Relative water content

Arndt, S.K., Irawan, A., Sanders G.J. (2015) Apoplastic water fraction and rehydration techniques introduce significant errors in measurements of relative water content and osmotic potential in plant leaves. *Physiologia Plantarum* **155**: 355-368

Relative water content is described as the amount of water in a leaf at the time of sampling relative to the maximal water a leaf can hold. It is an important parameter in water relation studies, e.g. it allows the calculation of the osmotic potential at full turgor.

The relative water content of a leaf (RWC) is calculated from the following parameters:

RWC = (FW – DW)/(TW – DW)

FW= leaf fresh weight
DW = leaf dry weight
TW = leaf turgit (re-saturated) weight

Relative water content can be measured in different ways. However, the critical part is the re-saturation of the leaves. Many agricultural scientists use leaf disks to re-hydrate leaves. But especially in stressed leaves that have a relative low relative water content the re-hydration of leaf disks can lead to an over-saturation of the leaf disk because the previously water depleted apoplast is over-saturated.
Please note that most leaves will rehydrate to full turgor in a short period of time (btw 1-3 hrs) and that rehydrating leaves for longer periods can lead to serious errors. Esp if rehydrated samples are used for subsequent osmotic relations.
A better way of re-saturating leaves is through the petiole of a leaf. The re-saturation follows the normal water pathway, there is no tissue damage (as it occurs with leaf disks), and over-saturation is minimized. Another advantage of re-saturation of intact leaves through the petiole is that the water potential of the leaves after re-saturation can be evaluated using a pressure bomb, thereby allowing an assessment of the success of re-saturation (a fully saturated leaf has a water potential close to zero).

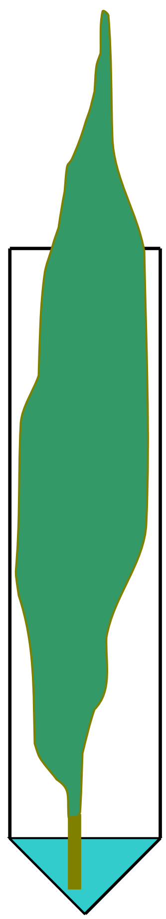
Materials

- Balance (0.000 g)
- Drying oven at 80ºC (can use microwave instead if oven is not available)
- Razorblades
- 50 ml Sarstedt tubes (or smaller vials, depending on leaf shape and size)
- Foam rack for 50ml Sarstedt tubes
- Water
- Tissues to dry leaves
- Secateurs or loppers to take branch leaf samples

Method

1. Cut a small twig from a tree

2. Put the twig immediately in a sealable plastic bag (zip-lock) and store in a cool and dry cooler box until further analysis.
3. In the lab fill 50 ml Sarstedt tubes with 5-7 ml of water (tap water is OK or use 50 mM CaCl). Sarstedt tubes are great for eucalypt leaves because the support the long and narrow leaf. It may be necessary to use different tubes or vials for other leaf forms.
4. Cut the leaf at the base of the petiole, i.e. where the petiole enters the twig or the branch using a sharp razor blade.
5. Determine the fresh weight of the leaf.
6. Place the leaf sample in the Sarstedt tube so that the petiole is submerged under the water in the tube. Place the tube in a dark and cool place (like a cupboard).
7. Let the sample re-saturate for some time (4 hours is enough for most samples).
8. Take the leaf out of the tube, dry it with a tissue and determine the turgit (saturation) weight.
9. Put the leaf sample in the drying oven for 24 hrs (oven at 80ºC)
10. Determine the dry weight of the leaf.



Relative water content

	Measure			*Calculate*		
Sample ID	FW (g)	TW (g)	DW (g)	RWC	FW/DW	FW/TW

FW = fresh weight
TW = turgit weight
DW = dry weight
RWC = (FW-DW)/(TW-DW)