Coexistence of Weedy Rice and Rice in Tropical America: Gene Flow Analysis

Luisa F. Fory, Rosana Pineda, Eliana González, Angela Mina, Juan C. Florez, Kiliany Arcia, Myriam C. Duque and Zaida Lentini,
CIAT (International Center for Tropical Agriculture) A.A 6313. Cali, Cali, Colombia.
E-mail: z.lentini@cgiar.org.

Introduction

One of the major obstacles to rice production in Latin America is the lack of stable yields due to several major diseases and pests, in addition to weeds, whose control accounts for about 30% of the production costs. Of the weeds affecting rice production, weedy rice is the main constraint. The presence of weedy rice in paddy fields is frequently the result of the predominant use of farmer-saved seed instead of certified seed source, and of direct seeding system for rice cultivation by farmers. The problem is exacerbated by the lack of crop rotation and the common practice of several crop cycles per year. In contrast to temperate regions where weedy rice is mainly composed by *Oryza sativa f. spontanea* (red rice), in tropical America the weedy rice complex is broadly diverse and apparently composed by numerous *Oryza* species (mostly annual and diploid, AA genome), usually with feral traits (taller, awned seeds with red pericarp, and shattering), varying degree of sexual compatibility (Oka and Chang, 1961; Vaughan and Tomooka, 1999), and flowering overlapping with the crop in different environments. There are indications that under temperate conditions genes placed in rice may transfer quickly into weedy rice (Langevin et al. 1990). Gene introgression not only presupposes physical proximity of the crop to its wild/weedy relative(s) and overlapping flowering so pollination can effect gene transfer, but also genetic compatibility of the crop and its immediate wild/ weedy relative(s) as well as fitness of the derived hybrid. 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. The current report summarizes the progress on setting up the use molecular markers to assess and trace gene flow from transgenic and non-transgenic rice into weedy rice under controlled conditions in confined field plots. The morphological, phenological, and genetic diversity of weedy rice collected from farmers fields in Colombia was initially analyzed to determine the genetic structure prior to gene flow, and to select the best combinations of transgenic or non-transgenic rice and weedy types to assess the gene flow.

Materials and Methods

Plant material

Six weedy rice accessions representing the diversity of types found in the plots of Colombian farmers (Figure 1) were selected to conduct gene flow analysis and identify indicators for easy tracing and monitoring of genetic introgression in the crop-weedy rice complex. The selection included weedy types susceptible to the rice hoja blanca virus (RHBV), with high flowering synchrony with rice, and easily distinguishable using specific microsatellite markers detecting polymorphism for various SSR specific alleles. Gene flow was assessed using as pollen donor a transgenic line (A3-49-60-12-3/Cica 8-2) carrying the RHBV-N protein-transgene conferring resistance to RHBV, and the *gus* and *hph* (hygromycin resistance) marker genes, and a non-transgenic rice variety locally known as Purple, characterized by having purple leaves, tillers, and grain apiculus, and dominant inheritance of anthocyanins.

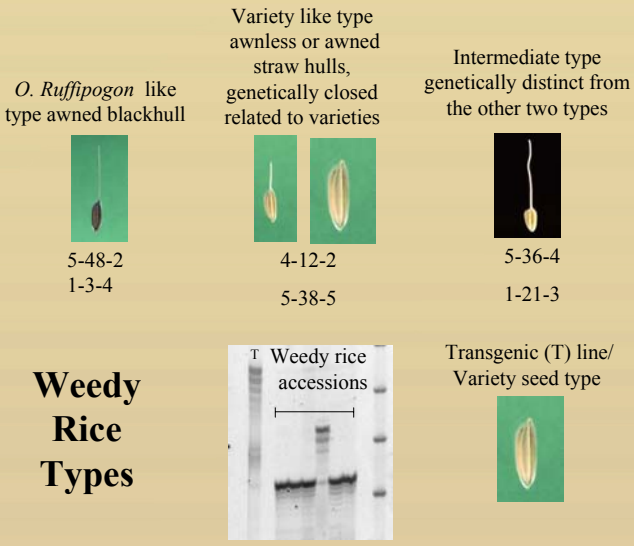

Figure 1. Seed traits and SSR polymorphism of weedy rice selected for gene flow analysis.

Experimental field designs to trace gene flow from transgenic and non-transgenic rice into weedy rice

Two experimental field-plot designs were used. The first design (multiple-square assay) consisted in randomized plots planting rice intermingled with 20% weedy rice in square plots, simulating farmers field conditions and reflecting the economic threshold level for weedy rice infestation in Colombia (Figure 2A). The second design (concentric circles assay) was used to measure gene flow distance by inter-planting pollen donor plants (transgenic and non-trasngenic) within a concentric-circle design at the middle of the plot. Weedy rice plants were planted in concentric circles from the center (the pollen source) using a Statistic Latin Square Design (Figure 2B).

Manual crosses

Manual reciprocal crosses were made between the different weedy rice types and the transgenic/non-transgenic rice varieties, according to Sarkarung (1996). These F_1 plants were used as controls.

Figure 2. General view of field plot designs for gene flow analysis: (A) Square plots and (B) Concentric circle plots.

Tracing gene flow

Gene flow was either traced at the phenotypic level by detecting gus expression or anthocyanin presence in plant tissues and putative hybrids were then confirmed by microsatellite molecular markers, or directly at the genetic level by bulking DNA samples of various plants to proceed with the molecular analyses without knowing the phenotypic profile. PCR analysis was conducted to trace the presence of transgenes and SSR analysis to confirm the hybrid nature of recovered F_1 plants (Figure 3).

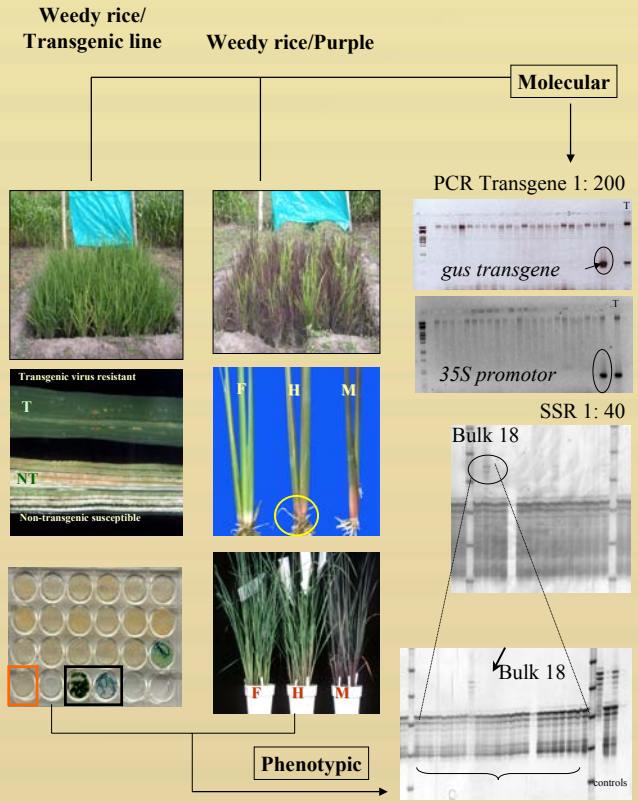

Figure 3. Analysis of gene flow at the phenotypic and/or molecular level.

Results and Discussion

Gene flow from rice into weedy rice

In the greenhouse, higher crossing rates was observed in manual-made crosses respect to the natural hybridization in the field. In general, higher hybridization rates in manual crosses was obtained (at least 2-fold) when rice was used as the male parent (pollen donor) and weedy rice as the female parent (pollen recipient). A lower hybridization rate was noted in the reciprocal crosses (using weedy rice as the pollen donor), probably suggesting a preferential gene flow rate from rice into the weedy rice. Hybridization rates from 7% up to 42% in manual crosses in the greenhouse were confirmed by microsatellite analysis.

In the field trials, flowering was synchronous between rice and the different weedy types. Hybridization rates of about 0% to 0.3% were obtained when either transgenes or the anthocyanin marker genes were used to trace gene flow in about 24,000 derived progeny plants, and confirmed by microsatellite markers (Figure 3). All derived transgenic hybrids confirmed by microsatellite express *gus* gene and display the region of the promoter 35S CaMV (Figure 3). Confirmed hybrids derived from the purple variety showed anthocyanins in the corresponding tissues. Outcrossing rates < 1% from transgenic/non-transgenic rice to weedy rice/rice varieties had recently been reported by other authors under temperate conditions (Noldin et al. 2001; Zhang et al. 2003 and Messeguer et al. 2004). But the cumulative hybridization rate (in consecutive years/period) may be higher under tropical conditions as compared with temperate conditions because of the lack of crop rotation and several crop cycles per year. Gene introgression dynamic over time and hybrid fitness analysis between rice/ weedy rice needs to be assayed at landscape level in farmers field. Towards that end our group will examine the gene flow/introgression dynamics in the crop/wild/weedy rice complexes using microsatellites complemented with organelle (maternal inheritance) polymorphism as a tool to trace the direction of gene flow.

Tracing gene flow with molecular markers

An optimized methodology using bulk DNA and PCR-based analysis allows the assessment of large number of seed samples with a high precision to detect hybrid candidates (Figure 4). This methodology is useful for tracking and monitoring gene flow at large scale in farmers' fields and in crop-to-wild contact zones. The scoring of phenotypic trait alone (i.e. gus expression, non-transgenic anthocyanin vegetative tissues) could either under or overestimate the level of hybridization rate. Similar results had been found in other works scoring herbicide resistance. Because of these potential errors, it is advisable that phenotypic data from putative out outcrossing events in a particular crop/year be confirmed with molecular techniques that can specifically identify the original parents or trait/gene(s). When handling thousands of samples, bulk DNA could first be assayed for the presence of the transgene(s) allowing the detection of 1 hybrid in 200 plant-bulk (Figure 4A and 4B), and the putative bulk then split in smaller samples allowing the detection of 1 hybrid in 40 plant-bulk with microsatellite (Figure 4C and 4D). SSR are valuable genetic markers because they are simple, co-dominant, allow detection of high levels of allelic diversity, and are easily and economically assayed by PCR. The analysis using microsatellites will give a better understanding of the gene flow/introgression dynamics in crop/wild/weedy complexes and of the potential impact on biodiversity.

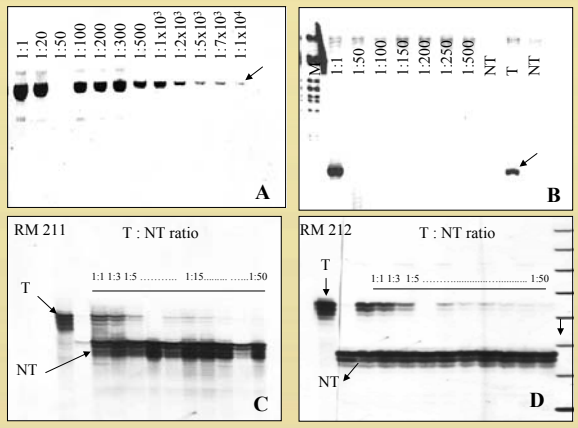

Figure 4. A) Detection of RHBV-N protein-transgene by PCR in various dilution ratio of plasmid *p*VR3. B) Detection of plants containing *gus* gene by PCR-bulk analysis (T *gus* gene : NT dilution). Microsatellite amplification with RM 211 (C) and RM 212 (D) in various dilution ratio T : NT DNA. T= transgenic. NT= not-transgenic

Conclusions

- In the greenhouse, hybridization rates of 7%-42% were found in manual crosses and confirmed by microsatellites. About two fold rates were obtained when rice was used as male parent.
- In the field, preliminary results suggest a natural gene flow rate from transgenic/non-transgenic rice to weedy rice of about 0.03 % to 0.4% that is significantly lower respect to manual crossing.
- There is a high correlation between phenotypic markers and SSR as tools to trace gene flow, however microsatellites allowed to detect some false negative/positive hybrid plants.
- Analysis of gene flow distance and direction respect to the pollen source, and effect of the wind on gene flow rate is currently in progress.
- F_2 plants derived from the confirmed hybrids will be evaluated for resistance to RHBV and fitness performance.

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