Optimizing Efficiency and Flexibility of *Agrobacterium*-Mediated Transformation of Red Clover (*Trifolium pratense*)

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Genetic modification of plants by the insertion of transgenes can be a powerful experimental approach to answer basic questions about gene product function. This technology can also be used to make improved crop varieties for use in the field. To apply this powerful tool to red clover (*Trifolium pratense*), germplasm with increased frequency of regeneration in tissue culture was developed (Quesenberry and Smith, 1993, *Crop. Sci.* 33:585) and utilized as the explant source for development of an *Agrobacterium*-mediated transformation protocol (Quesenberry *et al.*, 1996, *Crop Sci.* 36:1045; see http://www.dfrc.ars.usda.gov/DFRCWebPDFs/2006-31_Sull_369_384.pdf for a detailed protocol). We have been testing several variables within this original protocol to increase the efficiency and flexibility of the red clover transformation system. These tests include the use of hygromycin resistance as the plant selectable marker, increasing the duration of *Agrobacteria*-explant co-cultivation, and comparing *A. tumefaciens* strains LBA4404 (commonly used in transformation protocols) and EHA105, a kanamycin sensitive derivative of the EHA101 strain used in the original protocol. Hygromycin B at 25 mg/L was effective at selecting for successful transformation of binary vectors containing a hygromycin resistance gene (hpt) under the control of a Nos or double CaMV 35S promoter (Fig. 1). Developing embryos and fully regenerated red clover plants showed expression of a linked GUS transgene with few escapes. Hygromycin B was also highly effective at suppressing a fungal contaminant we often see on explants derived from greenhouse grown plant material. Preliminary data from experiments using *A. tumefaciens* strain EHA101 suggest that increasing *Agrobacteria*-explant co-cultivation time from 2 to 4 days results in a 30-50 % increase in transformation efficiency as assessed by embryo formation on selective media. Comparison of transformation efficiency of *A. tumefaciens* strains LBA4404 and EHA105 is currently under way. Preliminary results suggest that for red clover there is little difference in transformation efficiency between these two strains. These findings expand the options available to researchers wishing to transform red clover.

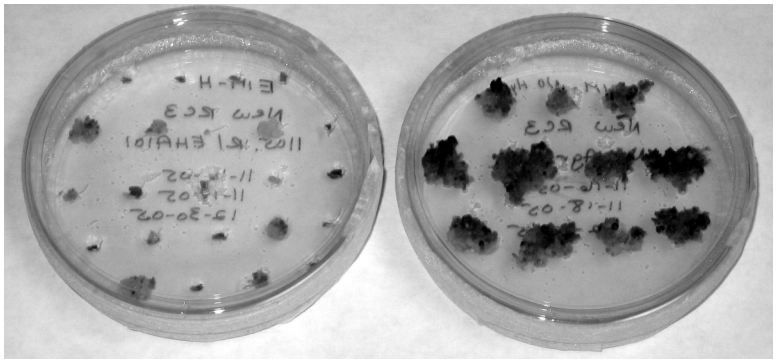

Figure 1. Callus and embryos form on several red clover explants in the presence of hygromycin B following Agrobacteria *co-cultivation (left). Expression of GUS linked to the hph gene was easily detected in most (7 out of 9) of the developing embryos tested in this experiment. A positive control (no* Agrobacteria*, no selection) is also shown (right). No callus or embryo formation is seen when uninfected explants are incubated on medium containing hygromycin B (not shown).*