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First insights on the intra-species diversity in *V. berlandieri*: environmental adaptation and agronomic performances when used as rootstock

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Abstract:
Context and purpose of the study - In grafted plants, such as grapevine, increasing the diversity of rootstocks available to growers is an ideal strategy to get adaptation to climate change. The rootstocks used for grapevine are hybrids of various American *Vitis*, including *V. berlandieri*. The rootstocks currently used in vineyards are derived from breeding programs involving very small numbers of parental individuals.

Material and method - In 2005, 78 wild female *V. berlandieri* plants were ampelographically identified in Texas, USA. The coordinates and elevation of each sampling site were recorded. After growing the plants in field, we selected 286 genotypes. A genotyping by sequencing allowed us to extract 104,378 SNP and to explore the genetic structure of the *V. berlandieri* population. After grafting, 846 plants were grown in pots and evaluated for root-related traits for two independent years. In our experimental design, four rootstocks (110R, 5BB, SO4, and Börner) widely used in vineyards were added to the study population in order to evaluate the agronomical performances of *V. berlandieri* wild rootstocks.

Results:

Two subpopulations were highlighted and related with variations in elevation, temperature and rainfall of sampling locations. A genome-environment association study highlighted 18 genetic markers associated with environmental variations. Root-related traits have shown a moderate variability (coefficient of variation from 0.15 to 0.45). Two genotypes were detected for their root-related traits performances when compared with commercial rootstocks. Moreover, 8 genetic markers were associated with four root related traits (the root average diameter, the number of small roots, the number of medium roots, and the total number of roots).

Our results shed new light on rootstock genetics and could open up possibilities for introducing greater diversity into genetic improvement programs for grapevine rootstocks in order to adapt grapevine facing climate change.

Keywords : root system architecture, genetics, QTL, water use efficiency, grafting

1. Introduction

Climate change is driving a need to adapt to new environmental conditions through many approaches, including the modification of plant material. Grapevine is a major horticultural crop around the world. Since the decimation of grapevine crops due to the phylloxera crisis in Europe, grapevine has been cultivated as a grafted crop. Most of the rootstocks used are hybrids between the American *Vitis* species *V. rupestris*, *V. berlandieri*, and *V. riparia* (Galet 1988). Thanks to the interactions that occur between the scion and the rootstock in grapevine, the rootstock is a precious tool for grapevine adaptation (Ollat et al. 2016). Despite the interest of using rootstock to adapt grapevine scion, the genetic diversity of Vitis American species remains poorly studied keeping its potential for grapevine breeding into the shade (Péros et al. 2021). The use of *Vitis berlandieri* as a rootstock has been shown to confer a high tolerance to limestone, drought and phylloxera on the scion (Boubals 1966; Galet 1988). However, the rooting and grafting performances of *V. berlandieri* are generally limiting for its direct use as a rootstock, and it is usually crossed with other American species to obtain hybrids, which are widely used in vineyards: 1103P, 110R, Fercal, SO4, and Gravesac (FranceAgrimer, 2018).

The aims of this study were: i) to describe the genetic structure of a natural population of *Vitis berlandieri*; ii) to characterize root-related traits in a wild American *Vitis* species used for grafting; iii) to compare the trait performances of wild genotypes with those of commercial rootstocks. Our results highlighted the diversity available in nature for grapevine rootstocks at the genetic and the phenotypic levels. In addition, a few genotypes have revealed high performances for agronomic traits of interest at the root level.

2. Material and methods

Plant material - The plant material used in this study consisted of 286 genotypes originating from 78 mother plants of wild *V. berlandieri* collected from the Edwards Plateau in Texas, USA (see Blois et al. in revision for further details). All the plants were used as rootstocks, onto which we grafted *Vitis vinifera* Riesling (clone 24-209 for two consecutive years, 2019 and 2020). The commercial rootstocks 110R (*V. berlandieri* cv. Boutin B × *V. rupestris* cv. Martin), SO4 (*V. berlandieri* Rességuier 2 × *V. riparia* Gloire de Montpellier), Börner (*V. riparia* 183 G × *V. cinerea* Arnold) and 5BB (*V. berlandieri* Resséquier 2 × *V. riparia* Gloire de Montpellier) (2020 only) were added to the population as control genotypes.

Population structure and environment association - A new whole genome sequencing allowed us to obtain a reference genome for *Vitis berlandieri* (*V. berlandieri* 10585). A genotyping by sequencing of the 286 genotypes led to a set of markers composed by 104,378 SNPs. We ran STRUCTURE v2.3.4 (Pritchard, Stephens, and Donnelly 2000) based on SNPs information (burn-in period of 100,000 and MCMC of 100,000 iterations). The altitude has been noticed for each sampling point and the TerraClimate plateform (Abatzoglou et al. 2018) was used to extract 10 climatic parameters for each location. A genome environment association study has been carried out on GAPIT using a BLINK model (Huang et al. 2018) in order to identify associations between genetic regions and environmental parameters.

Root phenotyping - Plants were pruned after 2 nodes, potted out, washed with pressurized water and stored in a cold room (3°C) at the end of November. The following year (May 2020 and January 2021 for plants grafted in 2019 and 2020, respectively), roots were cut 4 cm below the collar. Scions and rootstocks were measured with a semi-automatic caliper to determine their diameter and weighed. Primary roots were counted and all diameters were measured with the semi-automatic caliper. Roots were sorted according to their diameter. Those with a diameter of less than 1mm were considered to be *small* roots, those with a diameter of 1 to 2 mm were considered to be *medium-sized* and those with a diameter of more than 2 mm were considered to be *large* roots. The entire root system was dried in a drying oven at 80°C for three days and weighed. The traits measured were root dry weight (RDW), the total number of roots (Tot_Root_NB), total root diameter (Tot_diam), calculated as the sum of all primary root diameters for a single plant, average diameter (Av_Diam), calculated as the mean diameter of all primary roots from the same plant, the number of small roots (NB_Small, diameter < 0.1 cm), the number of medium-sized roots (NB_Medium, 0.1 cm > diameter <0.2 cm), the number of large roots (NB_Large, diameter > 0.2 cm), the proportion of small roots (Prop_Small), the proportion of medium-sized roots (Prop_Medium, I), the proportion of large roots (Prop_Large), scion diameter (SD), rootstock diameter on the thinner and wider sides (RSD_1 and RSD_2, respectively) and the weight of the woody part (PW). For each root related trait, a mixed linear model has been constructed in order to access the broad sense heritability, which represents the genetic part of the phenotypic variance. A PCA based on root-related traits was constructed. Then, a genome wide association study has been carried out on GAPIT using a BLINK model (Huang et al. 2018) for each trait in order to highlight genetic markers associated with root-related traits.

3. Results and discussion

Population structure and environment association - The STRUCTURE analysis revealed two subpopulations clearly separated geographically (Fig. 1) confirming the huge genetic diversity of wild *Vitis* species (This, Lacombe, and Thomas 2006). Based on environmental parameters, the two subpopulations evolved into distinct climatic areas. The subpopulation 1 was in a hot and wet region and the subpopulation 2 was in a cool and dry region. The genome environment association revealed 18 genetic markers associated with environmental variations. These results revealed genetic regions associated with local adaptation (Williams 1996) which constitute highly valuable information for grapevine breeding.

The high root-related traits variability in a natural population of V. berlandieri - Phenotypic variability was observed for root traits in the *V. berlandieri* population in 2020 and 2021. The H^2 of root-related traits was moderate to high (from 0.36 to 0.82). A PCA based on root traits variability revealed that the variables were organized similarly in 2020 and 2021 and the first two principal components explained 80% of the variability of traits (Fig. 2). The commercial rootstocks had similar coordinates in the two years, with 110R and Börner located close together on the graph. 5BB was present only in the 2021 panel and can be distinguished by its high RN. The 26186, 25436, and 24894 genotypes stood out on the individual PCA graph because they had similar extreme coordinates to the controls on the PCA, for both years of the experiment. The commercial rootstocks used have been reported to confer vigor on the scion, together with tolerance to drought, limestone and phylloxera, all of which are parameters of interest for grapevine rootstocks. The drought tolerance of commercial rootstocks is considered very high for 110R, high for Börner, moderate-to-high for SO4 and low for 5BB. The

widespread use of these commercial rootstocks reflects their good performance in the field. We therefore assumed that they perform better than other rootstocks in the field due to their specific root-system profiles. Then, *V. berlandieri* genotypes with root-system profiles similar to those of commercial rootstocks (24894, 25436 and 26186) therefore constitute promising candidates for use as parental material in breeding programs. Moreover, genetic markers were associated with the root average diameter, the total number of roots, the number of small roots, and the number of medium root (Fig. 3). These genetic markers could be used in breeding programs in order to improve the rooting ability of rootstocks.

4. Conclusions

Our results confirmed the existence of highly valuable genetic resources for grapevine breeding in nature. We have been able to highlight genetic markers associated with environmental adaptation and root-related traits in a natural *V. berlandieri* population. These genetic markers could be used in grapevine breeding in order to select more precisely new candidate genotypes. Then, our results revealed that the genetic diversity available in nature has to be studied and compared to the genetic diversity already used in the vineyard.

5. Acknowledgment

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6. Literature cited

Abatzoglou, John T., Solomon Z. Dobrowski, Sean A. Parks, and Katherine C. Hegewisch. 2018. "TerraClimate, a High-Resolution Global Dataset of Monthly Climate and Climatic Water Balance from 1958–2015." *Scientific Data* 5 (1): 170191. https://doi.org/10.1038/sdata.2017.191.

Boubals, Denis. 1966. "Etude de La Distribution et Des Causes de La Résistance Au Phylloxera Radicicole Chez Les Vitacées," no. 16: 145–84.

Galet, Pierre. 1988. *Cépages et Vignobles de France - Tome 1 Les Vignes Américaines*. IMPRIMERIE Charles DEHAN.

Huang, Meng, Xiaolei Liu, Yao Zhou, Ryan M Summers, and Zhiwu Zhang. 2018. "BLINK: A Package for the next Level of Genome-Wide Association Studies with Both Individuals and Markers in the Millions." *GigaScience* 8 (2): giy154. https://doi.org/10/gg5ws7.

Lynch, Jonathan. 1995. "Root Architecture and Plant Productivity." *Plant Physiology* 109 (1): 7–13.

Ollat, N., A. Peccoux, D. Papura, D. Esmenjaud, E. Marguerit, Jean-Pascal Tandonnet, L. Bordenave, et al. 2016. "Rootstocks as a Component of Adaptation to Environment." In . Chichester: John Wiley & Sons Ltd.

Péros, Jean-Pierre, Peter Cousins, Amandine Launay, Philippe Cubry, Andy Walker, Emilce Prado, Elisa Peressotti, et al. 2021. "Genetic Diversity and Population Structure in Vitis Species Illustrate Phylogeographic Patterns in Eastern North America." *Molecular Ecology* 30 (10): 2333–48. https://doi.org/10/gkgtr4.

Pritchard, Jonathan K., Matthew Stephens, and Peter Donnelly. 2000. "Inference of Population Structure Using Multilocus Genotype Data." *Genetics* 155 (2): 945–59.

This, Patrice, Thierry Lacombe, and Mark R. Thomas. 2006. "Historical Origins and Genetic Diversity of Wine Grapes." *Trends in Genetics* 22 (9): 511–19. https://doi.org/10/czzgc8.

Williams, George Christopher. 1996. *Adaptation and Natural Selection: A Critique of Some Current Evolutionary Thought*. Facsim. ed., with New pref. Princeton Science Library. Princeton, N.J: Princeton University Press.

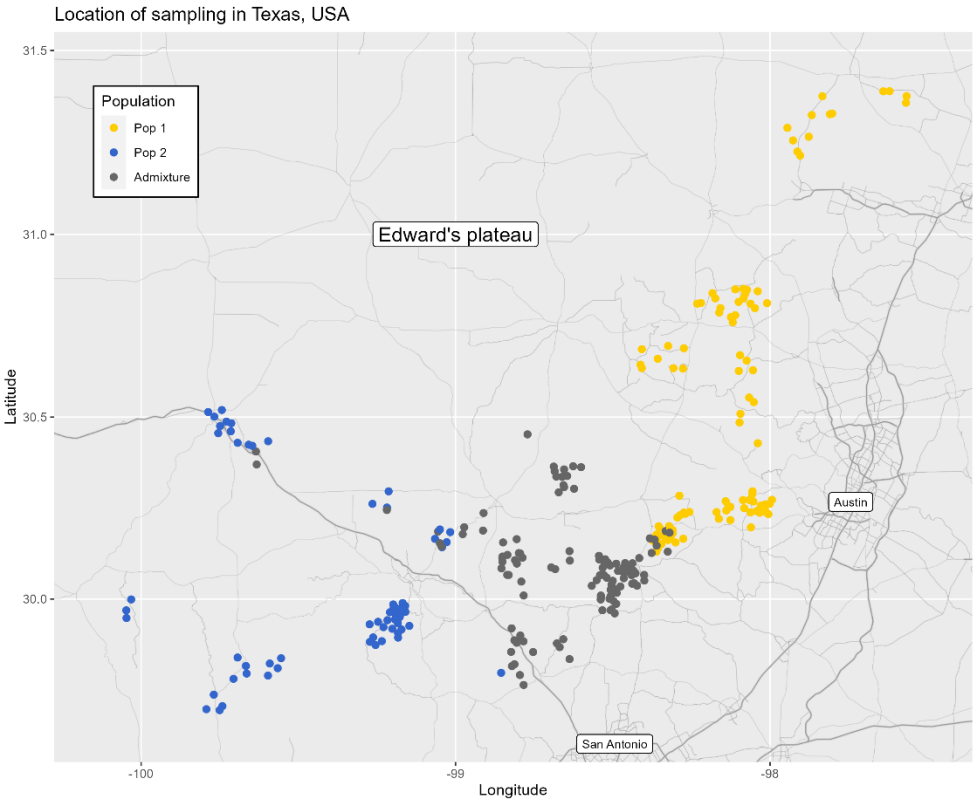

Fig. 1: Geographic position of the sites from which plants were sampled in Texas (the genotypes from the same mother plant have been jittered to facilitate observation). Population structure results from STRUCTURE K=2 have been used to distinguished the subpopulation 1 (yellow), the subpopulation 2 (blue) and an admixed group (grey).

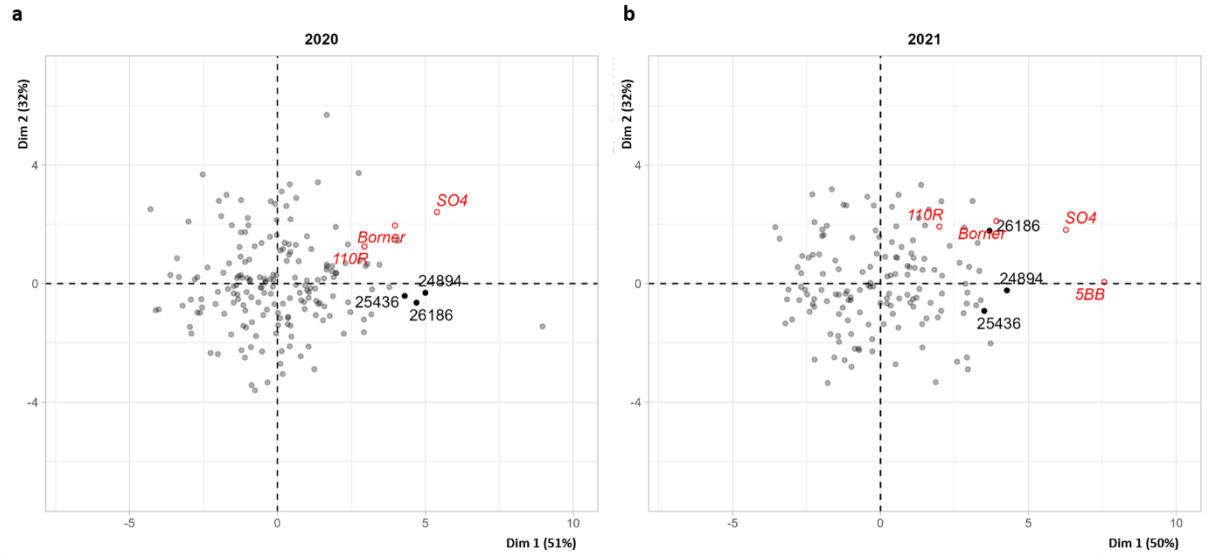

Fig. 2: Graph of individuals obtained from the PCA analyses in 2020 (a) and 2021 (b). Commercial rootstocks (110R, Börner, 5BB and SO4) are indicated in red as additional individuals. Numbers 24894, 25436 and 26186 indicate individuals with extreme performances for root-related traits similar to those of commercial rootstocks over the two years of the experiment.

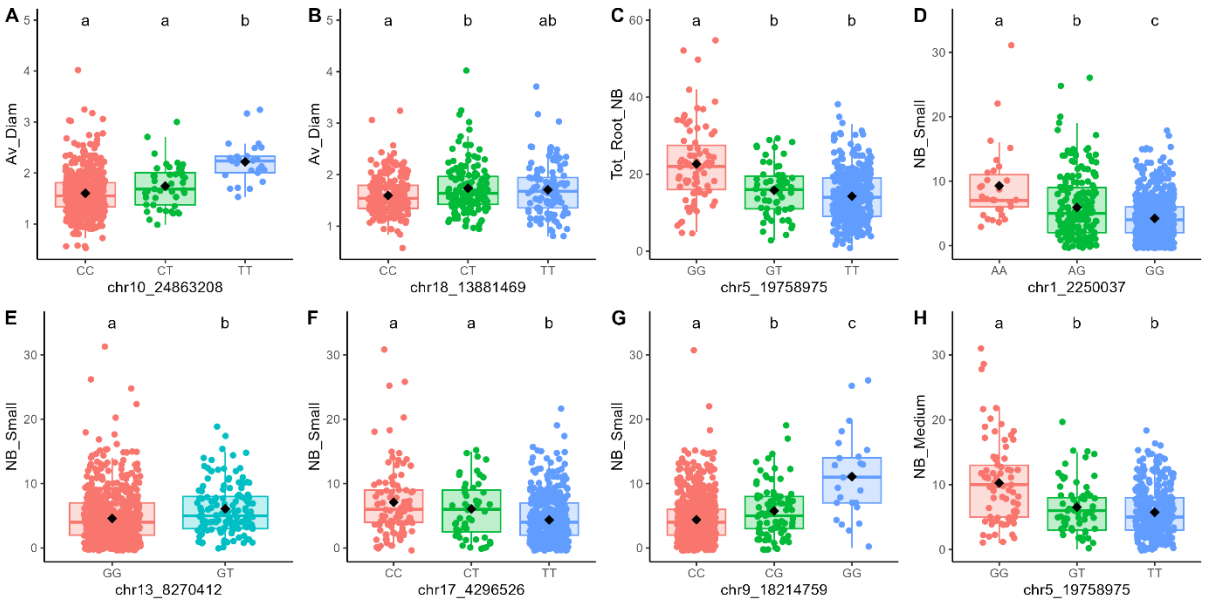

Figure 3: Boxplots of marker effects on root-related traits: chr10_24863208 (A) and chr18_13881469 (B) for mean root diameter (Av_Diam); chr5_19758975 (C) for total root number (Tot_Root_NB), chr1_2250037 (D), chr13_8270412 (E), chr17_4296526 (F), and chr9_18214759 (G) for the number of *small* roots (NB_Small, diameter < 1 mm), and chr5_19758975 (K) for the number of medium roots (NB_Medium, 1 mm > diameter < 2 mm).