

## Establishing Ground Rules for Sensor-like Application of NMR Relaxometry for the Study of Water and Dry Matter Dynamics in Living Plants

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The largest challenge in measuring living plant organs by means of time domain NMR (TD-NMR) is that they constitute complex systems characterized by strong natural variation and spatial heterogeneity in their physicochemical properties. In NMR relaxometry this is reflected by a species and organ specific continuous distribution of  $T_1$ - and  $T_2$ - relaxation constants. Advanced multidimensional approaches can deal with this complexity and take advantage of the information that is available in the NMR data, but are time-consuming and difficult to execute and interpret. Routine characterization of intact plant organs requires dealing with this complexity in a way that is fast and suitable for non-expert operators. This might be achieved by a more sensor-like approach.

A straight-forward way to quantify some of the most elementary parameters to evaluate plant growth and yield; fresh weight (FW), water weight (WW) and dry weight (DW) is to distinguish between proton pools of different mobilities based on  $T_2$  contrast. This can be done by comparing a measure of the total signal intensity representing both the liquid ( $PD_{liq}$ ) and solid ( $PD_{sol}$ ) proton fractions ( $=PD_{tot}$ ), to a  $T_2$ -weighted estimate that correlates with more mobile liquid proton fractions in the sample. In this study, we explore the basic ground rules for such a  $T_2$ -weighted approach for the study of water and dry matter dynamics in living plants.

As a starting point of our analysis, we employ the previously defined Solid Liquid Content (SLC) method<sup>1</sup> which is based on this general idea. To test its applicability, we analyzed the relaxometric data of samples of organs of various species with widely varying water and dry matter contents and degrees of lignification (intact leaves, wheat kernels and bean pods). Despite the simplicity of the approach and the extreme heterogeneity of the physicochemical properties of plant tissue, the SLC method yields surprisingly good and linear correlations between  $PD_{tot}$  and FW, and  $PD_{liq}$  and WW, respectively. Yet, our results indicate that the SLC parameters are (slightly) susceptible to the sample specific structural and/or physicochemical properties. This has two consequences. First, the method is not generic for all species and samples, and thus needs frequent recalibration. Second, due to inherent variation in the structural and physicochemical composition of plant organs of similar origin, the accuracy of the SLC method is limited. Depending on the sample type and biological process to be studied, the accuracy may suffice to resolve the key dynamics of interest. Indeed, our results indicate that the method works well for tracking the dynamics of moisture and solid content in seeds and bean pods that exhibit large developmental changes. Contrary, the dynamics of water content in leaves of similar origin are in general small compared to the spread in the SLC results.

To ensure proper application of the method, further insight into the physicochemical and micro- and macro structural features that define the SLC parameters is required. The latter is especially relevant in the context of long-term measurements during which plant organs are expected to change these features due to e.g. senescence or as a response to environmental (stress) factors. Here, we present a systematic assessment of the relaxometric sample features probed by the SLC method in dependence of the choice of  $T_2$ -weighting and explore if finetuning of the weighting can reduce deviations in the SLC parameters and make the method more generic.

**Reference:** [1] C.W. Windt, M. Nabel, J. Kochs, S. Jahnke, U. Schurr, A mobile NMR sensor and relaxometric method to non-destructively monitor water and dry matter content in plants, *Frontiers in Plant Science*, 12 (2021).