Pesticide Biochemistry and Physiology 138 (2017) 22–28

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Isoxadifen safening mechanism in sweet corn genotypes with differential response to P450-metabolized herbicides

Amit Paporisch *, Baruch Rubin

R. H. Smith Institute of Plant Science & Genetics in Agriculture, Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, Rehovot 76100, Israel

ARTICLE INFO

Article history:
Received 11 December 2016
Received in revised form 18 January 2017
Accepted 15 February 2017
Available online 16 February 2017

Keywords:
ben1
Cross-sensitivity
Herbicide detoxification
Herbicide-resistant crop
nsf1
Safener

ABSTRACT

Three sweet corn genotypes, two inbred lines (IBER001 and IBER002) and their hybrid (ER00X), differ in their phenotypic responses to several P450-metabolized herbicides, used in sweet corn, namely, foramsulfuron, iodosulfuron, rimsulfuron and tembotrione. Foramsulfuron is a sulfonylurea herbicide commonly formulated with the safener isoxadifen that is used for selective post-emergence weed control in corn. Our goal was to elucidate the mechanism of these genotypes' responses to foramsulfuron and safener isoxadifen and examine the heritability of those responses. IBER001 was sensitive to foramsulfuron + isoxadifen, with an ED_{50} of 3.6 g ai ha^{-1}, while IBER002 and ER00X were tolerant with ED_{50} values of 808 and 700 g ai ha^{-1}, respectively. ALS enzyme extracted from each of the different genotypes was equally sensitive to foramsulfuron. Pre-treatment with malathion, a known cytochrome P450 inhibitor, increased foramsulfuron injury in IBER002 and ER00X, but had no effect on those lines when isoxadifen was applied with the herbicide. Foramsulfuron-treated IBER001 was severely injured regardless of the presence of malathion and/or isoxadifen. Pre-treatment with malathion similarly increased the phytotoxicity of iodosulfuron + safener (mefenpyr) and rimsulfuron to the tolerant genotypes, but did not increase the level of injury caused by the tembotrione + isoxadifen treatment. Segregation of F2 and backcross progenies according to their responses to foramsulfuron + isoxadifen revealed a pattern of inheritance typical of a trait controlled by a single gene inheritance, with a recessive allele conferring sensitivity. Our results support the hypothesis that foramsulfuron selectivity is associated with P450 metabolism and that isoxadifen positively affects P450 activity. The sensitive genotype that does not respond to isoxadifen is presumably homozygous for a deficient or non-functioning P450 gene.

1. Introduction

Weed control in corn (*Zea mays* L.) is based on the use of several selective pre- and post-emergence herbicides from different chemical families with different modes of action. Corn's ability to avoid injury from sulfonylureas, such as nicosulfuron, chlorsulfuron and rimsulfuron, as well as herbicides with different modes of action, such as bentazon and clomazone, is primarily due to the rapid detoxification of those herbicides via cytochrome P450 (CYP P450) activity [1].

Studies have shown that some sweet corn genotypes are sensitive to several herbicides, with different modes of action, namely mesotrione and tembotrione (4-HPPD), foramsulfuron, rimsulfuron and nicosulfuron (ALS), dicamba (growth regulator) and carfentrazone (PPO). Sensitivity is controlled by a single gene, presumably a mutated CYP P450 gene [2–4].

Foramsulfuron is a sulfonylurea herbicide, used for selective post-emergence weed control in corn. It inhibits the activity of ALS, a key enzyme in the biosynthesis of branched-chain amino acids in plants. Foramsulfuron controls several broadleaf weeds, such as common lambsquarters (*Chenopodium album* L.), redroot pigweed (*Amaranthus retroflexus* L.) and velvetleaf (*Abutilon theophrasti* Medik.), as well as some grasses, including giant foxtail (*Setaria faberi* Herm.) and fall panicum (*Panicum dichotomiflorum* Michx.) [5]. Its common commercial formulations include the safener isoxadifen, in a 1:1 ratio by weight, with recommended field application rate ranging from 37 to 45 g a.i. ha^{-1}.

The term *safeners* refers to chemical agents used to selectively protect crops against herbicide injury. These chemicals reduce the toxicity of the herbicide to the crop without compromising weed control, mainly by enhancing herbicide detoxification in the crop plants [6]. Safeners have been shown to induce several enzymatic reactions related to metabolic detoxification of herbicides in grass crops, namely CYP P450 hydroxylation of nicosulfuron, primisulfuron and bentazon in corn [7],

Abbreviations: ALS, acetolactate synthase; BC, backcross; CYP, cytochrome; PPO, protoporphyrinogen oxidase; UTC, untreated control; 4-HPPD, 4-hydroxyphenylpyruvate dioxygenase.

* Corresponding author.
E-mail address: amit.paporisch@mail.huji.ac.il (A. Paporisch).

http://dx.doi.org/10.1016/j.pestbp.2017.02.002
0048-3575/

enhanced glutathione conjugation of fenoxaprop-ethyl in wheat and barley [8] and elevated activity of adenosine triphosphate sulfurylase and adenosine-5-phosphosulfate sulfotransferase which increase glutathione and cysteine levels in corn roots [9].

Isoxadifen has been shown to exhibit safening activity, protecting corn against herbicide injury [4,10,11], but evidence related to its mode of action is scarce. Bunting et al. [10] found a slight increase in foramsulfuron adsorption, as well as increased translocation to plant parts below the treated leaf, when the herbicide was combined with isoxadifen. In tolerant corn hybrids, isoxadifen application increased the detoxification/degradation of foramsulfuron to polar metabolites and those authors speculated that this effect might be due to induced CYP P450 or glycosyl transferase activity. In a more recent study, Behringer et al. [12] showed that applying a mixture of isoxadifen and mefenpyr to *Arabidopsis thaliana* induced the expression of endogenous xenobiotic-detoxification genes. However, these safeners are not known to protect *A. thaliana* plants from herbicide injury, thus it is not clear whether this gene induction is related to the safening activity.

In this study, we investigated the mechanism of selective responses to foramsulfuron and other selective post-emergence herbicides in three *sh2* sweet corn genotypes, including inbred lines and their hybrid. These genotypes are part of an Israeli breeding program and were found to have different phenotypic responses to foramsulfuron. Our objectives were: 1) to test the response of the above sweet corn genotypes to several CYP P450-metabolized herbicides and 2) to establish the role of the safener isoxadifen in sweet corn's response to foramsulfuron and examine its heritability.

2. Materials and methods

2.1. Plant material and growing conditions

During this study, three different *sh2* sweet corn genotypes were evaluated: two homozygous inbred lines (IBER001 and IBER002) and their hybrid (ER00X). Seeds were stored at 4 °C until use. Unless mentioned otherwise, in all of the experiments described below, corn seeds were planted in 6 × 6 × 7 cm plastic pots filled with commercial growth mixture (Pele-Shacham, Israel), germinated and grown in a net-house equipped with overhead irrigation sprinklers.

2.2. Herbicide applications

2.2.1. Responses of the sweet corn genotypes to different herbicides

Herbicides and safeners were applied at the V2–V4 corn growth stage using a chain-driven sprayer delivering 300 L ha^{-1}. Plants of each genotype were treated with the recommended rates of four commercial herbicide formulations (listed in Table 1). Each experiment included five replicates per herbicide treatment, as well as an untreated control (UTC). Plant injury was monitored weekly. Shoot fresh weight was recorded 14 days after treatment and is presented as the percentage of the fresh weight in the UTC.

2.2.2. Foramsulfuron dose response

Plants of all three genotypes were treated with different rates of a commercial foramsulfuron + isoxadifen in a 1:1 ratio by weight. Different sets of rates were applied to the sensitive and tolerant genotypes, based on preliminary experiments. Inbred line IBER001 was sprayed with 0.78, 1.56, 3.12, 6.24, 12.5, 25, 50 and 100 g foramsulfuron ha^{-1}, and inbred line IBER002 and hybrid ER00X were sprayed with 100, 200, 400, 800 and 1200 g foramsulfuron ha^{-1}.

2.2.3. Split herbicide and safener treatment

Isoxadifen and foramsulfuron analytical standards were obtained from Bayer CropScience, Germany. The responses of the different sweet corn genotypes to isoxadifen and foramsulfuron were determined by applying safener or herbicide, alone (split) or as a mixture. Formulations were prepared as a stock solution by dissolving 100 mg of isoxadifen or foramsulfuron in 1 mL of acetone and then diluting it to the required concentration with 20 g L^{-1} sorbitan monolaurate (Tween 20, Sigma-Aldrich, Israel) in deionized water. The final acetone concentration in the solution did not exceed 0.3%. Herbicide and safener solutions were applied alone or as a mixture, at rates of 22.5 g ha^{-1} each. A commercial formulation of foramsulfuron + isoxadifen was used as a positive control. However, since both the commercial formulation and the self-mixed formulation had similar effects on all three genotypes, only the data concerning the self-mixed formulation is presented in this paper.

2.2.4. Herbicide and malathion treatments

The involvement of CYP P450 in the responses of the different genotypes to the herbicides was tested using the organophosphate insecticide malathion, a known CYP P450 inhibitor [13,14]. Sweet corn plants were treated with 1000 g ha^{-1} malathion (Malathion 50, 500 g L^{-1}, ADAMA, Israel) and left for 1 h in a ventilated room before being treated with either foramsulfuron or isoxadifen applied alone or as a mixture. In addition, IBER002 and ER00X were treated with commercial formulations of 25 g a.i. ha^{-1} iodosulfuron + 75 g a.i. ha^{-1} mefenpyr, rimsulfuron and tembotrione + isoxadifen (as listed in Table 1), following malathion treatment.

2.3. In vitro ALS activity

Acetolactate synthase activity was measured in crude enzyme extracts from etiolated sweet corn seedlings using the method used by Ray [15] with some modifications as described by Sibony et al. [16]. All chemicals used for this procedure were purchased from Sigma, Israel. Sweet corn seeds were germinated in 1-L containers filled with vermiculite, which was treated with 10 g L^{-1} 20-20-20 N-P-K + microelements fertilizer (Deshen-Kol, Ecogan, ELGO, Israel) applied in the irrigation solution, and kept in a dark room at a constant temperature of 25 °C for 7–10 days. The etiolated seedlings were carefully removed from the vermiculite, rinsed with running water, blotted dry between two layers of paper towels and separated into shoots and

Table 1
Sweet corn genotypes (IBER001, IBER002 and ER00X) were evaluated for response to the following post-emergence herbicide formulations.

Herbicide	Safener	Trade name	Herbicide rate (g a.i. ha^{-1})	Safener rate (g a.i. ha^{-1})	Manufacturer
Foramsulfuron	Isoxadifen	Equip	45	45	Bayer CropScience
Iodosulfuron	Mefenpyr-diethyl	Hussar	10[a]	30	Bayer CropScience
Rimsulfuron	None	Titus	12.5		Du Pont de Nemours
Foramsulfuron	Cyprosulfamide	MaisTer Power	47.2	22.5	Bayer CropScience
Iodosulfuron			1.5		
Thiencarbazone			15		
Tembotrione	Isoxadifen	Laudis	91.5[b]	183	Bayer CropScience

[a] Hussar was applied with 0.4% of alkylaryl polyether alcohol (DX spreader, 800 g L^{-1}, ADAMA Agricultural Solutions Ltd., Israel).
[b] Laudis was applied with 1% mineral oil (Organic JMS Stylet Oil, 97.2%, JMS Flower Farm Inc., Florida, USA).