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PHASEOLIN: A MODEL PROTEIN FOR INVESTIGATION ON RECOMBINANT PROTEIN STABILITY IN THE CHLOROPLAST

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Plastid genetic engineering is a research area of plant biotechnology where, since the development of a chloroplast transformation protocol for tobacco, significant advances have been accomplished. Plastids offer considerable advantages as compared to conventional transgenic technologies, including high protein expression levels. Moreover, plastid transformation has been used to study the mechanisms of plastid gene expression and the plastid gene functions. However, the knowledge of the mechanisms regulating foreign protein folding, targeting, and accumulation in plastids is still quite limited. When a heterologous gene is inserted into the plastome and the corresponding protein is synthesized, the correct folding of this protein is very uncertain in the new environment. The stability of the foreign protein within the plastid environment seems to be the major determinant for accumulation. To investigate which are the most important molecular mechanisms that influence the stability of heterologous proteins in transplastomic plants, phaseolin has been used as reporter protein. Four gene constructs have been assembled and used to transform the tobacco plastome. One expresses the wild-type form of phaseolin with its endoplasmic reticulum (ER) signal peptide (spPhaseolin), which should target it to the thylakoids, as observed for zeolin (De Marchis et al., Plant Mol. Biol., 2010). The second gene construct is a mutated form of phaseolin which is able to form disulphide bonds thanks to a cysteine residue inserted at the C terminus (spPhaseolin*). We have preliminarily verified the formation of disulphide bonds between spPhaseolin* monomers in the ER. Moreover, other two phaseolin mutants have been prepared whose localization should be in the stroma because they will be devoid of their sp (ΔPhaseolin and ΔPhaseolin*). Based on previous results, the mutant ΔPhaseolin should be the most unstable form of phaseolin. The transplastomic plants will be analysed to investigate the stability of the phaseolin proteins, their folding and post-translational modifications.