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## ZEOLIN EXPRESSION IN TOBACCO CHLOROPLAST AND ALFALFA ENDOPLASMIC RETICULUM

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The major seed storage proteins of Zea mais and Phaseolus vulgaris, zein and phaseolin, accumulate in the endoplasmic reticulum and in storage vacuoles, respectively. Zeolin polypeptide, composed of phaseolin and the first 89 amino acids of y-zein, shown to form endoplasmic reticulum-located protein bodies in transgenic tobacco leaves (Mainieri et al., 2004, Plant Physiology 136: 3447-3456). Fusion with the gamma-zein domain which has been utilised to construct zeolin, could be a useful strategy to accumulate target proteins of pharmacological interest in transgenic plants. In order to verify if zeolin accumulates also in other plant species, alfalfa (Medicago sativa L.) plants have been transformed with the zeolin gene and the protein has been detected by western blot in alfalfa leaves. Moreover, zeolin forms endoplasmic reticulum-located protein bodies even in transgenic alfalfa leaves. Previously, it has been shown that zeolin is highly stable when accumulated in the endoplasmic reticulum. To investigate if the ability of zeolin to accumulate to very high levels was limited to this sub-cellular compartment, we inserted the zeolin gene in the tobacco chloroplast DNA. The zeolin gene was cloned in a plastid expression cassette into the chloroplast transformation vector pLD-CtV which targets the transgene cassette to the 16S-trnI/trnA-23S region of the chloroplast genome. A comparison between the two kinds of zeolin sub-cellular localization by western blot analysis shown that zeolin accumulated in the chloroplast to lower levels than in the endoplasmic reticulum. The considerable presence of phaseolin and gamma-zein polypeptides with respect to intact zeolin, suggests that in the chloroplast a high amount of zeolin is fragmented. No zeolin protein bodies were visualised in the chloroplast by electron microscopy.