Precision engineering of plant genes

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4. Abstract

The quality of transgene expression is influenced by the position of integration in the genome and the complexity of the integrated DNA. Today's methods cannot control both parameters, but gene targeting would be the solution. However, this technology is not available in higher plants. Gene targeting relies on homologous recombination. Progress in the general understanding of homologous recombination and the discovery of highly efficient gene targeting in a moss allow new approaches to improve gene targeting in plants. Key parameters that differ in efficient and inefficient systems are analysed and appropriate genes and /or features transferred to low efficiency gene targeting systems. Homologous recombination is analysed in the modified systems to confirm the function. Concepts shown to improve gene targeting are transferred to commercially important systems as a first step towards application.

5. Objectives

The identification of parameters which discriminate a system efficient in gene targeting from an inefficient system. The transfer of these parameters to a system with low gene targeting efficiency and analysis whether or not this results in an improvement of gene targeting. Establishment of a generally applicable strategy to improve gene targeting in plants. As a first step in commercialisation and to proof the concept, transfer of the

technology to crop plants and other biological systems. Marketing of key genes or features and methods to make this methodology useful for basic science, industries, and plant breeders.

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7. Description of the Work

The underlying process of gene targeting is homologous recombination. Somatic tissues of higher plants have an inefficient apparatus for homologous recombination. Other cells, like those of moss or meiotic or cells have high efficiencies of homologous recombination. The

difference in the recombination apparatus may be the presence of an active homologous recombination pathway or a relatively inactive illegitimate recombination pathway. To correlate these differences with the efficiencies of gene targeting, key representatives for both pathways are isolated from cellular systems that display high and low gene targeting efficiencies and expression patterns of such genes determined in high and low efficiency systems. In addition, parameters in transformation protocols contributing to high gene targeting frequencies will be investigated and novel transformation systems tested for their potential to yield high gene targeting frequencies. Biochemical features of key recombination proteins which may be decisive for high gene targeting efficiencies will be analysed from sources exhibiting high and low gene targeting efficiencies and compared to each other. The role of illegitimate recombination in gene targeting will be investigated in systems with an active homologous and inactive recombination apparatus and the results compared. Since the structure of chromatin determines the activity of genetic loci, the role of chromatin in gene targeting will be investigated. Key proteins, features of genes, or transformation protocols identified to correlate with high frequencies of gene targeting will be transferred to inefficient gene targeting systems or, vice versa, such items will be eliminated in the efficient systems. and the effect on gene targeting analysed. This analysis will directly lead to key features that determine gene targeting efficiencies. The final step transfer of concepts to crop plants leads into commercialisation of results.

8. Deliverables

Knowledge on recombination genes and their products in plants, knowledge on features discriminating gene targeting efficient systems from inefficient ones, functional analysis of key parameters differing in gene targeting efficient and inefficient systems, analysis of homologous recombination in a crop plant.