation strategies while respecting the economic, social, and environmental sustainability needs of consumers. In fact, working on rootstocks allows for not interfering with the territory by affecting its water reserves or biological fertility using exogenous compounds. In Europe, significant efforts are being made to obtain new genotypes with good tolerance to water and saline stress and the recent commercialization of the M series rootstocks has made a significant contribution to the sector (Bianchi & Brancadoro, 2021). At present, genetic research on rootstocks primarily relies on century-old material from wild American *Vitis* species, originally introduced by pioneering European researchers combating Phylloxera in the Old World. As a result, the repeated utilization of these original parent plants to generate new genotypes, coupled with the rigorous selection process applied to existing individuals in Europe, has resulted in a depletion of available genetic diversity. This has consequently diminished the efficacy of breeding programs. The project aims to find new alternative *Vitis* species to bring variability to rootstocks breeding. These new species will help improving the crossing and selection process, leading to the development of new rootstocks, tailored for the needs of modern viticulture.

5. Layout of the project (draft)

5.1. Materials & Methods:

The Department of Agricultural and Environmental Sciences at the University of Milan has at its disposal an extensive collection of American wild grapevines potentially applicable in rootstock breeding. This collection includes: i) a parental population of over 230 genotypes, encompassing most commercially available rootstocks. The entire genetic diversity of the parental population is represented by a core collection of 70 genotypes (Migliaro et al., 2019); ii) a new population of approximately 140 genotypes obtained in a recent breeding program; iii) a total of about 60 new uncharacterized genotypes belonging to 12 American progenies and provided by the USDA-ARS National Clonal Germplasm Repository at UC Davis. Phenotyping of both root and shoot systems is currently underway on genotypes within the core collection to identify morphological traits of

interest. Additionally, new genetic materials destined to enhance rootstock breeding programs are expected to be acquired from the United States of America.

Thus, for the final selection of candidate individuals for new breeding programs, three distinct steps will be taken on both the populations already available in the ampelographic collections of Milan and the seedlings obtained from the Viala Project:

- a. Phenotyping activities will aim to identify morphological traits of interest on both the root (root depth, geotropic angle, root density, etc.) and shoot (leaf biomass/root biomass ratio, Photochemical Reflectance Index, etc.) apparatus. Phenotyping will be conducted under controlled conditions to simulate stress conditions.
- b. Transcriptomic analysis will be performed to determine which genes are promoted or inhibited in expression under the same simulated stress conditions. Transcriptomic analysis will be conducted via RNA-seq using third-generation sequencing methodologies (NGS).
- c. Phenotypic data, in combination with accessible genetic profiles (assessed using SNP markers), will be utilized to pinpoint genetic regions linked to each analyzed trait through a genome-wide association approach (GWA).

5.2. Schedule and major steps (3 years):

The project will span a duration of three years. The initial year will be devoted to establishing the experimental framework based on analysis of existing scientific literature. Phenotyping of the root and shoot systems will take place during the vegetative growth seasons of the first and second years, with sampling for RNA-seq analysis occurring in the second year.

The third year will be exclusively dedicated to the Genome-Wide Association (GWA) study. Two scientific publications are expected to be produced, one in the second year and another in the third year of the project. Data analysis and statistical evaluations will be conducted at the conclusion of each year.

| | YEAR 1 | YEAR 2 | YEAR 3 |
|---------------------|--------|--------|--------|
| LITERATURE ANALYSIS | x | | |
| EXPERIMENTAL SETUP | x | | |
| PHENOTYPING | x | x | |
| RNA-SEQ | | x | |
| GWAS | | | x |
| DATA ANALYSIS | x | x | x |
| PUBLICATIONS | | x | x |

6. Available funds (to support research): CTE_NAZPR18LBRAN_01, €10.000

7. Co-Financing (to support the bourse): -

8. Literature:

- Bianchi, D., & Brancadoro, L. (2021). Water Use Efficiency and Nutritional Status of a New Grapevine Rootstock Selection. *Horticulturae*, 7(11), 503. <https://doi.org/10.3390/horticulturae7110503>
- Khan, M. M., Akram, M. T., Qadri, R. W. K., & Al-Yahyai, R. (2020). Role of grapevine rootstocks in mitigating environmental stresses: A review. *Journal of Agricultural and Marine Sciences [JAMS]*, 25(2), 1. <https://doi.org/10.24200/jams.vol25iss2pp1-12>
- Migliaro, D., De Lorenzis, G., Di Lorenzo, G. S., De Nardi, B., Gardiman, M., Failla, O., Brancadoro, L., & Crespan, M. (2019). Grapevine Non-*vinifera* Genetic Diversity Assessed by Simple Sequence Repeat Markers as a Starting Point for New Rootstock Breeding Programs. *American Journal of Enology and Viticulture*, 70(4), 390–397. <https://doi.org/10.5344/ajev.2019.18054>

Quénol, H., Barbeau, G., Hofmann, M., Foss, C., Grosset, M., Barbeau, G., Van Leeuwen, K., Irimia, L., Rochard, J., Boulanger, J.-P., Tissot, C., & Miranda, C. (2014). *Adaptation of viticulture to climate change: high resolution observations of adaptation scenario for viticulture: the adviclim european project* (Vol. 87). <https://www.researchgate.net/publication/274385639>