

## NITRIC OXIDE SYNTHESIS FROM NITRITE BY ISOLATED PLANT MITOCHONDRIA

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Nitrite is a potential source of nitric oxide (NO). Recent reports suggested that mitochondrial respiration is required for the nitrite reducing activity of *Arabidopsis thaliana* leaf extracts. The aim of this study was to characterize the synthesis of NO from nitrite in isolated mitochondria of *A. thaliana*. Using cells maintained in liquid cultures, ultrasound and differential centrifugation we established a protocol to isolate highly intact mitochondria. Respiratory controls of mitochondria energized with malate + glutamate, succinate, NADH, NADPH or TMPD, reached values of 3.4; 2.5; 2.9, 1.5 and 1.2 respectively, indicating coupling of the electron transport system with oxidative phosphorylation in these mitochondrial preparations. High membrane potential was established when mitochondrial suspensions were energized with different substrates, as determined with the optical probe safranine. NO and O<sub>2</sub> concentrations in the reaction medium were simultaneously followed with Clark type electrodes connected in a free radical analyzer. NO synthesis from nitrite was detected after mitochondria consumed oxygen of the medium. This anaerobic NO production was prevented by inhibitors of mitochondrial respiration. CPTIO, a NO scavenger, quenched the signal of the NO electrode. This mitochondrial nitrite reducing activity may be relevant for the metabolism of plant tissues subjected to low O<sub>2</sub> tensions, as it occurs in roots growing under flooded grounds. *Supported by Fapesp.*

Nitric oxide – Plant mitochondria - Nitrite