Plant Physiology and Biochemistry 115 (2017) 212–218

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

First confirmation and characterization of target and non-target site resistance to glyphosate in Palmer amaranth (*Amaranthus palmeri*) from Mexico

Jose Alfredo Dominguez-Valenzuela [a], Javid Gherekhloo [b], Pablo Tomás Fernández-Moreno [c], Hugo Enrique Cruz-Hipolito [d], Ricardo Alcántara-de la Cruz [e], Eduardo Sánchez-González [a], Rafael De Prado [c, *]

[a] Department of Agricultural Parasitology, Chapingo Autonomous University, Road México-Texcoco Km. 38.5, 56230 Texcoco, Mexico
[b] Department of Agronomy, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[c] Department of Agricultural Chemistry and Edaphology, University of Cordoba, 14071 Cordoba, Spain
[d] Bayer CropScience, Col. Ampl. Granada, 11520, México D.F, Mexico
[e] Universidade Federal de Viçosa (UFV), Brazil

ARTICLE INFO

Article history:
Received 27 January 2017
Received in revised form
27 March 2017
Accepted 28 March 2017
Available online 31 March 2017

Keywords:
Glyphosate
Herbicide resistance
Metabolism
Palmer amaranth
Translocation
Pro-106

ABSTRACT

Following the introduction of glyphosate-resistant (GR)-cotton crops in Mexico, farmers have relied upon glyphosate as being the only herbicide for in-season weed control. Continuous use of glyphosate within the same year and over multiple successive years has resulted in the selection of glyphosate resistance in Palmer amaranth (*Amarantus palmeri*). Dose-response assays confirmed resistance in seven different accessions. The resistance ratio based on GR_{50} values (50% growth reduction) varied between 12 and 83. At 1000 μM glyphosate, shikimic acid accumulation in the S-accession was 30- to 2-fold higher at compared to R-accessions. At 96 h after treatment, 35–44% and 61% of applied ^{14}C-glyphosate was taken up by leaves of plants from R- and S-accessions, respectively. At this time, a significantly higher proportion of the glyphosate absorbed remained in the treated leaf of R-plants (55–69%) compared to S-plants (36%). Glyphosate metabolism was low and did not differ between resistant and susceptible plants. Glyphosate was differentially metabolized to AMPA and glyoxylate in plants of R- and S-accessions, although it was low in both accessions (<10%). There were differences in 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme activity by 50% (I_{50}) between R- and S-accessions. However, no significant differences were found in the basal EPSPS activity (μmol inorganic phosphate μg^{-1} total soluble protein min^{-1}) between R- and S-accessions. A point mutation Pro-106-Ser was evidenced in three accessions. The results confirmed the resistance of Palmer amaranth accessions to glyphosate collected from GR-cotton crops from Mexico. This is the first study demonstrating glyphosate-resistance in Palmer amaranth from Mexico.

© 2017 Elsevier Masson SAS. All rights reserved.

1. Introduction

The *Amaranthus* genus includes many species some of which are major problematic weeds of warm-season crops, such as soybean (*Glycine* max (L.) Merr.), cowpea (*Vigna unguiculata* (L.) Walp.), corn (*Zea mais* L.) and sugarcane. *A. palmeri* is aannual broadleaf weed with erect growth, deep root system, high water use efficiency (Davis et al., 1964), and which is native to northwestern Mexico and southern California. Currently, *A. palmeri* has been reported as possessing resistance to herbicides with six different modes of action: acetolactate synthase (ALS) inhibitors, enolpyruvil-shikimate-3-phosphate synthase (EPSPS), mitosis inhibitors (dinitroanilines), photosystem II inhibitors (triazines), carotenoid biosynthesis (4-hydroxyphenylpyruvate) inhibitors, and protoporphyrinogen oxidase (PPO) inhibitors (Heap, 2017).

* Corresponding author.
E-mail addresses: jose_dv001@yahoo.com.mx (J.A. Dominguez-Valenzuela), gherekhloo@yahoo.com (J. Gherekhloo), pablotomas91@hotmail.es (P.T. Fernández-Moreno), hugo.cruzhipolito@bayer.com (H.E. Cruz-Hipolito), rychar419@gmail.com (R. Alcántara-de la Cruz), eduardosanchezgonzalez@hotmail.es (E. Sánchez-González), qe1pramr@uco.es (R. De Prado).

http://dx.doi.org/10.1016/j.plaphy.2017.03.022
0981-9428/© 2017 Elsevier Masson SAS. All rights reserved.

The adoption of glyphosate-resistant (GR)-cotton crops, and the dependence on glyphosate as the only weed control tool, have resulted in the appearance of herbicide-resistant weed species. Glyphosate (N-(phosphonomethyl)-glycine) is one of the world's most important non-selective post-emergence herbicides. Glyphosate (HRAC group G) inhibits the enzyme 5-enolpyruvilshikimate-3-phosaphate synthase (EPSPS), which prevents the biosynthesis of aromatic amino acids (Gianessi, 2005; Sammons and Gaines, 2014; James, 2015; USDA, 2017; HRAC, 2017).

In the recent years, the number of glyphosate-resistant weeds has grown rapidly, currently amounting to 37 species worldwide (Heap, 2017). Resistance to glyphosate is generally due to two mechanisms broadly classified as target site resistance (TSR), and non-target site resistance (NTSR) (Shaner et al., 2012; Fernández et al., 2015). TSR mechanisms found to confer resistance to glyphosate are the result of changes to the encoding of the EPSPS gene (González-Torralva et al., 2014; Yu et al., 2015; Fernández et al., 2015; Alarcon-Reverte et al., 2015), or overexpression of the EPSPS protein by gene amplification (Gaines et al., 2010; Salas et al., 2012, 2015; Ribeiro et al., 2014). On the other hand, NTSR mechanisms found to confer resistance to glyphosate consist of reduced absorption and translocation (Vila-Aiub et al., 2012; Fernández-Moreno et al., 2017), increased vacuolar sequestration (Ge et al., 2012), and metabolism to non-toxic compounds (Rojano-Delgado et al., 2012; de Carvalho et al., 2012), all of them resulting in lesser glyphosate translocation to EPSPS.

In 1998, GR-cotton crops were introduced into Mexico, and were easily adopted by Mexican farmers resulting in a complete transformation of weed management. To date, GR-cotton crops represent 85% of the total fields in Mexico. In these fields, two glyphosate applications per year (720 g ha^{-1}) during several years were generally used. In recent years, due to the weak control of *A. palmeri*, farmers have increased glyphosate dose to 1800 g ha^{-1} in some fields, applying it annually and without any rotation with another crop (Cruz-Hipolito, personal communication).

This is the first case of herbicide resistance by *A. palmeri* documented from Mexico. The main objectives of this study were: a) to determine the resistance index (RI) of the accessions collected, and b) to determine the mechanism(s) responsible for conferring glyphosate resistance in the *A. palmeri* accessions from Mexican fields.

2. Material and methods

2.1. Plant material

Mature seeds of resistant (R) *A. palmeri* accessions (Ch1, Ch2, Ch3, Ch4, Ch5, Ch6, and Ch7) used in this study were collected from fields in Chihuahua State (Northern Mexico) in 2015. Seeds of susceptible (S) accession were also collected in 2015 from an area of Durango State, in which herbicides had never been applied. To conduct the experiment, seeds were germinated in Petri dishes with filter paper moistened with distilled water and placed in a growth chamber at 28/18 °C (day/night) with a photoperiod of 16 h, 850 μmol m^{-2} s^{-1} photosynthetic photon flux, and 80% relative humidity. All seedlings were transplanted into pots containing sand/peat in a 1:2 (*v/v*) ratio, and placed in a greenhouse at 28/18 °C (day/night) with a 16 h photoperiod.

2.2. Dose-response assays

The experiment was conducted with ten replications (individual plants) per each accession at 8 doses including 0, 31.25, 62.50, 125, 250, 500, 1000, 2000, and 4000 g ae ha^{-1} of glyphosate (Roundup Energy® SL, 450 g ae L^{-1} as isopropylamine salt, Monsanto). The herbicide doses were applied at the 3- to 4- leaf growth stage of Palmer amaranth. Glyphosate was applied in a laboratory chamber (SBS-060 De Vries Manufacturing, Hollandale, MN, USA) equipped with 8002 flat fan nozzles delivering 200 L ha^{-1} at the height of 50 cm from plant level. Plant mortality (LD) and fresh weight reduction (GR) were measured 21 days after treatment.

2.3. Shikimic acid accumulation

Fifty 4-mm leaf disks were harvested from the youngest fully expanded leaf from a pool of 15 plants per accession at the 3- to 4-leaf stage and analyzed for shikimate. Five disks of fresh tissue were transferred to 2 mL Eppendorf tubes for each accession. One μL of glyphosate was added to each of the tubes at the following concentrations: 0, 0.1, 0.5, 1, 5, 10, 50, 100, 200, 400, 500, 600, and 1000 μM, following the methodology described by Fernández-Moreno et al. (2016). For each glyphosate concentration and accession, four replications were made and the assay was repeated twice.

2.4. ^{14}C-glyphosate absorption and translocation

The experiment was carried out according to the methodology of Fernández-Moreno et al. (2017). ^{14}C-glyphosate (American Radiolabeled Chemicals, Inc., Saint Louis, MO, USA) was added to commercial glyphosate with a specific activity of 0.834 KBq μ^{-1}. The final glyphosate concentration corresponded to 300 g ae ha^{-1} applied at 200 L ha^{-1}. The plants of each accession at the 3- to 4-leaf growth stage were treated with a 1-μL drop (0.834 KBq plant) placed with a micropipette (LabMate) onto the adaxial surface of the second leaf. At 12, 24, 48, 72, and 96 h after treatment (HAT), the treated leaf was washed with 3 mL of water: acetone (1:1 v/v) solution to remove the non-absorbed label. The rinsate was mixed with 2 mL of scintillation cocktail and analyzed by liquid scintillation spectrometry (LSS) on a scintillation counter (Beckman LS 6500, Fullerton, CA, USA.). The remainder of the plant was removed from the pot, and its roots were carefully washed with distilled water. The plant was divided into treated leaf, rest of shoots, and roots. The plant parts thus obtained were dried at 60 °C for 96 h and combusted in a Packard Tri Carb 307 biological sample oxidizer. Five replications (individual plants) of each accession were used, and the experiment was arranged in a completely randomized design. The assays were repeated twice. The proportion of absorbed herbicide was expressed as [kBq in combusted tissue/(kBq in combusted tissue + kBq in leaf washes)] × 100.

2.5. Glyphosate metabolism

Commercial glyphosate was applied at 300 g ae ha^{-1} as explained above. At 96 HAT, glyphosate and its metabolites, i.e., AMPA (aminomethylphosphonic acid), glyoxylate and sarcosine were determined by reversed-polarity capillary electrophoresis following the methodology described by Rojano-Delgado et al. (2012). The calibration equations were established using non-treated plants and known concentrations of glyphosate and its metabolites, which were determined from the enclosed areas under the peaks in the electrophoregram (Gonzalez-Torralva et al., 2012; Fernández et al., 2015; Fernández-Moreno et al., 2016). The experiment was arranged in a completely randomized design with five replications (individual plants) per accession and repeated three times.

2.6. EPSPS enzyme activity assays

The enzyme extraction was conducted according to Dayan et al.

Download English Version:

https://daneshyari.com/en/article/5515386

Download Persian Version:

https://daneshyari.com/article/5515386

Daneshyari.com