PAM analysis (NDH activity)

NDH activity can be monitored as a change in chlorophyll fluorescence using the PAM system (Mini-PAM, Warz, Germany). To optimize the measuring conditions, the appropriate controls are required (Arabidopsis or tobacco mutants defective in NDH activity).

Plant material should be adapted to the room light conditions for at least 15 minutes prior to the analysis. It is not necessary to conduct the analysis in the dark.

Measuring light (ML) intensity should be high enough (10 μmol photons m^{-2} sec^{-1}) to monitor the non-photochemical reduction of plastoquinone via NDH. In general, a much lower intensity of ML (0.01-0.5 μmol photons m^{-2} sec^{-1}) is used in PAM analysis so as not to cause photochemical reduction.

Apply a saturating pulse (3000 for μmol photons m^{-2} sec^{-1} 0.8 sec) under the ML background to monitor the Fm level, which is used to standardize the fluorescence levels.

Apply actinic light (AL, 100 μmol photons m^{-2} sec^{-1}) for 5 minutes. This light intensity is critical to form a sufficient reducing pool in the stroma. Higher AL intensity cannot be used as it may influence the fluorescence level after the actinic light illumination.

NDH activity can be monitored as a transient increase in chlorophyll fluorescence level within 1 minute.