STSM Report

Monitoring of iso/anisohydric grapevine embolism using NMR techniques.

COST Action: STReESS

COST STSM Reference number: COST-STSM-FP1106-15801

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Period: 06.05.2014-29.05.2014

Introduction: It is generally accepted that grapevines *(Vitis vinfera)* cultivars possess significant variability in their hydraulic behavior (Schultz, 2003), a feature reflected in the cultivar-specific responses to water deficit. Several publications characterized differences between near isohydric cultivars (Grenach, Cabernet Sauvignon) and near anisohydric cultivars (Chardonnay, Shiraz) (Schultz, 2003; Chalmers, 2007; Hochberg *et al.*, 2012; Tramontini *et al.*, 2013). Several differences between the iso/anisohydric groups were hypothesized to be the origin of the phenomena: aquaporins expression (Vandeleur *et al.*, 2009), stomata regulation through hormonal balance (Soar *et al.*, 2006) and embolism in respect to xylem architecture. Our previous research (some of it published as Hochberg *et al.*, (2012)) characterized Shiraz and Cabernet Sauvignon to differ in their hydraulic behavior. We found differences in stomatal regulation and xylem architecture. Additionally, differences in cavitation susceptibility have already been shown to exist between the cultivars through various measurements of PLC (percent loss of conductance) (Schultz, 2003; Alsina *et al.*, 2007; Figure 4); however, so far, comparison of these cavitation through direct imaging (in vivo) were never tested.

Purpose of the STSM: The purpose of this research was to evaluate cavitation susceptibility differences between Shiraz and Cs cultivars through (nuclear magnetic resonance (NMR) technology, and relate them to xylem architecture. In vivo visualization of embolism formation could greatly improve our understanding of the dynamics of embolism formation of these two cultivars. NMR technology used for plants is available in very few places around the globe and as such required traveling to Julich. Additionally we will test if it is possible to measure cavitation events by means of novel portable NMR (pNMR) sensors that can also be used in the field.

Description of the work carried out during the STSM:

Plant Material- This study was carried out on Shiraz and Cabernet Sauvignon one year old grapevines. The plants were grown in 6 L pots to a size of 25 leafs and were fully irrigated prior to the beginning of the experiment. Side branches were removed to shape the plant so it will fit the MRI tube.

Monitoring embolism appearance in MRI- We used the 4.7 Tesla MRI facility available in the Julich Forchungzsentrum. The MRI… The rootstock of each vine was cut under water and was transferred to the MRI with the open cut constantly submerged under water. The vine was placed on a special device built for the trellising of vines inside the MRI magnet. A 1 cm radio frequency coil was placed around a piece of stem and petiole, 50cm above the cut. MRI images were acquired every 21 minutes for a period of 20 hours. After the first image was taken the plants was moved out of the water and was left to dry while being imaged. At 4 time points during the dehydration process 4 leafs were sampled to estimate the reduction in Stem water potential using a pressure bomb. After completion of the 20 hours, the imaged petioles and stem were sampled for microscopy analyses.

Measuring vines with portable NMR- Two portable NMRs (Figure 1) were used to monitor the vines. At first, we observed the potential to measure air cavitation in stems and petioles. The tissue was placed in the center of the Magnets and its water content was continuously monitored. A cut directly under the measured tissue (presumably leading to significant cavitation) was made, and the reduction in water content was instantly measured.

Later we checked if this potential could be spotted in gradually drying plants. The plant's water content was continuously monitored while the stem was disconnected from the root stock to allow fast dehydration.

Last, we tested the feasibility of the portable NMR for monitoring the plants water statues while comparing it to the common shrinkage monitoring with dendrometer.

Figure 1- Portable NMR

Main results

NMR was shown to be an effective mean to observe large cavitation events, done through cutting below the measured area (Figure 2). However, in a gradual dehydration experiment, no such jumps were shown probably due to resolution of water content or time.

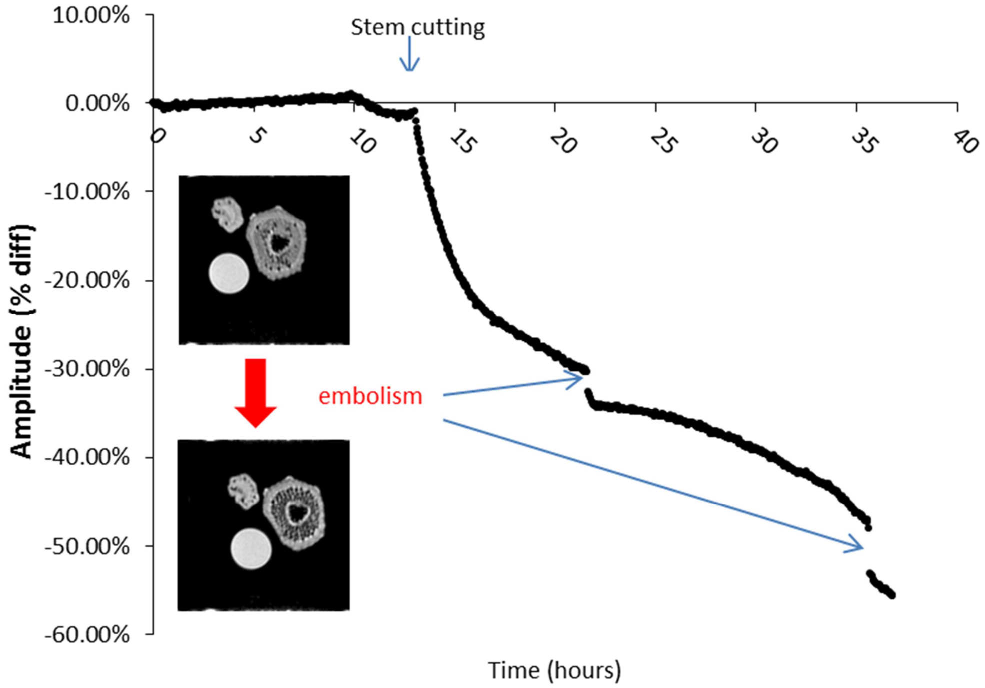

Figure 2- Stem dehydration (due cutting of its roots) monitored with portable NMR. Large embolism events, (marked in the image) were inflicted by cutting the stem 15cm and 2 cm below the measured spot, thus allowing air to penetrate the vessels (As also demonstrate by the MRI images in a stem and a petiole).

Portable NMR's measuring the petioles was shown as an effective mean to continuously monitor the plant water statues (Figure 3). The reliability of such measurement performed on the petiole (3 mm thick) could potentially reduce the size of the Magnet and its economical cost.

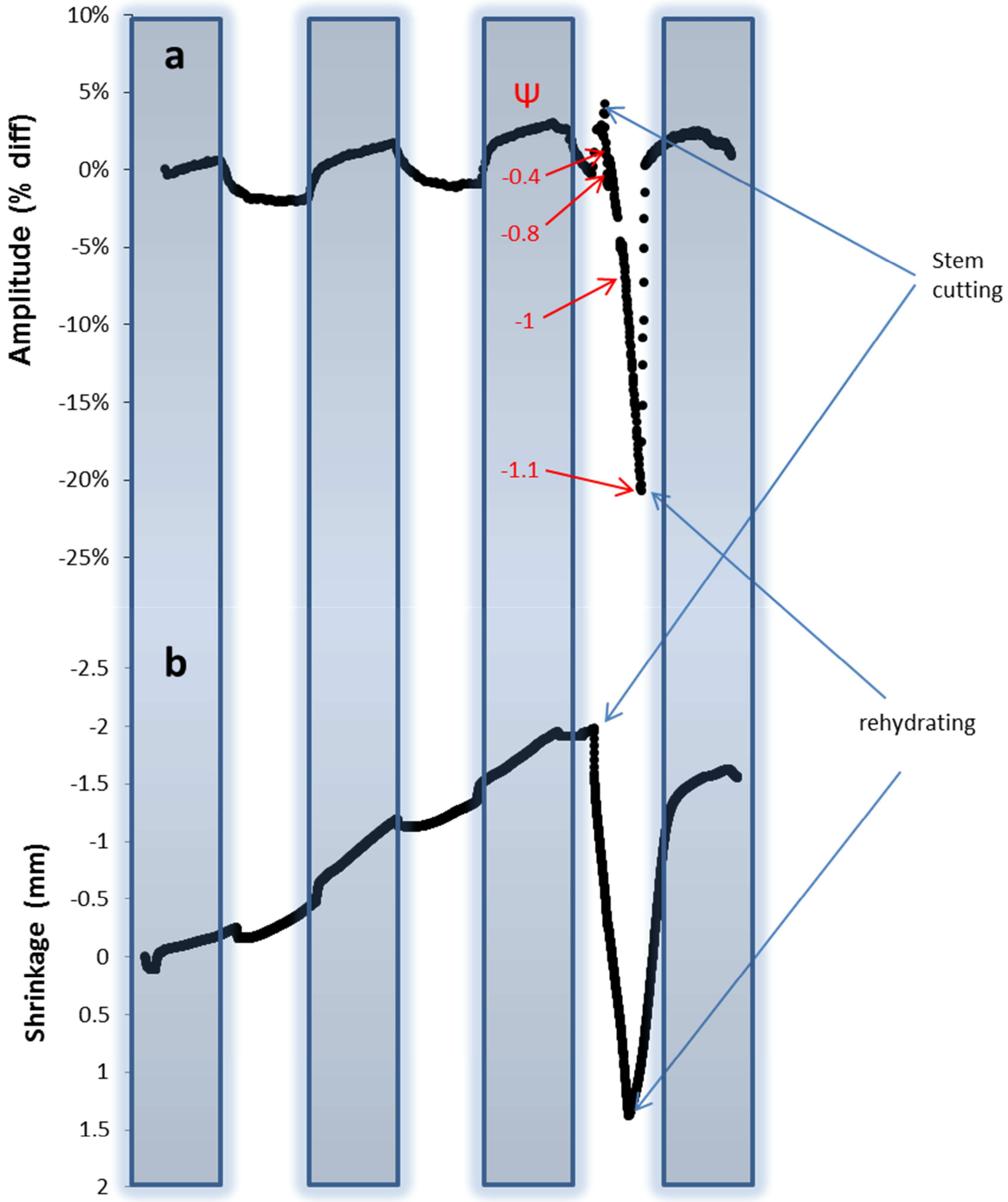

Figure 3- Monitoring grapevine petiole water statues through continuous NMR (a) and dendrometer (b) measurements for three consecutive days. Stem cutting, and it's rehydration through submergence in water were imposed to simulate water stress and its alleviation. Leaf water potential values (red) were taken after the stem cutting.

A large number of Images of embolism occurrences in Petioles and stems of both cultivars were taken. The images are yet to be processed before the data could be elaborated and processed. However, it is very clear that even under significant tensions (>-1 MPa), the rate of embolism is minor (much less than 50%) as compared with previous reports (Alsina et al., 2007) and the accompanied significant reduction in stomata conductance (more than 70%) (Hochberg et al., 2012).

Contribution to the action aims

As was stated in the action aims, this STSM enabled the collaboration between the beneficiary and Dr Carel Windt, sharing ideas and testing them in the unique infrastructure available in the Julich facility. The collaboration of labs focusing on water transport (Rachmilevitch lab, Ben Gurion University) and MRI water imaging (Windt lab, Julich) is complementary and essential for studing water statues in intact plants. This STSM started collaboration that will continue to further joint research.

Acknowledgments

First and foremost, I would like to thanks Dr Carel Windt for his hard work and the support (in all meaning of the word) he provided me during my visit. I would also like to thank Prof Hans Schultz from the Geisenheim Research Center for helping with the equipment and plant material, and to this cost action which financialy supported this scientific visit.

This report may be posted on the action website.

The confirmation letter by the host institution of successful execution of the STSM is attached separately.

<u>**Literature Cited**</u>

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