Measuring Arabidopsis, Tomato and Barley Leaf Relative Water Content (RWC)

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[Abstract] Measuring leaf relative water content (RWC) is a reliable and simple way to assess the water status of a leaf without any need for special equipment. Similar to leaf water potential, leaf RWC gives a strong indication of the plant's response to different environmental conditions; yet RWC has been shown to be a more stable parameter than leaf water potential (Sade *et al.*, 2009; Sade *et al.*, 2012). Although measuring RWC is destructive to the leaf, with proper planning, it need not affect the plant's behavior. This note will focus on three different model plants which are representative of plants with various leaf shapes (*e.g.*, Arabidopsis, tomato and barley). The technique for measuring RWC is the same for all three of these species (as well as for plants with many other types of leaves).

Materials and Reagents

1. 4-week-old Arabidopsis plants grown under short-day conditions (8/16 L/D; 22/22 °C)
2. 4-week-old tomato plants grown under moderate conditions (10/14 L/D; 25/20 °C)
3. 4-week-old barley plants grown under short-day conditions (8/16 L/D; 16/10 °C)

Equipment

1. Zipper-locked plastic bag (8 x 12 cm) for each sample
2. Paper bag (8 x 12 cm) for each sample
3. Sharp scissors
4. Scalpel
5. Paper towels
6. Analytical balance with 0.1 mg readability
7. 2-3 ml of 5 mM $CaCl_2$ in DDW water for each sample
8. Marker pen
9. Plastic box that can be sealed tightly

Software

1. Microsoft Excel

Procedure

1. Grow the plants.
2. Mark and weigh each plastic bag (BW) prior to the other measurements.
3. Typically, leaves are measured before dawn and/or at midday (Matin *et al.*, 1989). At the selected hour, select a fully expanded and mature leaf.
4. For Arabidopsis and tomato, cut the leaf with a scalpel leaving a 1-cm-long petiole.
5. For barley (or any other leaf without a petiole; *e.g.*, rice, wheat), use scissors to cut a 6- to 10-cm-long piece of leaf blade. It is important to harvest leaf pieces of the same size and from the same area (typically 6-10 cm starting from the leaf tip). It is recommended to use at least 4-5 biological replications especially when measuring plants under different treatments (*e.g.* drought stress).
6. Place the harvested leaf in a plastic bag immediately after cutting and close the bag (Figure 1).

Figure 1. Representative leaves in the Zipper-locked plastic bag (8 x 12 cm). A. Arabidopsis mature rosette leaf; B. Tomato mature fully expanded leaflet; C. Barley 6- to 10-cm-long leaf blade end. Bottom and right part of the pic show a roller for estimation of plastic bag size.

7. Make sure the bag is tightly sealed so that no vapor can escape. Put the bag in the closed (dark) box.
8. Weigh each bag with the fresh leaf inside to determine the total fresh weight (TFW).
9. Open the bag and gently make sure that the petiole is facing down. Insert 2-3 ml of 5 mM $CaCl_2$ into the bag and make sure that only the leaf petiole is immersed in the solution (Figure 2). Close the bag and put the sample back in the box at room temperature.

10. After 8 h, take the leaf out of the bag and put it between two paper towels to absorb excess water.
11. Weigh the turgid leaf to determine the turgid weight (TW).
12. Insert each sample into a paper bag and dry in a 60 °C dry oven for 3-4 days.
13. Weigh the dried samples to determine the dry weight (DW).
14. Calculate the relative water content of the leaf:

$$RWC = \frac{(TFW - BW) - DW}{TW - DW} \times 100$$

Notes

1. It is very important to use high-quality zipper-locked bags, to prevent humidity and liquids from leaking out of the bag.
2. Work quickly, but gently to maintain reliable results.
3. Maintain the same order of sampling to ensure that all samples are measured using the same protocol.
4. There are different zipper-locked bags that fit leaves of different sizes. Choose bags of a size appropriate for the species with which you are working.
5. Make sure that only the petiole is immersed in the solution (or the cut part of the leaf in the case of grass species; Figure 2).

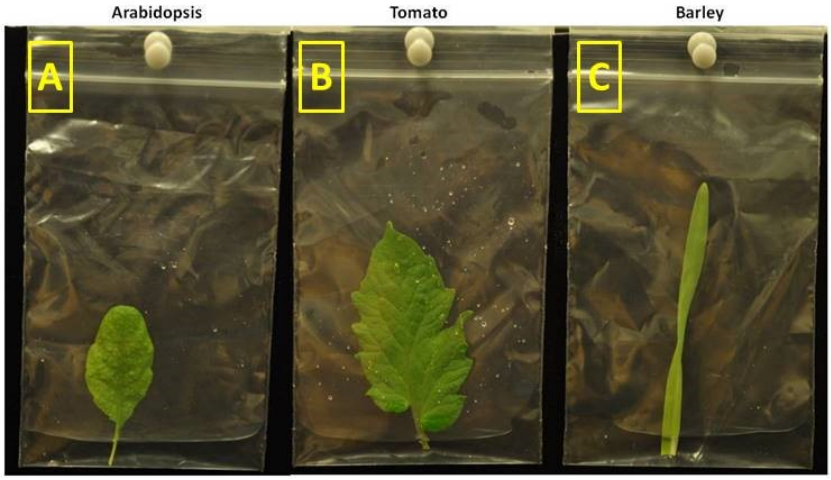

Figure 2. Leaves in the Zipper-locked plastic bag after insertion of 2-3 ml of 5 mM $CaCl_2$ into the bag. A. Arabidopsis mature rosette leaf; B. Tomato mature fully expanded leaflet; C. Barley 6- to 10-cm-long piece of leaf blade.
Note the solution in the bottom part of the plastic bag.

6. This protocol was used for measurements of 4 weeks old Tomato, Arabidopsis and Barley leaves under well irrigated conditions and short day photoperiod as stated in the Materials and Reagents section. However, it is suitable for different physiological age (as long as the leaves are fully expanded), different plants (as long as the morphological shape of the leaves is similar to those appear in this protocol), different photoperiod (both long and short day) and different irrigation regime (drought and salt).

Acknowledgments

This work was supported by the Israel Science Foundation (grant no. 1131/12) and the German-Israeli Project Cooperation (grant nos. FE 552/12–1 to A.R.F. and OR309/1-1.
This Protocol was adapted from Sade *et al.* (2014).

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