

Characterization of Genes Expressed During the Early Stages of Direct Somatic Embryogenesis in Alfalfa (*Medicago falcata* L.)

Yan Zhou, Mark R Fowler, Jenny Russinova, Cui-Ying Shao, Nigel W Scott, Malcolm C Elliott and A Slater

The Norman Borlaug Institute for Plant Science Research, De Montfort University, Leicester LE7 9SU, UK

A system for direct somatic embryogenesis has been developed in *Medicago falcata*. Young alfalfa leaf explants are induced to form pro-embryos directly after wounding and incubation in a liquid medium containing 2,4-D. After 15 days incubation, the organization of the swollen leaf tissue is disrupted, non-embryogenic cells enlarge and separate while globular clusters of small dividing cells (pro-embryos) are released into the medium. Embryo development proceeds through the heart and torpedo stages if the pro-embryos are transferred to a plant growth regulator-free medium. Subtractive cloning is a powerful method for detecting and isolating gene sequences that are differentially expressed. This approach was used to isolate genes expressed during the early induction period in the alfalfa direct somatic embryogenic system, and nearly 100 different clones were identified. After DNA sequence characterization, the majority of these clones were identified by sequence homology and categorized by function. The largest group of genes encodes a range of proteins involved in ribosome biosynthesis, translation and post-translational modification. This group of growth-related genes included twenty different ribosomal proteins, which appeared very rapidly during the induction period. Other groups of genes coded for stress-related proteins, cytoskeletal components and regulatory proteins.

One of the regulatory genes found in this study encodes a transcription factor of the homeodomain/leucine zipper (HDzip) class. This gene is of particular interest because similar genes have been associated with somatic embryogenesis in other systems. In order to characterize the role of this gene in direct somatic embryogenesis in alfalfa, constructs containing regions of the HDzip gene in sense and antisense orientation, under the control of the CaMV35S promoter, have been introduced into alfalfa tissue by *Agrobacterium*-mediated transformation procedures. Transformed plants are currently being regenerated. The analysis of these transformed plants should provide important insights into the role of this HDzip gene in somatic embryogenesis.

This work was supported, in part, by the EU INCO-Copernicus Programme.