

## Correct targeting of proinsulin in protein storage vacuoles of transgenic soybean seeds

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**ABSTRACT.** Soybean plants are promising bioreactors for the expression of biochemically complex proteins that cannot be produced in a safe and/or economically viable way in microorganisms, eukaryotic culture cells or secreted by transgenic animal glands. Soybeans present many desirable agronomic characteristics for high scale protein production, such as high productivity, short reproductive cycle, photoperiod sensitivity, and natural organs destined for protein accumulation in the seeds. The significant similarities between plant and human cells in terms of protein synthesis processes, folding, assembly, and post-translational processing are important for efficient accumulation of recombinant proteins. We obtained two transgenic lines using biolistics, incorporating the human proinsulin gene under control of the monocot tissue-specific promoter from sorghum  $\gamma$ -kafirin seed storage protein gene and the  $\alpha$ -coixin cotyledonary vacuolar signal peptide from *Coix lacryma-jobi* (Poaceae). Transgenic plants expressed the proinsulin gene and accumulated the polypeptide in mature

seeds. Protein targeting to cotyledonary protein storage vacuoles was successfully achieved and confirmed with immunocytochemistry assays. The combination of different regulatory sequences was apparently responsible for high stability in protein accumulation, since human proinsulin was detected after seven years under room temperature storage conditions.

**Key words:** Human proinsulin; Protein storage vacuole; Protein stability; Molecular farming