Regeneration of GUS,Bt-toxin and HNP transgenic plants of *Lotus corniculatus*

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*Lotus corniculatus*L (birds-foot trefoil) is an important forage legume with rich nutrients and "blot safety". However,some characteristics of *L. corniculatus*,such as deficiency of sulfur amino acid need to be improved.Content of sulfur amino acids in forage plants is positively related to wool growthin sheep.

Previous reports of transformation of *L. corniculatus* described the incoporation of NPTll (Yu and Shao 1990),cat(Hansen et al.1987,Petit et al.1987,Jorgensen et el.1988),luc(Jensen et al.1986,Hansen et al.1989),GUS(Hansen et al.1989). Here we report regeneration of GUS,Bt-toxin and HNP (sulfursrich amino acid)transgenic plants from seedling cotyledon explants of *L.corniculatus* via an *Agrobacterium* vector.

Materials and Methods

Axenic seedling cotyledons (7-10 days) of *L. corniculatus* were cut transversely into two pieces,and placed into a suspension of *A.tumefaciens* strain A3 with plasmid pBI121 containing NPTll gene(nos promoter) and GUS gene (CaMV35s Promoter),LBA4404 with plasmid pB48.215 containing NPTll gene and Bt-toxin gene(CaMV35s promoter) or A281 with plasmid pBF649 containing NPTll gene(nos promoter) and HNP(sulfursrich amino acid) gene (CaMV35s promoter) for 0.5h,and co-culured for 4 days on MS1-2(Lu et al.1986).Then the segments were transferred to MS1-2with 25mg/l kanamycin and 300mg/l cefotaxime.The control were not infected by A281 with other conditions same as the treatment samples.Shoots and plants from cotyledon explants wer removed and cultured on fresh MS1-2 with 100mg/l kanamycin and 300mg/l cefotaxime at one month interval, for 5-6 times.Roots or stems from kanamycin-resistant plants were cut into 0.5-1 cm long and cultured on MS1-2 with 25-50mg/l kanamycin.Samples of cotyledon,leaf and stem segments were made for observation of scanning electron microscopy according to report of Ho and Vasil(1983).The kanamycin-resistant plants were cut into small pieces and inoculated to YEB medium for axenic examination of the plants.The plants were transferred to pots and greenhouse.

Determination of NPTll enzyme activity was carried out according to the method of angenon et al(1987),Schreier et al,(1985), and Roy and Nirmala(1990).

DNA molecular hybridization (dot blot and southern blot)was carried out referring to the method of Maniatis et al(1982).

Results and Discussions

Plants were regenerated from cotyledon explants on selection medium with

about 10% regeneration frequencies,and from root or stem segments of the regenerated plants on MS1-2 with 25-50mg/l kanamycin.Untreatment cotyledon explants all died on selection medium.It will shorten period of selecting the transformation plants to obtain plants from root or stem segments of the regeneration plants.

Scanning electron microscopic observation showed that *Agrobacterium* number attached on cut face of cotyledon segments were much more than it on surface of them.

There was no *Agrobacterium* in and out kanamycin-resistant plants after 5-6 times selection.

Enzyme assay showed NPTll or GUS enzyme activity in the plants(Fig.1-1,3).Results of dot and southern blotting of the plant DNA demenstrated that there were homogenic fragments of GUS,Bt-toxin and HNP genes in the plants,but there were not them in the controls(Fig.1-2,4). These confirmed that GUS,Bt-toxin and HNP genes had been integrated into genomes of *L.corniculatus* cells.The transformed plants have survived , continued grow since being transferred to pots and greenhouse,and showed no phenotyphic abnormalities.

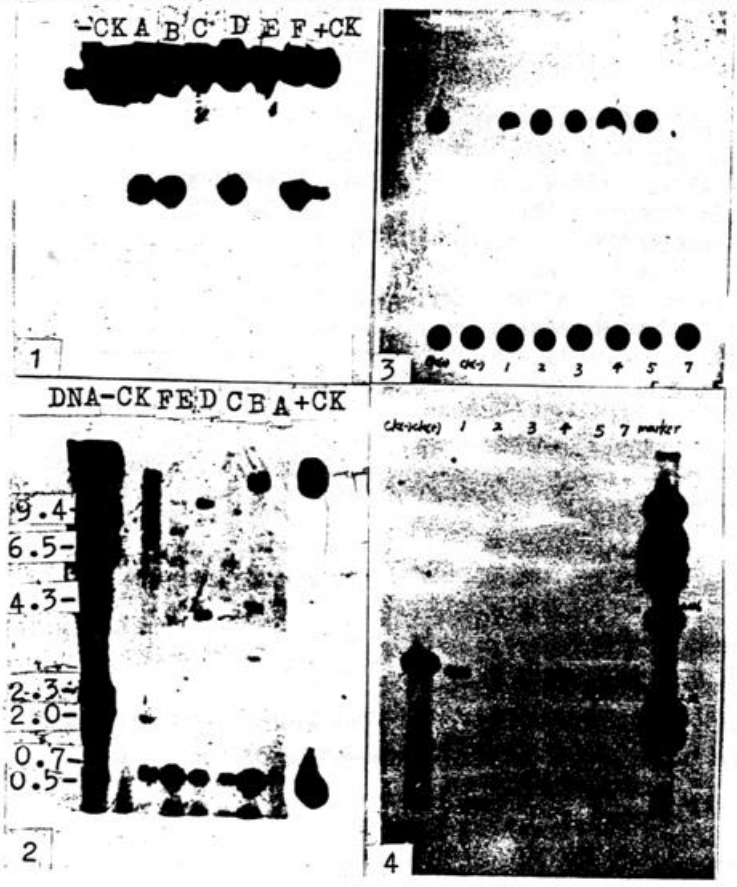

Fig.1-1 Examination of NPT 11 enzyme (Schreier et al,1985) from Bt-toxin transgenic plants. Plants A,B,D,F showed strong NPT11 activities.

2 Southern blotting of DNA from Bt-toxin transgenic plants. Plant F,E,D,C,B,A showed 0.7kb band (Bt-toxin gene).

3 Examination of NPT11 enzyme (Roy et al,1990) from HNP transgenic plants. Plant 1,2,3,4,5,7 showed strong NPT11 activities.

4 Shouthern blotting of DNA from HNP transgeneic plants. Plant 1,4,7 showed 3.0kb band (HNP gene).

References

1 Angenon G et al.(1987)EMBO Course on Plant Molecular Biology Genet.
2 Hansen J et al. (1988). Plant Cell Rep.8:12-15.
3 Ho WJ and Vasil IK.(1983). Ann Bot. 51:719-726.
4 Lu DY et al.(1986) Kexue Tongbao(Chinese Sci Bul).31:1647-1650.
5 Jensen JS et al.(1986). Nature. 321:669-674.
6 Jorgensin Jan-Elo et al.(1988). Nucl Aci Res. 16:40-50.
7 Petit A et al.(1987). Mol Gen Genet.207:245-250.
8 Roy Pand Nirmala S.(1990).Plant Mol Biol.14:873-876.
9 Schreier P et al .(1985).EMBO J.4:25-32.
10 Yu JP and Shao QQ.(1990). Science in China(Series B).34:932-937.

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