Molecular Phylogenetics and Evolution 55 (2010) 136–142

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Multiple patterns of rDNA evolution following polyploidy in *Oryza*

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ARTICLE INFO

Article history:
Received 26 June 2009
Revised 6 October 2009
Accepted 18 October 2009
Available online 24 October 2009

Keywords:
Oryza
Polyploidy
Concerted evolution
Ribosomal DNA
Internal transcribed spacer sequences

ABSTRACT

Ribosomal ITS sequences are commonly used for phylogenetic reconstruction because they are included in rDNA repeats, and hence are ubiquitous and present in high copy number. Ribosomal rDNA repeats often undergo rapid concerted evolution within and between arrays. Interspecific hybridization merges divergent repeat types in a single nucleus, setting in motion evolutionary processes leading to coexistence, maintenance of paralogs, origin of novel sequence variants, loss of arrays, or inter-array sequence homogenization via concerted evolution. Here we examined ITS polymorphism within and among six *Oryza* tetraploids of varying genomic composition to infer the extent and direction of concerted evolution following allopolyploid speciation. We demonstrate that different polyploids have experienced varying fates, including maintenance or homogenization of divergent arrays, even among allopolyploids having the same genomic origins but in different geographic locations. Bidirectional concerted evolution, in which arrays become homogenized to alternative progenitor diploid types in different allopolyploid derivatives, is evident among species in one clade. Our results exemplify the panoply of outcomes for ribosomal DNA evolution following allopolyploid speciation.

1. Introduction

Ribosomal ITS sequences are commonly used for phylogenetic reconstruction because they are included in rDNA repeats, and hence are ubiquitous and present in high copy number. Ribosomal rDNA repeats often undergo rapid concerted evolution within and between arrays (Baldwin et al., 1995; Álvarez and Wendel, 2003; Nieto Feliner and Rosselló, 2007). The result of concerted evolution is that individual copies are identical or nearly so, such that repeats within a lineage appear to evolve more or less in unison because inter-repeat sequence variation is reduced to a negligible level due to sequence homogenization. Nevertheless, in recent years a number of studies have shown that ITS polymorphism within individuals is quite common (Baldwin et al., 1995; Wendel et al., 1995; O'Kane et al., 1996; Buckler-IV et al., 1997; Denduangboripant and Cronk, 2000; Mayol and Rosselló, 2001; Bailey et al., 2003; Rosselló et al., 2006, 2007; Nieto Feliner and Rosselló, 2007; Zhang and Ge, 2007; Kim et al., 2008; Göker and Grimm, 2008; Grimm and Denk, 2008; Pilotti et al., 2009). The presence of multiple paralogs, recombinant mosaic sequences or a mixture of both within a genome are phenomena of ITS evolution in plants that need to be considered when conducting phylogenetic analysis (reviewed in Álvarez and Wendel, 2003). This complexity may be increased following allopolyploidization, which merges divergent repeat types in a single nucleus, thereby giving rise to at least an evolutionarily ephemeral coexistence of divergent ITS repeats (Volkov et al., 2007). A number of studies demonstrated the diversity of outcomes of this process, including maintenance of biparental rDNA repeats (e.g., Soltis and Soltis, 1991; O'Kane et al., 1996; Popp and Oxelman, 2001; Zhang et al., 2002), to loss of one parental copy (e.g., Wendel et al., 1995; Volkov et al., 1999; Kotseruba et al., 2003; Kovarik et al., 2005, 2008; Matyasek et al., 2007; Kim et al., 2008), to the origin of chimeric repeats (e.g., Volkov et al., 1999; Nieto Feliner and Rosselló, 2007).

To better understand rDNA repeat interactions following genomic mergers it is important to study the process in different natural polyploid systems. The genus *Oryza* is particularly appropriate in this regards, as approximately half of the extant *Oryza* species are considered to be allopolyploids derived from interspecific hybridization (Gopalakrishnan et al., 1965; Vaughan, 1989,1994; Ge et al., 1999; Lu and Ge, 2005). Based on interspecific crossing, subsequent cytogenetic analysis (review in Nayar, 1973), total genomic DNA hybridization (Aggarwal et al., 1997), and comparisons of homoeologous DNA sequences (Ge et al., 1999), four different genomic constitutions have been recognized among allopolyploid species (i.e., BBCC, CCDD, HHJJ and HHKK). Species with the genomic compositions of BBCC and CCDD have been especially well-studied phylogenetically. Cladistic analyses of multiple gene sequences or microsatellite markers have clarified phylogenetic

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doi:10.1016/j.ympev.2009.10.023

relationship among these *Oryza* polyploids and their diploids donors (Ge et al., 1999; Bao and Ge, 2004; Bao et al., 2006). These data show that BBCC species had multiple origins, with B genome maternal parents for Asian species and C genome for African species. All CCDD species derive from a maternal CC genome parent and a paternal DD genome parent. Although no diploid, DD genome parent has been discovered to date, sequence analysis has shown that EE genome species are closely related to the DD genome progenitor that gave rise to the CCDD species (Ge et al., 1999; Bao and Ge, 2004). These clear relationships facilitate an analysis of the pattern and direction of ITS homogenization following allopolyploid formation.

Based on rDNA-FISH analysis, Chung et al. (2008) recently localized rDNAs on chromosomes of *Oryza* species. They demonstrated that allopolyploid species had four to eight rDNA loci in their haploid genomes, and that these rDNA sites were not always additive of their parental diploid rDNA array numbers.

In the present study, using cloning and sequencing of multiple repeats per individual, we examined ITS polymorphism within and among six *Oryza* allopolyploids having BBCC or CCDD genomes, assessing nucleotide diversity among homologous ITS repeats and their origins. Our objective was to infer the evolutionary outcome of reuniting two divergent ITS repeat types in these allopolyploids, thereby providing some insight into the consequences of allopolyploidization on ITS evolution in *Oryza*.

2. Materials and methods

2.1. Plant materials

A total of 25 *Oryza* accessions were used in this study, representing six allopolyploids and five diploids (Table 1). The allopolyploids included three species with BBCC genomes and three with CCDD genomes. For the diploids, we selected one species each with BB and EE genomes, and three with CC genomes. Also studied were individual representatives of a diploid with a GG genome. The latter taxon was selected as the phylogenetic outgroup, based on earlier evidence that the species with GG genomes are sister to the remainder of the genus (Wang et al., 1992; Ge et al., 1999; Zou et al., 2008). In most cases, two accessions were selected per taxon; exceptions were *O. malampuzhaensis* (three accessions) and *O. latifolia* (four accessions).

Seeds were kindly provided by the Internal Rice Research Institute (IRRI, Manila, Philippines), except that the outgroup accession (*O. granulata*) was collected from China. Seeds from each accession were germinated following methods described previously by Bao and Ge (2004) and kept in the greenhouse for DNA-extraction. Further identities and genomic identification of these accessions are described in Bao et al. (2005).

2.2. DNA-extraction, PCR, cloning, sequencing and ITS copy searching

Total DNA was extracted from fresh leaves, following extraction methods described by Bao and Ge (2004). PCR amplification was performed in 25 μl volumes of 10 mM Tris buffer (pH 8.3) containing 2.5 mM $Mgcl_2$, 0.2 mM of each dNTP, 5 pM of each primer, 100 ng of template DNA and 0.75 U of Taq polymerase (Takaya). In addition, 8% dimethylsulfoxide (DMSO) was added to the reaction mixture to facilitate denaturation during PCR (Baldwin et al., 1995). Two primers were used to amplify the ITS region, i.e., ITS5 (5′AGAAGTCGTAACAAGGTTTCCGTA3′) near the 3′ end of the 18S rRNA gene, and ITS4 (5′TCCTCCGCTTATTGATATGC3′) near the 5′ end of the 26S rRNA gene. The procedure for 35 cycles of amplification was: 1 min denaturation at 94 °C, 1 min annealing at 52 °C,

Table 1
Materials used in the study, and nucleotide diversity of ITS sequences.

Taxa	Acc. no.	Genome	Origin	Seq. no.	ITS type	GenBank no.	π	*Hap.*
O. officinalis	105085 106159	CC	Philippines Papua New Guinea	6[b] 1[a]	C	AF479067, EU574633–EU574637 AF479063	0.0000 0.0005*	H_1 H_2
O. eichingeri	101422 105159	CC	Uganda	2[a]	C	AF479069, AY181995	0.0000	H_3
O. rhizomatis	103410 105448	CC	Sri Lanka	2[a]	C	AF479065, AF479066	0.0000	H_4
O. punctata	104071 105607	BB	Chad	10[b] 1[a]	B	AF479070, AF479071 EU574638–EU574646	0.0023 0.0022*	H_7–H_10
O. punctata	104059 105158	BBCC	Nigeria Kenya	2[b] 2[b]	B C	AF479074, AF479075 AF479077, AF479078	0.0017 0.0034	H_11, H_12 H_5, H_6
O. minuta	104674 101082	BBCC	Philippines	31[b]	B	AY188606–AY188636	0.0082	H_13– H_31
O. malampuzhaensis	105223 105328 80764	BBCC	India	27[b]	B	AY188637–AY188663	0.0035	H_32– H_38
O. alta	100161 105143	CCDD	Brazil Guyana	15[b]	C	AF513601–AF513606 AF514909–AF514917	0.0058	H_39– H_46
O. grandiglumis	105664 105669	CCDD	Brazil	8[b]	C	AF520780, AF520781 AY151363–AY151368	0.0061	H_46– H_48
O. latifolia	100914 100167 105145 105141	CCDD	Mexico Costa Rica Colombia Costa Rica	45[b]	D	AF513577–AF513600 AF513553–AF513564 AY126015 AY151355–AY151362	0.0044	H_49– H_63
O. australiensis	105263 101144	EE	Australia	2[a]	E	AF520777, AF520778	0.0050	
O. granulata	C0024	GG	Hainan, China	1[a]	G	AF520779	–	

π refers to nucleotide diversity, according to Nei and Li (1979). *Hap.* refers to different haplotypes (cf. Fig. 1).
[a] Refers to the sequences obtained by PCR sequencing.
[b] Denotes sequences obtained following cloning.
* Refers to the values obtained based on all sequences from species of *O. officinalis* or *O. punctata*.