Rapid Communication

Tryptophan Decarboxylase, Tryptamine, and Reproduction of the Whitefly

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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 (ZuAiga 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 T1 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, and the material was suspended at 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 C18 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.

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.

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.
The number of empty pupal cases after 30 d experiments. TDC or control (35S-uidA-expressing).

Aspersia (Vehovszky and Walker, 1991). Other indole-alkaloids collected from cotton plants and placed on 6-week-old tobacco plants. monoamine oxidase stimulation in the brown snail. Figure 3. labeled Tdc contained tryptamine. Results are from four independent experiments.

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 selection. In null plants no tryptamine could be detected. All plants labeled Tdc contained tryptamine. Results are from four independent experiments.

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 selection. In null plants no tryptamine could be detected. All plants labeled Tdc contained tryptamine. Results are from four independent experiments.

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