Australian Plant Phenomics Facility

The Plant Accelerator®

| Pilot Project Report – DroughtSpotter Platform | |
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| Project Title: | Investigating the relationship between hydraulic and stomatal conductance and its regulation by root and leaf aquaporins under progressive water stress and recovery, and exogenous application of ABA in grapevine |
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**General information about the project design**

A set of 72 vines from two different cultivars (36 Syrah and 36 Grenache) were used to test the different irrigation treatments and 8 more pots without plants were used as controls (total=80 vines). One-year-old vines grown in 4.5 L pots were transferred to the DroughtSpotter and allowed them to acclimate 4 or 5 days to the chamber climatic conditions. The treatments applied were i) well-watered (WW) vines; ii) watered stressed (WS) vines; iii) root fed-ABA (ABA) vines and iv) recovery from water stress (RE) vines.

Well-watered plants were irrigated to field capacity every day, according to the plant weight loss by transpiration, replacing the exact amount of water consumed. The water stress treatment was established according to a defined value of leaf maximum daily stomatal conductance ($g_s$) of about 50 mmol $m^{-2}s^{-1}$ (Flexas et al., 2002). Irrigation was withheld in these vines until the desired $g_s$ value was achieved, and maintained constant for a week by replacing the amount of water consumed. After a week under water stress, half of the vines were rehydrated (RE) by irrigating the pots to field capacity. Another set of vines, were root-fed with ABA by applying 50 µM every day to the root system, concomitantly with the irrigation, until leaf stomatal conductance achieved a similar value to those in WS plants (50 mmol $m^{-2}s^{-1}$).

Each treatment was randomly assigned to the vines and replicated 6 times. Three time points of measurements were performed during the course of the experiment: at the beginning of the experiment (Day 0), when water stress and ABA treatments achieved the desired level of stomatal conductance (Day 3-4) and 5-7 days after rewatering RE vines.

Measurements: leaf gas exchange measurements (stomatal conductance, transpiration and photosynthesis), leaf water potential (predawn and midday) and stem water potential, hydraulic conductance of the leaves and roots, samples for xylem sap ABA concentration, leaf and roots aquaporins (AQPs).

**Aims of the experiment**

1. Determine the gas exchange responses of the two cultivars to progressive water stress and recovery from water stress.
2. Measure the hydraulic conductance of the root, leaves, and whole-vine (soil-to-leaf) during various levels of drought stress and recovery therefrom.
3. Quantify the expression of aquaporin transcript levels using real-time quantitative PCR (RT-PCR) at various stages of drought stress and recovery.
4. Study the physiological responses of the cultivars to different imposed stress conditions; by water and by ABA exogenous application

**Key results and outputs**

The leaf hydraulic conductance ($K_{leaf}$) decreased by 75% in the water-stressed plants compared to the well-watered plants, but it was unchanged with exogenous ABA. Upon rewatering, the water-stressed plants reached 66% of the initial $g_s$ and had $K_{leaf}$ values similar to the well-watered plants.

Differences between cultivars were observed in the relationship between gs and leaf water potentials (predawn and stem), where Grenache had a steeper (and significantly different) curve than Syrah, thus showing a more sensitive response of $g_s$ to water stress.

Positive linear relationships were observed between root hydraulic conductance ($L_o$) and $g_s$, and between $L_o$ and transpiration for Syrah that were related to vine water status and ABA application. These coupling found between these variables suggest $L_o$ might be responding to the transpirational demand from the leaves. In contrast, a significant relationship was found between $g_s$ and $K_{leaf}$ in Grenache when plotting all the treatments together, but no relationship was found for Syrah. This decreased in $K_{leaf}$ observed in Grenache as compared to Syrah, could be associated to some of the AQPs expressed in the leaves, where PIP 2;1 that was down-regulated under WS conditions.

Finally, both cvs. showed a significant relationship with $g_s$ which confirms the role of ABA on stomatal regulation. ABA concentration in the xylem sap was higher Grenache as compared to Syrah which explains with its higher sensitivity to WS.

A better understanding of these mechanisms is highly relevant to irrigation scheduling and to ensure a sustainable vineyard management in a context of water scarcity.

<u>Preliminary Conclusions</u>

-The more drought-tolerant Grenache showed a more conservative behaviour than Syrah under WS through reductions in $L_o$, $g_s$, $K_{leaf}$ that were linked to changes in leaf hydraulics probably mediated by AQPs.

-Syrah showed a more optimistic behaviour maintaining $L_o$ under WS mediated by an upregulation root AQPs, but had a lower $L_o$ under well-watered conditions

-Our data suggests that for Grenache, both hydraulic and chemicals signals are involved in WS responses, whereas in Syrah hydraulic signals predominate.

**Statement on how data obtained from the DroughtSpotter provided new insights into your research**

The DroughtSpotter platform allowed us to achieve precise control over soil moisture and vine water stress, which was the most critical aspect to the success of this project. This platform allowed us to calculate whole-vine transpiration (water use) based on the differences in pot weights during a specific time interval at each stage of water stress and recovery. The high resolution vine water use data that was collected continuously on the DroughtSpotter platform was highly valuable to this project.