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Rapid Communication

Tryptophan Decarboxylase, Tryptamine, and Reproduction of the Whitefly[1]

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Tryptophan decarboxylase (TDC) from *Catharanthus roseus* (periwinkle) converts tryptophan to the indole-alkaloid tryptamine. When the TDC gene was expressed in transgenic tobacco, the 55-kD TDC enzyme and tryptamine accumulated. *Bemisia tabaci* (sweetpotato whitefly) reproduction on transgenic plants decreased up to 97% relative to controls. Production of tryptamine, its derivatives, or other products resulting from TDC activity may discourage whitefly reproduction and provide a single-gene-based plant protection strategy.

The process by which insects select a host plant for feeding and reproduction begins with the sensing of structural and metabolic components of the plant. Alkaloids are one of several plant-produced substances known to greatly influence insect recognition, feeding, and oviposition. Levels of the alkaloid gramine are inversely related to the extent of aphid infestation (Zuñiga et al., 1988). Furthermore, polyphagous aphids colonize only low-alkaloid-producing plants, whereas aphids with restricted host specificity prefer high-alkaloid producers, using the ingested alkaloids in their own defense (Niemeyer, 1990).

It has been difficult to examine how alkaloids affect insect feeding, because to do so host plant lines must differ only in alkaloid content. Testing alkaloids in an artificial insect diet provides some information, but not all behavioral aspects of plant-insect interactions in situ can be reproduced exactly.

To directly assess insect feeding on alkaloid-containing plants, we transferred and expressed in *Nicotiana tabacum* (tobacco) the gene encoding the TDC enzyme (EC 4.2.1.27) of *Catharanthus roseus* (L.) (periwinkle). In several species with TDC activity (Robinson, 1979), tryptamine is thought to participate in the protection of young seedlings against insects, particularly in newly emergent seedling stems and cotyledons (McKenna et al., 1984; De Luca et al., 1988; Aerts et al., 1991; Bracher and Kutchan, 1992). *N. tabacum* was chosen for transformation because it does not contain significant TDC activity, providing a background for producing and testing the effects of tryptamine and tryptamine-based alkaloids on insect reproduction.

MATERIALS AND METHODS

Transgenic Plants

A full-length TDC cDNA from *Catharanthus roseus* (L.) was placed under control of the cauliflower mosaic virus 35S promoter, transferred into *Agrobacterium tumefaciens*, and transformed into tobacco (*Nicotiana tabacum* cv SR1) (Horsch et al., 1985; Songstad et al., 1990). Twenty plants were regenerated and self-pollinated, and progeny plants appeared normal. All subsequent analysis was performed using T_1 generation plants.

Tryptamine Isolation

Samples were ground in ice-cold 100 mM Tris-HCl, pH 8.0, 100 mM NaCl, 20 mM EDTA, 10 mM DTT. Following centrifugation, the supernatant was extracted for 2 to 3 h at 4°C with 5 volumes of methanol:chloroform:water (4:5:1), with 100 μM norleucine added as internal control. After a second extraction of the supernatant (with 0.75 volume of chloroform), samples were dried and the material was suspended in 0.5% HCl in absolute methanol overnight at 4°C. The aqueous extract was separated from particles with centrifugation, the samples were dried, dissolved in 70% methanol, passed through a SepPac C_{18} cartridge (Millipore), and subjected to amino acid analysis using a Beckman 7300 amino acid analyzer (ninhydrin method) at the Biotechnology Core Facility, University of Arizona (Tucson). Phloem-derived amino acids from single leaves (6 weeks old) were isolated according to King and Zeevaart (1974). Samples were recovered and the volume was recorded, dried, extracted as above, and subjected to amino acid analysis.

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Abbreviation: TDC, tryptophan decarboxylase.

Protein Analysis

Antibodies against TDC were a gift of V. DeLuca (Institute Botanique, Université de Montreal, Canada). Six-week-old leaves from 35S-*uidA* and 35S-TDC tobacco transformants were extracted in 100 mM Tris-HCl, pH 8.0, 100 mM NaCl, 20 mM EDTA, 10 mM DTT. Following centrifugation, 30 μg of total protein/lane (Ghosh et al., 1988) were separated by 12.5% SDS-PAGE, electroblotted to Hybond N^+ (Amersham), and incubated in primary TDC-antiserum (1:2000) followed by secondary goat anti-rabbit antiserum conjugated to peroxidase. Peroxidase development was with an ECL detection system as specified by the manufacturer (Amersham).

Whitefly Tests

Reared on *Gossypium hirsutum* L., adult whiteflies (*Bemisia tabaci*) were collected and 10 pairs were placed in clip cages on the third leaf from the apex of 6-week-old kanamycin-resistant plants grown at 25°C in 16 h of light at 250 μmol m^{-2} s^{-1}. Feeding adults were observed during the initial 3 d, and mortality (due to handling) did not exceed 10% of the insects per clip cage. Based on initial observations of courtship and feeding behavior, adults reacted similarly when placed on either the control or TDC host. Leaves were not significantly damaged. After 30 d, leaves were scored for hatched pupal cases.

RESULTS AND DISCUSSION

Trp levels were similar in whole-leaf extracts of TDC and control plants and tobacco previously transformed with the *uidA* (GUS) gene (Jefferson et al., 1987) (Fig. 1). In phloem extracts, concentrations of Trp and tryptamine in TdC-7 were 6.4 and 2.8 μg/g fresh weight, respectively. The presence of tryptamine in phloem was expected because the cauliflower mosaic virus 35S promoter is active in phloem-associated tissue, albeit less strongly than in other tissues (Benfey et al., 1990). In leaves of some transformants, tryptamine levels exceeded those of Trp (Fig. 1), suggesting great synthetic flexibility in amino acid biosynthesis.

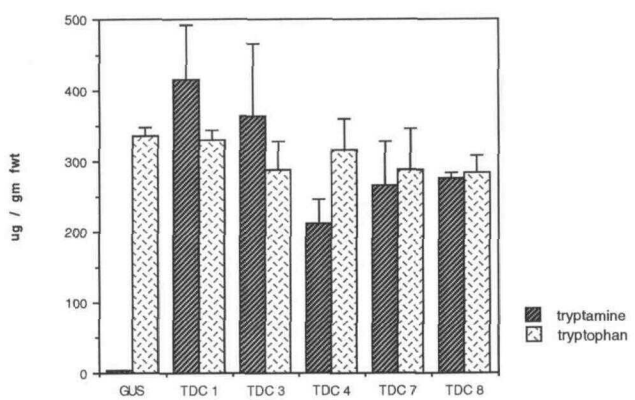

Figure 1. Amounts of tryptamine and Trp in TDC-expressing and control (35S-*uidA*-expressing) tobacco. fwt, Fresh weight.

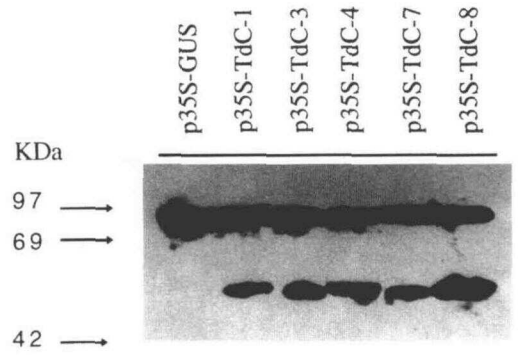

Figure 2. Immunological detection of TDC protein in transgenic tobacco. TDC protein of the apparent molecular weight 55,000 was observed only in 35S-TdC transformants and not in 35S-GUS controls.

TDC expression was demonstrated immunologically with antisera raised against purified TDC (Fernandez et al., 1989) (Fig. 2). A 55-kD protein reacted with the TDC antibody only in TDC-transformed plants. The antiserum also recognized a larger protein of an apparent molecular mass of 80 kD, perhaps a glycosylated form of TDC. More likely, this band represents an endogenous plant peroxidase reacting with the peroxidase substrates, since control (GUS) plants also contained the 80-kD band (Fig. 2). TDC expression in tobacco did not alter growth rate. However, blackening of wounded stems of TDC transformants was observed in vitro (not shown), suggesting an altered secondary metabolism compared to controls. Seeds from TDC-expressing plants contained a smaller protein (30 kD), which reacted with the TDC antibody, perhaps a result of proteolysis in this tissue.

B. tabaci, the sweetpotato whitefly, was used to test the effect(s) of tryptamine on insect feeding and development. The whitefly, like the aphid, uses a stylet to pierce phloem cells and obtain carbohydrates and amino acids from the vascular system of the host plant. The natural resistance of this insect to many commonly used pesticides and the widespread crop damage caused by whitefly (Henneberry and Toscano, 1993) justified the use of this insect model.

Tryptamine-containing plants and nonexpressing control (GUS) plants were compared in whitefly emergence tests (Smith, 1989) (Fig. 3). Detectable TDC and tryptamine in tobacco plants was coincident with a decrease in whitefly pupae emergence, as much as 97% compared to control plants (Fig. 3).

The mechanism by which tryptamine may act against insects is unknown. Perhaps TDC mediated a decrease in essential amino acids, such as Trp, needed for insect yolk production and oogenesis (Chapman, 1969). Arguing against this hypothesis, Trp levels did not decrease in leaves containing TDC activity (Fig. 1).

Although this is preliminary, we suggest that tryptamine may exert anti-whitefly effect(s) during either larval and pupal development and/or adult selection of a leaf for feeding and oviposition. Tryptamine has been reported to inhibit development in planaria and *Tetrahymena pyriformis*

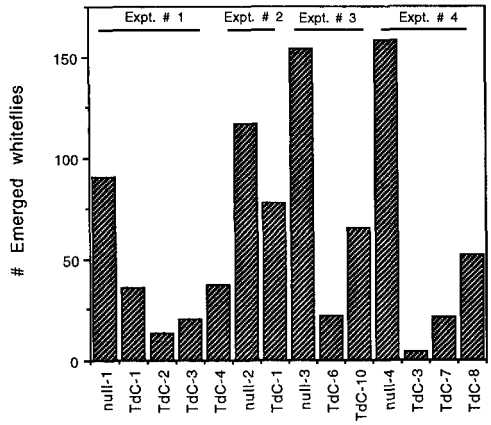

Figure 3. Whitefly emergence on transgenic tobacco expressing TDC or control (35S-*uidA*-expressing). *B. tabaci* whiteflies were collected from cotton plants and placed on 6-week-old tobacco plants. The number of empty pupal cases after 30 d is shown for control and TDC-expressing plants. null, Control (35S-*uidA*) plants; Tdc (No.), kanamycin-resistant plants from different transformation events after selfing. In null plants no tryptamine could be detected. All plants labeled Tdc contained tryptamine. Results are from four independent experiments.

(Csaba, 1993), to block neuromuscular transmission and glutamate transmission on the postsynaptic nerve in insects (Piek, 1985), and to inhibit serotonin-mediated brain monoamine oxidase stimulation in the brown snail *Helix aspersia* (Vehovszky and Walker, 1991). Other indole-alkaloid derivatives of tryptamine may affect whitefly feeding (Zuñiga et al., 1988; Niemeyer, 1990). Surface glucosinolate concentrations, which greatly influence insect host selection (Niemeyer, 1990), decreased dramatically when TDC was expressed and tryptamine accumulated in transgenic plants (Chavadej et al., 1994). Tryptamine and tryptamine-derived alkaloids, when expressed in plants, may act as anti-oviposition and anti-feedant agents and/or inhibitors of larval and pupal development, providing an example of a single-gene-based approach to further our understanding of plant-insect interactions.

Clearly, more study is needed to determine the exact mechanism of tryptamine action on whitefly reproduction. Detailed studies of the feeding and mating behavior of whiteflies on control and tryptamine-containing plants and artificial feeding tests using larvae and pupae will help determine how tryptamine may affect reproduction. Expression of the TDC gene in a strong and tissue-specific manner may also help determine where these insects obtain feeding and oviposition cues. Making plants less acceptable for feeding and interfering with insect development should slow insect population growth. Plant biosynthesis and deposition of epicuticular waxes and *trans*-aconitic acid deter insect feeding (Woodhead and Padgham, 1988; Eigenbrode and Espelie, 1995). Toxic constituents of glandular trichomes also adversely affect feeding, growth, and survival of insects (Jackson et al., 1986). Thus, combinations of TDC, *Bacillus thuringiensis* toxins (Perlak et al.,1990), protease inhibitors (Hilder et al., 1987; Johnson et al., 1989), and perhaps genes that affect surface structure and composition (Shade et al., 1994) will confer significant insect protection and further our understanding into the relationships of plants with their associated insects.

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LITERATURE CITED

Aerts RJ, Snoeijer W, Aerts-Teerlink O, van der Meijden E, Verpoorte R (1991) Control and biological implications of alkaloid synthesis in *Cinchona* seedlings. Phytochemistry **30:** 3571–3577

Benfey PN, Ren L, Chua N-H (1990) Tissue-specific expression from CaMV 35S enhancer subdomains in early stages of plant development. EMBO J **9:** 1677–1684

Bracher D, Kutchan TM (1992) Strictosidine synthase from *Rauvolfia serpentina*: analysis of a gene involved in indole alkaloid biosynthesis. Arch Biochem Biophys **294:** 717–723

Chapman RF (1969) The Insects: Structure and Function. The English Universities Press, London

Chavadej S, Brisson N, McNeil JN, DeLuca V (1994) Redirection of tryptophan leads to production of low indole glucosinolate canola. Proc Natl Acad Sci USA **91:** 2166–2170

Csaba G (1993) Presence in and effects of pineal indoleamines at very low level of phylogeny. Experientia **49:** 627–634

De Luca V, Alvarez-Fernandez F, Campbell D, Kurz WGW (1988) Developmental regulation of enzymes of indole alkaloid biosynthesis in *Catharanthus roseus*. Plant Physiol **86:** 447–450

Eigenbrode SD, Espelie KE (1995) Effects of plant epicuticular lipids on insect herbivores. Annu Rev Entomol **40:** 171–194

Fernandez JA, Owen TG, Kurz WGW, De Luca VD (1989) Immunological detection and quantitation of tryptophan decarboxylase in developing *Catharanthus roseus* seedlings. Plant Physiol **91:** 79–84

Gosh S, Gepstein S, Heikkila JJ, Dumbroff EB (1988) Use of a scanning densitometer or an ELISA plate reader for measurement of nanogram amounts of protein in crude extracts from biological tissues. Anal Biochem **169:** 227–233

Henneberry TJ, Toscano NC (1993) First Annual Review of the 5-Year National Research and Action Plan for Development of Management and Control of the Sweetpotato Whitefly. USDA-ARS Bulletin

Hilder VA, Gatehouse AMR, Sheerman SE, Barker RF, Boulter D (1987) A novel mechanism of insect resistance engineered into tobacco. Nature **330:** 160–163

Horsch RB, Fry JE, Hoffmann NL, Eicholtz D, Rogers SG, Fraley RT (1985) A simple and general method for transferring genes into plants. Science **227:** 1229–1231

Jackson DM, Severson RF, Johnson AW, Herzog GA (1986) Effects of cuticular duvane diterpenes from green tobacco leaves on tobacco budworm (Lepidopera:*Noctvidae*) oviposition. J Chem Ecol **12:** 1349

Jefferson RA, Kavanaugh TA, Bevan MW (1987) GUS fusions: β glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J **6:** 3903–3907

Johnson R, Narvaéz J, An G, Ryan CA (1989) Expression of proteinase inhibitors I and II in transgenic tobacco plants: effects on natural defense against *Manduca sexta* larvae. Proc Natl Acad Sci USA **86:** 9871–9875

King RW, Zeevaart JAD (1974) Enhancement of phloem exudation from cut petioles by chelating agents. Plant Physiol **53:** 96–103

McKenna DJ, Towers GHN, Abbott F (1984) Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and beta-carboline constituents of *Ayahuasca*. J Ethnopharmacol **10:** 195–223

Niemeyer HH (1990) The role of secondary plant compounds in aphid-host interactions. *In* RK Campbell, RD Eikenbary, eds, Aphid-Plant Genotype Interactions. Elsevier, Amsterdam, pp 187–205

Perlak FJ, Deaton RW, Armstrong TA, Fuchs RL, Sims SR, Greenblate JT, Fischoff DA (1990) Insect resistant cotton. Bio/Technology **8:** 939–943

Piek T (1985) Neurotransmission and neuromodulation. *In* GA Kerkut, LI Gilbert, eds, Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol 11. Pergamon Press, Oxford, UK, pp 55–117

Robinson T (1979) The evolutionary geology of alkaloids. *In* GA Rosenthal, DH Janzen, eds, Herbivores: Their Interaction with Secondary Plant Metabolites. Academic Press, London, pp 413–448

Shade RE, Schroeder HE, Pueyo JJ, Tabe LM, Murdock LL, Higgins TJV, Chrispeels MJ (1994) Transgenic pea seeds expressing α amylase inhibitor of the common bean are resistant to bruchid beetles. Bio/Technology **12:** 793–796

Smith CM (1989) Plant Resistance to Insects. John Wiley & Sons, New York, pp 109–157

Songstad DD, DeLuca V, Brisson N, Kurz WGW, Nessler CL (1990) High levels of tryptamine accumulation in transgenic tobacco expressing tryptophan decarboxylase. Plant Physiol **94:** 1410–1413

Vehovszky A, Walker RJ (1991) An analysis of the 5-hydroxytryptamine (serotonin) receptor subtypes of central neurons of *Helix aspersa* Comp. Biochem Physiol **100:** 463–476

Woodhead S, Padgham DE (1988) The effect of plant surface characterization in resistance of rice to the brown plant hopper, *N. laparvata lugens*. Entomol Exp Appl **47:** 15–22

Zuñiga GE, Varanda EM, Corcuera LJ (1988) Effect of gramine on the feeding behavior of the aphids *Schizaphis graminum* and *Rhopalosiphum padi*. Entoml Exp App **47:** 161–165