APX2 induction via herbicide infiltration (DCMU, DBMIB, Methyl Viologen)

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Abstract

Infiltration of leaves with various herbicides, targetting photosynthetic electron transport, were utilized to mimic changes in redox state (otherwise caused by changes in light irradiance) of photosynthetic electron transport components and study the response of APX2.

This was successfully utilized in the following publication:

Jung, H., Crisp, P.A., Estavillo, G.M., Cole, B., Hong, F., Mockler, T.C., Pogson, B.J., and Chory, J. (2013). Subset of heat-shock transcription factors required for the early response of Arabidopsis to excess light. Proc. Natl. Acad. Sci. 110: 14474–14479.

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Guidelines

Plants are ideally low/moderate light acclimated (< 150 µ mol m^{-2} s^{-1}) that should allow induction of redox change (e.g. oxidation vs reduction) of targetted photosynthetic electron transport components (e.g. cytochrome bf complex, PQ pool).

Before start

Make sure healthy, low-moderate light acclimated plants are growing (of desired genotypes) with well expanded leaves (leaf # 4 - 7 are generally good; ~3 - 4 week old plants w/ 12-hr day/night cycle).

Materials

Petri Dish LI-PD01100 by P212121
ethanol by Contributed by users
Tweezers by Contributed by users

DCMU D2425 by Sigma – Aldrich

Methyl viologen 856177 by Sigma Aldrich

vacuum chamber by Contributed by users

vacuum pump by Contributed by users

DBMIB 271993 by Sigma Aldrich

surgical scissors by Contributed by users

Protocol

Step 1.

Grow plants under low - moderate light conditions (60 – 100 µM, make sure this is measured).

Step 2.

Excise leaf of plant (use surgical scizzors if possible to ensure a clean cut, alternatively can perform hole puncture) and float on the surface of water (in petri dish with known water level so that concentration of herbicide used is accurate = 15 mL water used for concentrations below).

Step 3.

Incubate excised leaves at light conditions used in #1 for approximately 5 hours.

Step 4.

Make fresh herbicide solutions (dissolve in ethanol):

- 50 mg DBMIB in 15 mL ethanol = 10 mM
- 20 mg DCMU in 40 mL ethanol = 2 mM
- 26 mg MV in 40 mL ethanol = 2.5 µM
- Control = ethanol only.

Step 5.

Add the following volumes of prepared herbicide solution to 36 mL ethanol:

- 36 µL of 10 mM DBMIB (C_f = 24 µM)
- 75 µL of 2 mM DCMU (C_f = 10 µM) or
- 15 µL of methyl viologen (C_f = 2.5 µM).

Add 36uL (or largest volume used) of ethanol only as a **control**.

Step 6.

Transfer leaves to new petri dish with desired herbicide solution or control.

Step 7.

Transfer entire petri dish with leaves into vacuum chamber and apply pump for 3 min to setup a vacuum. Susbequently, slowly release pressure to facilitate uptake of herbicide solution into leaves (or control).

Step 8.

Incubate leaves (still in herbicide solution or contorl) at light conditions used in #1 for at least 2 hours.

Step 9.

Check chlorophyll fluorescence using PAM (ΦPSII, qP, NPQ) to infer photosynthetic performace and/or harvest (snap-freeze & grind & store at -80 °C) leaves for further experiments.

An important decision should be made here regarding whether leaves measured by PAM are also harvested for measuring gene expression (or desired emeasurement). Investigator should be consistent, but be aware that running under a PAM is essentially light stress for the leaf and will likely induce additional changes to gene expression. Previously, we harvested independent leaves from those measured under PAM.

Chlorophyll fluorescence is extracted with the PAM as an average across individual leaves. Thus, each leaf is considered a single biological replicate (with petri dish as a blocking factor). Single leaves (100 mg) should give ample mRNA.

Warnings

Be careful when handling herbicides, particularly methyl viologen. Consider wearing face mask (although no aerosols should be produced).