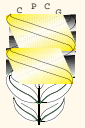


Relationships Among Wild Relatives of Rice Determined by Ribosomal Gene Sequencing

Mahin Abedinia, Robert J. Henry, and Simon Cripps Clark

CENTRE FOR PLANT CONSERVATION GENETICS

Southern Cross University
PO Box 157 Lismore 2480, Australia

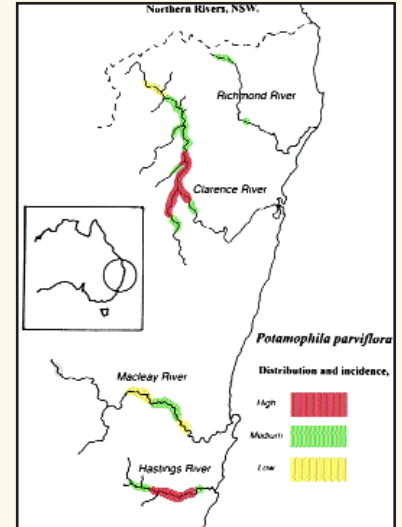


Introduction

The wild relatives of cultivated rice are important sources of useful genes for genetic improvement of rice production and quality. Cultivated rice belongs to the genus *Oryza* in the tribe *Oryzaceae*. There are 12 genera and about 70 species in this tribe (Vaughan 1994), most of which have not been the subject of detailed molecular genetic studies. We have investigated species from 2 genera, *Zizania* from North America and *Potamophila* from Australia, and related them to *Oryza*. *Zizania* is a native North American plant that grows in the

eastern and east central parts of that continent. *Zizania palustris* L. is an edible species which is now cultivated (Oelke *et al.*, 1997). *Potamophila parviflora* R.Br. which is the only species in this genus is restricted to rivers of the east coast of Australia.

We have examined the distribution of *Potamophila parviflora* and assessed the relationship between *Potamophila*, *Zizania* and *Oryza*, by sequencing the first internal transcribed spacer (ITS 1) between the 17S and 5.8S of their ribosomal genes.



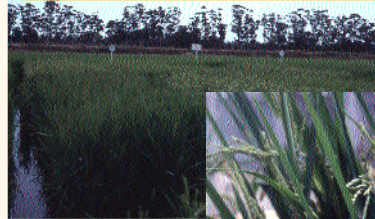
The Tribe Oryzaceae (Poaceae)

Genus	Number of Species	Distribution	Chromosome Number (2n)
<i>Oryza</i>	22	Pantropical	24
<i>Zizania</i>	3(4)	North America Asia, Europe	30,34
<i>Potamophila</i>	1	Australia	24
<i>Prostopychloa</i>	1	Southern Africa	Unknown
<i>Maltebrunia</i>	5	Tropical and South Africa	Unknown
<i>Leersia</i>	17	Worldwide	24,48,60,96
<i>Zizaniopsis</i>	5	North and South America	24
<i>Porteresia</i>	1	South Asia	48
<i>Luziola</i>	11	North and South America	24
<i>Rhynchoriza</i>	1	South America	24
<i>Hygroryza</i>	1	Asia	24
<i>Chikusochloa</i>	3	China, Japan	24

Plant Materials

Potamophila: *Potamophila parviflora* is a large clumped semiaquatic grass which grows mainly in and around the rivers of the east coast of Australia. The map shows the collecting locations, distribution and abundance of *Potamophila parviflora*. Leaf and seed samples were collected from various sites in this area.

Zizania: plant samples have come from a commercially grown field in southern NSW.



Australian rice cultivar, Jarrah



Potamophila parviflora, Upper Eden Creek NSW



North American wild rice, *Zizania*



Genetic Analysis

Total DNA was extracted from the leaf samples of each individual wild rice plant and used for PCR amplification and direct PCR sequencing. The ITS was amplified using primers which were designed from conserved regions of the 17S and 5.8S ribosomal genes based on the published sequence of rice DNA (Takaiwa, 1985).

Resulting sequences together with published ITS 1 region sequences of rice, *Oryza sativa* (Takaiwa *et al.* 1985), and sequences of four other grasses from the tribe Triticeae (Hsiao *et al.* 1994) were used as the input data in phylogeny analyses. These sequences were aligned using the CLUSTAL V computer programs. Phylogenetic analyses of ITS 1 were carried out in PAUP 3.1.1 using the exhaustive search option.

Rice	TCGTGACCCCT -GACCAAAAC AGACCGGAGAA -CGGTCACCC CCGTCCCGCC GAGCGCTCCG
Potamophila A..T.....T..G..A.G.C..
ZizaniaT...G...C.TAC.G..GCGCGA.GG
BarleyT..CT.....T..AAT..T...CGATGGCA
WheatT...C.....T...AAT..T...GCGA.GCCA
WildwheatT..C.....T..AAT..T...GCGATGGCA
SorghumTA.A.....T...AT...T...A.CT...T...TT.G...
Rice	CGCGGAGCCA ACCGAGGCC CGG-----
Potamophila	CG.G.C.TTT GAGAC...T GCGCCGCC CGCCCGAAG CCGCCGCC T CG.GCGCGG
Zizania	CA.G.GCCT...AA-...T.A.CCGGTC CGGC.ATAGG CTCTCCCTCT CCAGGGGGG.
Barley	TT.TTC.T.G TT..GCCAAT T--CCTGAC CGCTCCACT CCT..AGAG C...GGGGGT
Wheat	C..TCC.T.G CT..GCCAAT G--CCTGAC CACTCCCT CCTCGGAGG GGTGGGGCT
Wildwheat	TTCTCC.T.G CT..GCCAAT AT.CCTGAC CACTCCCT CCTTGGAGT GGTGGGGCT
Sorghum	T..GC.CAAG GT..CCCTTG. T.CAAGCT... ..GGGCA
Rice	-GGC----C AACGAAACC AGCGCGCGA GGCGT-CRAG GAA-CACAG ATACCCCGC
Potamophila	A...AC.....T.....AT.....TTC T.CT.AG...T
ZizaniaC.....T.....AT.....TT TACT.A...
Barley	C.TG.T...A.....AT.....TT GCCTAA..CG
Wheat	C.GG.T...A.C.....TT GCCTAA..CG
Wildwheat	C.GG.T...A.....TT GCCTAA..CG
Sorghum	GAGG.GCCA...A.....TT.....TA TGTT...TT
Rice	CGCGGCCCG TCGGCCCTG CGTCCGGCG GCBC
Potamophila	...T.C----C .A...GCAC GACG.GATAA .AC.
Zizania	...C----C .A...GCAC GCGTGTATTA .AC.
Barley	G.GA.ATG.C .A.CTTCCT. GTCTT.AC.T.T.T
Wheat	G.G.CATTC .A.CTTCCTA G.CGT.CCTC .T.T
Wildwheat	G.G.CATG.C .A.CTTCCTA G.CATTCCT .T.T
Sorghum	GCACG.G.A GT..T.GCA GCGCTCCCG T.C.

Aligned sequences of the ITS 1 from rice, two wild rices and the outgroups. Complete sequences between two amplifying primers (ITS 1 and ITS 2) are aligned to maximum identity with minimal gap adjustments. Nucleotide positions start at the beginning of the ITS 1. Dots (.) indicate matched sequences to the rice and bars (-) indicate the gaps.

Conclusion

Molecular evidence concerning the phylogeny of rice and related taxa were obtained by comparing sequences from the first internal transcribed spacers (ITS 1) of nuclear ribosomal RNA genes. Phylogenetic trees of these species generated from the ITS 1 sequence data were in general agreement with phylogenies based on the traditional taxonomic classification by Clayton and Renvoize (1986). This study confirmed that sequences of the ITS region are useful for phylogenetic studies among closely related species. Within the *Oryzaceae* clad, our data suggest that, despite a widely different habitat, a close relationship exists between the North American wild rice, *Zizania* and the Australian wild rice *Potamophila*. The ITS 1 sequence data also provide evidence that *Potamophila* is more closely related to rice and potentially a better source of useful gene for the improvement of cultivated rice as they both share the same number of chromosomes.

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Phylogenetic tree derived from ITS 1 sequence analysis

