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Over-expression of tryptophan decarboxylase gene in poplar and its possible role in resistance against *Malacosoma disstria*

RISHI I.S. GILL[1,*] and BRIAN E. ELLIS[2]
*[1]Department of Forestry & N.R., Punjab Agricultural University, Ludhiana 141 001, India; [2]UBC Biotechnology Laboratory and Faculty of Agricultural Sciences, University of British Columbia, BC Vancouver V6T 1Z4, Canada; *Author for correspondence (e-mail: rishigill@rediffmail.com; rishipau@yahoo.com; phone: 098-148-13070; fax: +91-161-2400945)*

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Abstract. Amines and their derivatives are known to influence insect behavior involved in feeding and reproduction. In order to examine the feasibility of improving the resistance of poplar to insect pests by the introduction of a plant-derived amine-generating transgene, explants from the hybrid poplar clone 'INRA 717 1B4' (*P. tremula* × *P. albo*) were transformed with a *Camptotheca acuminata* tryptophan decarboxylase (TDC, EC 4.1.1.28) cDNA driven by the CaMV 35S promoter. The enzyme TDC catalyzes the decarboxylation of tryptophan to tryptamine, which, in addition to being a bioactive amine itself, is known to act as a precursor of various other indole derivatives. Putative transgenic lines were confirmed by PCR for the *TDC1* gene sequence and by the expression analysis of the transgene mRNA and encoded protein. No visible phenotypic changes were associated with ectopic *TDC1* expression. Chemical and radiotracer analyses of the transgenic plants revealed tryptamine accumulation as high as 4 mM in leaf tissue, and suggested that the tryptamine produced by ectopically expressed *TDC* was not further metabolized. Insect bioassays with the *TDC* transgenic plants showed that the tryptamine accumulation was consistently associated with adverse effects on feeding potential and physiology of *Malacosoma disstria* (forest tent caterpillar).

Abbreviations: 717 – Poplar hybrid 717 (*Populus tremula* × *P. alba*); FLAG – Bacterial flagellar protein; FTC – Forest tent caterpillar; MS – Murashige and Skoog medium; PCR – Polymerase chain reaction; TDC – Tryptophan decarboxylase gene; TLC – Thin layer chromatography

Introduction

Plants produce a rich diversity of secondary metabolites. These chemicals do not appear to be necessary for basic metabolism, but are thought to contribute to the plant's environmental fitness and adaptability. Consistent with this role, plants often accumulate specific secondary metabolites during the vulnerable stages of their life cycle (Thomas et al. 1995). The proposed protective roles for plant secondary metabolites have made them popular targets for metabolic

engineering. It is generally felt that the production of particular secondary metabolites could be enhanced greatly via genetic manipulation if a 'rate-limiting' enzyme in the biosynthetic pathway could be over-expressed (Berlin et al. 1993).

In order to examine the feasibility of improving the resistance of poplar to insect pests by increasing the plant's level of aromatic amines, we have tested the effectiveness of ectopically expressing an aromatic amino acid decarboxylase transgene. For this purpose, we used a *Camptotheca acuminata* Decne. tryptophan decarboxylase (*TDC*) cDNA that had been previously isolated and characterized by Lopez-Meyer and Nessler (1997). TDC catalyzes the decarboxylation of the amino acid L-tryptophan to form tryptamine. This bio-active amine and its metabolites have been shown to affect behavior, development and physiology in some insects (Vehovszky and Walker 1991; Csaba 1993; Thomas et al. 1998).

Given the centrality of indole metabolism for plant physiology, constitutive expression of high levels of TDC activity in transgenic plants has the potential to be detrimental to growth (Thomas et al. 1995; Yao et al. 1995). In an earlier study of *TDC* transgenic tobacco, blackening of wounded stems was observed *in vitro* and attributed to an alteration of secondary metabolism caused by the increased levels of TDC (Thomas et al. 1995). Likewise, in *TDC* transgenic potato plants, high levels of TDC caused an imbalance in metabolic flux within the shikimate and phenylpropanoid pathways, which was correlated with the increased susceptibility to *Phytophthora* infection (Yao et al. 1995).

On the other hand, *TDC*-transformed tobacco plants have been reported to appear morphologically normal, and to display levels of indole-3-acetic acid that did not differ significantly from controls (Songstad et al. 1990). *TDC*-transgenic *Petunia hybrida* Vilm. plants also did not show any morphological or growth abnormalities, despite accumulating 8-fold more tryptamine than wild-type plants (Thomas et al. 1999).

To establish whether protective levels of amine accumulation could be induced in woody perennials, the *TDC1* gene was introduced into hybrid poplar (*P. tremula* L. × *P. alba* L.) using an *Agrobacterium*-based transformation system.

Materials and methods

Plant material and growth conditions

Hybrid poplar, *Populus tremula* × *P. alba*, clone 'INRA 717 1B4' plants were maintained *in vitro* in Magenta boxes (Sigma) containing MS medium (Murashige and Skoog 1962). The shoots were incubated under a 16-h photoperiod of coolwhite fluorescent light (25–32 μmol/s/m^2) and 25 ± 1 °C unless otherwise mentioned.

TDC1 vector construction

The *Camptotheca acuminata TDC1* cDNA was kindly provided by Dr. Craig Nessler, Virginia Technical University, USA. The coding region of the *TDC1* gene was PCR-amplified, and *Bam*HI restriction enzyme sites were added to the 5′ and 3′ end of the *TDC1* cDNA sequence using *Bam*HI-*TDC1*-Forward (5′ CGGGATCCCAAATGGGTAGCCTTGATTCCAATTACGACACTG AA 3′) and *Bam*HI-FLAG-*TDC1*-Reverse (5′ CGGGATCCTCA CTTGTCA TCGTCGTCCTTGTAGTCATCCTCTTTCAGG AGAACATCCGCTCCT TC 3′) primers. The underlined sequences (GGATCC and CTTGTCATCGTC GTCCTTGTAGTC) are *Bam*HI cut site and FLAG sequence, respectively, in the forward and reverse primers. The reverse primer also incorporated a C-terminal FLAG epitope. The amplicon was ligated into a binary vector, Bin19/pRT101 (Ro et al. 2001), which contains an *npt II* selectable marker. The recombinant binary vector was used to transform competent *A. tumefaciens* (EHA105) cells by a freeze–thaw transformation procedure (Holsters et al. 1978).

Plant transformation and PCR analysis

A published method for *Agrobacterium*-mediated transformation of poplar hybrid 717 (Leple et al. 1992) was employed. After co-cultivation with *A. tumefaciens*, leaf disks were first cultured on MS medium without any antibiotics for 2 days and then transferred onto MS medium containing 500 mg/l cefotaxime and 100 mg/l kanamycin for the selection of transformants. Putative *TDC1* transgenics were detected using PCR and a forward primer specific to the CaMV 35S promoter sequence (ATGACGCACAATCC CACT) together with a gene-specific reverse primer (ACGCCTACGATCTCA AAGTG). The PCR-positive transgenic poplar plantlets were transferred to soil and grown in a growth chamber.

Northern blotting and RT-PCR analysis

Total RNA (8 μg) was resolved on 1% agarose–formaldehyde gels, blotted onto Hybond XL membrane (Amersham Pharmacia Biotech) using the capillary transfer method and probed as described previously (Orvar et al. 1997), using ^{32}P-labeled 1.35-kb fragment of the *TDC1* open reading frame (ORF) as probe. Hybridization signals were scanned and analyzed using the Storm 860 Phosphor Imager (Amersham Pharmacia Biotech) and ImageQuant software (Molecular Dynamics).

Gene-specific primers were used to amplify the ORF of *TDC1* (forward: 5′ GTTCTCAGCCAAGTTGATCC 3′; reverse: 5′ CCTCTTTCAGGAGAA-CATCC 3') using cDNA as the template. The cDNA was synthesized from

total RNA extracted from control and transgenic poplar lines, using a first-strand cDNA synthesis kit (Invitrogen). RT-PCR was performed using gene-specific primers designed to amplify *TDC1* (20 cycles). The number of cycles was adjusted so that the amplification was within the linear range. As an internal control, 18S ribosomal cDNA was amplified using a 1:1 ratio of 18S-specific primers to competimers mix (Ambion).

Protein extraction and Western blotting

Total protein extracts were prepared and aliquots (15 μg) were used for Western blotting as described earlier (Samuel et al. 2000). A primary antibody dilution of 1:5000 was used for anti-FLAG (Sigma), and of 1:8000 for mouse peroxidase-conjugated secondary antibody (DAKO Diagnostics).

Analysis of TDC enzyme activity

An assay for the enzyme *TDC* was performed, as reported earlier, based on direct fluorometry of the enzymatic reaction product (tryptamine) selectively removed in ethyl acetate from the reaction mix (Sangwan et al. 1998). Soluble proteins were extracted and the reaction mixture was incubated for 30 min in a shaking water bath (30 °C) and the reaction was terminated by the addition of 2.0 ml 4-N NaOH. The alkalinized reaction was extracted with 3.5 ml ethyl acetate, and the ethyl acetate phase was used directly to measure tryptamine fluorescence intensity in a LS50 Luminescence spectrometer, with excitation and emission wavelengths set at 280 and 350 nm, respectively. In the buffer control, all steps of the enzyme assay procedure were followed except that no crude homogenate protein was added to the reaction mixture.

Analysis of tryptamine and amino acids

Soluble metabolites were extracted from the fully expanded leaves (harvested from the top of 10-week-old plants) of each *TDC1* poplar line by grinding the tissue (~250 mg) to a fine powder in liquid nitrogen. Repeated extraction of the powder was carried out using 5 ml (twice) and then 2 ml 10 mM sodium acetate buffer (pH 6.42). Each time, the slurry was vortexed for 1 min at maximum speed and centrifuged (9300 × *g*; 4 °C; 10 min). The combined supernatants were transferred to a new tube and subjected to three rounds of freeze–thaw. After every thawing step, the solution was centrifuged (9300 × *g*; 4 °C; 10 min) and the supernatant was separated and filtered through 0.45-μm and 0.20-μm hydrophobic PTFE 4-mm Millex syringe filters before being sent to the Advanced Protein Technology Center, Sick Children's Hospital, Toronto, Canada, for amino acid and amine analysis.

^{14}C-tryptophan feeding experiments

L-(side chain-3-^{14}C)-Tryptophan (50 mCi/mmol) [NEN Life Science Products] was obtained in an ethanol:water (2:98) solution and used without radiochemical dilution. The ethanol was removed by passing a slow stream of argon through the solution for 30 min. One leaf each from a *TDC1* poplar transgenic line (# 12) and wild type poplar (10-week-old plant) was cut from the plant and the petiole immediately dipped into a solution of labeled tryptophan (3.2 μCi) in a 1.5-ml microfuge tube. The leaf was held in constant light at 24 °C until all of the solution was absorbed (~1 h). Distilled water was then added to the tube and transpiration allowed to continue for another 50 min.

Extraction and identification of indole compounds

The extraction and fractionation of indole compounds from the ^{14}C-tryptophan-fed leaves was performed by solvent partitioning as described earlier (Schneider et al. 1972). The recovery of radiolabeled tryptophan products in the different fractions (neutral, acidic, basic, butanol and aqueous) was monitored by scintillation counting. Each fraction was concentrated to dryness under a slow stream of argon, and subsequently redissolved in an equal volume of methanol (200 μl). One-third of each fraction (~65 μl) was then analyzed by thin layer chromatography (TLC).

TLC were run on microcrystalline cellulose plates (150-μm layer) and silica gel 60A-K6 plates (250-μm layer) using three different solvent systems: BAW [*n*-butanol:glacial acetic acid:water (60:15:25 v/v)], IAW [isopropanol:concentrated ammonia:water (8:1:1 v/v)], and AA [acetone:concentrated ammonia (100:1 v/v)]. Distribution of radioactive products on the developed chromatograms was analyzed using a Storm Phosphoimager 860 (Amersham Pharmacia Biotech) and ImageQuant software (Molecular Dynamics).

To detect indole compounds on the TLC plates, DMAC [*p*-dimethylaminocinnamaldehyde] (0.1 g) was dissolved in 10 ml concentrated HCl, and diluted to 200 ml with acetone immediately before use. The developed chromatograms were dipped quickly in this solution, followed by brief air drying and then heating for 2.5 min in an oven (65 °C).

Insect material

Egg bands of the forest tent caterpillar (FTC), *Malacosoma disstria* Hub., (Lepidoptera, Lasiocampidae) were kindly provided by Dr. Bob McCron, Insect Production Unit, Canadian Forest Service, Sault Ste. Marie, Ontario. Eggs hatched in 4–5 days and the neonates were maintained for about 10 days on an artificial diet [(No. 9795, BioServe Inc., Frenchtown, NJ) supplemented with finely ground alfalfa to improve acceptability, and with a vitamin mix (No. 8045, BioServe Inc., Frenchtown, NJ) for added nutritional value

(Bomford and Isman 1996)]. They were then fed cut leaves of wild type poplar 717 for the remainder of their larval development.

Nutritional analysis

This bioassay was carried out in order to differentiate behavioral feeding responses from any toxic effects resulting from the activity of TDC. Early 4th instar larvae of *M. disstria* were used to study the effect of *TDC1* expression in poplar plants on nutritional indices. On the day of the consumption and growth assay, early 4th instar larvae were selected and starved for 4 h. Larvae and leaf material were weighed and one larva was placed in a Petri dish along with a known amount of leaf material from transgenic or control poplars. Larvae were then allowed to feed on leaves of transgenic and control plants for 4 days. More leaf material was added when larvae had consumed about 70% of the initially added leaves. At the end of the experiment, the larvae were weighed, and the residual leaves and frass were dried at 60 °C for 24 h and also weighed. Twelve replications were performed with each genotype. All nutritional indices were calculated on a dry weight basis. To determine the fresh weight:dry weight ratios, 15 leaf samples (ca. 1.2 g each) and 15 early 4th instar larvae were dried for 24 h at 60 °C and weighed.

Once dry weights had been determined for larvae, diet and frass, and initial dry weights calculated for larvae and diet, nutritional indices were calculated using the following formulae (Farrar et al. 1989): Relative growth rate (RGR_i = weight gained (mg)/initial larval weight (mg)/number days), Relative consumption rate (RCR_i = leaf consumed (mg)/initial weight (mg)/number days), Efficiency of conversion of ingested food (ECI = [weight gained (mg)/leaf ingested (mg)] × 100), Efficiency of conversion of digested food (ECD = [weight gained (mg)/leaf ingested−frass (mg)] × 100) and Approximate digestibility (AD = [leaf ingested−frass (mg)/leaf ingested (mg)] × 100).

Results and discussion

A gene construct containing the *C. acuminata TDC1* gene was successfully introduced into poplar hybrid 717 plants. Out of 178 poplar leaf disks co-cultivated with *A. tumefaciens* carrying the *TDC1* plasmid, a total of 15 PCR-positive lines were recovered (Figure 1). A total of four poplar lines were recovered from leaf disk co-cultivation with *A. tumefaciens* carrying an empty vector pBin19/PRT101, and these served as control plants for the subsequent analysis.

TDC1 *gene expression*

Through RT-PCR analysis, expression of the *TDC1* gene was confirmed in poplar hybrid. The level of gene expression varied among the different

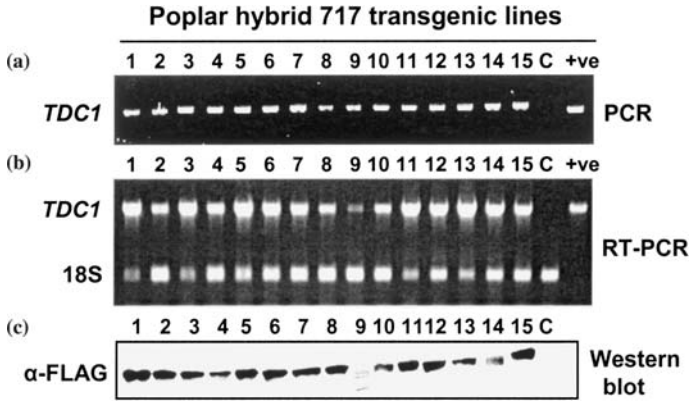

Figure 1. PCR and expression analysis of transgenic poplar hybrid 717 lines. Positive (+ve) line represents results of PCR and RT-PCR using *TDC1* plasmid DNA as a template in the reaction mix. (a) Genomic DNA extracted from the 15 transgenic and control (Wt, wild type) poplar lines was used as a template in PCR with 35S-forward and *TDC1* gene-specific reverse primers. (b) RT-PCR was performed with RNA extracted from the young leaves of transgenic and control (Wt) poplar lines. The upper and lower bands correspond to the *TDC1* and 18S rRNA genes, respectively. (c) SDS-PAGE and Western blot analysis were performed with proteins extracted from the leaves of transgenic and control (Wt) poplar plants. Each lane contained 15 μg total protein. The membrane was probed using anti-Flag antibody.

transgenic lines (Figure 1), a phenomenon that may be due to position effects or different gene copy number, or both. Generation of a range of expression levels in a series of transgenic lines provides useful information on the effects of different levels of gene expression in an isogenic background on a particular phenotype (Gelvin 1998). In the extreme case, there can be an apparent absence of transgene expression.

Northern blot analysis of a subset of poplar transgenic lines yielded an overall expression pattern similar to that obtained by RT-PCR (Figure 2). The highest gene expression was detected in transgenic line # 1 and the lowest in line # 9. No transcript was detected in total RNA isolated from non-transformed control plants.

TDC protein expression

A prominent reactive band (~55 kDa) was detected by anti-FLAG immunoblotting in almost all of the transgenic poplar lines, while no protein of this size was detected in extracts from non-transgenic control plants (Figure 1). The pattern of differential accumulation of TDC–FLAG protein was generally correlated with the relative levels of the *TDC–FLAG* mRNA in these same lines (Figure 1b). However, there were some exceptions (e.g. lines # 12 and 15) in which only a modest level of transcript was detected by RT-PCR, but the

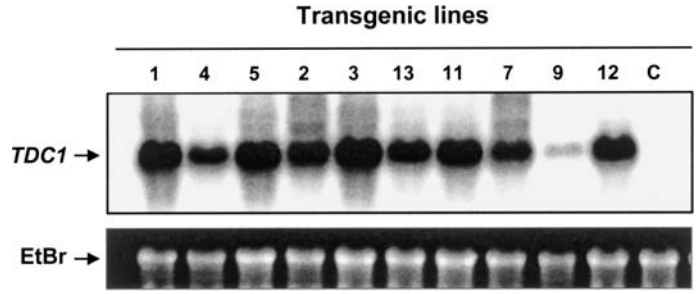

Figure 2. Northern blot analysis of *TDC1* poplar hybrid 717 lines. Leaf RNA (8 μg) was analyzed from transgenic and control poplar plants. RNA blots were probed with a ^{32}P-labeled 1.35 kb *TDC1* gene fragment.

protein levels were as high as in other lines. This suggests that, in some cases, post-transcriptional mechanisms could increase the stability or translational activity of the transgene mRNA.

Enhanced TDC enzyme activity in transgenic lines

TDC activity ranged from 12.5 to 45.5 pkat/mg protein in TDC1 transgenic poplar lines (Figure 3). These values are lower than those detected in earlier *TDC*-tobacco studies (19.03–69.2 pkat/mg protein) (Songstad et al. 1990; Goddijn et al. 1993; Poulsen et al. 1994). Such variations in enzyme activity could be due to the species differences, or to the position or age of leaf tissue used for the TDC activity assay. TDC enzyme activity can vary among the leaves of the same plant, as shown in an earlier tobacco study where the highest levels were detected at the shoot tip and the lowest near the shoot base (Songstad et al. 1990).

Since wild-type hybrid poplar plants are believed to lack a *TDC* gene, neither TDC activity, nor tryptamine-derived secondary products are expected to occur in wild-type plants. As anticipated, no *TDC* gene expression could be detected in wild type poplar hybrid 717 plants. However, a basal level of apparent TDC enzyme activity was detected in the leaf tissues of wild type poplar plants (Figure 3), and a very small peak eluted in the amino acid analysis profiles at the position expected for tryptamine. The exact identity of the metabolites involved in this case was not pursued further.

Amino acid and amine profile in transgenics

Tryptophan and phenylalanine are both biosynthetically derived from chorismate through a branch point controlled by the key enzymes anthranilate synthase (AS) and chorismate mutase (CM). Chorismate is either converted to

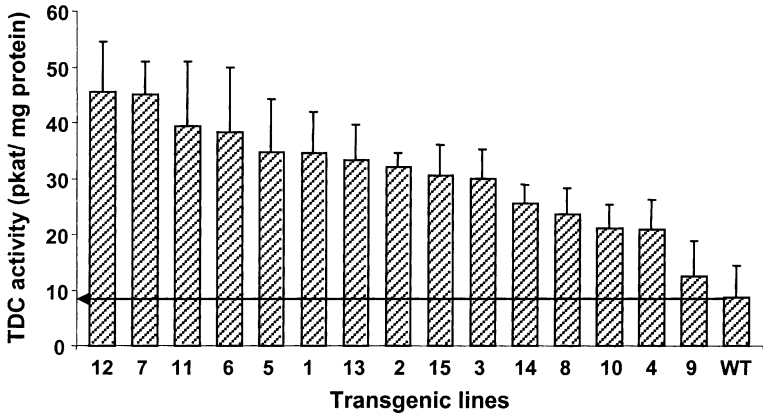

Figure 3. TDC activity in the leaves of hybrid poplar transgenic lines. A direct fluorometry procedure was used to measure the catalytic activity of 20 μg crude homogenate protein. Bars represent the means ± SE of three experiments.

anthranilate, the precursor to tryptophan, or to prephenate, which can be converted to phenylalanine and tyrosine (Yao et al. 1995). In a number of cases, ectopic TDC expression in transgenic plants (canola, potato or tobacco) has been reported to drastically reduce the levels of tryptophan and phenylalanine accumulated in the transgenic tissues (Chavadej et al. 1994; Yao et al. 1995; Guillet et al. 2000). Where a more detailed analysis was reported, the accumulation of other, non-aromatic amino acids was largely unaffected (Yao et al. 1995). However, this impact of ectopic TDC expression is clearly not consistent, since other studies have found no change in the size of the tryptophan pool in TDC-transgenic petunia or tobacco (Thomas et al. 1995, 1999), The over-expression of a *C. roseus TDC* cDNA in tobacco was also reported to have no effect on the specific activity of AS and CM enzymes (Poulsen et al. 1994).

In the present study, the mean amount of each amino acid accumulated in the different *TDC1* expressing groups was compared with the mean amount accumulated in untransformed control plants. Several of the amino acids (serine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, cysteine, isoleucine, leucine, phenylalanine, tryptophan) showed a similar trend of accumulation; namely, a higher level of the amino acids in plants belonging to the high *TDC1* expression group, lower levels in low expression groups and still lower accumulation in untransformed control (Figure 4). All the aromatic amino acids (tyrosine, phenylalanine and tryptophan) displayed this general trend of accumulation. In contrast, a reverse trend was observed in accumulation of aspartate, glutamate, asparagine and glutamine. However, the amount of these amino acids accumulated was significantly greater than that of untransformed control plants, except in the case of asparagine.

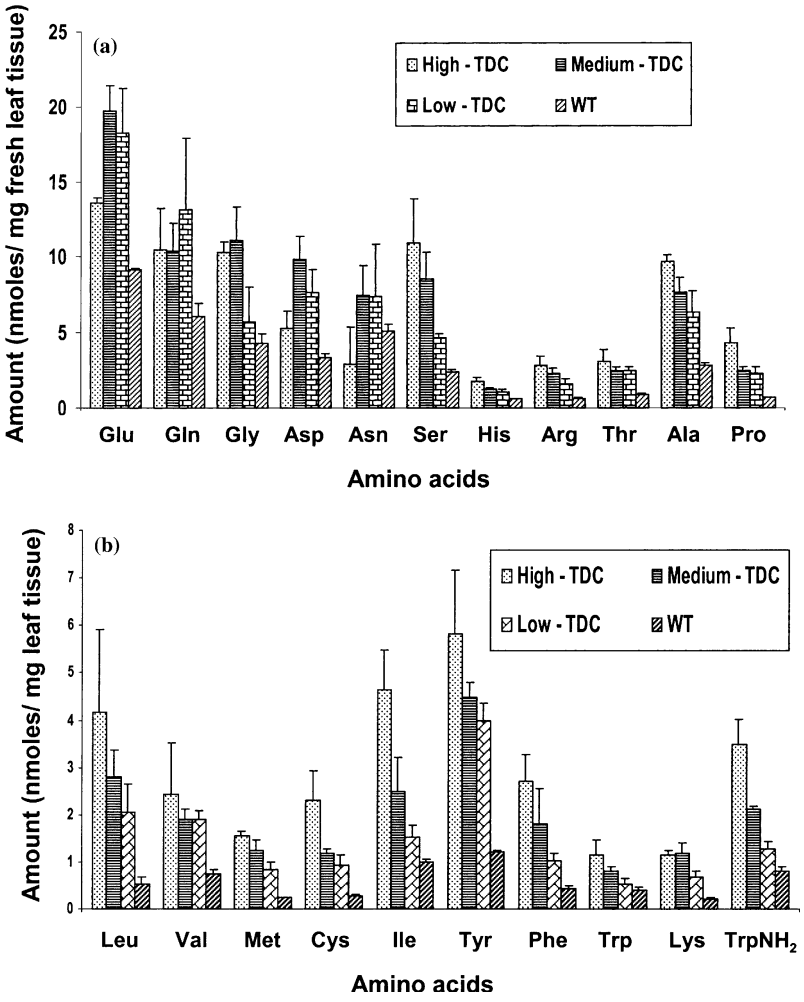

Figure 4. Analysis of amino acids and amine in wild type and *TDC1* over-expressing hybrid poplar lines. Soluble metabolites were extracted from fully expanded leaves of high, medium and low-expressing (defined on the basis of the level of tryptamine accumulation) *TDC1*- transgenic poplar hybrid lines. Amino acids and amines were converted to phenylisothiocyanate derivatives and analyzed by reversed-phase HPLC with UV detection. Peak areas were quantified and are shown in the clustered bar diagram along with the corresponding values for their respective controls. Values represent the means ± SE from analysis of the triplicate samples.

The tryptamine accumulating in the various *TDC1* transgenic poplar lines ranged from ~1 to ~4 mM (Figure 4). This is somewhat lower than the levels reported for transgenic tobacco lines over-expressing *TDC*, which were in the range of ~8 mM tryptamine (Songstad et al. 1990). Since the levels of TDC activity, and of tryptamine accumulation, were lower in transgenic poplar than in transgenic tobacco plants, the relative lack of impact on amino acid

metabolism in poplar may simply reflect a lower flux through the newly created branch pathway in this species. However, since different indirect homeostatic mechanisms become involved in different plants, when attempts are made to perturb cell physiology (Westerhoff 1995), it is difficult to assign cause-and-effect relationships. Even similar levels of expression of the same heterologous gene will very likely generate different endogenous metabolic profiles in divergent genetic backgrounds.

Identification of indole compounds

Tryptophan is the metabolic precursor of many different indole compounds in plants, so over-expression of TDC has the potential to broadly affect the indole metabolite composition of the plant. In order to establish the extent of change in the profile of indole compounds in *TDC1*-transgenic poplars compared to wild type plants, the extractable indole derivatives were compared using chromatography, chromogenic reactions and radiotracer studies. Autoradiography of chromatographically resolved extracts from *TDC1*-transgenic poplar leaves fed ^{14}C-tryptophan revealed a newly labeled product that could be tentatively identified as tryptamine. A 30-fold higher percentage of recovered label was found in the 'basic' fraction of transgenic line extracts, in comparison to the 'basic' fraction of wild type plant extracts (data not shown). The indole compounds expected to be recovered in this basic 'fraction' are tryptamine and *N*-methyltryptamine (Schneider et al. 1972). Analysis by TLC in three different solvent systems demonstrated that no label from tryptophan was incorporated into compounds that had R_f values corresponding to either 5-hydroxytryptamine or *N*-methyl tryptamine (data not shown), whereas heavy labeling was associated with tryptamine itself.

When sprayed with the indole-reactive reagent, DMAC, tryptamine generates a distinctive blue-purple reaction product (Schneider et al. 1972). In all three solvent systems, the new product detected in the basic fraction of transgenic line extracts reacted with DMAC exactly as tryptamine (data not shown). These results are in agreement with several previous reports on the over-expression of *TDC* in herbaceous crops, which demonstrated an increase in accumulation of tryptamine in transgenic lines without further metabolism (Songstad et al. 1990; Poulsen et al. 1994; Goddijn et al. 1995). Even in poplar transgenic lines that were accumulating relatively large amounts of tryptamine, the plants appeared to grow normally and no obvious morphological changes were observed in leaves or stem.

Nutritional analysis

The RGR_i, ECI and ECD of FTC larvae feeding on the leaves of high *TDC1*-expressing poplar line (# 12) were significantly reduced in comparison to larvae

feeding on leaves of empty vector control (EV) line (Table 1). However, no significant reductions in the RCR_i and AD were observed. The ECI is an overall measure of an insect's ability to utilize food that is ingested for growth and it is a product of both the AD and ECD. The AD represents the portion of food assimilated, and ECD reflects the efficiency with which the assimilated food is converted to body substance. Parallel differences in reduction of ECI and in the growth rate of the insects, suggests interference with the physiology of food conversion as a mechanism of action.

While the FTC larvae feeding on high *TDC1* expression poplar plants showed a significantly lower ECD, no differences were observed in the AD between the same larvae. This demonstrates that the reduction in ECI was due to a significantly lower efficiency of conversion of the digested food to body tissues i.e. growth, rather than to problems with digestion and absorption from the gut. The lower growth rate of FTC larvae feeding on high *TDC1* plants may therefore be due primarily to a higher metabolic cost encountered when feeding on leaves containing elevated levels of tryptamine. The development times and growth rates of gypsy moth larvae feeding on a diet containing phenolic glycosides were also strongly affected, consistent with the higher metabolic cost associated with dealing with toxic metabolites (Hemming and Lindroth 2000).

In addition to toxic metabolites, physical factors and bioavailability of plant nutrients can affect the growth rate of insects. Comparative behavioral studies of *Chrysomela scripta* (Coleoptera: Chrysomelidae) adults feeding on mature and immature leaves of *Populus* × *euramericana*, illustrated that low nitrogen content and high amounts of fiber and tannins in mature leaves resulted in longer developmental times and lower prepupal weights in larvae feeding on mature leaves (Harrell et al. 1982). Eastern tent caterpillars were shown to grow faster and process food stored in their gut more rapidly at high temperatures (35 °C) as compared to larvae maintained at lower temperatures (20 °C) (Schroeder and Lawson 1992).

In summary, stable *Agrobacterium*-mediated transformation was achieved in hybrid poplar using a heterologous *TDC1* gene, with recovery of a large number of transgenic lines. Their transgenic status was confirmed on the basis

Table 1. The effect of *TDC1* over-expression on nutritional indices of forest tent caterpillar larvae after feeding for 4 days on the leaves of poplar *TDC1* transgenic and control plants.

Transgenic plants	RGR_i (mg/mg/day)	RCR_i (mg/mg/day)	ECI (%)	ECD (%)	AD (%)
High-*TDC*	0.21^{a}	2.70^{a}	8.33^{a}	13.87^{a}	64.03^{a}
Control	0.37^{b*}	2.93^{a}	14.09^{b}	24.70^{b}	65.20^{a}

Each value represents mean of 12 replications.

*Means followed by the same letters within columns do not differ significantly ($p > 0.05$) in LSD test.

Abbreviations: RGR_i, Relative growth rate; RCR_i, Relative consumption rate; ECI, Efficiency of conversion of ingested food; ECD, Efficiency of conversion of digested food; AD, Approximate digestibility.

of the presence of the inserted *TDC1* gene sequence, expression of transgene mRNA, expression of the encoded protein, activity assays for TDC function, and metabolite analysis. Amino acid and amine analysis of the transgenic lines revealed accumulation of up to 4 mM tryptamine with no change in the size of the endogenous tryptophan pool, and no reduction in the level of other aromatic amino acids. No visible phenotypic changes were associated with this accumulation of tryptamine. It is clear from the experimental data that the elevated levels of tryptamine accumulated in the *TDC1* transgenic poplar leaf tissues are capable of reducing the growth rate of FTC. The physiological studies suggest that the origin of this effect is probably a form of chronic toxicity, at least under laboratory conditions. Although the inhibition is not dramatic, small differences in feeding rates could have important ecological consequences. Whether *TDC* should be considered for development as a potential insect resistance gene will depend upon several factors, including its performance under field conditions and long-term stability of transgene expression. Finally, the ecological consequences of adding *TDC* to the array of genetic defences available in the poplar genome need to be carefully examined. Elevated tryptamine levels can be expected to have positive and/or negative impacts on other organisms that depend on their interactions with poplar to successfully complete their life cycle.

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