The effect of drought memory on grapevine physio logical responses to water deficit and recovery

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Abstract. To cope with water stress, grapevine developed efficient adaptation mechanisms to transfer water from roots to shoots, mediated by an efficient stomatal control of transpiration. Each variety has a specific water use regulation response under drought, which is important to study. In the present work, we explored the influence of the field-grown genotypes' drought memory in the drought-response phenotype of their vegetative progenies, in Trincadeira (isohydric) and Castelão (anisohydric) cultivars under a drought event followed by recovery. These progenies were subjected to full irrigation (FI) and non-irrigation (NI) for five years in the field, and their progenies were in grown in a glasshouse and subjected to a well-watered and a water- stress treatment, followed by recovery. NI progenies from both cultivars had improved gas exchange parameters, better total plant hydraulic conductance under drought, and faster recovery than FI progenies. Nocturnal transpiration was affected both by progeny and treatment. Leaf wax content was significantly enhanced by WS both in FI and NI progenies, but it was higher in NI progenies. Although isohydric and anisohydric genotypes exhibited different drought acclimation responses due to their inner genetic behavior, their underlying hydraulic, stomatal and photosynthetic regulatory mechanisms were also affected by historical origin.

1 Introduction

To cope with water stress, plant species, including grapevine, developed efficient adaptation mechanisms to transfer water from roots to shoots, mediated by an efficient stomatal control of transpiration [1]. This water use regulation response under drought is specific of the variety [2-4], and it is still unclear whether this regulation results from innate genotypic behavior (iso- and anisohydric characteristics), or is a response to environmental factors, namely recurrent water stress priming effects.

In the present work, we explored the influence of the field-grown genotypes' drought memory in the drought- response phenotype of their vegetative progenies, in Trincadeira (isohydric) and Castelão (anisohydric) cultivars under a drought event followed by recovery.

2 Materials and methods

2.1 Plant material

The study was performed under greenhouse conditions at Instituto Superior de Agronomia, University of Lisbon in July 2022. Cuttings from Trincadeira (TR) and Castelão (Cs) cultivars were obtained from the ampelographic collection located at the commercial vineyard of Herdade do Esporão, Reguengos de Monsaraz (latitude: 38.394696, longitude: -7.553158). Canes were collected at the beginning of January 2022 from 11-year-old grapevines grafted on 1103 Paulsen rootstock and were selected from plants subjected to two different irrigation regimes for five consecutive years): full irrigated (FI, irrigated at 100% ETc), and non-irrigated but rain-fed (NI). Three to four node cuttings were rooted in a nutrient solution for rooting

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(with 2 µM indolebutyric acid) under controlled conditions in a growth chamber (26 ºC – 22 ºC; 16 h light – 8 h dark). Cuttings were then placed in 2.5 L pots filled with mix of soil, peat and coconut fiber (2:1:1), transferred to a glasshouse and regularly irrigated until the beginning of the experiment. After acclimation, plants from each variety and irrigation origin were selected according to their homogenous growth. Two irrigation schemes were applied in each variety (TR and Cs) and provenance (FI and NI): Well Watered (WW) in which the soil was kept at field capacity; and Water Stress (WS), in which the plants were not irrigated for 8 days. In total, 8 different treatments were made, and in each, 9 plants were used. Three plants from each treatment were selected to be constantly, and non- destructively, monitored and weighted (total of 24 plants), while the rest were used for the destructive measurements.

2.2 Experimental method

One day before the beginning of the trial (T-24h), all the plants were irrigated at field capacity and let to drain all their gravitational water until it stopped dripping. Afterwards, all pots were covered with a plastic bag to remove soil evaporation from the plant weight loss calculations. Each pot of the non-destructive group was placed on an individual manually built scale. The experiment began at T0 (July 4th, 2022) and ended at Trec72h (July 14th, 2022). WW plants were irrigated every 2 days to replace the water lost by transpiration. After 168h (T2, July 11th, 2022), WS plants were re-watered and kept at field capacity for 72 h, the end of the experiment. Measurements were done at five key moments: i) T0; ii) T1 (72h after water withhold), expected mild stress in the WS treatment group, which coincided with the first day of the heatwave; iii) T2, expected severe stress in the WS treatment group, before the WS re-watering; iv) Trec24h, 24 hours after the recovery; v) Trec72h, 72 hours after the recovery and the last day of the experiment.

2.3 Climatic conditions

Temperature, relative humidity and radiation were regularly recorded (10 min interval) in the glasshouse using CS215 probe (Campbell Scientific, Inc, USA) and a KIPP & ZONEN CMP 3 pyranometer, respectively. Probes were coupled to a Campbell CR1000 datalogger (Campbell Scientific, Inc, USA). Vapor-Pressure Deficit (VPD) was then calculated using the temperature (T) and relative humidity (RH) data.

2.4 Plant water status

Leaf water potential (Ψleaf) measurements were made with a pressure chamber (Model 600, PMS Instruments Company, Albany, OR, USA) at predawn (Ψpd), and at midday (Ψmd). These measurements were made at T0, T1, T2, Trec24h and Trec72h. For each measurement, one to two fully expanded leaf per plant of three to four plants per treatment were used.

2.5 Total hydraulic conductance

Total hydraulic conductance (Kh) was obtained using Darcy's law [5-6], with the formula Kh = E / (Ψmd-Ψpd).

2.6 Gas exchanges

Net photosynthesis (An), transpiration (E) and water use efficiency (WUE) were assessed on the 24 non-destructive plant group, using a CO_2/H_2O gas analyzer (CIRAS-3 DC, PP SYSTEMS, Inc, Massachusetts, USA), while stomatal conductance (*gs*) was measured using a Porometer/Fluorometer (LI-600, LI-COR Inc, Lincoln, Nebraska). These measurements were made at T0, T1, T2, Trec24h and Trec72h, between 11:00 am and 1:00 pm.

3 Results

3.1 Plant water status

Predawn leaf water potential (ΨleafPD) was similar in WW and WS plants on T1 in both TRFI and TRNI (Fig. 1). When water stress was severe (T2), ΨleafPD of WS plants decreased significantly in both progenies, returning to WW levels during recovery. In Cs, there were also no differences between progenies in the response to WS. The major difference in ΨleafPD) was observed between TR and Cs under severe WS (T2), when TR showed values of *circa* -1.2 MPa and Cs did not decrease from - 0.7 MPa.

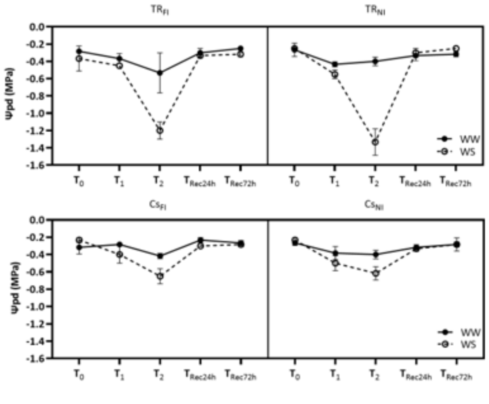

Figure 1. Predawn leaf water potential (ΨleafPD) in the five time points of the experiment (T0, the beginning of the experiment; T1 and T2, the water stress treatment; Trec24h and Trec48h, the recovery), in Well Watered (WW) and Water Stressed (WS) plants of Trincadeira and Castelão of Full Irrigated (FI) and non Irrigated (NI) progenies. Values are expressed in MPa and are mean±SE.

3.2 Leaf gas exchanges

Stomatal conductance (*gs*) values were higher in FI progenies in well watered conditions in both varieties, and were higher in Cs than TR (Fig. 2). Upon WS, *gs* decreased in both progenies, reaching lower values in FI progenies than in NI. This decrease was faster in TR than in Cs. *gs* recovered quickly after plant re-watering (30 min) in water stressed TRNI, that presented values higher than TRFI. In Cs the differences between progenies were not so significant, with CsNI progenies showing higher values of *gs* at the end of recovery (Fig. 2).

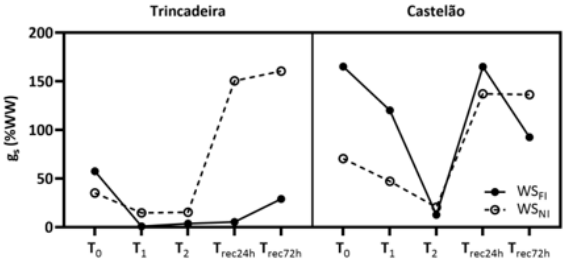

Figure 2. Stomatal conductance (*gs*) in the five time points of the experiment (T0, the beginning of the experiment; T1 and T2, the water stress treatment; Trec24h and Trec48h, the recovery), in Trincadeira and Castelão plants of the Full Irrigated (FI) and non Irrigated (NI) progenies. Values were measured in mM H2O m^{-2} s^{-1}, and are presented as percentage of the respective Well Watered (WW) treatment value.

Photosynthesis (An) drops to null values upon water stress (T1) in the water stressed FI progenies of the isohydric TR, while in NI progenies, An decreases progressively and only reaches null values at the maximum stress (T2) (Fig. 3). The An behaviour is consistent with *gs* results in both progenies (Fig. 1), and can be explained by them. In the anisohydric Cs, An kinetic along the experiment was almost the same in FI and NI progenies, with two differences: while FI progenies reached null photosynthesis at T2, NI progenies still presented positive An values, and at the end of recovery An was higher in NI progenies (Fig. 3).

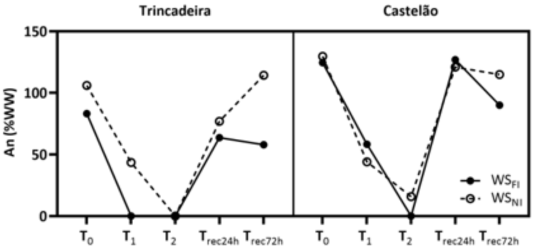

Figure 3. Rate of Apparent Photosynthesis (An) in the five time points of the experiment (T0, the beginning of the experiment; T1 and T2, the water stress treatment; Trec24h and Trec48h, the recovery), in Tricadeira and Castelão plants of the Full Irrigated (FI) and non Irrigated (NI) progenies. Values were measured in mM CO2 m^{-2} s^{-1}, and are presented as percentage of the respective Well Watered (WW) treatment value.

As expected, plant transpiration (E) decreased in WS treatment in both varieties and progenies (Fig. 4), and differences between varieties and progenies were also observed. E rate was higher both at WW and WS in TRNI as compared with TRFI. In Cs, the decrease of E in WS was only observed at T1 in CsFI. In CsNI, no significant differences were observed between water treatments. After re-watering, E increased in WS plants.

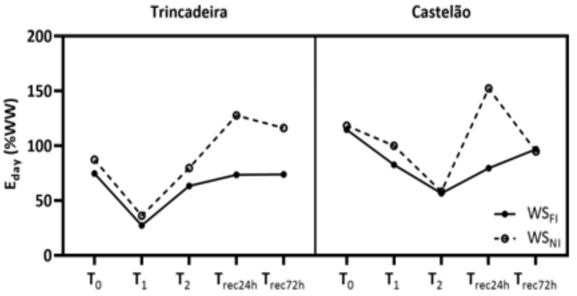

Figure 4. Rate of Transpiration (E) in the five time points of the experiment (T0, the beginning of the experiment; T1 and T2, the water stress treatment; Trec24h and Trec48h, the recovery), in Trincadeira and Castelão plants of the Full Irrigated (FI) and non Irrigated (NI) progenies. Values were measured in g H2O cm^{-2} d^{-1}, and are presented as percentage of the respective Well Watered (WW) treatment value.

3.3 Hydraulic conductance

Total plant hydraulic conductance (Kh) was lowest in WS vines of TRFI from the onset of stress (Fig. 5). In contrast in TRNI, no differences between WW and WS were observed, nor were there changes during WS; however, during recovery, at Trec24h, Kh was higher in WS than in WW. In Cs, Kh decreased in WS at T2 in both progenies. After re-watering, Kh increased in WS vines in both progenies, but while Kh did not differ between WW and WS in CsFI, in Ni progenies, Kh was higher in WS than in WW (Trec24h and Trec72h).

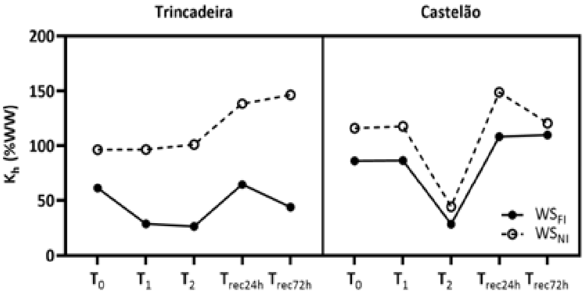

Figure 5. Total plant hydraulic conductance (Kh) in the five time points of the experiment (T0, the beginning of the experiment; T1 and T2, the water stress treatment; Trec24h and Trec48h, the recovery), in Tricadeira and Castelão plants of the Full Irrigated (FI) and non Irrigated (NI) progenies. Values were measured in kg m^{-2} d^{-1} MPa^{-1}, and are presented as percentage of the respective Well Watered (WW) treatment value.

3.4 Wax content

Leaf wax content, quantified at the end of the experiment, was significantly enhanced by WS both in FI and NI progenies, but it was higher in NI progenies (Fig. 6).

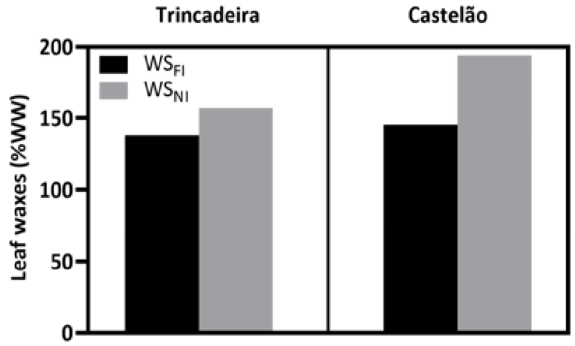

Figure 6. Leaf wax content at the end of the experiment, in Trincadeira and Castelão plants of the Full Irrigated (FI) and non Irrigated (NI) progenies in the WS treatment. Values were measured in µg cm^{-2}, and are presented as percentage of the respective Well Watered (WW) treatment value.

4 Discussion

Although isohydric (Trincadeira) and anisohydric (Castelão) genotypes had different drought responses related to their genetic background, the hydraulic, stomatal and photosynthetic regulatory mechanisms underlying their response to water stress were also affected by their historical origin.

The Ψ_{leafPD} showed differences between the varieties, being much lower at maximum stress in TR (isohydric) than Cs (anisohydric). However, no influence of historical origin was observed. In contrast, historical origin effect was observed in g_s and An in addition to varietal differences. In TR, NI progenies had consistently higher g_s and E when compared with FI progenies. While in Cs, NI progenies showed lower g_s than FI with no differences in E. These data indicate that historical drought occurrence impacts differently among varieties. The isohydric variety TR, showed higher drought memory effect to deal with water stress imposition as well as recovery, while the anisohydric Cs showed less differences between progenies. In any case, regardless of the genotype and progeny origin, stomatal regulation and photosynthetic capacity were deeply altered as reported in other grapevine varieties [7]. In terms of photosynthesis, in both varieties the FI progenies showed more carbon limitation that NI. These results point out the role that plant stress ‘memory’ plays to induce more plasticity to future exposure to the same stress factor. Previous studies showed anatomical changes in the vascular system of grapevines, such as reduction in vessel size with increased density and lignifications, under multi-annual drought stress [8]. This could explain the higher K_h observed in NI progenies compared with FI when subjected to WS. Corroborating these results, upon recovery K_h and g_s increased more as compared with FI progenies. The quick increase in g_s and K_h upon water recovery was also reflected in An, in line with recent reports by [9], who showed a post-rehydration photosynthesis almost immediate (a few hours and less than 24 h). Nonetheless, the recovery of An was faster in TR than in Cs. K_h and E showed higher values in NI progenies after recovery than FI. This later result could be explained by a higher need for water refilling in stressed plants from NI progenies and may indicate that NI progenies operate at xylem pressures close to critical thresholds when exposed to WS [10]. This later result opens the way for interesting research on how the drought priming influences the process of hydraulic recovery in grapevine and how it is modulated among varieties. Moreover, although additional data and analyses are required to conclude whether the changes observed in this study are truly an effect of drought priming, the alteration of the different physiological and anatomical (e.g. wax content) parameters is potentially an indication of the establishment of drought memory in grapevine progenies.

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