

CHARACTERIZED ONE OF THE LARGEST COLLECTIONS OF GRAPEVINE ROOTSTOCKS (NON-VINIFERA) BY MOLECULAR MARKERS

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The non-vinifera germplasm: the stranger!

Since the first decade of the 19th century, grapevine has been grown on rootstocks, represented by a mix of non-vinifera grapevine species, such as *Vitis berlanderi*, *Vitis rupestris*, *Vitis riparia* and hybrids. Rootstocks contribute to control other soil borne pests (e.g., phylloxera and nematodes) and different environmental conditions (e.g., drought and salinity) (Galet, 1998; Whiting, 2005).

To date, rootstock genetic background is extremely narrow to the extent that less than ten rootstocks are used worldwide for grafting (Keller, 2010). Moreover, these genotypes, released at the end of the 19th century, seem to be inadequate to cope with the emerging challenges in viticulture related to climate change (Huglin and Schneider, 1998).

Since 1985, the DiSAA (Department of Agricultural and Environmental Sciences) research group operating at the University of Milan (UMIL) has been working on the selection of new grapevine rootstocks. M-series (M1, M2, M3 and M4) rootstocks were selected and, recently, registered at the National Register of *Vitis* varieties. These rootstocks showed tolerance to iron-limited conditions (M1 > M3) (Porro et al., 2013; Vannozzi et al., 2017), moderate resistance to salinity (M2 and M4) (Porro et al., 2013; Meggio et al., 2014), and high tolerance to drought (M4) (Porro et al., 2013; Meggio et al., 2014; Corso et al., 2015; Bianchi et al., 2020b).

Because we are far from solving the climate change challenges, new genotypes dealing with unfavorable environmental conditions should be released in the next future. Our group is keeping

working on this in way. In order to select the most promising genotypes for forthcoming rootstock breeding programs, we established one of the largest non-*vinifera* germplasm collections at UMIL, collecting plant materials from Italy, Spain, and USA, and assessed the genetic variability in the new repository.

The step-by-step methodologies

The first step towards the selection of new pre-breeding materials was to genotype the new non-*vinifera* collection. In collaboration with the Research Centre for Viticulture and Enology, (Conegliano, Italy) the collection was genotyped using 22 SSR (Simple Sequence Repeat) markers (Migliaro et al., 2019). These markers are the same universally applied to genotype the *vinifera* germplasm (Laucou et al., 2011). The SSR profiles were used to identify unique profiles, to investigate the genetic diversity of the collection and to sort out a core collection. Finally, the core collection was genotyped using the new high-throughput genotyping array holding more than 18kK SNP markers (Laucou et al., 2018). The SNP profiles were used to compare the genetic variability between non-*vinifera* and *vinifera* compartments (Bianchi et al., 2020a).

The genetic variability of non-*vinifera* collection at UMIL

The non-*vinifera* is located in the winegrowing region called Oltrepò Pavese (9°05'E; 44°58'N; 144 m asl) collection and, to date, it accounts for more than 400 accessions. Three hundred and seventy nine individuals were genotyped with 22 SSRs. As expected, approximately 39% of accessions represented redundant germplasm and 232 unique genotypes were detected. Among them, 111 genotypes were identified according to information available in literature and databases cross-checking. More than 50% of genotypes were unknown genotypes. Since the collection hosts genotypes belonging to different species and hybrids, a large number of alleles was detected, as well as a large genetic diversity. The genetic analyses identified three main ancestral groups: i) one cluster included mainly *V. rupestris* and *V. riparia* genotypes; ii) one grouped the progenies of the three varieties most used in the past for rootstock breeding programs (e.g. *V. berlandieri* Ressayguiere 2, *V. riparia* Gloire de Montpellier, *V. rupestris* du Lot); another one grouped mostly *V. labrusca* hybrids. A fourth, admixed group accounted for individuals with complex pedigree, where more than two species were involved (Migliaro et al., 2019).

A core collection feasible to handle

Large germplasm collections are difficult to manage for accurate phenotyping. In order to overcome this problem, we extrapolated a core collection capturing the entire genetic diversity in a restricted number of genotypes feasible to handle (Brown, 1995). Our core collection accounted for 70 genotypes. Interestingly, only 7% of genotypes were *berlandieri* × *rupestris* and *berlandieri* × *riparia* genotypes. The majority of core collection individuals were unknown genotypes, with few information in terms of ampelographic and agronomic traits. Moreover, this core collection included the putatively novel pre-breeding materials.

The SNP are a useful tool to study the genetic diversity of non-vinifera germplasm

So far, the Vitis18kSNP genotyping array has been extensively used to investigate the genetic diversity of *vinifera* germplasm (De Lorenzis et al., 2015, 2019; Mercati et al., 2016; Ruffa et al., 2016; Laucou et al., 2018; Sunseri et al., 2018; D'Onofrio et al., 2021). The array, set up by the GrapeReSeq Consortium, holds around 18k SNP markers. Some of them (around 13.5k) were detected re-sequencing 47 *V. vinifera* genotypes, while the others (around 4.5k) were detected in the genome of non-vinifera species (*Vitis aestivalis*, *V. berlandieri*, *Vitis cinerea*, *Vitis labrusca*, *Vitis lincecumii*, and *Muscadinia rotundifolia*) (Laucou et al., 2018). Recently, the array has been validated on non-vinifera genotypes as well, using the core collection as training population (Bianchi et al., 2020a). More than 14k loci were successfully amplified, with 61% of markers identified in the *V. vinifera* genome and around 39% of SNPs identified in the genome of other species, confirming the usefulness of this tool in genotyping non-vinifera germplasm. The uniqueness and preciousness of core collection has been proved by genetic analyses. Four ancestral groups have been identified: i) group 1 clustered only three known genotypes (*V. berlandieri* × *V. riparia*, *V. vinifera* × *V. rupestris* and *V. labrusca*); ii) group 2 clustered *V. cordifolia*, *V. labrusca*, *V. rupestris*, and *V. vinifera* genotypes; iii) group 3 clustered genotypes with *V. candicans*, *V. labrusca*, *V. longii* and *V. rupestris* in their pedigree; iv) group 4 clustered pure *V. riparia* genotypes and most of unknown genotypes (Bianchi et al., 2020a). Moreover, when the non-vinifera SNP profiles were compared with the *vinifera* ones, a clear differentiation among the two compartments has been highlighted (Bianchi et al., 2020a).

The promising novel breeding materials

In order to identify rootstocks breeding materials not commonly used so far, a new non-vinifera collection was established at UMIL. The collection showed high genetic variability, summarized in a core collection encompassing 70 genotypes. Most of this variability has not explored yet and

preliminary results highlighted that some genotypes showed promising behavior to cope with drought, with performances sometimes better than the one detected in tolerant reference rootstocks (Bianchi et al., 2018). We are on the good way to get the most promising genotypes for forthcoming rootstock breeding programs.

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References

- Bianchi, D., Brancadoro, L., and De Lorenzis, G. (2020a). Genetic Diversity and Population Structure in a *Vitis* spp. Core Collection Investigated by SNP Markers. *Diversity* 12, 103.
- Bianchi, D., Caramanico, L., Grossi, D., Brancadoro, L., and De Lorenzis, G. (2020b). How do novel M-rootstock (*Vitis* spp.) genotypes cope with drought? *Plants* 9. doi:10.3390/plants9101385.
- Bianchi, D., Grossi, D., Tincani, D. T. G., Simone Di Lorenzo, G., Brancadoro, L., and Rustioni, L. (2018). Multi-parameter characterization of water stress tolerance in *Vitis* hybrids for new rootstock selection. *Plant Physiol. Biochem.* 132, 333–340. doi:10.1016/j.plaphy.2018.09.018.
- Brown, A. H. D. (1995). “The core collection at the crossroads,” in *Core Collections of Plant Genetic Resources*, eds. T. Hodgkin, A. H. D. Brown, T. J. L. van Hintum, and E. A. V. Morales (Chichester: A Wiley-Sayce Publication), 3–19.
- Corso, M., Vannozzi, A., Maza, E., Vitulo, N., Meggio, F., Pitacco, A., et al. (2015). Comprehensive transcript profiling of two grapevine rootstock genotypes contrasting in drought susceptibility links the phenylpropanoid pathway to enhanced tolerance. *J. Exp. Bot.* 66, 5739–5752. doi:10.1093/jxb/erv274.
- D’Onofrio, C., Tumino, G., Gardiman, M., Crespan, M., Schneider, A., Bignami, C., et al. (2021). Parentage Atlas Of Widely And Locally Cultivated Italian Grapevine Varieties As Inferred From SNP Genotyping. *Front. Plant Sci.* 11, 605934.
- De Lorenzis, G., Chipashvili, R., Failla, O., and Maghradze, D. (2015). Study of genetic variability

- in *Vitis vinifera* L. germplasm by high-throughput Vitis18kSNP array: The case of Georgian genetic resources. *BMC Plant Biol.* 15, 154. doi:10.1186/s12870-015-0510-9.
- De Lorenzis, G., Mercati, F., Bergamini, C., Cardone, M. F., Lupini, A., Mauceri, A., et al. (2019). SNP genotyping elucidates the genetic diversity of Magna Graecia grapevine germplasm and its historical origin and dissemination. *BMC Plant Biol.* 19, 7. doi:10.1186/s12870-018-1576-y.
- Galet, P. (1998). *Grape varieties and rootstock Varieties.* , ed. T. J. Smith Chaintré, France: Oenoplurimedia.
- Huglin, P., and Schneider, C. (1998). *Biologie et Écologie de la Vigne.* , ed. T. & D. Lavoisier Paris.
- Keller, M. (2010). Managing grapevines to optimize fruit development in a challenging environment: A climate change primer for viticulturists. *Environ. Sustain. Vitic. Pract. Pract.*, 259–292. doi:10.1201/b18226.
- Laucou, V., Lacombe, T., Dechesne, F., Siret, R., Bruno, J.-P., Dessup, M., et al. (2011). High throughput analysis of grape genetic diversity as a tool for germplasm collection management. *Theor. Appl. Genet.* 122, 1233–45.
- Laucou, V., Launay, A., Bacilieri, R., Lacombe, T., Adam-Blondon, A.-F., Bérard, A., et al. (2018). Extended diversity analysis of cultivated grapevine *Vitis vinifera* with 10K genome-wide SNPs. *PLoS One* 13, 1–27.
- Meggio, F., Prinsi, B., Negri, A. S., Simone Di Lorenzo, G., Lucchini, G., Pitacco, A., et al. (2014). Biochemical and physiological responses of two grapevine rootstock genotypes to drought and salt treatments. *Aust. J. Grape Wine Res.* 20, 310–323. doi:10.1111/ajgw.12071.
- Mercati, F., De Lorenzis, G., Brancadoro, L., Lupini, A., Abenavoli, M. R., Barbagallo, M. G., et al. (2016). High-throughput 18K SNP array to assess genetic variability of the main grapevine cultivars from Sicily. *Tree Genet. Genomes* 12, 59.
- Migliaro, D., De Lorenzis, G., Simone Di Lorenzo, G., De Nardi, B., Gardiman, M., Failla, O., et al. (2019). Grapevine non-vinifera genetic diversity assessed by SSR markers as a starting-point for new rootstock breeding programs. *Am. J. Enol. Vitic.* in press.
- Porro, D., Pedò, S., Bertoldi, D., Bortolotti, L., Failla, O., and Zamboni, M. (2013). *Evaluation of new rootstocks for grapevine: Nutritional aspects.*
- Ruffa, P., Raimondi, S., Boccacci, P., Abbà, S., and Schneider, A. (2016). The key role of “Moscato bianco” and “Malvasia aromatica di Parma” in the parentage of traditional aromatic grape varieties. *Tree Genet. Genomes* 12, 50.
- Sunseri, F., Lupini, A., Mauceri, A., De Lorenzis, G., Araniti, F., Brancadoro, L., et al. (2018).

- Single nucleotide polymorphism profiles reveal an admixture genetic structure of grapevine germplasm from Calabria, Italy, uncovering its key role for the diversification of cultivars in the Mediterranean Basin. *Aust. J. Grape Wine Res.* 24, 345–359. doi:10.1111/ajgw.12339.
- Vannozzi, A., Donnini, S., Vigani, G., Corso, M., Valle, G., Vitulo, N., et al. (2017). Transcriptional Characterization of a Widely-Used Grapevine Rootstock Genotype under Different Iron-Limited Conditions. *Front. Plant Sci.* 7, 1–17. doi:10.3389/fpls.2016.01994.
- Whiting, J. R. (2005). “Grapevine rootstocks,” in *Viticulture, volume 1: Resources, 2nd Edition*, eds. P. R. Dry and B. G. Coombe (Ashford, Australia: Winetitles Pty Ltd), 167–188.