Pressure volume curves (bench drying method)

Sanders G, **Arndt SK** (2012) Osmotic adjustment under drought conditions. In: Aroca Alvarez R ed. Plant Responses to Drought Stress: From Morphological to Molecular Features. Springer. p 199-229.

Materials

- Pressure bomb (PMS 1000)
- Razor blade
- 50mL Sarstedt tubes for rehydration
- Foam rack for 50 mL tubes
- MilliQ water
- Tissues to dry leaves
- Marker pen to mark leaves
- Drying oven
- Microwave (for fast drying)
- Glass beaker (250 mL) with glass beads or a glass rod (to avoid spontaneous boiling)
- Graphics paper
- Pencil
- Ruler
- Scientific calculator

1. Plant sample rehydration
    - Cut a leaf petiole with a sharp razorblade very close to the branch so that you have a long petiole.
    - Label the leaf using a marker pen, you can write on the leaves
    - Put the leaf in a 50ml Sarstedt tube that is filled with 5ml of distilled water. Make sure the petiole of the leaf is in the water
    - Put the samples in a dark and cool place (a cupboard is good) and rehydrate the samples for at least 3 hours (**NOTE:** rehydration time is different for different plant species and dependent on drought stress of plants)
2. Leaf weight
    - Take the leaves out of the Sarstedt tube and dry the using a tissue
    - Determine turgit (or saturation) weight (0.000 balance) of each leaf and write down weight
3. Water potential measurement
    - Determine water potential of leaf (see above)
    - Make sure the pressure is increased slowly and also released slowly. Fast increase of pressure can lead to incorrect water potential readings. Fast release of pressure can lead to problems with the pV curve in the later stages, probably due to issues with an imbalanced translocation of water inside the leaf.

4. Dry leaf on bench

- Put the leaf on a bench and leave there to dry. Avoid direct sunlight or rapid airflow.
- For initial drying the leaf may be put in a zip-lock bag to slow the drying of the leaf. Initial water loss leads to large decreases in water potential. In order to obtain enough measurements in the initial parts of the curve the drying may be slowed down by placing the leaf in a small zip lock bag. It can be taken out once enough points have been obtained in the initial slope.

5. Repeat steps 2-4
   - Repeat steps 2-4 until enough measurements are taken. There need to be at least five measurement points past the turgor loss point on the linear section of the pV curve. A chart (MPa vs weight) will help to identify how many more measurements are necessary

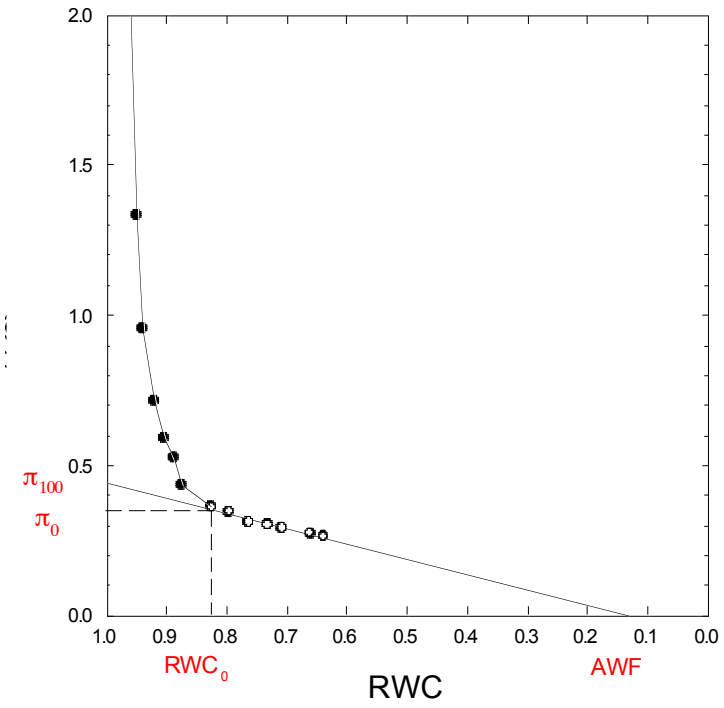
6. Dry leaf sample
   - Put the leaf into a drying oven and dry at 80ºC overnight to determine dry weight of the leaf
   - Alternatively, put the leaf into a microwave that contains a glass beaker with water and dry at maximum power. Please make sure there is always water in the glass beaker and that the glass beaker contains either glass beads or a glass rod to avoid spontaneous boiling. If there is no water in the beaker the leaf will burn! Dry the leaf for 5 min in the microwave, weigh, dry for an additional 2 min and weigh again. Record the dry weight once the weight does not change anymore.

Calculations

Note: An **Excel spreadsheet** that allows an automated assessment of pV curves can be downloaded from Kevin Tu's Landflux webpage: http://landflux.org/Tools.php

Manual calculation:

1. Convert the Ψ readings into 1/Ψ
2. Calculate the relative water content of the leaf for each 1/Ψ datapoint
   RWC = (FW-DW)/TW-DW)
   FW = fresh weight
   TW = turgit or saturation weight
   DW = dry weight
3. Use the obtained data to graphically determine the following parameter (see graph):
   π_{100} = osmotic potential at full turgor
   π_0 = osmotic potential at zero turgor
   RWC_0 = relative water content at turgor loss point
   AWF = apoplastic water fraction


2.0
1.5
1.0
0.5
0.0
π_{100}
π_0
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
RWC_0
RWC
AWF

Example of a pV curve:

Turgit weight: 1.107g
Dry weight: 0.473 g

Ψ (MPa)	FW (g)	RWC	1/Ψ
-0.1	1.107		
-0.2	1.095		
-0.45	1.084		
-0.75	1.075		
-1.05	1.068		
-1.4	1.056		
-1.7	1.046		
-1.91	1.036		
-2.3	1.027		
-2.77	0.996		
-2.9	0.978		
-3.22	0.957		
-3.29	0.937		
-3.42	0.922		
-3.67	0.892		
-3.78	0.878		

π_{100}	
π_0	
RWC_0	
AWF	