Plant Biotechnology, 16(1), 89–91 (1999)

Oleosin Partitioning Technology for the Production of Recombinant Proteins in Oilseeds

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In recent years, it has become apparent that plants may act as convenient and inexpensive production systems for proteins. Uses for these proteins include biopharmaceuticals, vaccines, industrial and food enzymes and food or feed additives. Exploiting plants in this way may impart many advantages other than cost. Plants are generally not susceptible to mammalian pathogens. Thus, production of blood proteins such as albumin or factor IX in plants could isolate the source of the protein from cryptic infections which might be discovered or characterized later. When proteins are expressed in a storage organ such as a seed, the desired product may be stockpiled as a very inexpensive and stable form of inventory. This, in turn, leads more readily to continuous flow systems of production rather than batch fermentation. Plants, being eukaryotes, carry out post-translational modifications not performed by prokaryotes such as *E. coli*.

One of the key problems to be resolved in using plants as a protein production system is the development of inexpensive recovery and purification schemes. We have approached this using the unique properties of seed oil-bodies and their proteins oleosins. Oleosins are targeted with high-efficiency to developing oil-bodies in seeds. On extraction in an aqueous environment, the oleosins partition specifically to the oil-body fraction. This floats to the surface of an extract under gravity or low-speed centrifugation.

If recombinant oleosins are made in transgenic plant seeds through extensions to their C- or N-terminal ends, the new polypeptide is also found to accumulate with high avidity on the oil-body. In consequence, it is possible to produce a recombinant protein in seeds as an oleosin fusion. This is easily recovered and can be purified to homogeneity in a few steps post-cleavage.

We have shown that this technology can be used for production of a wide variety of proteins and polypeptides. These proteins accumulate in a manner such that refolding is not normally required. The process has been scaled-up to permit continuous flow separation of the components. This technology appears to provide a versatile and robust system for the production of valuable proteins in plants. The advantages and challenges ahead for this system and plant-based production methods in general will be discussed.

Genetically Engineered Insect and Disease Control: From Fledging Science to Commercial Products

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The insect pests and pathogens cause substantial losses in crop yield worldwide despite the use of intensive and sophisticated crop protection measures. The gllobal losses due to insect pests and diseases is still 12–13% despite the use of pesticides and disease-resistant crop varieties. There is an urgent need for new sources of resistance in many crops. During the past decade, significant advances have been made toward the development of insect- and disease-resistant crops through genetic transformation. The transgenic cotton, corn and potato plants containing the 1st-generation insect-control-proteins from *Bacillus thuringiensis* have been successfully commercialized. Rapid progress has also been made toward the identification and cloning of 2nd-generation insect-control-proteins with novel modes of action. The genes conferring resistance to a range of fungal and bacterial pathogens have been identified and shown to confer agronomically useful level of resistance to these pathogens in field tests of genetically engineerred crops. During my talk, I will provide an overview of the advances made in our laboratories at Monsanto Company toward the development and commercialization of insect- and disease-resistant transgenic crops.