Plant Physiol. (1993) 103: 329–334

Biochemical Basis of Resistance of Tobacco Callus Tissue Cultures to Hydroxyphenylethylamines

Jonathan Negrel*, Francine Javelle, and Michel Paynot

Laboratoire de Phytoparasitologie, Station de Génétique et d'Amélioration des Plantes, Institut National de la Recherche Agronomique BV 1540 21034, Dijon Cedex France

It has been reported that hydroxyphenylethylamines, such as tyramine and octopamine, are toxic to tobacco (*Nicotiana tabacum* L.) callus cultures grown in the presence of auxins, whereas calli grown in the presence of cytokinins and crown gall cultures are resistant to these amines (P. Christou and K.A. Barton [1989] Plant Physiol 89: 564–568). In an attempt to understand the underlying mechanism of this resistance, we compared the fates of tyramine in tyramine-sensitive and tyramine-resistant tobacco tissue cultures (cv Xanthi nc). The very rapid formation of black-colored oxidation products from tyramine in sensitive tissues suggested that the toxicity might be caused by the oxidation of tyramine by phenol oxidases present in the tissues or released into the medium after subculture. This was confirmed through many indirect procedures (effect of exogenously added tyrosinase, induction of polyphenol oxidase [PPO] activity by auxin, etc.). The study of tyramine structure-activity relationships further suggested that the toxicity of tyramine might be due to the formation of indolequinones after oxidation by PPO. Subculture of calli grown on 2,4-dichlorophenoxyacetic acid in a medium containing benzyladenine triggered a slow decrease in PPO activity and dramatic increases in peroxidase and tyramine hydroxycinnamoyl transferase (THT) activities. THT was undetectable in calli grown on 2,4-dichlorophenoxyacetic acid but very active in tyramine-resistant crown gall cultures. Moreover, when [^{3}H]tyramine was fed in vivo to tyramine-resistant tissues, it was rapidly integrated into cell walls in the wound periderm formed at the periphery of the calli. Both the conjugation of tyramine and its integration into cell walls could compete with the formation of toxic quinones and therefore play a part in the resistance. Thus, it seems likely that the control of the toxicity of hydroxyphenylethylamines by cytokinins results primarily from changes in the metabolism and the compartmentation of these amines.

It has been reported that tyramine, the decarboxylation product of Tyr, together with octopamine (β-hydroxytyramine) is toxic to tobacco (*Nicotiana tabacum* L.) callus cultures grown in the presence of auxins, whereas the toxicity of these compounds can be overcome by prior cultivation of the calli in the presence of high concentrations of exogenous cytokinins (Christou and Barton, 1989). Tobacco crown gall cultures grown on hormone-free medium are also highly resistant to octopamine, and it has been suggested that this resistance may result from the synthesis of cytokinins in the transformed cells. Attenuated crown gall cultures generated by transformation of tobacco with modified *Agrobacterium* strains are not resistant to octopamine when they lack the isopentenyl transferase gene involved in the synthesis of cytokinins. Therefore, it has been suggested that cytokinins may activate a resistance mechanism to the toxicity of phenylethylamines in tobacco callus cultures (Christou and Barton, 1989).

The purpose of the present work was to identify this mechanism in order to evaluate whether the control of tyramine toxicity resulted from a change in the metabolism of phenethylamines following the application of the hormone. We report here that tyramine itself is not toxic, but it can generate toxic products in the presence of enzymes that are present in wounded tissues after subculture. Preliminary results suggest that these enzymes may be PPOs; hence, exposure of the calli to exogenous cytokinins may prevent the appearance of toxicity by decreasing the extent of oxidation of hydroxyphenethylamines by phenol oxidases. Exposure to cytokinins also induces a rapid metabolism of tyramine in resistant tissues, resulting in the integration of tyramine into cell walls at the periphery of the wound periderm formed after subculture. The rapid cytokinin-induced metabolism of tyramine could play a part in the resistance by leading to a reduction in the production of toxic quinones.

MATERIALS AND METHODS

Plant Material and Tissue Culture

Tobacco callus cultures were initiated from *Nicotiana tabacum* cv Xanthi nc pith tissue grown on MS medium (Murashige and Skoog, 1962), supplemented with 0.5 mg/L of 2,4-D and 0.05 mg/L of BA at 25°C with 16 h of light. After 1 month, the callus regions were subcultured on MS medium containing 0.5 mg/L of 2,4-D. At this concentration, significant callus growth occurred in the absence of exogenous cytokinin (Witham, 1968). To study the effect of cytokinins, the calli were transferred to MS medium supplemented with 0.05 mg/L of BA. Calli were taken at different time intervals after the transfer, frozen in liquid nitrogen, and stored at −80°C before analysis. Suspension cultures were grown from the callus cultures maintained on 2,4-D in MS medium containing 2 mg/L of 2,4-D. In the study of the toxicity of hydroxyphenethylamines, tyramine and tyramine analogs

* Corresponding author; fax 33–80–63–32–63.

Abbreviations: homotyramine, [4-(3-aminopropyl)phenol]; ME, mercaptoethanol; MS, Murashige and Skoog; PPO, polyphenol oxidase; THT, tyramine hydroxycinnamoyl transferase.

were added to the sterile culture medium after filtration through Millex filters (Millipore).

Crown gall cultures were obtained from stem sections of *N. tabacum* cv Xanthi nc as described by Barton et al. (1983), using the *Agrobacterium tumefaciens* strains T37, A6, B6s3, and ACH5. After 1 week of incubation, callus regions that had developed at the upper surface of the stem segments were transferred to hormone-free MS medium containing carbenicillin (200 μg/mL). After three transfers at 1-month intervals on this medium, followed by one transfer on MS medium containing cefotaxime (Claforan, Roussel Uclaf Industries, Romainville, France) (50 μg/mL), the calli, free of bacteria, were cultivated on hormone-free MS medium. Tyramine-resistant crown gall cultures were obtained after subculture on a medium containing 10 mM tyramine (Christou and Barton, 1989). The sectors of the primary galls that did not become necrotic were subcultured on the same medium for more than 2 years.

Chemicals

Tyrosinase (extracted from mushroom) was purchased from Sigma. *p*-Hydroxybenzylamine was synthesized from 4-methoxybenzylamine (Aldrich) (Negrel and Jeandet, 1989). Homotyramine was a gift of Roussel Uclaf Industries. *p*-Coumaroyl- and feruloyl-tyramine were synthesized as described by Villegas and Brodelius (1990). [ring-^{3}H]Tyramine (1.5 TBq/mmol) was purchased from New England Nuclear, and [7-^{14}C]tyramine (1.96 GBq/mmol) was obtained from Amersham.

Extraction Procedures, Enzyme Assays, and Protein Assay

Parallel experiments were run to monitor peroxidase and PPO activities and THT activities. To assay peroxidase and PPO activities, the calli were ground in 0.1 M phosphate buffer (pH 6) (2 mL/g fresh weight) in the presence of activated charcoal (50 mg/g fresh weight). After centrifugation, the supernatant solutions were dialyzed against 0.01 M sodium phosphate buffer (pH 6) overnight and used directly as a source of enzymes. Peroxidase activity was assayed in 0.02 M guaiacol, 0.1 M sodium phosphate buffer (pH 6), and 0.03 M H_2O_2. The increase in absorbance was monitored at 420 nm in a Beckman DU70 spectrophotometer. PPO activity was assayed in 0.1 M sodium phosphate buffer (pH 6) using chlorogenic acid as substrate (0.02 mg/mL). The decrease in absorbance was monitored at 330 nm. Enzyme activity is expressed as units per mg protein, where one unit is defined as the amount of enzyme causing a decrease in absorbance of 0.01 per s for PPO and 1 per s for peroxidase.

To assay THT activity, the calli were ground in 0.2 M Tris-HCl buffer (pH 7.5) containing 1 mM EDTA, 10 mM ME, and 2% ascorbic acid. After centrifugation, proteins were precipitated with solid ammonium sulfate (65% saturation) and centrifuged and the pellet was dissolved in extraction medium (1 mL/g fresh weight) and dialyzed against 0.01 M Tris-HCl buffer (pH 7.5) containing 10 mM ME. THT activity was then measured spectrophotometrically at 356 nm using feruloyl-CoA and tyramine as substrates in 0.1 M Tris-HCl (pH 7.5) buffer (Negrel and Martin, 1984). Protein content was determined using the Bradford reagent (Bradford, 1976).

Feeding Experiments

[7-^{14}C]Tyramine was fed in vivo to each tissue type (approximately 1 g fresh weight) 15 d after subculture. Calli were transferred into Petri dishes and droplets of the radioactive solution (74 kBq, 200 μL) were applied at the periphery of each callus. After 4 h, the callus was ground in methanol. After centrifugation, the pellet was reextracted twice in methanol and combusted in a Packard oxidizer to produce $^{14}CO_2$ for measurements of radioactivity. The methanol extracts were pooled, evaporated in vacuo, and partitioned between water and ethyl acetate. The ethyl acetate phase was concentrated and analyzed by TLC on Kieselgel 60 in $CHCl_3$:methanol (24:1, v/v). Feruloyl- and *p*-coumaroyl-tyramine were detected by autoradiography and tentatively identified by co-chromatography with synthetic standards before and after irradiation of the sample with UV light (Negrel and Jeandet, 1987).

Light Microscopy and Autoradiography

After feeding [ring-^{3}H]tyramine (20 μCi in 200 μL) to calli for 4 h as described above, portions of the treated calli were cut in the vicinity of the wound periderm. These portions were fixed for 2 h at room temperature in phosphate-buffered glutaraldehyde (2.5%, pH 6.8, 0.1 M), dehydrated in ethanol, and embedded in glycol methacrylate. Sections (approximately 4 μm) were cut with glass knives and examined directly or after autoradiography under a Leitz fluorescence microscope. The sections were covered with Amersham LM1 emulsion using the dipping technique. After exposure for 1 week in the dark at 4°C, the autoradiographs were developed in Kodak Microdol X.

Oxidation of Homotyramine by Tyrosinase

Homotyramine hydrochloride (5 mg) was dissolved in 1 mL of 0.1 M sodium phosphate (pH 6), and 0.5 mg (1000 units) of tyrosinase was added. The tube was filled with O_2, stoppered, and incubated at 30°C overnight. The reaction medium was then analyzed by preparative TLC on Kieselgel 60 F_{254} plates developed in $CHCl_3$:methanol (1:1). The main product of the reaction appeared as a purple band of R_F 0.47. The product did not react with ninhydrin or with $FeCl_3$. After elution in methanol, it was further purified by HPLC on a C_{18} column (4.6 × 250 mm; 5 μm) using a methanol:water gradient (20–100% methanol in 30 min) with a flow rate of 1 mL/min (retention time = 4.96 min). It was detected at 517 nm (λ_{max}). The purified product could be readily reduced with ascorbic acid, but reoxidation of the product occurred spontaneously after separation of ascorbic acid by TLC. The product extracted from calli grown on MS medium supplemented with 2,4-D and 10 mM homotyramine had the same properties (R_F in TLC, reaction with ninhydrin and $FeCl_3$, retention time in HPLC, UV spectrum after purification by HPLC). The toxicity of the purified product was not tested because of the limited amount of homotyramine available.

RESULTS AND DISCUSSION

Cause of the Toxicity of Tyramine

To understand how cytokinins control the resistance to tyramine, it was first necessary to determine the cause of the toxicity of tyramine. The effect of tyramine on tobacco calli grown on MS medium supplemented with 0.5 mg/L of 2,4-D was consistent with the effect of octopamine described by Christou and Barton (1989). Both the calli and the culture medium in the vicinity of the calli turned black a few hours after subculture. The toxic effect of tyramine was dose dependent and could be detected at concentrations ranging from 1 to 10 mM. Below 1 mM, the growth of the callus was not altered. At 10 mM, growth stopped and the cells died 3 to 7 d after subculture. The fact that blackening of the culture medium always appeared prior to the toxicity suggested that phenol oxidases may play a role in the toxicity. This was supported by the finding that tyramine was not at all toxic to tobacco cell suspensions grown in liquid medium in the presence of 2,4-D. Addition of 10 mM tyramine to the medium did not affect the growth of the cell-suspension cultures over a 15-d period. However, addition of commercial tyrosinase in the culture medium (20 units/mL) before subculture triggered a rapid oxidation of tyramine and the appearance of toxicity. Similar results were obtained with callus cultures grown on solid medium supplemented with tyrosinase. In this case, the toxicity of tyramine appeared more quickly than in controls.

The involvement of phenol oxidases was further suggested by comparing the toxicity of tyramine for tobacco calli grown in the presence of 2,4-D to that for calli grown in IAA. Both the extent of blackening and the level of PPO activity were very sensitive to the presence of auxin in the medium. PPO activity in calli maintained for 1 month on MS medium supplemented with IAA (1 mg/L) reached 1250 units/mg of protein, i.e. 10-fold the activity in controls maintained on 2,4-D. This result was in agreement with previous work (Vernon and Strauss, 1972). When calli grown in IAA were transferred onto a tyramine-containing medium, toxicity appeared more quickly than in controls.

An interesting indication of the cause of the toxicity arose indirectly from the study of tyramine structure-activity relationships. Previous work has established that the substances whose addition results in toxicity contain both the phenolic hydroxyl and the primary amino groups of tyramine (Christou and Barton, 1989). We found that the length of the aliphatic side chain has a dramatic effect on the toxicity of tyramine analogs (see Fig.1 for structures). *p*-Hydroxybenzylamine was not at all toxic when added at 10 mM, whereas homotyramine was highly toxic even at 1 mM. The toxicity

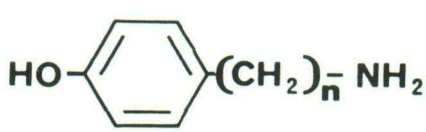

Figure 1. Structures of tyramine analogs referred to in the text: *p*-hydroxybenzylamine ($n = 1$), tyramine ($n = 2$), and homotyramine ($n = 3$).

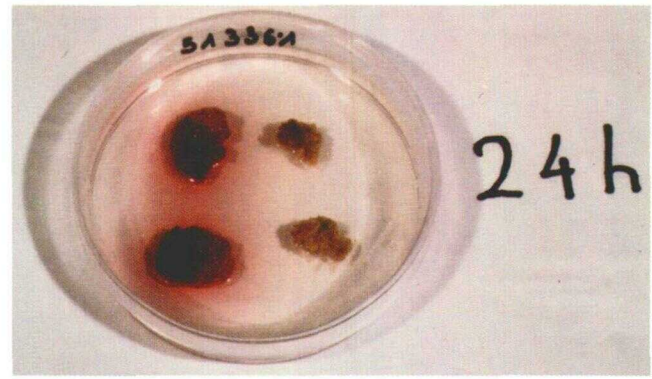

Figure 2. Differential behavior of tobacco calli subcultured on a medium supplemented with 10 mM homotyramine. Left, Calli grown for 1 month in the presence of 2,4-D (0.5 mg/L) before subculture. Right, Calli grown for 1 month in the presence of BA (0.05 mg/L). The photograph was taken 24 h after subculture.

of tyramine was intermediate. When calli grown on 2,4-D were plated on MS medium supplemented with 10 mM homotyramine, the calli and the medium turned purple within minutes and no further growth was observed. The medium turned black only after 1 to 2 months.

p-Hydroxyphenylethylamines are known to be transformed by tyrosinase to *o*-quinones, which undergo nonenzymic ring closure to form purple indolequinones that polymerize to form black melanin-like compounds (Mazur et al., 1956; Sugumaran, 1991). This intramolecular reaction, which cannot occur in the case of *p*-hydroxybenzylamine, could lead to a stable six-membered ring in the case of homotyramine. Since quinones are known to be highly toxic (Mayer and Harel, 1979), the ability of hydroxyphenethylamines to form relatively stable quinones may be the basis for both the toxicity of tyramine and the differences in toxicities of tyramine analogs.

The purple product formed from homotyramine was surprisingly stable and could be extracted into methanol from calli maintained on homotyramine for 48 h. Although we did not attempt to determine its structure using physical methods, we found that its properties were the same as those of the main oxidation product of homotyramine obtained after incubation with commercial tyrosinase (see "Materials and Methods"). The same product was also formed in vitro in enzymic extracts of tobacco calli incubated in the presence of homotyramine. Moreover, diethyldithiocarbamate, a known inhibitor of PPO (Mayer and Harel, 1979; Mayer, 1987), completely blocked the formation of this product in vitro when added at 1 mM.

Thus, homotyramine proved very useful for the detection of PPO activity in situ in the calli after subculture. In this way, we found that calli grown on BA for 1 month and transferred directly without wounding onto homotyramine-containing medium did not release oxidases exhibiting PPO activity in the medium (Fig. 2). However, when the calli were cut during subculture, oxidation was evident in the vicinity of the wound, but the upper part of the calli survived, suggesting that an additional resistance mechanism might have been induced by exposure to cytokinins.

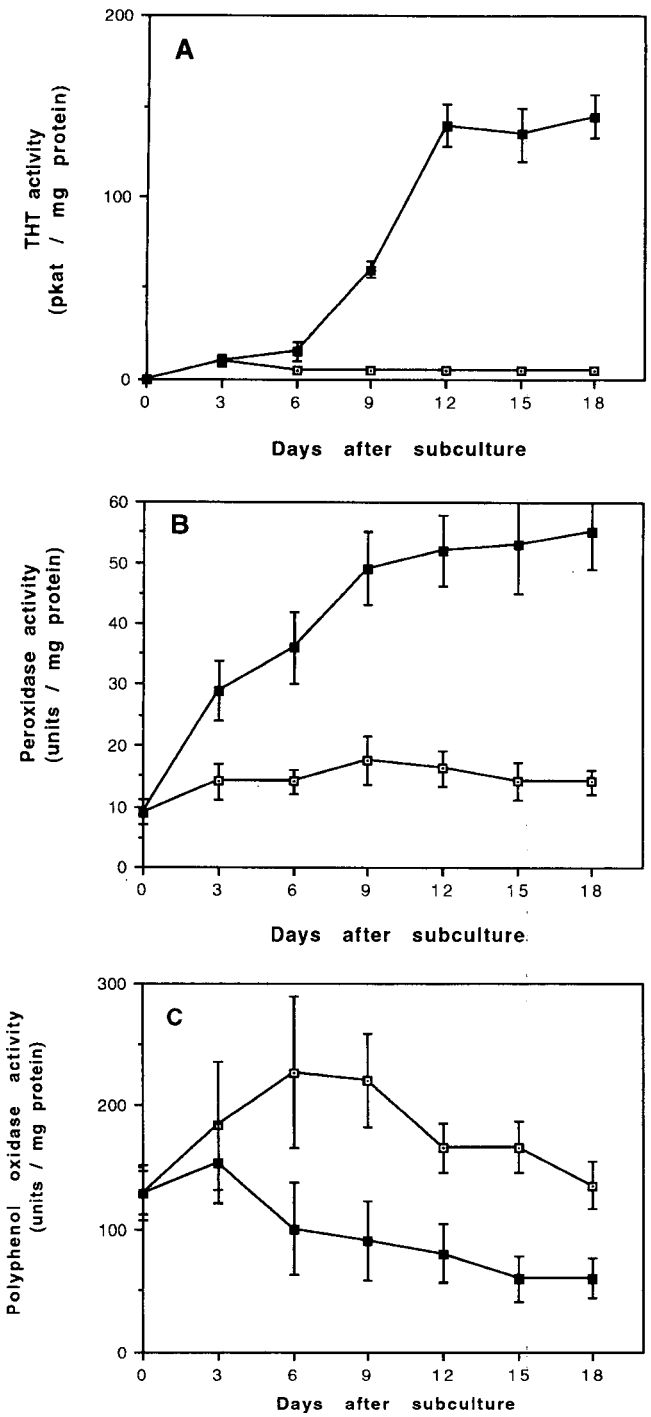

Figure 3. Time course of THT (A), peroxidase (B), and PPO (C) activities in tobacco calli subcultured from a MS medium supplemented with 0.5 mg/L of 2,4-D to a medium containing 0.05 mg/L of BA (■) or 0.5 mg/L of 2,4-D (⊡, control). All measurements were done in triplicate (mean ± SE)

Metabolism of Tyramine in Resistant Calli

These results prompted us to compare the metabolism of tyramine in calli grown on 2,4-D or on BA. In tobacco, hydroxyphenylethylamines often occur conjugated to hydroxycinnamic acids (Smith, 1977; Negrel and Martin, 1984). When the amino group of tyramine is acylated, the intramolecular addition leading to the formation of indolequinones cannot take place (Sugumaran, 1991). Therefore, it appeared possible that the conjugation to cinnamic acids could play a role in the detoxification of tyramine. This was supported by the finding that feruloyl- and *p*-coumaroyltyramine were not toxic, even in the presence of tyrosinase. In tobacco, the conjugation of hydroxyphenethylamines is catalyzed by the enzyme THT (Negrel and Martin, 1984).

Figure 3A shows the time course of changes in THT activity in tobacco callus tissue grown on MS medium in the presence of 2,4-D and subcultured on a MS medium supplemented with BA. One week after the transfer, THT activity rose dramatically. Soluble peroxidase activity also rose after transfer to BA (Fig. 3B). By contrast, PPO activity in the calli decreased slowly after subculture (Fig. 3C). None of these changes occurred in control cells subcultured on a 2,4-D-containing medium.

Peroxidase and THT activities were not uniformly distributed in the callus. Most of the THT activity was concentrated in the tissues at the periphery of the calli. Two weeks after the transfer, the center of the calli consisted of nonaggregated cells surrounded by a periderm about 2 mm thick that could be separated from the callus with a scalpel. About 80% of total THT and peroxidase activities could be extracted from this periderm.

Moreover, THT was found to be very active in all the crown gall tissues tested (Table I). Again, activity was located essentially in the wound periderm formed after subculture. When [^{14}C]tyramine was fed in vivo to calli grown on BA or to crown gall tissues, 4 to 7% of the supplied radioactivity was recovered in *p*-coumaroyl- and feruloyl-tyramine, whereas up to 40% was recovered in the pellet after methanol extraction. By contrast, only 3% was recovered in this pellet in the case of calli grown on 2,4-D. Furthermore, in these calli no incorporation of tyramine in the amides was detected.

The distribution of radioactivity in tyramine-resistant calli fed with [ring-^{3}H]tyramine was then studied by light autoradiography. Radioactivity was detected essentially in the cell walls of the wound periderm. Figure 4 shows a light micrograph of the periderm formed after subculture of crown gall tissue. Similar results were obtained in calli grown on BA for

Table I. *THT activity in crown gall tissues*

Activity was measured 2 weeks after subculture using tyramine and feruloyl-CoA as substrates.

Tissue Type	THT Activity
	pkat/mg protein
Wild type	<1
T37	124
B6s3	100
A6	93
ACH5	103

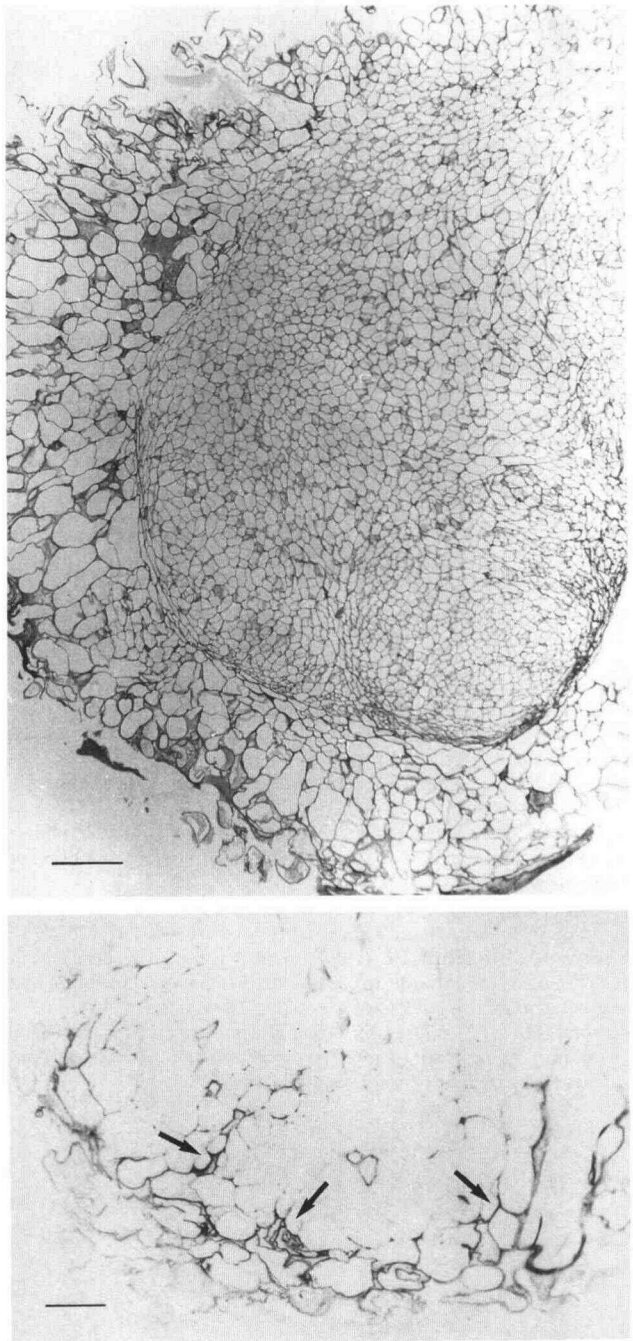

Figure 4. Upper, Longitudinal section of a tyramine-resistant crown gall culture (generated by inoculation with *A. tumefaciens* strain B6S3) 3 weeks after subculture. Bar = 200 μm; ×43. Section stained with toluidine blue. Lower, Autoradiograph showing the integration of [ring-^{3}H]tyramine in the cell walls of the wound periderm (arrows). Longitudinal section bar = 100 μm; ×100.

2 weeks, whereas no integration of radioactivity into cell walls was detected in control calli grown on 2,4-D. The cell walls of the wound periderm in resistant calli were highly autofluorescent and showed intense staining with the suberin stains Sudan black B and Nile blue (Graham and Graham, 1991).

The fact that phenolics can generate toxic compounds in plant tissue culture media is well established (Reinert and White, 1956; George and Sherrington, 1984). In this regard, the fact that hydroxyphenethylamines can turn toxic to tobacco cells when added to a culture medium is not surprising, although these amines are natural plant products (Smith, 1977) that are known to occur in tobacco callus tissues (Mitchell et al., 1984).

It is generally assumed that tissue blackening occurs as the result of the action of phenol oxidases that are synthesized or released when tissues are injured during subculture (George and Sherrington, 1984). Results presented here show clearly that tyramine is not directly toxic, but can generate toxic products, most probably quinones, in the presence of phenol oxidases. It is likely that cytokinins act indirectly by decreasing the rate of formation of these products, which could be achieved by a reduction of the release of phenol oxidases in the culture medium and a concomitant activation of tyramine metabolism within resistant tissues (Fig. 5). Both events are probably associated with the histological changes occurring in calli transferred from 2,4-D to BA.

In tobacco callus cultures, cytokinins are known to induce cellular aggregation (Halperin and Minocha, 1973) and lignification (Kuboi and Yamada, 1978). In our experimental system, cellular aggregation took place first at the periphery of the callus and was accompanied by the deposition of lignin-like compounds in the wound periderm. The fact that two enzymes that can use hydroxyphenethylamines as substrates, i.e. THT and peroxidase (Negrel and Lherminier, 1987), are active in this periderm may indirectly increase its efficiency as a diffusion barrier against the quinones formed in the culture medium from tyramine. In this respect, it is noteworthy that tyramine has been shown to be a constituent of the wound periderm of potato tubers, in which it is found

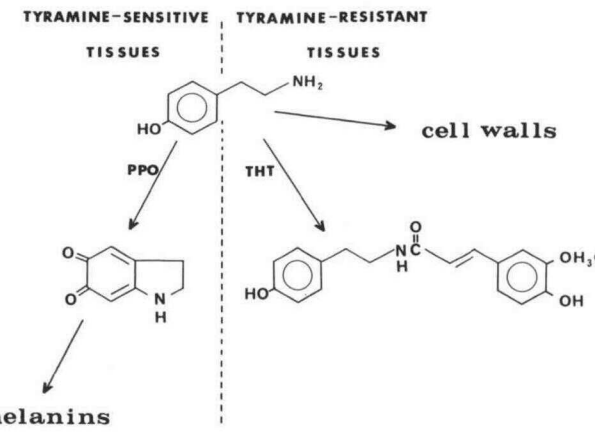

Figure 5. Metabolism of tyramine in tyramine-sensitive or -resistant callus cultures of tobacco.

together with lignin in suberified cell walls (Borg-Olivier and Monties, 1989).

In conclusion, our results suggest that the antagonist activity of cytokinins in the growth medium to the toxicity of hydroxyphenethylamines described by Christou and Barton (1989) is primarily due to the effect of cytokinins on the metabolism and the compartmentation of these amines. However, further work is necessary to better characterize both the toxic products formed from hydroxyphenethylamines and the enzymes involved in the formation of these products in tobacco calli, although preliminary results presented in this paper strongly suggest that these enzymes could be PPOs.

ACKNOWLEDGMENTS

We are grateful to Dr. Annik Petit (Institut des Sciences Végétales, Gif-sur-Yvette, France) for the gift of *Agrobacterium tumefaciens* strains.

Received January 19, 1993; accepted June 9, 1993.

LITERATURE CITED

Barton KE, Binns AN, Matzke JM, Chilton MD (1983) Regeneration of intact tobacco plants containing full length copies of genetically engineered T-DNA and transmission of T-DNA to R1 progeny. Cell **32:** 1033–1043

Borg-Olivier O, Monties B (1989) Caractérisation des lignines, acides phénoliques et tyramine dans les tissus subérisés du périderme naturel et du périderme de blessure de tubercule de pomme de terre. C R Acad Sci **308:** 141–147

Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72:** 248–254

Christou P, Barton KA (1989) Cytokinin antagonist activity of substituted phenylethylamines in plant cell culture. Plant Physiol **89:** 564–568

George EF, Sherrington PD, eds (1984) Plant Propagation by Tissue Culture. Handbook and Directory of Commercial Laboratories. Exergetics Ltd, Edington, UK, pp 334–340

Graham MY, Graham TL (1991) Rapid accumulation of anionic peroxidases and phenolic polymers in soybean cotyledon tissues following treatment with *Phytophtora megasperma* f. sp. *glycinea* wall glucan. Plant Physiol **97:** 1445–1455

Halperin W, Minocha S (1973) Benzyladenine effects on cell separation and wall metabolism. Can J Bot **51:** 1347–1354

Kuboi T, Yamada Y (1978) Regulation of the enzyme activities related to lignin synthesis in cell aggregates of tobacco cell culture. Biochim Biophys Acta **542:** 181–190

Mayer AM (1987) Polyphenol oxidases in plants—recent progress. Phytochemistry **26:** 11–20

Mayer AM, Harel E (1979) Polyphenol oxidases in plants. Phytochemistry **18:** 193–215

Mazur A, Green S, Shorr E (1956) The oxidation of adrenaline by ferritin iron and hydrogen peroxide. J Biol Chem **220:** 227–235

Mitchell SD, Firmin JL, Gray DO (1984) Enhanced 3-methoxytyramine levels in crown gall tumors and other undifferentiated plant tissues. Biochem J **221:** 891–885

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant **15:** 473–494

Negrel J, Jeandet P (1987) Metabolism of tyramine and feruloyltyramine in TMV inoculated leaves of *Nicotiana tabacum*. Phytochemistry **26:** 2185–2190

Negrel J, Lherminier J (1987) Peroxidase-mediated integration of tyramine into xylem cell walls of tobacco leaves. Planta **172:** 494–501

Negrel J, Martin C (1984) The biosynthesis of feruloyltyramine in *Nicotiana tabacum*. Phytochemistry **23:** 2797–2801

Reinert J, White PR (1956) The cultivation in vitro of tumor tissues and normal tissues of *Picea glauca*. Physiol Plant **9:** 177–189

Smith TA (1977) Phenethylamine and related compounds in plants. Phytochemistry **16:** 9–18

Sugumaran M (1991) Molecular mechanisms for mammalian melanogenesis: comparison with insect cuticular sclerotization. FEBS Lett **293:** 4–10

Vernon SL, Straus J (1972) Effects of IAA and 2,4-D on polyphenol oxidase in tobacco tissue cultures. Phytochemistry **11:** 2723–2727

Villegas M, Brodelius PE (1990) Elicitor induced hydroxycinnamoyl-CoA:tyramine hydroxycinnamoyltransferase in plant cell suspension cultures. Physiol Plant **78:** 414–420

Witham FH (1968) Effect of 2,4-dichlorophenoxyacetic acid on the cytokinin requirement of soybean cotyledon and tobacco stem pith callus tissues. Plant Physiol **43:** 1455–1457