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Australian *Oryza*: Utility and Conservation

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Abstract Australian *Oryza* are an understudied and under-exploited genetic resource for rice improvement. Four species are indigenous: *Oryza rufipogon*, *Oryza meridionalis*, *Oryza australiensis* are widespread across northern Australia, whereas *Oryza officinalis* is known from two localities only. Molecular analysis of these wild populations is required to better define the distinctness of the taxa and the extent of any gene flow between them and rice. Limited

collections of these wild populations are held in seed and DNA banks. These species have potential for domestication in some cases but also have many traits of potential value in the improvement of domesticated rice. Stress tolerance (biotic and abiotic) and grain quality characteristics in these populations may be useful.

Keywords Germplasm · Conservation · Diversity · Wild rice

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Introduction

Rice (*Oryza sativa* L.) was domesticated in Asia (Vaughan et al. 2008). Asian wild rice populations have coexisted with domesticated rice for a long period with significant potential for gene flow between wild and domesticated rice in both directions. Domesticated rice was introduced to Australia only very recently (in the last 200 years) and has not been grown near many of the wild populations. Production has been mainly in southern New South Wales and Victoria rather than in the tropics. As a result of this genetic isolation, *Oryza* in Australia may provide important information on the genetics and biology of rice prior to domestication. Australian *Oryza* species are a diverse and poorly characterized plant group. These populations probably represent an important and underutilized genetic resource for use in rice improvement. Four *Oryza* species are native to Australia: *Oryza meridionalis* N.Q.Ng, *Oryza rufipogon* Griff., *Oryza australiensis* Domin, and *Oryza officinalis* Wall. ex G.Watt (Fig. 1). *O. sativa* is naturalized in some limited areas (possibly shattering forms of *O. sativa*). The Oryzeae tribe in Australia also includes the endemic and monospecific *Potamophilia* and two *Leersia* species one of which is native (*Leersia hexandra* Sw.).

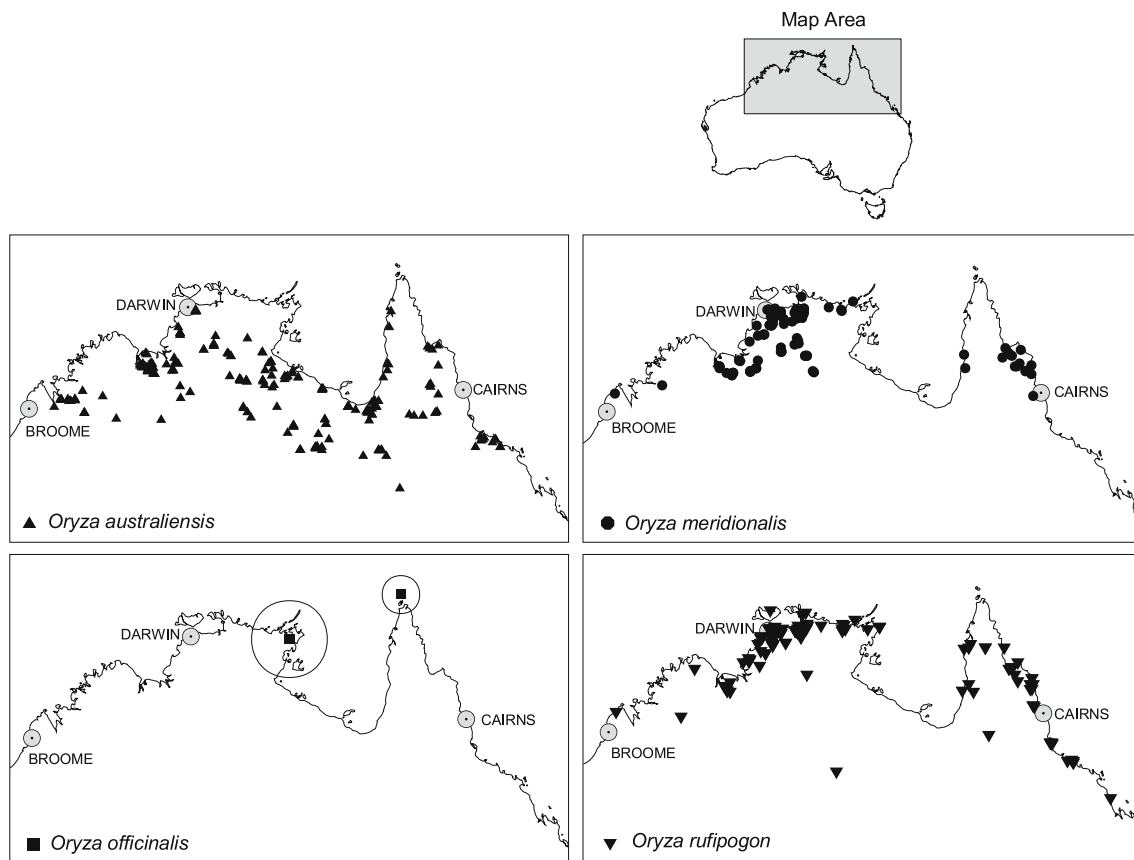


Fig. 1 Distribution of *Oryza* species in Australia as indicated by Australian Herbarium records.

Taxonomy of Australian *Oryza*

The Ehrhartoideae subfamily of the Poaceae includes three tribes and 120 species with two tribes and 18 species including the *Oryzaceae* tribe found in Australia (Table 1).

Table 1 *Oryza* and Other Members of the Ehrhartoideae Subfamily in Australia (Flora of Australia, Kodala 2009)

Tribe	Genus	Species
<i>Oryzaceae</i>	<i>Leersia</i>	<i>hexandra</i>
		<i>oryzoides</i> ^a
	<i>Oryza</i>	<i>australiensis</i>
		<i>officinalis</i>
		<i>sativa</i> ^a
		<i>rufipogon</i>
		<i>meridionalis</i>
<i>Potamophila</i>	<i>parviflora</i>	
<i>Ehrhateae</i>	<i>Ehrharta</i> ^a	6 introduced species
	<i>Tetrarrhena</i>	6 endemic species
	<i>Microlaena</i>	<i>stipoides</i> <i>tasmanica</i>

^a Species introduced and naturalized in the Australian environment

Native *Oryza* species are distributed across northern Australia, typically in monsoonal rainfall wet season areas.

O. rufipogon

O. rufipogon is a native perennial found in wet or swampy locations in northern Australia from Queensland and the Northern Territory to Western Australia. Recent molecular evidence (Hao and Ryuji, unpublished) suggests that the Australian populations of *O. rufipogon* may be distinct from the Asian populations.

O. meridionalis

O. meridionalis (Fig. 2) is a species that was thought to be endemic to Australia until the recent report of a population in Irian Jaya, Indonesia. This is an annual species that grows in wet areas and survives as seed in the dry season. This species has been reported from Queensland, Northern Territory, and Western Australia. The relationships between *O. meridionalis* and *O. rufipogon* populations require further investigation to establish the extent to which these populations are genetically distinct especially where they are reported from the same location (Fig. 3).

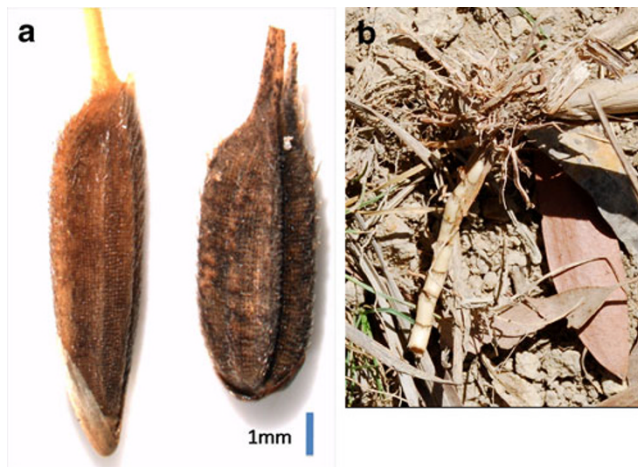


Fig. 2 a *O. australiensis* (right) compared to *O. meridionalis* (left). b Rhizomes of *O. australiensis* that allow survival in the dry season.

O. australiensis

O. australiensis (Fig. 2) is an endemic Australian species found across northern Australia in Queensland, Northern Territory, and Western Australia. This is a perennial that grows in relatively dry areas for an *Oryza* species. It is found in seasonally wet areas and apparently survives the dry season as rhizomes or seeds. This species has the largest genome in the genus due to apparent expansion with retrotransposons which has effectively doubled the genome size (Piegu et al. 2006).

O. officinalis

O. officinalis is a native species that has been reported from only two locations in the north of Queensland and the Northern Territory. The distribution of this species in Australia requires more investigation as it has been found in two remote and poorly collected regions only: Moa Island in Torres Strait and eastern Arnhem Land in the Northern Territory.

Oryza sativa

Rice has been cultivated in several regions in Australia and has become naturalized in some locations. Plants may also be found near to cultivation in the main production areas in New South Wales but are not likely to become naturalized in these areas. It is not clear if any weedy rice, shattering forms of *O. sativa*, are present.

Oryza nivara

The status of *O. nivara* S. D. Sharma & Shastry in Australia is uncertain (Kodela 2009).

Oryza minuta

Reports of *O. minuta* J. Presl. from Australia have probably been due to confusion with *O. officinalis* or other species (Kodela 2009). Further molecular analysis of Australian populations will clarify these reports.

Other related plants in the Australian flora

Potamophila parviflora

P. parviflora R. Br. is the sole species of the genus and is restricted to the rivers of northern New South Wales (Abedinia et al. 1998). It has not been reported from Queensland despite being found close to the border. This taxon is apparently more closely related to *Zizania* than to *Oryza* based upon ribosomal gene sequence analysis (Abedinia et al. 1998). Furthermore, *Potamophila* shares with *Zizania* the possession of separate sex flowers (Wheeler et al. 2001). The seeds are very small but the morphology of the seed is also similar to that of *Zizania*.

Microlaena stipoides (Labill.) R.Br.

M. stipoides is a widespread native species found in all states but not in the Northern Territory. Some genotypes of the species have large seeds that may be suitable for consumption as a whole-grain food as an alternative to rice. This species may be suitable for production in colder areas and with much less water than that required for domesticated rice. Attempts to domesticate this species as an alternative to rice that can be grown with less water are currently in progress. The strategy for accelerated domestication of this species involves the use of targeted mutagenesis (Cross et al. 2008) of domestication genes that have been characterized in the rice genome.



Fig. 3 Wild rice habitat *O. meridionalis* lodged in the dry season at Nifold Plain, Lakefield National Park.

Progress in characterizing the genomes of Australian *Oryza*

Physical maps of the genomes of *O. rufipogon* (439 Mb), *O. officinalis* (651 Mb), and *O. australiensis* (965 Mb) have been produced (Kim et al. 2008). Whole shotgun sequencing of *O. australiensis* and *O. meridionalis* has been undertaken recently (Henry et al., unpublished). Assembly of reference genome sequences for these species will await sequencing of bacterial artificial chromosome tiles of these genomes.

Conservation of Australian *Oryza*

Ex situ

Genebanks

Sixty-two Australian wild *Oryzae* accessions are stored as seed in the Australian Tropical Crops and Forages Germplasm Centre, comprised of 54 *Oryza* and eight *Potamophila* collections (Table 2; www.dpi.qld.gov.au/auspgris). These *Oryza* were collected across northern Australia and were along the known geographic spread for each species. The collections of *Potamophila* are from all known populations of the species and are therefore representative both genetically and geographically. The *Oryza* collections are from a relatively wide geographic distribution; however, the local environment is vastly different across the known geographic ranges of these species. Future targeted collecting missions need to be undertaken to actively conserve seed from the ecotypes within the geographic range of each species so that the genetic diversity of these species is appropriately conserved and available for future research and breeding activities.

Ex situ seed collections of Australian collected *Oryza* species are also conserved in some international germplasm collections. The two largest collections are at the International Rice Research Institute (IRRI) in the Philippines and the National Institute of Genetics and National Bioresource Project, Japan (Table 2). The IRRI collection consists of a total of 83 accessions from the three species *O. australiensis*, *O. meridionalis*, and *O. rufipogon* (<http://www.irgicis.irri.org:81/grc/irgicishome.html>). The collection conserved in Japan consists of a total of 84 accessions from the two species *O. australiensis* and *O. meridionalis* (<http://www.shigen.nig.ac.jp/rice/oryzabase/wild/coreCollection.jsp>).

Recent DNA sequence analysis has shown that some Australian samples held in the National Institute of Agrobiological Sciences genebank in Japan as *O. rufipogon* are distinct from other *O. rufipogon* and similar to *O. meridionalis* (Ishikawa, unpublished). Further molecular analysis of plants from sites where both of these taxa are reported may clarify the distinctness of these species and the extent of any gene flow between them.

DNA banks

Genetic variation within a species has been demonstrated to be sensitive to environmental change and is a significant marker for changes in biodiversity (Forest et al. 2007). The Australian Plant DNA Bank holds DNA and associated tissues of small numbers of accessions of the Australian *Oryza* species (Table 2) as a reference against which genetic change in wild populations could be monitored. These samples represent materials collected in collaboration with other research groups and agencies, e.g., Australian Tropical Crops and Forages Collection and Hirosaki University. More than one individual plant per species are held and the original specimens were collected from more

Table 2 Ex Situ Conservation of Australian Species from the *Oryzae* Tribe in the Australian Plant DNA Bank and as Seed Under Long-Term Conservation Conditions in the Australian Tropical Crops and

Forages Germplasm Centre, the International Rice Research Institute in the Philippines, and the National Institute of Genetics and National Bioresource Project in Japan

Taxon	Australian Plant DNA Bank	Australian Tropical Crops and Forages Germplasm Centre		IRRI, Philippines	National Bioresource Project, Japan
	Total	Total	State of origin		
<i>Oryza australiensis</i>	33	27	NT 20, QLD 6; WA 1	34	37
<i>Oryza meridionalis</i>	11	18	NT 18	45	47
<i>Oryza officinalis</i>	3	0			
<i>Oryza rufipogon</i>	3	5	NT 4, QLD 1	4	
<i>Potamophila parviflora</i>	8	8	NSW 8		
Total	58	62		83	84

T Northern Territory, QLD Queensland, WA Western Australia

than one geographical location ensuring that geographical diversity is held. Much more detailed sampling will be required to adequately represent the genetic diversity of Australian *Oryza*. These samples provide a central repository of DNA for genomics applications and research (Rice et al. 2006). Plant DNA banks have the ability to conserve the genetic fingerprint defining the species as well as the diversity within, and in the future, it is possible that they will act as “molecular snapshots” (Rice et al. 2008).

It is also highly desirable that the plant specimen vouchers be lodged with a recognized herbarium such as those listed in the Index Herbariorum (Thiers, continuously updated; <http://sweetgum.nybg.org/ih/>). Strong linkages between DNA Banks and traditional germplasm collections have merit in that they allow plant breeders and researchers to screen the DNA prior to selecting germplasm accessions for further investigation. With the recent advances in DNA sequencing and molecular analysis, the sequencing of whole genomes is an achievable short-term goal, and it is likely that DNA collections will hold the original samples from which the published sequence was derived. Linking of DNA vouchers with global positioning system data and herbarium vouchers and other data about the site of collection is desirable to add scientific value to collections.

In situ

O. officinalis is known from only two widely separated populations in remote areas of extreme northern Australia (Fig. 1). Little is known of the extent and size of these two populations and even less of the degree to which these are genetically distinct from individuals in neighboring countries to the north. In the absence of this knowledge, the conservation status of the material in Australia cannot be assessed.

The widespread distribution of the other three species in Australia (Fig. 1) indicates that these species are secure in

the wild. However, the limited knowledge of the genetic diversity within and between widely separated populations suggests the possibility that the loss of local populations might be significant. These species are found both within and outside protected areas such as national parks. Novel genetic variants and even undescribed species may be present. More extensive collection and careful molecular analysis are required to ensure that valuable genetic resources are not lost before they are discovered or described.

Risks for local populations include competition with weeds and land clearing for agriculture. Grazing by livestock may damage populations. The site depicted in Fig. 4 at Abattoir Creek in Queensland illustrates a population of *O. rufipogon* that was being grazed by livestock and threatened by altered surface water flows from a nearby sugarcane plantation. Weeds were also abundant at this site.

Climate change poses a range of threats to *Oryza* species in Australia. Changes in rainfall intensity altering water flows may alter suitable habitats for *Oryza* in Northern Australia. Rising sea levels may allow salt to move further inland in some extensive wetland that currently support significant *Oryza* populations.

Analysis of the genetic diversity in these wild populations is required to determine the best way to manage their conservation. Genome sequencing is expected to provide a reference sequence for each species to use as basis for measuring diversity within these species.

Conservation of these populations in situ can only be assured if we have more knowledge of the distribution of diversity within populations. This will establish the need or otherwise to protect specific populations especially in the very widespread species. Effective conservation plans depend on obtaining this genetic information. Conservation in reserves would appear adequate, but view this could change in genetic variations between populations were identified.

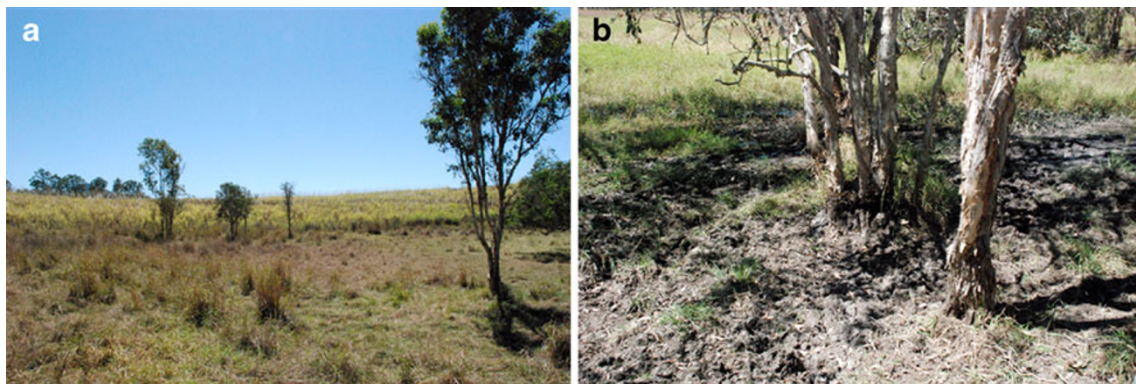


Fig. 4 Wild rice habitat *O. rufipogon* population at Abattoir Creek environmental park. **a** Population with adjacent sugarcane plantation nearby perennial type habitat. **b** Grazed *O. rufipogon* population under *Melaleuca* sp. at the same site in the dry season.

Utilization of Australian *Oryza*

Australian rice breeding programs have made very limited use of rice outside the *O. sativa* gene pool. *Oryza* species which include the Australian *Oryza* have been a source of both biotic and abiotic resistance genes (Brar and Khush 1997). *O. officinalis* has provided genes for bacterial blight, whitebacked planthopper, and brown planthopper resistance (Brar and Khush 1997). *O. australiensis* has been a source and of bacterial blight resistance (Brar and Khush 1997), and brown planthopper resistance has been introgressed from *O. australiensis* into rice cultivars (Jena et al. 2006). *O. australiensis* is also a source of durable blast resistance and genes for this important trait have been incorporated into advanced breeding lines (Jeung et al. 2007; Suh et al. 2009). *O. meridionalis* (Sundaramoorthi et al. 2009) and *O. australiensis* have been suggested as sources of drought tolerance. *O. rufipogon* has been used as a source of both biotic, tungro resistance, and abiotic, acid sulfate soil, and aluminum tolerance, stress-response genes (Brar and Khush 1997; Nguyen et al. 2003; Ram et al. 2007). Quantitative trait locus analysis of *O. rufipogon* × *O. sativa* and *O. minuta* × *O. sativa* crosses suggests both *O. minuta* and *O. rufipogon* harbor loci which enhance yield potential in an *O. sativa* background (Xie et al. 2008). *O. minuta* and *O. nivara* have provided a range of disease resistance genes (Brar and Khush 1997; Gu et al. 2004); however, the presence of these species in Australia or their relationship to Australian populations is uncertain and would benefit from further collection and genetic analysis.

The wild relatives of cultivated rice are yet to provide genes for grain quality, unlike the wild relatives of cultivated wheat and tomato which have been utilized extensively as donors of quality traits (Hajjar and Hodgkin 2007). The grain characteristics of Australian *Oryza* have been investigated to establish their potential as domesticated food crops and to evaluate the likely impact that they might have on rice grain quality if used as genetic resources for rice (*O. sativa*) breeding. The whole grain appearances of *O. rufipogon*, *O. meridionalis*, and *O. australiensis* suggest these species have potential as whole grain foods that could be consumed as alternatives to *O. sativa* (Kasem et al. 2010). The grains are generally of a similar size but have a darker appearance when compared with cultivated rice. The endosperm structure and starch granule morphology of *O. rufipogon* and *O. australiensis* was found to be similar to that of *O. sativa* (Shapter et al. 2008). However, the starch granules in *O. australiensis* were slightly smaller and the protein matrix more obvious in *O. rufipogon*.

The starch synthesis gene GBSS has been compared in *O. rufipogon* and *O. australiensis* (Shapter et al. 2009). The genes all showed close homology with rice and with other wild grasses with similar starch granule morphology. Genes

for grain traits that have been selected by humans such as aroma (Bradbury et al. 2005) and texture and cooking time (Waters et al. 2006) have been characterized in rice using the resources of the genome sequence. The diversity of these genes can now be explored in wild relatives of rice. These species are diverse genetic resources for use in rice improvement and may be especially useful in adapting rice to future pest and diseases and to abiotic stress. The more diverged relatives of rice in the Australian flora, such as *Potamophila* and *Microlaena*, may contribute to food security by providing more radical options to adapt rice to different or changing environments and to a wider range of environments.

Despite the demonstrated value of wild rice introgressions (Ballini et al. 2007), rice breeders are often reluctant to disturb favorable linkage blocks which they have constructed over many years by crossing locally adapted cultivars with wild rice relatives. New high output sequencing and high throughput genotyping platforms now mean it is possible to obtain cross specific genome sequence. These data can be converted to molecular markers which saturate the background of both parents which then allows the background of the recurrent parent to be efficiently and quickly regained which contains very narrow introgressed segments of the wild rice genome.

The Australian *Oryza* are a poorly characterized but potentially very important part of the genepool for rice. The conservation of these genetic resources both in situ and ex situ requires further effort to ensure that this diverse resource remains available to support rice improvement.

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