Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract – 5.17

PLANT UNCOUPLING MITOCHONDRIAL PROTEIN (PUMP): AN ENERGY DISSIPATING SYSTEM ACTIVATED IN RESPONSE TO OSMOTIC STRESS IN DURUM WHEAT

D.TRONO*, M. SOCCIO*, **, A.M. MASTRANGELO*, N. DI FONZO*, L. CATTIVELLI*, Z. FLAGELLA**, D. PASTORE**

*) Istituto Sperimentale per la Cerealicoltura - CRA, SS 16 Km 675, 71100 Foggia, Italy - trono@iscfoggia.it
**) Dipartimento di Scienze Agroambientali, Chimica e Difesa Vegetale, Facoltà di Agraria, Università di Foggia, Via Napoli 25, 71100 Foggia, Italy

durum wheat mitochondria, uncoupling protein, salt stress, osmotic stress

Plant uncoupling mitochondrial protein (PUMP) represents an energy dissipating system able to uncouple mitochondria through a free fatty acid (FFA)-activated H^+ cycling process and to control mitochondrial production of reactive oxygen species (ROS). Whereas it is generally agreed that PUMP acts as a defense mechanism against oxidative stress, data concerning its involvement in plant response to environmental stresses, which lead to oxidative stress, are still lacking.

In order to shed some light into this aspect, an investigation was carried out concerning PUMP functioning in durum wheat mitochondria purified from etiolated early seedlings submitted to moderate and severe salt (NaCl) and osmotic (mannitol) stress.

Results on "proton leak" kinetics of mitochondria, demonstrated an increase in PUMP functioning, expressed as proton conductance ("proton leak"/$\Delta\Psi$) at 100 mV, that was stress-intensity-dependent and more evident under salt than under osmotic stress, but this result did not allow to establish whether PUMP functioning increase was due to protein activation and/or to an increase in protein amount.

To investigate about this point, a new method, based exclusively on $\Delta\Psi$ measurements, was properly developed to evaluate both *i)* PUMP functioning due to the presence of endogenous FFA and *ii)* maximal PUMP activity, which may estimate protein amount, induced by externally added FFA to succinate-energized mitochondria. Values concerning PUMP functioning due to the presence of endogenous FFA highly correlated with values obtained as proton conductance at 100 mV, thus showing the suitability of the new method. As regards maximal PUMP activity, $\Delta\Psi$ measurements showed no or little (below 30%) increase, thus suggesting that protein content under stress conditions remains almost unchanged. Consistently, no increase in the expression level of plant UCP-related genes under stress conditions was observed in terms of transcript amount. The above results suggest that the increase in PUMP functioning observed under stress conditions is due to protein activation by modulators rather than to an increase in protein amount. In line with previous findings showing PUMP activation by ROS and by the increase in FFA availability, we found that under all stress conditions *i)* an increase in mitochondrial ROS production and FFA content occurred, with the latter being due to an increase in mitochondrial phospholipase A_2 activity (PLA_2), whose existence was demonstrated here for the first time; *ii)* PUMP activation was prevented by catalase plus superoxide dismutase and by bovine serum albumin, which remove ROS and FFA, respectively; *iii)* once activated, PUMP may lower ROS production, probably according to a feed-back mechanism.

In conclusion, an increase of ROS production, PLA_2 activity and FFA content occurs in mitochondria from early seedlings suffering hyperosmotic stress, leading to activation of PUMP, that in turn, may act as a defense mechanism by controlling further large scale ROS production. On the contrary, trancript levels of plant UCP-related genes and maximal PUMP activity remain unchanged.