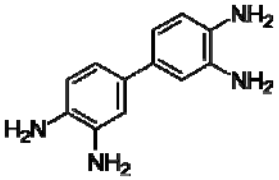
Hydrogen peroxide detection with 3,3'-Diaminobenzidine

Gyula Czégény
Demonstrator

Studies on the role of hydrogen peroxide (H_2O_2) in both oxidative stress and signaling pathways in plants require reliable, specific and sensitive detection methods. Direct measurements of hydrogen peroxide in intact leaves can provide more precise information on the real *in vivo* situation in the plant than any experiment with artificial system (leaf extract, cell suspension, etc.). However, the correct determination of H_2O_2 in a leaf is problematic and an optimal H_2O_2 probe is currently missing. (Šnyrychová et. al.)

Detection of H_2O_2 is possible with **3,3'-diaminobenzidine (DAB)**. It forms brownish insoluble polymer when reacts with hydrogen peroxide. The presence of peroxidase enzyme is also needed for this reaction. This precipitation becomes visible after chlorophyll removing with 96% EtOH.



Plant materials:

6 weeks old tobacco plants (*Nicotiana tabacum L. var Xanthi*) were grown in light chambers under approx. 200 µmol m^{-2} s^{-1} Photosynthetically Active Radiation (PAR: 400-700 nm) only without additional UV-B.

There are three samples:

1. Control → The plant gets PAR only, not stressed.

2. MetV → The leaf infiltrated with paraquat (Methyl Viologen) in the evening before the practical. Paraquat inhibits photosynthesis. It accepts electrons from PS I and transfers them to molecular oxygen. Superoxide radical is the final product of this reaction.

3. UV-B → The plant gets 6 hours additional UV-B for two days before the practical.

Method:

1. Dissolve 7.5 mg DAB in 10mL 0.1N HCl and set the pH to 6.5 – 7. Keep the solution at room temperature.

2. Infiltrate the solution into the tobacco leaf after making a small hole on the epidermis with a needle.

3. Cut the infiltrated areas from the leaf and put them into a glass tube filled with 96% ethanol.

4. Heat them for two hours in a 70°C water bath to remove chlorophyll. Changing of the ethanol is needed when the original turns to green.

5. The samples turn to rigid because of this treatment. Put the samples into 50% EtOH, so they become flexible again.

Pitfalls:

The DAB solution has been infiltrated into the leaf with a syringe. Unfortunately this technique sometimes more stressful for the leaf than the original stressor itself, so the production of hydrogen peroxide can be more intensive at the outline of the infiltration points. Additional pitfall of this method that the contrast of the brown staining isn't quantitative, it does not on the concentration of H_2O_2 solely.

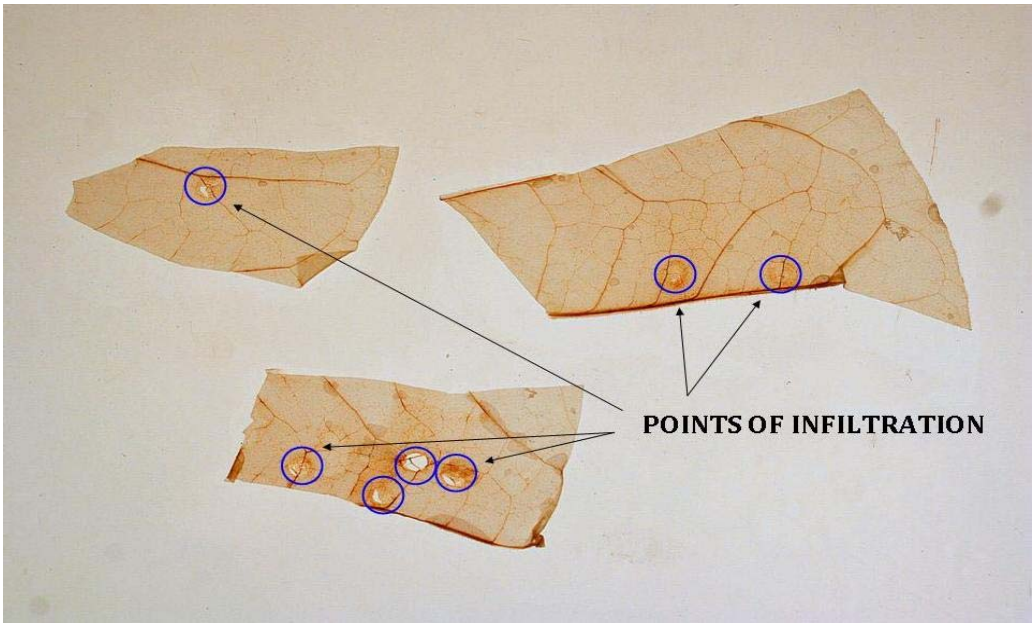


Figure 1. Difficulties of the infiltration technique.

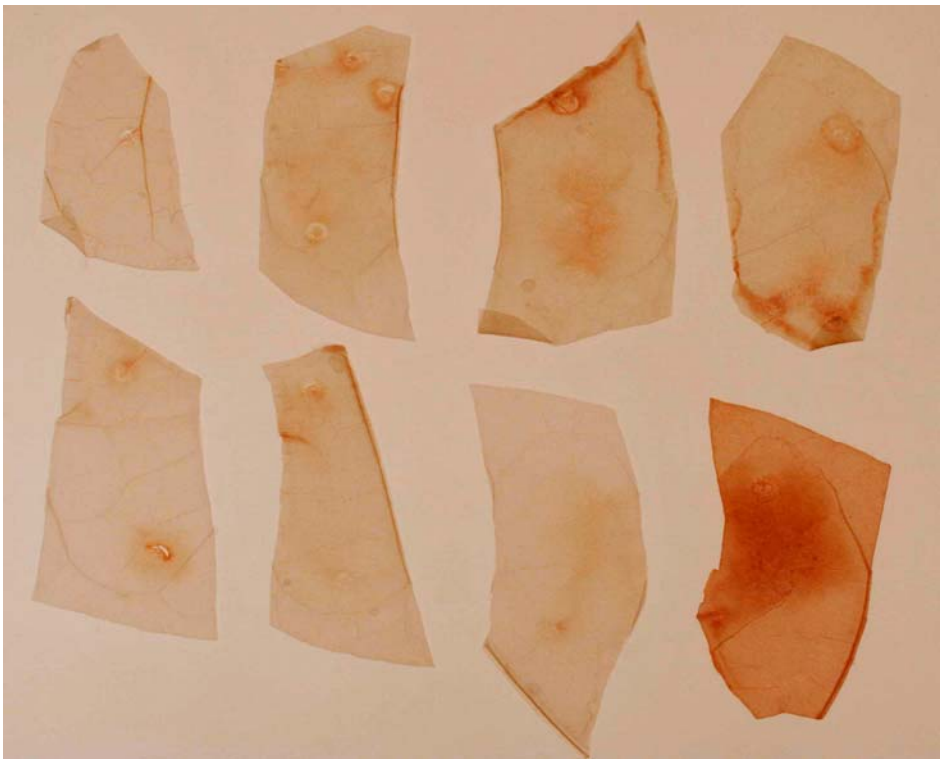


Figure 2. Calibration with different concentrations of hydrogen peroxide.

Šnyrychová I., Ayaydin F., Hideg É. (2009) Detecting hydrogen peroxide in leaves in vivo – a comparison of methods. Physiol. Plantarum 135:1-18