Dissecting the interaction of photosynthetic electron transfer with mitochondrial signalling and hypoxic response in the Arabidopsis rcd1 mutant


Supplementary figures S1-S5
Dissecting the interaction of photosynthetic electron transfer with mitochondrial signalling and hypoxic response in the Arabidopsis rcd1 mutant. Shapiguzov A et al. 2020

Supplementary figure S1. Gas exchange in Col-0 and rcd1 leaf discs treated or untreated with MV, as monitored by MIMS. The kinetics of O₂ evolution in Col-0 is presented in the main figure 2g. O₂ evolution in rcd1 is indistinguishable from the wild type. Kinetics of CO₂ evolution can be used to estimate the rate of mitochondrial respiration. Treatment with MV did not affect dark CO₂ evolution either in Col-0 (left) or in rcd1 (right). When plants were exposed to light, photosynthetic carbon fixation reabsorbed a fraction of CO₂ produced by respiration, thus lowering net CO₂ emission [1]. Treatment with MV prevented light-dependent CO₂ reabsorption in Col-0, but not in rcd1. This suggested that both photosynthesis and respiration were active in MV-treated rcd1, while only respiration was active in MV-treated Col-0.

Supplementary figure S2. The effects of MV on chlorophyll fluorescence during exposure to low light. (A) Kinetics of chlorophyll fluorescence in Col-0 and in rcd1 mutant measured as in the main figure 2a,b. The reads are normalized to Fo. (B) Quantification of Fm’ parameter measured in (A). Mean values ± standard deviations are shown. *** P value < 0.001, one-way ANOVA with Bonferroni corrected post hoc test. (C) Long illumination led to recovery of Fm’ in MV-treated rcd1, but not in MV-treated rcd1 npq4. The reads are normalized to Fo. (D) The response of rcd1 to MV is “reset” in darkness. Introducing dark periods (“d”) in the course of light exposure temporarily restored NPQ (as seen by lowered Fm’) in MV-treated rcd1. Thus, light-dependent inhibition of physiological activity of MV in rcd1 was reversible by darkness.
Supplementary figure S3. Measurements of chlorophyll fluorescence in hypoxic atmosphere.

(A) Chlorophyll fluorescence of leaf discs was recorded after 20-min treatment with nitrogen gas in darkness. In all the tested lines except rcd1, leaf discs pre-treated with MV demonstrated quenched chlorophyll fluorescence as compared to MV-untreated controls. The presence of this effect in ptox, npq4, and stn7 mutants suggested that it was not mediated by PTOX chloroplast terminal oxidase, NPQ, and chloroplast state transitions, accordingly. The reads are normalized to Fo obtained in dark-adapted hypoxic conditions.

(B) OJIP kinetics was recorded using 1-second flash of saturating light in dark-acclimated plants that were pre-treated with hypoxic atmosphere for 20 min. The response of the stn7 mutant deficient in state transitions was similar to wild-type. (C) The rcd1 ntrc mutant with compromised CET was indistinguishable from rcd1. (D) Dynamic response of OJIP kinetics in MV-treated Col-0 and rcd1 leaf discs subjected to hypoxia in AnaeroGen (N = 2). The extracted parameter φRE1o = 1 – Fi/Fm is presented in the main figure 4d. The OJIP kinetics are double normalized to fluorescence at Fo and Fi (20 µsec and 40 msec, accordingly).
Supplementary figure S4. Response to MV is sensitive to hypoxia in plants treated with antimycin A. Pre-treatment of wild-type plants with 2.5 µM antimycin A (AA) followed by 20-min hypoxic treatment abolished the effect of MV on PET. Chlorophyll fluorescence kinetics was normalized to Fo obtained under dark-adapted hypoxic conditions. Quantification of Fm’ is presented in the main figure 5a (N = 3).

Supplementary figure S5. Response to MV in the mutants deficient in AOX isoforms. (A) In the aox1a, aox1c and aox1d mutants deficient in AOX isoforms, and in the line overexpressing AOX1a, chlorophyll fluorescence under hypoxia was sensitive to MV similarly to Col-0. (B) In the rcd1 aox1a double mutant chlorophyll fluorescence under hypoxia was insensitive to MV similarly to rcd1. The OJIP kinetics are double normalized to fluorescence at Fo and Fi (20 µsec and 40 msec, accordingly) (N = 2).