Agrobacterium tumefaciens-mediated transformation of perennial ryegrass (*Lolium perenne* L.)

H. Sato, M. Fujimori, Y. Mano, T. Kiyoshi and T. Takamizo
National Institute of Livestock and Grassland science, 768 Senbonmatsu, Nasushiobara, Tochigi, 329-2793, Japan. Email: s.hiroko@affrc.go.jp

Keywords: *Agrobacterium tumefaciens*, transformation, perennial ryegrass, GUS

Introduction An *Agrobacterium tumefaciens*-mediated transformation method has several advantages. However, this method has no example of success in perennial ryegrass (*Lolium perenne* L.). Since *Lolium* species are outcrossing, one cultivar consists of many genotypes. Each genotype can show a different ability for callus formation and plant regeneration (Takahashi *et al*., 2004). Thus, it is important to select a good genotype for efficient and stable transformation. If the plant is maintained *in vitro*, we can perform transformation using calli induced from shoot tips of the same genotype at any time. Our objective is to confirm an *A. tumefaciens*-mediated transformation method for perennial ryegrass and to screen for suitable genotype.

Materials and methods Embryogenic calli were induced from shoot tips of each 50 genotypes of perennial ryegrass cultivars Saturn and Norlea. These calli were infected with *A. tumefaciens* strain EHA101 harboring a binary vector pIG121Hm and co-cultured in the presence of 100μ M acetosyringone for 4 days. The infected calli were cultured on MS selective medium containing 250mg^{-1} carbenicillin and 100mg^{-1} hygromycin. After 6-8 weeks, the hygromycin-resistant calli were transferred to MS regeneration medium containing 0.2mg^{-1} kinetin. Transformation was confirmed by histochemical GUS assay and PCR analysis of HPT and GUS gene.

Figure 1 Structure of the T-DNA region of pIG121Hm

RB and LB, right and left border; Pnos and Tnos, promoter and terminator of nopaline synthase gene; P35S, promoter of CaMV 35S RNA gene; NPT‖, neomycin phosphotransferase ‖ gene; GUS, β-glucuronidase gene; HPT, hygromycin phosphotransferase gene

Results Hygromycin-resistant calli and regenerated plants were obtained from several genotypes of Saturn and Norlea. Histochemical GUS assay showed that calli after 4 days co-cultivation and hygromycin-resistant calli were both GUS positive. Regenerated plants were subjected to PCR analysis and a single fragment of both 643bp for GUS and 375bp for HPT were detected at the equivalent sizes to the fragment from pIG121Hm. No amplification product was recognized from the non-transformed plant.

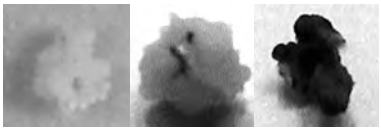

Figure 2 Expression of GUS gene (Saturn No.7)
A: non-transformed callus. B: callus after 4 days co-cultivation. C: hygromycin-resistant callus

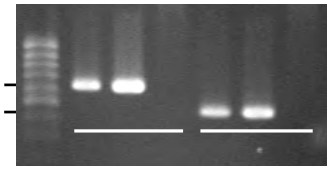

Figure 3 Detection of GUS and HPT gene by PCR (Norlea No.35)
M, 100bp DNA ladder; TP, transformed plant; P, pIG121Hm plasmid DNA; NP, non-transformed plant

Conclusions We established an *A. tumefaciens*-mediated transformation method of perennial ryegrass using selected genotypes with high transformation efficiency and plant regeneration.

References

Takahashi, W., T. Komatsu, M. Fujimori & T. Takamizo (2004). Screening of regenerable genotypes of Italian ryegrass (*Lolium multiflorum* Lam.). *Plant Production Science*, 7: 55-61.