17. Development of Herbicide Resistant Tall Fescue Plant by *Agrobacterium tumefaciens*-mediated Transformation

Gi-Won Lee, Hyun-Sook Woo, Jin-Suk Kim[1] and Byung-Hyun Lee

(College of Agriculture and Life Science, Gyeongsang National University, Jinju 660-701, Korea.
Korea Research Institute of Chemical Technology, Daejeon, 305-600, Korea.[1])

*Agrobacterium*을 이용한 제초제 저항성 톨 페스큐의 개발

이기원 · 우현숙 · 김진석[1] · 이병현

(경상대학교 동물자원과학부, 한국화학연구원[1])

Key words : *Agrobacterium*, EPSPS, Tall fescue.

<Objectives>

Herbicides are widely used for the purpose of crop cultivation and are usually removed from the environment by natural degradation and degradation by bacteria and plants.

In order to develop herbicide resistant forage plants, efficient plant regeneration and transformation systems for tall fescue (*Festuca arundinacea* Schreb.) have been established. Using these systems, expression vector containing herbicide (glyphosate) resistance gene, EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), under the control of ubiquitin promoter was introduced into tall fescue plants via *Agrobacterium*.

<Materials and Methods>

1. Plant cultivars: Tall fescue cv. Kentucky-31, Cajun, Fawn, Hokuryo.
2. Expression vectors: *Agrobacterium* EHA105 carrying vector pCAMBIA1300 encoding the EPSPS genes and hygromycin phosphotransferase (HPT) in the T-DNA region.
3. Transformation: Embryogenic calli were immersed on *Agrobacterium* suspension supplemented with 100 μM acetosyringone, and transferred to the co-culture medium. Co-cultivated calli were washed with 250 mg/L cefotoxime and transferred to selection medium (N6 medium, 1 mg/L 2,4-D, 3 mg/L BAP, 50 mg/L hygromycin), and cultured for 4-6 weeks. Hygromycin resistant shoots were rooted on a plant growth regulator-free MS medium, and transferred to soil.

<Results and Discussion>

We have produced glyphosate resistant tall fescue by *Agrobacterium*-mediated transformation. Various factors were checked to improve the transformaton efficiency, such as phytohormone, pH, acetosyringone concentration, infection time, and co-cultivation period. Effects of several factors affecting on transformation efficiency were investigated (Fig 1). Using this system, expression vectors containing EPSPS were introduced into the genome of tall fescue. PCR analysis revealed that the transgene was successfully integrated into genome of regenerated plants (Fig 2). Herbicide resistance of transgenic tall fescue plants is under investigation. < This work was supported by upland crops project of BioGreen 21 Program, RDA >

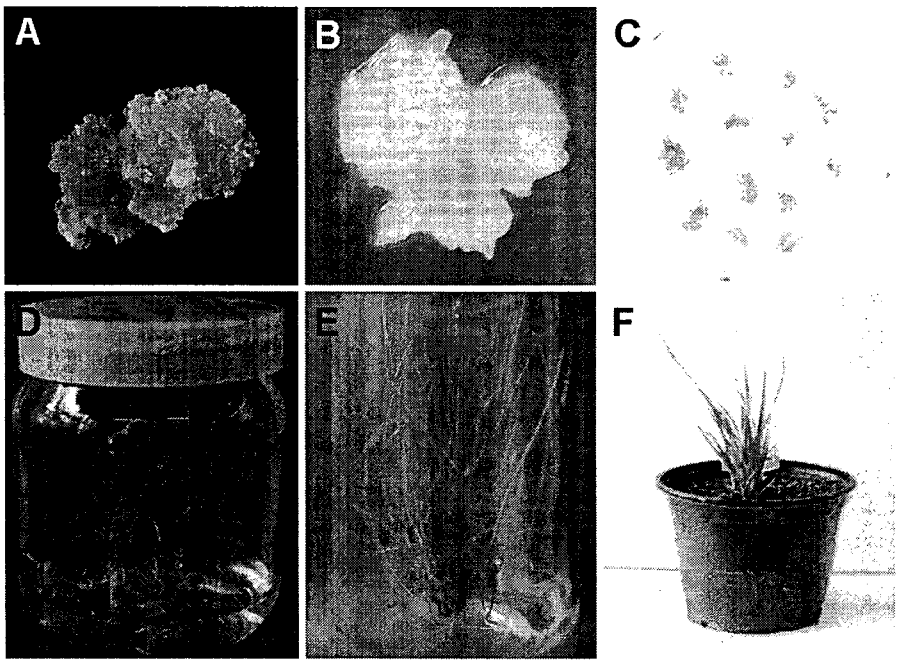

Fig. 1. *Agrobacterium*-mediated transformation of tall fescue.
(A) A compact embryogenic callus. (B) calli were co-cultured for 5 days. (C) Regeneration of the transgenic plants. (D) Resistant shoots developed new green leaves and non-resistant shoots remained small and turned brown. (E) Regenerated plantlets resistant to hygromycin. (F) Complete transgenic plantlets growing in soil.

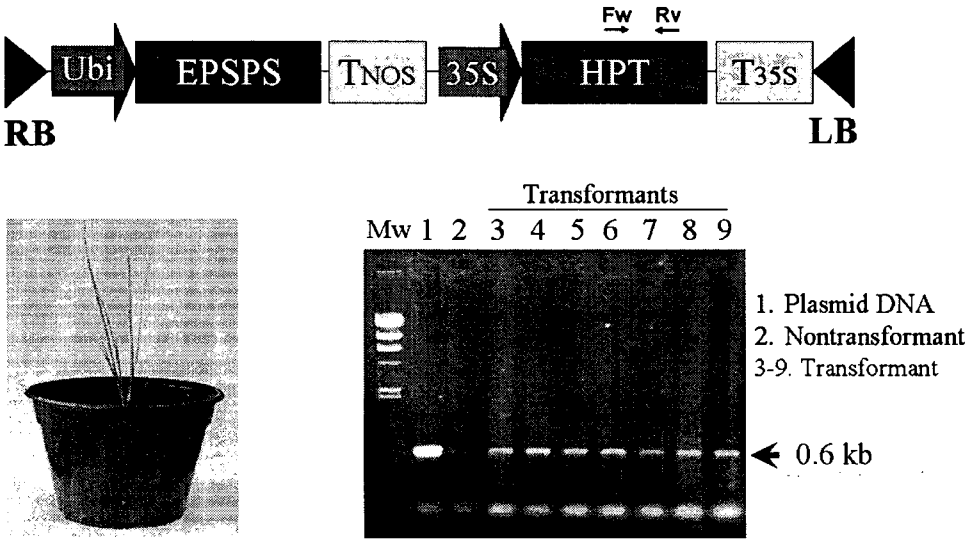

Fig. 2. PCR analysis of genomic DNA from transgenic tall fescue.