

## EFFECT OF K<sup>+</sup> CHANNEL ACTIVITY ON THE OXIDATIVE PHOSPHORYLATION IN DURUM WHEAT MITOCHONDRIA FROM CONTROL AND HYPEROSMOTIC-STRESSED SEEDLINGS

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Durum wheat mitochondria (DWM) possess an ATP-inhibited K<sup>+</sup> channel, the Plant mitoK<sub>ATP</sub> (PmitoK<sub>ATP</sub>), that catalyses the electrophoretic uniport of K<sup>+</sup> through the inner mitochondrial membrane; the co-operation between PmitoK<sub>ATP</sub> and the K<sup>+</sup>/H<sup>+</sup> antiporter, very active in plant mitochondria, allows the operation of a K<sup>+</sup> cycle, whose capacity in DWM is so high to collapses membrane potential ( $\Delta\Psi$ ), the major component of protonmotive force ( $\Delta p$ ) in plant mitochondria. Under environmental stress conditions the decrease in  $\Delta\Psi$  (and  $\Delta p$ ) due to PmitoK<sub>ATP</sub> activation may dampen the generation of reactive oxygen species (ROS) that is known to increase under these conditions. This suggests that the channel may play a role in the defence of cell from oxidative stress occurring when plants suffer adverse environmental conditions.

In order to establish whether activation under stress of PmitoK<sub>ATP</sub> entails a loss of ATP synthesis, here, we investigated how this channel may affect oxidative phosphorylation (OXPHOS) in DWM purified from control seedlings and from seedlings subjected both to severe mannitol and NaCl stress.

ATP synthesis via OXPHOS (oligomycin sensitive) by succinate-oxidising DWM was followed in continuous, by using a spectrophotometric ATP-detecting system, in the absence or presence of KCl to activate the channel. As expected, severe osmotic and salt stress caused a decrease in the rate of ATP synthesis compared to the control condition. This result is in line with the decrease in the ATP content observed in seedling tissues as a consequence of the hyperosmotic stress imposition. When PmitoK<sub>ATP</sub> was activated by KCl the ATP synthesis via OXPHOS was about 90% inhibited in severely stressed DWM. Contrarily, in control DWM, although PmitoK<sub>ATP</sub> collapsed  $\Delta\Psi$ , ATP synthesis as well as coupling (RC and ADP/O ratios) checked by oxygen uptake experiments were found to be unaffected. Similar results were obtained using DWM from moderately mannitol stressed seedlings. Measurements of  $\Delta p$ H and  $\Delta\Psi$  demonstrate that no  $\Delta p$ H increase occurs able to compensate the  $\Delta\Psi$  decrease and maintain  $\Delta p$  and suggest that the driving force for ATP synthesis is a no-bulk phase  $\Delta p$ , retained by DWM as a consequence of braking of PmitoK<sub>ATP</sub> by its inhibitor ATP.

We suggest that PmitoK<sub>ATP</sub> may play an important defensive role at the onset of the environmental/oxidative stress by preserving energy in a crucial moment for cell and mitochondrial bioenergetics. Consistently, under moderate mannitol stress, miming an early stress condition, the channel may efficiently control ROS generation (about 35 fold from fully open to closed state)

without impairing ATP synthesis. Anyway, if the stress significantly proceeds, the  $P_{\text{mitoK}_{\text{ATP}}}$  becomes fully activated by decrease of ATP concentration (25-40%) and increase of activators (FFAs and superoxide anion), thus impairing ATP synthesis.