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Efficient *Agrobacterium*-Mediated Transformation of Tall Fescue (*Festuca arundinacea*), a Forage Crop

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Objectives

Tall fescue is a perennial cool-season grass species widely used for forage and turf in worldwide. Traditionally, forage improvement has depended on conventional breeding programs. Developments in biotechnology are now permitting those involved in forage improvement to use useful genetic materials from many sources, rather than from those species which are sexually compatible. The development of a simple, economical and high efficient transformation system is the basis for genetic manipulation of tall fescue. To introduce better and useful genes into genome of tall fescue, we have developed an efficient transformation system using *Agrobacterium*-mediated gene transfer system.

Materials and Methods

1. Plant cultivars: Tall fescue cv. K-31, Hokuryo, Cajun.
2. Plasmid and *Agrobacterium*: pIG121Hm, EHA105.
3. Callus induction: Dehusked mature seeds were cultured for 4 weeks on MS medium supplemented with 9 mg/L 2,4-D and 0.1 mg/L BAP.
4. Transformation: Embryogenic calli were immersed on *Agrobacterium* suspension supplemented with 100 uM acetosyringone, and transferred to the co-culture medium.
5. Selection and regeneration of transgenic plants: Co-cultivated calli were washed with 500 mg/L cefotoxime and transferred to selection medium (MS medium plus 0.5 mg/L 2,4-D, 2.5 mg/L BAP and 25 mg/L hygromycin) for 4 weeks.
6. GUS assay: Histochemical GUS assay was performed one week after cocultivation treatment according to standard method.

Results and Discussion

An efficient transformation system for tall fescue (*Festuca arundinacea*) has been developed via *Agrobacterium*-mediated transformation of embryogenic callus. Mature seed-derived embryogenic calli were inoculated with *A. tumefaciens* carrying plasmid pIG121Hm (*intron-gus, nptII* and *hpt* genes). Transgenic tall fescue plants were regenerated from hygromycin-resistant calli and showed stable expression of *gus* gene. Vacuum treatment during *Agrobacterium* infection and addition of acetosyringone to the co-culture medium increased the transformation efficiency of embryogenic calli of tall fescue. Addition of a surfactant, Tween-20, to the inoculation medium showed positive effect on transient expression of *gus* gene. Most of hygromycin-resistant calli were developed into phenotypically normal plants. Using this rapid and efficient transformation system, introduction of multiple abiotic stress resistant genes into the genome of tall fescue is currently in progress. <This work was supported by upland crops project of BioGreen 21 Program, RDA>

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