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Linking the genetic diversity of root traits and drought responses in wild *Vitis* species☆

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ABSTRACT

Drought impacts viticulture reducing yield, and grape and wine quality. Roots play a key role in maintaining water status both through constitutive traits and through morphological, physiological and biochemical adjustments in response to drought. For this reason, the root system is a novel target in crop breeding programs aiming to produce drought adapted varieties. However, the relationships between roots traits and drought responses in perennial crops are poorly known. To address this, we aimed to link root syndromes of genetically controlled traits with genetic variation of shoot drought responses across a previously unexplored range of wild *Vitis* species. We identified three constitutive root syndromes that partially explained shoot drought responses. The first root syndrome had root systems with few ramifications, the second syndrome had a higher number of adventitious roots and thicker ramifications, and third syndrome was characterized by higher root biomass and higher cumulative root length. Those genotypes of the first and second syndrome had higher stem elongation rates and transpiration during early stages of the drought treatment compared to the third one. The accessions associated with the first syndrome were North American *Vitis* species, those with the second syndrome mostly the Eurasian *V. sylvestris*, and those associated with the third syndrome were two Asian species and the North American species *V. labrusca*. We also explored genetic variation in root functional trait plasticity in response to drought. Notably, two North American species, *V. acerifolia* and *V. doaniana*, showed high osmotic adjustment, while Asian species showed high plasticity in polyphenol concentrations. The originality of this work lies in the evaluation of root traits in previously uncharacterized *Vitis* accessions and the identification of constitutive root syndromes that could aid in the selection of more drought adapted rootstocks.

1. Introduction

The root system is central to whole-plant functioning, playing key roles in water and nutrient uptake, anchorage, and interaction with the soil microbiome. Consequently, understanding how roots adapt and respond to environmental constraints is crucial for improving plant resilience (Lynch and Brown, 2012). Roots are the first organ to detect the onset of drought conditions and consequently they play a key role in maintaining plant hydration through both constitutive traits and through morphological, physiological and biochemical adjustments in response to the stress (*i.e.* plasticity; Kano et al., 2011; LaRue et al., 2022). Considering that drought is a major limitation for plant growth and productivity, in both natural and agricultural ecosystems, and that it will be exacerbated in some regions as a consequence of climate change (IPCC, 2023), identifying the root traits that underlie the ability of plants to cope with drought will provide essential knowledge to develop

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drought resilient crops.

The root system is formed by many interactions between root traits across different spatial, temporal and biological scales that collectively influences root function (Bernardo et al., 2025). This complexity hampers our understanding of the relationship between root traits and water deficit responses. For instance, root system architecture, which defines the spatial distribution of roots in the soil, determines the soil exploration capacity conditioning the access of roots to water reservoirs (de Dorlodot et al., 2007; Fichtl et al., 2023). Functional traits, such as root osmotic adjustment and the antioxidant capacity, are also important to maintain root apical elongation (Blum, 2017; Munns, 1988; Voothuluru et al., 2024) and drought-survival (Hanzouli et al., 2024), respectively. Root morphology-related traits, such as diameter, root-specific length, and branching density, have also been related to the ability of plants to take up water and to recover after drought events (Alonso-Forn et al., 2025; Lynch, 2015; Peccoux et al., 2017). Root morphology-related traits can be described according to the hierarchical branching order of daughter roots. This is challenging but essential to understand their roles in drought adaptation (Iversen, 2014). In order to take into account the multiple traits interacting in a root system that determine drought responses, it is essential to move beyond the traditional approach of studying individual root traits in isolation because it limits our understanding of drought tolerance capacity. In this sense, root trait syndromes, which refer to the coordinated expression of multiple root traits, allow adopting a more integrative approach that considers synergistic effects between traits (Zheng et al., 2009). This shift in focus is critical for identifying root trait combinations that can be targeted for crop improvement.

The first step to breeding root trait syndromes with enhanced drought resilience is to understand the genetic variance underlying root traits and the genetic correlations between them (Turner-Hissong et al., 2020). By partitioning genetic variance, we can identify how much of the variation in root traits is heritable and can be targeted in breeding programs. Genetic correlations inform the intertwined genetic architecture of these traits, *e.g.* because of pleiotropy or linkage disequilibrium between genes affecting different traits. In this sense, genetic correlations indicate that the same set of genetic variants influence diverse phenotypic traits, that do not necessarily imply a mechanistic relationship (*i.e.* their biological pathways may be independent). Despite the importance of genetic studies for breeding, few studies have quantified genetic variation for perennial root systems (but see Alahakoon and Fennell, 2023; Blois et al., 2023; Chen et al., 2021; Tandonnet et al., 2018). As a result, breeding strategies aimed at improving drought tolerance in perennials are hindered by a significant gap in our understanding of how root traits are genetically controlled and how they interact to overcome drought conditions (Lynch and Brown, 2012).

In addition to the constitutive traits, plants can adjust their phenotypes in response to the environment through plasticity. Both processes can vary significantly within and across species (Matesanz and Ramírez-Valiente, 2019). Plasticity in root anatomy and root system architecture has been documented as beneficial to overcome drought (e.g. Prince et al., 2016; Cuneo et al., 2020; Schneider et al., 2020b, 2020a). However, whether root plasticity may be beneficial or maladaptive can vary according to growing conditions (Lynch, 2019, 2013). Interestingly, there is evidence that constitutive traits, such as the turgor loss point and water-use efficiency, are useful predictors of the plant tolerance to drought, even more performant than stress-induced traits or plastic responses (Bartlett et al., 2014; Plantevin et al., 2022). In the context of breeding root syndromes adapted to drought, the evaluation of root constitutive traits to predict drought responses would have the advantage to allow stress-free evaluations and simplify phenotyping, which could be especially useful to evaluate the high numbers of genotypes needed to estimate representative genetic variances.

In this study, we used grapevine as a model perennial, grafted fruit crop. Rootstocks are accessions or hybrids of American *Vitis* species naturally resistant to Phylloxera, an insect pest affecting most viticultural regions (Rispe et al., 2020). Most of the currently used rootstocks were selected more than 100 years ago and they have a narrow genetic background that may compromise their long-term suitability in new environments (Riaz et al., 2019). Grapevine yield and berry composition is currently limited by drought in some wine-growing regions (Santos et al., 2020) and these negative impacts are predicted to increase in the current context of global change (van Leeuwen et al., 2024). In this sense, breeding and selecting rootstocks better adapted to drought represents a leverage for the sustainable production of vineyards without modifying the grape varieties and consequently, wine typicity (Marín et al., 2021). Therefore, there is a need to provide new genetic resources and develop next-generation breeding tools to improve the performance of grapevine rootstocks both now and in the future.

The objective of this work was to link root trait syndromes of genetically controlled traits with genetic variation of shoot drought responses, using grapevine wild relatives to maximize genetic variability. We hypothesize that constitutive root traits can be linked to shoot drought responses. This approach is a significant step forward for breeding grapevine rootstocks, by identifying target root traits with significant genetic variation which would not require evaluation under drought conditions. First, we aimed to quantify the amount in genetic variation of root traits among and within *Vitis spp* in order to better understand evolutionary divergence and their potential for improvement through selection; Then, we aimed to identify genetically controlled root trait syndromes for morphological and functional traits. Finally, we aimed to link root syndromes to shoot drought responses through a multi-trait approach. To address these objectives, we studied a panel of 45 accessions belonging to 12 different *Vitis spp* in a controlled drought experiment. Constitutive root morphological traits were phenotyped using image analysis, while the estimation of plasticity in functional root responses was done for the osmotic adjustment and the quantification of polyphenols as antioxidant metabolites. Coordinated genetic variation in root traits was linked to shoot drought responses using cophenetic correlations. The findings obtained shed light on how to design breeding targets to provide viticulture with new drought-adapted grapevine rootstocks.

2. Material and methods

2.1. Plant material

Twelve wild *Vitis spp*. were selected in order to maximize genetic diversity (nine North American, two Asian and one Eurasian species) for a total of 43 accessions (Table S1). The three species most commonly used as parental genotypes for commercial rootstocks, *i.e. V. berlandieri, V. rupestris* and *V. riparia* (Riaz et al., 2019), were represented by two or three different accessions. Species less used in rootstock breeding, such as *V. acerifolia, V. amurensis, V. candicans, V. coignetiae, V. doaniana, V. labrusca, V. monticola and V. sylvestris* were represented by three to five accessions. All the accessions were chosen from European germplasm collections (FRANCE: The INRAE collections from the Grapevine Biological Research Center (Vassal, Montpellier), Ecophysiology and Functional Genomics of Grapevine (EGFV, Bordeaux), and Grapevine Health and Wine Quality (SVQV, Colmar); SPAIN: The germplasm center “El Encín” (IMIDRA, Alcalá de Henares, Madrid); GERMANY: The Julius Kühn Institute grapevine collection from the Institute for Grapevine Breeding (Geilweilerhof, Siebeldinge). The plant material was provided as one-meter-long bundles of dormant wood, which were stored in a cold room at 4 °C for several weeks. For each accession, ten cuttings of 10 cm were taken from this wood. The cuttings were rehydrated in water overnight for 12 h before being planted in pots filled with sand, placed on a heating pad, and watered for several weeks. Six replicates per accession, with a well-established root-system, were selected for transplantation. Then, plants were grown in 7.5 L containers, each of them filled with 6 kg of a 1:2 (v:v) mixture of sand and peat-coco compost . All

plants were grown in a glasshouse equipped with a cooling system for 3 months under well-watered conditions. They were irrigated daily in non-limiting conditions with a nutritive solution (KNO_3: 2.45 mM; K_2HPO_4: 0.00 mM; KH_2PO_4: 0.57 mM; $MgSO_4$: 0.69 mM; $CaCl_2$: 1.27 mM; $CaSO_4$: 0.6 mM) through an automatic drip irrigation system. Plants were pruned to one stem and trained, with lateral (axillary) branches removed once a week throughout the duration of the experiment.

2.2. Drought experiment

The experiment was conducted from August 20 to September 16, 2022. A total of 270 6-month old plants were placed in a greenhouse with 150 scales equipped with an automatic irrigation system at the National Research Institute for Agriculture, Food and Environment in Bordeaux, France (44° 47′28 N, 0° 34′55 W) five weeks before the start of the experiment. Environmental conditions in the greenhouse, including global radiation, air temperature and humidity were recorded at three different locations using pyranometers (LI-Q23022, Li-Cor, Lincoln, NE, USA) and thermohygrometers (HPM 155, Vaisala) connected to a data logger (CR1000; Campbell Scientific Ltd, Shepshed, Leicestershire, UK). The six replicates per accession were randomly divided into six blocks, conceived to capture temperature and humidity gradients inside the greenhouse. Three blocks were assigned to the well-watered treatment (WW) and 3 blocks to the water deficit treatment (WD). As a result, there was one replicate per accession per block. The main stem of all the plants in the experiment were pruned to 1 m long on July 7, 2022. To facilitate management of plants in the greenhouse, those plants in the WW treatment were pruned again to a height of 1 m on August 18, 2022. For this reason, we did not consider stem growth of the WW treatment in this study. Plants on the WD treatment had mean stem height of 149.3 ± 33.2 cm (mean ± standard deviation) at the start of the experiment.

Plants in the WD treatment were initially watered to field capacity and then water availability was progressively decreased to reach a target soil water content (SWC) of 40% (Fig. S1). To estimate the water retention capacity of the soil, we weighed 15 soil samples at field capacity and after drying to constant weight. We determined the weight of each pot evaluated in the experiment at field capacity by fully irrigating and then allowing to drain overnight. Pots were covered with a plastic bag to prevent water losses by evaporation. At field capacity, the water content of the soil was, on average, 49% of its dry weight (DW). Throughout the experiments, the amount of water in the soil was determined by weighing the pot daily in an experimental setup of 135 balances (CH15R11, OHAUS type CHAMP, Na¨nikon, Switzerland, precision 1 g) (Sadok et al., 2007). WD pots were irrigated, with a nutritive solution, automatically mid-morning, to achieve the target water content. A circular water distribution system was installed around each cutting to favor homogeneous distribution of watering in the pot. Plants were maintained under 40% SWC for 15 days. Plants in the WW treatment were manually irrigated with the same nutritive solution four times per week to meet not limiting conditions.

At the end of the experiment, harvest was done during 5 consecutive days in order to allow all plants to spend 15 days under the fixed SWC. Every WD replicate per accession was harvested at the same time than a replicate of the same accession from the WW treatment.

2.3. Root constitutive traits

We measured root constitutive traits for plants harvested from the WW treatment. One adventitious root (*i.e.* root of order 0) per plant was randomly sampled at the harvest from the cuttings base and cleaned with tap water. Individual roots were scanned with a flatbed scanner (Epson Perfection V850 Pro, resolution 1200pp) by placing them in a Plexiglas tray (22 cm × 30 cm, 1.5 cm). The Plexiglas tray was filled with a few millimeters of water, which was covered with a rigid transparent plastic sheet to disentangle the roots and minimize overlapping. Images were captured in TIFF format with a resolution of 1200 dots per inch (dpi) using the transparent mode. Then, we measured root dry weight after the root was oven dried at 80 °C for 48 h. Thus, root traits for every accession were estimated from one adventitious root per pot from three independent pots containing the same accession.

The scanned image was analyzed with two softwares: i) RhizoVision (Seethepalli and York, 2020) allowed to estimate whole root traits, such as individual root volume (IRV), individual root area (IRA), individual root length (IRL). These three characteristics were measured at the level of a single adventitious root with all its daughter roots (see Table 1 for trait definitions). Then, we estimated specific root length (SRL) and specific root area (SRA) as the ratio of IRL and IRA to the scanned root dry weight, respectively, and root tissue density (RTD) was calculated as the ratio of the scanned root dry weight to IRV. ii) SmartRoot v4.21 (https://smartroot.github.io/, Lobet et al., 2011) that allows to precisely characterize roots according to their developmental classification (Freschet et al., 2021): the adventitious root was rated as order 0, and then consecutive daughter roots of order 1 and 2. As this task is time-consuming, we focused our analysis of the last 14 cm of the apical part from the root apex digitalizing ramifications until order 2. Roots were semi-automatically vectorized in a RSML format using the Smartroot v4.21 plugin of the ImageJ v1.52 software (https://imagej.net/ij/). Each vectorized root was defined by a set of nodes characterized by 2D spatial coordinates, diameters, root orders and parental nodes. By this method we were able to estimate diameter of the root (DR) (directly extracted from SmartRoot), interbranching distance (IBD), length of the apical unbranching zone (LAUZ), diameter of the root at the insertion point (DRI) and at the root tip part (DRT) (were estimated from the RSML file by in-house pipeline developed by Larrey et al. (2025), Fig. S2). DRI, DRT, IBD and DR values for roots of any order corresponded to the mean of the distribution of all ramifications by order or over all the points per root.

In addition, the dry root system was weighted after 48 h at 80 °C to measure the dry root biomass (TRDW).

2.4. Osmotic adjustment

In order to estimate osmotic potential at full-turgor we sampled non-lignified apical roots portions of 3 cm long representative of the whole root system and preserved in 5 ml tubes filled with purified water for 24 h at dark. Then, the excess water was removed and root fragments were transferred to a holed 1 ml Eppendorf tube that was embedded in 1.5 ml Eppendorf and immerged in liquid nitrogen for 1 h. Samples were stored at −80 °C until osmotic potential estimation. Prior to osmotic potential (Ψ_{root}; $\Psi_{root_}$WW when is only measured in WW treatment) estimation, samples were defrosted and centrifuged during 5 min at 1300 rpm. Then, 10 µl aliquots of the extracted root tip content were analyzed by freezing point osmometry using an Osmomat 030 osmometer (Gonotec GMBH, Berlin, Germany).

2.5. Root polyphenols quantification

In order to estimate polyphenol content, we sampled non-lignified apical root portions of 3 cm long representative of the whole root system and stored the samples at −80 °C. Samples were ground with a cryogenic mill (Retsch 25 ml 1.4112) and freeze-dryed (Retsch MM400). We weighted 10 mg of root powder to be used in the robotized extraction of metabolites using an 80% ethanol extraction protocol (Luna et al., 2020).

High-performance liquid chromatography coupled with an Orbitrap QExactive+ mass spectrometer analysis (Thermo Scientific) was performed with a Phenomenex Luna® Omega Polar C18 column (50 mm × 2.1 mm, 1.6 µm). Solvents A (Milli-Q water–0.1% formic acid) and B (acetonitrile–0.1% formic acid), were used with a gradient: 0–7.5 min: 1–36% solvent B; 7.5–8.5 min: 36–95% solvent B; 8.5–10 min: 95% solvent B; 10–12 min: 1% solvent B. The flow was 0.5 mL.min^{-1} and the

Table 1
Results of linear mixed models testing the inter-specific, intra-specific and block effects. The proportion of explained variance, and the broad sense heritability (H^2 with 95% confidence interval) for 16 morphological constitutive root traits (measured in WW treatment) are presented. $n = 127$ plants, belonging to 43 accessions and 12 wild *Vitis sp*.

Abbreviation	Trait	Units	Block effect	Proportion of variation			H^2	Confidence interval	
				Inter-specific	Intra-specific	Residuals		Min	Max
IRL	Individual root length	mm	NS	**0.18** ***	**0.31** ***	0.52	**0.37**	0.21	**0.49**
IRV	Individual root volume	mm^3	NS	**0.17** ***	0.18	0.65	**0.22**	0.00	0.36
IRA	Individual root area	mm^2	NS	**0.18** ***	**0.27** ***	0.55	**0.33**	0.15	0.46
LAUZ	Length of the apical unbranching zone	cm	NS	0.00	0.00	1.00	0.00	0.00	0.16
SRL	Specific root length	$mm.g^{-1}$	NS	0.04	0.18	0.78	**0.19**	0.00	0.33
SRA	Specific root area	$mm^2.g^{-1}$	NS	0.02	**0.24** *	0.74	**0.25**	0.03	0.36
RTD	Root tissue density	$g.cm^3$	NS	0.02	0.16	0.82	**0.16**	0.00	0.29
IBD_{order0}	Interbrancing distance order 0	cm	NS	**0.27** ***	0.00	0.73	0.00	0.00	0.10
IBD_{order1}	Interbrancing distance order 1	cm	NS	0.16	0.11	0.74	0.13	0.00	0.31
DRT_{order1}	Diameter Root tip order 1	cm	NS	0.04	0.13	0.83	**0.14**	0.00	0.28
DRT_{order2}	Diameter Root tip order 2	cm	NS	**0.16** ***	**0.27** **	0.57	**0.32**	0.13	0.45
DRI_{order1}	Diameter root insertion point order 1	cm	NS	0.06	0.10	0.85	**0.10**	0.00	0.26
DRI_{order2}	Diameter root insertion point order 2	cm	***	0.00	**0.27** *	0.73	**0.27**	0.06	0.37
DR_{order0}	Mean diameter of root order 0	cm	NS	**0.21** ***	0.00	0.79	0.00	0.00	0.13
DR_{order1}	Mean diameter of root order 1	cm	NS	0.08	0.06	0.87	0.06	0.00	0.23
DR_{order2}	Mean diameter of root order 2	cm	**	**0.11** *	**0.25** *	0.63	**0.29**	0.07	0.41

Bold values stand for significant differences according to the following code: NS = Not significant; *$p < 0.05$; ** $p < 0.01$; ***$p < 0.001$.

oven was set at 40 °C. Full scan HRMS data was acquired in negative polarity with data dependent MS/MS fragmentation (normalized collision energies: 15,30 and 40) (Mairata et al., 2025). A calibration curve was built with polyphenol standards (Table 3), including six flavanols (Catechin, epicatechin, procyanidin B1, procyanidin B2, epigallocatechin, gallocatechin), one phenolic acid (salycilic acid), and one flavonol (quercetin 3-glucoside), all purchased at Extrasynthese (France), and nine stilbenes previously purified at the laboratory (trans resveratrol, trans piceid, ε viniferin, miyabenol, hopeaphenol, isohopeaphenol, pallidol, vitisin A, vitisin B). Additionally, forms of procyanidin B4, cis-resveratrol and cis-piceid were detected and quantified as Procyanidin B2, trans-resveratrol and trans-piceid equivalents, respectively. The results were expressed as mg of polyphenol/mg root.

2.6. Shoot drought responses

Stem height and biomass was only considered for the WD treatment to avoid bias from the pruning of the main stem shortly before the beginning of the experiment in the WW treatment. Stem height was measured three times per week. The growth rate (GR) was calculated using Eq. (1).

$$GR_n = \frac{Height_n - Height_{n-1}}{Number\ of\ days\ between\ n\ and\ n-1} \quad (1)$$

Growth rate was estimated for each of the three weeks of the experiment: for the first week between August 22th and 29th, the second week between August 29th and September 5th, and for the third week between September 5th and the harvest date.

At harvest, stem biomass of WD plants was measured through dry weight after 48 h at 70 °C. Stem biomass was used to estimate the root-to-stem ratio using Eq. (2):

$$root - to - stem\ ratio = \frac{root\ dry\ biomass}{stem\ dry\ biomass} \quad (2)$$

Number of adventitious roots (NAR) roots was measured on both watering treatments.

We estimated water use-related traits from two approaches: i) an integrated estimation of the amount of water used through daily transpiration at the whole plant level (estimated through pot weight loss after one day of transpiring). Daily transpiration was standardized by total leaf area. Total leaf area per plant was estimated once a week. For this purpose, we used the length of the main vein as a predictor of leaf area. The correlation between the length of the main vein and leaf area was estimated by measuring 30 leaves of different sizes per species (to account for leaf morphological differences), scanning the leaves in a scanner Expression 1640XL (Seiko Epson Corp., Suwa, Nagano, Japon) and estimating leaf area with imageJ. A power curve fitting allowed to establish the relationship between length of the main vein and leaf area per species ($R^2 > 0.85$, p_value<0,05, Fig. S3). The amount of transpiration by unit of leaf area was cumulated per week (*i.e.* sum of the transpiration of seven consecutive days). Cumulated transpiration during the last week (TR_{week3}), was also standardized by unit of dry root biomass (TRB). Instantaneous water use was estimated through stomatal conductance (gs) measured once per week (3 blocks per day during 2 days per week) in WD and WW treatments. gs was measured with a porometer (LICOR LI600, Lincoln, NE, USA) in a time slot between 10 a.m. and 12 pm (at local time) on a fully developed leaf.

In this work, we estimated plasticity as the difference (Δ) between WD and WW treatment using accession BLUPs (see Section 2.7).

2.7. Data analysis

All the statistical analysis was performed using R software (R Development Core Team 2024; R version 4.4.0; http://www.r-project.org/). Linear Mixed Models were used to estimate the effect of factors influencing trait variation. For those traits measured in WW and WD treatments the model in Eq. (3) was fitted.

$$Y_{ijkl} = \mu + W_i + W(B)_{ij} + S_k + S(A)_{kl} + W \times S + \varepsilon_{ijkl} \quad (3)$$

where Y_{ijkl} is the mean phenotypic value, Wi the random effect of watering treatment, $W(B)_{ij}$ the random block effect nested within watering treatment, S_k the random inter-specific effect, $S(A)_{kl}$ the random intra-specific effect nested with species, $W \times S$ the species by treatment interaction, and ε_{ijkl} the residual variance.

For those traits only measured in WW or WD watering treatments models were fitted according to Eq. (4), where B_j was considered as a fixed factor.

$$Y_{ijkl} = \mu + B_j + S_k + S(A)_{kl} + \varepsilon_{ijkl} \quad (4)$$

Genetic variance explained by inter-specific and intra-specific factors were obtained from linear mixed models according to Eqs. (5) and (6) depending on whether the trait was estimated in both watering treatments or only in one of them. Eq. (7) was used to estimate broad-sense heritability. Confidence interval (CI) of the broad sens heritability was determined by using CI for the variance of the accession factor, extracted with "confint" function from 'stats' package (R Core Team and

contributors worldwide).

$$Prop.Var = \frac{\sigma^2_{Factor}}{\sigma^2_{species} + \sigma^2_{Accession} + \sigma^2_{residuals}} \quad (5)$$

$$Prop.Var = \frac{\sigma^2_{Factor}}{\sigma^2_{block} + \sigma^2_{treatment} + \sigma^2_{species} + \sigma^2_{Accession} + \sigma^2_{residuals}} \quad (6)$$

$$H^2 = \frac{\sigma^2_{Accession}}{\sigma^2_{Accession} + \sigma^2_{residuals}} \quad (7)$$

The package lme4 was used for fitting linear mixed model (Bates et al., 2015). Best Linear Unbiased Predictor (BLUP) was extracted for each trait and computed as the sum of the inter-specific and intra-specific BLUPs. BLUPs are considered as the genetic effects for each analyzed trait. Consequently, they were used for subsequent analyses, *i.e.* estimation of trait plasticity (see previous section), genetic correlation between traits (*i.e.* Pearson correlation) and the estimation of trait syndromes.

We estimated trait syndromes following the Bodner et al. method (2013) for the classification of root constitutive system and shoot drought response syndrome, using those traits with significant genetic effects (*i.e.* intra-specific and/or inter-specific) and for the plastic response of roots, using those traits with significant genetic effects and watering treatment or watering treatment per species interaction effect. This method consisted on a Principal Component Analysis (PCA) using the 'FactoMiner' library and conserving the Principal Components with eigenvalue > 1, which explained more than 70% of total variation. With the extracted PCA loadings, a hierarchical clustering analysis was made with Euclidean distance and Ward's method to determine different clusters. The number of clusters was determined with the packages 'NbClust' v3.0.1 (Charrad et al., 2014). Then, we performed linear and a Student-Newman-Keuls test (SNK, derived from Tukey's test), to look for significant differences in traits explaining the identified syndromes. Finally, we tested the correlation between the different syndromes through cophenetic correlation using the packages dendextend (Galili, 2015). The cophenetic distance is the measure of similarity necessary for two objects to be grouped in the same cluster. The cophenetic correlation is therefore the correlation between the cophenetic distance matrices of the two dendrograms (Sokal and Rohlf, 1962). The packages used to create all the graphics were 'ggplot2' and 'ggcorrplot'.

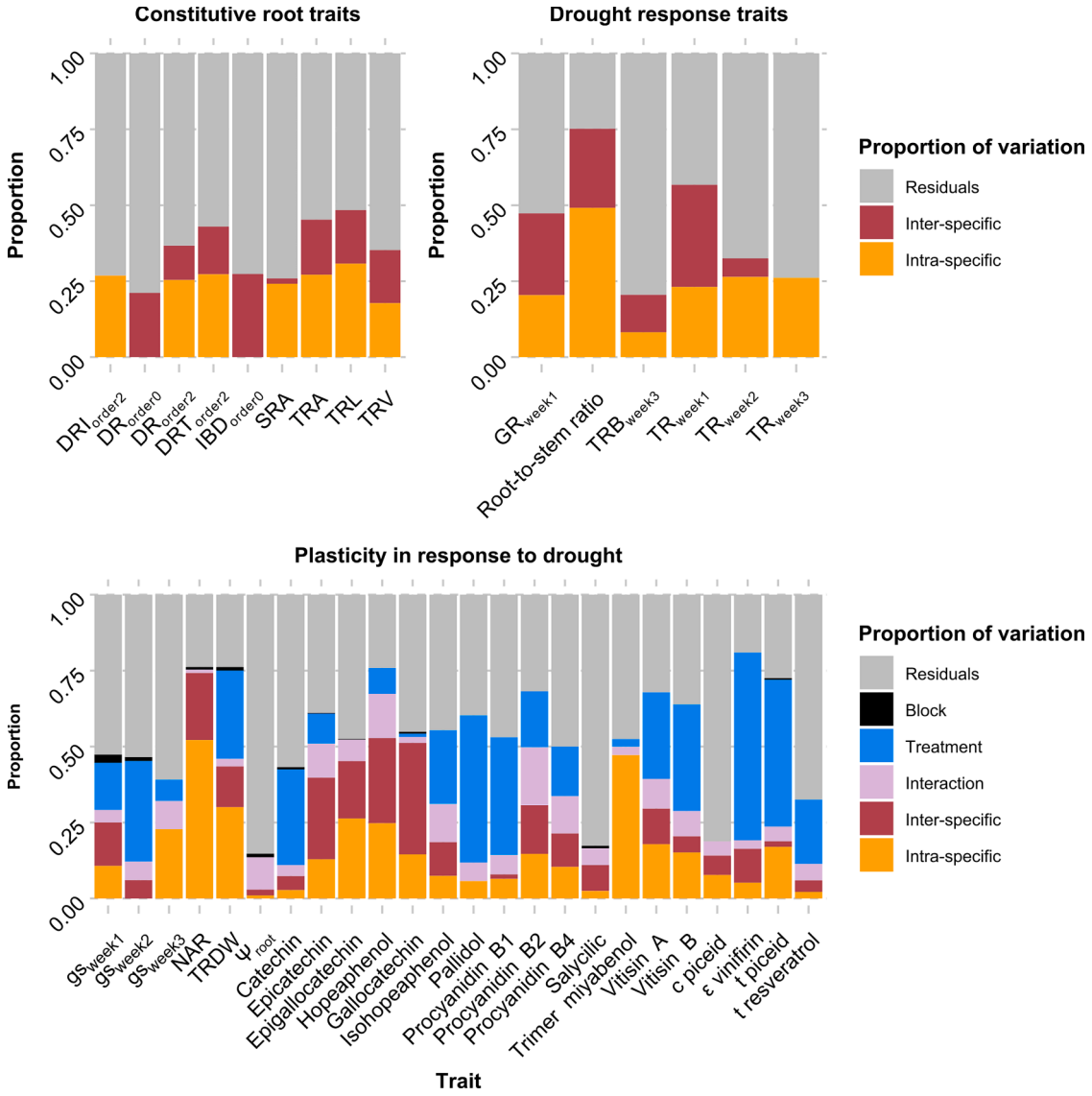

Fig. 1. Proportion of explained variance extracted from random factors in linear mixed models testing the inter-specific (red) and intra-specific (orange) effects of (A) root constitutive traits and (B) drought response traits. and in linear mixed models testing also the watering treatment (blue) and inter-specific × watering treatment (pink) effects of (C) the plasticity of shoot and root traits in response to drought.

3. Results

3.1. Genetic variation of constitutive root traits

Nine out of 16 root morphological traits showed significant inter and/or intra-specific variation (Table 1). Interestingly, those traits with significant genetic variance represented global traits of the analyzed adventitious root and its ramifications, such as IRA, IRL, IRV and SRA but also traits specific of the last root ramification studied (order 2), such as DRT_{order2}, DR_{order2} and DRI_{order2}. Most of the root traits specific to ramifications of order 0 and order 1 did not present significant genetic variance, except for DR_{order0} and IBD_{order0} which presented the lowest genetic variances and showed no significant heritability (Table 1). IRL, IRA, and DRT_{order2} showed the highest percentage of the variance explained by genetic factors (intra and inter-specific variation) 49%, 45% and 43%, respectively (Fig. 1). Root traits with significant intra and inter -specific genetic variance had heritability estimates from 0.29 to 0.35 (Table 1). LAUZ, IBDorder1 and DR_{order1} showed no significant genetic effects nor heritability estimates.

3.2. Genetic variation in shoot and root drought responses

During the first week of the experiment, soil water content (SWC) decreased progressively and most of the plants reached the drought threshold of 40% SWC after 10 days (Fig. S1). The growth rate (GR) was maintained during the first week but was greatly decreased during the second week, and was stopped in the last week (Table S3). The transpiration rate (TR) also decreased throughout the experiment for every species (Table S3).

Six out of eight drought-response traits had significant genetic variation. The genetic effects on growth rate were also significant for the first week. All the other drought response traits showed significant variation at both the intra and inter-species level, with the exception of TR_{week3}, that was significant only at the intra-specific level and TRB_{week3} that were significant only at the inter-specific level (Table 2). During the experiment, the genetic variation of TR changed from a significant inter and intra-specific effect in the first and the second week, to only significant intra-specific effect at the end of the experiment (Table 2). Consequently, its heritability decreased from 0.35 (week 1) to 0.26 (week 3) (Table 2). Overall, the root-to-stem ratio showed the highest heritability with a value of 0.66.

3.3. Plasticity in response to drought

Plants under WD showed higher TRDW than plants in WW treatment. However, the degree of variation was different among species, as revealed by the significant interaction between species and water treatment (Table 3). In addition, TRDW also showed significant intra-specific variation. NAR did not change in response to drought but showed a higher proportion of genetic variation (Fig. 1) with approximately 2/3 explained by the intra-specific level and 1/3 by the inter-specific level. Consequently, NAR and TRDW had medium to high heritability estimates of 0.69 and 0.56 respectively (Table 3). Similarly, Ψ_{root} changed differently in response to water deficit according to the species (Table 3, Fig. S4). This strong species by treatment interaction did not allow to estimate a general trend of Ψ_{root} in response to water deficit. In addition, there was a high residual variability that resulted in low levels of variance explained by our models for this trait (Fig. 1). Nevertheless, *V. acerifolia, V. doaniana* and *V. sylvestris* showed most of the accessions relying on osmotic adjustment in response to water deficit (Fig. S4). *V. acerifolia* and *V. sylvestris* showed also the higher increment in TRDW (Table S2).

Stomatal conductance (gs) decreased in response to water deficit only for the second week of the water deficit treatment. At this time-point, gs_{week2} changed differently in response to water deficit according to the species (Table 3). We did not detect significant changes in gs_{week1} and gs_{week3} in response to water deficit. However, significant intra-specific genetic variation was estimated at these time-points. As a result, at the beginning (week 1) and at the end (week 3) of the water deficit period genetic factors explained a higher proportion of the phenotypic variance than the environment (water deficit treatment). On the contrary, at the middle of the experimental period (week 2) the higher part of the phenotypic variance was explained by the water deficit treatment.

Thirteen out of 20 root polyphenols changed in response to water deficit (Table 3). They all varied depending on the species with 12 metabolites varying differently in response to water deficit according to species (*i.e.* significant water treatment by species interaction, Table 3) and 12 of them showed also significant genetic variation at the intra-specific level. We did not detect neither significant genetic variance nor water deficit effect for c-resveratrol, quercetin glucoside and gallocatechin. In the flavanols group, epigallocatechin and trimer miyabenol showed the highest heritability and a proportion of variance explained by the genetic factors of *ca.* 0.5 (Table 3, Fig. 1). Similar values were estimated for the most heritable stilbene, *i.e.* hopeaphenol (Table 3, Fig. 1). Epigallocatechin, salicylic acid, c-piceid and trimer miyabenol did not varied in response to water deficit whereas ε-viniferin, pallidol, procyanidin B1, and t-piceid showed the strongest part of the phenotypic variance explained by the water deficit treatment (close to or above 0.4, Fig. 1).

3.4. Root constitutive syndromes versus drought response strategies

We linked constitutive root traits and shoot drought responses through two approaches: trait-by-trait genetic correlations and multivariate cophenetic correlation. Trait-by-trait genetic correlations revealed that root-to-stem ratio, TR_{week1} and TR_{week2} presented the higher number of significant correlations with root constitutive traits and root plasticity in response to drought. Transpiration rate was negatively correlated with most of the global root constitutive traits:

Table 2
Results of linear mixed models testing the inter-specific, intra-specific effects and block effects. The proportion of explained variance, and the broad sense heritability (H^2 with 95% confidence interval) for 8 shoot drought response traits (measured in WD treatment) are presented. $n = 127$ plants, belonging to 43 accessions and 12 wild *Vitis sp*.

Abbreviation	Trait	Units	Block effect	Proportion of variation			H^2	Confidence interval	
				Inter-specific	Intra-specific	Residuals		Min	Max
TR_{week1}	Transpiration by leaf area week 1	$g.cm^{-2}.week^{-1}$	***	**0.34** ***	**0.23** ***	0.43	0.35	0.21	0.46
TR_{week2}	Transpiration by leaf area week 2	$g.cm^{-2}.week^{-1}$	NS	**0.06** *	**0.26** ***	0.68	0.28	0.11	0.40
TR_{week3}	Transpiration by leaf area week 3	$g.cm^{-2}.week^{-1}$	NS	0.00	**0.26** **	0.74	0.26	0.09	0.36
GR_{week1}	Growth rate week 1	$cm.day^{-1}$	NS	**0.27** ***	**0.2** ***	0.53	0.28	0.11	0.41
GR_{week2}	Growth rate week 2	$cm.day^{-1}$	NS	0.01	0.05	0.94	0.05	0.00	0.21
GR_{week3}	Growth rate week 3	$cm.day^{-1}$	NS	0.00	0.00	1.00	0.00	0.00	0.12
TRB_{week3}	Transpiration by root biomass for week 3	$g.g^{-1}.week^{-1}$	NS	**0.12** **	0.08	0.79	0.09	0.00	0.24
Root-to-stem ratio	Root-to-stem ratio	$g.g^{-1}$	NS	**0.26** ***	**0.49** ***	0.25	0.66	0.61	0.73

Bold values stand for significant differences according to the following code: NS = Not significant; *$p < 0.05$; ** $p < 0.01$; ***$p < 0.001$.

Table 3
Results of linear mixed models testing the inter-specific, intra-specific, block, treatment and inter-specific x treatment effects. The proportion of explained variance, and the broad sense heritability (H^2 with 95% interval confidence) for 26 root and shoot traits measured in WW and WD treatments are presented. $n = 254$ plants, belonging to 43 accessions and 12 wild *Vitis sp*.

Abbreviation	Trait	Phenotyped level	Proportion of variance						H^2	Confidence Interval	
			Inter-specific	Intra-specific	Block	Treatment	Interaction	Residual		Min	Max
	Salycilic acid	Phenolic acid	**0.09** ***	0.02	0.00	0.00	0.06	0.83	0.03	0.00	0.13
	Epicatechin	Flavanols	**0.27** ***	**0.13** ***	**0** ***	**0.1** *	**0.11** ***	0.39	0.25	0.14	0.36
	Epigallocatechin	Flavanols	**0.19** ***	**0.26** ***	**0** ***	0.00	**0.07** ***	0.47	0.36	0.26	0.47
	Gallocatechin	Flavanols	**0.37** ***	**0.14** ***	0.00	0.01	0.02	0.45	0.24	0.14	0.36
	Procyanidin B1	Flavanols	**0.02** ***	**0.06** *	**0** ***	**0.39** **	**0.06** *	0.47	0.12	0.02	0.22
	Procyanidin B2	Flavanols	**0.16** ***	**0.15** ***	**0** ***	**0.18** ***	**0.19** ***	0.32	0.32	0.21	0.43
	Procyanidin B4	Flavanols	**0.11** ***	**0.10** ***	**0** ***	**0.16** **	**0.12** ***	0.50	0.17	0.07	0.28
	Trimer miyabenol	Flavanols	**0** **	**0.47** ***	0.00	0.03	0.03	0.48	0.50	0.42	0.57
	Quercetin glucoside	Flavonols	0	0.04	0.01	0.00	0.00	0.95	0.04	0.00	0.12
	c_Piceid	Stilbene	**0.06** ***	0.08	0.00	0.00	0.05	0.81	0.09	0.00	0.19
	c_Resveratrol	Stilbene	0.00	0.00	0.00	0.20	0.00	0.98	0.00	0.00	0.07
	Catechin	Stilbene	**0.05** ***	0.03	**0.01** ***	**0.32** *	0.04	0.57	0.05	0.00	0.15
	Hopeaphenol	Stilbene	**0.28** ***	**0.25** ***	**0** **	**0.09** **	**0.14** ***	0.24	0.51	0.41	0.6
	Isohopeaphenol	Stilbene	**0.11** ***	**0.07** *	**0** ***	**0.24** ***	**0.12** ***	0.45	0.14	0.04	0.25
	Pallidol	Stilbene	**0** ***	**0.06** *	**0** ***	**0.48** ***	**0.06** **	0.40	0.13	0.03	0.22
	t_Piceid	Stilbene	**0.02** ***	**0.17** ***	**0** ***	**0.48** ***	**0.05** ***	0.27	0.38	0.28	0.49
	t_Resveratrol	Stilbene	**0.04** ***	0.02	**0** ***	**0.21** *	0.05	0.67	0.03	0.00	0.12
	Vitisin A	Stilbene	**0.12** ***	**0.18** ***	**0** ***	**0.29** ***	**0.10** ***	0.32	0.36	0.26	0.46
	Vitisin B	Stilbene	**0.05** ***	**0.15** ***	**0** ***	**0.35** ***	**0.08** ***	0.36	0.30	0.19	0.41
	ε-Viniferin	Stilbene	**0.11** ***	**0.05** ***	**0** ***	**0.62** ***	**0.03** **	0.19	0.22	0.11	0.33
NAR	Number of adventitious roots	Root trait	**0.22** ***	**0.52** ***	0.01	0.00	0.01	0.24	0.69	0.62	0.76
Ψ_{root}	Osmotic potential	Root trait	**0.02** *	0.01	0.01	0.00	**0.11** *	0.85	0.01	0.00	0.1
TRDW	Root biomass	Root trait	**0.13** ***	**0.30** ***	**0.01** ***	**0.29** **	**0.02** *	0.24	0.56	0.47	0.65
gs_{week1}	Stomatal conductance week 1	Shoot trait	**0.14** ***	**0.11** **	**0.03** ***	0.15	0.04	0.53	0.17	0.06	0.28
gs_{week2}	Stomatal conductance week 2	Shoot trait	**0.06** ***	0.00	**0.01** ***	**0.33** *	**0.06** *	0.53	0.00	0.00	0.07
gs_{week3}	Stomatal conductance week 3	Shoot trait	0.00	**0.23** *	0.00	0.07	0.09	0.61	0.27	0.11	0.37

Bold values stand for significant differences according to the following code: *$p < 0.05$; ** $p < 0.01$; ***$p < 0.001$.

IRL, IRA, SRA, TRDW_WW, and Ψ_{root}_WW. TR_{week1} was also positively correlated with osmotic adjustment (*i.e.* the magnitude of change in Ψ_{root} in response to water deficit) and negatively correlated with the delta of procyanidin_B2 under WD. We observed a similar trend of relationships between root constitutive traits and TR_{week2}, with additional correlations on the roots of order 0, but no link could be established between TR_{week3} and constitutive or plastic root traits. In general, constitutive root traits showed more significant correlations with shoot drought responses than plastic root traits (Fig. 2).

For the multivariate cophenetic correlation we estimated three dendrograms based on the loadings of PCA. The first dendrogram was estimated for the root constitutive traits with significant genetic variation, the second one for drought response traits with significant treatment effect and genetic variation, and the third one for root plasticity using the delta of traits with a significant treatment or species by treatment interaction and genetic variation.

Twelve root constitutive traits, with a significant genetic variance, were included in the PCA: DR_{order0}, IBD_{order0}, SRA, IRA, IRL, IRV, DRT_{order2}, DR_{order2}, DRI_{order2}, NAR_WW, TRDW_WW, and Ψ_{root}_WW. The first three components of the PCA were retained for clustering the different accessions, with 38.9%, 27.3% and 10.2% of explained variance, respectively. Following the hierarchical clustering, three root constitutive clusters were identified (Fig. 3). This clustering represented different root syndromes based on constitutive traits (Fig. S5). The first root constitutive syndrome was composed of 24 accessions, mainly species from North America, such as *V. acerifolia, V. berlandieri, V. californica, V. candicans, V. doaniana, V. monticola, V. rupestris, and V. riparia*, except the accession rip_1, which is not in this cluster. In the second root constitutive syndrome, there were 7 accessions mainly from *V. sylvestris*. Three other accessions (berl_4, acer_2 and lab_2) isolated from their species were also found in the second root constitutive syndrome. The third root constitutive syndrome was composed by Asian species including *V. coignetiae* and *V. amurensis*, with the exception amu_2, and the North American species *V. labrusca*.

Eleven out of the twelve constitutive root traits showed significant differences between the two syndromes. Ψ_{root}_WW was the only one with no significant differences. The first root constitutive syndrome showed less developed root systems with lower IRA and TRDW_WW and less ramified adventitious roots (higher IBD_{order0}) The second root constitutive syndrome showed more adventitious roots and thicker ramifications of order 2 (DRT_{order2}, DRI_{order2} DR_{order2}). No significant difference between the first and the third root constitutive syndrome were found for these traits. The third root constitutive syndrome showed the more developed root systems with the highest TRDW_WW, SRA, IRV, IRL and IRA.

We studied differences between the two root constitutive syndromes for those drought response traits with significant genetic variation, using a linear model and a SNK test. Root-to-stem ratio, TR_{week1}, TR_{week2}, GR_{week1}, Δgs_{week2}, and $TRBWD_{week3}$ were significantly different (Fig. 4). At the beginning of the WD treatment, the third root constitutive syndrome had lowest GR_{week1}, TR_{week1}. For the first and second root constitutive syndrome, there is no significant differences. During the rest of experiment, the same trend was observed for transpiration but no difference between the root constitutive syndrome was detected. TRB_{week3} was higher in the first root constitutive syndrome, but root-to-stem ratio was the lowest (Fig. 4). The second root constitutive syndrome stands out by having significantly higher values of Δcatechin, ΔIsohopeaphenol, Δprocyanidin B1 (Fig. 4).

Shoot drought response syndromes were identified using those traits measured in water deficit conditions with significant genetic variance at the inter-specific or intra-specific level. Thus, the root-to-stem ratio, TR_{week1}, TR_{week2}, TR_{week3}, GR_{week1}, $TRBWD_{week3}$, and Δgs_{week2} were

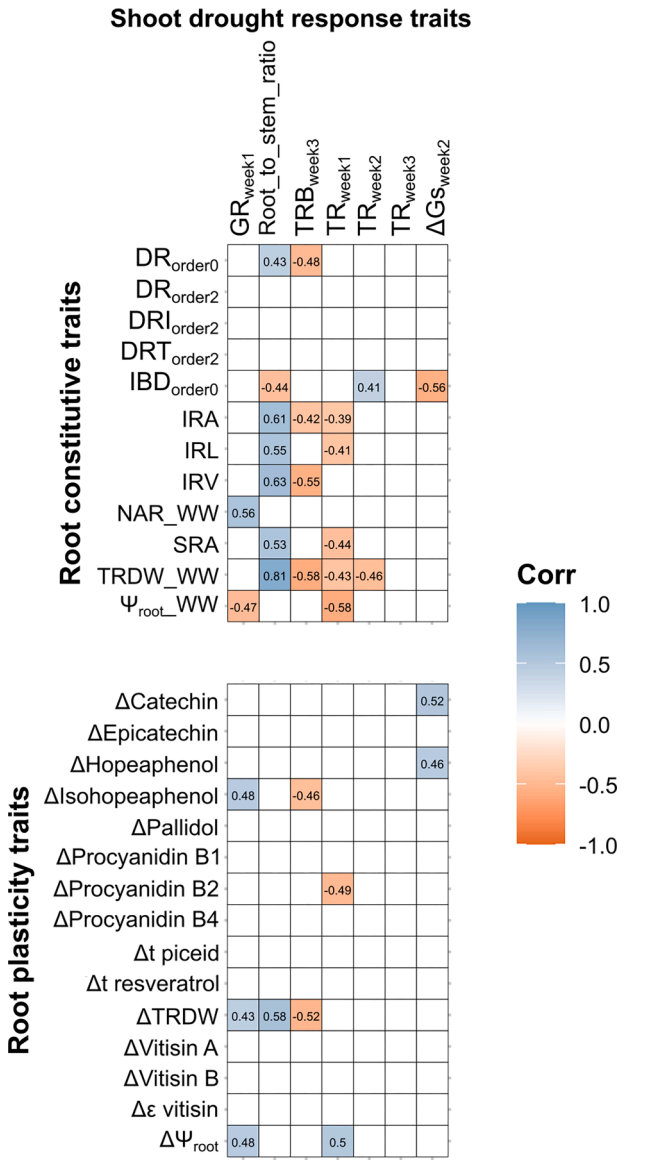

Fig. 2. Genetic correlations between root constitutive traits and root plasticity with shoot drought response traits. For shoot drought response traits, genetic correlations were estimated from BLUPs (Best Linear Unbiased Predictor) and for root plasticity traits ΔBLUPs ($BLUPs_{WD}$ -$BLUPs_{WW}$) were used. Correlation was made with Pearson's method with a FDR correction (q_value< 0.05). Only the significant correlations were presented.

used to determine the shoot drought response syndrome. The three first dimensions were retained for the clustering, explaining in total 78% of the variance. We identified three clusters. The first one, with 30 out of 45 accessions, was mainly composed of *V. acerifolia, V. californica, V. candicans, V. doaniana, V. monticola, V. rupestris* and *Vitis coignetiae.* The second shoot drought response syndrome was composed by 8 accessions from *V. labrusca* and *V. amurensis* and by isolated accessions from other species. The third shoot drought response syndrome is exclusively composed from all *V. sylvestris* accessions (Figs. 5, S6).

V. sylvestris differed from the other shoot drought response syndromes with the highest genetic values of GR_{week1}, TR_{week1} and Δgs_{week2}. On the contrary, the second shoot drought response syndrome showed the lowest genetic values of standardized transpiration per leaf area during all the experiment (TR_{week1}, TR_{week2}, TR_{week3}). The first shoot drought response syndrome stood out for having the smallest genetic values of root-to-stem ratio and the highest TRB_{week3} values.

Finally, we identified plastic strategies in response to water deficit considering root traits (Δ between WD and WW BLUPs for Ψ_{root}, TRDW, and from 13 polyphenols) that had significant genetic variances and showed significant changes in response to water deficit. The first four dimensions were selected for the clustering analysis, which explained 76% of the variance. The accessions were separated in three syndromes with 24, 14 and 5 accessions, respectively (Figs. 6, S7). The first root plasticity syndrome comprised *V. acerifolia, V.amurensis, V.doanina, V. californica.*, *V. coignatie V. monticola and V. riparia.* The second root plastic syndrome was composed of *V. labrusca, V. candicans and V. berlandieri* (Figs. 6, S7). The last root plasticity syndrome was exclusively composed of all *V. sylvestris* accessions (Fig. S7).

A linear model and a SNK test revealed that *V. sylvestris* had the highest genetic values of ΔCatechin, ΔHopeaphenol, ΔIsohopeaphenol, ΔProcyanidin B1 and ΔTRDW. On the other hand, the first root plasticity syndrome showed the lowest genetic value for most plasticity traits, such as ΔCatechin, ΔHopeaphenol, ΔProcyanidin B1, ΔProcyanidin B4, ΔVitisin A, ΔVitisin B, and Δε viniferin. The second root plasticity syndrome showed no signs of osmotic adjustment ($\Delta\Psi_{root}$) (Fig. 6).

The comparison between the root constitutive syndrome and the shoot drought response syndrome revealed a coefficient of cophenetic correlation of 0.30 (Fig. S8). The comparison between the shoot drought response syndrome and the root plasticity syndrome resulted in a coefficient of cophenetic correlation of 0.25 (Fig. S8). The comparison between the root constitutive syndrome and the root plasticity syndrome showed the lowest coefficient of cophenetic correlation, which was 0.14 (Fig. S8).

4. Discussion

Roots are the first plant organ detecting soil water deprivation. Consequently, its role is crucial in detecting soil water limitation, stress-signaling and optimization of water acquisition (Duan et al., 2023; Kalra et al., 2024). However, the link between root phenotypes and shoot drought responses is hard to be established because of the complexity of interactions at different spatial and temporal scales both below and above-ground (Chaves et al., 2003; Kou et al., 2022). This gap of knowledge hampers breeding new varieties with adapted root systems to more drought prone environments (especially for perennial crops), which is highly necessary for crop adaptation to climate change (van Leeuwen et al., 2024). In this work we focused on natural variation of root traits genetically correlated with shoot drought responses in an iconic perennial crop such as grapevine. We studied a diverse panel comprising 12 different *Vitis* species with the potential to be used in grapevine rootstock breeding programs. We considered several accessions per species to compare genetic variability at the inter-specific and intra-specific level. We measured 16 root traits using specialized image analysis software and at the same time, we evaluated drought responses and the plasticity of root and shoot traits, in young plants grown under controlled conditions. This approach allowed us to identify root syndromes of genetically controlled traits and to partially link them to shoot drought responses from a multi-trait approach. Our results prove that genetic diversity of root syndromes is not only structured by the phylogenetic relationship and that functional adaptations may have converged for genetically distant species. We also show that constitutive genetic variation in root strategies may be adaptive for drought responses and consequently it could be considered to breed new grapevine rootstocks adapted to drought.

4.1. Genetic variability of root traits

Identifying genetic variation is essential to assess evolvability in natural populations and the potential of improvement in breeding programs (Gao et al., 2023). Thus, one of the objectives of this study was to quantify genetic variation at the intra et inter-specific level for root traits and drought responses. We found significant variation at the inter-specific level for most root traits. However, intra-specific genetic

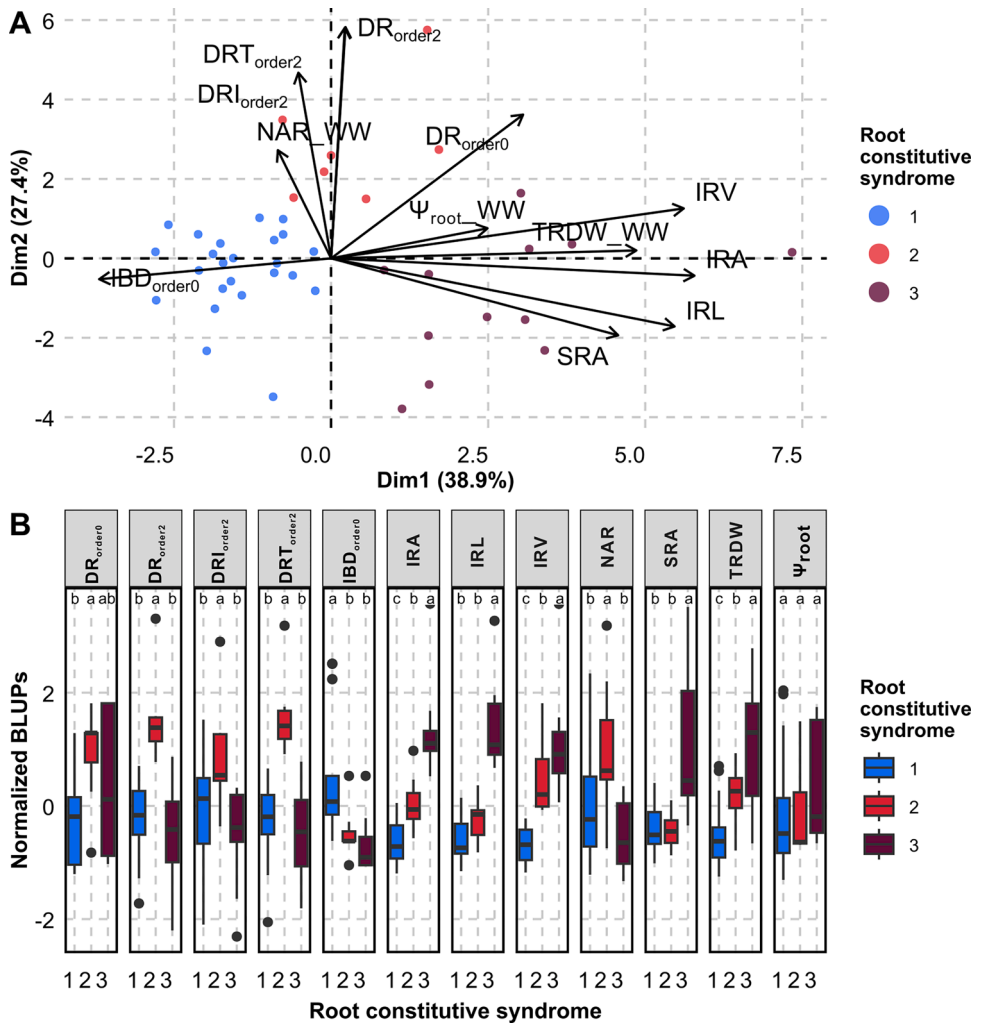

Fig. 3. Distribution of the 43 wild *Vitis* accessions according to their root constitutive syndrome, determined for 12 root traits with significant genetic variation. (A) Biplot of the first two components of the principal components analysis (PCA) clustered according to their root constitutive syndrome (syndrome 1 in blue, syndrome 2 in red, and syndrome 3 in purple). (B) Boxplot of the normalized BLUPs of the same 12 root traits according to the determined root constitutive syndrome. Significant differences between the three root constitutive syndromes were determined by a linear model and Student-Newman-Keuls test with the raw BLUPs.

variation was comparable, even higher than differences among species for several traits. This trend has already been observed in different species of the *Solanaceae* family for root inter-branching distance (Bui et al., 2015). The maintenance of intraspecific variation has already been identified as an important component of functional structure contributing to community assembly and ecosystem functioning for shoot traits in woody species (Vilà-Cabrera et al., 2015). To our knowledge, quantification of intra-specific variation in root traits with a large panel of woody species have not been done previously. In this sense, our results allowed to quantify below-ground natural variation in an economically important complex of species. This knowledge is crucial to assess the potential for evolution of root traits under future climate conditions for this group of endangered species (Heinitz et al., 2019). The high levels of intra-specific variation found for root traits points to the importance of considering different accessions within species in grapevine rootstock breeding programs because very different phenotypes can be produced from the same species. The similar levels of genetic variation at the inter-specific and intra-specific levels could be explained by the frequent hybridizations events occurring between *Vitis spp* that results in genetic introgression among them (Morales-Cruz et al., 2021).

Estimating heritability have allowed to answer important breeding questions (Dudley and Moll, 1969). The estimates of heritability for roots traits obtained in this study suggest that there is potential for improvement in some root traits through selection. This is especially important for those traits that showed a significant correlation with drought response traits, such as the cumulated root length, area, specific root area and total root biomass that were related to transpiration at the beginning of drought stress. The highest heritability estimates were obtained for the number of adventitious roots and the total root biomass. Previous studies in grapevine showed similar heritability estimates for these traits (Blois et al., 2023; Tandonnet et al., 2018). However, these same studies also highlighted higher heritability estimates for the root diameter than our results. In addition, our results showed that heritability for root diameter increases according to the hierarchical order of ramification (*i.e.* root diameters of order 2 are more heritable than root diameter of the primary root). This may be explained by the high intra-genotype variability in root morphology, which is better captured in roots of order 2 (*i.e.* estimated by the mean of several order 2 roots in the scanned image per plant) compared to the estimate of a single primary root per plant.

In this work we characterized order-based roots and we estimated root morphology both along the entire root, such as root length, area or mean diameter, and at specific regions, such as diameter at root tip and at the insertion point. Although this task is time-consuming, we were able to measure a total of 255 plants, corresponding to 43 different

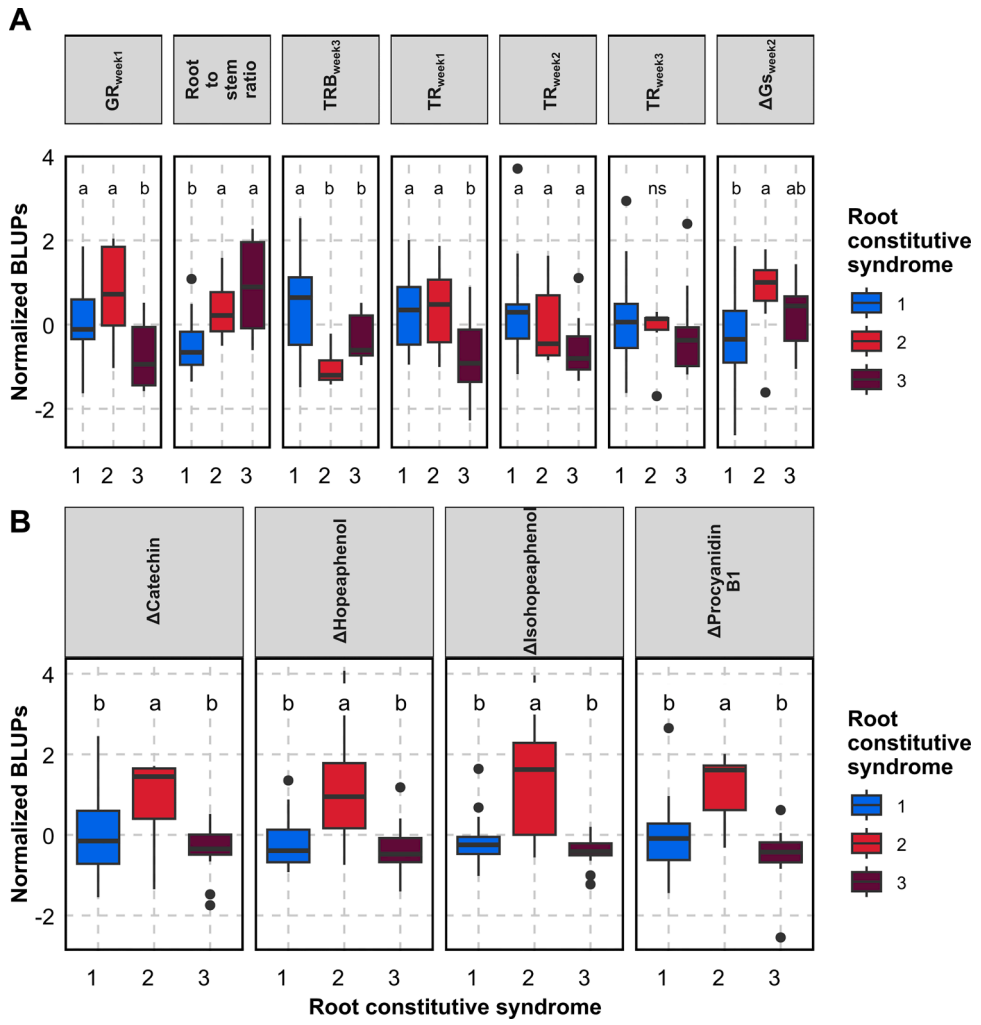

Fig. 4. (A) Boxplot of the normalized genetic values for 7 traits related to root and shoot drought responses according to root constitutive syndromes (syndrome 1 in blue, syndrome 2 in red, and syndrome 3 in purple) identified for the 43 wild *Vitis* accessions. (B) Boxplot of the genetic value of fourth root plasticity traits, measured in WW and WD treatments for 43 wild *Vitis* accessions, significant to root constitutive syndrome separation. Data used for the visualization was normalized BLUPs. Significant differences between the three root constitutive syndromes were determined by a linear model and Student-Newman-Keuls test with the raw BLUPs.

accessions belonging to 12 different *Vitis spp*. Dissecting root morphology based on hierarchical order allows to improve our understanding of dynamic root processes and a better comparison between studies and different species than other kind of classifications based on diameter classes (McCormack et al., 2015). In addition, it has been shown that root order results in different root functions. For instance, lower order roots are more involved in transport functions and higher order in absorptive process (Iversen, 2014). Besides the order-based characterization we explored two key dimensions of root traits: diameter and branching intensity (Kong et al., 2014). Variation of root diameter-related traits across species may represent distinct strategies of root production and persistence (Lynch, 2015). Inter-branching distance plays an important role in nutrient and water acquisition (Kong et al., 2014; Zhan et al., 2015). Our results, shows that the genetic variation for these two dimensions for adventitious roots is mainly found among species. This suggests that introducing genetic variation in rootstock breeding programs to improve root diameter and branching intensity of adventitious roots would require developing interspecific hybrids with species carrying the desired traits. Nevertheless, our results suggest that potential for improvement diameter-related traits in higher order roots exists within species.

4.2. Genetic variability of drought responses

Having assessed the patterns of genetic variation in constitutive root traits, we did a step forward to quantify genetic variation in root drought responses and plasticity. For this purpose, we focused on root biomass and functional traits, such as osmotic adjustment and polyphenol content. Surprisingly, osmotic potential did not change according to water treatment. This is explained by the differential response of species to water deprivation, as revealed by the significant interaction effect. Indeed, some species, such as *V. acerifolia and V. doaniana*, showed more pronounced osmotic adjustment in response to water deficit (Fig. S4). Osmotic adjustment in roots is a well-proven drought adaptation mechanism that allows to maintain cell elongation under water-stress (Blum, 2017; Munns, 1988; Sharp and Davies, 1979; Voothuluru et al., 2024; Westgate and Boyer, 1985). However, inter and intra-species variation in osmotic adjustment has already been reported for other species (Bell et al., 2007; Pang et al., 2011). *V. acerifolia*, one of the species with higher osmotic adjustment, showed also the higher increment in total root biomass in response to drought. All the accessions strongly reduced their shoot growth rate under WD. Consequently, we could not estimate genetic differences in growth rate, with the exception of the first week of water deprivation. *V. doaniana* and *V. sylvestris*, followed by *V. monticola* and *V. acerifolia*, were the species with higher shoot growth rate during the early stages of water deficit, which is in

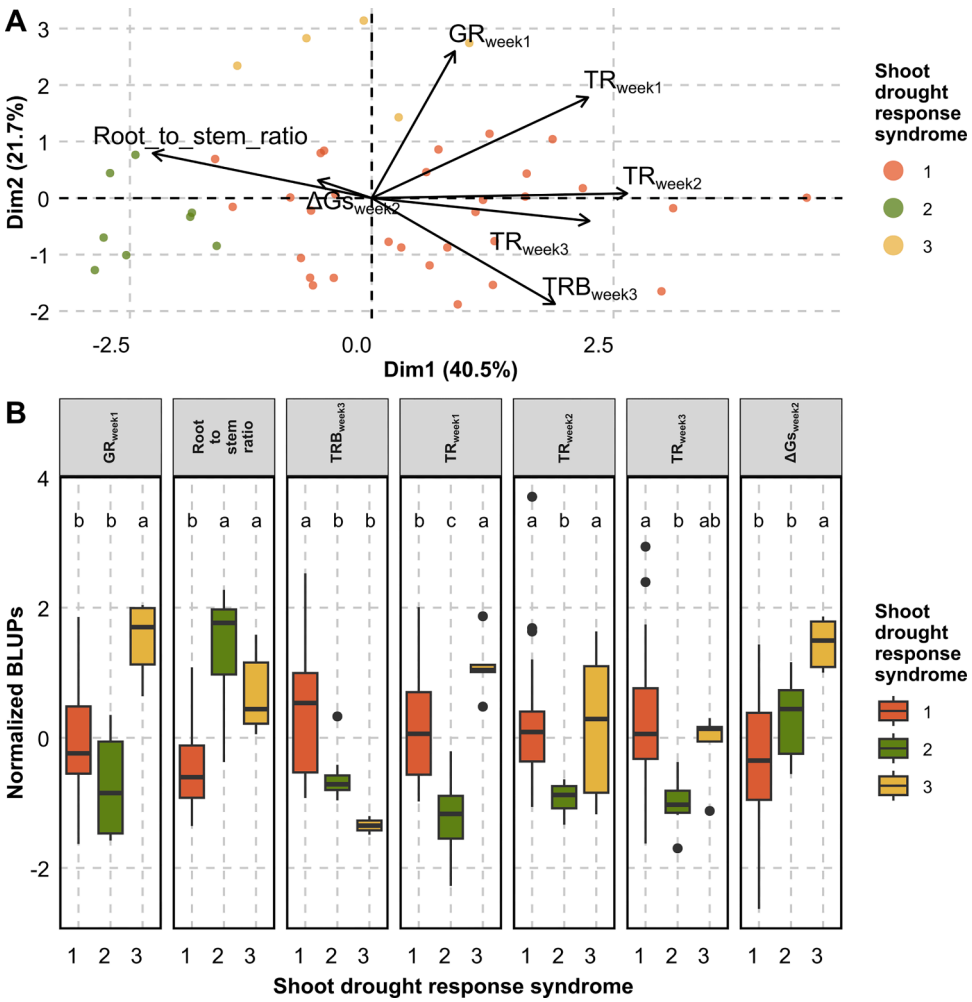

Fig. 5. Distribution of the 43 wild *Vitis* accessions according to their shoot drought response syndrome, determined for 7 traits with significant genetic variation. (A) Biplot of the first two components of the principal components analysis (PCA) clustered according to their shoot drought response syndrome (shoot drought response syndrome 1 in orange, syndrome 2 in green and syndrome 3 in yellow), and (B) Boxplot of the normalized BLUPs of the same 7 root traits according to the determined shoot drought response syndrome. Significant differences between the three shoot drought response syndromes were determined by a linear model and Student-Newman-Keuls test with the raw BLUPs.

accordance with their higher root osmotic adjustment.

One of the usual consequences of drought stress is a greater production of Reactive Oxygen Species (ROS; Tarkowski et al., 2022). This enhanced ROS production is compensated by an antioxidant mechanism that modulates ROS concentration and arrange the cell redox-status (Cruz De Carvalho, 2008; Lin et al., 2023). In this study, we focused on polyphenols because of their antioxidant activity in the grapevine roots during water stress (Hanzouli et al., 2024). Genetic differences in the expression of t-resvatrol, ε-viniferin, and t-piceid were already found between genotypes, with the more drought-resistant presenting higher concentrations (Hanzouli et al., 2024). In our study, all the measured polyphenols increased in WDs with few exceptions, mainly in those species that presented lower osmotic adjustment (Table S2). In this sense, our results points to the existence of a trade-off between the production of osmoprotectans and antioxidants in *Vitis* spp. under moderate water deficit (Fig. 6) that should be confirmed in future studies by specific quantification of osmolytes.

4.3. Root syndromes of genetically controlled traits

Characterizing root system diversity is important for several reasons, such as crop improvement, prediction of changes in species distribution under global change or interpretation of different root functions (Bodner et al., 2013). In this work, we identified three root syndromes that captured diversity in root constitutive traits. The first syndrome comprised American species (*V. acerifolia, V.berlandieri, V. candicans, V. doaniana, V. monticola, V. riparia and V. rupestris*) and was characterized by a lower investment in their root systems, with less ramified primary roots (adventitious roots) resulting in less biomass of the root system. The second syndrome comprised all the accessions from the Eurasian *V. sylvestris* which had more adventitious roots and thicker ramifications. The third cluster, comprised Asian Vitis spp. (*V. amurensis* and *V. coignetiae*) and most *V. labrusca* accessions. The accession rip_1 (*Vitis riparia* cv. Gloire de Montpellier), a commonly used progenitor in rootstock breeding (Riaz et al., 2019), also belonged to this cluster. This third cluster had higher specific root area, specific root length and total biomass. Most of the species had all their accessions grouped within a single root constitutive syndrome. The strong phylogenetic structuring of root traits suggests that root trait syndromes have evolved slowly since speciation events (Valverde-Barrantes and Blackwood, 2016). Only the clustering of *V. labrusca* with the two Asian species did not strictly correspond to the phylogenetic classification of *Vitis* spp (Péros et al., 2021, 2010; Zecca et al., 2012). However, this outcome is in accordance with reports from Galet (1988) based on morphological traits but also habitat and biogeography characteristics. Galet (1988) identified the Series Labruscae containing together *V. labrusca*

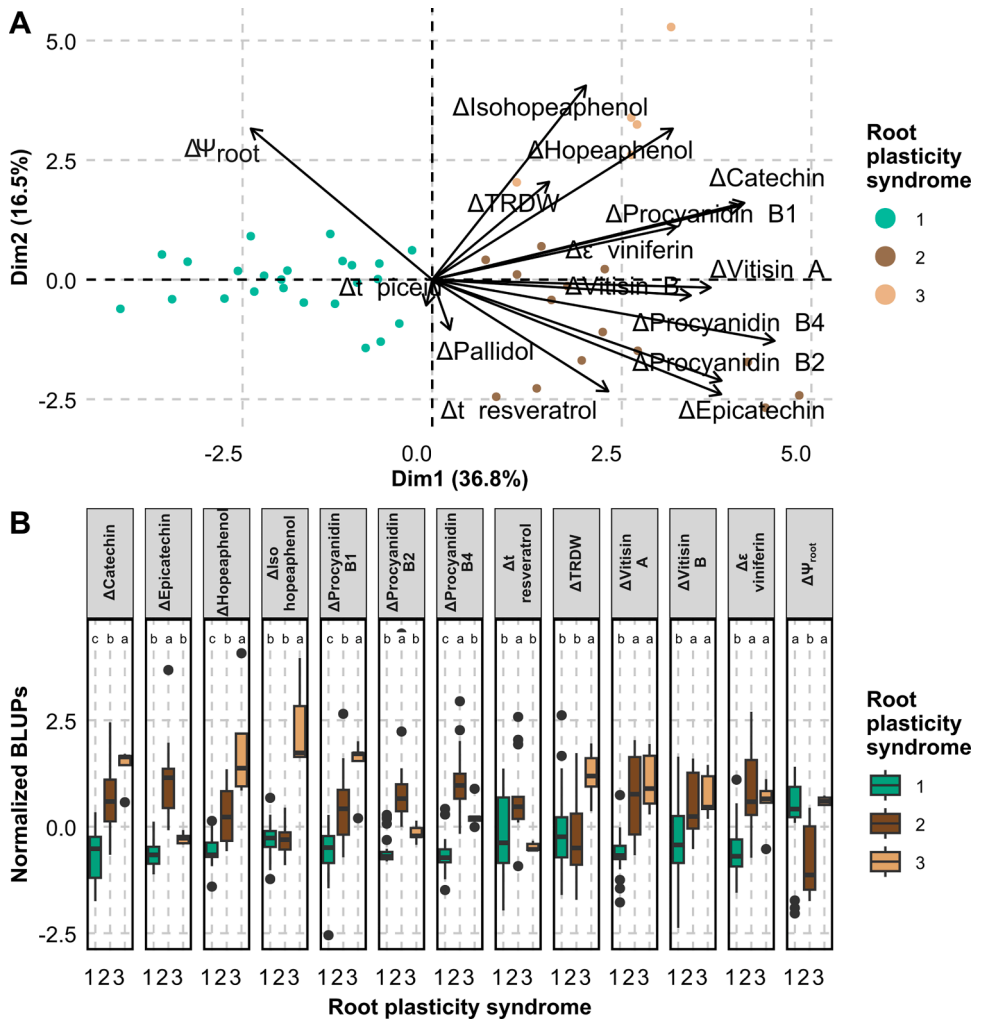

Fig. 6. Distribution of the 43 wild *Vitis* accessions according to their root plasticity syndrome, determined for 13 root traits with significant watering treatment effect and genetic variation. (A) Biplot of the first two components of the principal components analysis (PCA) (B) Boxplot of the normalized ΔBLUPs ($BLUPs_{WD}$ -$BLUPs_{WW}$) of the same 13 root traits according to the determined root plasticity syndrome (root plasticity syndrome 1 (green), 2 (brown), and 3 (dust)). Significant differences between the three root plasticity syndromes were determined by a linear model and Student-Newman-Keuls test with the raw BLUPs.

(American species) and *V. coignetiae* (Asian species). According to Galet (1988), our results point to recurrent root phenotype evolution not constrained by the phylogeny for Asian species and *V. labrusca* (Li and Wu, 2023; Wedger et al., 2019).

The accessions studied in this work were introduced in European germplasm collections at the beginning of the past century (Bordenave et al., 2018). For this reason, precise location of their origin is missing for most of them which hampers our understanding of their native environments with the exception of *V. sylvestris* accessions, more recently introduced from South Spain and France. Accordingly, genetic resource centers do an intensive work to fingerprint their germplasm collections and solve misclassification problems, which is a special issue for the grapevine rootstock material (Andrés et al., 2007). Generally speaking, American *Vitis spp*. occupy warmer and drier climates (Heinitz et al., 2019) while Asian *Vitis spp*. are reputed for their cold tolerance (Wang et al., 2020). In this sense Laughlin et al. (2021) found that forest species occupying warm and dry environments had a higher chance to have low specific root length and large root diameters, which would match with the first and second root constitutive syndrome that we have identified, comprising most of the American *Vitis* species and *V. sylvestris*. On the other hand, the third root syndrome, comprising Asian species and *V. labrusca*, presented higher specific root area and lower root diameter, which would be adaptive in cold environments (Laughlin et al., 2021). However, there is controversy on which root traits favor plant productivity under drought and some studies suggests that fine root diameters may be advantageous (Comas et al., 2013).

4.4. Link between root traits and shoot drought responses

Fine characterization of roots in response to water deficit is challenging for perennial species. As a consequence, there is few genetic studies in perennial root systems (Alahakoon and Fennell, 2023; Blois et al., 2023; Chen et al., 2021; Tandonnet et al., 2018) and few of them phenotyped order-based roots separately. This gap of knowledge hampers our understanding on how genetic variation of the below ground part contribute to plant adaptation to drought. As a result, breeding programs focusing on resilient root systems able to face climate change scenarios in woody perennials are practically unavailable.

The main reasons for the lack of genetic studies in perennial root systems rely on the difficulty of access to the belowground organs and the time-consuming phenotyping of root system architecture, morphology and function. For these reasons, we tested whether genetic variation of root constitutive traits was linked to shoot drought responses. Validating this hypothesis could help saving phenotyping time in future genetic studies or breeding programs. We addressed this question from two approaches: genetic correlations trait-by-trait and based on constitutive root syndromes. Both of them resulted in similar outcomes. What stands out from these analyses, is that species with less

developed root systems had more adventitious roots but less dense tissues and lower ramifications but thicker. These root syndromes resulted in higher growth rate and transpiration during early stages of the drought period and lower root-to-stem ratio. American *Vitis* species and *V. sylvestris* belonged to these root constitutive syndromes. A reduced lateral root branching density can improve drought tolerance by reducing inter root competition and the metabolic cost of soil exploration (Zhan et al., 2015). This is in line with the lower specific root area that suggests a reduction in tissue maintenance costs (Schneider and Lynch, 2020). At the same time, increasing the diameter of higher order roots (order 2 in this study), which are involved in water absorption (Iversen, 2014) may enable these roots a greater capability to explore hard drying soils, because of higher mechanical support (Peralta Ogorek et al., 2025). As a consequence, the accessions with less developed root systems resulted in lower root to stem ratio, which indicates that shoot development was not reduced for these groups of species. However, our results should be validated in field conditions, and whether the detected root syndromes could be advantageous for drought responses to more severe stresses should also be addressed in further studies.

In this study, we did not measure the morphological plastic response of roots. Nevertheless, we analyzed the plastic drought response of roots for functional traits, that is osmotic adjustment and the accumulation of antioxidant molecules, such as polyphenols. The syndrome observed for root plastic responses revealed that osmotic adjustment was negatively correlated with the increment of some polyphenols, such as resveratrol, pallidol and t_piceid but les related to the increment of the other polyphenols. Root plasticity was poorly related with shoot drought responses. Similar results has been previously obtained for drought resistance traits, such as turgor loss point, in a wide panel of wild and crop species (Bartlett et al., 2014). This can be explained by the significant genotype by environment interaction found between water treatment and species for most plastic traits indicating that the magnitude of plasticity may be considerable only for some species. In addition, it is possible that the variation of some polyphenols is just driven by the environment (*i.e.* passive plasticity) and does not reflect the manifestation of a physiological response mechanisms activated by the plant to cope with drought (*i.e.* active plasticity) (Forsman, 2015). In order to evaluate whether the plasticity of root functional responses to drought should be considered as a target in breeding, precise estimations of the adaptive plasticity have to be addressed in further studies (Brooker et al., 2022). This information is crucial to estimate the environments under which the candidate accessions should be evaluated (Dudley and Moll, 1969).

The cophenetic correlation between root constitutive syndrome and shoot drought responses was moderate but was higher than the cophenetic correlation between root plastic strategy and shoot drought responses. This correlation allows comparison of the variation in the multi-trait matrix of root constitutive traits with that of the multi-trait matrix of shoot drought response traits. Several studies proved the importance of constitutive traits to deal with stressful environments (de María et al., 2020; Liu et al., 2023). For instance, de María et al. (2020) identified that drought-tolerant genotypes of a woody species (*Pinus pinaster* Ait.) constitutively expressed stress-related genes that were detected only in latter stages on sensitive individuals subjected to drought. At the end, our results constitute a first step towards the inference of shoot drought responses from root constitutive traits. However, drought tolerance is determined by a diversity of traits (Gambetta et al., 2020). In this work, we focused on root-related traits, transpiration related-traits and growth of the main stem, but many other traits, such as leaf morphology, stomatal regulation, hydraulic traits and phenology should be also evaluated to better asses drought tolerance of *Vitis spp*.

5. Conclusions

Providing viticulture with new rootstocks able to cope with drought is urgent in the context of climate change. However, most of the currently used rootstocks rely on a narrow genetic background. Natural variation of crop wild relatives is useful to introduce adaptive traits in breeding programs. In this work, we quantified genetic variation of root traits and shoot drought responses for 43 accessions, belonging to 12 North American, Asian and Eurasian wild *Vitis* species, that were previously understudied. We identified three constitutive syndromes of root traits with genetic variation that showed contrasted shoot drought responses. Two syndromes allowed to maintain transpiration and growth for the first stages of the drought period. Within these clusters, the species *V. acerifolia, V. doaniana* and *V. sylvestris* stood out because they also activated osmotic adjustment, a mechanism of drought tolerance. These results points to their potential interest to be used in breeding programs. Accordingly, *V. sylvestris,* the wild ancestor of domesticated grapevine, has been recently proposed as a candidate for breeding programs because of their ability to adapt to various abiotic stresses (Daldoul et al., 2025). Functional responses of roots, based on osmotic adjustment and polyphenol content showed moderate heritability, highlighting their potential use in breeding programs. However, multivariate analysis revealed that constitutive morphological root syndromes were more closely linked to shoot drought strategies than root plasticity syndromes, which advocates for the evaluation of constitutive traits to select root phenotypes favorable for drought-prone environments. In the end, our results contribute to the understanding of root systems ideotypes with a potential for breeding.

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Supplementary material

Supplemental Table S1. Germplasm origin, name and code of each accession used.

CRediT authorship contribution statement

E.R. Patin: Writing – original draft, Investigation, Formal analysis, Data curation. **U. Pérez-López:** Writing – review & editing, Investigation. **A. del Sol Iturralde:** Investigation. **J. Valls-Fonayet:** Writing – review & editing, Investigation. **P. Pétriacq:** Writing – review & editing, Investigation. **P. Gastou:** Investigation. **J.P. Tandonnet:** Investigation. **M. Larrey:** Writing – review & editing, Formal analysis. **P. Vivin:** Writing – review & editing, Investigation. **E. Marguerit:** Writing – review & editing, Investigation. **N. Ollat:** Writing – review & editing, Conceptualization. **M. de Miguel:** Writing – original draft, Supervision, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2025.100964.

Data availability

Data will be made available on request.

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