

GENETIC DIVERSITY ANALYSIS OF WEEDY RICE AND WILD *Oryza* SPECIES COLLECTED IN CROP-NATURAL ECOSYSTEMS CONTACT ZONES

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INTRODUCTION

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). 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). This work describes the use of chloroplast and nuclear molecular markers for the characterization of weedy and wild rice populations collected in commercial rice fields and natural environments 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

Collection and characterization of wild rice species and weedy rice populations from natural environments and crop-contact zones in Venezuela.

State	Site Collection	Species/Suggested	Genome Type	Collected Samples	# Accessions Analyzed
Portuguesa	Clavellinas	<i>O. latifolia</i>	CCDD	35	21
	El Estuerzo	<i>O. latifolia</i>	CCDD	26	21
	El Estuerzo	<i>O. glumaepatula</i>	AA	31	11
	El Estuerzo	Weedy rice/Hybrid	AA	22	20
	Santa Lucia	<i>O. rufipogon</i> /arrocillo	AA	13	8
Guarico	Carretera-Km 133	<i>O. glumaepatula</i>	AA	14	14
	Carretera Calabozo	<i>O. glumaepatula</i>	AA	51	43
	Lecherito	<i>O. glumaepatula</i>	AA	44	27
	Estero de Camaguan	<i>O. glumaepatula</i>	AA	87	11
	Via Herrera	<i>O. glumaepatula</i>	AA		

IRRI accessions

- O. latifolia* (20)
- O. rufipogon* (21)
- O. alta* (4)
- O. grandiglumis* (4)
- O. glumaepatula* (8)
- O. nivara* x *O. rufipogon* (2)
- O. nivara* (2)



Fig 1. Wild *Oryza* species growing in the swamp "Estero Camaguan", Southern Guárico state



Fig 2. Tetraploid wild *Oryza* growing intermingled with rice crop in Portuguesa State

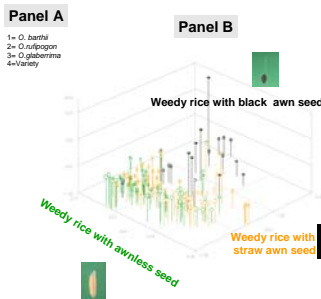


Fig 3. Wild *Oryza* population characterized by spikes with red long awns growing next to rice field in Portuguesa State

Weedy rice collected in Colombia



Fig 4. Panel A, weedy rice types showing diversity in grain colors (from husk to dark/black hulls), variation in awn length and pericarp color (from dark red to white). Panel B, Multiple Correspondence Analysis based on SSR that show the Population structure of the weedy rice in Colombia.



Use of chloroplast and nuclear molecular markers for the characterization of weedy rice population collected in commercial farmers fields in Colombia and Venezuela

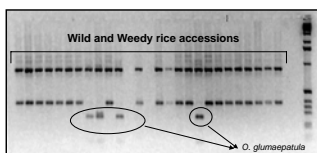


Fig 5. PCR amplified cpDNA trnS [TRNA-Ser- (GGA)] and trnT [TRNA-Thr (UGU)] sequences of several *O. sativa* wild species, weedy rice and rice varieties. Arrows indicate polymorphism found in *O. glumaepatula* and in wild *Oryza* collected from Estero de Camaguan.

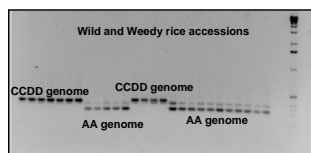
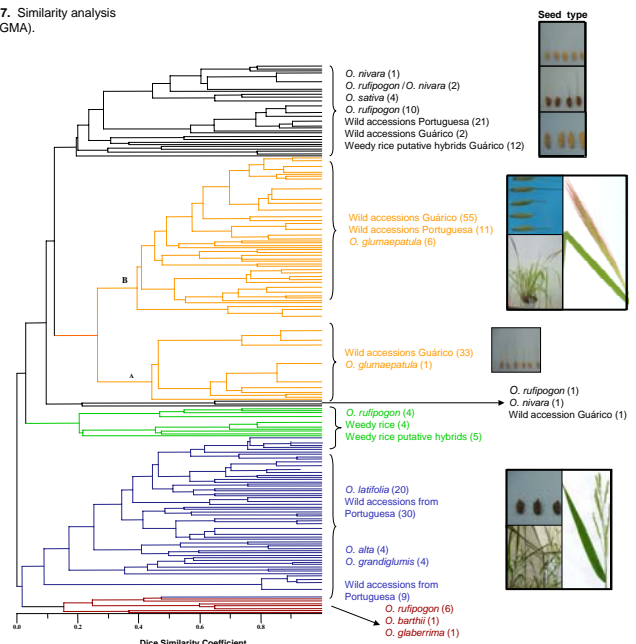


Fig 6. PCR amplified cpDNA TrnL-TrnF sequence from weedy rice and various wild *Oryza* species AA and CCDD genome.

RESULTS AND DISCUSSION

Fig 7. Similarity analysis (UPGMA).



Characterization of wild and weedy rice accessions. Molecular analysis using four SSR markers generated seven clusters (Fig 7). Wild accessions, weedy rice and putative hybrids were clearly separated from the cluster including *O. glaberrima*, *O. barthii* and some *O. rufipogon* accessions. Tetraploid accession from IRRI and CCDD wild accessions identified according to cpDNA TrnL-TrnF sequence (Fig. 6) were also clear cut separated from the AA genome samples. Thirty CCDD wild accessions were closely related to *O. latifolia* and nine were related to all tetraploid group (*O. latifolia*, *O. alta* and *O. grandiglumis*). These wild CCDD accessions were characterized by being tall plants with truncated ligules, small seeds with short awns and the spikes were <7 mm, taxonomy traits used reproducibly to distinguish *O. latifolia* from *O. alta* and *O. grandiglumis* in Venezuela (D. Vaughan, NIAS, Japan, personal communication). Accessions. 99 wild accessions (AA genome) collected from Guárico and Portuguesa, identified as *O. glumaepatula* according to cpDNA trnS [TRNA-Ser- (GGA)] and trnT [TRNA-Thr (UGU)] sequences clustered with 7 of the 8 (88%) *O. glumaepatula* accessions from IRRI. All the weedy rice, putative hybrids and the remaining (44) AA genome wild accessions clustered with all the *O. rufipogon* IRRI accessions (14) from a diverse number of Asian countries. All the weedy rice accession analyzed so far are diploid AA genome and most of them (87%) are of *indica* type according to cp ORF 100 sequence.

CONCLUSIONS

The morphological and molecular analysis of the Colombian weedy rice samples allowed the discrimination of three main groups within the weedy rice population. The main group includes weedy rice variety type with awnless seeds and straw glumes very similar to commercial varieties, a small group characterized by having black awns and glumes clustering closely with *O. rufipogon* accession (*O. rufipogon* type); and an intermediate type with straw glumes and awns.

Analysis with chloroplast (cp) and nuclear DNA specific sequences indicate that weedy rice accession collected in Colombia and Venezuela are AA genome. Likewise in Colombia, there are some weedy rice accessions closely related to *O. rufipogon* and the genetic identity of the putative weedy rice hybrids collected in the farmers fields still needs further analysis. Most of the CCDD wild accessions are closely related to *O. latifolia*. AA genome wild accessions from "Estero de Camaguan", "Via Herrera", "Lecherito" and some from Portuguesa are genetically related to *O. glumaepatula* according to the cpDNA sequence and SSR analysis. Specific SSRs and cpDNA markers are being used to identify potential hybrids between *O. sativa* and wild *Oryza*, to determine the reproductive biology and direction of gene flow.

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