Gene flow analysis in rice wild/weedy relatives in Tropical America: Understanding crop-biodiversity interactions

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Abstract

Agriculture in developing countries is currently facing major challenges compelling the need for increased crop productivity with efficient systems that reduce inputs, are environmental friendly, provide social equity, and increase revenues to small farmers as a gateway to reduce hunger and poverty. Different alternatives of agricultural systems and consumer acceptance worldwide are shaping domestic and international food trade with direct economic effects on the most deprived sectors of society in developing countries. This complex and recent scenario requires knowledge allowing the development of practices for the coexistence of different types of agriculture satisfying the needs of diverse sectors of society while ensuring economic growth and environmental protection. To address these multiple constraints tropical agriculture will have to cope with diversity of agricultural systems: subsistence agriculture, productive systems helped by conventional breeding and/or biotechnology, organic farming for specialty markets, among others.

This research is part of a project which main goal is to generate baseline genetic information for the development of guidelines on the safe introduction and use of novel agriculture traits (biotechnology derived or not native from the place of introduction), while reducing potential environmental impact on native biodiversity in the Neotropics.

Background

Rice (*Oryza sativa* of Asian origin, AA genome) is an introduced domesticated species that has become one of the most important staple grains for human consumption in tropical America in recent decades. The rice genus, *Oryza* has a pan-tropical distribution. Four species have been recorded in tropical America. *Oryza glumaepatula* (diploid, AA genome) classifies within the primary gene pool (Akimoto, 1998; Vaughan, 1994), whereas *Oryza grandiglumis*, *O. alta* and *O. latifolia* are allotetraploid (CCDD) and included in the secondary gene pool. Due to its morphological similarity with *O. rufipogon*, *O. glumaepatula* was originally classified as the American strain of *O. rufipogon* (Vaughan, 1994). Nevertheless, *O. glumaepatula* is a distinct AA species based on morphological traits, molecular markers, has compatibility barriers with *O. rufipogon*, and is closer related to the African species *O. glaberrima*, *O. barthii* and *O. longistaminata* than to the Asian *O. rufipogon* (Akimoto 1998, Ge et al., 2001; Juliano et al., 1998). With the exception of Costa Rica and Brazil that had conducted a complete analysis of the *Oryza* wild relatives composition and spatial frequency distribution, the information for the rest of the region is incomplete and scattered in few herbarium records (Lentini and Espinoza, 2005).

Weedy rice (commonly known as red rice) is sympatric with the rice crop. In tropical America, the weedy rice complex is broadly diverse and maybe composed by various *Oryza* species (mostly annual and diploid, AA genome) that yet have not been fully identified. Weedy rice usually has feral traits (taller, awned seeds with red pericarp, and shattering), varying degree of sexual compatibility and flowering overlapping with the crop in different environments (Oka and Chang, 1961). Weedy rice appears to be the main candidate for gene flow and introgression from cultivated rice, since it is compatible and usually intermingled with the rice crop.

Previous results from experiments conducted under controlled-confined conditions showed that gene flow occurred predominantly from the crop into weedy rice (Lentini and Espinoza, 2005). We are currently evaluating introgression of genes that confer a positive advantage against selection and monitoring the potential impact at landscape level in weedy populations. This work also describes the use of chloroplast and nuclear molecular markers for the characterization of weedy rice population collected in commercial farmers fields in Colombia and Venezuela, and their utility for tracking gene flow at landscape level (rate and direction) in weedy/ wild *Oryza* species populations.

Materials and Methods

Tracking gene flow at landscape level in farmers fields

Herbicide resistance in rice derived from mutagenesis (imidazolinone resistance) was bred into elite local materials and released as improved variety Clearfield CF205® in Central America and Colombia. The herbicide-rice model is convenient because of easy tracking of the resistance trait in the weedy rice complex, for which herbicide is used as a form of chemical control (positive selection), and in the wild *Oryza* populations found in natural environment in the crop contact zones (neutral selection). Total of 520 accessions of weedy rice were collected from commercial rice fields after planting 2-3 cycles with the variety Clearfield CF205® tolerant to the herbicide Imazapic (IMI) in Tolima, and 556 accessions of weedy rice collected from farmers fields prior and after planting 1 cycle with the same herbicide tolerant variety in Jamundi, Valle del Cauca (Colombia). Each plot was sub-divided into squares of 52 x 52 m (Fig. 1A). Flowering panicles of weedy rice in contact with flowering panicles of CF205® were randomly selected from areas of 200-250 m^2 in each plot and enclosed in paper bags for collecting seeds (Fig. 1B). The *acetolactate synthase* (ALS) gene that confers resistance to the imazapic herbicide was sequenced and gene specific primers were designed to detect the single point mutation (single nucleotide polymorphism, SNP) in the ALS gene, set of primers were tested, selected and used to trace this gene in the weedy populations at landscape level using molecular bulk analysis.

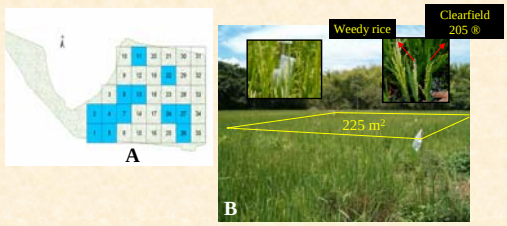

Fig. 1.- Sampling weedy rice from commercial plots planted with CF205®. Blue squares represent the sampled areas within a plot.

Collection and characterization of wild *Oryza* species and weedy populations from natural environments and crop-contact zones

Total of 331 wild *Oryza sps* accessions were collected in Guárico and Portuguesa States, the two main rice cropping areas in Venezuela (Figure 2). A total of 5.373 seedlings were characterized phenotypically and then subjected to molecular analysis. Specific chloroplast DNA (cpDNA) and nuclear sequences proven useful for identification of *Oryza* species (Ge et al., 2001; Ishii et al. 2001, Ying and Ge, 2003) were used to identify the genome type and ploidy level, and discern if either any of the American *Oryza* species (*O. latifolia, O. grandiglumis*, *O. alta* and *O. glumaepatula*) may be part of the weedy rice complex. Total of 146 Colombian accessions of weedy rice and 61 accessions wild *Oryza* species from IRRI Genetic Resources were used as controls and to standardize the use of chloroplast markers for tracking gene flow.

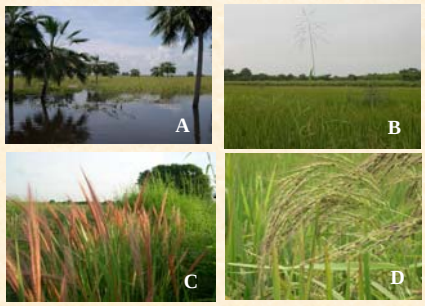

Fig. 2.- Types of accessions collected in natural environments and crop-contact zones in Venezuela. **(A)** Wild *Oryza* species growing in the swamp "Estero Camaguan", Southern Guárico state. **(B)** Tetraploid wild *Oryza* growing intermingled with rice crop in Portuguesa State. **(C)** Wild *Oryza* population characterized by spikes with red large awns growing in natural environment- rice crop contact zones in Portuguesa. **(D)** Weedy rice with red awns growing intermingled with rice commercial crop in Guárico.

Results and Discussion

Collection and characterization of wild rice species

The TrnL-TrnF spacer sequence allowed to clearly distinguish AA diploids from CCDD tetraploids (Figure 3A). This result is consistent using large diversity of AA and CD accessions from IRRI, including *O.glumaepatula, O. rufipogon, O. barthii, O. glaberrima, O. alta, O. grandiglumis* and *O. latifolia* species among others. None of the weedy rice accessions analyzed so far are tetraploids, all are AA diploids. Similar results were obtained by amplifying the nuclear Adh-2 gene and digested with EcoNI. This combination gene/restriction enzyme also allows a clear differentiation of *Oryza sativa* (AA) from the complex from *Oryza officinalis* (CD). A clear specific polymorpfism was detected in *O. glumapatula* accessions from Colombia, Costa Rica and Venezuela, and the wild *Oryza* sample collected from Estero de Camaguan in Venezuela, when amplifying specific regions of the cpDNA TrnS-TrnT sequence followed by digestion with Dra I restriction enzyme (Figure 3B). The analysis of this cpDNA region has been selected to analyze the rest of accessions from Estero de Camaguan including a large diversity of *O. glumaepatula* accessions from various countries.

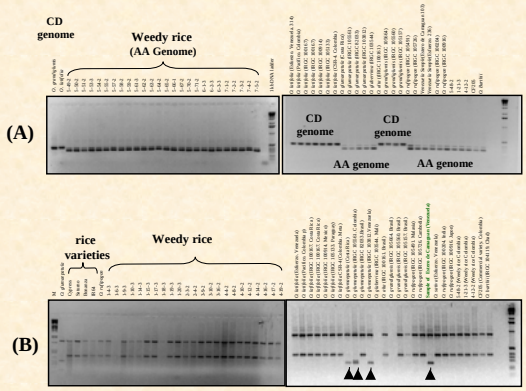

Fig. 3.- (A) PCR amplified cpDNA TrnL-TrnF sequence from weedy rice and various wild *Oryza* species AA and CCDD genome. **(B)** PCR amplified cpDNA trnS [TRNA-Ser- (GGA)] and trnT [tRNA-Thr (UGU)] sequences of several *O. sativa* wild species, weedy rice and rice varieties. Arrows indicate polymorphism found in *O. glumaepatula* and in wild *Oryza* collected from Estero de Camaguan (Fig.2A).

Tracking herbicide resistance gene introgressed into weedy rice from CF205® variety in commercial farmers fields

Progeny (54.385 plants) derived from weedy rice panicles collected in commercial rice fields, planted with Clearfield CF205® in Tolima and Valle del Cauca, were first assayed for its herbicide resistance in replicated field trials using lethal herbicide concentrations according to the commercial manufacturer (Masterkey DG® system) and optimized to avoid potential escapes. DNA of herbicide resistant plants was bulked, and then analyzed for the presence of the ALS resistant gene from CF205®. Resistant bulk samples were subsequently analyzed to identify the number of individual plant(s) per bulk containing the ALS resistant gene. In the case of Valle del Cauca fields which had been planted just 1 cycle with CF205®, 9% (19.128 plants) of the samples showed resistance to imazapic herbicide, and of these plants <1% contained the ALS gene from Clearfield CF 205® confirming the outcross of the herbicide resistance gene from the variety into weedy rice. This outcross rate is in accordance to previous results obtained in our group, and elsewhere, assaying out-crossing from either transgenic or not transgenic rice under controlled experimental conditions. In contrast in the case of the Tolima fields, 28% of the samples (38.257 plants) (3 fold higher than in Valle del Cauca) are resistant to Imazapic even after three applications of lethal concentration of the herbicide. The molecular analysis of these plants to detect the presence of the ALS gene from CF 205® is in progress. Detail analysis should be conducted to rule out the possibility of cross-herbicide resistance causing an over-estimation of the out-cross rate. However, it might be possible that these results suggest high rates of accumulative hybridization (in consecutive years/period) because of the lack of crop rotation and several crop cycles per year.

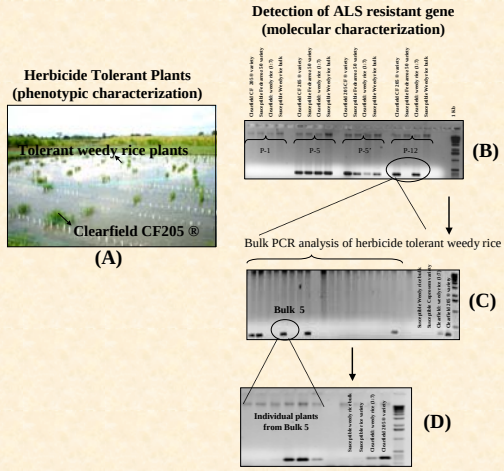

Fig 4.- Assessment of outcross and introgression of ALS resistant gene from Clearfiled CF205 ® into weedy rice collected from farmers fields. **(A)** Evaluation of imazapic herbicide resistance. **(B)** Selection of suitable primers designed -single nucleotide polymorphism (SNP) to specifically amplified the ALS gene from Clearfiled CF205 ® . **(C)** DNA-bulk analysis of herbicide resistant plants. **(D)** Molecular analysis of individual plants per each bulk to detect the ALS gene from Clearfiled CF205 ®.

Conclusions

- Our previous work showed that outcross of ≤0.3 % is predominantly from non-transgenic or transgenic rice into weedy rice under controlled experimental conditions. Similar rates were obtained in the first cycle of outcross at landscape level in Valle del Cauca.
- Methods and tools were adapted to assess out-cross at landscape level using SNP molecular markers by bulk analysis, allowing to analyze large populations of samples (about 19.128 plants) in about 1 month. This protocol will also be applicable for analysis at ecological level.
- The analysis with chloroplast and nuclear DNA specific sequences indicate that weedy samples analyzed so far are AA diploid. Wild *Oryza* sample from Estero de Camaguan appeared to be *O. glumaepatula*, including the morphological taxonomic traits. Final taxonomy should be confirmed by International referenced expert. The complete molecular analysis of wild *Oryza* accessions and weedy rice is in progress. Specific SSRs and cpDNA markers are being used to identify potential hybrids between *O. sativa* and wild *Oryza*, determine the reproductive biology and direction of gene flow.
- The use of non-transgenic herbicide resistance model will give information on impact of introgressed non-transgenic resistance genes that may affect fitness of derived hybrids, invasiveness, population dynamics and genetic structure of the corresponding wild/weedy, and for anticipating a potential impact from a transgenic situation. This information will be useful for *in situ* conservation, and could be applicable to develop guidelines for environmental safety and co-existence of different types of agriculture systems in the Neotropics.

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