Reproductive development in Australian wild rice: a source of genetic variation for cultivated rice?

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Abstract

Oryza meridionalis is a wild relative of *Oryza sativa* endemic to tropical Australia. As a vigorous summer-growing plant, it has potential for agriculture. We report a number of novel findings relating to the reproductive development of wild rice. The photoperiod requirement of three *O. meridionalis* accessions from tropical Australia were compared, based on observations that flowering times differed under natural daylengths. While panicles initiated in the accession from Western Australia after just 53 d, even with a 13-h daylength, accessions from the Northern Territory and Queensland required a 12-h day or less to initiate and took three weeks longer to do so. That is, plants from the driest region were photoperiod-insensitive, maybe as an adaptive phenomenon. Grain yield was compromised in the wild rice by small individual grains and a harvest index of about 10%. The grain of *O. meridionalis* had high inorganic nutrient levels, particularly copper and zinc. Nitrogen levels were also high, suggesting a protein-rich grain in wild rice. Amino acid composition was not remarkable except for a higher methionine level in the wild rice relative compared to *O. sativa* and wheat.

Introduction

Rice (*Oryza sativa* L.) is one of the oldest cultivated cereal crops in the world. The centre of radiation of *Oryza* species is probably Austral-Asia (Vaughan *et al.* 2005), spreading throughout southeastern Asia, into Australia and north into Asia. *Oryza glaberrima* is a widely cultivated rice species in Africa that evolved from Asian ancestors. Various theories have been postulated regarding the radiations of *Oryza* species (Chang, 1976; Vaughan *et al.*, 2005) and about 22 wild *Oryza* relatives have been identified (Khush 1997; Vaughan *et al.* 2003; Vaughan *et al.* 2005). *Oryza* now occurs as a pan-tropical grass, with distributions which are sometimes broad (e.g. *O. rufipogon*) but in other species are narrow (e.g. *O. schlechteri*). More than 10,000 years after rice was first used as a human food source, it has evolved into a complex of more than 500,000 landraces and 100,000 distinct genotypes held at the International Rice Research Institute collection.

Modern rice retains many characteristics of the primitive grasses from which it arose, with numerous tillers producing open panicles in a relatively short and flood-tolerant plant. Its range is considerable, from upland plantings where it grows on a sometimes limited water supply, to floating rices which are harvested from deep water by boat. The temperature range to which it has been adapted is also marked, with rice grown in cool regions of north Asia and southern Europe through to hot savannahs and tropical zones. However, in spite of the phenotypic variation seen in *O. sativa*, at most 15% of the genetic variation across all 24 rice species is found in *O. sativa* (Zhu *et al.* 2007). In this study, we investigated *O. meridionalis*, a rice species native to northern Australia and Papua New Guinea. *Oryza meridionalis* shares the AA genome with *O. sativa* (Naredo *et al.*, 1997) but strong divergence within *O. meridionalis* has produced distinct genetic groups across northern Australia. For example, populations in Queensland and the Northern Territory are distinct (Juliano *et al.* 2005).

The imperative for human nutrition demands a broadening of the genetic base of *O. sativa*. Phenotypic features in wild rice relatives that would have to be improved are seed shattering, tillering and the vegetative–floral switch that is controlled through photoperiod. Shattering is obviously incompatible with seed retrieval, while excessive tillering might be an adaptation to a long growing season that is incompatible with high yields in a cropping cycle. Duration to heading is determined by the basic vegetative phase (BVP) and subsequent sensitivity of the floral switch to temperature and photoperiod.

Photoperiod is a critical agronomic feature that particularly influences time of panicle emergence in rice, even at low latitudes. However, photoperiod sensitivity is genetically variable, with cultivars from north-eastern China being photoperiod insensitive while many rices require short days to flower (Vergara and Chang, 1985). This characteristic is under the control of several key genes that vary in expression among japonica rice varieties (Wei *et al*., 2007). Using rice cultivars from low latitudes in a study in Senegal, Sié *et al*. (1998) identified a photoperiod-insensitive cultivar and undertook crosses to determine heritability. A number of interacting genes responsible for photoperiod sensitivity have been identified (see Sié *et al*., 1998). Through these studies, it is hoped to identify quantitative trait loci which can be used to identify agronomic lines that do not require short days to initiate flowering. Photoperiod sensitivity has also been observed in sub-tropical and tropical species such as sorghum (Clerget *et al*., 2004) and mung bean (Damayanti *et al*., 2010).

The adaptive significance of photoperiod in natural plant communities is complex but relates to seasonal conditions and particularly the timing of the monsoon in the tropics. Accessions of a wild mungbean species (short-day plants) were studied from different regions of northern Australia and adjacent regions, showing that those from more arid zones flowered earliest, possibly to achieve maturity before water supply was exhausted (Rebetzke and Lawn, 2006). Absence of sensitivity of floral initiation to photoperiod could be a more general phenomenon that is expressed widely across tropical Australia, where summer rainfall is erratic inland and in westernmost regions. On the other hand, species from wetter regions might be photoperiod sensitive, flowering only after the summer solstice. By this mechanism, plants such as wild *Oryza* spp. could optimise water utilisation through variation in photoperiod.

Another plant characteristic that is vital to agronomic success is grain composition. Both inorganic (Zhao and McGrath, 2009) and organic (e.g. protein level, amino acid profile) composition are critical for human nutrition and the possibility that some nutritionally essential constituents are enriched in wild relatives of crop plants should be pursued though screening of a range of germplasm. Seed size and the ratio of aleurone to endosperm will influence the overall nutritional qualities of rice grain by affecting the balance between starchy and ‘metabolic’ components of the seed. Furthermore, natural variation in inorganic nutrient storage in grain tissues would be a valuable tool in biofortification (Zhao and McGrath, 2009)

Finally, the proportion of plant biomass that is invested in grain is captured in the quotient called harvest index and is critical to domestication of plants. Wild species have not been selected for harvest, having evolved biomass partitioning patterns consistent with survival rather than yield. Thus, consideration of harvest index must be a high priority in any breeding programme aimed at incorporating germplasm from wild relatives into modern rice.

The aims of this study were to quantify for the first time these characteristics in the wild rice relative, *O. meridionalis*. Specifically, we report on the findings of an initial experiment on the photoperiod sensitivity of three wild accessions, as well as the grain quality in this wild rice species when compared to the agricultural species, *O. sativa*.

Materials and Methods

Photoperiod experiment - *Oryza meridionalis* was grown in 10 kg pots (400mm tall) containing a fine clay loam collected from Camben NSW (34ºS 150°E). Seed of three accessions of *O. meridionalis* Ng. were sown into Jiffy Pots® and after germination they were transferred to soil. Accessions were from wild populations collected in Keep River, Western Australia (16ºS 129ºE), Howard Springs Northern Territory (12ºS 131ºE) and Cape York, Queensland (15ºS 145ºE). These accessions were selected to represent a longitudinal gradient from similar latitudes across northern Australia.

Plants were grown to maturity in three replicated growth cabinets with eight pots of each accession in each cabinet. Light intensities were 600 µmol m^{-2} s^{-1} at the top of the canopy. Day temperature was 27ºC and continued for 13 h from the moment the lights switched on in all photoperiod treatments. Night temperature was 22ºC. Daylength was maintained at 13 h through the first 28 d post-germination. This long daylength was maintained for the first month of the basic vegetative phase (BVP) in order that decreasing daylength did not commence until close to the photoperiod-sensitive phase. Across many rice species, the BVP varied from 10 - 63 d (Vergara and Chang, 1985). After 28 d, daylength was shortened in two of the growth cabinets by 1 and 2 min d^{-1} while the third cabinet was maintained at a 13-h day.

Initiation of inflorescences was checked regularly using two criteria. Plants were dissected by making longitudinal slices through the region of the first node, peeling back leaf sheathes and thereby exposing the apical meristem (Fig. 1). Initiation was defined as the time when an inflorescence was clearly visible, by which time the tiny panicles were only 5 - 10 mm in length. Initiation was confirmed by elongation of stem internodes, which is characteristic of all grasses once floral development begins. Invariably, anatomical evidence for floral initiation was confirmed by stem elongation in the remainder of the same cohort of plants. The combination of these phenomena was used to estimate the moment that the floral switch occurred, within an error of ± 2 d.

Grain productivity (harvest index and grain quality) - Growth of plants to maturity was carried out in naturally lit glasshouses at temperatures of 29ºC (day) and 21ºC (night) from June to October. This experiment compared *O. sativa* cv. Amaroo (japonica subspecies) to *O. meridionalis* ('Cape York' accession, above). Plants were grown in the soil described above with 80 mg kg^{-1} of soil added as single superphosphate (8.8P:11S:20Ca) while nitrogen was added as urea at rates of 0 to 600 mg kg^{-1}. The native nitrate levels in this fine-textured loam were approx. 10 mg kg^{-1}. Grain was mature within four months of sowing except for some late panicles in *O. meridionalis* which developed slowly after maturity of the first heads. These represented a small proportion of total grain in other experiments.

Grains were harvested and de-hulled using a rubber abrasion technique. Grain was weighed and prepared for analysis. Inorganic nutrient contents were determined by inductively coupled plasma spectroscopy (ICP) by grinding seed to a fine powder before analysis at Waite Analytical Services at Adelaide University. Amino acid concentrations were determined at the Australian Proteome Analysis Facility at Macquarie University using the ground sample described above. Protein was hydrolysed in 6 *N* HCl at 110ºC for 24 h. After hydrolysis, the samples were derivatised using AccQTag reagent (Waters Corporation, Milford, MA, USA) then analysed using a high resolution RP-HPLC column on a UPLC system with 10min run times.

Results

Reproductive initiation in O. meridionalis

Initiation of the Keep River accession began with panicle initiation 53 d after germination (Fig. 2), even in control plants where daylength was constantly 13 h. This accession also initiated by 53 d after sowing when the daylength had been shortened incrementally to just 12 h, indicating that this variant had no photoperiod sensitivity within the sub-tropical latitudes.

Initiation of the other two *O. meridionalis* accessions followed 75 d after sowing but only when days had shortened. The Howard Springs accession did not flower until daylength had shortened to about 12 h 10 min while a daylength as short as 11 h 25 min was required to initiate the Cape York accession. Neither the Howard Springs nor the Cape York lines flowered in the course of the experiment if a 13 h day was maintained.

Panicle maturation and grain quality

Harvest indices (HI) of plants grown to maturity in glasshouses are shown in Table 1. The HI of *O. meridionalis* never exceeded 10%, indicating a modest partitioning of dry matter to grain. Furthermore, HI declined as the external N supply increased, contrasting with harvest indices that ranged up to 50% in *O. sativa*, depending upon N supply (data not shown).

Seeds of the three wild rice accessions were about half the mass of *O. sativa* (22.5 ± 1.7 mg). Seed harvested from glasshouse experiments using *O. meridionalis* accessions from Cape York, Keep River and Howard Springs weighed 9.4 ± 2.3, 11.4 ± 1.2 and 7.8 ± 0.9 mg (± SE), respectively.

Nutrient concentrations were distinctly different between grains of the two species (Table 2). Nitrogen levels were about 30% greater in *O meridionalis* when compared with *O. sativa*. Seed of *O. meridionalis* also had 16% more P but almost 30% less K than *O. sativa* (data not shown).

Levels of most key micronutrients were also higher in *O. meridionalis*. Zinc levels in particular were twice as high as in *O. sativa* while boron and copper levels were almost three times greater in *O. meridionalis*. Iron and manganese levels were very similar in the two species, indicating that the large changes observed for some inorganic nutrients was not due to a simple change in seed structure such as a shift in the proportion of bran layers. The differential inorganic levels are made most stark by the almost seven-fold higher levels of selenium in *O. sativa* seeds (data not shown).

Nutrient levels are not shown for all three *O. meridionalis* accessions used in the study of photoperiod. However, the levels of all nutrients reported in Table 2 were not substantially different among the various accessions of *O. meridionalis*.

Total amino acid concentrations on a dry mass basis appear in Table 3, with a recalculation of *percentage* amino acids in both rice and wheat for easier comparison. The dominance of glutamic acid is notable with levels considerably lower in rice than in wheat meal. The result is higher levels of most amino acids in rice, particularly arginine and aspartic acid. Proline was the most depleted amino acid in rice compared to wheat.

Discussion

The aims of this study were limited to some primary questions about characteristics of Australian wild *Oryza* species. Phenology is a critical characteristic for domestication of wild relatives of modern crops through hybridisation and it was in this context that the current study of phenology and grain properties was undertaken. Improvement of cultivated varieties of rice through introduction of genes from wild Australian relatives has been attempted, with *O. australiensis* as a donor for disease resistance (Jeung *et al*., 2007). However, the broader use of these wild plants as a food source has not been achieved in spite of their high photosynthetic capacity and growth rates (Zhao *et* al. 2008; Zhao *et al*., 2010). Three characteristics that are critical to domestication are: (a) timing of initiation of flowering in relation to season; (b) conversion of dry mass to grain (harvest index) and (c) the content of micronutrients and protein quality in grain.

Photoperiod is one of the two major environmental triggers controlling the timing of the floral switch in plants (Yoshida, 1981). Rice is a short-day plant and even at low latitudes where seasonal variation is slight and daylength almost invariant, domesticated and wild rice is often sensitive to daylength change (Vergara and Chang, 1985). Tropical sorghum is also sensitive to photoperiod, possibly even sensing the rate of daylength change (Clerget *et al*., 2004) and mung beans are most photoperiod sensitive when originating near the equator (Damayanti *et al*., 2010). In tropical Australia (latitude: 12 - 15°S) where the wild rice species used in this study originated, there appeared to be variation in photoperiod sensitivity. We first detected this in glasshouse experiments in Sydney (latitude: 33ºS) where the flowering times of different Australian individual accessions of *O. meridionalis* and *O. australiensis* were starkly obvious: some accessions seemed to require rapidly shortening days to flower. Plants were thereafter grown under highly controlled daylength that was set to mimic the latitude of origin, showing that as observed previously, the accession from Keep River National Park (far north-eastern Western Australia) commenced floral initiation less then eight weeks after sowing whereas the other accessions did not flower this soon, regardless of whether days were 13 h long or shortening. The Howard Springs accession from near Darwin required days to shorten to almost 12 h in order for panicles to initiate while the easternmost accession from central Cape York in Queensland had to experience daylength to be shorter than night in order to flower. The ecological basis for these observations is a matter of speculation but it is thought that daylength might act as a proxy for rainfall reliability in the Australian Big wet. In *Vigna* species, Rebetzke and Lawn (2006) showed that in wild mung bean, those accessions from the driest inland areas had the shortest reproductive phases, speculating that this was to achieve reproduction during the monsoon. For *O. meridionalis* from Keep River in Western Australia, this would be a credible survival strategy.

The initiation of flowering is only the first step in pathway to grain yield. Any efforts to make wild relatives of rice agronomically viable will require a shift in harvest index (HI), which is consistently more characteristic of crop domestication than any other feature. The wild rice relative *O. meridionalis* (Cape York accession) studied here was typical of the other Australian wild rice species that we have grown, having harvest indices not exceeding about 10% at any nitrogen level and with adequate water supply. The components of yield that contribute to this low HI are numerous and not reported in detail here but include a 50% lower individual grain weight. The Cape York accession produced many tillers in our experiments but did not produce ripe heads synchronously and retains the grain shattering phenotype typical of wild plants. Thus, it has some important limitations to commercial exploitation but these are general tractable through breeding (e.g. seed shattering - Konishi *et al*. (2006); Vaughan (2008)).

Finally, the quality of grain from novel crops is critical for human nutrition, especially in the Third World where supplementation is often impracticable and biofortification is a high research priority (Mayer *et al*., 2008). *Oryza meridionalis* offers some potential, with significantly higher concentrations of copper, zinc and iron on a dry mass basis. The cause of variation between *Oryza* species is now worth exploring, with the location of the inorganic nutrients within the various grain layers a critical question. Specifically, it is likely that micronutrients critical for nutrition are concentrated in the aleurone layer and thus are susceptible to being lost on polishing of rice grains. This would in turn diminish the nutritional superiority of the wild relative. However, it should also be noted that inorganic nutrient levels are not uniformly elevated in *O. meridionalis*, ranging from one-third higher for manganese to a 3.5-fold increase for copper. Nitrogen levels were 30% higher in *O. meridionalis*, suggesting a more protein-rich seed that must bring nutritional benefits. The amino acid composition of this protein-rich grain was not dramatically different from *O. sativa* (Wu *et al*., 2002) except that methionine levels appeared to be higher in *O. meridionalis*: methionine is en essential amino acid. The amino acid composition of wheat diverges from that seen within *Oryza* spp. but follows the same general patterns seen in rice. Proline, was notably more abundant in wheat while 13 amino acids were more abundant in wild rice that in wheat.

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Table 1: Harvest index of *O. meridionalis* (accession Cape York). Plants were grown in a naturally lit glasshouse with a range of inorganic nitrogen levels supplied, from 0 to 600 kg ha^{-1}. At panicle maturity, seeds were harvested before the panicles shattered. In that these plants are somewhat indeterminate, the seed from late-developing panicles could not be harvested but the major seed set was captured. Spikelets were de-hulled prior to weighing Standard errors of four replicates are quoted in brackets.

Nitrogen supply (mg kg^{-1})	Harvest index (%)
0	11 (1.2)
200	10 (0.8)
300	8 (0.6)
400	7 (0.5)
600	6 (0.8)

Table 2: Nutrient concentrations (% DM or mg kg^{-1}) in the grain of *O. sativa* and *O. meridionalis*. Nitrogen concentrations and levels of various inorganic constituents are reported on a dry mass basis. Means are quoted with standard errors in brackets.

	Nutrient concentration (DM basis)					
Species	*N**	*Fe***	*Mn***	*Zn***	*Cu***	*B***
O. sativa	1.57 (0.28)	10 (0.2)	36 (3)	17 (0.4)	2.2 (0.01)	5.2 (1.6)
O. meridionalis	2.02 (0.09)	16 (0.2)	48 (4)	49 (0.1)	10.0 (0.2)	19 (0.7)
% ↑ in wild rice	29	60	33	188	355	265

* g $100g^{-1}$ DM ** mg kg^{-1} DM

Table 3: Amino acid concentrations in protein of grain on *O. meridionalis* per unit of total grain dry matter (first data column). The proportions of each amino acid are converted to percentages of the total complement in the last three columns. Values for *O. meridionalis* are means of four replicate samples. Values for *O. sativa* were taken from Wu *et al*. (2002) and wheat from Vaníček and Turek (1983). Note that tyrosine was not measured in wheat and tryptophan/cysteine could not be measured in *O. meridionalis* and were thus omitted from the percentage calculations.

Amino acid	**Concentration (mg g DM^{-1})**	**% in total protein (*O. meridionalis*)**	**% in total protein (*O. sativa*)**	**% in total protein (*T. aestivum*)**
Histidine	2.1	2.6	2.8	2.2
Serine	4.3	5.3	5.9	4.3
Arginine	7.9	9.7	6.5	4.3
Glycine	3.6	4.5	8.3	4.0
Aspartic acid	7.8	9.6	9.8	6.0
Glutamic acid	16.1	19.9	17.9	32.8
Threonine	3.1	3.8	4.2	3.1
Alanine	4.8	5.9	8.9	4.2
Proline	3.8	4.6	5.6	11.9
Lysine	3.2	3.9	3.6	3.4
Tyrosine	3.2	-	-	-
Methionine	1.9	2.3	1.3	1.8
Valine	5.6	6.9	7.5	5.1
Isoleucine	4.0	4.9	4.8	4.0
Leucine	7.8	9.6	8.6	7.6
Phenylalanine	5.1	6.3	4.3	5.8

Figure 1: Dissection of two newly initiated panicles of *O. meridionalis* (Keep River accession). Panicles were enclosed in many layers of leaves that had been peeled away. Each division represents 1 mm.

Figure 2: Panicle initiation in three *O. meridionalis* accessions from Keep River (Western Australia), Howard Springs (Northern Territory) and Cape York (Queensland). Seedlings were grown in soil in growth cabinets for 28 d before being exposed to three daylength regimes: (a) continuing 13 h of daylight (b) days shortening by 1 min d^{-1} (red line) and (c) days shortening by 2 min d^{-1} (yellow line). Panicles initiated in all three treatments after 53 d in the Keep River line and by 73 d in the other lines, but only when daylength had shortened to 12 h (Howard Springs) and about 11½ h (Cape York). Based on multiple replicates that were sampled every 3-4 d close to initiation and at least 4 replicates when panicle emergence was first noted.