

Introduction

Hybridization between crops and their wild relatives sometimes brings genes into wild populations, occasionally resulting in the evolution of aggressive weeds and/or endangerment of rare species. Transgenic crops may also result in similar outcomes. The likelihood of crop-to-wild hybridization depends on the out-cross rate, and on distance and direction between wild and crop populations. Cultivated rice, *O. sativa* L., is an autogamous plant, with a low out crossing rate of 0-1% (Roberts et al. 1961). Rice is an introduced species in the Neotropics from Africa and Asia, but with wild/weedy relatives including wild native species in Central and South America. Hybridization can be expected within the genomic group that includes *O. sativa*, viz., the AA group. The wild relatives of AA genome, which are found in Central and South America and may hybridize with the rice crop, include *O. rufipogon* and *O. glumaepatula* (Oka and Chang, 1961; Vaughan and Tomooka, 1999). Red rice (*Oryza sativa* f. *spontanea*) is a weedy rice with a red pericarp and dark-colored grains. The seeds shatter readily and possess dormancy characteristics. The plants typically are tall, late maturing, and have pubescent leaves and hulls (Figure 1). In contrast to Asia where manual transplanting is still predominant, in tropical America direct seeding of red rice-contaminated seed source is common, making red rice the most serious weed problem. Genes from rice varieties may transfer quickly into red rice (1% to 52% hybridization rate) (Langevin et al. 1990). However, most of the hybridization rate estimates have been done under temperate conditions. This work is part of a project directed to analyze the gene flow from non-transgenic or transgenic rice into wild/weedy relatives in the Neotropics, and its effect(s) on the population genetic structure of the recipient species.



Figure 1. (A) Rice variety; (B) Red rice plant; (C) Seed shattering from red rice panicle; (D) Red rice plant showing red ligules and tiller.

Objective

Setting up tools to assess gene flow from transgenic and non-transgenic rice into red rice under field conditions. As a first step a morphological and phenological characterization was conducted from field specimens collected from farmer's fields in the main rice-cropping region of Colombia, South America.

Materials and methods

Collection of Red Rice Samples in the Field

Various commercial rice field plots were selected at Tolima, the major rice-cropping area in Colombia. Plots with known cropping history were selected. Plots had been planted with the same variety for at least the last eight growing seasons (two years), and included one of the 4 more widely grown commercial varieties: Fedearroz 50 currently grown by 80% of farmers; Oryzica 1 the previous most popular variety cultivated before Fedearroz 50 was released; and Coprosem 1 or Cimarron. A total 158 red rice plants and their corresponding seeds were harvested from each plot. Samples were grouped in populations based on their origin according to the commercial variety grown in the field. The Fedearroz 50 population is comprised by samples from different locations. Plant and seed data was collected from field collections, and a sample was stored for herbarium record. Seeds were increased for field experiments.

Morphological and Phenological Characterization

The morphological characterization was conducted using 16 qualitative and 9 quantitative descriptors. The phenology characterization included 6 descriptors. Plant and seed morphological evaluations were conducted using the samples collected from the field (F0 generation). The phenological characterization was performed in the greenhouse (F1 generation) and in the field (F2 generation) using progeny plants obtained through selfing of each F0 plant. The corresponding rice varieties grown in the field at the moment of the field sample collection, a transgenic Cica 8 rice variety, and the wild species *O. rufipogon*, *O. glaberrima*, and *O. barthii* were used as controls.

Data Analysis

Principal component and coordinate analysis were conducted using a SAS statistical package program.

Results and Discussion

Seed parameters allowed a better characterization of the red rice populations collected. The analysis of the 1650 seeds indicated broad husk color diversity, varying from pale yellowish color (like the commercial varieties) to dark brown color (like the wild rice species) (Figure 2). The widest diversity was noted in the populations derived from the Oryzica 1 and Fedearroz 50 plots, where about 50% of the red rice biotypes showed grains with husk color alike the corresponding commercial variety, from 2% to 15% of biotypes had husk color alike the wild rice species, and the other biotypes with husk colors corresponding to either golden, golden stripes, or brown stripes. In contrast, about 40% of the red rice biotypes derived from the Coprosem 1 and Cimarron plots showed dark brown grains alike the wild species, 0% and 10% of the red rice with grains alike the varieties, and only one or two of the other husk color categories. Similar patterns were noted when the awn, the apiculus, and the pericarp were characterized.

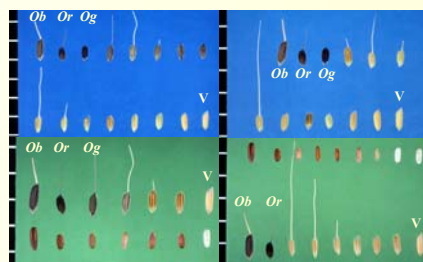


Figure 2. Seed color variation in red rice from field samples. Ob = *O. barthii*; Or = *O. rufipogon*; Og = *O. glaberrima*; v = rice variety

A correlation analysis between grain length and grain width also showed that the red rice populations derived from the Coprosem 1 and Cimarron field plots were the least diverse, and clearly distinct from the rice varieties. The red rice population derived from the Oryzica 1 plot was the most diverse. While some biotypes showed shorter and thicker grains alike the wild species *O. rufipogon* and *O. glaberrima*, others showed long and slender grains alike the crop (Figure 3). Few of the samples from the Fedearroz 50 plot had long and slender grains. *Oryza barthii* had extra long and thick grains (Figure 3). Those red rice biotypes undistinguishable from the variety by their grain traits still showed seed shattering characteristic of the weedy rice.

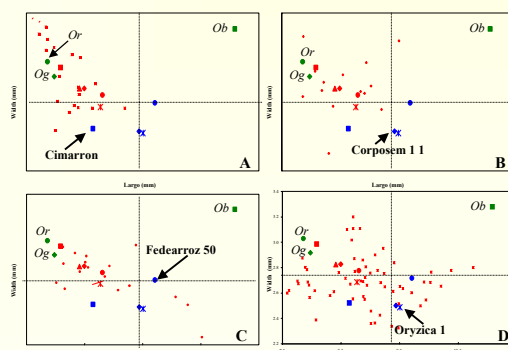


Figure 3. Distribution of grain size according to length and width of red rice samples (red points) collected from each plot (A) Cimarron; (B) Coprosem 1; (C) Fedearroz 50; and (D) Oryzica 1. Arrows indicate each corresponding variety (blue points). Wild species (green points) *O. rufipogon* (Or), *Oryza glaberrima* (Og), *Oryza barthii* (Ob)

Principal component analysis using the 9 quantitative morphological descriptors indicated that 77% of the variability could be explained by 4 main components, where grain length, width and thickness, jointly with length between nodes, and width of the flag leaf and the previous leaf were the most important. The coordinate principal component analysis using the 16 qualitative morphological parameters allowed to group the 158 red rice biotypes in three major clusters mainly characterized by the presence or absence of grain awn, and dark brown husk color, respectively (Figure 4). Results indicated a significant similarity between some red rice biotypes and rice varieties. Likewise a tight association was noted between various red rice samples and the wild *Oryza* species, especially with *O. rufipogon* (Figure 4). Of the phenological traits evaluated days to flowering (DTF, 50% anthesis) is the most relevant when considering likelihood of gene flow. The Ryan-Einot-Gabriel-Welsch multiple range test ($p > 0.005$) discriminated three groups: early flowering (*O. glaberrima*, mean DTF 85; and *O. barthii*, mean DTF 88), intermediate flowering (all red rice populations, rice varieties and *O. rufipogon*, mean DTF from 91 to 111), except rice varieties Cica 8 and Fedearroz 50 which were classified as late flowering according to this test (mean DTF from 112 to 116). Most red rice biotypes collected from the Fedearroz 50 plots were earlier flowering respect to this variety, but flowering of various individual biotypes overlapped with both Fedearroz 50 and Cica 8. No clear flowering pattern was noted respect to the variety previously grown in the plot (Figure 5).

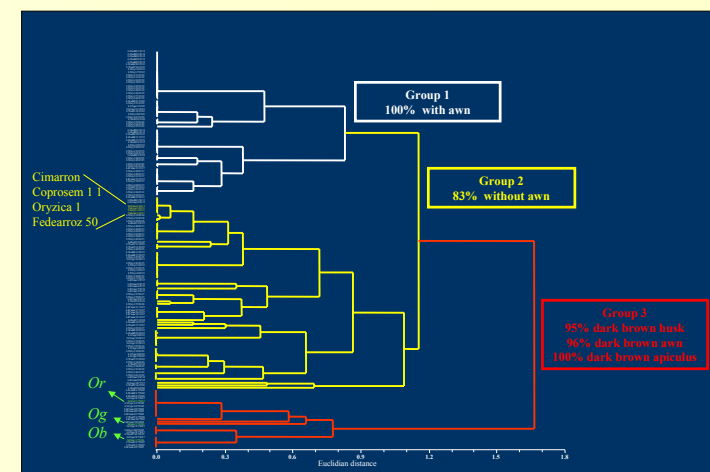


Figure 4. Coordinate principal component analysis using 16 qualitative morphological parameters. Ob = *O. barthii*; Or = *O. rufipogon*; Og = *O. glaberrima*

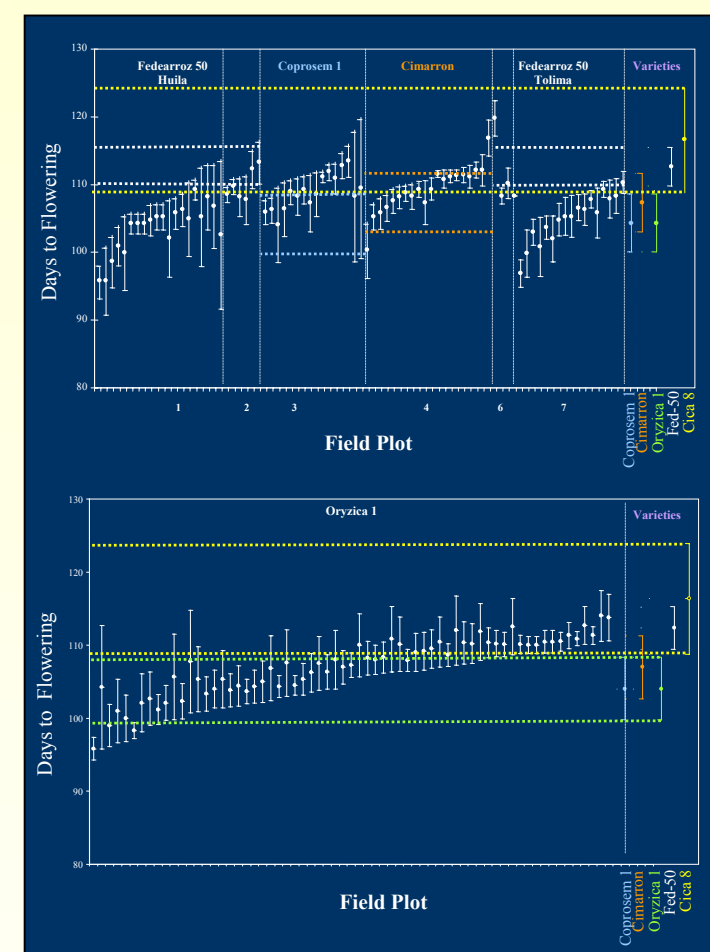


Figure 5. Days to flowering of each red rice biotype collected and corresponding variety being grown in the field at sampling time. Flowering of transgenic Cica 8 variety was included as a reference.

Conclusions

- Seed traits significantly accounted for most of the variability found in the red rice populations collected. The presence/absence of awn and husk color grouped the biotypes in three major clusters. Seed qualitative traits jointly with the relation grain length to width easily allows to identify the red rice varietal types and those similar to wild species from the regular weedy types.
- Large number of red rice biotypes overlap in flowering with cultivated varieties. No clear influence in flowering pattern was noted respect to the variety previously being grown in the field.
- Based on this morphological and phenological characterization, some red rice biotypes had been selected to conduct gene flow analysis and identify indicators for an easy trace of gene flow in the field over subsequent generations.

References

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