Crop Protection 78 (2015) 15–19

Contents lists available at ScienceDirect

Crop Protection

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

Resistance to imazamox in Clearfield soft wheat (*Triticum aestivum* L.)

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ARTICLE INFO

Article history:
Received 11 February 2015
Received in revised form
5 August 2015
Accepted 9 August 2015
Available online 24 August 2015

Keywords:
Clearfield wheat
Imazamox resistance
ED_{50}
I_{50}
Resistance mechanisms
Photosynthesis activity

ABSTRACT

Imidazolinone (IMI)-resistant crops exhibit relative tolerance to herbicides that inhibit the enzyme acetolactate synthase (ALS). The principal objective of this work was to evaluate the different resistance levels to imazamox between five Clearfield wheat cultivars. The IMI-resistant wheat cultivars (Bicentenario, Dollinco, Impulso, Invento, and Ikaro), widely planted in large areas in Latin America, were compared to a sensitive cultivar (Pandora) using several *in vivo* and *in vitro* experiments. The imazamox dose, expressed as g ai ha^{-1} that reduced the wheat fresh biomass by 50% (ED_{50}), ranged from 151.0 (Ikaro) to 1.6 (Pandora). The herbicide concentrations (μM) that inhibited the ALS activity by 50% (I_{50}) were in agreement with the ED_{50} values, suggesting that imazamox resistance could be due to a mutation in the ALS enzyme. The order of Clearfield wheat cultivars by the level of resistance to imazamox was: Ikaro > Impulso > Invento > Bicentenario = Dollinco >>> Pandora.

The finding that IMI-resistant wheat cultivars regained their photosynthetic activity with time and the fact that Ikaro plants showed an increased level of resistance over time suggest that other resistance mechanisms might be involved in the differential tolerance to imazamox in these wheat cultivars.

1. Introduction

The wheat is one of the major world crop with a production exceeding 650 million tons. Weeds are included among the major biotic limitations to crop productivity (Shennan, 2008). Unfortunately, weeds have been and are a barrier which reduce the quality and quantity of this important food. The weeds establish a relationship of competition with the main crop to obtain water and nutrients, essential for growth and development of plants. This competition can reduce more than 50% of the yield (Munsif et al., 2014).

The control of several weeds harmful to wheat has improved since the 1980's with the release of herbicides that inhibit the enzyme acetolactate synthase (ALS). This group of herbicides comprises five chemical families: imidazolinones (IMIs), sulfonylureas, triazolopyrimidines, pyrimidinylthiobenzoates, and sulfanilamide-carbonyl-thiazolidinones (Devine et al., 1993; Pang et al., 2003; McCourt et al., 2006; Yu and Powles, 2014).

In partnership with the BASF (Badische Anilin und Soda Fabrik) (BASF, 2010) company, agricultural research institutes in Chile have developed, through classical plant breeding methods (mutagenesis, plant selection, and back crosses with elite cultivars), several wheat cultivars resistant (R) to IMI herbicides, commercialised under the trade name "Clearfield crops" (Newhouse et al., 1992). Some of these are Bicentenario, Dollinco, Ikaro, Impulso, and Invento.

In general, the possible herbicide resistance mechanisms in plants comprise altered herbicide retention on the crop leaf surface, impaired uptake, impaired translocation and/or vacuolar sequestration, herbicide detoxification by the plant, as well as insensitivity of the target enzyme to the herbicide (Park and Mallory-Smith, 2004; Tan et al., 2005; Ge et al., 2010; Powles and Yu, 2010; Rosario et al., 2011; Yu and Powles, 2014).

The development of Clearfield wheat cultivars that are resistant to IMI herbicides is one approach to increasing wheat grain yields. A resistant crop to imazamox is normally attributed to a mutation on the target site enzyme acetolactate synthase (ALS or EC 2.2.1.6) (Shaner et al., 1996; Anderson et al., 2004). However, other resistance mechanisms cannot be excluded (Tan et al., 2005).

Due to the ALS enzyme functions at the chloroplast level, it has also been hypothesised that variables such as photosynthesis

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http://dx.doi.org/10.1016/j.cropro.2015.08.004
0261-2194/

activity and chlorophyll content might be indirect indicators of other herbicide resistance mechanisms in these wheat cultivars. Profound changes occur shortly after the application of ALS-inhibiting herbicides (Blair and Martin, 1988; Devine, 1989; Gaston et al., 2003). Photosynthesis is not considered a primary target of ALS-inhibiting herbicides, but changes in photosynthesis-related parameters and responses have been observed in treated plants (Riethmuller-Haage et al., 2006). The measurements of the changes in chlorophyll content and photosynthetic activity provide a rapid, non-destructive and simple method for monitoring the physiological status of the photosynthetic apparatus in the plant. This method can be used to study the effects of PSII-inhibiting herbicides as well as those of herbicides with other modes of action (Christensen et al., 2003).

Thus, the specific objectives of this work were to evaluate the resistance level to imazamox (IMI) in five Clearfield wheat cultivars through: a) whole plant assays and herbicide retention experiments; b) measurements of ALS activity and c) measurements of photosynthetic activity. The results obtained in the specific objectives may suggest different resistance mechanisms those already known in these cultivars, which would also entail a further objective to the principal one.

2. Material and methods

2.1. Chemical

The herbicide and reagents used in this study were the following: A technical grade imazamox (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid), a commercial herbicide formulation (imazamox, Pulsar 40, 4% w/v SL) and all other reagents were with analytical grades.

2.2. Plant material and dose–response experiments

One hundred fifty seeds of Bicentenario, Dollinco, Ikaro, Impulso, Invento and Pandora S cultivars were placed in 1 L capacity pots containing a peat:sand mixture (1:1) and the pots were kept in the growth chamber with day/night temperature of 27/18 °C and 14/10 h photoperiod, a light density of 250 mmol m^{-2} s^{-1}, and 80% relative humidity. Plants were treated at 3–5 leaves growth stage. In subsequent experiments, herbicides were applied using a laboratory track sprayer delivering 250 L ha^{-1} at 250 kPa. The herbicide concentrations were expressed as the active ingredient (ai). The adjuvant used was methylated seed oil at 435 g ai ha^{-1} (DASH, 34.8% w/v, CE, BASF) in all treatments and the shoot fresh biomass was evaluated 21 days after treatment (DAT).

Imazamox (4% w/v, SL, BASF) doses were 0, 1, 2.5, 10, 20, 40, 80, 120, 160, and 240 g ai ha^{-1}. The herbicide dose that caused a 50% reduction in growth with respect to the untreated control (ED_{50}) was determined for all cultivars. The treatments were replicated ten times and the assays were conducted twice. The resistance factor (R/S ratio) was computed as ED_{50}(cultivar R)/ED_{50}(cultivar S).

2.3. ALS enzyme activity assays

The ALS activity was measured by determining the formation of the acetoin product after the acid decarboxylation of acetolactate in an acidic medium, which was detected as a coloured complex and determined at 520 nm following the protocol described by Osuna and De Prado (2003). The plant tissue for the assays was obtained from wheat plants established and grown under the conditions described previously (Rosario et al., 2011). It was assayed in triplicate for each concentration and cultivar. The assay was conducted twice. The herbicide concentration (μM) required to inhibit 50% (I_{50}) of the ALS activity was calculated as previously described by Osuna and De Prado (2003). The resistance factor was computed as I_{50}(cultivar R)/I_{50}(cultivar S).

The total protein content in the crude extract was measured using the colourimetric method described by Bradford (1976) following the instructions provided in a commercial kit for protein determination (Sigma; Procedure No. P5656).

2.4. Retention assays

Herbicide retention assays were made using a method adapted from Menendez et al. (2011) with 10 replicates and the assay was conducted twice. The plants were sprayed at the two-leaf stage in the same way as in the bioassays with a spray solution containing imazamox at 40 g ai ha^{-1} plus adjuvant (DASH, 34.8% w/v, CE, BASF) was labelled with a dye (fluorescein at 100 mg L^{-1} of 5 mM NaOH) and applied to plants with the equipment already described under whole plant assays. After the spray had dried on the foliage, the plants were cut at ground level and shaken for 30 s in 50 mL of 5 mM NaOH. Readings were made with a spectrofluorimeter at 490/510 nm. Plants were then placed at 80 °C for 24 h, and dry matter was weighed.

2.5. Photosynthetic activity

The greenhouse experiment was established to know the effect of imazamox on the photosynthesis activity of wheat cultivars (Ikaro, Impulso, Invento, and Pandora S). The Imazamox doses were 0, 40, 80 and 120 g ai ha^{-1}. The herbicide was applied under the same conditions as above and when the cultivars were at the tillering stage. The adjuvant DASH at 1.25 L ha^{-1} was used in all treatments.

Photosynthetic activity was measured using a portable gas analyser (LiCor Inc., Model 6400, Lincoln, USA). The instrument was adjusted to have constant conditions of CO_2 concentration (400 ppm), flow (500 cm^3 min^{-1}), leaf temperature (25 °C) inside the leaf chamber, and PAR (photosynthetic active radiation) of 1500 μmol photons m^{-2} s^{-1}. The collection of photosynthesis data began one week after the application of the treatment at the tillering stage and was conducted for four weeks. Measurements were made using the second leaf below the flag leaf. Ten measurements were taken per leaf, and the average value was calculated. The treatments were replicated ten times and the assays were conducted twice.

2.6. Statistical analyses

The ED_{50} and I_{50} were calculated using a log-logistic model using Sigma Plot software (version 11.0) from Systat Software, Inc; the data were adjusted to a non-linear regression curve (Seefeldt et al., 1995; Rosario et al., 2011) whose statistical model is:

$$Y = c + \left\{ (d - c) \Big/ \left[1 + (x/g)^b \right] \right\}$$

Where *Y* is expressed as a percentage of the value for untreated plants; *c* and *d* are the lower and upper asymptotes, respectively; *b* is the slope of the curve; *g* denotes ED_{50} or I_{50} (which coincided with the point of inflection halfway between the upper and lower asymptotes); and x is an independent variable representing the herbicide rate.

The data obtained in the spray retention assays were subjected to ANOVA. The Tukey HSD (honestly significant difference) test at 5% probability was used to separate means. Statistical analyses were performed using Statistix software (version 9.0) (also the