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Genetic transformation of tomato pollen lysine-rich gene to *Elytrigia intermedium* and regeneration of transgenic plants

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Key words : *Elytrigia intermedia*, lysine-rich gene, transformation, regeneration

Introduction *Elytrigia intermedium* (Hest) Nevski is a perennial Triticeae grass that has high biomass production, high palatability and digestion, but low lysine content. A pollen-specific lysine-rich gene cDNA sequence from tomato was obtained by RT-PCR. A plant expression plasmid pBIUB-TSB was constructed with the ubiquitin promoter. Plant regeneration was constructed using the mature seed of *Elytrigia intermedia* as the explant. Plasmids of pBIUB-TSB were transferred into the calli by the particle gun and the laser microbeam methods. The regenerated plants were selected for kanamycin resistance.

Materials and methods The plant regeneration of *E. intermedium* was accomplished by use of a: 1) calli inducing medium of MS + 2.4-D (4.5mg/L) + BA 0.1mg/L + Sucrose 30g/L + Agar 6.5g/L PH 5.8~6.0, 2) subculture medium of 1) + Vc 5mg/L, 3) differentiation medium of MS + BA 2mg/L + NAA 1mg/L + CoCl₂ 5mg/L + ZT 0.5mg/L, and 4) rooting medium of 1/2 MS + IAA 0.5mg/L + NAA 0.5mg/L + AC 0.1%. The particle gun and the laser microbeam methods were used in transformation.

Results Some regenerated plants were obtained by both the particle gun and laser microbeam under selection for kanamycin resistance of 300mg/L. A total of 14 plants were expressed positively by genomic PCR detection and southern blotting analysis, 9 plants were transformed with the particle gun, and 5 plants were transformed with the laser microbeam (Figure 1, Figure 2).

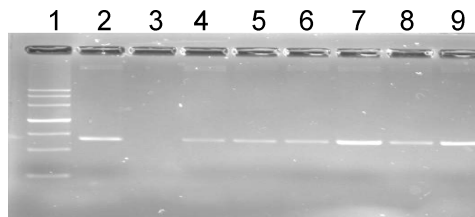


Figure 1 Electropherogram of PCR of transgenic plants: 1= DNA marker, 2= positive control; 3= negative control, 4-7= particle gun transformed plants, and 8-9= laser microbeam transformed plants.

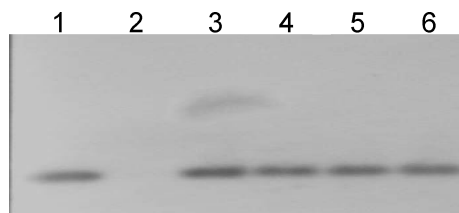


Figure 2 Southern blot of transgene plants: 1= plasmid pBIUB-TSB, 2= negative control, 3,4= transformed plants with the particle gun method, 5,6= transformed plants with the laser microbeam method.

Conclusions A regeneration system of *E. intermedium* (Hest) Nevski was obtained with seed explant. Both the particle gun and the laser microbeam methods were used successfully in transformation of calli of *E. intermedium*; PCR and southern blotting proved that the lysine-rich gene was integrated into the genome of *E. intermedium*.

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