TRANSFORMATION OF *POPULUS TREMULOIDES* USING *AGROBACTERIUM RHIZOGENES*.

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Abstract

Several factors affecting *A.rhizogenes*-mediated transformation of *Populus* were studied. The leaf section of *Populus* was more sensitive to kanamycin used for selection of transformant than the stem section. The soaking period for inoculation did not affect gall formation up to 2 hours. The optimum concentration of acetosyringone and pH of bacterial culture medium for inoculation were 50μM and 5.5, respetively. One day cocultivation after inoculation gave highest transformation rate. The visible hairy roots were formed from the transformed leaf sections within 3 weeks after culture on both of the media with and without growth regulators. The plantlets were regenerated from the infected leaf sections within 6 weeks after culture on the medium containing 0.005mg/l of NAA and 0.5mg/l of BA. The expression of the introduced opine genes in the plantlets were confirmed by analysis of agropine and mannopine.

INTRODUCTION

Agrobacterium rhizogenes has the T-DNA on Ri plasmid. Under the control of a virulence region, two seperate T-DNA region, T_L and T_R are transfered into the plant genome. The T_R T-DNA contains genes for the synthesis of opine and auxin. The T_L T-DNA codes for a set of genes which increase auxin sensitivity. The T-DNA of Ri plasmid causes hairy root formation in the infected plant tissues. *A. rhizogenes* is very useful in propagation and transformation of plant types where the whole plant can be easily regenerated from root.

Since the long generation time of woody species makes traditional breeding stratergies difficult, the genetic engineering of trees has recently generated a great deal of interest. Among trees, *Populus* has a number of characteristics that makes them well suited as a model system for the transformation of woody plants. They are fast growing, produce valuable products, and the whole plant can be easily obtained by root culture.

In this experiment several factors affecting *A.rhizogenes*-mediated transformation of *Populus* were investigated to establish a system for woody species transformation.

MATERIALS AND METHODS

Plant: *Populus tremuloides* was used.

Strain: *Agrobacterium rhizogenes* R1601 was used, which has auxin/cytokinin, nptII, and agropine/manopine genes.

Tissue culture and opine analysis were carried as descibed by Ahuja(1983) and Rogers(1986), respectively.

RESULTS AND DISCUSSION

The leaf sections did not form any callus on kanamycin medium at the concentration of 100 μg/ml or higher, the stem sections, on the other hand formed callus by 95% at the same concentration of kanamycin(Fig.1). Therefore the leaf section was appeared to be better for screening of transformant on kanamycin medium.

The normal callus was induced on the medium containing NAA and/or BA. The *A. rhizogenes* incited galls were formed mainly from the wounded edge and abaxial surface of leaf section, while the normal calluses were formed from vein. The formation of this normal callus was not observed on the growth regulator free medium, but the gall induction by *A. rhizogenes* occurred

on the growth regulator free medium(Table 1.). This result shows that the growth regulator free medium can be also used for transformant screening.

The bacterial density, over the range from $4x10^5$cfu to $7x10^9$cfu, in soaking suspension did not affect gall formation rate. The gall formation was over 90% up to 2 hour soaking period, but the gall formation was decreased at 4 hour soaking. Just dipping of tissue in bacterial suspension appeared to be enough for the infection.

The gall formation was increased from 73% to 88% with the period of cocultivation up to 4 days, but the bacterial contamination was also increased 0% to 38%. The optimum cocultivation period was 1 day, and the % of gall forming sections and contaminated sections at this cocultivation condition were 85% and 5%, respectively.

The treatment of acetosyringone in both of the liquid bacterial culture medium and the soaking medium seemed to promote gall induction, especially at low pH. The highest gall formation was obtained at the pH 5.5 of medium containing 50 μM of acetosyringone(Fig.2). This result was consistant with the fact that acetosyringone could activate virulence gene expression of *Agrobacterium*.

After cocultivation the bacteria were eliminated from the leaf sections on the culture medium containing 250 ug/ml of cefotaxime and ampicillin. The selective growth of transformed galls were observed on the medium containing 100μg/ml of kanamycin or on the growth regulators-free medium. The visible hairy roots were formed from the transformed leaf sections within 3 weeks after culture on both of the media with- and without-growth regulators. The plantlets were regenerated from the transformed leaf sections within 6 weeks after culture on the medium containing 0.005mg/l of NAA and 0.5mg/l of BA. The expression of the introduced opine genes in the gall, root, and shoot were confirmed by analysis of agropine and mannopine(Fig.3.).

REFERENCES

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Table 1. Formation of the normal callus and the gall on the medium with or without growth regulators. The gall was incited by A. rhizogenes. NAA(0.5 mg/ml) and BA(0.5 mg/ml) were used as auxin and cytokinin, respectively. The formations of callus and gall were measured after 2 week culture. The total number of sections tested pereach treatment were more than 40.

Growth regulator	Free	NAA	NAA+BA
Callus formation(%)	0	98	100
Call formation(%)	74	47	60

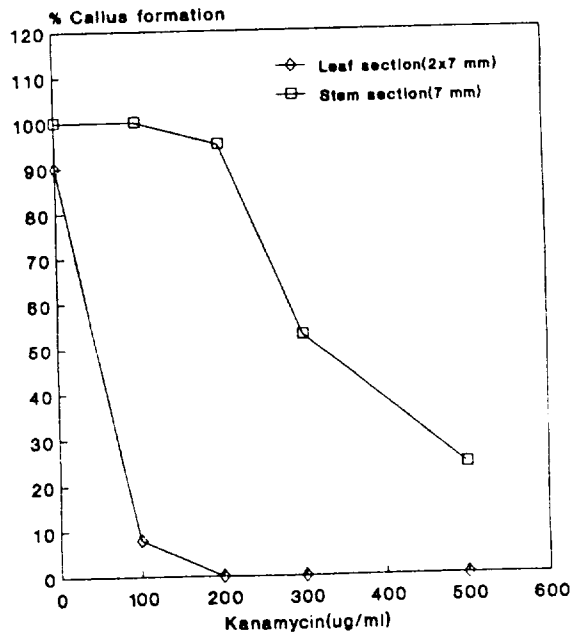

Figure 1. Sensitivity of tissue to kanamycin.

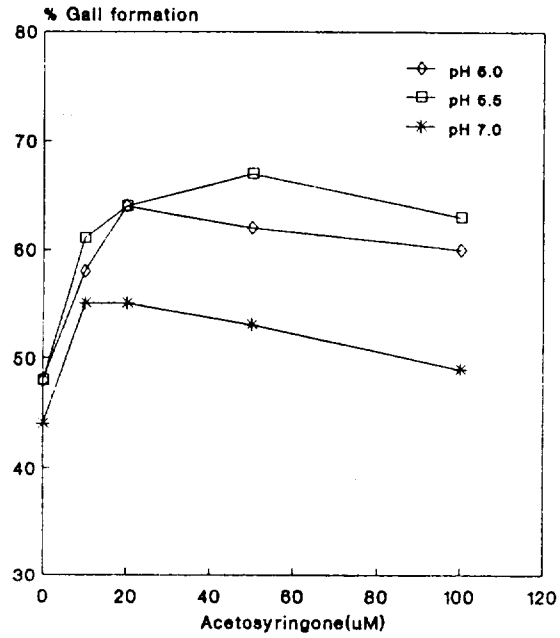

Figure 2. Effect of acetosyringone treatment on call induction.

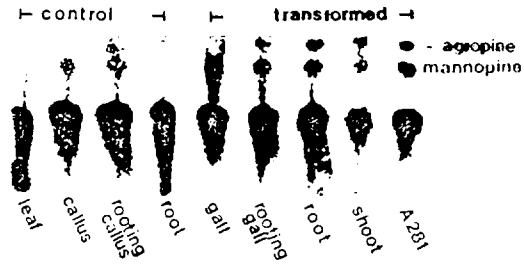

Figure 3. Opine analysis of non-transformed and transformed tissues.