Phytochemistry, Vol. 21, No. 8, pp. 1885–1887, 1982.
Printed in Great Britain.

0031-9422/82/081885-03$03.00/0

PHENOLIC OXIDASE ACTIVITIES IN GLANDULAR TRICHOMES OF *SOLANUM BERTHAULTII*

JAMES D. RYAN, PETER GREGORY and WARD M. TINGEY*

Department of Plant Breeding and Biometry; and *Department of Entomology, Cornell University, Ithaca, NY 14853, U.S.A.

(*Received* 23 *October* 1981)

Key Word Index—*Solanum berthaultii*; Solanaceae; wild potato; glandular trichome; enzymology; polyphenol oxidase; peroxidase.

Abstract—Polyphenol oxidase and peroxidase activities were detected in foliar glandular trichomes of the wild, insect-resistant potato species, *Solanum berthaultii*. These enzyme activities may provide the basis for conversion of clear, viscous trichome exudate into a hard, brown substance which is formed rapidly after insect attack.

INTRODUCTION

The foliar epidermis of the wild insect resistant Bolivian potato species *Solanum berthaultii* (Hawkes) is covered with glandular trichomes (Fig. 1), secretions of which entrap several species of insect pests [1, 2]. Upon contact by the insect, the shorter, four-lobed hairs are ruptured and a clear exudate is discharged onto the insect's body. This exudate is rapidly converted to a hard, brown substance which accumulates on the tarsi and mouthparts, thus impeding mobility and preventing feeding. Exudate from the longer, simple trichomes remains clear and viscous.

At present little is known about the biochemical mechanism of this defensive browning reaction. Gibson [3] suggested that the reaction might involve production of a polymeric phenol by action of polyphenol oxidase (PPO) because: (a) The reaction is inhibited in the absence of air; (b) Sodium diethyldithiocarbamate (DEDTC), which inhibits PPO activity, retards the reaction; and (c) Use of Folin–Denis's reagent indicated the presence of phenols in the glandular trichomes. However, this suggestion was very tentative because it was based on evidence at the *in vivo* level. Here, we present direct biochemical evidence for PPO and peroxidase (PO) in glandular trichomes of *S. berthaultii*.

RESULTS AND DISCUSSION

Glandular trichome exudates were collected and assayed for PPO and PO activities. Readily detectable levels of activity were found in both the PPO and PO assays (Table 1). Boiled extracts showed no activity.

Substrate specificities of activities in the two assays were markedly different (Table 1). In the PPO assay catechol was rapidly oxidized whereas several other phenolic substrates were either oxidized at much lower rates (guaiacol, pyrogallol, gallic acid, caffeic acid, protocatechuic acid, *p*-hydroxybenzoic acid) or remained unoxidized (orcinol, phloroglucinol). In the PO assay all of the substrates except catechol oxidized more rapidly than had been observed in the PPO assay, but the most dramatic differences were observed with guaiacol and pyrogallol in which oxidation rates were 12 and 7 times higher respectively, and with orcinol and phloroglucinol which had not been oxidized in the PPO assay but were oxidized slowly in the PO assay.

The inhibitors DEDTC, phenylthiourea (PTU) and 2,3-dimercaptopropanol[British Anti-Lewisite (BAL)], which can complex with the copper prosthetic group of PPO or the heme group of PO are known inhibitors of PPO activity [4] and PO activity [5–7]. DEDTC and PTU caused similar concentration-dependent inhibition of PPO and PO activities in the trichome exudates, whereas BAL was a more potent inhibitor of PO activity than of PPO activity (Table 2).

Our demonstration of enzyme activities in the PPO and PO assays and of concentration-dependent inhibition of these activities with DEDTC, PTU and BAL strongly suggests the presence of phenolic oxidase activities in the glandular trichomes of *S. berthaultii*. The finding that substrate specificity was markedly different in the PPO vs PO assay indicates the presence of two types of enzymic activity, one driven by oxygen (PPO), the other by hydrogen peroxide (PO), rather than a single activity which can be observed in either assay system. The differential sensitivities of activities in the PPO vs PO assays to BAL also support this concept.

The observed PPO and PO activities do not neces-

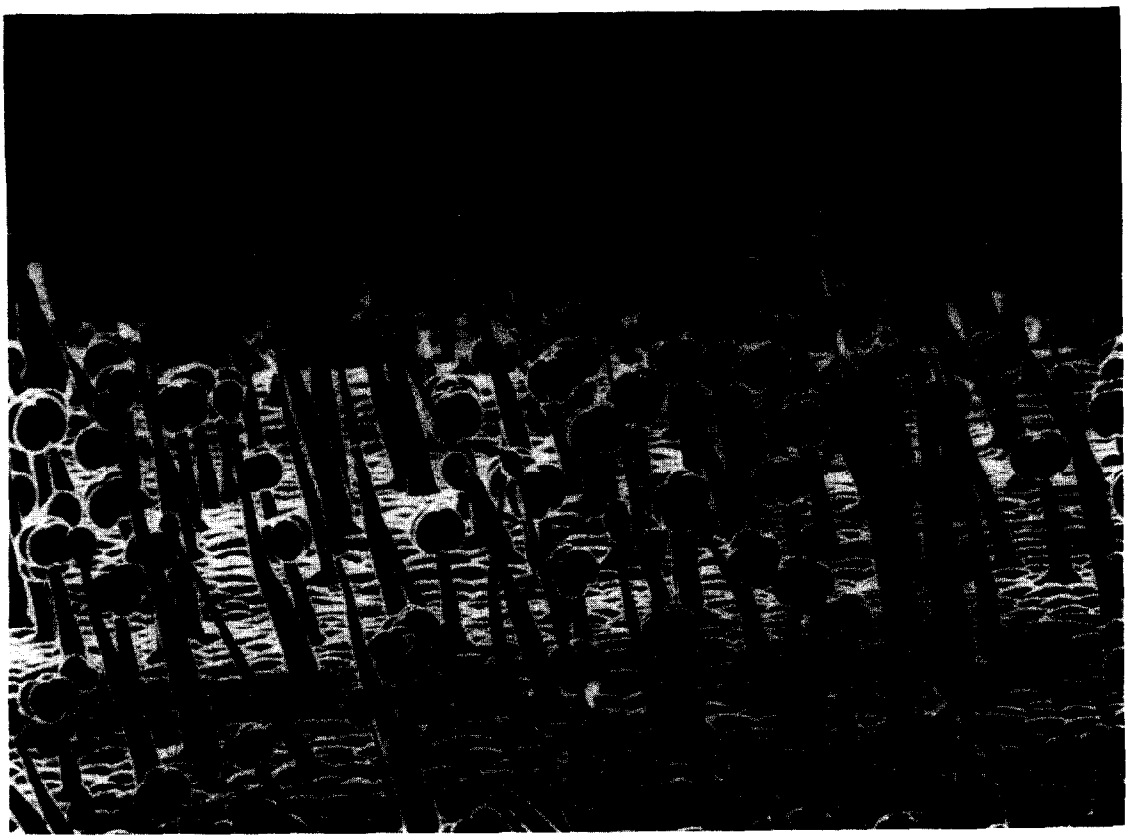

Fig. 1. Simple and four-lobed glandular trichomes on foliage of the *Solanum berthaultii* clone, PI 310927-11 (122 ×).

Table 1. Phenolic oxidase activities of *Solanum berthaultii* glandular trichomes

Reaction mixture	Phenolic oxidase activities (ΔA_{470}/2 min)*	
	PPO assay	PO assay
Extract + 2.9 mM catechol	0.60	0.50
Boiled extract + 2.9 mM catechol	0.00	0.00
Extract + 4.0 mM guaiacol	0.05	0.62
+ 2.9 mM pyrogallol	0.10	0.70
+ 2.9 mM gallic acid	0.20	0.45
+ 2.9 mM caffeic acid	0.10	0.40
+ 0.9 mM protocatechuic acid	0.20	0.30
+ 0.9 mM *p*-hydroxybenzoic acid	0.15	0.25
+ 2.9 mM orcinol	0.00	0.15
+ 0.9 mM phloroglucinol	0.00	0.10

*ΔA_{470} for boiled extracts + other phenolic substrates was 0.00.

sarily imply the presence of separate PPO and PO enzymes, as oxidative and peroxidative phenolic reactions can be catalysed by a single enzyme [6]. Studies are underway to determine the number and properties of the enzymes involved and to characterize the substrate(s), product(s) and initiation mechanism of the browning reaction.

EXPERIMENTAL

Trichome exudate collection. *Ca* 3 g healthy leaflets were removed from greenhouse-grown plants of the *S. berthaultii* clone PI 310927-11. Very young or senescent foliage was avoided. A cotton swab was dampened with cold (4°) Pi buffer (70 mM; pH 7.0). Trichomes were discharged by 3–5

Table 2. Effects of inhibitors on phenolic oxidase activities of *Solanum berthaultii* glandular trichomes

Reaction mixture	Phenolic oxidase activities (ΔA_{470}/2 min)	
	PPO assay (O_2/catechol)	PO assay (H_2O_2/guaiacol)
Control (extract − inhibitor)	0.60	0.62
Boiled extract	0.00	0.00
Extract + 0.1 mM DEDTC	0.45	0.55
0.3 mM DEDTC	0.35	0.30
0.5 mM DEDTC	0.10	0.05
0.1 mM PTU	0.55	0.50
0.3 mM PTU	0.30	0.35
0.5 mM PTU	0.10	0.10
0.008 mM BAL*	0.58	0.50
0.17 mM BAL*	0.40	0.20
0.33 mM BAL*	0.25	0.10
0.50 mM BAL*	0.20	0.00
0.67 mM BAL*	0.15	0.00

*Extract was pre-incubated with BAL for 20 min. before initiation of reaction.

light passes of the damp swab over the abaxial leaf surfaces as described by Gibson [8]. Trichome exudate was removed from the swab by washing with 6 ml cold Pi buffer. The resulting crude enzyme mixture was immediately assayed for PPO or PO activities or stored on ice.

Enzyme assays. PPO activities were assayed by a modification of the method described by Hori[9]. To a reaction mixture containing 0.5 ml Pi buffer (70 mM; pH 7.0), 0.25 ml H_2O and 0.25 ml 14.5 mM catechol, was added 0.25 ml crude enzyme mixture. Change in A_{470} was monitored in a Varian Cary 219 spectrophotometer. PO activities were assayed as described for PPO except that the reaction mixture contained 0.5 ml Pi buffer (70 mM; pH 7.0), 0.25 ml 0.3% H_2O_2 and 0.25 ml 20 mM guaiacol.

Acknowledgements—We wish to thank Professors P. P. Feeny and C. F. Wilkinson of Cornell University for valuable discussions. This work was supported by the Science and Education Administration of the U.S. Department of Agriculture under Grant No. 7800454 from the Competitive Research Grants Office, the International Potato Center, Lima, Peru and a USDA Special Grant.

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