Molecular characterization of rice and wild/ weedy relatives by microsatellites and their use to assess gene flow in the Neo-Tropics

Eliana González[1], Luisa F. Fory[1], Juan J. Vásquez[1], Paola Ruiz[1], Adriana Mora[1], James Silva[1], Myriam C. Duque[1], Edgar Corredor[2], and Zaida Lentini [1].
[1]International Center for Tropical Agriculture (CIAT). A.A. 6313. Cali, Colombia. [2]Fedearroz. Bogotá, Colombia. E-mail: z.lentini@cgiar.org
Funding from GTZ, Germany. Project No. 99.7860.2-001.00

Introduction

A careful assessment of potential impacts of gene flow from transgenic plants on population genetics of natural crop plant biodiversity is needed in other to design strategies for the safe and durable use of these crops in the Neo-tropics. This work is part of a project directed to analyze the gene flow from non-transgenic or transgenic rice into wild/weedy relatives, and its effect(s) on the population genetic structure of the recipient species. The current report summarizes the progress on setting up the use of microsatellites markers to assess and trace gene flow from transgenic and non-transgenic rice into wild *Oryza* species and red rice under controlled confined field plots, and under local agricultural field conditions in Colombia. A genetic diversity analysis was first conducted in order to determine the genetic structure prior gene flow, and to select the best combinations of transgenic or non-transgenic rice, and wild/weedy populations to assess the gene flow.

Objectives

To assess the gene flow from non-transgenic or transgenic rice into wild/weedy relatives, and its effect(s) on the population genetic structure of the recipient species. To assess the potential changes due to introgression, it is necessary to determine the genetic structure of the donor and recipient populations prior gene flow in order to select the best crop Vs wild/weedy combinations. Crop and wild/weedy specific molecular markers will be used to facilitate tracing gene flow of at least 1% hybridization rate in the field.

Materials and Methods

Plant Material

The materials included: 9 rice commercial varieties (Cica 8, Cimarron, Fedearroz 50, Fedearroz 2000, Fedearroz Victoria 1, Iniap 12, Oryzica 1, Oryzica Llanos 5, and Palmar). Sixteen homozygous transgenic Cica 8 rice lines resistant to RHBV virus. Four handmade crosses each between one transgenic Cica 8 line and non-transgenic Cica 8, Iniap 12, Fedearroz 50 or Oryzica 1, respectively. One hand made cross each between non-transgenic Cica 8 and Iniap 12, Fedearroz 50 or Oryzica 1, respectively (controls). One hundred and fifty eight accessions of red rice collected from various commercial rice field plots in Tolima, the major rice-cropping area of Colombia, previously characterized morphologically and phenologically. One accession each of *O. barthii, O. glaberrima, O. glumaepatula,* and *O.rufipogon.* All these genotypes were included in order to select the microsatellites detecting the highest level of polymorphisms among genotypes, and the most polymorphic pairs from each class to conduct gene flow analysis.

Genetic Analysis using Microsatellite Markers

A set of 50 microsatellite markers (at least 4 per each chromosome) were screened. The microsatellite selection was based on their location in the chromosome (McCouch et al., 1997). At least two markers located distal from the centromere per each chromosome arm were chosen to increase the likelihood of finding recombination between the experimental genotypes. The markers were amplified at different annealing temperatures according to the estimated melting temperatures of the primers. The PCR products were resolved on silver-stained polyacrylamide gels and microsatellite alleles were sized by comparison to the 10 and 25 bp molecular weight standards (Promega). The genetic distance between samples was calculated using the Dice algorithm, and a dendogram was constructed using the Unweighted Pair-Group Mean Average (UPGMA). The genetic distances and dendogram were built using NTSYS-PC software version 2.02 (Rohlf, 1997).

Results and Discussion

Genetic diversity of rice varieties

Results suggest that the average rate of polymorphism between Cica 8 and the different varieties ranged from 48% respect to the variety Iniap 12, to 96% respect to the variety Palmar (Table 1). Between 85% and 92% of the microsatellite markers analyzed were polymorphic between Cica 8 and *O. rufipogon, O. glaberrima*, and *O. barthii*, respectively (Table 1). *O. barthii* and *O. glaberrima* were also included in this study to generate baseline information that may be useful for Africa. As expected, results indicated that no polymorphism is detected between non-transgenic Cica 8 variety and transgenic Cica 8 lines. Results suggest that the transgenic lines are true-type Cica 8 with the exception of the transgenes introgressed in the rice genome. In contrast, polymorphism from 30% to 39% is detected in hand made crosses between either Cica 8 or transgenic Cica 8 and the selected varieties. Crop-specific microsatellite markers segregated in a co-dominant fashion in the hybrids as expected. Therefore, Cica 8 specific microsatellite could be used to trace gene flow from transgenic Cica 8 into other rice varieties and wild/weedy relatives.

Table 1. Average rate of polymorphism using microsatellite molecular markers

Variety / Wild species	Cm	I-12	*Ob*	*Og*	OL5	Pal	*Or*	Vic	F2000	Ory
Cica 8	50	48	85	88	52	96	92	68	57	52
Cimarrón		46	84	92	44	89	92	66	52	46
Iniap 12			96	92	60	84	88	46	33	42
O.barthii				57	84	92	92	88	88	92
O.glaberrima					92	67	100	100	92	92
O. Llanos 5						73	85	64	55	23
Palmar							89	75	79	78
O.rufipogon								92	89	96
Victoria 1									40	60
Fedearroz 2000										46

Cica 8 (C8); Cimarron (Cm); Iniap 12 (I-12); Oryza barthii (Ob); Oryza glaberrima (Og); Oryzica Llanos 5 (OL5); Palmar (Pal); Oryza rufipogon (Or); Fedearroz Victoria 1 (Vic); Fedearroz 2000 (F2000); Oryzica 1 (Ory)

Genetic characterization of rice varieties, red rice, and wild Oryza species

The dendrogram obtained showed thirteen groups with a similarity of 0.43. *O.glumaepatula* was the most distant group, followed by *O.glaberrima* and *O. barthii*. *O.rufipogon*, was found within the red rice group (Figure 1). For the red rice population, in some cases it was possible to associate the genetic clusters with some phenological and morphological traits. The first group was mainly composed by red rice accessions with pale yellowish husk (91%) and early to intermediate flowering (64%). The second and third groups contained accessions with grains of pale yellowish husks (95%), but with intermediate to late flowering (93%). Most of the red rice-variety biotypes and commercial varieties are in this group. The groups 8, 9,10, and 11, were mainly composed by red accessions with grain awn (69%), and intermediate to late flowering (85%). Cluster 9 grouped the wild species *O. rufipogon* with some red rice biotypes with significant similarity on morphological and phenological traits. The fourteen most polymorphic microsatellites (Figure 2) detected a high number of alleles (106). In general the size of the alleles ranged from 108 to 252 bp. This analysis allowed the identification of 46 specific alleles for red rice; 17 specific alleles for the wild species; and only two specific alleles for the rice varieties. *O.glumaepatula* showed larger number of specific alleles (8) compared to *O.rufipogon*, which shared all their alleles with red rice. A low number of heterocigotes was found in the red rice population as a whole, with a range of 0-19 heterocygotes per microsatellite. Total genetic diversity index of Nei (0.637) was intermediate to high. In contrast, the genetic diversity index between the red rice populations collected from the different field plots was 0.55, which is considered an intermediate value. The value obtained for Gst (0.136) reflects a lower genetic diversity inter-population (collected from different field plots) than intra-population (individuals collected from the same field plot analyzed as one population).

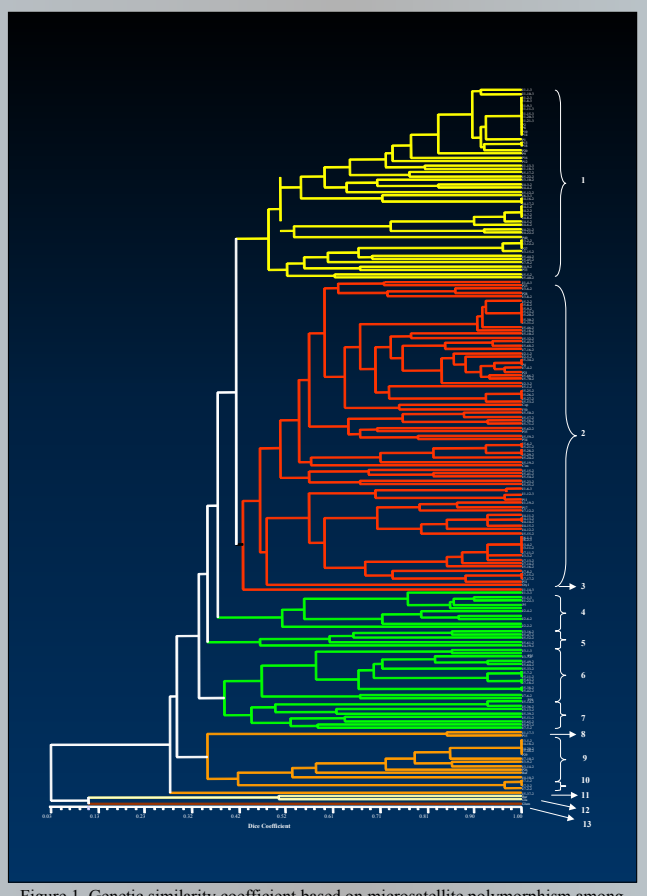

Figure 1. Genetic similarity coefficient based on microsatellite polymorphism among red rice accessions, 4 commercial varieties, and 4 wild *Oryza* species.

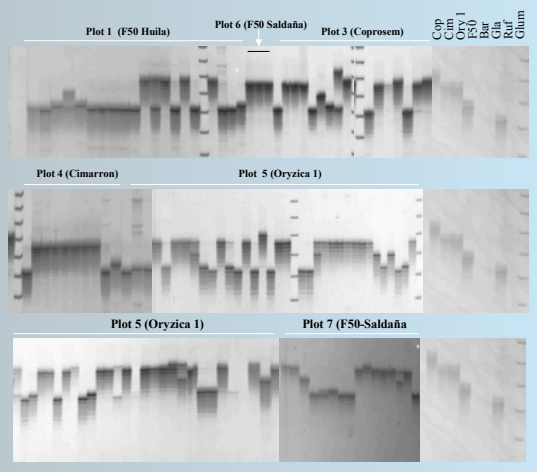

Figure 2. Polymorphic segregation pattern noted between different red rice populations derived from different field plots, using RM 222 microsatellite marker. Cimarron (Cim); Coprosem 1 (Cop); Fedearroz 50 (Fed 50); Oryzica 1 (Ory 1); *Oryza barthii* (Bar); *Oryza glaberrima* (Glab); *Oryza glumaepatula* (Glum); *Oryza rufipogon* (Ruf)

Tracing gene flow with microsatellite molecular markers

Clearly distinct combinations of crop, red rice, and wild species had been selected for gene flow and introgression follow up, based on the morphological, phenological, and molecular genetic characterization using microsatellite markers. Conditions had already been standardized for detection of 2% out-cross using genotype specific markers (Figure 3). Conditions are being optimized to detect 1% of out-crossing rate.

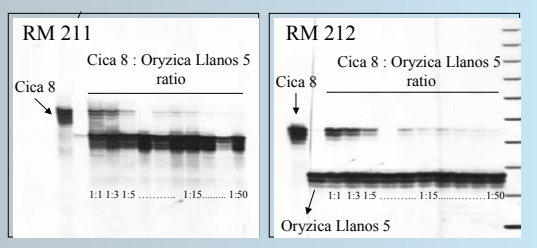

Figure 3. Microsatellite amplification of various DNA Cica 8 : Oryzica Llanos 5 ratio.

Conclusions

· Specific microsatellite alleles were identified in different commercial varieties, red rice accessions and wild species, which can be used to trace gene flow and introgression. As expected, wild species *O. glaberrima* with *O. barthii* clustered together in a group, and in a separate cluster with *O. glumaepatula* of low similarity with the rest of the individuals. According to this cluster analysis, some red rice accessions showed high genetic similarity to the varieties and others to the wild species *O.* rufipogon. This genetic similarity coincide with the morphological and phenological characterization as well. The red rice-*O.rufipogon* biotypes will be subject of taxonomic classification to elucidate if they are introduced accessions of the wild species from Asia. The red rice-variety biotypes might be indicators of hybridization between red rice and the crop, and thus better candidates as receptors of gene flow. These materials could be ideal materials to trace transgene(s) flow. Clearly distinguishable red rice biotypes are recommended to trace gene flow from non-transgenic rice, in order to provide a broad understanding of the hybridization and introgression dynamic on this population over time.

References

McCouch S.R., X. Chen, O. Panaud, S. Temnykh, Y. Xu, Y.G. Cho, N. Huang, T. Ishii & M. Blair. 1997. Microsatellite marker development, mapping and applications in rice genetics and breeding. Plant Molecular Biology 35: 89-99.

Promega Corporation. 1999. Technical Manual: SILVER SEQUENCE™ DNA Sequencing System. 19 p.

Rohlf, F. 1997. NTSYS-PC. Numerical Taxonomy and Multivariate Analysis System version 2.02.