

Safeners, herbicides, and grain sorghum microsomes

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Summary

Microsomes from excised shoots of 3-day-old, dark-grown, grain sorghum [*Sorghum bicolor* (L.) Moench, Funk G522DR and DK 41Y] seedlings metabolized cinnamic acid, lauric acid, metolachlor, bentazon, and diazinon. Treatment of G522DR seed with safeners (naphthalic anhydride, dichlormid, flurazole, BAS 145138, oxabetrinil, CGA 133205, and CGA 154281) enhanced metabolism of lauric acid, bentazon, and diazinon. However, metabolism of cinnamic acid was not affected and that of metolachlor was depressed. Microsomes isolated from DK 41Y seedlings tended to have higher endogenous levels of oxidative activity than microsomes isolated from G522DR seedlings.

Introduction

The two main detoxication pathways that determine susceptibility or tolerance of plants to most herbicides are the glutathione and the microsomal cytochrome P-450 monooxygenase systems. Both glutathione-S-transferases and monooxygenases exist in multiple isoforms. For the most part, the monooxygenase isoforms remain to be identified and characterized. Safeners enhance the activity of both systems, however, specificities of the enhancement remain to be documented especially for the monooxygenase system.

The general objective of the study being discussed here was to compare the effects of seven safeners (naphthalic anhydride, dichlormid, flurazole, BAS 145138, oxabetrinil, CGA 133205 and CGA 154281), applied as seed treatments, on the metabolism of five substrates (cinnamic acid, lauric acid, metolachlor, bentazon, and diazinon) by a microsomal fraction obtained from grain sorghum shoots.

Materials and Methods

Microsomal preparations were isolated from shoots of sorghum cultivars Funk G522DR and DK 41Y which have bronze and cream-colored endosperms, respectively. Seed were germinated, microsomes isolated, and protein contents determined with previously published procedures (4,5). All seed were treated with Captan (0.2%, w/w). Safeners were applied to the Captan-treated seed at the rates indicated parenthetically: naphthalic anhydride (0.5%, w/w); dichlormid, flurazole, BAS 145138, and CGA 154281 (0.2%, w/w); oxabetrinil (0.12%, w/w); and CGA 133205 (0.04%, w/w). Composition of the microsomal reaction mixtures, and analytical procedures that included separation and quantification of metabolites were conducted following previously reported procedures (4,5) with adjustments being made to accommodate the different substrates.

Results

The sorghum microsomes readily oxidized the five substrates and formed the metabolites that are indicated parenthetically: cinnamic acid (4-hydroxycinnamic acid), lauric acid (possibly 8-, 9-, and 10-hydroxylauric acid, identity not confirmed), metolachlor (O-desmethylmetolachlor), bentazon (6-hydroxybentazon), and diazinon (identity not established). Effects of the different seed treatments on the formation of metabolites are compared in Table 1 relative to oxidative activity associated with the Captan controls for sorghum cultivar G522DR. A ratio of 1 indicates that microsomes

isolated from safened seedlings metabolized the substrates at the same rate as microsomes isolated from Captan-control seedlings. Values less than 1 indicate that metabolism was depressed by the safeners below control rates, and values greater than 1 indicate that metabolism was increased above control rates by the safeners. The safeners are grouped with respect to the magnitude of the responses elicited. As a group, BAS 145138, flurazole, and dichlormid expressed a lower level of response than did NA, oxabetrinil, and the two CGA compounds. None of the safeners had a marked effect on the oxidation of cinnamic acid relative to unsafened seed. All of the safeners depressed the oxidation of metolachlor, with the second group of safeners causing considerably more depression. The first group of safeners slightly enhanced the oxidation of lauric acid and bentazon, whereas the second group provided a much greater enhancement not only of lauric acid, but also of bentazon and diazinon oxidation.

Table 1. Comparison of the responses induced by safener seed treatments relative to unsafened controls on the oxidation of metabolites by sorghum shoot microsomes (cv G522DR). The values, shown as ranges, were obtained by dividing the nmol metabolites formed/mg microsomal protein/h by microsomes isolated from safened seedlings by nmol metabolites formed/mg microsomal protein/h by microsomes from unsafened seed.

Substrate	Safeners	
	BAS 145138 Flurazole Dichlormid	Naphthalic anh. Oxbetrinil CGA 133205 CGA 154281
Cinnamic acid	0.9 - 1.1	
Metolachlor	0.8 - 0.9	0.4 - 0.7
Lauric acid	1.3	3.0 - 8.8
Bentazon	1.1 - 1.5	3.3 - 4.4
Diazinon	0.9 - 2.5	2.6 - 3.4

All of the safeners were evaluated only with seed of the bronze endosperm cultivar G522DR. For comparative purposes, exploratory studies have been conducted with seed of the cream-colored endosperm cultivar DK 41Y. The endogenous oxidative activities of microsomes isolated from DK 41Y were somewhat higher than for G522DR microsomes (Table 2). In Table 2, the ratio in the last column was obtained by dividing the nmol of metabolite formed by the DK 41Y microsomes by the nmol of metabolite formed by the G522DR microsomes. A ratio > 1 indicates that the endogenous activity was higher in DK 41Y than in G522DR. Except for the oxidation of metolachlor and possibly cinnamic acid, the DK 41Y microsomes were more active than the G522DR microsomes by factors that ranged from 1.43 for diazinon to 3.49 for bentazon. Responses obtained following safener treatment of DK 41Y seed paralleled those reported herein for G522DR seed.

Discussion

The monooxygenases that catalyzed the oxidation of the five substrates have different properties (based on differential responses to inhibitors, the data for which were not included herein) and may be different isoforms. Enhancement of cytochrome P-450 monooxygenase activities by various chemicals, including safeners, has been reported by other investigators for the oxidation of herbicides, lauric acid, and cinnamic acid (1,2,3,4,6,7).

Table 2. Comparison of oxidative activity between microsomes isolated from grain sorghums with bronze (G522DR) and cream-colored (DK 41Y) endosperms.

Substrate	Cultivar		Ratio DK/G522
	G522DR nmol metab./mg protein/h	DK 41Y	
Cinnamic acid	113	130	1.15
Lauric acid	16	34	2.13
Metolachlor	0.50	0.47	0.94
Bentazon	0.41	1.43	3.49
Diazinon	0.14	0.20	1.43

Differences in the level of the endogenous monooxygenase activities of the two sorghum cultivars suggests that the tolerance of crops to herbicides could be increased through conventional breeding techniques. Increasing the activity of specific monooxygenase isoforms by chemical agents could increase the tolerance of marginally tolerant crop varieties to herbicides and/or provide an additional safety factor for varieties that are already tolerant.

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