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Agrobacterium-mediated genetic transformation of *Elymus breviaristatus* with pseudomonas pseudoalcaligenes insecticidal protein gene

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Key words : Agrobacterium-mediated transformation , callus regeneration , mature embryo , ppip , transgenic plant

Introduction *Elymus breviaristatus* is the predominant type of pasture grass in cold season . This forage grass , however , is a favorable food for grasshoppers (*Locusta Orthoptera*) . Grasshopper plague has become a major damage to the maintenance of pasturage . The development of transgenic technology provides an alternative and effective method for reducing the damage of grasshoppers by the products of pesticidal genes (Akutsu *et al* , 2004) .

Materials and methods Embryogenic calli of *Elymus breviaristatus* were induced from mature seeds , and proliferated on callus induction and maintenance medium containing kinetin 0 .05 mg/L and 2 ,4-D 8 .0 mg/L under dim-light condition at 26°C . The embryogenic calli produced were used for transformation mediated by EHA105 which carries the pCAMBIA 1304-ppip . The calli transformed were selected and grown in the presence of 60 mg/L hygromycin (Li *et al* , 2006) .

Results MS medium was more favorable for callus induction and regeneration of *Elymus breviaristatus* than other media when mature embryos of seeds were used as explants (data not shown) . The calli of three-and four-month-old what had higher frequency of hygromycin-resistant callus when co-cultivated at 19-23°C (data not shown) . The transformation frequency of the calli reached 10 .52% (data not shown) . Calluses induction and regeneration of *Elymus breviaristatus* are shown in Figure 1 . The presence of ppip gene in the genomic DNA of regenerated plants was detected by means of Southern hybridization (Figure 2) , and the expression of the trans-genes was verified by reverse transcription-PCR (Figure 3) .

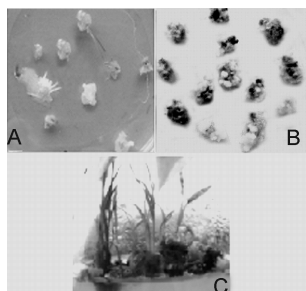


Figure 1 Calluses induction and regeneration of *Elymus breviaristatus* .

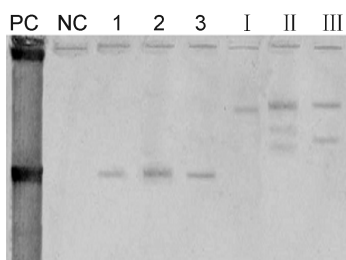


Figure 2 DNA gel blots analysis of transgenic *Elymus breviaristatus* .

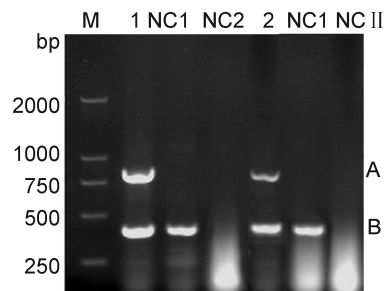


Figure 3 RT-PCR analysis of transgenic *Elymus breviaristatus* .

Conclusions *Agrobacterium-mediated* transformation in the conditions tested was effective for transferring foreign genes into *Elymus breviaristatus* . These results may set an example for the *Agrobacterium* method that can effectively transfer foreign genes into *Elymus breviaristatus* .

References

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Li DX , Zhang J , Zhao J , Zhang Y , Li L , Liu SJ , Chen F , Yang ZR (2006) . Transformation of calli of siberian wildrye grass (*Elymus sibiricus* L . cv . chuancao No .2) mediated by *agrobacterium* . *Journal of Plant Physiology and Molecular Biology* . 2006 , 32 (1) : 45-51 .