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Organ Specific Expression of the *nos-NPT II* Gene in Transgenic Hybrid Poplar[1]*

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형질 전환된 포플러에 대한 *nos-NPT II* 유전자의 기관별 발현 특성[1]*

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ABSTRACT

To effectively modify tree function with genetic engineering, transgenes must be expressed at the proper level in the appropriate tissues at suitable developmental stages. Toward understanding the spatial and temporal expression of transgenes in woody plants, transgene expression was evaluated in three greenhouse -grown, transgenic lines of *Populus alba* X *P. grandidentata* hybrid clone 'Hansen'. All transgenic poplar lines possess constructs containing the bacterial nopaline synthase(*nos*) promoter linked to a neomycin phosphotransferase *II* (*NPT II*) selectable marker gene. In addition, each transgenic poplar line contains one of the following gene constructs : 1) a wound-inducible potato proteinase inhibitor II (*pin2*) promoter linked to a chloramphenicol acetyltransferase(*CAT*) reporter gene ; 2) *a nos* promoter linked to a *PIN2* structural gene ; or 3) a Cauliflower Mosaic Virus 35s promoter linked to a *PIN2* structural gene. Polymerase chain reaction(PCR) was used to verify the presence of foreign genes in the poplar genome. Enzyme-linked immunosorbent assays(ELISAs) were used to evaluate organ specific expression of the *nos-NPT II* construct. *NPT II* expression was detected in leaves, petioles, stems, and roots of transgenic poplar, thereby indicating that the *nos* promoter is potentially effective for general constituative expression of transgenes. *NPT* expression varied among transgenic poplar lines and among organs for one transgenic line, Tr15. With Tr15, *NPT II* levels were highest in older leaves and petioles. These results indicate that screening of several transgenic lines may be required to identify lines with optimal transgene expression.

Key words : *Populus*, *transgene expression*, *neomycin phosphotransferase*, *nos* promoter, ELISA, PCR

要 約

임목을 대상으로 삽입된 외래 유전자의 공간적, 시기별 발현 특성을 이해하기 위한 기초연구로서 온실에서 생육 중인 형질전환된 2년생 잡종 포플러(*Populus alba* X *P.grandidentata*) Hansen 클론을 대상으로 삽입된 외래 유전자의 발현정도를 각 기관별로 조사하였다. *Agrobacterium* binary

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vector pRT45, pRT102 및 pRT104에 의해서 형질전환된 3계통의 형질전환체 Tr15, Tr345, Tr665 모두는 선발가능한 표식 유전자로서 *nos* promoter-*NPT II* 유전자가 대상 식물체의 genome에 삽입되어 있으며, 그외에, *pin2* promoter-*CAT* 유전자(pRT45), *nos* promoter-*PIN2* 유전자(pRT102), Cauliflower Mosaic Virus 35s promoter-*PIN2* 유전자(pRT104)가 3계통의 형질전환체에 제각각 삽입되어 있는 잡종 포플러이다. 이들 3계통의 형질전환 포플러 식물체의 DNA를 PCR 검정 기법을 이용하여 분석해 본 결과 선발 가능한 표식 유전자인 *NPT-II*가 삽입되어 있음이 입증되었다. 형질전환된 3계통의 식물체간에, 그리고 각 식물체의 기관별 *NPT II* 유전자의 발현 정도를 비교 분석하기 위해서 NPT-ELISA 검정을 실시하였다. 삽입된 NPT *II*유전자는 형질전환된 포플러의 잎, 엽병, 형성층 조직, 줄기의 목질부, 뿌리에서 발현되었으며, 발현 정도는 형질전환된 식물체의 계통에 따라서, 그리고 형질전환된 식물체의 부위에 따라서 다양하게 나타났다. pRT45에 의해서 형질전환된 Tr15 형질전환체의 경우, 늙은 잎과 엽병에서 *NPT II* 유전자가 가장 높은 수준으로 발현되었으며, 어린 잎과 뿌리 조직에서 가장 낮게 발현되었다. 삽입된 외래 유전자가 각 식물체간에, 각 기관에 따라서 각각 상이한 발현 정도를 나타내는 이와같은 결과는 형질전환된 식물체에 대한 효과적인 선발 과정이 요구됨을 의미함은 물론이고, 형질전환 식물체의 발달 과정에 따라서 삽입된 외래 유전자가 공간적, 시기적으로 각각 다르게 발현할 수 있다는 것을 나타낸다.

INTRODUCTION

The lack of specific genetic information regarding diverse woody plants has required the development of model systems for molecular biological studies of woody plants. Current molecular biological studies are focused on *Populus* species as a model system for deciduous woody plants. Features of *Populus* species that have contributed to its development as a model system include 1) *Populus* species are grown worldwide and valued for wood products, fiber, energy, and environmental protection (Dickmann and Stuart, 1983) ; 2) physiological and genetic traits of *Populus* species are well studied ; 3) tissue culture systems for *in vitro* propagation and regeneration of *Populus* are well developed (Chun et al., 1988a ; Chun 1993) ; 4) transformation of *Populus* species can be achieved with *Agrobacterium*-based systems (Chun et al., 1988b ; Confalonieri et al., 1994 ; Fillatti et al., 1987 ; Howe et al., 1994 ; Klopfenstein et al., 1991 ; Parsons et al., 1986 ; Pythoud et al., 1987 ; Schwartzenber et al., 1994) ; and 5) screening at the molecular level is facilitated by the small genome (c=0.7pg) of *Populus* species (Dhillon et al., 1984 ; Parsons et al., 1989). A saturated genetic map of a *Populus* pedigree is nearing completion (Bradshaw, 1994).

Studies that use genetic transformation of *Populus* to characterize or improve genetic function are becoming more common. Previous studies have been directed toward understanding transgene regulation in *Populus* species (De Block, 1990 ; Klopfenstein et al., 1991 ; Leple et al., 1992). Plant growth regulator effects in *Populus* species have been investigated with genetic transformation (Charest et al., 1992 ; Parsons et al., 1986 ; Schwartzenberg et al., 1994 ; Wang et al., 1990). Other studies have been directed toward transforming *Populus* species with genes that confer herbicide resistance (Brasileiro et al., 1992 ; De Block, 1990 ; Devillard, 1992 ; Fillatti et al., 1987) or insect resistance (Ellis et al., 1994 ; Howe et al., 1994 ; Klopfenstein et al., 1993 ; McCown et al., 1991).

Even though several transgenic poplars have been successfully derived from *Agrobacterium*-mediated transformation, little information is available regarding organ specific expression of inserted foreign genes in various transgenic poplar species.

Molecular biological studies aimed at understanding the genetic mechanisms of regulation and expression of inserted foreign genes in poplars can facilitate the development of genetically engineered trees by 1) providing unique opportunities to increase knowledge of gene expression in woody plants, and 2) utilizing promoter characteristics to design genes that are expressed in specific tissue at specific developmental stages under specific environmental conditions.

Previous studies have shown that a poplar phenylalanine ammonia-lyase(*PAL*) gene is developmentally regulated, with highest expression occurring in developing vascular tissues and sub-epidermal cells of developing leaves(Subramaniam et al., 1993). In transgenic tobacco plants, it was demonstrated that the expression of the *nos* promoter is developmentally regulated and organ specific(An et al., 1988). Recently, developmental and environmental regulation of the CaMV *35s* promoter in transgenic poplar was evaluated using a *GUS* reporter gene(Ellis et al., 1994).

In an attempt to characterize the organ specific expression of an inserted foreign gene in woody plants, hybrid poplar trees were transformed with gene constructs containing a neomycin phosphotransferase(*NPT II*) structural gene and a proteinase inhibitor *II*(*PIN2*) or chloramphenical acetyltransferase(*CAT*) structural gene. In this paper, we describe the organ specific expression of the selectable marker gene, *NPT II*, under the control of a bacterial nopaline synthase(*nos*) promoter in transgenic 'Hansen' clones of *Populus alba* L. X *P. grandidentata* Michx.

MATERIALS AND METHODS

Plant Material

Three lines, Tr15, Tr345, and Tr665, of 2-year-old greenhouse-grown, transgenic 'Hansen' clones of *Populus alba* X *P. grandidentata* were chosen as plant material for studying organ specific expression of *nos-NPT II* gene. The transgenic poplar lines were derived from separate transformation events of the 'Hansen' clone. Tr15, Tr345, and Tr665 lines were derived via by transformation with *Agrobacterium tumefaciens* containing binary vectors pRT45, pRT102, and pRT104, respectively(Table 1, Fig. 1). The transformation of the 'Hansen' clone by *Agrobacterium* binary vector systems and subsequent expression of a wound-inducible potato *proteinase inhibitor II-CAT* gene and *nos-NPT* selectable marker gene was previously described(Klopfenstein et al., 1991).

Table 1. *Agrobacterium* strain and binary vectors used for transformation of 'Hansen' poplar clone

Strain/ vectors	Selectable marker gene	Target foreign gene
A281/ pRT45	*nos-NPT II*	*pin2-CAT*
A281/ pRT102	*nos-NPT II*	*nos-PIN 2*
A281/ pRT104	*nos-NPT II*	*35s-PIN 2*

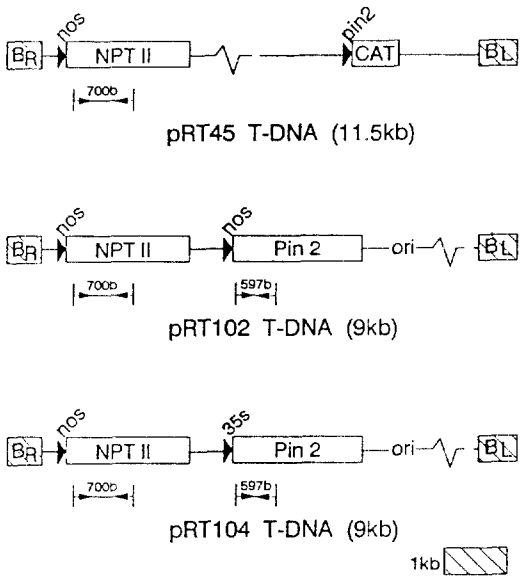

Fig. 1. T DNA maps of pRT45, pRT102, and pRT104
nos=bacterial nopaline synthase promoter (Hoekema et al., 1983)
35s=Cauliflower Mosaic Virus 35s promoter (An, 1987)
pin2=potato proteinase inhibitor II promoter (Thornburg et al., 1987a ; b)
NPT II=neomycin phosphotransferase structural gene(Hoekema et al., 1983)
CAT=chloramphenicol acethyltransferase structural gene(An, 1987)
PIN2=potato proteinase inhibitor II structural gene(Thornburg et al., 1987a ; b)
B_R & B_L=border right and border left

DNA Analyses

Total poplar DNA was isolated from fresh leaf tissue using the protocol of Rogers and Bendich (1988). The presence of the *NPT II* gene in the greenhouse-grown transgenic poplar was confirmed using polymerase chain reactions(PCR) to amplify a

target sequence within the *NPT II* selectable marker gene. Amplifications were performed with Perkin Elmer DNA Thermal Cycler. Based on the sequence of *NPT II* and work by others, the following primers were selected for amplification of a 700-base pair region : npt1, 5'-GAGGCTATTCGGC-TATGACTG-3' ; npt2, 5'-ATCGGGAGCGGC-GATACCGTA-3'(Hammill et al., 1991). The respective position of these primers in the *NPT II* gene is at nucleotides 201-222 and 879-900. PCR reactions were conducted as 100μl reaction mixtures. Reaction mixtures contained 10mM Tris-HCl, pH8.3 ; 50mM KCl ; 3.0mM $MgCl_2$; 0.015% (w/v) gelatin ; 200μM of each dNTP ; 2μM of each primer ; 0.5 units AmpliTaq polymerase(Perkin Elmer Ceuts, Norwalk, CT) ; and 400ng of DNA template from transgenic or untransformed control poplar or 40ng pRT102 plasmid template DNA. Each time PCR was performed, a negative control was included that contained no DNA template in the reaction mixture. The negative control demonstrates that PCR reagents are free of DNA template contamination. Ampliwax PCR gems(Perkin Elmer Cetus) were used as a vapor barrier to prevent evaporation from the reaction mixture. After preheating to 94℃ for 5 min, PCR cycling was performed for 1 min at 94℃ (duplex denaturation), 1 min at 62℃ (annealing), and 1.5 min at 72℃ (DNA synthesis) for 30 cycles. The amplification products were visualized under uv light after 1.6% (w/v) agarose gel electrophoresis and staining with ethidium bromide(Sambrook et al., 1989).

NPT II ELISA

Leaves of three different physiological ages based on the Leaf Plastochron Index(Larson and Isebrands, 1971) (at LPI's of 1 to 5, 11 to 15, and 21 to 25), petioles(at LPI 1 to 25), stem tips(at LPI 1 to 5), outer stems(outside of phloem at LPI 11 to 15), inner stem(inside the phloem at LPI 1 to 25), and roots were used to evaluate differential and developmental expression of the *nos-NPT II* selectable marker gene in three greenhouse-grown transgenic lines, Tr15, Tr345, and Tr665.

Approximately 10g of sample tissues were pooled from 3 plants of similar age and quickly frozen in liquid nitrogen before grinding with an equal volume of extraction buffer containing 100mM Tris-HCl (pH7.5), 0.1% (v/v) Tween 20, and 1.0% (w/v) polyvinlypyrrolidone(MW = 40, 000). Insoluble debris was removed by centrifugation and protein concentration was determined according to the method of Bradford(1976). Protein concentrations were adjusted to 1.0mg/ml with extraction buffer in preparation for enzyme-linked immunosorbent assays(ELISAs).

NPT II protein was detected using rabbit polyclonal antibodies and other immunoassay components from 5 prime-3 prime, Inc.(Boulder, CO, U.S.A.) and following general recommendations of the manufacturer. Briefly, each well of immunoassay plates was coated with anti-NPT II antibody, rinsed, and blocked with Phosphate Buffered Saline (PBS) (137mM NaCl, 2.6mM KCl, 10mM Na_2-KPO_4, and 1.7mM KH_2PO_4 at pH7.2) containing 5% (w/v) nonfat dry milk to prevent nonspecific adsorption. After rinsing, 200μl of plant sample or NPT II-containing standard curve dilutions was added to each well and allowed to bind for 2 hr at room temperature(RT) before rinsing again. A secondary biotinylated anti-NPT II antibody was applied in blocking buffer for 1 hr at RT, then plates were rinsed. Streptavidin-conjugated alkaline phosphatase was applied for 30min at RT. After washing, the p-nitrophenyl phosphate substrate was added, and the subsequent colorimetric reaction was quantified spectrophotometrically at 405nm.

Each sample was replicated three times on each immunoassay plate, and each immunoassay plate was replicated three times. Quantification of NPT II was obtained using readings from the standard curve, in which purified NPT II was diluted in leaf (LPI 11-15) extract(1μg protein/ml) of untransformed control 'Hansen'. Statistical analysis was conducted on the basis of multiresponse permutation procedures(MRPP) by Mielke et al.(1981) and Mielke(1984).

RESULTS AND DISCUSSION

Polymerase Chain Reaction

The presence of the *NPT II* gene in the separate

lines of transgenic poplar was confirmed using PCR to amplify the target sequence within the *NPT II* selectable marker gene. Fig. 2 shows the results of PCR amplification of genomic DNA from the three different transformants using primers designed to amplify a 700-base pair region of the *NPT II* gene, npt1 and npt2. There were no visible PCR products from reactions with DNA from untransformed control 'Hansen' clone or with that containing no added DNA template. Lanes 5, 6, and 7 contain PCR products of template DNA from transformed poplar lines as follows : Tr665(lane 5), Tr15(lane 6), and Tr345(lane 7). Lane 2 contains PCR products using plasmid(pRT102) template DNA that contains the *NPT II* structural gene. Bands of the same mobility were observed with genomic template DNA from the 3 separate lines of transgenic 'Hansen' clone as well as the *NPT II* containing plasmid, pRT102. Lane 3 demonstrates the negative control for this PCR set using PCR reagents, *Taq* polymerase, and dNTP's, but no template DNA. The negative control demonstrates that PCR reagents were not contaminated with target DNA. Lane 4 shows the lack of an amplified PCR product using template DNA from untransformed, control 'Hansen' poplar.

PCR provides a relatively simple and consistent method for demonstrating the transformation of poplar with foreign genes. The PCR products from amplification of *NPT II* sequences in the transgenic poplar often showed amplified bands of varying intensity(Fig. 2). Tr665 and Tr345 showed both amplification products of PCR from the *NPT II* and *pin2* gene sequences. Tr15 was transformed by T-DNA that did not contain the *pin2* structural gene and as expected Tr15 demonstrated only PCR product corresponding to the *NPT II* sequence(Table 2). The expression results of the NPT II and PIN II ELISA assays of Tr665 and Tr345 indicate co-expression of the selectable marker gene(*NPT II*) with the target gene(*PIN2*) (Klopfenstein et al., submitted).

PCR and Southern hybridization(Klopfenstein et al., 1991) of three transgenic poplar lines(Tr15, Tr345, Tr665) and several other transgenic lines has demonstrated that both *NPT II* and the second foreign gene were present, thereby indicating that

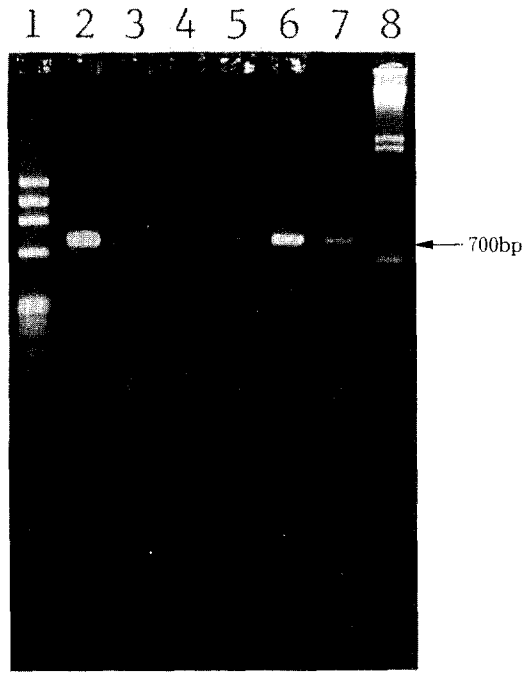

Fig. 2. Confirmation of nos-*NPT II* gene in transgenic poplar using polymerase chain reaction. The presence of the *NPT II* gene in the transgenic poplar was confirmed using polymerase chain reaction(PCR) by amplifying a target sequence within the *NPT II* selectable marker gene. Primers were selected for amplification of a 700-base pair region. After temperature cycling, the PCR products were electrophoresed on a 1.6% agarose gel. The molecular weight marker was Φ174/Hae III and lambda/Hind III markers on lane 1 and 8, respectively. The visible DNA molecular marker bands on lane 1 are 1,353, 1,078, 872, 603, and 310 bp and on lane 8 are 23,130, 9,416, 6,557, 4,361, 2,322, 2,027, and 564 bp. Lane 2 contains PCR products using plasmid (pRT102) DNA template that contains the *NPT II* structural gene. Lane 3 contains the negative control for this PCR set using PCR reagents, *Taq* polymerase, dNTP's, and *NPT II* primers, but no template DNA. The negative control demonstrates that the PCR reagents were not contaminated with target DNA. Lane 4 demonstrates a lack of PCR product amplification using template DNA from untransformed, control Hansen poplar. Lane 5 through 7 contain PCR products of template DNA from transformed poplar lines of Tr665(Lane 5), Tr15(Lane 6), Tr345(Lane 7).

Table 2. NPT II, PIN II ELISA, and PCR analysis of transformed 'Hansen' clone

Transgenic line	NPT II[a]	PIN II[b]	PCR amplification products nptl, 2	pinl, 2
Control	0[c]	0[c]	−	−
Tr15	6,938	*	+	*
Tr345	238	40	+	+
Tr665	5,010	81	+	+

[a] Values represent pg of NPT II soluble protein per mg leaf tissue protein (LPI 11 to 15) as determined by the mean three ELISA's

[b] As Klopfenstein et al. (submitted). Values represent ng of PIN II partially purified protein per mg tissue protein as determined by the mean three ELISA's from LPI 3 to 13

[c] This value was used as a baseline to determine NPT II or PIN II concentrations in transgenic plants.

* Tr15 was transformed by T-DNA that did not contain the *pin2* structural gene.

Table 3. Organ specific expression of *nos-NPT II* in transgenic poplar as detected by ELISA. Values represent pg of NPT II protein per mg tissue protein as determined by the mean three ELISA's.

Tissue	Transgenic Hansen poplar line						Control
	Tr15		Tr345		Tr665		
Leaf (LPI 1 to 5)	3,098 (294) d		1,448 (223) b		477 (247) a		0*
Leaf (LPI 11 to 15)	6,938 (604) b		238 (84) b		5,010 (3,143) a		0
Leaf (LPI 21 to 25)	11,963 (1,027) a		362 (189) b		4,527 (4,071) a		0
Petiole	11,577 (1,744) a		2,862 (2,483) b		5,000 (4,010) a		0
Stem tip	4,229 (767) cd		436 (229) b		4,073 (3,764) a		0
Outer stem	5,123 (489) bcd		319 (170) b		4,319 (4,051) a		0
Inner wood	6,225 (727) bc		0 (0) b		2,541 (2,541) a		0
Root	2,955 (145) d		7,398 (1,110) a		5,067 (3,060) a		0

* This value was used as a baseline for determining NPT II concentrations in untransformed control plants.

** Means in a column followed by different letters are significantly different ($P<0.05$). Standard errors of the mean are parenthesized.

Populus was transformed by intact T-DNA from the *Agrobacterium* binary vector system. *Agrobacterium* transformation systems are a desirable method to transform *Populus* because they are relatively inexpensive and easy to use, produce an acceptable transformation rate, transfer a limited copy number of T-DNA, and typically transfer intact T-DNA (Chun et al., 1988b ; Confalonieri et al., 1994 ; Howe et al., 1994).

NPT ELISA

Organ-specific expression of the *nos-NPT II* gene construct for three different lines of greenhouse-grown, transgenic 'Hansen' poplar were evaluated with enzyme-linked immunosorbent assay (ELISA) tests. Leaves of three different physiological ages based on the Leaf Plastochron Index (LPI's of 1 to 5, 11 to 15, and 21 to 25), petioles (at LPI 1 to 25), stem tips (at LPI 1 to 5), outer stems (outside phloem at LPI 1 to 25), inner stems (inside phloem at LPI 1 to 25), and roots were assayed for *NPT II* expression. ELISA tests demonstrate variable levels of *nos-NPT II* expression among organs and among three different transgenic lines (Table 3). Measured *NPT II* expression of all transgenic lines ranged from 238 to 11,963pg NPT II protein per mg of leaf protein ;

from 2,862 to 11,577pg per mg of petiole protein ; from 319 to 5,123pg per mg of outer stem protein ; from 0 to 6,225pg per mg of inner stem protein ; and from 2,955 to 7,398pg per mg of root protein.

Quantification of NPT II was obtained by comparison to standard curve readings, in which purified NPT II was diluted in leaf(LPI 11-15) extract(1μg protein/ml) of untransformed control 'Hansen'. Overall MRPP comparison(Mielke et al., 1981 ; Mielke, 1984) of all tissues showed significant($p>0.046$) differences for the NPT protein among three transgenic lines. Because the absolute values of NPT II protein differ significantly in independent NPT II assays, organ specific NPT levels among different transgenic lines cannot be compared directly. Therefore, we conducted a separate Duncan's Multiple Range Test to determine statistical differences among various organs for each transgenic line.

The Tr15 line demonstrated more stable and consistent expression of the inserted *nos-NPT II* gene in various organs than the Tr345 or Tr665 line. NPT II protein concentrations in various organs of the Tr15 line ranged from 2,955 to 11,963pg/mg tissue. A Duncan's Multiple Range Test determined that *nos-NPT II* expression in 8 different tissues of Tr15 was significantly different(alpha=0.05). Tr665 line demonstrated no significant difference among 8 different tissues of each line by Duncan's Multiple Range Test. This is perhaps attributable to the very high variation in NPT II protein among organs and replications. With Tr345, a significant difference (Alpha=0.05) between root and all other tested organs was shown.

For the transgenic 'Hansen' Tr15 line, leaves from LPI 21 to 25 and petioles were similar in NPT II protein content. Also for Tr15, leaves at LPI 11 to 15, inner stems, and outer stems exhibited similar NPT II content, as did outer stems, stem tips, leaves at LPI 1 to 5, and roots. Among 8 different organs tested for the Tr15 line, leaves from LPI 21 to 25 and petioles yielded the highest NPT II protein content with 11,963 and 11,577pg NPT II protein per mg tissue protein, respectively. Leaves at LPI 1 to 5 and root tissue demonstrated the lowest NPT II protein among 8 organs tested for Tr15 line, with 3,098 and 2,955pg NPT II protein per mg tissue protein, respectively.

Previous methods to assay for enzyme activity of NPT II involved nondenaturing polyacrylamide-gel electrophoresis and radioactive labeling of reaction products(Klopfenstein et al., 1991 ; Reiss et al., 1984). Using a radioactive assay, it was previously demonstrated that *nos-CAT* was expressed in an organ specific and developmentally regulated manner in transgenic tobacco(An et al., 1988). The *nos* promoter was most active in lower leaves, lower stems, and roots of transgenic tobacco plants. A simpler radioisotopic assay developed by McDonnell et al.(1987) was subsequently used to compare promoter effects on *NPT II* expression in callus and leaves of a transgenic poplar hybrid(Leple et al., 1992), embryos of transgenic *Citrus* embryos(Vardi et al., 1990), and shoots of transgenic Kiwi fruit (*Actinidia deliciosa*)(Uematusu et al., 1991).

In comparison to radioisotopic assays, ELISA quantification of NPT II expression is less tedious and involves fewer safety considerations. In previous work, ELISA was used to demonstrate *NPT II* expression in leaves of transgenic papaya(*Carica papaya* L.)(Fitch et al., 1993). Using ELISA, three transgenic plantlets of *Liriodendreon tulipifera* L. that contained a *nos-NPT II* gene were previously determined to contain NPT II levels ranging between 1,500 and 5,800pg/mg protein in the roots and between 1,400 and 4,500pg/mg protein in the leaves(Wilde et al., 1992). Our preliminary work indicates that *nos-NPT II* expression in transgenic poplar is similar to that in transgenic *L. tulipifera*.

Variability of *nos-NPT II* gene expression among transgenic poplar lines may indicate a positional effect of gene insertion on foreign gene expression (Peach and Velten, 1991). Such variability in gene expression levels demonstrates that obtaining an optimum level of gene expression may require screening of several lines of transgenic poplar. Because stability of the *NPT II* gene product in *Populus* tissue is not established, it is unknown whether higher NPT II levels in older tissue represent higher gene expression levels or merely reflect NPT II accumulation. Although general differences were noted among *nos-NPT II* expression levels in various poplar organs, the *nos* promoter displayed

the general capacity to promote gene expression in all organs tested. The *nos* promoter could be effective with foreign genes where general, constituative expression is warranted.

Additional information is needed regarding environmental influences on the regulation by the *nos* promoter in woody plants. Factors such as plant water status, wounding, plant growth regulator status, or other environmental and developmental conditions may profoundly affect *nos* promoter function in woody plants. Because genetic engineering of trees has occurred relatively recently, mature transgenic trees are unavailable. As plantings of transgenic trees are allowed to mature over time, continued studies are needed to evaluate transgene expression in mature woody plants under natural environmental conditions. Genetic manipulation that affects organ specific gene expression could provide increased pest resistance, higher stress tolerance, improved pulping characteristics, enhanced wood quality, or other beneficial features that would increase the value or survival of planted trees.

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