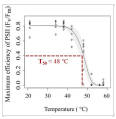

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Heat tolerance in plant leaves

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1 *Works for me* dx.doi.org/10.17504/protocols.io.29fgh3n

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ABSTRACT

The protocol is based on Krause (2010) with some modifications proposed by the Feeley Lab in 2016. This protocol is for finding heat tolerance or the critical thermal maximum temperature of plant leaves. Plants can undergo heat damage that can be repaired as long as the critical value is not exceed. This value is called critical thermal maximum (CT_{max}) or heat tolerance (T_{50}) and is the highest temperature a leaf can take before and irreversible damage occurs, and when exceeded leads to necrosis. The way we measure this is using chlorophyll fluorescence, more specifically, the maximum efficiency of PSII value or F_v/F_m, which optimal values are around 0.83 in non stressed leaves. F_v/F_m can be obtained with a fluorometer or with an Irga, in both cases using dark adaptation clips. Plant collection is recommended to be done on the day of the procedure or, as long as leaves are kept well hydrated, the material can be even measured a day after the collection. The total time required for the measurement to be compleated is 2 days (including a 24h wait). Apart from the fluorometer, the equipment needed includes a water heating device (stable water temperature is imperative), such as a water bath or if possible a Sous Vide (recommended to us by Ken Feeley; works great!).

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0224218

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Krause GH, Winter K, Krause B, Jahns P, García M, Aranda J, et al. High-temperature tolerance of a tropical tree, Ficus insipida: Methodological reassessment and climate change considerations. Funct Plant Biol. 2010;37: 890–900. doi:10.1071/FP10034

MATERIALS TEXT

- Fluorometer (we use the OS30P+ Opti-Sciences) and the dark adaptation clips
- Water heating device (i.e. water bath, Sous Vide Anova precision cooker (recommended))
- Buckets or any other recipient (only needed if you are using a Sous Vide)
- Tea cloth bags (can also be made with tea bags or Miracloth)
- Zip lock bags (medium and small size)
- Bobby pins
- Weigths (i.e. scale weights, rocks.. etc)
- Circle puncher (diameter depends on leaf size)
- Petri dishes
- Tissue paper

BEFORE STARTING

- Take into account that to complete one curve in order to find the T_{50} value you need to heat 9-10 disks of a leaf or plant
- The procedure takes 2 days in total (since there is a 24h wait for data collection)
- Ideally leaf samples should be collected the day of the heat tolerance test
- Prepare tea cloth bags
- Make sure the zip lock bags are watertight

Plant collection

1 Collect young fully expanded leaves (sample should be enough to cut 9-10 leaf disks).

- Make sure after the collection and before the heating that your samples are kept well hydrated. Ideally cut them under water in order to maintain the water column and keep it that way until leaf disks are cutted. Otherwise cut a bigger branch in order to make a second underwater cut in the lab.

Before heating leaf disks

2 Cut 9-10 leaf disk with a circle puncher.

- If there are too many samples and just one or two heating devices, make sure you keep the disks properly label in a petri dish with wet paper towel, this ensures all disks are kept hydrated while you settle the heating temperatures.

- You can replace the circle puncher with any other tool, just make sure leaf disks are always the same size, this prevents some of them dessicating faster than the others.

Authors tip: Plants with very thick leaves or very strong parallel veins can be damaged by the circle puncher, for species with this caracteristics we cutted same size squares.

2.1 Start heating the water.

- We recommend you start with the highest temperature since cooling water is easier and can save you some time.

Authors tip: We own just two Sous Vide, so we set the firts one to 58 °C (highest temperature) and use it for the 56 and 52 °C baths and the second one to 48 °C and use it for the 42, 38 and 34 °C baths.

3 Dark adapt with the clips at least 2 or 3 untreated leaf disks in order to record the initial value of Fv/Fm. 25m

- The time of dark adaptation can be from 20 to 30 minutes, run an initial test to make sure you are getting the maximum efficiency your healthy leaf can achive.

4 Record Fv/Fm from non-heated disks to obtain the initial value for each species and to ensure sampled leaves are healthy.

Near 0.83

- Very low initial values can be a sign of stressed leaves, make sure you change the leaf sample if this occurs.

- This value is the initial F_v/F_m from which you calculate T_{50}. It can slightly change between species.

Authors tip: The initial F_v/F_m can be slighty different among species, between our 21 species, we found one with lower initial values; even when several healthy leaves were collected, values were always near 0.75.

5 Place samples inside a tea cloth bag, with one cloth layer in the adaxial side and three layers in the abaxial side of the leaf disks to prevent anaerobiosis.

Author tip: Several leaf disks can be enclosed in the same bag, just make sure you prevent them from clustering during the water bath. For this purpose we use bobby pins.

6 Put the disks enclosed in the tea cloth in a zipped bag and into another watertight zipped bag containing a weight of 100 grams to ensure complete immersion of the leaf disks and to avoid damping them.

- To ensure the bag does not float, remove all air from the second zip bag.

Heating treatment and control

7 Heat leaf disks at the following temperatures; 34 °C, 38 °C, 42 °C, 48 °C, 52 °C, 56 °C and 58 °C, and keep for fifteen minutes at each temperature. 15m

7.1 Control disks are not heated but enclosed in the bags for fifteen minutes at ambient temperature (lab). 15m

- This step ensures the bags are not adding any other stress that can alter the F_v/F_m measure.

Author tip: Lab temperature can change during the day, so we recommend doing the control treatment always at the same hour of the day.

8 Place heated and control disks on petri dishes (properly labelled with the treatment) with wet paper towel and store in the dark for 24h. 24h

F_v/F_m measure

9 The next day, dark adapt all your leaf disks with the clips for at least 20 minutes (see note in Step 3). 20m

10 Record Fv/Fm of all leaf disks with the modulated fluorometer.

Curve fitting

11 In order to find the T_{50} value, the temperature at which 50% reduction of initial F_v/F_m occurs, fit a logistic curve for each species. Start by graphing temperature vs. F_v/F_m.

Author tip: For curve fitting, we used “fitplc” R package, with the Weibull model and a 95% confidence interval modifying the “Kmax” argument so that it corresponded to the mean initial value for each species.