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Poster Abstract – F.18

LOCALIZATION OF THE RECOMBINANT PROTEIN ZEOLIN IN THE CHLOROPLAST

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Plants are potentially useful for the production of many therapeutic proteins and several strategies have been exploited to maximize heterologous protein accumulation in the plant cell. Recently, a fusion protein made between the bean storage protein phaseolin and the N-terminal portion of maize prolamin storage protein γ-zein was shown to accumulate in novel ER-derived protein bodies of vegetative tissues, while γ-zein and phaseolin usually accumulates in the ER and in storage vacuoles respectively. The γ-zein fragment, on the basis of the knowledge acquired on zeolin, could be exploited for the production of large amounts of recombinant pharmaceuticals in transgenic plants. In this study we investigate if zeolin could be also expressed in the chloroplast and not only in the ER. In fact, together with others numerous advantages such as high-level of transgene expression, in many crop species chloroplast DNA is not transmitted through pollen thus eliminating the risk of transgene spreading in not transgenic crops or relative wild species. The *zeolin* gene has been cloned in three plastid expression cassettes. Two of them have been assembled to evaluate the utility of including in the zeolin gene the region that encodes the signal peptide. In the third construct the *BiP* gene, which encodes an ER chaperone, has been cloned downstream of the zeolin gene. The presence of the signal peptide seems to promote zeolin accumulation in the chloroplast, even though a comparison between the two zeolin sub-cellular localizations shows that zeolin has accumulated in the chloroplast to lower levels than in the ER. Immunoblotting of the transplastomic plant protein shows the considerable presence of phaseolin and γ-zein polypeptides with respect to intact zeolin. This suggests that in the chloroplast a higher amount of zeolin is fragmented in comparison to zeolin accumulated in the ER. Moreover, no protein bodies have been detected in the chloroplast. In order to promote zeolin folding into protein bodies in this organelle, we have integrated the *BiP* gene together with the *zeolin* gene in the tobacco plastome but even in this case transplastomic plants has not accumulated zeolin in protein bodies. We hypothesize that the disulfide bonds necessary for zeolin assembly have not been correctly formed in the chloroplast. This can have caused zeolin misfolding and not-stable proteins that reach poor expression levels due to chloroplast proteases activity.