Euphytica (2006) 152:75–86
DOI 10.1007/s10681-006-9180-x

Risk assessment of the transfer of imazethapyr herbicide tolerance from Clearfield rice to red rice (*Oryza sativa*)

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Received: 27 October 2005 / Accepted: 10 May 2006

Abstract Hybridization between Clearfield rice and weedy red rice would have a direct impact on management and long-term strategies of imazethapyr technology for rice weed control. The objective of this research was to determine rates and agronomic consequences for outcrossing between Clearfield rice and red rice. Red rice populations showed extensive variation for plant height, panicle length, tillers/plant, seeds/plant, seed set and grain weight. Outcrossing was detected from all Clearfield rice cultivars ('CL121', 'CL141', 'CL161', and 'CLXL8') to red rice and was confirmed by phenotypic and DNA marker analyses. An overall outcrossing frequency of 0.17% was observed in 2002 red rice samples with a range from 0% to 0.46%. Tolerance of 2002 red rice samples to imazethapyr corresponded to levels of acetohydroxyacid synthase (AHAS) activity. A majority (94%) of the progeny from the 2002 samples segregated 3 resistant:1 susceptible for tolerance to imazethapyr, indicating that a single dominant gene from Clearfield rice was associated with tolerance in the hybrid material. The remaining samples did not segregate for tolerance, suggesting that spontaneous mutations for tolerance were present in this material before or after crossing with Clearfield rice. A four-fold increase in outcrossing frequency of 0.68% was observed in 2003 red rice samples with the highest outcrossing frequency for a single location at 3.2%. Results from this study indicate that outcrossing between Clearfield and red rice will occur rapidly at rates that warrant early-season field scouting and a crop rotation scheme to prolong usefulness of the Clearfield technology.

Keywords Clearfield rice · Imazethapyr · Outcrossing · Risk assessment · Red rice

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Introduction

Red rice (*Oryza sativa* L.), with the same genus and species as cultivated rice (Gianessi et al. 2002), is a troublesome weed in most rice growing regions of the world (Fisher & Ramirez 1993), and a noxious pest for rice production in the southern United States (Oard et al. 2000). U.S. red rice biotypes consist primarily of straw-hulled awnless and black-hulled strains, but brown-hulled, golden-hulled (Noldin et al. 1999), and gray-hulled types have also been observed in other regions of the world (Constantin 1960). Red rice decreases the yield of cultivated rice due to its competition

for sunlight, water and nutrients. Seed shattering and dormancy result in prolonged viability in the soil that ultimately affects production and quality of the commercial crop (Lago 1982). Presence of a red pericarp in the weed seed reduces market value of the commercial product during the milling process (Dilday et al. 1990).

Although rice is considered a self-pollinating crop, spontaneous hybridization has been documented between the African cultivated rice *O. glabberima Steud.* and its weedy relative *O. breviligula Chev.et Roehr* (Oka & Morishima 1971). Asian cultivated rice *O. sativa* L. was reported to hybridize with *O. perennis Moench* (Oka & Morishima 1971) and *O. rufipogon Griff.* (Chen et al. 2004; Song et al. 2004). Frequency of hybridization between cultivated rice and weedy biotypes in small plot studies was reported to be markedly affected by different cultivars. Chen et al. (2004) and Rong et al. (2004) detected variable outcrossing rates between traditional and hybrid rice under different planting designs. Langevin et al. (1990) found that the highest outcrossing rates occurred during overlap of flowering time between cultivated and weedy plants. Zhang et al. (2003) detected a 0.33% outcrossing rate from red rice to a transgenic line expressing the *bar* gene under small research plot conditions. No outcrossing was detected from the transgenic line to red rice. Recent small-plot research showed that the ALS gene from Clearfield cultivar CL161 was transferred by natural outcrossing to produce imazethapyr-tolerant red rice hybrids (Rajguru et al. 2005).

AHAS is a critical enzyme in plants catalyzing the first step in the biosynthesis of branched-chain amino acids leucine, isoleucine and valine (Tan et al. 2005). Single nucleotide substitutions in the gene encoding AHAS were found to confer imazethapyr tolerance in rice and other crops. The rice cultivar '93AS3510' was previously treated with the chemical mutagen ethyl methyl sulfonate (EMS) for selection of an imazethapyr-tolerant line that was crossed with the cultivar 'Cocodrie' to produce the Clearfield cultivar 'CL121' (Croughan 1994). The imazethapyr-tolerant line was also crossed with the cultivar 'Maybelle' to produce Clearfield 'CL141' while Clearfield 'CL161' was derived from EMS-treated seed of the cultivar 'Cypress' (Gealy et al. 2003; Wenefrida et al. 2004). The hybrid cultivar CLXL8 was developed and released from the RiceTec Corporation (Anonymous 2005). Clearfield rice in combination with imidazolinone herbicides is an effective management tool to control red rice in cultivated rice (Tan et al. 2005). Because red rice and cultivated rice belong to the same species, transfer of imazethapyr tolerance from Clearfield rice to red rice should be minimized or prevented to preserve this weed control technology. No comprehensive study concerning hybridization between Clearfield rice and red rice in commercial fields and the consequences for red rice control has been conducted. The objectives of this study were to determine the frequency of outcrossing from Clearfield rice to red rice under commercial field conditions and to formulate management strategies to prolong utility of Clearfield technology for red rice control.

Materials and methods

Plant material, traits evaluated, and field locations

Twenty-four commercial locations in southwest Louisiana, each ∼12 to 20 hectares in size, were selected for outcrossing studies in 2002 and 2003 (Table 1). Ten locations were planted in 2002 with CL121 and two with CL141. Location 11 was sampled from the first and second (ratoon) crop plants. The following year, 10 locations were evaluated with CL161 and one location each with CL121 or CLXL8. No field site in 2002 was common with those evaluated in 2003, and no Clearfield rice was planted previously at any of the 24 sites. The first flowering date and visual estimates of percent red rice infestation after imazethapyr application were recorded for all locations across both years. Data for the following traits were collected in both years for 100 randomly-selected red rice plants and 10 Clearfield plants at each location: plant height, measured at maturity from the soil surface to the tip of panicle of main stem, panicle length, seeds per panicle, seeds per plant, spikelets/panicle, seed set rate (seeds/panicle divided by spikelets/panicle) and 100 grain weight calculated by 100 × seed weight/plant divided by seeds/plant. Harvested panicles were dried at 50°C for four days to ∼12% moisture. All seeds were placed into paper bags and stored at 4°C in the dark until planting the following year.

The identification and evaluation of putative hybrids produced between Clearfield and red rice in both years were conducted in field plots at the Ben Hur Farm, Baton Rouge, Louisiana. The field layout in 2003 was 73.2 m × 30.5 m in a completely randomized design of 2.4 m single row plots. Approximately 3.5 g seeds