Plant-based strategies aimed at expressing a synthetic human adenosine deaminase at high levels

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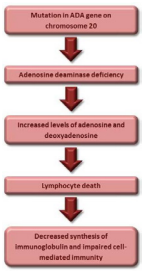
An inherited disorder, ADA deficiency is a form of severe combined immunodeficiency, which is ultimately caused by an absence of adenosine deaminase (ADA), a key enzyme of the purine salvage pathway. The absence of ADA-activity in sufferers eventually results in a dysfunctional immune system due to the build-up of toxic metabolites. To date, this has been treated with mixed success, using PEG-ADA, made from purified bovine ADA coupled to polyethylene glycol. It is likely however, that an enzyme replacement therapy protocol based on recombinant human ADA would be a more effective treatment for this disease. Therefore, as a preliminary step to produce biologically active, synthetic human ADA in transgenic tobacco plants and tobacco BY-2 cell suspensions a human ADA cDNA has been inserted into a plant expression vector under the control of the CaMV 35S promoter and terminator. In an attempt to maximise the yield various recombined gene constructs containing compartmental targeting sequences were tested along with different translational regulatory sequences, such as TMV omega and RUBISCO untranslated regions.

Tobacco plants and BY-2 cells transformed with cytosolic constructs showed levels of recombinant ADA of up to 97 ng mg-1 TSP. By comparison, transgenic calli expressing constructs containing apoplast-directing signals showed higher levels of recombinant ADA expression of up to 140 ng mg-1 TSP. The most significant ADA activities, however, were measured in the media of transgenic BY-2 cell suspensions prepared from transformed calli: where incorporation of a signal for arabinogalactan addition to ADA, led to a recombinant protein yield of approximately 16 mg L-1. A 336-fold increase over ADA produced by cell suspensions transformed with a cytosolic construct.



Biography

David Bringloe has completed his PhD and his current research interests involve heterologous gene expression systems and plant biotechnology, his main focus is to control of foreign gene expression in plants, particularly the production of therapeutic proteins and now also prions. To date, plant-based strategies have been employed to express various therapeutic enzymes and proteins at high levels in whole plants and plant cell suspensions.

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