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**Australian Wild *Oryza* Species: Growth, Canopy
Structure and Atmospheric CO₂ Effects**

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Summary

The canopy structure of *O. sativa* and the Australian wild *Oryza* relatives, *O. australiensis* and *O. meridionalis*, were compared developmentally and with digital models. Species differences were found in average leaf angle, self-shading, leaf dispersion and leaf area index (*LAI*). There was no advantage of the near vertical leaf angles that are associated with high leaf area index in *O. sativa* and therefore, *O. sativa* did not have superior light interception efficiency (*LIE*). There was also no clear difference between the species in light-saturated photosynthetic rates; all species had higher rates in the vegetative than the reproductive phase of growth. Elevated CO₂ enhanced photosynthesis, accelerated development of total leaf area, shoot and root weights, tiller number and plant height during vegetative development but at the reproductive stage of development, there were no longer any significant species differences. *Oryza meridionalis* produced the greatest biomass, with substantial variation between *O. meridionalis* accessions collected from an arc across tropical Australia. *Oryza sativa* produced more grain and had much higher harvest indices than *O. meridionalis* or *O. australiensis*. It was concluded that the wild *Oryza* relatives were as efficient as *O. sativa* in photosynthetic carbon fixation. However, radical re-design of *O. sativa* canopies to resemble the wild *Oryza* archetypes might confer advantages in heat dispersal or efficient water use.

Declaration

The work presented in this thesis was carried out between February 2010 and February 2014 on a part-time basis. This work represents original research that has not been submitted for any other degree. All work was carried out by the author unless otherwise acknowledged.

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Abbreviations and definitions

α	Elevation angle, angle between a structure and a horizontal line
β	Leaf dispersion parameter
Φ	Apparent quantum yield
ε	Coefficient in <i>STAR</i> equation
a	Elevation angle, angle above the horizon, complement of zenith angle
Ω	Viewing angle (elevation, azimuth pair)
A	CO ₂ assimilation rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)
A_c	Canopy crown surface area
A_L	Plant leaf area
A_{max}	Maximum net photosynthetic rate at light saturation
Accession	A genetically distinct strain (or line) within a species (in this case, rice), collected from a known location, with distinct morphological properties and true breeding to these characteristics.
ANOVA	Analysis of variance
c_i	Intracellular [CO ₂]
CGR	Crop growth rate
CY	<i>O. meridionalis</i> from Cape York, Queensland
<i>DAP</i>	Displayed leaf area perpendicular to incident sunlight not shadowed by other leaves of the same plant
<i>DAP:PAP</i>	Ratio of displayed (unshaded) leaf area over projected area
<i>DAP:TLA</i>	Ratio of displayed (unshaded) leaf area to total leaf area directed towards a given sky region
<i>DAS</i>	Days after sowing
DF	Degrees of freedom
E_d	Light capture efficiency= DAP/TLA
GLA	Gap light analysis
<i>HI</i>	Harvest index, grain weight divided by the sum of shoot and grain weight
HS	<i>O. meridionalis</i> from Howard Springs, Northern Territory

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IRGA	Infrared gas analyzer
k	Relative growth rate
KR	<i>O. meridionalis</i> from Keep River, Northern Territory
L_D	Leaf area displayed (not shadowed)
L_P	Leaf area projected (shadowed + not shadowed)
L_T	Total leaf area (TLA)
LAI	Leaf area index
LCP	Light compensation point
LIE	The ratio of mean light intercepted by leaves to light intercepted by a horizontal surface of equal area.
mya	million years ago
PAP or L_P	Projected (leaf) area perpendicular to incident sunlight
PAR	Photosynthetically active radiation
R_d	Dark respiration rate
RuBP	Ribulose-1,5-bisphosphate carboxylase/oxygenase
SEM	Standard error of the mean
SLA	Specific leaf area
S:R	Shoot weight to root weight ratio
$\overline{STAR}$	Silhouette to total area ratio, averaged over all viewing angles
	STAR segmented into 15° segments instead of being averaged over the whole hemisphere
TLA	Total leaf area
Zenith angle	Angle between an object and a vertical line, complementary to elevation angle

The following abbreviations for accessions and species are used:

OS = *O. sativa*

OM CY = *O. meridionalis* CY

OA = *O. australiensis*

OM HS = *O. meridionalis* HS

OM KR = *O. meridionalis* KR

Chapter 1 – Introduction

1.1 Biogeography

The distribution of wild¹ *Oryza* has been greatly affected by world climate. The range at 20,000 years BP, a glacial period, is much more restricted than the distribution 9,000 years BP, a relatively warm period (Figure 1.1). In the warmer, wetter climate of the late Pleistocene-early Holocene, rice distribution was at its most extensive (Fuller 2011). Figure 1.2 shows reported modern sites of wild *Oryza*, which is closer to the 9,000 years BP distribution than to the 20,000 years BP distribution. The distribution of rice now is a little less than it was in 9,000 BP.

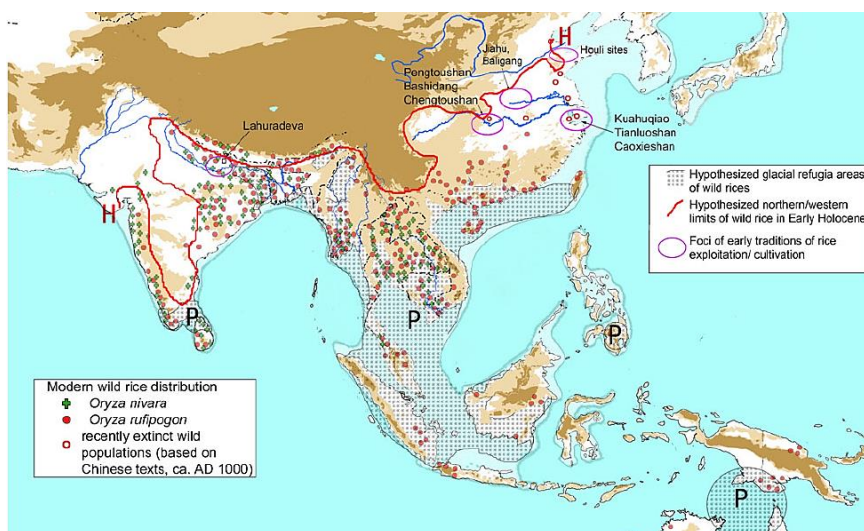


Figure 1.1 Red line is maximum distribution of *Oryza* at 9,000 BP; Shaded areas are rice that survived the last glacial period 20,000 BP; solid circles and crosses represent the distribution of modern rice (Fuller 2010)

¹ The term ‘wild rice’ is sometimes applied to *Zizania palustris* or *Z. aquatica*, also called ‘Indian rice’, which occurs in North America. However, these species are within the Tribe Oryzeae but not the genus *Oryza* (Tang et al. 2010). In this paper, we refer to the ‘wild *Oryza* species’ or ‘wild relatives of rice’ to denote those *Oryza* species that are not normally cultivated for food.

Oryza is found mainly between 37° N and 37° S (Figure 1.2) and up to an altitude of 1,000m (Vaughan 1989). Each of the 21 wild species has a wide but patchy distribution, often left to waste land at the fringe of cultivated rice growing areas. *O. rufipogon*, usually considered the ancestor of *O. sativa*, is widely distributed from southern China to India, Southeast Asia, Indonesia and New Guinea (Figures 1.1 and 1.2; Huang et al. 2012a). Large areas of three wild *Oryza* species grow in Northern Australia where there is little competition from cultivated crops and they can form extensive grasslands. Wild *Oryza* species also grow in Africa, South America, Southeast Asia, China, Indonesia and India (Figure 1.2).

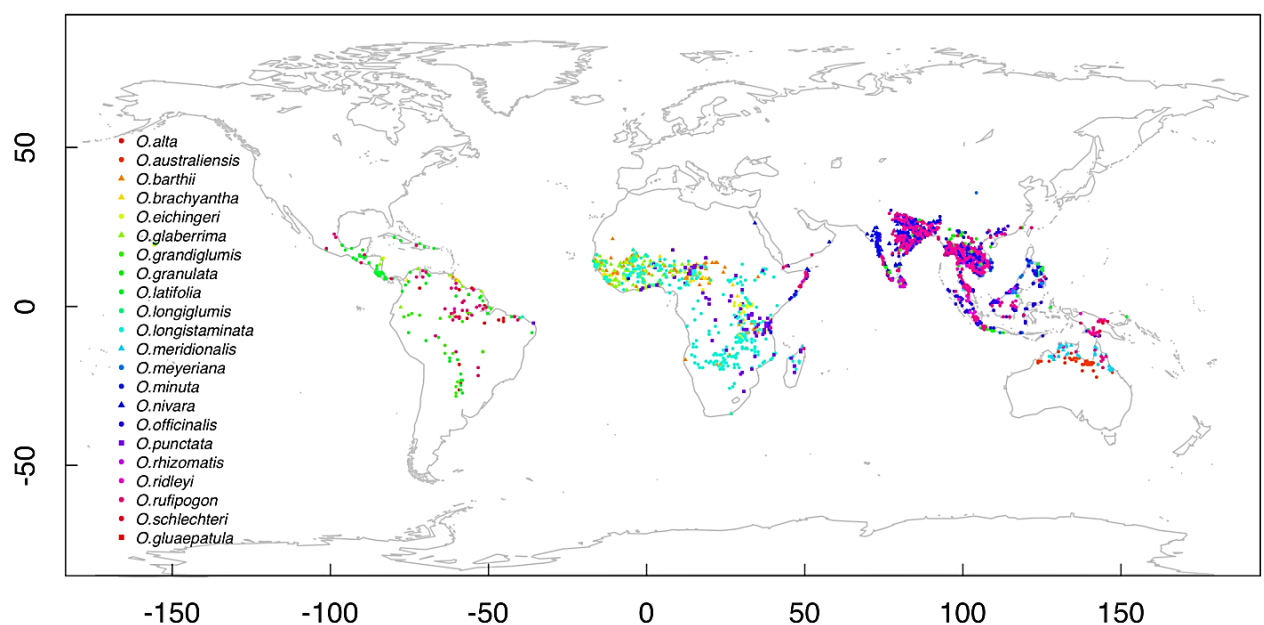


Figure 1.2 Collection points for wild *Oryza* species (Atwell et al. 2014). Latitudes and longitudes are represented on the axes.

Wild *Oryza* species grow on savanna, savanna woodland, floodplains, seasonally flooded land, stagnant water and deep water. Some rice grows in shaded conditions or in woodland, although species preferring full sun are more common (Vaughan et. al. 2008; Zhao et al. 2008). Australian wild *Oryza* mostly grows on seasonally flooded ground. *Oryza australiensis*, a perennial plant, survives the dry season as rhizomes and *O. meridionalis*, an annual plant, survives as seed; both survive in seasonally dry areas (Henry et al. 2010). Wild rice species are often relatively drought tolerant compared to cultivated (Vaughan et al. 2008; Atwell et al. 2014).

Rice is cultivated on every continent except Antarctica (Table 1.1) and grows over a range of latitudes from 0° to 53° (Liu et al. 2013), from sea level to an altitude of over 3,000 m (Poutel and Kotani 2013). *Oryza sativa* is dependent on high water availability and grows best in sub-tropical and tropical climates although it can grow in dry regions if it is irrigated. Some rice can grow in temperatures as low as 20°C, but typical rice-growing temperatures are in the range 25 - 30°C (Yoshida 1981).

Table 1.1 Modern rice growing regions, area and production in 2012². Mean yield = 4.4 t ha⁻¹

Region	Area (‘000 ha)	Area (%)	Production (‘000 t)	Production (%)	Production (t ha ⁻¹)
European Union	466	0.29	3,053	0.4	6.7
Central America	298	0.19	1,027	0.1	3.5
East Asia	33,562	21.23	224,681	32.2	6.7
Northern Africa	756	0.48	6,857	1.0	9.2
Northern America	1,113	0.70	9,240	1.3	8.3
South America	4,575	2.89	23,429	3.4	5.1
South Asia	60,565	38.31	220,461	36	3.7
South-East Asia	46,388	29.34	180,860	25.9	3.9
Sub-Saharan Africa	9,339	5.91	19,863	2.8	2.1
Caribbean	470	0.30	1,405	0.2	3.3
Oceania	110	0.07	1,111	0.2	9.2
Central America	298	0.19	1,027	0.1	3.5

² Modified from International Rice Research Institute (2012) based on USDA estimates.

1.2 Origin of the genus *Oryza*

Oryza species are postulated by Vaughan et al. (2005) to have developed from *O. ridleyi*, probably via *O. granulata* (considered to be the closest species to the ancestral *Oryza*), in an area including Southeast Asia and New Guinea and possibly eastern India, and then spread to South Asia, China, Africa and South America to the west and Australia to the south-east (Figure 1.3). Transport by bird and animal vectors has been suggested as a mechanism of intercontinental movement of rice genotypes from the Centres of Diversity from which the genus is believed to have radiated. *Oryza sativa* is usually considered to have developed from *O. rufipogon* (Huang et al. 2012a).

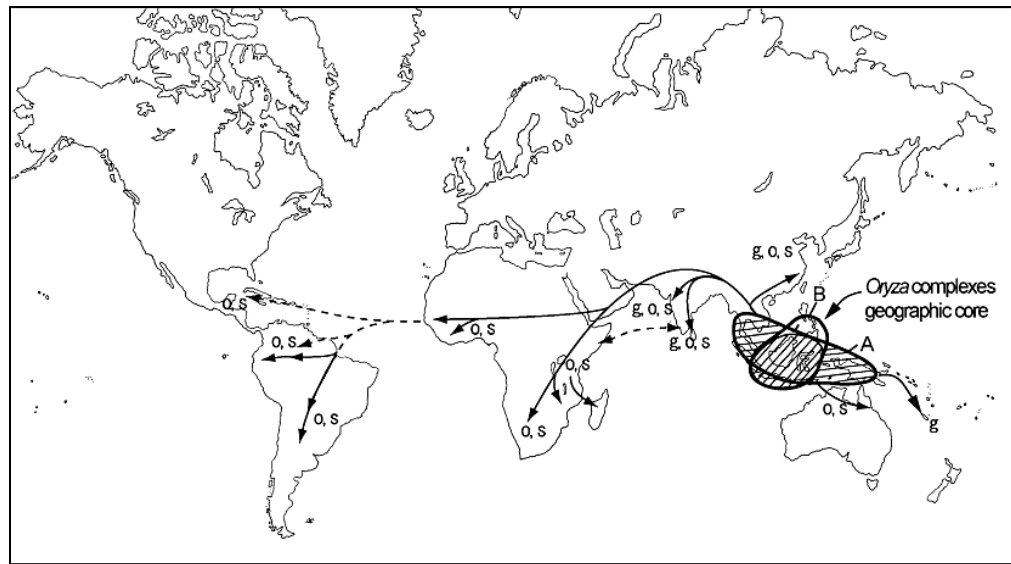


Figure 1.3 Centres of Diversity for (A) *O. ridleyi* and (B) *O. granulata* and extent of intercontinental migration of *O. sativa* (s), *O. officinalis* (o) and *O. granulata* (g); (Vaughan et al. 2005)

1.3 The evolution of genus *Oryza*

Grasses evolved 55 - 60 mya (Kellogg 2001) and the genus *Oryza* evolved from ancestral grasses later, although the dating is uncertain. Levy and Feldman (2002) used the time of genome duplication to estimate this event at 10 - 60 mya and Kristas et al. (2012) used 'ultra-conserved

elements' to estimate divergence at 50 mya. Vaughan et al. (2005) considered the likely site of origin as in modern day Indonesia or New Guinea because these are sites of high genetic diversity (Figure 1.3). There is great variation in genome size, with tetraploid species having the largest genomes (Table 1.2) but the diploid *O. australiensis* genome is very large, 965 MB, compared to *O. glaberrima*, about 357 MB. The *O. australiensis* genome expansion probably occurred about 3 mya by the addition of many retrotransposon copies (Piegu et al. 2006). *Oryza australiensis* and *O. sativa* separated from their common ancestor about 8.5 mya (Piegu et al. 2006). Speciation of commercially important rice seems to have been much more recent, with *O. sativa* and *O. glaberrima* separating 0.64 mya and the *indica* and *japonica* subspecies of *O. sativa* just 0.4 mya (Ma and Benettzen 2004). The timing of these events remains a matter of disagreement (Vaughan et al. 2005). Of *Oryza* with AA diploid genome, *O. meridionalis* is considered the closest to the ancestral type (Zhu and Ge 2005).

1.4 The rice genome

The rice genome was first sequenced in 2005 (International Rice Genome Sequencing Project 2005) consisting of 389 Mb and 37,500 - 50,000 possible genes. There has since been some downward revision of the number of transcribed genes and with so many of unknown function, the process is not complete (Jiang et al. 2012a). Sakai et al. (2013) found 37,869 'loci with expression evidence', that is, probable genes. Genome sequencing of wild *Oryza* species has revealed some larger genomes in the wild relatives than observed in *O. sativa* (Kim et al. 2007, 2008; Jacquemin et al. 2013), particularly for tetraploid species but also to a lesser extent for diploid species such as *O. australiensis* (965 Mb) and *O. granulata* (882 Mb; Table 1.2).

There are 23 species of *Oryza*, some diploid and some tetraploid. However there is some uncertainty about whether some species are truly distinct (e.g. *O. grandiglumis* and *O. alta*) or 'one complex species with different ecotypes' (Vaughan et al. 2003). Similarly, *O. sativa* and *O. rufipogon* are sometimes thought to be members of the same species. Most rice accessions are diploid, as are the rice accessions studied in this investigation (*O. australiensis*, *O. meridionalis* and *O. sativa*) but some are tetraploid, of which the South American varieties are notable for

having a genome that does not occur in a diploid genotype (Table 1.2; Ge et al. 1999). The *O. sativa* genome size is ~389 Mb, small compared to other crop species such as barley ~5,300 Mb or hexaploid wheat ~17,000 Mb. Gene numbers are more consistent than base pair numbers, as the cereal genomes contain many repeats increasing genome size but not gene number (Jiang 2012a; Morrell et al. 2012).

Table 1.2 Accessions of the *Oryza* species, genome and distribution

Genome	Section/species	Locality	Genome size (Mb)		
	Section <i>Oryza</i>		*	**	***
AA	<i>O. sativa</i> ssp. <i>indica</i>	China - now worldwide	389	~400	
AA	<i>O. sativa</i> ssp. <i>japonica</i>	Japan	389	~400	
AA	<i>O. glaberrima</i>	Chad	357	~354	809
AA	<i>O. barthii</i>	Cameroon		~411	
AA	<i>O. glumaepatula</i>	Brazil,		~464	
AA	<i>O. longistaminata</i>	Kenya, south and west Africa		~352	
AA	<i>O. meridionalis</i>	Australia		~435	
AA	<i>O. nivara</i>	Laos	448	~448	760
AA	<i>O. rufipogon</i>	China, Thailand	439	~445	760
BB	<i>O. punctata</i>	Cameroon	425	~423	539
CC	<i>O. officinalis</i>	Philippines		~653	1021
CC	<i>O. rhizomatis</i>	Lanka		~650	
BBCC	<i>O. minuta</i>	Philippines	1124		1691
BBCC	<i>O. eichingeri</i>	Uganda		~650	
CCDD	<i>O. alta</i>	Guyana, Brazil	1008		1000
CCDD	<i>O. grandiglumis</i>	Brazil			
CCDD	<i>O. latifolia</i>	Costa Rica			
EE	<i>O. australiensis</i>	Australia	965	~960	1054
	Section <i>Ridleyanae</i>				
FF	<i>O. brachyantha</i>	Sierra Leone	362	~260	343
HHJJ	<i>O. longiglumis</i>	Indonesia			
HHJJ	<i>O. ridleyi</i>	Malaysia	1283		1568
Unknown	<i>O. schlechteri</i>	Papua New Guinea			
	Section <i>Granulata</i>				
GG	<i>O. granulata</i>	China, Vietnam	882	~862	907
GG	<i>O. meyeriana</i>	Malaysia			
	Outgroup				
	<i>O. perieri</i>			~323	
HHKK	<i>O. coarctata</i>	Bangladesh			1568

*Kim et al. 2007; ** Jacquemin et al. 2013; ***Wing et al. 2005.

1.5 Domestication

Oryza sativa was domesticated 8,000 - 13,000 ya, possibly in the Yangtze valley in China (Molina et al. 2011, but see Shomura et al. 2008 who argue for a Southeast Asian origin for *japonica* rice), and *O. glaberrima* more recently, perhaps 300 BC in Africa (McIntosh (1995) quoted by Sweeney and McCouch (2007)) although Vaughan (1989) and Agnoul et al. (2012) maintain domestication was much earlier. *Oryza sativa* was probably derived from *O. rufipogon* (Huang et al. 2012a) and the African *O. glaberrima* from *O. barthii* (Sweeney and McCouch 2007). There is dispute about the number of instances of domestication of *O. sativa* (Kovach et al. 2007; Molina et al. 2011; Yang et al. 2012). Did *O. sativa* ssp. *japonica* and *O. sativa* ssp. *indica* separate independently or did *japonica* separate first then subsequently *indica* diverged from *japonica*? The evidence favours *japonica* first, then *indica* but is not yet conclusive (Sweeney et al. 2007; Huang et al. 2012b).

Resistance to shattering (the premature shedding of seed from the inflorescence after ripening) is a key difference between wild and cultivated rice because shattering makes wastage in harvesting very high (Harlan et al. 1973). He et al. (2011) claim at least 13 genes may be involved in domestication. Shattering is largely controlled by two genes, *sh4* the key shattering gene that distinguishes cultivated from wild rice, and *qSH1* which controls the difference in the degree of shattering between *indica* and *japonica* varieties of rice (Vaughan et al. 2007).

Sweeney and McCouch (2007) argues pericarp colour change was important in domestication as it provided farmers with a readily observable way of distinguishing varieties of rice that are otherwise hard to tell apart. White rice came to be widely dispersed by trade after it first appeared in *japonica*. They list features that may account for human preference for white rice such as ease of cooking and easier detection of contaminants in white rather than red rice (*O. rufipogon*).

Other factors associated with domestication are noted, such as hull colour and tiller angle (Huang et al. 2012a). Wide tiller angle and prostrate form, found in *O. rufipogon*, is regarded as an undesirable trait that was selected against in domestication, and is affected by genes *PROG1*, whilst *LA1* and *TAC1* affect tiller number only (Jin et al. 2008). Tan (2008) note inactivation of

one gen, *PROG1*, reduced tiller angle, produced more upright plant structure and increased grain yield. Some differences between wild and domesticated rice are listed in Table 1.3.

Table 1.3 Characteristics of wild and domesticated rice (Sweeney and McCouch 2007)

Character	Wild	Domesticated
Awns	Long	Short
Shattering	Severe	Reduced
Dormancy	High	Low
Pericarp	Pigmented	White
Hulls	Dark	Straw colored
Reproduction	Outcrossing	Inbreeding
Grain size	Small	Large but variable
Panicle	Open, few branches	Densely packed

1.6 Growth and development of cultivated *O. sativa*

Germination of cultivated *O. sativa* may occur at temperatures from 8 - 44°C but it is optimal at 37°C when 90 - 97% of seeds will germinate. Below 18°C, the rice spikelet may not be fertile. Optimum growth temperature range is 30 - 32°C. Cultivated rice may develop in 80 - 150 d but can take much longer and many wild *Oryza* species are perennial (Yoshida 1981). In aerobic or mildly hypoxic conditions, the coleorhiza emerges before the coleoptile, while the reverse occurs in anaerobic conditions. From the coleoptile a prophyll develops, then the first true leaf. This shoot forms the culm, from which branches called tillers and adventitious roots develop (Smith and Dilday 2003). Rice growth stages are vegetative phase, vegetative lag phase, reproductive phase as outlined below.

Vegetative phase (numbers in brackets refer to labels in Figure 1.4A)

S0 Dry seed

S1 Coleorhiza (2) emerges (or coleoptile (1), when submerged)

S2 Both coleoptile and coleorhiza have emerged

S3 Prophyll (4) emerges; leaf (6) emergence; tillering (10) commences after fifth leaf emerges; the first tiller comes from the axillary bud of the second leaf, the second from the axillary bud of the third leaf (Figure 1.4A). Tiller numbers vary from 5 - 30 for *O. sativa*.

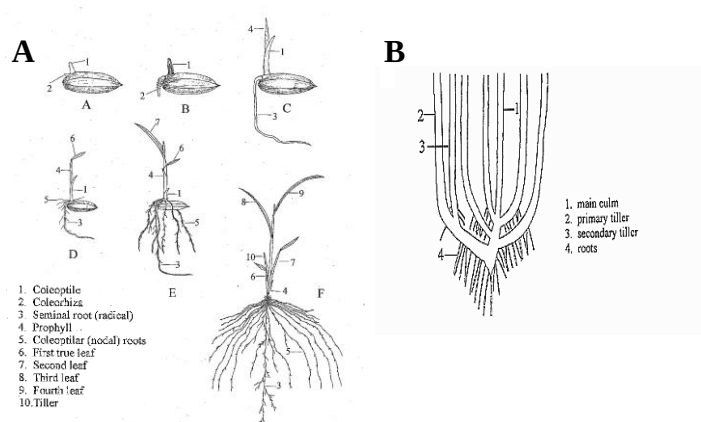


Figure 1.4 Vegetative growth stages of rice: **(A)** Rice seedlings in aerobic conditions; **(B)** tiller development (figure from Smith and Dilday (2003))

Vegetative lag phase - after tillering and before reproduction.

Reproductive phase

Panicle initiation is not apparent without fine dissection, and even then only after the event has occurred at a developmental level. It is characterised by elongation of the first internode within the main stem and therefore displacement of the first node away from the stem base. This can be observed as a ridge in the stem base followed by booting when the leaf sheath thickens to accommodate the pre-emergent head. The panicle then ‘exserts’ (that is, emerges from the leaf sheath) and an inflorescence is observed, followed by anthesis and fertilization (Hardke, 2013).

Grain (caryopsis) filling and ripening begins when 50% of florets are pollinated. *Oryza sativa* is largely determinate, with panicles maturing over a few weeks, after which leaves begin to senesce. A mature flower is shown in Figure 1.5.

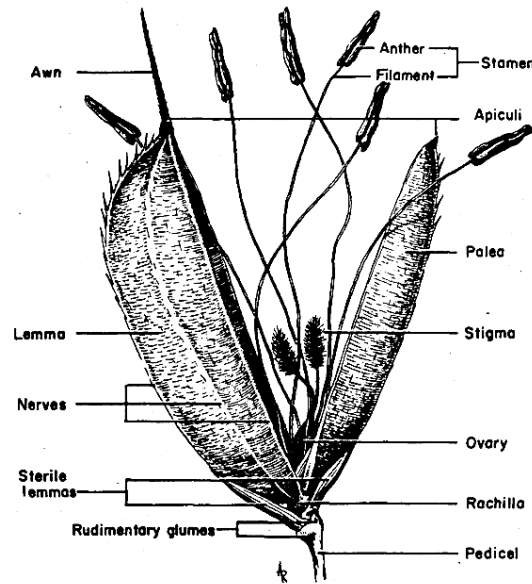


Figure 1.5 Morphology of the rice spikelet (Chang 1965)

Flowering begins at the tip of the panicle and moves progressively towards the base of the rachis. Pollination may be by wind for wild *Oryza* species, while *O. sativa* is mostly self-pollinated as a result of the lemma and palea not opening and releasing pollen onto surrounding stigmata. Pollen is viable for only a few hours in *O. sativa* but longer in wild species which also have spikelets open for a longer period (Figure 1.6).

The timing of each developmental stage from seedling emergence to maturity is variable and may be advanced or retarded by temperature, hours of sunlight and nutrient availability. In general, from days after sowing (DAS) to panicle initiation is about 60 d and a further 60 - 80 DAS is required to reach plant maturity for a growing season of four to five months (Smith and Dilday 2003; Whitworth 2006). Developmental stages are shown in Figure 1.6. The growth over time can be represented by a sigmoid curve, the early phase of which is closely related to final biomass.

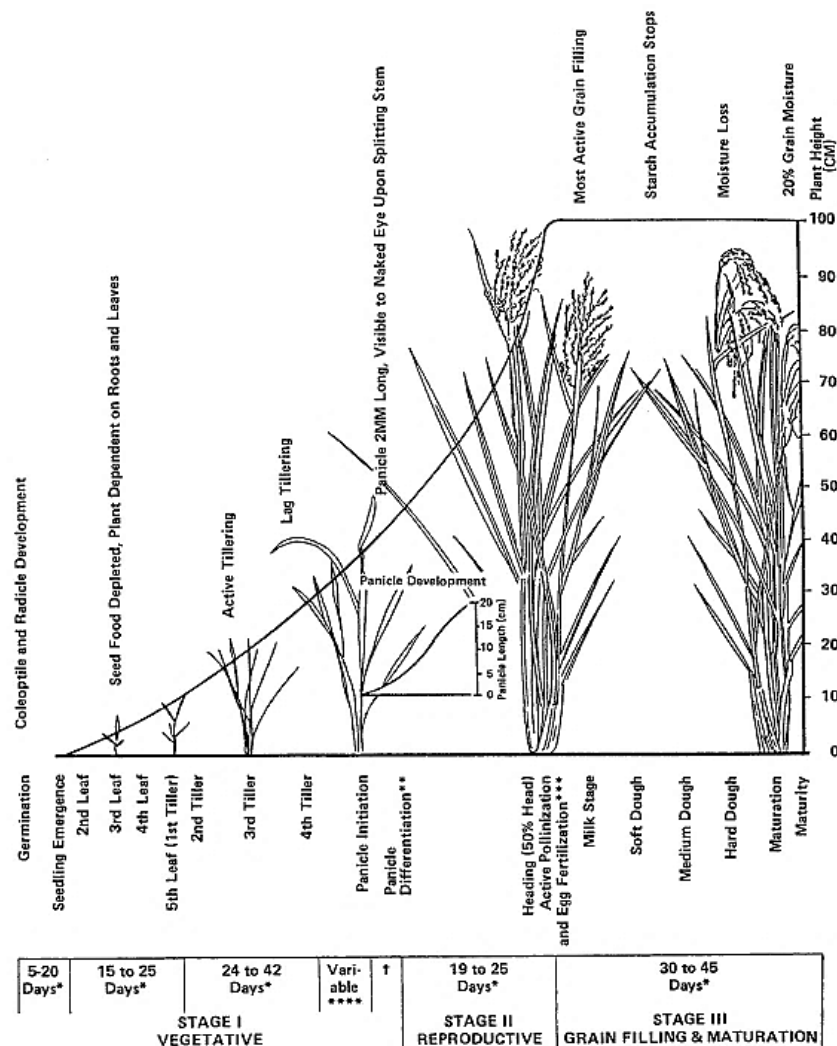


Figure 1.6 Developmental stages of rice (from Moldenhauer and Gibbons in Smith and Dilday 2003).

Rice growth conforms broadly to a sigmoidal pattern, for which there are several mathematical models (Yin et al. 2003). The duration of the exponential phase is closely related to final biomass.

There are few data for Australian wild *Oryza* species but it is believed that vigorous vegetative growth (Hamaoka et al. 2013) but relatively modest grain yields are typical (Wurm 1998). Data for *O. sativa* are usually at an aggregated crop level. For example, Dong et al. (2011) report aboveground biomass of *O. sativa* of 16 - 23 t ha⁻¹ and grain yields ranging from 6.2 - 7.4 t ha⁻¹.

The average harvest index was about 35%. The crop growth rate (CGR) of *O. sativa* was calculated by San-oh et al. (2006) over two two-month periods and shown to decline from 21.5-11.7 g m⁻² d⁻¹. This contrasts with less than 0.265 t ha⁻¹ for *O. meridionalis*, reported by Wurm (1998). Individual plant data are mostly reported in stress or toxicology studies (Boonjung and Fukai 1996).

1.7 Prerequisites for rice cultivation

Copious sunlight and water are essential requirements for vigorous growth of this C₃ grass, with other factors such as inorganic nutrition frequently becoming limiting when these inputs are satisfied. The wet tropics, typically with monsoonal rainfall (rainfed rice) or irrigation (in drier climates), are favored for rice growing. Full sunlight is generally required for peak growth rates in *O. sativa* but some *Oryza* species (*O. granulata* and *O. ridleyi*) tolerate some shade (Vaughan et al. 2005). Of itself, latitude is not important as higher latitudes have longer sunlight hours during the growing season than tropical latitudes. Latitude change from 28° to 48° made little difference to estimated photosynthesis rates of model plants as differences in day length compensated for lower radiation intensity at the higher latitude (Duncan et al. 1967). For example, Figure 1.7 shows that in the growing season, Griffith, in the rice-growing Riverina at latitude 34°S, slightly exceeded the solar radiation levels in tropical northern Australia (Cairns 17°S, Howard Springs 12°S and Keep River 16°S) although the annual radiation at ground level at 34°S is lower than the tropical sites. During September to April, the different sites have similar radiation levels; Riverina 25.2 MJ m⁻², Northern Territory 24.2 MJ m⁻², and North Queensland 23.8 MJ m⁻². The northern Australia radiation levels are similar to typical rice growing regions like Thailand, Japan and the International Rice Research Institute (IRRI) wetland site in the Philippines where the summer months average 19.4, 21.7 and 20.0 MJ m⁻², respectively (Sealite 2013).

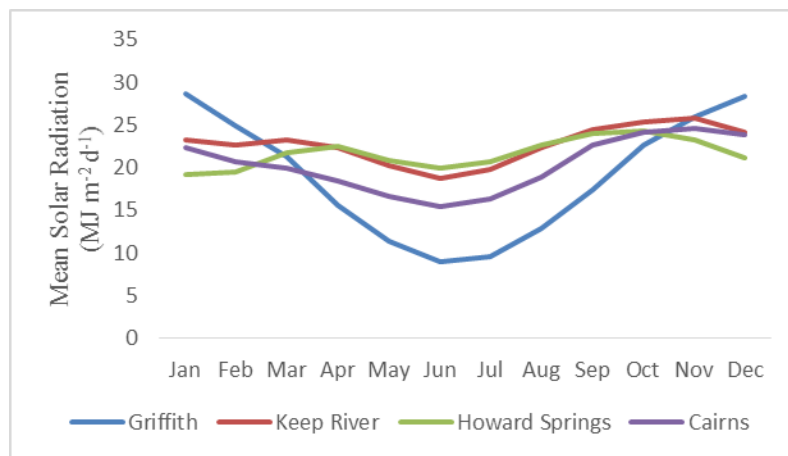


Figure 1.7 Average daily radiation in a temperate region, Griffith, NSW compared to the tropical regions of Keep River, NT, Howard Springs NT and Cape York, Qld (Bureau of Meteorology www.bom.gov.au/climate/data/). Australian rice growing season September to March.

Terjung (1984) estimates the irrigation water requirements for cultivated *O. sativa* per season are 5-10 ML ha⁻¹ in China and Whitworth (2006) estimates 10 - 15 ML ha⁻¹ in Australia, where rice is grown in a high evapotranspiration environment. Water requirement may also be expressed as the number of litres of water needed to produce a kilogram of rice. Estimates range from 600 - 1700 L of water per kilogram of rice harvested (Bouman et al. 2006; Bauman 2009; Chapagain and Yamaji 2010).

1.8 Rice cultivation methods

While most rice production occurs under irrigated conditions, large amounts are also grown in rainfed systems that are at the mercy of droughts and flooding. Most rice cultivation is based on *O. sativa*, which is grown principally in Asia but also in Africa, Europe, North and South America and Australia (Office of the Gene Technology Regulator 2005). *Oryza sativa* ssp. *japonica* is grown widely in California, Southeast Australia, Italy, Spain and North Africa.

There are several ways of growing rice - rainfed upland, irrigated lowland and rainfed lowland which includes deep water, flooded and floating types. At locations such as the Riverina in

Australia, rainfall is low so irrigation is essential, with the relatively cloudless skies, low humidity and high solar radiation allowing high yields compared to the tropics. Rice is the predominant food crop in Asia, where the largest producers are found. Most rice production is flood irrigated lowland (paddy) rice in the monsoonal wet tropics but some is rain-dependent (rainfed lowland and upland rice; Table 1.4; IRRI 2013). Because of the rather specific physical requirements of rice, rice-growing regions are susceptible to climate change and were more extensive over 7,000 years ago, when the climate was warmer and monsoons stronger than they are now. The distribution of wild *Oryza* species is likely to have been restricted by loss of available land to cultivated rice and by weeding in rice cultivation areas, to avoid crop contamination by weedy rice (Fuller 2011).

Rice has also been harvested in Africa for about 3,500 years – although natural stands of *O. glaberrima* were collected rather than deliberately cultivated domesticated rice (Plant Resources of Tropical Africa 2013). Cultivation expanded in the 15th century with the introduction of *O. sativa* by Portuguese settlers. The elaborate cultivation methods, building dykes and transplanting seedlings, suggest rice cultivation was long-established but no definite dates are available. *O. sativa* is replacing *O. glaberrima* in recent times as it is more productive although less hardy (Linares 2002).

Table 1.4 World rice production by type of cultivation

Type of cultivation	% world output	Yield (t ha ⁻¹)	Best yield (t ha ⁻¹)
Irrigated lowland	72-75	5.4	8-10
Rainfed lowland	17-20	1 - 2.5	4.5
Deepwater, floating	2 - 4	~2	3.5
Upland	4	1	3.5

After Kende et al. 1998; Vaughan, Lu and Tomooka 2008; Filho et al. 2013; Nguyen et al. 2013; Worou et al. 2013., Ashwathi and La, 2014. Varying tables are not equivalent so totals do not add exactly to 100%.

1.9 Importance of rice crops

Rice is a major food staple, particularly in Asia. Across all developing countries, rice provides 27% of dietary energy supply because of its high starch content but only 20% of dietary protein. Its amino acid profile is non-optimal for humans but the grain contains limited amounts of essential micronutrients (FAO 2004). Yields have increased substantially since the 1960s but in recent years the rate of increase has slowed (Normile 1999; Lu and Zou 2005). Factors contributing to increased grain yields include government policies, supply of fertilizer, irrigation and higher yielding varieties of grain (Johnson 1997). Rice productivity is highly variable: Papademetriou et al. (2000) note production varying from 8.2 t ha⁻¹ in Australia to 1.8 t ha⁻¹ in Cambodia, with world average yield being 3.9 t ha⁻¹ in 1997, somewhat less than the more recent figures in Table 1.1. Breeding programs, particularly at IRRI, have produced high-yielding varieties, the best known being the dwarf varieties like IR8 that were produced by modulation of the synthesis of or sensitivity to the growth regulator gibberellin (Sakamoto et al. 2003). This cultivar had a yield potential of 9.7 t ha⁻¹ when introduced although in practice that yield was often not achieved (Virk et al. 2004).

While the rate of human population increase has slowed in the last half century, absolute world population still increases faster than rice production. Price has been much more volatile than production (Figure 1.8). Prices for cereals have increased, for rice in particular, and can be expected to continue to increase if per person calorie consumption continues to increase at the high end of its projected future use. Projections of future rice consumption are speculative – as the world's poor become better off, their consumption will increase but the middle classes of Asia are also switching to other cereals and food stuffs (European Commission Agricultural Commodity Markets Outlook 2008 - 2017). Figure 1.8 shows annual *rate* of change in world rice production, human population and the price of rice. The impact on price of small variations in the supply-demand relationship is dramatic (Clarete et al. 2013).

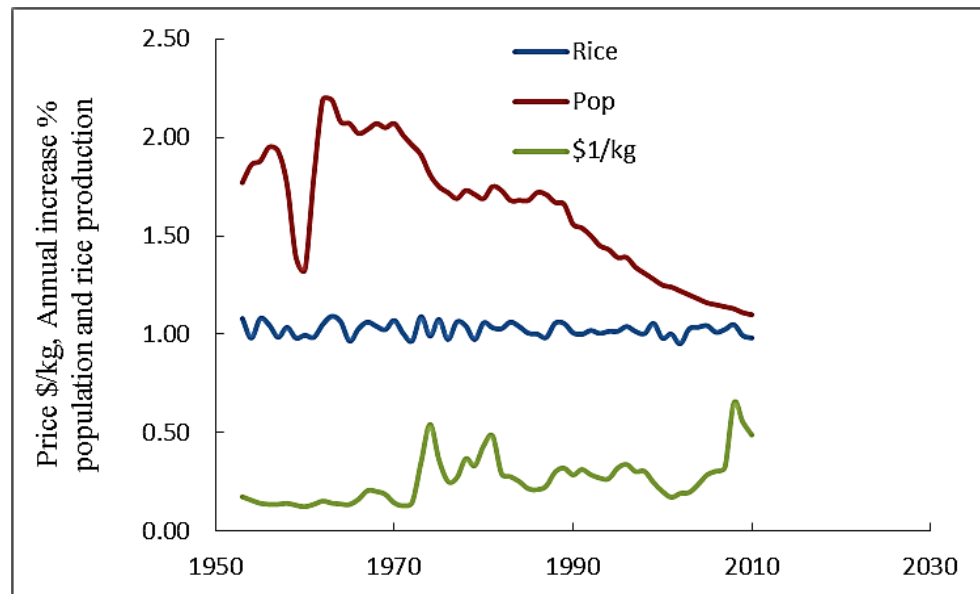


Figure 1.8 The rate of change of world rice production, price (\$US/kg) and percentage population increase (Pop) year on year (IRRI: <http://ricestat.irri.org:8080/wrs/#Select>) (U.S. Bureau of the Census, Current Population)

1.10 Possible methods to increase rice production

Cereal production can be increased by increasing resources devoted to production, namely land, water, labour and capital, by improving the efficiency of the use of resources and the productivity of the crop through genetic advances. Although increasing land devoted to crop production is possible, competition from other enterprises such as forestry, urban development and infrastructure projects will constrain the conversion of less productive land to rice production. Rice cultivation in flooded areas does not require highly fertile soil but is widely affected by acid sulfates during flooding. Costs of irrigation and fertilizer, and availability of water for more extensive irrigation are also significant constraints on increasing inputs (FAO 2002), while less arable land is available as urbanisation competes for space (FAO 2013). Johnson (1997) maintains government policies are the single most important factor determining productivity. However, given this inevitability, the only way to achieve yield progress is through the widest possible application of breeding to *O. sativa*.

One critical means of yield increase is through increased harvest index (the proportion of plant conversion to food). The ambition in 1995 was to achieve a 20% increase in harvest index, from 0.5 to 0.6, equating to a yield of 12 t ha⁻¹ (Khush 1995), by optimisation of input use and new strains developed through hybridisation or direct gene manipulation (Lu and Zou 2005). Features sought were fewer tillers (only about 70% of tillers produce grain), sturdy stems and large panicles (Khush 1995; Virk et al. 2004). Problems include the high cost of seed and resistance to the use of ‘genetically modified’ crops, infertility of some hybridised rice and the unsatisfactory taste of hybrid rice (Khush 1995; Nomile 1999; Virmani and Kumar 2004; Sahai 2012). Initial gains from the ‘Green Revolution’ were reversed in some regions, such as India where the major factor in declining output was thought to be declining investment in irrigation (Bhalla and Singh 2010), but farmers taking land out of production due to unprofitability was also a factor (Sahi 2012). Paddy rice requires at best 1,100 - 1,200 litres of water to produce a kilogram of grain but ironically, much lower water use efficiency is achieved in less well-managed regimes, imposing a major constraint on rice production (Table 1.4).

Attention has turned to wild rice as a source of genetic variation that may lead to greater productivity of cultivated rice (Zhu and Ge 2005; Wang et al. 2013, Atwell et al. 2014). Knowledge of the *japonica* genome is of great value in this endeavor (Barabaschi et al. 2012) and the *Oryza* Map project (Jacquemin et al. 2013) will provide many of the sequences of wild relatives. This in turn will open the door for functional genomics and related techniques in gene discovery to be applied. There are ecological hazards that need to be observed in the environments in which rice grows. For example, Jiang et al. (2012b) note that hybridisation and introgression between wild and cultivated rice may also risk increasing the invasiveness of weedy rice (Jiang et al. 2012b).

Yield and productivity depend on development of new rice varieties, resource input, custom, attitudes and institutions, particularly regulations, tax and subsidies (Bhalla and Singh 2010). The underlying biological mechanisms affecting rice production are the focus of this study but economic factors and competition for water resources are likely to become increasingly important in rice production world-wide.

1.11 Plant morphology, light capture and growth

In recent years, attention has turned from the basic questions of tiller number and development to more subtle aspects of morphology of the rice plant. For example, are some canopies more efficient at light capture than others (Doust 2007)? Yuan Longpin (quoted in Normile 1999) favours ‘long narrow very erect top leaves’. In optimal environments, erect leaves and high leaf area index (*LAI*) are important for maximum productivity by optimising photosynthetic light capture (Sakamoto et al. 2006) but this is disputed (Sinclair 1999; Sinclair and Sheehey 1999) who argue a high *LAI* is necessary primarily to store enough nitrogen (N) for transfer from leaves to grain at maturation. To obtain high-yielding rice, an *LAI* of ca. 7 m² m⁻² is needed to obtain a yield of 10 t ha⁻¹ (Sinclair and Sheehey 1999). Simplistically, a high *LAI* may be achieved by higher planting density (San-oh et al. 2006) but shading may also increase and any gains may be negated.

Monteith found that if *LAI* is high, light capture by horizontal leaves occurs mainly in the top leaf layer and lower layers get little light whereas more erect leaves allow more light to reach lower leaf layers; this effect applied for *LAI* > 5 (Monteith 1965). Senescence induced by low light sets limits on *LAI*, as self-shading becomes greater as *LAI* increases (Weei-Pirng et al. 1986). Therefore, to achieve light intensities high enough to prevent premature senescence, higher *LAI*s require a higher leaf angle – that is, closer to vertical (Duncan et al. 1967; Trenbath and Angus 1975). A ‘higher’ leaf angle equates to a smaller zenith angle.

Duncan et al. (1967) modeled potential photosynthesis and leaf angle, showing greater efficiency as leaf angle approaches vertical and *LAI* increases from 2 to 4 to 8. Leaf angle made little difference until *LAI* exceeded 3 but thereafter more vertical leaves were more efficient. San-oh et al. (2006) found light attenuation through the canopy was least with leaves at small zenith angles (angle between the leaf and a vertical line). Zheng et al. (2008) used three dimensional (3-D) computer modeling of hybrid rice concluding that rice with more nearly vertical leaves in the upper leaves of the canopy had deeper penetration of light into the canopy with sun elevation 70° (nearly overhead) but not with lower sun elevation angles. Conversely, rice with fewer near-

vertical leaves in the upper canopy had less light penetration in latitudes where it might be grown, consistent with the modeling of Duncan et al. (1967).

However, in a later paper, Zheng et al. (2009) considered ‘steep’ leaves could be a disadvantage. Their computer simulation showed that leaf angle and *LAI* interacted. At sun elevation angles less than 50° (angle above horizon), leaf angle made little difference; differences emerged with sun elevation angles 50° - 73°, with 73° being the maximum sun elevation at the site where the plants were digitised. Close to vertical leaves let more light reach the bottom of the canopy but this shape did not maximise photosynthesis. Optimal photosynthesis was achieved when leaves in the upper canopy were close to vertical but lower leaves were pitched at about 60° above horizontal. These contrasts in canopy shape interacted with *LAI*: plants with very high (simulated) *LAIs* of 9.5 were most efficient when most leaves were vertical whereas the most efficient morphology at an *LAI* of about 7.5 was the ‘mixed mode’ of zenith angle, increasing above zero with penetration deeper into the canopy.

Weed suppression is also influenced by *LAI*, being most effective when developing rice plants have a high *LAI* and droopy, widely spread canopies (Jones et al. 1997). These observations suggest that tiller and leaf angles might be more horizontal in young plants and closer to vertical in older plants (Ouyang et al. 2011). The ideal leaf angle is different at different levels in the canopy. Near vertical leaves are effective at the top of the canopy but leaves lower down in the canopy should ideally be closer to horizontal to better intercept light. Leaves in a canopy should be close to vertical high in a dense canopy with good light exposure, reducing leaf exposure to damaging levels of radiation, but close to horizontal deep in the canopy where the little light available comes more or less vertically (Niinemets 2012). The ideal leaf orientation changes with both the developmental stage of the plant and the position of the leaf in the canopy.

Photosynthetic rates in some wild *Oryza* species are high compared to *O. sativa*, the differences being related to a diploid genome, perennial growth and growth in full sun (Table 1.5; Zhao et al. 2010). Photosynthesis is affected by leaf thickness, leaf nitrogen, exposed mesophyll cell surface area, mesophyll cell CO₂ conductance and stomatal CO₂ conductance (Gu et al. 2012; Giuliani et al. 2013).

The conclusions of Duncan et al. (1967) and Trenbath and Angus (1975) indicate the canopy shape of wild *Oryza* should be less efficient than the canopy shape of *O. sativa*, but this does not seem to be reflected in measured photosynthetic rates found by other investigators (Yeo et al. 1994, Zhao et al. 2008, Kiran et al 2013). Perhaps physiological advantages in wild *Oryza* counterbalance architectural disadvantages or perhaps the canopy shape is not a major determinant of photosynthetic capacity.

Table 1.5 Photosynthesis level and plant characteristics

Species	Genome	Habit	Habitat	Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)		
				Zhao et al. (2008)	Yeo et al. (1994)	Kiran et al. (2013)
<i>O. sativa</i>	AA	A	S	21.6	19.1	
<i>O. meridionalis</i>	AA	A/P	S	34	11.2, 14.7	
<i>O. australiensis</i>	EE	P	S	33.8	19.8	
<i>O. australiensis</i>	EE	P	S	34.8		
<i>O. australiensis</i>	EE	P	S	35.6		
<i>O. australiensis</i>	EE	P	S	36.7		
<i>O. glumaepatula</i>	AA	P	S	14.3	10.6	6.8
<i>O. eichingeri</i>	CC	A	S	21.1	8.47 - 11.4	15.6
<i>O. punctata</i>	BB	A	Sh	22.8	10.4	
<i>O. latifolia</i>	CCDD	P	Sh	23.4	9.7	
<i>O. officinalis</i>	CC	P	Sh	23.5	12.8, 10.1	
<i>O. alta</i>	CCDD	P	Sh	24.2	10.3	
<i>O. nivara</i>	BBCC	P	S	24.5	12.2 - 18.7	11.8 - 24.2
<i>O. grandiglumis</i>	CCDD	P	Sh	25.3	10.7	

<i>O. glaberrima</i>	AA	A	S	27.2	13.3 - 16.5	11.6 - 14.4
<i>O. longistaminata</i>	AA	P	S	28.8		11.6 - 13.2
<i>O. longistaminata</i>	AA	P	S	34.4	18.0	
<i>O. minuta</i>	BBCC	P	S	29.9	12.5	
<i>O. minuta</i>	BBCC	P	S	31.2		
<i>O. barthii</i>	AA	A	S	31.5	17.9	11.6
<i>O. barthii</i>	AA	A	S	27.2		
<i>O. rufipogon</i>	AA	P	S	25.6	11.1	9.6
<i>O. rufipogon</i>	AA	P	S	34.4	12.5	17.9
<i>O. rufipogon</i>	AA	P	S	37.6	11.1	
<i>O. brachyantha</i>	FF	A	S		19.3	
<i>O. longiglumis</i>	HHJJ	P	Sh		9.17	
<i>O. meyeriana</i>	GG	P	Sh		9.27	
<i>O. rhizomatis</i>	CC	P	S		17.7	
<i>O. granulata</i>	GG	P	Sh		7.9	
<i>O. granulata</i>	GG	P	Sh		7.9	

Habit: A = annual, P = perennial; Habitat: S = sun, Sh = shade. Photosynthesis calculated from light response curves with PAR 0-2500 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (Zhou et al. 2008), 0-1500 , PAR up to 1500 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (Yeo et al. 1994) and Kirin et al. (2013) used one level of PAR, 1000 $\mu\text{mol m}^{-2}\text{s}^{-1}$. The three papers use different methods so they are not comparable, but it is evident they do not rank the *Oryza* species consistently.

1.12 The impact of canopy architecture on plant productivity

Valladares et al. (2002) report plants in a similar environment (forest understory) have similar LIE (the ratio of mean light intercepted by leaves to light intercepted by a horizontal surface of equal area), which they referred to as convergence, despite different plant architecture, showing different structures could achieve similar LIE results. Increasing age and maturation of a plant increases the number of leaves and causes more pronounced self-shading, which reduces *LIE* (Niinemets et al. 2005). Delagrangé et al. (2006) found shade tolerant plants had less leaf area, with less leaf overlap, than shade intolerant plants in higher irradiance environments which had

greater leaf area and more leaf overlap. Shaded plants typically had leaves close to horizontal (excluding some climbers, which also had low LIE compared to other shade plants). More vertical leaf inclination (greater leaf angle to horizontal) produced better light interception efficiency than leaves at lesser angles to horizontal in canopies with many leaves. Trees grown in higher light intensity was associated with a higher proportion of leaves with higher leaf elevation angle than trees grown in lower intensity light (Delagrange et al. 2006). Other adaptations to optimise light capture are structural such as optimizing leaf separation by control of leaf and tiller orientation, or changes in leaf-level anatomy and biochemistry such as increased resource investment in chlorophyll (Niinemets 2012). For example, Duursma et al. (2012) found the most important variables in determining *LIE* were leaf dispersion – the average distance between the midpoint of a leaf and the midpoints of the five nearest leaves – and total plant leaf area and crown surface area, which accounted for 85% of the variation in *LIE* (high leaf area-to-crown surface area ratios indicate a more dense crown and more self shading). By inference, canopy structure in dense stands of either cultivated or wild rice will be under intense selective pressure to optimise light capture. However, some *Oryza* species grow in high-radiation environments in which disposing of excessive energy from light is important; capacity to deal with excess light is poor in short-lived, fast growing crops (Demmig-Adams and Adams 2006). Near vertical leaves would give some protection against strong sunlight, as would adaptations to cope with high temperatures.

Another aspect of plant architecture is tiller number. Increase in tiller number leads to an increased crown density as leaf numbers increase and also to an increase in number of panicles but late developing panicles yield less grain (Yoshida 1981). Tiller number is influenced by auxin (reduces tiller development) and genes that modify the expression of auxin (Lin et al. 2009; Choi et al. 2012). Late emerging tillers are less productive (Mohapatra and Kariali 2008). Possibly the increased crown density is associated with lower LIE which may be related to the decreased grain production but age effects may also apply, the later tillers having inadequate time to develop.

1.13 Plant growth

Annual plants typically have a sigmoid growth curve, with a maximum at maturity then senescence (Yin et al. 2003), shown clearly in plant height change over time (Chapagain and Yamaji 2010).

The growth of wild *Oryza* varieties has been little studied but has been reported to be high compared to cultivated *Oryza* and might be related to characteristics that could be transferred to cultivated rice (Henry et al. 2010) Hamaoka et al. (2013) compared growth rates in domesticated and some wild rice at 56 DAS, offering limited support for this idea (Table 1.6).

Table 1.6 Comparison of wild and cultivated rice. Dry weight of roots, leaves and stems measured four weeks after transplanting, eight weeks after sowing (Hamaoka et al. 2013)

Species	Subspecies	Cultivar	Origin	Biomass (g)
<i>O. sativa</i>	<i>japonica</i>	Akenohoshi	Japan	13
<i>O. sativa</i>	<i>japonica</i>	Nipponbare	Japan	16
<i>O. sativa</i>	<i>indica</i>	IR24	Philippines	21.5
<i>O. sativa</i>	<i>indica</i>	Kaslath	India	26.1
<i>O. glaberrima</i>		IRGC1077	Senegal	30
<i>O. glaberrima</i>		IRGC 10403	Mali	22.8
<i>O. barthii</i>		IRGC 10410	Chad	26.7
<i>O. meridionalis</i>		W1625	Australia	20
<i>O. nivara</i>		IRGC 10571	Cambodia	30
<i>O. rufipogon</i>		W106	India	24

1.14 Plant modeling

Early studies of leaf orientation, shadowing and ability to intercept light concentrated on mathematical modeling (Monteith 1965; Duncan et al. 1967; Trenbath and Angus 1975), with attempts to deal with curved leaves using approximations based on geometry (Chen and Black 1992; Chen et al. 1997). These investigators established that the significant variables were the angle of the sun above the horizon (elevation, usually designated α , but also measured from the vertical, the *zenith angle*), the angle between the incoming light ray and the longitudinal axis of the leaf in the vertical plane, the angle of rotation about the long axis of the leaf and the angle between the incoming light ray in the horizontal plane and the leaf axis (azimuth angle) - which determined the radiation reaching the silhouette of the leaf. To deal with leaves that were curved, the flat or silhouette leaf area had to be related to the total surface area of one side of the leaf. Several authors relate the surface area of a body to its silhouette area, the simplest result being that the average silhouette area, when all angles of projection are considered, is one-quarter the leaf surface area (Figs 1.9 and 1.10; Lang 1991; Chen and Black 1992).

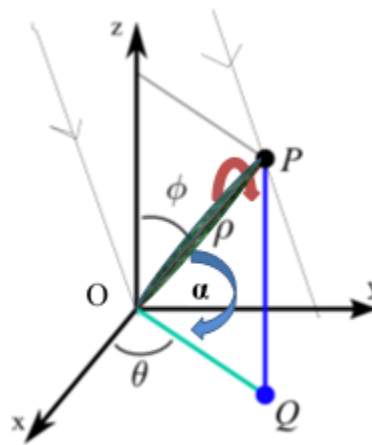


Figure 1.9 The x - y plane is horizontal, z is vertical. If x is north, θ is degrees from north or azimuth, O - P is a leaf (green), Q is a point in the x - y plane immediately below the leaf tip (P), P - O - Q is elevation angle of the leaf, α . The angle between the z -axis and the leaf (O - P) is zenith

angle, (ϕ), and the leaf can rotate about the axis O-P (red arrow). Incoming sunrays (faint arrows) project the silhouette of the leaf on a plane perpendicular to the light ray.

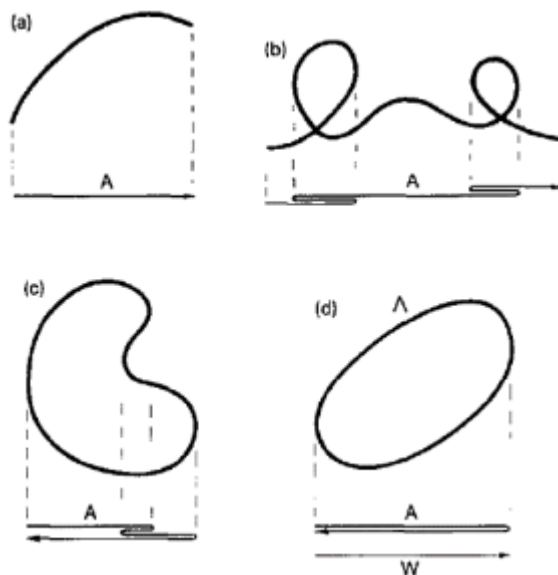


Figure 1.10 The one-dimensional line depicted along the base of each shape is a projection of the two-dimensional figure above. These examples are analogous to the two-dimensional silhouette of 3-D structures such as leaves. A curved or complex line is reduced to a straight line projection on a plane, A, of length W (Lang 1991).

Several techniques have been developed to compute unshaded leaf area. For example, Yplant (Pearcy et al. 2012), a software program that analyses digitised plant data, can be used to compute the ratio of displayed leaf area perpendicular to an incoming light ray to total leaf area ($DAP:TLA$ or E_d) or silhouette area to total leaf area ratios (*STAR* ; Oker-Blom and Smolander 1988). The two methods do not seem to have been directly compared. *STAR* differs from *STAR* by being averaged over the sky hemisphere and weighted by various factors (Duursma et al. 2012). For small plants in pots, a scanning technique that can cope with curved leaves exists but not yet generally available (PlantScan, CSIRO Plant Industry Black Mountain Laboratories Clunies Ross Street Canberra ACT 2601). This development may change imaging standards for small plants in the future.

The projection of three dimensional figures is relevant to the effect of digitization of plants by Yplant which has some measurement errors when curved surfaces are involved. New technology provided another approach. Digitization has been used to record and compare plant morphology. The 3-D coordinates of points on plants allows representations of plant structure that enable computation of leaf area exposed to sunlight in complex plant structures with self-shading which would be too labour intensive to measure directly. The aim of these systems was to assess light capture and carbon gain by plants (Pearcy and Yang 1996). The 3-D model is used to estimate the projection of the leaf silhouette onto a plane perpendicular to an incoming light ray (Figures 1.9 and 1.10). Light rays from a predetermined set of elevation and azimuth angles are applied then the total area projected and the overlapping silhouettes of multiple leaves calculated to find the leaf area exposed to light.

Digitization has been used mainly for cultivated rice (*O. sativa* L; Watanabe et al. 2005; Zheng et al. 2008). Canopy morphology distinguished sub-varieties within the same species of *O. sativa* (Zheng et al. 2008) and may also distinguish accessions of *O. meridionalis*, the subject of Chapter 4, which asks whether canopy structures of three rice species influence light capture efficiency enough to be of practical importance.

1.15 Climate and CO₂ effects

Climate change may also affect rice crop productivity. Atmospheric CO₂ concentration is rising, but it is uncertain how much this will affect growth, photosynthetic efficiency and canopy architecture in broad acre farming (Kull et al. 2003; Ainsworth and Long 2005). Effects demonstrated in glasshouses were greater than effects in FACE open air field studies. Several studies of rice and [CO₂] indicate high ambient atmospheric [CO₂] usually but not always accelerates panicle initiation, reduces days to heading and plant biomass accumulation. These effects diminish as the plants aged in a FACE study (Shimono et al. 2009). Zhu et al. (2012) note that elevated [CO₂] increases grain production in rice but diminishes grain quality. Enhanced

photosynthesis with elevated [CO₂] may not be maintained, due to ‘acclimation’, which may reflect less Rubisco and or slower Rubisco regeneration (Baker and Allen 1993). Zhu et al. (2012) question that acclimation to increased [CO₂] occurs and suggest accelerated senescence in rice to account for declining photosynthesis after long exposure to elevated [CO₂].

Global temperature increases are expected to have generally adverse effects on agriculture, particularly in the tropics, due to increased heat and drying, whereas temperate crops may not be so badly affected (Battisti and Naylor 2009); they did not specifically evaluate possible effects on rice. Shimono et al. (2010) found stomatal conductance diminished with elevated [CO₂] which should have a water-saving effect, as some earlier reports had suggested (Widodo et al. 2003; Leahey et al. 2009). However, some studies suggest drought stress would have adverse effects very early in plant development, before stomatal change could occur, so the overall effect of climate change would be negative (Powell et al. 2012). Climate change factors influence productivity in opposing ways so net effects may be slight (Korner 2006; Long et al. 2006).

1.16 Photosynthesis and carbon gain in wild and cultivated *Oryza*

Published carbon assimilation rates for rice are highly variable; it is not always evident how the reported measurements were made. For *O. sativa* rates in the order of 20 - 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ are reported, with lower rates occurring in the reproductive phase of the lifecycle than in the vegetative phase (Yeo et al. 1994; Shimono et al. 2009). Both Yeo et al. (1994) and Zhao et al. (2008) report photosynthetic rates higher in perennial, sun-growing and diploid rice species than in annual, shade-grown and tetraploid rice species (Table 1.6). Photosynthetic rates in *O. australiensis* and *O. sativa* were high compared with *O. meridionalis* according to Yeo et al. (1994) but *O. meridionalis* was reported to be high compared to *O. sativa* in Zhao et al. (2008), indicating a large effect of growth conditions and maybe growth stage on gas exchange.

Some investigators have failed to find good correlations between photosynthetic efficiency and crop yield. For example, modern wheat has lower photosynthetic rates than wild *Triticum* relatives but higher grain yield (Evans and Dunstone 1970). Sinclair et al. (2004) note additional

examples and calculate a 50% increase in photosynthetic yield would produce a 6% increase in crop yield and would require additional nitrogen input.

1.17 Aims of this investigation

This study aims to describe the growth and developmental characteristics of *O. australiensis* and three accessions of *O. meridionalis* relative to a commercial cultivar of *O. sativa*. This led into an analysis of growth rates of all three species when grown in current ambient CO₂ concentrations (~390 ppm) and elevated CO₂ (~700 ppm). Photosynthetic performance was then tested in the same CO₂ concentrations to assess long-term effects of growth conditions on photosynthesis. The impact of clearly contrasting canopy morphology between genotypes was assessed by analysing light penetration by two methods. This was followed by making digital plant models which permitted comparisons of architecture, light capture efficiency and photosynthetic potential using data from light-response curves. In this way, aggregate carbon gain by whole plants could be assessed.

Chapter 2 – Growth experiments

2.1 Introduction

Annual crops typically grow in a sigmoidal pattern when growth is plotted against time. From this growth curve, a number of physiological dimensions of plant performance can be derived. For example, the length of the exponential phase is a critical determinant of final crop (or forest) biomass. Knowledge of the growth pattern is important in selecting wild rice varieties for potential cultivation.

Goudriaan and Monteith (1990) distinguish the phases of biomass vs time as: (i) exponential; (ii) linear; and (iii) saturation phase as the biomass reaches its peak. More recently Yin et al. (2003) report a mathematical model for a sigmoid growth function but note that many models give good results. Chapagain and Yamaji (2010) show a sigmoid curve for the height of a rice cultivar, Koshihikari. Leaf area, aboveground biomass, root biomass and number of tillers are growth measures highly relevant to productive potential. The reproductive phase can confound the simpler analysis of earlier vegetative growth (Yin et al. 2003). In their mathematical analysis, an exponential decay function of declining vegetative growth as plants mature contrasts with grain production, which accelerates rapidly as vegetative growth slows. Such is the pattern expected in the annual species, *O. sativa* and *O. meridionalis*. However, this picture is different in perennial plants such as *O. australiensis*, where the final decline in growth might be confounded by accelerated growth of ramets.

There are few data for Australian wild *Oryza* species but it is believed that vigorous vegetative growth (Hamaoka et al. 2013) but relatively modest grain yields are typical (Wurm et al. 1998). Dong et al. (2011) report aboveground biomass for individual *O. sativa* plants of 16 - 23 t ha⁻¹ and grain yields ranging from 6.2 - 7.4 t ha⁻¹. The average harvest index was about 35%. However, the growth on entire crops still conforms broadly to the sigmoidal patterns for individual plants discussed above. For instance, the crop growth rate (CGR) of *O. sativa* was

calculated by San-oh et al. (2006) over two two-month periods and shown to decline from 21.5 to 11.7 g m⁻² d⁻¹.

Tiller development is another aspect of the growth of grasses because tillers constitute quasi-independent plants that still compete for resources of light and nutrients. For example, Mohapatra and Kariali (2008) showed that rice in pots with surplus nutrients generally had more tillers than plants in the field. In all cereals including rice, increasing tiller number is not directly related to grain yield, as later tillers may not develop panicles (Yoshida 1981). On these grounds, excessive tillering is not desirable in cultivated rice (Mohapatra and Kariali 2008) as late developing tillers, secondary and tertiary tillers, produce less grain of lower quality than early, primary tillers. The yield of grain per tiller decreases acropetally as late tillers emerged higher on the main stem (culm or higher order tillers on lower order tillers; Figure 1.4).

As a C₃ annual, rice would be expected to sustain a large vegetative growth and yield response to elevated CO₂. Shimono and Okada (2013) found tiller number, plant height and plant biomass in the early vegetative stage of development increased under an elevated CO₂ atmosphere compared with plants grown at ambient [CO₂]; the effect diminished with time. They also varied temperature and found tiller numbers were greater with elevated glasshouse temperature compared with control plants. By contrast, there are no data reporting on the response of wild *Oryza* species to rising CO₂ levels even though this is unquestionably the key feature of the atmospheric changes that now confront the planet.

The aims of this investigation are to follow the growth trajectory of a representative strain of cultivated rice, *O. sativa* cv. Amaroo, and compare an Australian annual wild rice, *O. meridionalis* and an Australian perennial wild rice, *O. australiensis*. The measures of growth used are plant height, shoot weight, root weight, leaf area and number of tillers. Leaf area index (LAI) and canopy density are considered in Chapter 4.

2.2 Methods

2.2.1 Plant growth

Seeds were prepared for germination by soaking them in water for 30 min. To prevent fungal infection, seeds were then soaked for 5 min in bleach diluted to 50% and washed three times with distilled water. Seeds were primed at 36°C for 48 h to facilitate germination then grown at 28°C/22°C (day/night; 12 h each). Pots were filled with finely sieved soil of a type which had been used successfully in rice experiments previously (a soil from Robertson, NSW). Plants were watered daily and fertilized with Aquasol™ 5 g L⁻¹, 200 mL per pot weekly. Plants were grown in square section pots, 20 × 20 cm, height 25 cm, one plant per pot. Pots contained 9 kg of soil and tubs (Expt 3b) contained 50 kg of soil. Pots were placed on wire mesh tables approximately 100 cm above the glasshouse floor. Pots were arranged randomly in single blocks within glasshouses.

The rice accessions used were *O. sativa* cv. Amaroo, *O. australiensis* and *O. meridionalis* accessions Keep River (KR), Howard Springs (HS) and Cape York (CY). For two growth studies, autumn 2010 and winter 2011, seeds were sown and grown at two levels of atmospheric [CO₂], 390 ppm and 700 ppm in four greenhouses, two with high and two with ambient [CO₂]. Plants were grown in four experiments:

- 1 Autumn 2010 (28 February to 6 June 2010), harvested 90 - 95 DAS;
- 2 Summer (20 December 2010 to 18 March 2011), harvested 42 - 84 DAS at approximately 10-day intervals; growth rates were calculated at harvest.
- 3 Winter (28 May to 29 June 2011), (a) plants grown in pots, harvested 18 - 41 DAS (+ supplementary lights) harvested at 7-day intervals and (b) plants grown at densities of 36 and 72 plants m⁻² in tubs, harvested at 81 DAS; growth rates were calculated at harvest
- 4 Summer-autumn (20 January to 15 June 2012), harvested at 30 - 145 DAS (supplementary lights). Harvest intervals were increased as the plants matured from weekly (twice) to fortnightly (twice) then approximately monthly (twice); growth rates were calculated at harvest.

Replicates in these experiments were: Expt 1, 4 - 6 replicates; Expt 2, 3 replicates; Expt 3, 4 or 5 replicates; Expt 4, 6 replicates. Dry weights and leaf area were measured on harvested plants, randomly selected from the available plants, with replicate numbers as noted above. Harvest sessions for early growth (Expt 3) were every seven days, intervals between harvest for older plants (Expts 2 and 4) were less frequent as the older plants did not grow so rapidly.

Experiment 3 was grown under artificial 400 W Philips Contempra high pressure sodium lamps (20 per glasshouse of 27 m², mounted at height 2.4 m). Lamps switched on automatically if the ambient light level fell below 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and supplementary lights added 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The same lighting was used for Experiment 4 because it was an overcast summer. All experiments were at 30° and 22° (12-h day/night). For canopy-density studies, plants were also grown in 30 L rectangular tubs containing 50 kg of the soil described above with eight or sixteen plants evenly spaced in each tub, to produce a dense growth of plants and simulate crop densities. Plants were watered liberally throughout.

Plant height measurements, leaf angles and tiller angles were measured on intact plants. Plant growth was assessed by leaf area measurement immediately after harvesting (LAI2000 Plant Area Meter) and plant dry weight was measured after drying in an oven for 72 h at 70°C.

Plant growth was assessed by shoot and root dry mass, leaf area, plant height and number of tillers per plant. Plant growth rates were estimated by weighing dried plants harvested 18 - 41 and 42 - 84 DAS, then calculating k from the growth equation $A_1 = A_0 e^{kt}$; where A_0 = weight at the beginning of the growth period, A_1 = weight at the end of the growth period and t = days between measurements.

Leaf and tiller angles from vertical, zenith angles (ϕ in Figure 4.1) were measured with a protractor. The angle between the leaf and the supporting culm or tiller was measured. For *O. sativa* this was a good measure of the zenith angle of the leaf, but for *O. australiensis* and *O.*

meridionalis the angle at the leaf/stem junction was less than the average for the leaf as a whole. The measured angle was the angle between vertical and the leaf axis for each leaf.

2.2.2 Statistics

Minitab16 was used for statistical analysis. Figures show actual measurements, but a natural log transformation was used for statistical analysis. The raw data were not normally distributed and a natural log transformation produced a distribution more satisfactory for statistical testing. When natural log transformation did not produce a normal distribution, Levene's test for difference of variance was used, as recommended in Minitab. If variances were significantly different, a general linear model version of ANOVA was used, as recommended in the Minitab instructions. The main statistical method was analysis of variance (ANOVA), with one-way, two way and multiple (MANOVA) methods, mainly using the general linear model and Tukey tests for multiple comparisons of means.

2.3 Results

2.3.1 Morphology

The rice species *O. sativa*, *O. meridionalis* and *O. australiensis* showed different morphology. *Oryza australiensis* were the tallest plants, had the fewest tillers and the most curved leaves. They were slowest to reach maturity, and at the dates of comparison with the other rice accessions, they were less mature plants. They were the only plants considered perennial, although re-growth from older plants, ratooning, can occur with other rice accessions. When *O. australiensis* was allowed to develop for longer, additional shoots were found, often springing from rhizomes rather than new tillers. This was not observed with the other species in this study.

Early plant growth was similar on all rice accessions, the accessions developing distinctive features which become evident as tiller development proceeded. First leaves were nearly vertical and straight, remaining like that in *O. sativa*, but the leaves of *O. meridionalis* were more curved and both leaves and tillers were inclined further from vertical than *O. sativa* leaves. *Oryza*

australiensis had fewer, larger and much more curved leaves; tillers were close to vertical unless lodged. *Oryza australiensis* was more prone to lodging than the other accessions. Younger leaves were closest to vertical and became more curved and further from vertical as they aged, particularly in *O. australiensis*.

2.3.2 Leaf area

Early leaf area growth was rapid and could be modeled by an exponential equation (leaf area = $0.7687e^{0.1129DAS}$, $R^2 = 0.96$). Figure 2.1A shows an average growth curve for all plants from 18 - 41 DAS, in the vegetative phase. Figure 2.1B shows the early growth for all rice accessions. Figure 2.1C extends this into the reproductive phase. As the plants approached the reproductive phase, vegetative growth slowed for the annual species but the perennial, *O. australiensis*, continued to grow. The long term growth pattern shows a plateau of leaf area in older plants, producing a sigmoid growth curve (average leaf area all species = $-0.0005 DAS^4 + 0.0847 DAS^3 - 4.6962 DAS^2 + 107.16 DAS - 853.63$, $R^2 = 0.9939$). When all rice accessions are plotted separately, the same patterns are found but with more variability. Growth in older plants was by addition of new tillers, with enlargement in the horizontal plane rather than increasing plant height.

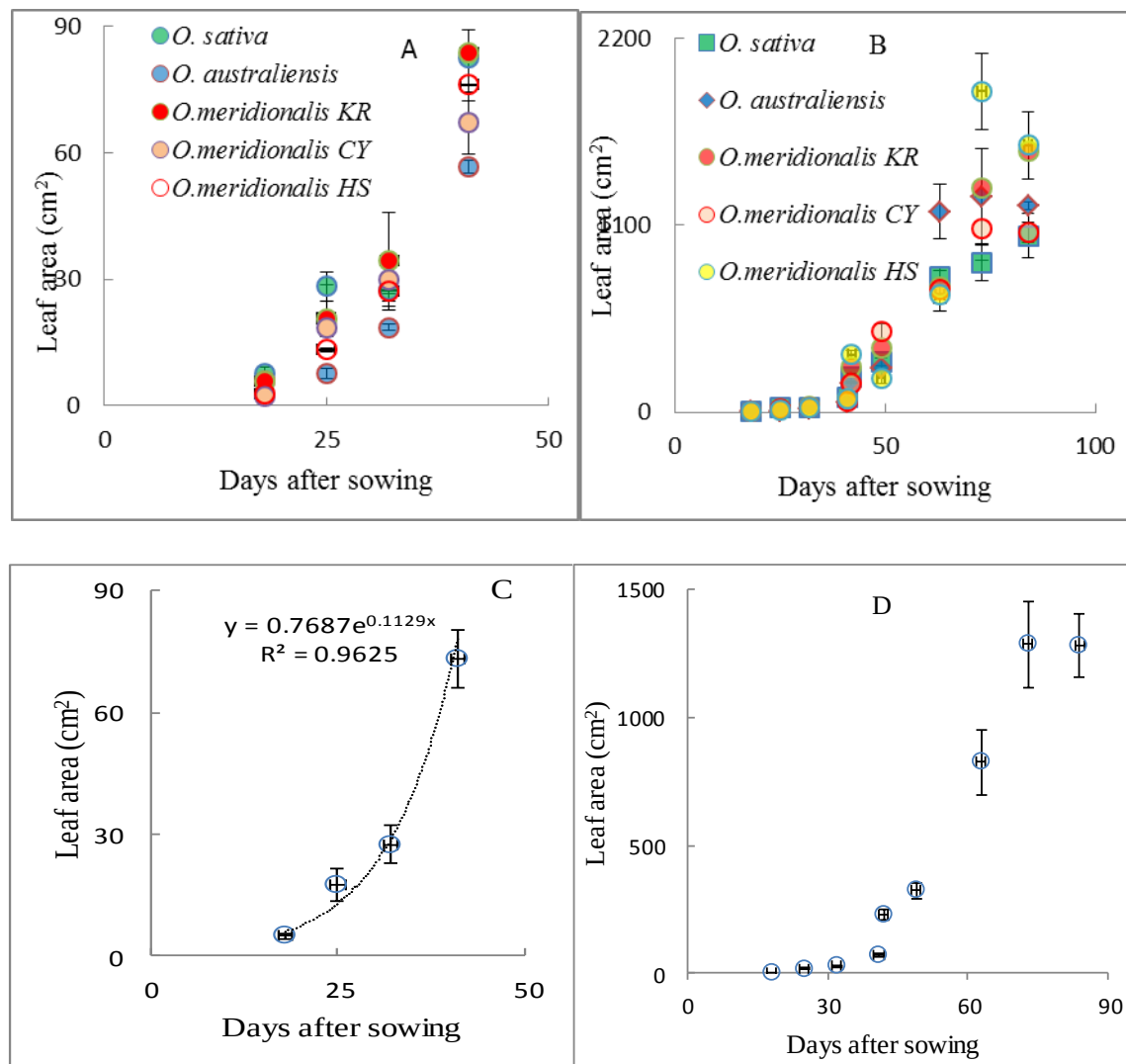


Figure 2.1 (A) Leaf area ($\pm$ SEM) of all accessions 18 - 41 DAS (Expt 3a); effect of [CO₂] shown in Figure 2.14, 2.15 and 2.16. **(B)** Leaf area ($\pm$ SEM) of all accessions 18 - 84 DAS (Expt 2 & 3a); **(C)** All species leaf area ($\pm$ SEM) 18 - 41 DAS (Expt 3a) with best fit polynomial; effect of [CO₂] shown in Figure 2.14, 2.15 and 2.16. **(D)** All species leaf area ($\pm$ SEM) 20 - 84 DAS with best fit polynomial (Expt 2 & 3a)

The leaf areas of the rice accessions were considerably different at harvest time in most comparisons but differences did not always reach statistical significance as shown in Table 2.1. Rice grown in 2012 with supplementary lights had greater leaf area than plants grown in 2010 and 2011 without supplementary lights (DF_{2,80}, F = 18.16, $p < 0.001$). *Oryza meridionalis* KR

generally had greater leaf areas than *O. australiensis* and *O. sativa* but results were variable in and were also affected by planting density (Table 2.1). The *O. australiensis* plants, which are perennial, continued growing when *O. sativa* and *O. meridionalis* reached maturity and had a different structure as new shoots developed from rhizomes while old shoots withered and the eventual size of the plant depended on the size of the pot. Some plants not harvested continued growing over six months, filling the whole of the container in which they were planted. The distribution of leaf area for all reproductive phase plants is shown in Figure 2.2. It is highly skewed to the left.

Table 2.1 Total leaf area (cm² per plant) of rice species and accessions at maturity. Plants in the 2011 experiment were grown at two densities (36 and 72 plants m⁻²) denoted in brackets.

Experiment [CO ₂]	<i>O. sativa</i>	<i>O. australiensis</i>	<i>O. meridionalis</i>		
			KR	CY	HS
Expt 1 [390]	1192 a	950 ab	982 ab	1316 a	1124 b
Expt 1[700]	1519	1316	1280	1663	911
Expt 2	784 b	1063 ab	1536 a	1056 ab	1374 ab
Expt 4	2033 ab	1639 b	2789 a		
Expt 3b (36)	1699 b	911 b	2189 a		
Expt 3b (72)	960 b	821 b	1685 a		

Plants grown in [CO₂] 390 ppm except for Expt 1, in which some were grown at [CO₂] 390 and some at CO₂[700]. Although the high CO₂ plants had greater leaf area, the difference was not significant. Means that do not share a letter are significantly different (Tukey test, comparisons are across rows). All samples were at maturity, young plants from Expt 3a are not included in this table. Expt 3b - plant density 36 or 72 plants m⁻². Expt 2 plants were measured At 84 DAS., Expt 4 plants measured at 90 DAS.

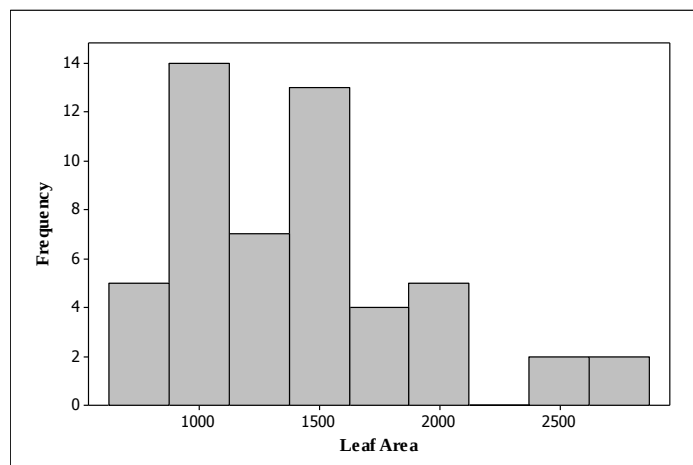


Figure 2.2 Frequency of total leaf area per plant pooled for all species and accessions at maturity (90 DAS) (Expt 1), high and low]CO₂] growth plans pooled..

Leaf area increased as tiller number increased; the correlation was 0.85 (Figure 2.3; leaf area = $67.45 \times \text{tiller number} - 12.88$, $R^2 = 0.73$).

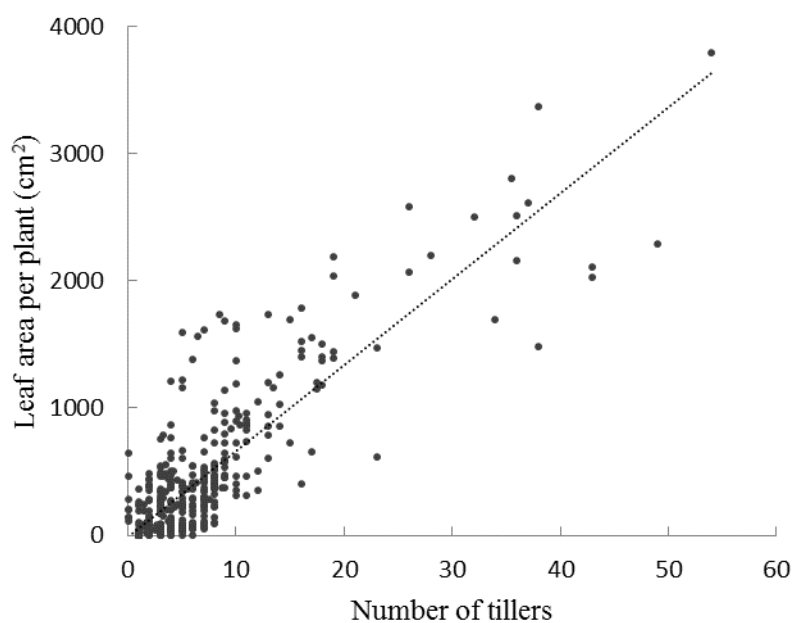


Figure 2.3 Leaf area and tiller number for all species/accessions combined (Plants from Experiments 1, 2 and 3a, 18 DAS to 90 DAS).

2.3.3 Plant Height

Plant height over time had a sigmoid pattern similar to the change in plant leaf area over time (Figure 2.4). *Oryza sativa* and *O. meridionalis* reached 1 - 1.2 m while *O. australiensis* grew to about 1.8 m (Figure 2.5). Further plant growth was by the addition of new tillers. *Oryza australiensis* plants were tallest (DF_{4,63}, $F = 12.24$, $p < 0.0005$, Tukey test); there was no significant difference between *O. meridionalis* and *O. sativa* (Tukey test). There was no significant increase in plant height after 60 DAS (Figure 2.4; see Appendix).

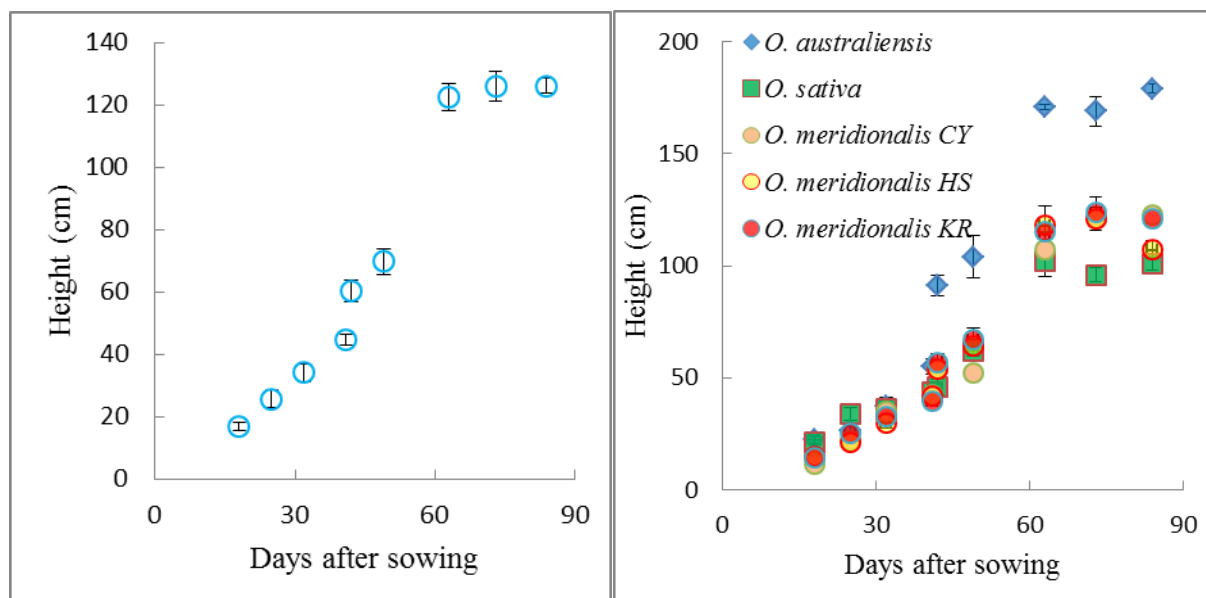


Figure 2.4 Plant height for all species and accessions 18 - 84 DAS. Means $\pm$ SEM.

Figure 2.5 Plant height individual species and accessions from 18 - 84 DAS. Means $\pm$ SEM.

2.3.4 Shoot and Root Biomass

For the period 18 - 42 DAS, root and shoot dry weights increased exponentially (Figure 2.6), but later growth slowed. There was no clear difference between the species in plant weight up to 41 DAS. Growth slowed after about 40 DAS and at harvest, *O. meridionalis* plants were largest. Growth after 40 DAS was no longer a good fit for an exponential function (Figure 2.7).

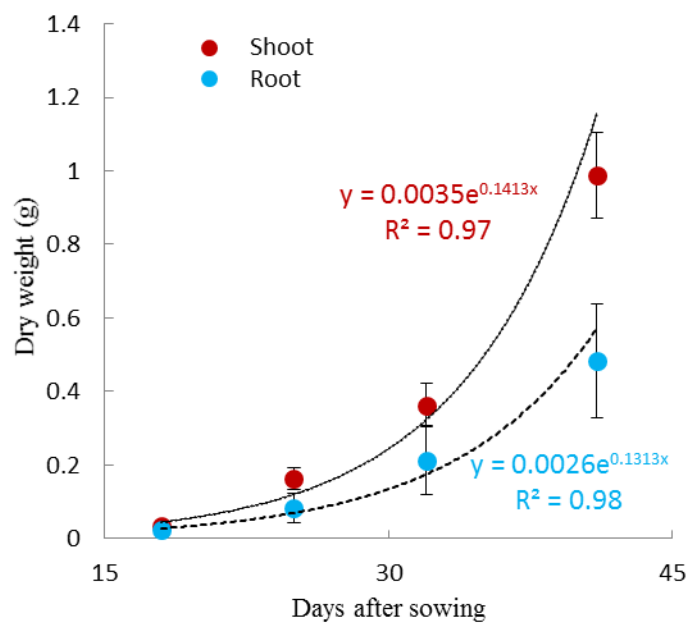


Figure 2.6 Shoot and root weights (18 - 42 DAS) of all species and accessions combined in the earliest stages on vegetative growth. Means $\pm$ SEM.

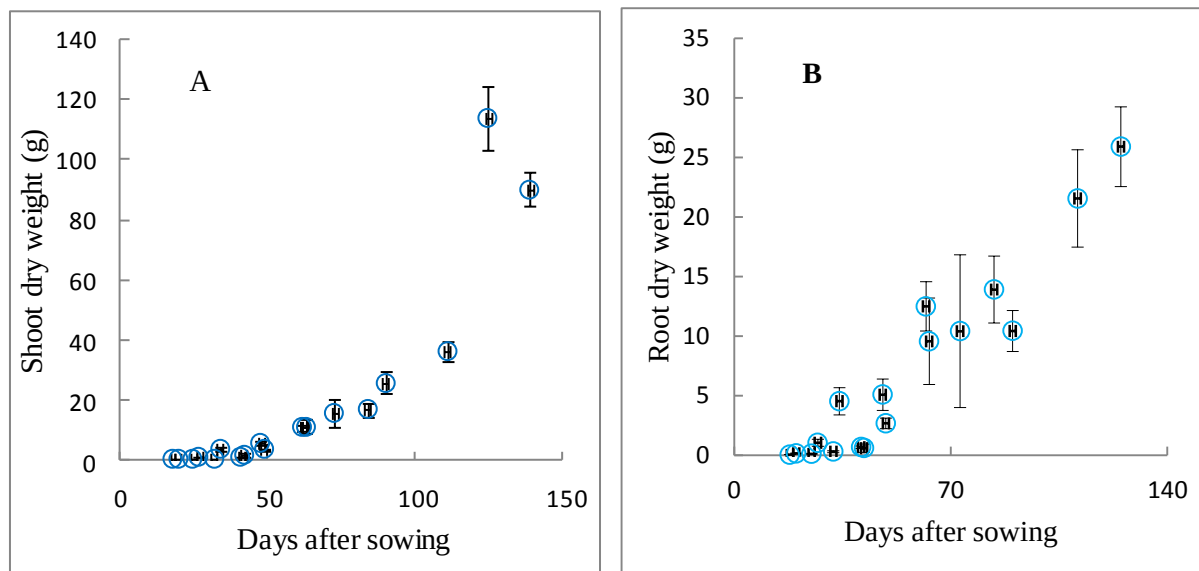


Figure 2.7 (A) Shoot and **(B)** root dry weights 18 - 125 DAS. Data are for all species combined and presented as means ($\pm$ SEM).

Most of the rice accessions are annual plants so a classical sigmoidal growth curve was expected. At the time of grain development, the *O. meridionalis* plants were the largest but *O. australiensis* were still immature; they continued to grow and to develop new shoots, from rhizomes, well after the other plants had completed growth (Figure 2.8). Root weight declined in older *O. sativa* plants but continued to increase in *O. australiensis* and *O. meridionalis*. Shoot mass data for mature plants across three growing sessions were compared and the data were converted into Z scores (value/standard deviation). This was done to reconcile different light regimes and final biomass by allowing pooling of trends (Figure 2.9). Differences between accessions were only apparent in mature plants at 90 DAS, when *O. meridionalis* shoots were heaviest (Tukey test); no other accession comparisons reached significance.

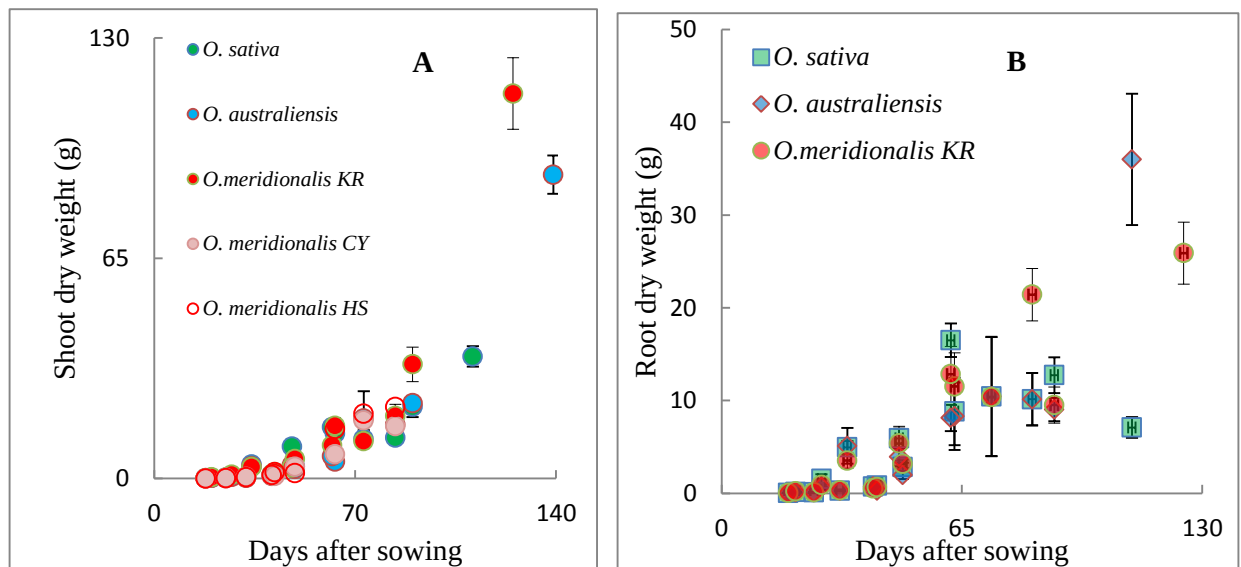


Figure 2.8 (A) Shoot and (B) root dry weight for all species and accessions. Means $\pm$ SEM.

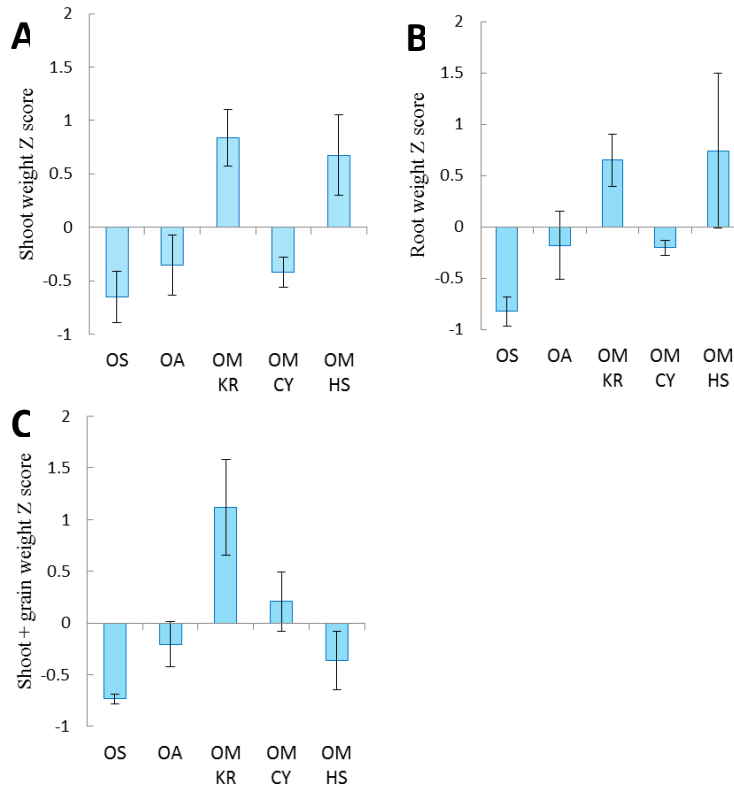


Figure 2.9 (A) Shoot mass Z scores ($\pm$ SEM); **(B)** root mass Z scores ($\pm$ SEM); and **(C)** shoot + grain weight Z scores ($\pm$ SEM) for all accessions (OS = *O. sativa*; OA = *O. australiensis*; OM KR = *O. meridionalis* KR; OM CY = *O. meridionalis* CY; OM HS = *O. meridionalis* HS). A Z score measures how far an observation lies from its mean in units of standard deviation.

Root and shoot relative growth rates declined with age. Growth rates were calculated using the equation $A_1 = A_0 e^{kt}$ where A_1 = final weight, A_0 = original weight, t = time in days, k = growth constant (relative growth rate) = $(\ln A_1 - \ln A_0)/t$ and are shown in Figure 2.10. Weights measured in Experiments 2, 3a and 4 were used to calculate growth rates.

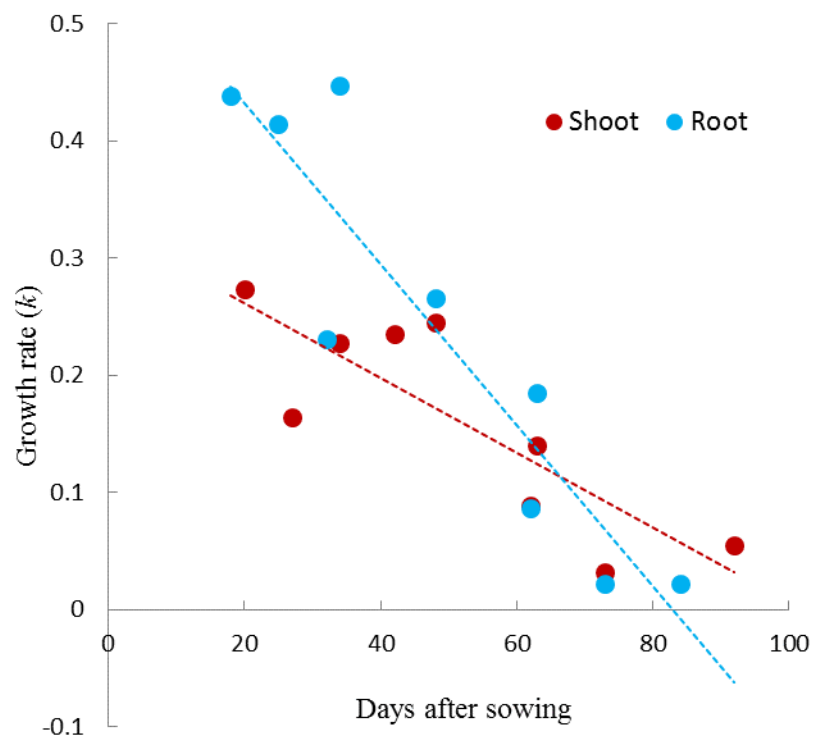


Figure 2.10 Relative growth rate (k) for shoots and roots in all genotypes over the duration Experiments 2, 3a and 4, using successive measurements of shoot and root mass.

There was no significant difference in shoot-to-root weight ratios between rice accessions ($DF_{2,45}$, $F = 0.29$, $p = 0.75$). Shoot/root dry weight averaged 1.3:1 and declined slightly as plants aged.

Shoot dry weight (shoots m^{-2}) was derived from tubs sown with rice at two densities. Shoot mass was greater in the densely planted tubs ($DF_{1,12}$, $F = 5.22$, $p = 0.04$), with *O. meridionalis* having the heaviest canopy, *O. sativa* intermediate and *O. australiensis* lightest ($DF_{2,12}$, $F = 6.08$, $p = 0.015$; Table 2.2).

The increase in dry weight at twice the low planting density ranged from 10 - 54%. Tukey tests show significant comparisons of species (across both densities) and densities (across all species), showing that the denser planting had significantly higher biomass. Shoots of *O. australiensis* were significantly lighter than those of *O. meridionalis*.

Table 2.2 Shoot dry weight per unit area (g m⁻²) of three species at two planting densities (36 and 72 plants m⁻²).

Species	Planting density		High/low density (%)	Tukey (species)
	36 (plants m ⁻²)	72 (plants m ⁻²)		
<i>O. sativa</i>	682	782	115	AB
<i>O. australiensis</i>	467	720	154	B
<i>O. meridionalis</i>	824	908	110	A
Tukey (density)	B	A		

Variables not sharing a letter are significantly different (Tukey test).

The *O. sativa* plants produced the largest inflorescences and a much greater proportion of plant mass was converted to seed. Harvest index (*HI*), grain weight divided by the sum of total shoot mass, was about 50% for *O. sativa* but only about 15% for *O. meridionalis* and about 10% for *O. australiensis*. The wild rice matured later and over a longer period than *O. sativa*. Wild rice species also shattered, making grain collection more difficult whereas the *O. sativa* heads retained their grain (Figure 2.11 and see Appendix).

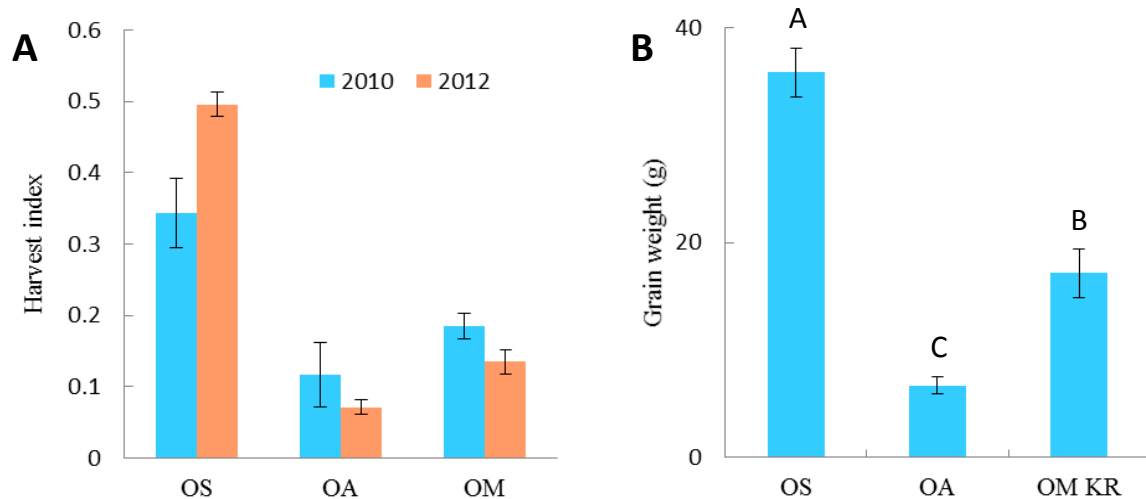


Figure 2.11 (A) Harvest index of plants grown in 2010 and 2012; **(B)** grain weight of plants from these two experiments combined. Means $\pm$ SEM. Variables not sharing a letter are significantly different (Tukey); OS = *O. sativa*; OA = *O. australiensis*; OM = *O. meridionalis* (all accessions); OM KR = *O. meridionalis* KR). Grain weights not significantly different in 2010 and 2012 (df 1, 5, F8.9, P= 0.8).

2.3.5 Tiller Development

Tiller development continued at a near-constant rate from initiation until over 90 DAS (Figure 2.11). *Oryza australiensis* had significantly fewer tillers at the time of harvest than the other rice accessions (Table 2.3). *Oryza australiensis* continued to develop and add new structures long after the other plants had ceased to grow, inflorescence was complete and the plants were senescent. *Oryza australiensis* added new shoots from rhizomes rather than tillers originating from the culm.

Table 2.3 Tiller number at 90 *DAS*. Means $\pm$ SEM.

<i>Oryza species</i>	Tillers	Tukey test
<i>O. sativa</i>	26 (3.5)	B
<i>O. australiensis</i>	8 (1)	C
<i>O. meridionalis</i> KR	37 (5)	A

Variables not sharing a letter are significantly different (Tukey test).

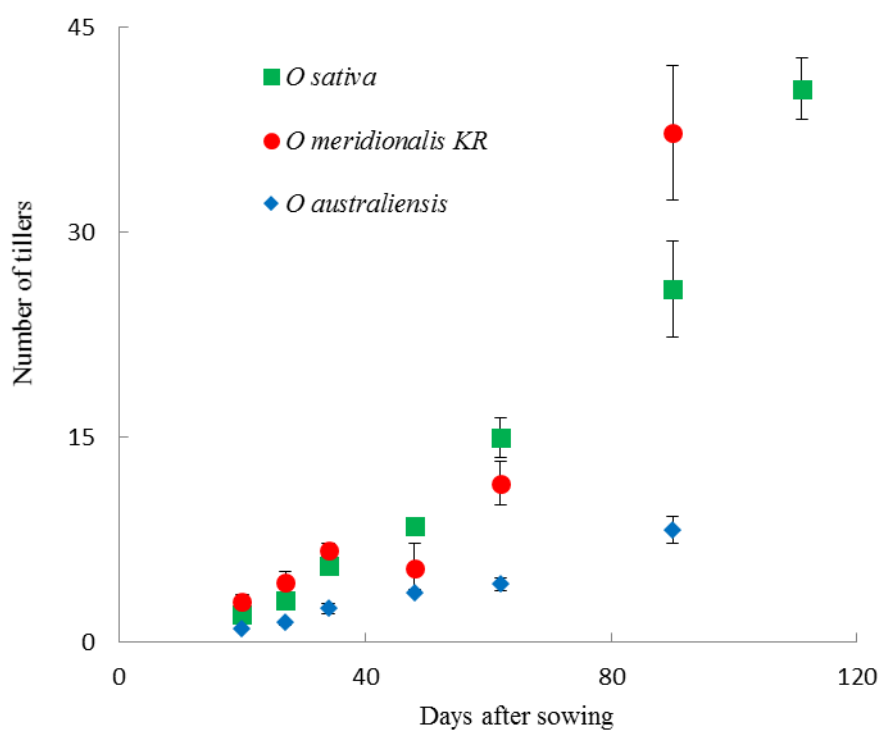


Figure 2.12 Tiller number for each species, including one accession of *O. meridionalis*. Means $\pm$ SEM.

The *O. sativa* plants had an average of 26 tillers at 90 *DAS*, mostly oriented at small angles to the vertical with leaves that were relatively straight until panicles initiated. On the other hand, the three *O. meridionalis* accessions had widely splayed tillers and leaves, with a large angle from the vertical compared to *O. sativa* tillers and leaves, which were closer to vertical. At 90 *DAS*, *O.*

meridionalis KR had 37 tillers and *O. australiensis* had 8 tillers (Table 2.4). *Oryza australiensis* also had near vertical tillers and both *O. sativa* and *O. australiensis* had significantly different zenith angles from *O. meridionalis* (Table 2.7). Leaf zenith angle was smallest in *O. sativa* but angles in *O. australiensis* and *O. meridionalis* overlapped (Table 2.4). *Oryza meridionalis* leaves were more curved and further from the vertical. Figure 2.13A & B show the similarity of *O. sativa* and *O. australiensis* in tiller angle but contrasts in leaf angles.

Table 2.4 Leaf and tiller angles (degrees from the vertical) for species and accessions.

	<i>O. sativa</i>	<i>O. australiensis</i>	<i>O. meridionalis</i>	<i>O. meridionalis</i>	<i>O. meridionalis</i>
			CY	HS	KR
Tiller	11(1) B	15((2) B	37(2) A	30(3)(A	36(2) A
Leaf	29(2) C	43(3) AB	54(3) A	38(3) B	47(2) A

Variables not sharing a letter are significantly different (Tukey test). SEM in brackets.

3.6 Specific Leaf Area

There was no significant difference in specific leaf area (SLA) in the rice species and accessions, but leaves growing at 700 ppm [CO₂] had lower SLA than leaves at ambient [CO₂] 93 DAS (212 cm² kg⁻¹ vs 258 cm² kg⁻¹; DF_{1,46}, F = 9.99, *p* = 0.003). SLA showed little change over time (Figure 2.13).

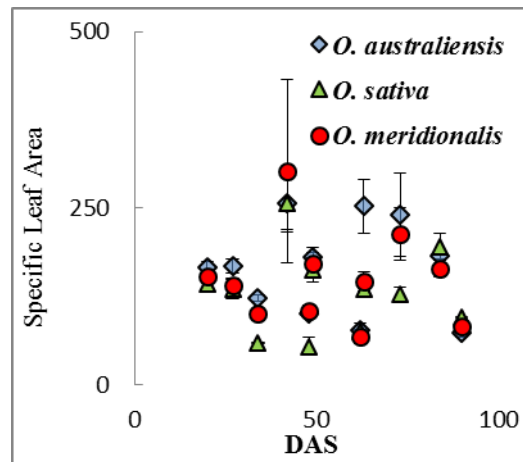


Figure 2.13 Specific leaf area for all species and accessions at both ambient and elevated CO₂ (± SEM).

2.3.7 Effects of atmospheric CO₂ concentration of growth

High atmospheric [CO₂] (700 ppm) consistently accelerated growth in all three rice species, with no interaction effects between species and [CO₂] when assessed by leaf area, tiller number, shoot dry weight, root dry weight and plant height. These stimulatory effects were not sustained until maturity, with no significant difference at harvest between plants grown in high and low atmospheric [CO₂]. In early growth stages, data for shoot and root dry weight were combined to produce a good fit to an exponential growth equation, but tiller number and plant height did not fit this pattern. Figures 2.14 - 2.16 show growth curves, with equations of best fit in Table 2.5 and statistics for leaf area, tiller development, shoot and root dry weight and plant height are summarised in Appendix 2.1.

Although grain weight was higher in the plants grown in 700 ppm CO₂ compared with plants grown in 390 ppm CO₂ (see figure 2.11 above) the difference was not statistically significant.

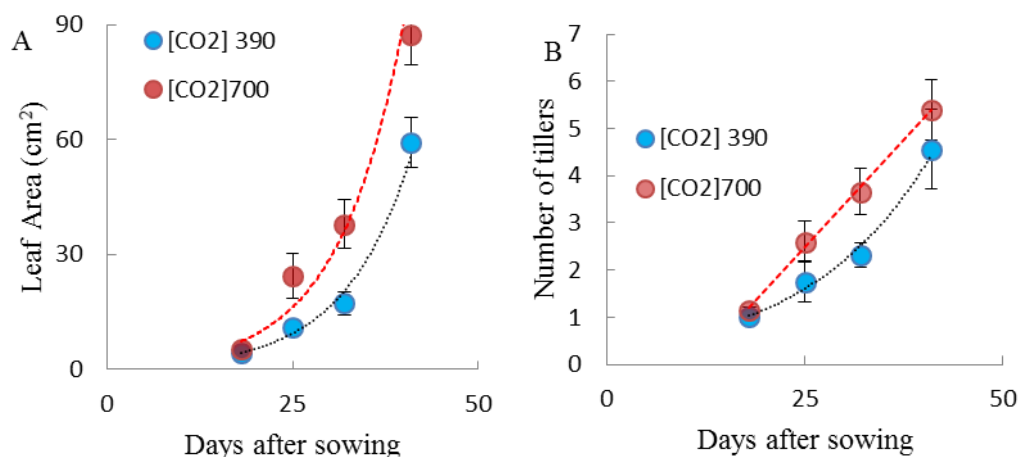


Figure 2.14 Effects of 700 ppm CO₂ on leaf area and tiller number. **(A)** Leaf area 18 - 41 DAS and **(B)** number of tillers 18 - 41 DAS for all species and accessions combined. Mean ± SEM

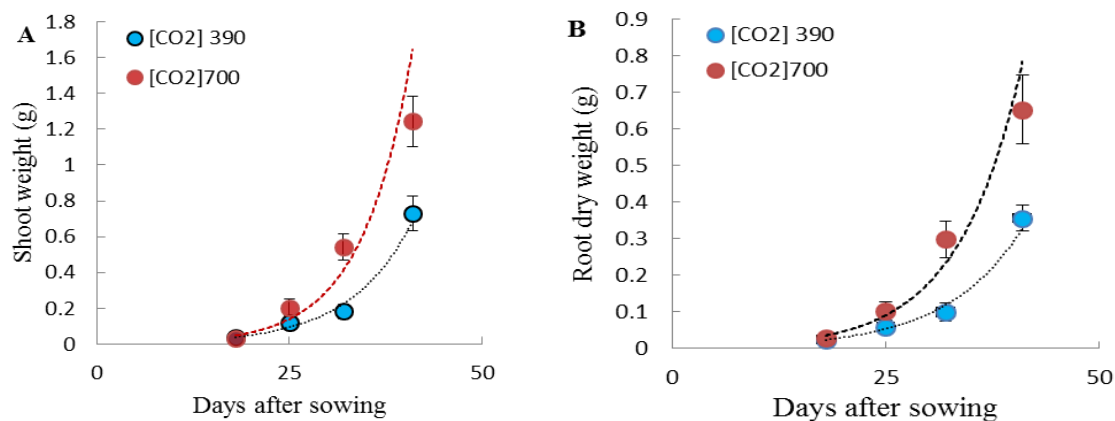


Figure 2.15 Effects of CO₂ on shoot and root weights. **(A)** Shoot dry weight 18 - 41 DAS and **(B)** root dry weight 18 - 41 DAS for all species and accessions combined. Means $\pm$ SEM.

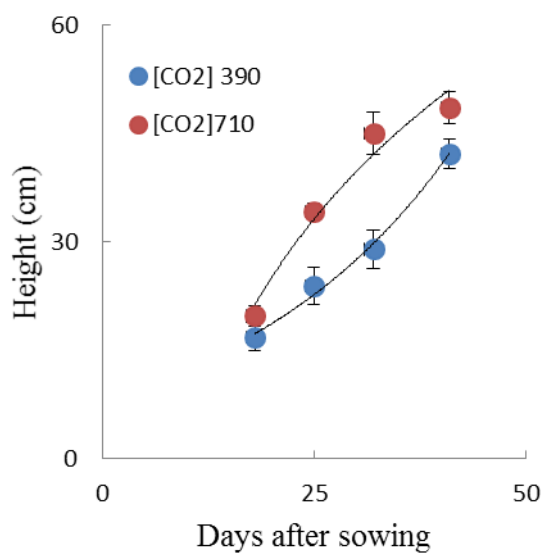


Figure 2.16 Plant height 18 - 41 DAS for all species and accessions combined. Means $\pm$ SEM.

Plant height was greater for plants 18 - 41 DAS grown in high [CO₂] (DF_{2,168}, F 10.1, $p < 0.0005$), with *O. australiensis* being tallest, but older plants showed no difference between [CO₂] treatments (Appendix 2.6).

Table 2.5 Regression equations for various growth variables against time over the period 18 - 41 DAS for all species and accessions combined. Equations of best fit are included.

CO ₂ (ppm)	Regression equation of best fit	R ²
390	Leaf Area = $0.5889e^{0.1109DAS}$	0.98
710	Leaf Area = $0.9246e^{0.1146DAS}$	0.93
390	Tiller no = $0.3541e^{0.0603DAS}$	0.997
710	Tiller no = $0.1846DAS - 2.2007$	0.999
390	Shoot weight = $0.0042e^{0.124DAS}$	0.99
710	Shoot weight = $0.003e^{0.1358DAS}$	0.94
390	Root weight = $0.0031e^{0.1134DAS}$	0.99
710	Root weight = $0.0031e^{0.1346DAS}$	0.97
390	Height = $8.6168e^{0.0388}$	0.99
710	Height = $36.198 \ln DAS - 83.343$	0.96

2.3.8 Discussion

- The morphological characteristics typical of modern cultivated high-yielding rice have been described by Yoshida (1972) and Sinclair and Sheehey (1999), with erect structures (leaves, tillers and culms) and illustrated in Figure 1.6 and images reported by Zheng et al. (2009). This archetype is characterised by the narrow zenith angles of *O. sativa* reported above (Table 2.4). No similar descriptions are available for *O. australiensis* and *O. meridionalis*, which showed much larger leaf zenith angles (both species) and larger tiller zenith angles (*O. meridionalis*).
- Total canopy leaf areas are rarely reported in published accounts of *Oryza* development. Tivet et al. (2001) published individual leaf areas but not leaf area for whole plants. Ahmad et al. (2009) measured leaf area but reported only LAI. Sakagami et al. (2009) report leaf areas for *O. sativa* and *O. glaberrima* of 3.7 to 8.3 m² plant⁻¹ for semi-submerged plants - substantially larger than any plants reported in my study, where leaf areas were close to those reported for *O. sativa* by Yang and Heilman (1990) (800 cm²) and Huang et al. (1997) (470 to 668 cm²) after just four weeks growth. Baker et al. (1992) found maximum leaf areas in mature plants (80 - 115 d after

sowing) was only 450 - 550 cm², indicating that conditions of cultivation must be highly influential on productivity. Mature plants in this study had mean leaf area 940 cm² (Figure 2.2), reflecting the rapid leaf area development typical of cultivated rice in ideal growth conditions. No reports of leaf areas relating to *O. australiensis* and *O. meridionalis* are published to our knowledge, making this the first coherent set of data on the Australian wild rice. The leaf areas per plant in three accessions of *O. meridionalis* were not statistically significantly different. While *O. meridionalis* plants generally had higher leaf areas than either *O. sativa* or *O. australiensis*, the variability in canopy areas between individual plants was large and therefore leaf areas did not differ at maturity between the species.

- Plant height plateaued at about 80 to 90 DAS (Yoshida 1981; Chapagain and Yamaji 2010), later than reported here but consistent with the observations of Moldenhauer and Slaton (in Hardke 2013) who described considerable variation in developmental rates in *O. sativa*. Growth in optimal conditions of highly lit glasshouses facilitated rapid development in our experiments. No comparable developmental information is available for *O. australiensis* and *O. meridionalis*. The three species reached peak heights at the same times (~60 d - Figure 2.5B).
- Typically plant biomass in rice experiments is reported as mass per unit area (Ahmad et al. 2009) rather than individual plant biomass, excepting a few studies. Baker et al. (1992) found maximum plant aboveground biomass at 105 - 115 d after planting was about 8 - 9 g (dry weight) plant⁻¹ for generally small plants (see previous section). Sakagami et al. (2009) reported shoot biomass of 6 - 9 g for *O. glaberrima* and 3 - 11g for *O. sativa*. It is difficult to reconcile the very large plant leaf areas reported above (Sakagami et al. 2009) with such small shoot biomasses. Up to 90 DAS in this study, shoot (dry) biomass was about 22 g for *O. sativa* and *O. australiensis* and 34 g for *O. meridionalis* with *O. australiensis* and *O. meridionalis* continuing to grow beyond 90 DAS.
- Root biomass is relevant to crop species because it can account for much of the carbon budget of the plant and therefore potential yield. Srivastava et al. (2014) found root biomass 7 g plant⁻¹ in uncontaminated (control) soil. Several authors give root mass per unit ground area; for example, Boonjung and Fukai (1996) but there are again fewer data available for individual plants, with none for *O. australiensis* and *O. meridionalis*. The results indicate that root mass of

O. sativa declines at about 90 DAS whereas in *O. meridionalis* it seems to reach a plateau and in the highly perennial *O. australiensis*, it is still increasing (Figure 2.8B).

- The harvest indices published for *O. sativa* range from 0.44 to 0.51 (Yoshida 1981) to 0.39 (Sudhir-Yadav 2011), consistent with the finding in this study of about 0.5 for *O. sativa*. The harvest indices of wild rice relatives are a pivotal factor in their ultimate utility. The only reported yield data for *O. meridionalis* gives grain yield per hectare, with extremely wide boundaries, and no data are available for *O. australiensis*. The upper bound estimate of yield in *O. meridionalis* (Wurm 1998) was 0.26 t ha⁻¹, with reported harvest index in cultivated rice of about 0.5, compared with average world yield in *O. sativa* 4.4 t ha⁻¹ and best practice yields of 10 t ha⁻¹. The figures reported here suggest higher potential yields in the Australian wild relatives, because harvest indices were around 0.1 - 0.2 for *O. meridionalis* KR, and plant biomass was about 800 to 900 gm⁻², (Table 2.2) suggesting a *possible* gain yield of about 0.8-0.9 t ha⁻¹.
- *Oryza sativa* in field and glasshouse conditions was reported had 10 to 30 tillers per plant (Yoshida 1981; Baker et al. 1992; Choi et al. 2012) compared with a mean of 26 tillers per plant in this study. There are no published data for *O. australiensis* and *O. meridionalis* although tiller development is a critical aspect of plant performance in dry land field conditions. *Oryza australiensis* had an average of only 8 tillers per plant at 90 DAS, reflecting a consistent pattern of slow tiller production in the first season of growth. However, tiller numbers continued to increase, arising from rhizomes and reaching 40 by 180 DAS. *Oryza meridionalis* averaged 37 tillers at 90 DAS, and was consistently an abundant producer of tillers.
- Zhao et al. (2010) report SLA for 15 rice species including *O. sativa* (SLA 9 - 18), *O. australiensis* (SLA 22 - 26) and *O. meridionalis* (SLA 29). Specific leaf area did not differ significantly between the species in our study but was higher in plants grown in a low CO₂ atmosphere as is widely reported in the literature (Ainsworth and Long 2004) suggesting that plants grown in high ambient [CO₂] shifted the investment of biomass to optimize photosynthetic function. SLA values were high compared with the results of Zhao et al. (2008) but comparable to the results of Caton et al. (2003) and Luquet et al. (2005).
- Many studies have shown enhanced growth in *Oryza* and other plants grown in an atmosphere of elevated [CO₂] (Baker et al. 1993; Ziska et al. 1996; Ainsworth and Long 2004;

Korner 2006; Norby and Zak 2011). In this study, early growth was enhanced by 700 ppm CO₂ in all species but tiller numbers began to accelerate in ambient conditions after 30 d and surprisingly, final plant size was not increased significantly by raising atmospheric CO₂ levels ($p = 0.982$). Ziska et al. (1996) found [CO₂] increased *O. sativa* biomass at harvest by 22 - 70%; Baker et al. (1993) noted an acceleration of development (biomass at harvest 20% higher in a high [CO₂] atmosphere compared with ambient [CO₂]), with fewer leaves in ambient CO₂ conditions produced before panicle initiation. We speculate that another factor constrained the CO₂ response even though plants were grown with unlimited supply of water and nutrients. Plants were grown in summer in high-light conditions but perhaps self-shading within the dense canopies at maturity (see Chapter 4), prevented maintenance of peak photosynthetic rates.

Chapter 3 – Photosynthetic characteristics of cultivated and wild *Oryza* species

3.1 Introduction

Photosynthesis provides the energy that powers fixation of carbon and thereby growth, so it is a central factor in investigations of plant productivity. Gross photosynthesis is estimated by measuring CO₂ assimilation, the first step in transforming atmospheric CO₂ into plant biomass. Estimates of CO₂ assimilation for wild *Oryza* range from 8-35 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Yeo et al. 1994; Zhao et al. 2010) while rates for cultivated rice fall in a narrower range close to 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Ziska and Teramura (1992) found cultivars of *O. sativa* with assimilation rates as high as 40-50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in atmospheres of 360 ppm [CO₂]. Rates increased further to 50-60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in an atmosphere of 660 ppm [CO₂]. This indicates that rice, typical of C₃ species, had enhanced photosynthesis at elevated CO₂ concentrations; net assimilation increased by 50% at [CO₂] of 550-600 ppm (Baker et al. 1990; Shimono et al. 2009). Among Australian species, *O. australiensis* has been reported to fix CO₂ at 19 - 37 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with most estimates at the higher end of the range, while *O. meridionalis* had CO₂ assimilation rates of 11 - 34 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Yeo et al. 1994; Zhao et al. 2008). There are some indications that both *O. australiensis* and *O. meridionalis* may have greater CO₂ assimilation rates than *O. sativa* but the estimates are not conclusive as there is a great deal of variation in published results (Table 1.5).

The effects of elevated [CO₂] on photosynthetic behaviour are important because of the inexorable rise in global atmospheric levels. However, the data still give an inconclusive picture when the timing of CO₂ exposure is considered. In particular, the relationship between photosynthetic rate at the CO₂ concentration in which the plants were grown as opposed to the [CO₂] at which the photosynthesis rate was tested was complex. The photosynthetic rate in plants grown at ambient [CO₂] exceeded that of plants grown at high [CO₂] when they were tested at the same [CO₂]. This effect was most marked at low [CO₂] test levels, 160 ppm, but was still evident when tested at [CO₂] of 660 ppm (Baker et al. 1990). Conversely, Ziska and Teramura (1992)

grew two varieties of *O. sativa* at elevated [CO₂] of 660 ppm, showing increased CO₂ assimilation rates at extremely high intracellular [CO₂] (above 750 ppm), exceeding the rates observed for plants grown at 360 ppm CO₂.

As well as these effects of CO₂ supply on photosynthesis, there are also effects that appear to be specific to developmental stage. This is presumably caused by changes in source-sink relations as plants generated reproductive organs. Evidence for this phenomenon comes from Shimono et al. (2009), who found clear differences in the photosynthetic rates of plants in vegetative and reproductive life stages, with vegetative plants having higher rates; approximately 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and reproductive stage plants averaging 18 $\mu\text{mol m}^{-2} \text{s}^{-1}$. CO₂ assimilation rates in younger plants were also more responsive to elevated atmospheric [CO₂], increasing 39% in elevated [CO₂] compared with an increase of 17% in reproductive-stage plants in an elevated CO₂ atmosphere (in this study elevated [CO₂] is ambient [CO₂] + 200 ppm CO₂).

The aim of this project was: (1) to establish the photosynthetic capacity of *O. sativa*, *O. australiensis* and *O. meridionalis* when grown and measured at 390 ppm [CO₂] and 700 ppm [CO₂]; (2) to determine whether plants acclimate to elevated [CO₂] by lowering photosynthetic rates and (3) effects of developmental stage on steady-state photosynthesis.

3.2 Methods

3.2.1 Plant cultivation

Rice plants were grown as described in Chapter 2. Plants were grown in glasshouses at about 390 ppm and 700 ppm atmospheric [CO₂], with supplementary lights when natural irradiance fell below 400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. CO₂ was injected into two glasshouses and [CO₂] levels were controlled by solenoids and monitored continuously (The Canary Company Pty Ltd gas detection and monitoring systems). Temperature was maintained at 28°C in daytime and 22°C at night on

12-hour cycles. Measurements were made at average of 105 *DAS* (range 90-124 *DAS*) on sunny days. Light response curves were calculated from leaves using a LiCor 6400³ at two stages of development (Vegetative at 34 ± 1.5 *DAS* and reproductive at 101 ± 1.2 *DAS*) (carried out by Dr Andrew Scafaro). Using these measurements, $A_{\max}$ was calculated by Dr Remko Duursma using a modified YPlant program (YPlant QMC Pearcy et al. 2012)).

3.2.2 Photosynthesis

Photosynthesis was measured with a LiCor 6400. In short, CO₂ and water vapour concentrations were measured in a stream of air before and after being passed over a leaf sealed in a gas-tight leaf chamber using an infrared gas analyzer (IRGA). A photosynthesising leaf removes CO₂ from the gas and permits calculation of the rate of photosynthesis by the rate at which CO₂ is removed from the gas passed over the leaf (Figure 3.1). To ensure stable input gases, CO₂ was ‘scrubbed’ from incoming air and a specific concentration of CO₂ set by adding CO₂ from a canister. Measurements were all made between 9 am and 12 noon.

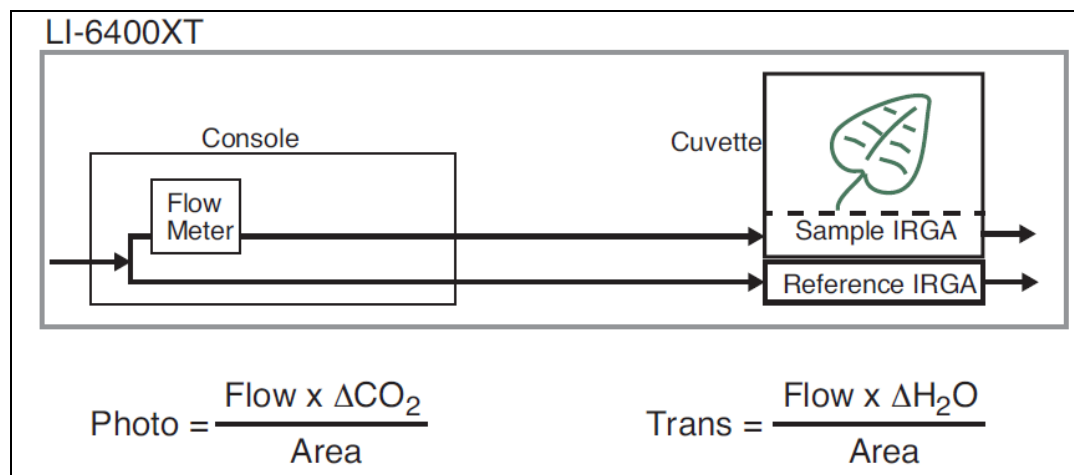


Figure 3.1 Function of LI-6400 (Photo = photosynthesis; Trans = transpiration;
From *Using the LI-6400 /6400XT Portable Photosynthesis System*.

³ Li 6400 Portable Photosynthesis System LI-COR Biosciences, Inc. • 4421 Superior Street • Lincoln, Nebraska 68504.

3.2.3 Statistics

Minitab16 was used for statistical analysis. Raw data were not normally distributed but testing for equal variance showed a significant difference (Levene's test statistic = 4.79, $p < 0.0005$) so a standard general linear model ANOVA was used. Johnson's transformation was applied to the data to obtain a normal distribution and then confirmed (see Appendix 3.1). The two methods gave the same results. The main statistical method was analysis of variance (ANOVA), using the general linear model.

3.3 Results

3.3.1 CO₂ assimilation rates

Experiment 1: mature plants in the reproductive stage of development 91 to 98 DAS. Rice plants grown at elevated (700 ppm) CO₂ had significantly higher levels of photosynthesis (30.9 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) than plants grown at ambient CO₂ levels (390 ppm) - 25.6 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ($\text{DF}_{1,129}$, $F = 25.33$, $p < 0.0005$). The differences between rice accessions were not significant ($\text{DF}_{4,129}$, $F = 2.0$, $p = 0.1$) nor was interaction significant ($\text{DF}_{4,129}$, $F = 0.3$, $p = 0.9$; Appendix note 3.2). All *Oryza* species studied showed increased CO₂ assimilation when grown in 700 ppm [CO₂] compared with *Oryza* grown in 390 ppm [CO₂].

Experiment 2: CO₂ assimilation was measured at 400 and 700 ppm [CO₂]. Regardless of what [CO₂] the plants were grown in, plants measured at 700 ppm CO₂ had faster assimilation than those tested at 400 ppm CO₂ ($\text{DF}_{1,138}$, $F = 155$, $p < 0.0005$) (Table 3.1, Figure 3.2). The interaction term was significant as the plants grown in a low [CO₂] had considerably higher assimilation rates when transferred to a high CO₂ test chamber, whereas the plants grown at high [CO₂] did not ($\text{DF}_{1,138}$, $F = 4.6$, $p = 0.034$). There were species differences in assimilation by the rice accessions, with *O. australiensis* being lowest and *O. meridionalis* CY significantly higher but with rates for the other accessions overlapping ($\text{DF}_{4,138}$, $F = 2.6$, $p = 0.04$) as shown in Table 3.1. Figure 3.3 shows the distribution of photosynthetic rates for all plants combined after being

grown at 390 ppm [CO₂] and 700 ppm [CO₂], illustrating the similarity of photosynthetic rates across Experiments 1 and 2.

Table 3.1 CO₂ uptake ($\mu\text{mol m}^{-2} \text{s}^{-1}$) in plants grown and tested at different atmospheric CO₂ (ppm) levels ($\pm$ SEM).

	[CO ₂]	[CO ₂] during growth (ppm)		% increase at
	(during test)	390	700	700 ppm
<i>O. sativa</i>	400	24 (1)	27 (1)	13
<i>O. sativa</i>	700	42 (1)	29 (2)	-31
<i>O. australiensis</i>	400	23 (2)	20 (1)	-13
<i>O. australiensis</i>	700	34 (1)	30 (1)	-12
<i>O. meridionalis</i> KR	400	22 (2)	27 (2)	23
<i>O. meridionalis</i> KR	700	33 (2)	34 (2)	3
<i>O. meridionalis</i> CY	400	23 (1)	27 (1)	17
<i>O. meridionalis</i> CY	700	39 (1)	40 (2)	3
<i>O. meridionalis</i> HS	400	23 (1)	26 (1)	13
<i>O. meridionalis</i> HS	700	35 (2)	43 (2)	23

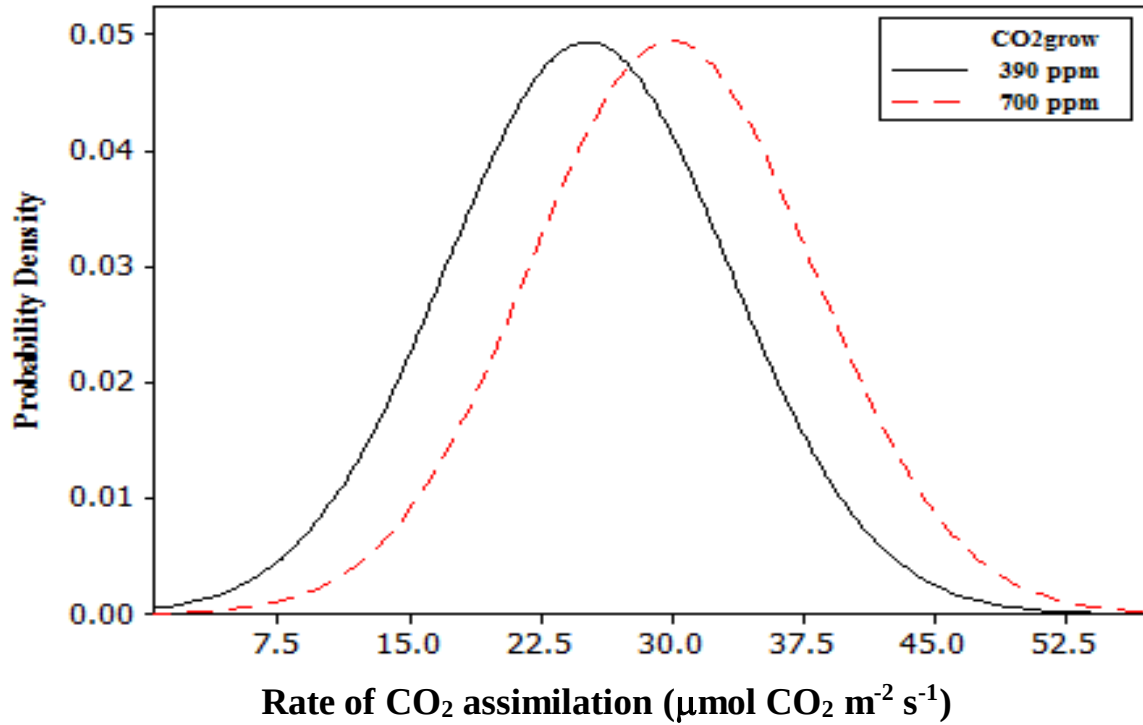


Figure 3.2 Photosynthetic rates of plants grown at 390 and 700 ppm CO₂ (black and hatched red lines respectively). Rates were measured at 400 and 700 ppm CO₂ but pooled to generate the two curves. Smoothed probability curves (normal distribution around means (25 and 30 μmol CO₂ m⁻² s⁻¹ for 390 and 700 ppm respectively) with standard deviations of 7.1 and 7.7 μmol CO₂ m⁻² s⁻¹. Data for all accessions were also pooled.

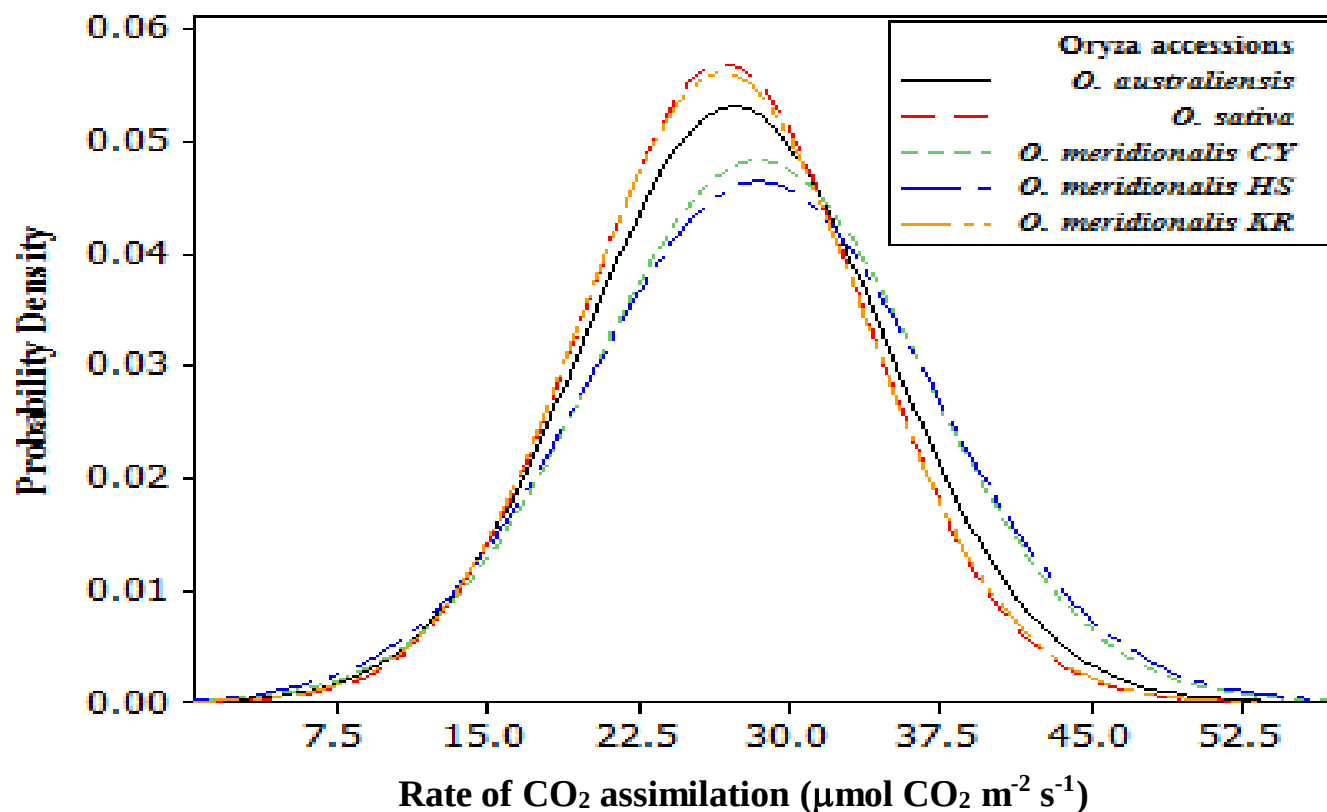


Figure 3.3 Experiment 2: Rates of CO₂ assimilation ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in rice accessions show no significant difference. Measurements made at [CO₂] 400 ppm and 700 ppm pooled, results for plants grown in 390 and 700 ppm CO₂ pooled.

3.3.2 Light-response curves

Light-response curves were drawn from measurements of CO₂ assimilation at eight photosynthetically active radiation (*PAR*) levels and maximum calculated rate of photosynthesis (A_{max}) computed using a non-rectangular hyperbola. The curves shown in Figure 3.4 are the original measurements which were used to compute A_{max} , results shown in Chapter 4.

The initial (light-limited) slope of the light-response curves in Figure 3.4 show steeper slopes in vegetative phase plants than in reproductive phase plants. However, initial slopes of the light-

response curves of plants in the vegetative phase are very close together while plants in the reproductive phase had steeper slopes for *O. australiensis* than for *O. sativa* and *O. meridionalis*.

$A_{\max}$ was estimated from the models based on the light-response curves and generated higher estimates of CO₂ assimilation than the peak rates measured by gas exchange (see Section 4.3.19 and Table 4.7).

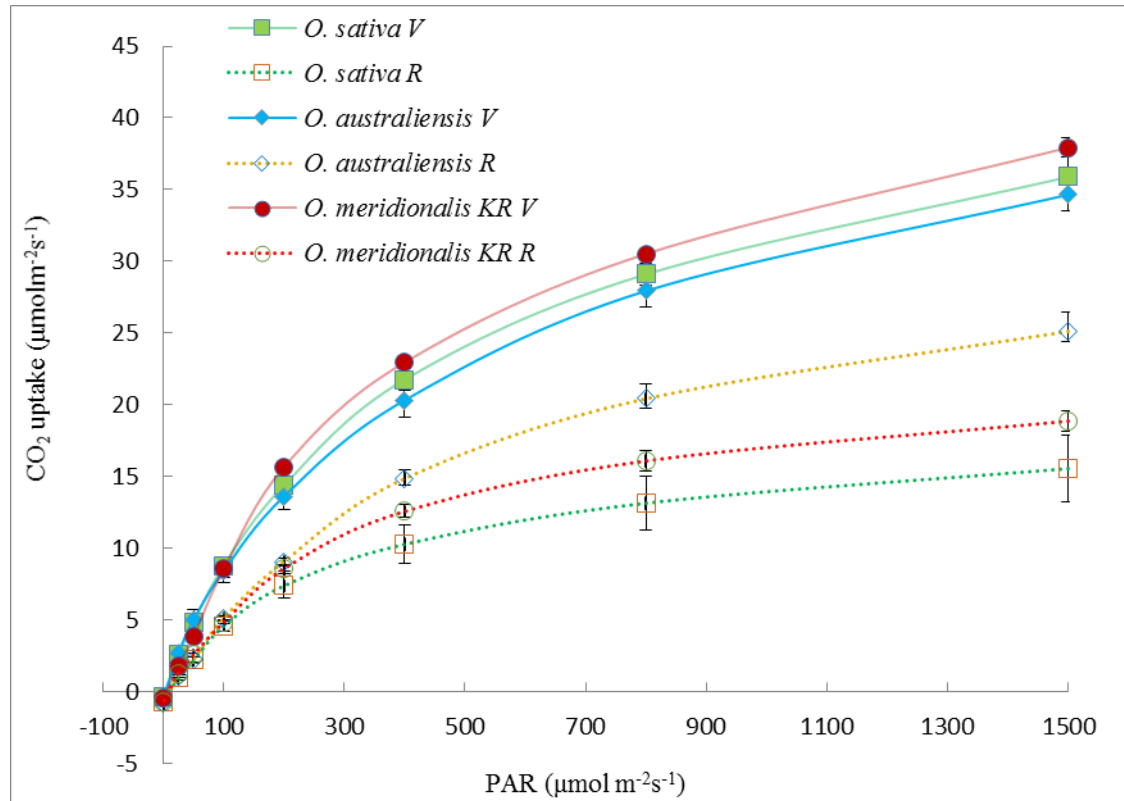


Figure 3.4 Light response curves for *O. sativa*, *O. australiensis* and *O. meridionalis* KR in vegetative (V) and reproductive (R) developmental stages. PAR indicates light levels supplied in the LiCor (μmol photons m⁻² s⁻¹). Errors shown as ± SEM.

3.3.3 Developmental stage and photosynthesis

When plants at different stages of development were compared (Figure 3.5), plants in the ‘vegetative’ stage (mean 34 DAS) were more photosynthetically active than older plants in the ‘reproductive’ stage (91 - 98 DAS) ($F_{1,13} = 178$, $p < 0.0005$). *Oryza australiensis* were notably more active in the reproductive stage than *O. sativa* and *O. meridionalis* at the same stage and there was a significant interaction between lifestage and species ($DF_{3,13}$, $F = 12.4$, $p = 0.001$). Photosynthesis rates for *O. sativa* fall to a greater extent as the plants matured from vegetative to reproductive phase than do the rates in the wild *Oryza* species.

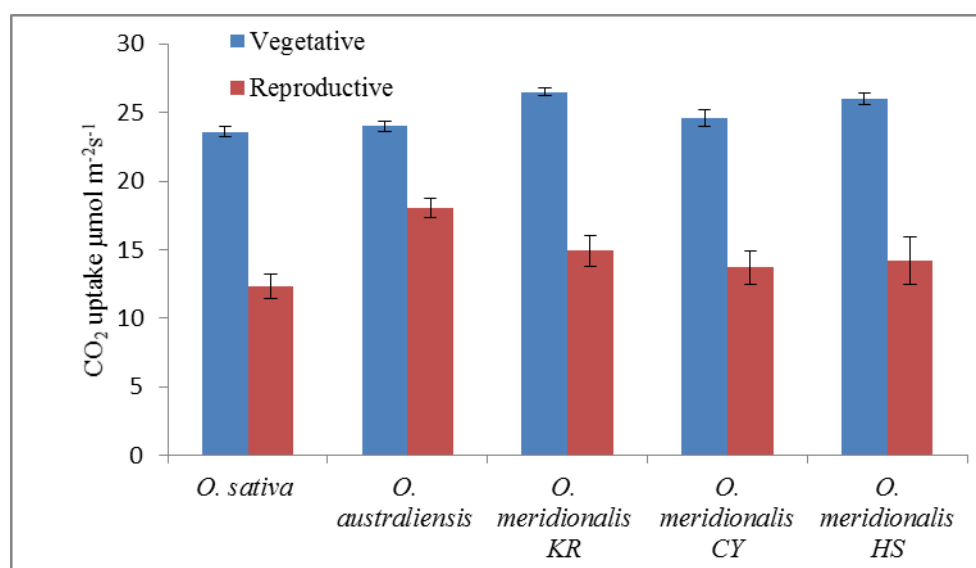


Figure 3.5 Vegetative and reproductive phase plants CO₂ assimilation.

3.4 Discussion

- There are small but non-significant differences between the *Oryza* species or accessions in photosynthetic rates, regardless of whether they were measured at ambient or elevated CO₂. Published photosynthetic rates (Table 1.5) also show considerable intra-specific variation within *Oryza* species (Yeo et al. (1994); Jiang et al. (2002); Zhou et al. (2008)). In this study, photosynthetic rates were comparable to rates reported by Yeo et al. (1994) and Zhao et al. (2008). All measurements were made between 9 am and 12 noon, removing any effect of the

diurnal decline photosynthesis (Chen et al. 2002). Surprising, the selection processes used to develop cultivated rice accessions have not resulted in greater photosynthetic capacity in *O. sativa* than the wild *Oryza* species and yet grain yield has increased dramatically through a rise in harvest index. This is discussed further in Chapter 5.

- We predict $A_{\max}$ using rectangular hyperbolae without measuring it at light saturation and as such, it does not take account the possibility of photoinhibition. Early data relied upon the measuring $A_{\max}$ rather than using mathematical estimates (Ziska and Teramura 1992). Jiang et al. (2002) report a saturating photon flux density for *O. sativa* hybrids of about $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$, well below the test range we used ($1500 \mu\text{mol m}^{-2} \text{s}^{-1}$). While these levels are less than those typically seen in the middle of a sunny day at low latitudes ($2000 - 2200 \mu\text{mol m}^{-2} \text{s}^{-1}$; see Demmig-Adams and Adams (2000); Vaz and Sharma (2010)). However, light levels above $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ exceed those that are typically observed in tropical Australia.
- There are clear differences in photosynthetic rates at different phases of plant development; plants in the vegetative stage had markedly higher rates of photosynthesis than plants in the reproductive phase. There was also a strong species interaction whereby the decline in assimilation rate was smaller for *O. australiensis* than for *O. sativa* or *O. meridionalis*, probably because *O. australiensis* is a perennial plant which retains vegetative characteristics in some parts of the plant even after flowering and grain filling. Shimono et al. (2009) also reported differences in photosynthetic rates with developmental stage in rice and also that reproductive stage plants responded less to elevated [CO₂] than did vegetative stage plants.
- Growth in an elevated CO₂ atmosphere always produced plants with an enhanced photosynthetic capacity, regardless of the [CO₂] at which they were measured. This was a somewhat unexpected result with respect to those plants that were measured at ambient CO₂ levels because the common dogma is that such plants would have suppressed photosynthetic rates when suddenly exposed to 400 ppm. It should be noted that plants were acclimated to the lower [CO₂] for 2 h and the possibility of upregulation of the photosynthetic machinery in this time must be considered.
- Plants grown at lower levels of atmospheric CO₂ had an increased rate of photosynthesis when measured at 700 ppm CO₂ and did not consistently photosynthesise faster than those

maintained at 700 ppm throughout. There was a species interaction (see above, *Experiment 2*), with *O. sativa* the only species that clearly acclimated to ambient CO₂ and produced a burst in photosynthesis when CO₂ levels were raised to 700 ppm. Baker et al. (1990) noted an increase in photosynthetic rate in *O. sativa* with high atmospheric [CO₂] regardless of the growth history of the plant. However, this CO₂ response saturated at about 500 ppm CO₂, with CO₂ assimilation rates scarcely changing between 500 and 900 ppm. They attributed this to decreasing Rubisco and carboxylase activity as CO₂ levels were raised over the whole growing season (Baker and Allen, 1993).

- During reproductive development, the boost to photosynthesis when plants were switched from 390 ppm (growing conditions) to 700 ppm CO₂ (test conditions) was about 20%. Under similar regimes, Ziska and Teramura (1992) found increases of 0 - 25%, Baker et al. (1990) 25 - 50% in plants 19 - 41 DAS and Shimono et al. (2009) 37% in vegetative plants and 17% in reproductive stage plants. Vu et al. (1997) found the increase in CO₂ assimilation was approximately 50% but Wang et al. (2006) found the increase was only about 6%. Clearly, there are large differences between experiments even in *O. sativa*, probably more likely to be because of experimental conditions than genetic contrasts.

Chapter 4 – Canopy architecture

4.1 Introduction

Canopy models have been used to estimate carbon assimilation by entire plant canopies. Because plants are complex structures, models are required to estimate light interception for three-dimensional structures and when combined with light-response curves, plant carbon gain can be estimated. Such models use measures of leaf area and leaf orientation to incoming photosynthetically active radiation (*PAR*), with adjustments for shading of leaves by other leaves of the same plant. Plants and leaves are three-dimensional structures, so it is important to know the angle at which incoming light strikes a leaf; for example, Figure 4.1 shows leaf angle to the horizontal (elevation, α), angle to north (azimuth, θ) and rotation on the petiole (ρ), all of which affect leaf exposure to light. Early studies concentrated on the geometry of leaves and incoming light (Monteith 1965; Duncan et al. 1965; Chen and Black 1992; Cheng et al. 1997), but as three-dimensional (3D) digital positioning methods were developed, 3D plant simulations based on coordinates from real plants were used (Pearcy and Yang 1996) which account for the actual spatial distribution of leaves in plant crowns.

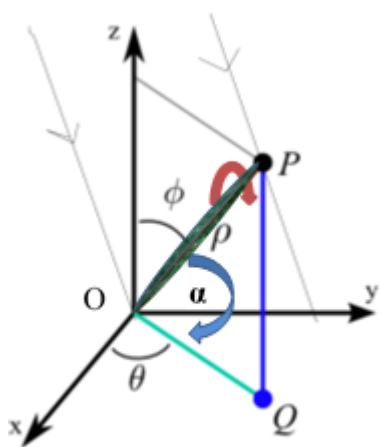


Figure 4.1 Representation of three-dimensional co-ordinates, elevation, azimuth and rotation about the axis O–P (α , θ , ρ respectively) of a hypothetical structure depicted by the green leaf (origin $\rightarrow P$) which were used in FLORADIG as described by Pearcy and Yang (1996). In addition, the zenith angle, ϕ , (z–O–P) the complement of α , is shown. The arrowed lines are incoming light rays.

Two 3D digital reconstructions of plants, both using projections giving flat leaves, are considered here. Pearcy and Yang (1996)

estimated total leaf area (L_T) and displayed leaf area (L_D) to calculate efficiency of light capture (E_D , $E_D = L_D/L_T$) (see Figure 4.2). The ratio of leaf silhouette area to total leaf area was developed by Oker-Blom and Smolander (1988), which has been modified to compute an averaged figure for a whole canopy, $\overline{STAR}$ (Delagrange et al. 2006). More recently, a model for rice that has a curved leaf has been developed (Song et al. 2013).

Australian wild relatives of rice are morphologically distinct from each other and from cultivated rice but it is not clear how these differences affect LIE (The ratio of mean light intercepted by leaves to light intercepted by a horizontal surface of equal area) or computed carbon gain. If wild *Oryza* has any advantage over *O. sativa*, can the mechanism be identified and transferred to cultivated *Oryza*? Could re-design of crop species to accommodate climate change factors such as extreme heat be aided by incorporation of features from wild *Oryza*? This approach is likely to require introgression of genes that encode an alteration in canopy shape (e.g. to reduce heat load). This study was important as a preamble to manipulation of genes for architecture.

Wild and cultivated rice accessions were compared with respect to canopy shape, distribution of leaf area in the canopy, shading by other leaves of the same plant and leaf area index to assess light interception efficiency and potential photosynthesis. Most plants were grown in pots without competition but to simulate a crop or field condition, some were grown in tubs at two different plant densities, which also allowed calculation of leaf area index (LAI) and the distribution of leaf area at different levels of the leaf canopy. Canopy architecture was recorded with photography as well as the digitisation. Leaf/stem zenith angles were measured. Leaf and tiller characteristics and light interception efficiency (LIE the ratio of mean light intercepted by leaves to light intercepted by a horizontal surface of equal area) was assessed in digitised plants; *Oryza* species at vegetative stage and reproductive stage were compared by two methods. The effect of the atmospheric [CO₂] in which the plants were grown was investigated. CO₂ assimilation by whole plant canopies was modelled with Yplant.

4.2 Methods

4.2.1 Plant cultivation

The same three *Oryza* species were used in the experiments reported in this chapter - *O. sativa*, *O. meridionalis* and *O. australiensis*. These species were grown in the conditions described in Chapter 2, either in 10 kg pots (plants used for digitisation and [CO₂] comparisons) or in tubs containing 30 kg soil (density experiments) in glasshouses at 30°C (day) and 22°C (night) under natural and artificial light regimes as indicated in legends. Digitised plants were aged between 30 to 113 d and derived from four separate experiments, commencing 28 February and 20 December 2010, 28 May 2011 and 20 January 2012. The last two experiments were conducted under lights (described in Chapter 2.2.1 Plant Growth) to overcome the effects of short-days and cloud that could have retarded growth.

4.2.2 Digitization

Canopy morphology was recorded by digital photography for a qualitative record to show the overall shape of the plants and optical measurements of *LAI*. Digitisation allowed numerical modeling of light capture and shading of leaves by other leaves of the same plant. A Polhemus FASTRAK digitiser⁴ was used to collect canopy architecture coordinates (plant base, nodes, leaf tip and leaf margins) which were then recorded with Floradig software (Hanan 2000). When digitising plants, it was necessary to find a site not subject to magnetic field interference due to metal or electrical currents. Before recording, the equipment was tested to check the digitiser gave consistent accurate readings in three-dimensions; readings accurate to ± 2 mm were obtained before plants were digitised. A windbreak was used to avoid drafts on the leaf canopies. Plant images were inspected during the digitisation process to check that images were consistent with plant form. Equipment was set up as shown in Appendix 4.14.

⁴ Polhemus 40 Hercules Drive, PO Box 560 Colchester, Vermont, 05446-0560, USA.

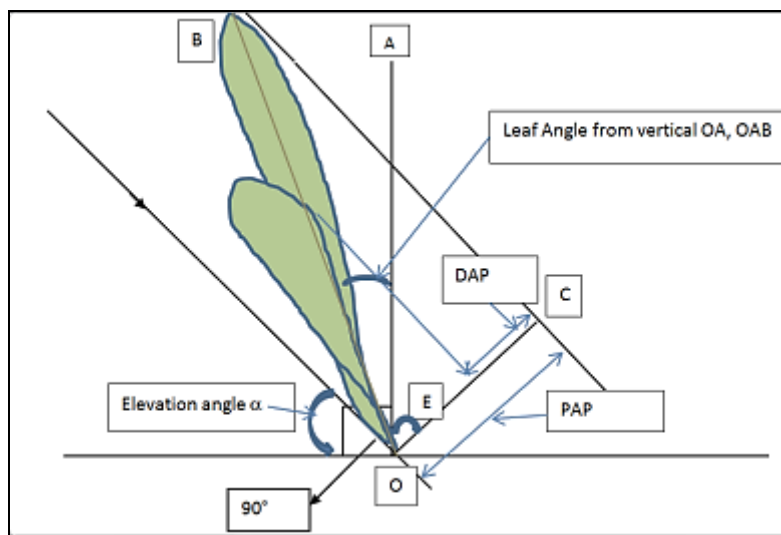


Figure 4.2 *PAP* is the area projected on a plane perpendicular to an incoming light ray at elevation angle α and *DAP* is the displayed leaf area projected onto a plane perpendicular to an incoming light ray at elevation α less the area shaded by other leaves, that is *PAP*, minus the area shadowed by another leaf.

The output from Floradig was processed in Yplant (Pearcy and Yang 1996) to compute leaf areas (L_P , L_D , L_T). The leaf area projected onto a plane perpendicular to an incoming light ray, perpendicular area projected (*PAP*), was modelled in eight steps of azimuth (compass direction, 0, 45, 90, 135, 180, 225, 270 and 315 degrees) and 20 steps of elevation (from 2.25°, just above horizontal, in steps of 4.5° to 87.75°, almost vertical), generating 160 data points for each plant. The displayed leaf area (*DAP*) is the area projected onto a plane perpendicular to an incoming light ray (*PAP*), less the area shadowed by other leaves; as for *PAP*, this was measured in 160 steps. Figure 4.2 shows *PAP* and *DAP*. Total leaf area (*TLA*) was used to estimate light interception efficiency (*LIE*) by calculating $DAP:TLA$. Projection efficiency was estimated by measuring $DAP:PAP$; for example a canopy in which no leaves shaded their neighbours would have a $DAP:PAP$ of one. Areas were recorded using Yplant (E_D , the fraction of unshaded foliage projected towards a given sky region – is sometimes used for $DAP:TLA$).

An alternative but related measure of light interception efficiency used in this analysis is the silhouette to total area ratio averaged over all viewing angles, $\overline{STAR}$ (Figure 4.3; Valladares et al. 2002; Duursma et al. 2012).



Figure 4.3 ‘Envelope’ containing the outermost leaves of a plant prepared with YplantQMC (Duursma and Cieslak 2012). This envelope gives rise to crown surface area A_c .

The distribution of leaves in space (leaf dispersion), whether clumped or uniformly distributed, was also calculated with YplantQM (Duursma Cieslak and Pearcey 2012) by measuring the average distance between the mid-point of a leaf and the mid-points of the five nearest leaves and compared to a random distribution. This measurement is β in the STAR calculation. Values < 1 are clumped, values > 1 are dispersed (Duursma et al. 2012).

4.2.3 Light exposure and latitude

The natural growing season falls within the period from the spring equinox to the autumn equinox. At the equinoxes the noon sun elevation angle will be 76° (and the sun will be to the North) at Keep River (Northern Territory savanna) and 56° at the Riverina (SE temperate zone); these are the locations in which the rice used in this study would usually be naturally found or cultivated. At the summer solstice, the sun elevation angle will be 97.5° (to the South) at Keep River and 81° at the Riverina, so the average noon sun elevation in the growing season (between the equinoxes) at Keep River will be about 86° while it is only about 68° in the Riverina (Figure 4.4).

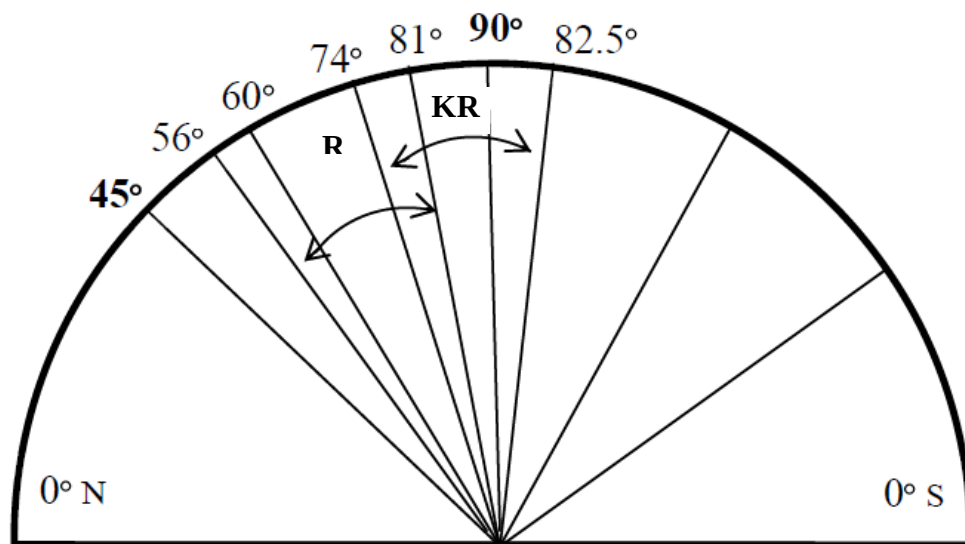


Figure 4.4 Noon sun elevation at Keep River (KR - 74° N at the equinox to 7.5° S at the summer solstice, average over the growing season 86° N) and the Riverina (R - 56° N at the equinox to 81° N at summer solstice, average 68° N over the growing season) from spring equinox to summer solstice to autumn equinox.

4.2.4 Plants grown in competition and leaf area index

Competition between plants in a crop or natural grassland was simulated by growing plants in large rectangular tubs containing 30 kg of soil at two planting densities, eight and 16 plants per tub (surface area 0.2183 m²), equating to 36 and 72 plants m⁻². Leaf area was measured at booting stage. *LAI* was estimated using two light interception measures, the LAI2000 Plant Canopy Analyzer⁵ which compares light intensity above the canopy with light intensity below the canopy and hemispherical canopy photographs, taken vertically from ground level⁶, which were used in

⁵ The LAI2000 Plant Canopy Analyzer, LiCor Biosciences, 4421 Superior St, Lincoln, Nebraska, 68504, USA..

⁶ Nikon Coolpix and 'fisheye' 185° extreme wide angle lens.

Gap Light Analysis (GLA) software⁷. GLA calculations require the original hemisphere photographs to be adjusted to remove shadows not due to vegetation, such as the shadows of light fittings, shown in Figure 4.5. Plant Canopy Analyzer and GLA estimates were compared with leaf area measured at harvest using LAI2000 Plant Area Meter⁸ which passes flattened leaves over a light detector, divided by tub area, 0.2183 m². Leaves overlapping tubs clipped before measurement. Figure 4.6 shows the results.

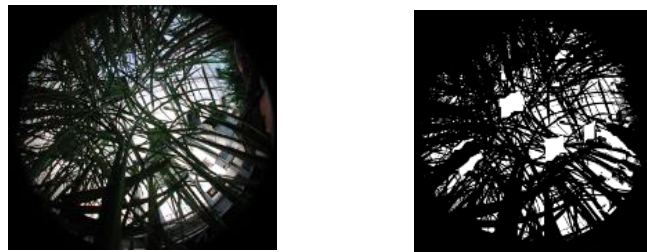


Figure 4.5 Original hemispherical photograph, the ‘registered image’ (left) and a ‘working image’ prepared from the photograph (the registered image) adjusted to remove the shadows of light fittings (right) using the Gap Light Analyzer editing facility, with the unwanted part of the image erased.

4.2.5 Statistics

Statistics were calculated using Minitab 16. A general linear model for two-way analysis of variance and MANOVA were used to compare *O. sativa*, *O. australiensis* and *O. meridionalis*, the effect of [CO₂] and the effect of sun elevation on the leaf area exposed to direct sunlight. Data were not normally distributed but using data transformed to a normal distribution or raw data made no difference to the statistical results. ANOVA (general linear model) was used. Days after

⁷ Gap Light Analyzer (GLA) Imaging software to extract forest canopy structure and gap light transmission indices from true-colour hemispherical (fisheye) photograph. Simon Fraser University, Burnaby, British Columbia, CANADA and Institute of Ecosystem Studies, Millbrook, New York, USA.

⁸ LAI2000 Plant Area Meter, LiCor Biosciences, 4421 Superior St, Lincoln, Nebraska, 68504, USA.

sowing (*DAS*) and total leaf area (*TLA*) were grouped and averaged to simplify the calculations involving these variables.

4.3 Results

4.3.1 Leaf characteristics

Digitised images of plants showed some differences to those found by direct measurement previously noted in Chapter 2. Estimates of projected leaf area were smaller in digitised plants than direct measurements (see Table 2.1). Estimates of areas of individual leaves were possible for a whole plant using Yplant. Again, *O. australiensis* was notable for having fewer but larger leaves compared with the other rice accessions (Table 4.1) although the differences between species were not statistically significant except for mean leaf area which was greatest in *O. australiensis*. Leaf number per plant was readily assessed by the digitised model and development over time is shown in Appendix 4.3.

Table 4.1 Characteristics of leaves of the three *Oryza* species.

Vegetative phase*	<i>O. sativa</i>	<i>O. australiensis</i>	<i>O. meridionalis</i>	Means
Number of leaves	17 (3) A	10 (4) A	10 (1) A	11 (1) b
Individual leaf area (cm ²)	16 (2) B	23 (5) A	8 (1) B	13 (2) b
Mean plant leaf area (cm ²)	322 (102) A	429 (272) A	114 (33) A	231 (7) b
Reproductive phase*				Means
Number of leaves	52 (11) A	27 (7) A	51 (11) A	47 (7) a
Individual leaf area (cm ²)	17 (3) B	51 (11) A	20 (3) B	25 (30) a
Mean plant leaf area (cm ²)	962 (292) A	1906 (700) A	1155 (303) A	1241 (225) a

Tukey test - variables not sharing a letter are significantly different; upper case comparing species, lower case comparing growth phase; Means ($\pm$ SEM). *Vegetative phase up to 40 *DAS*, Reproductive phase > 80 *DAS*. Samples between 40 and 80 *DAS* were not used to avoid classification problems.

4.3.2 Tiller and leaf orientation

Oryza sativa had smallest zenith angles for leaves and tillers, *O. meridionalis* had large zenith angles for leaves and tillers and *O. australiensis* had small zenith angles for tillers but large zenith angles for leaves (Table 2.7). See Section 2.3.5 for details.

4.3.3 Effects of intraspecific competition on vertical distribution of leaf area within canopies

Plants were grown in tubs at two densities (36 and 72 plants m⁻²). At harvest, *O. meridionalis* plants had significantly greater total leaf area per tub than *O. sativa*, while *O. australiensis* had a total leaf area one-third smaller than *O. sativa* and less than half of that observed in *O. meridionalis* (DF_{2,12}, F = 54.34, *p* < 0.0005 and Tukey test). The greatest proportion of leaf area was found in the lowest third of the vertical height of the canopy for *O. meridionalis* but *O. australiensis* and *O. sativa* had greater proportions of leaf area in the middle third of the canopy (Table 4.2; Figure 4.6). ‘Low density’ plantings (36 plants m⁻²) had greater leaf area per plant (1600 cm²) than tubs with 72 plants m⁻² (1156 cm²) (DF_{2,12}, F = 18.74, *p* < 0.0005; Figure 4.7), although the total leaf area of all plants in a tub was greater when planted more densely. However, leaf area in the less densely planted tubs almost matched the leaf area in the densely planted tubs (Figure 4.6, Table 4.2; see Appendix 4.2).

Table 4.2 Leaf area (cm²) per tub of plants grown at high and low density at 81 DAS

Canopy level	Plants (m ⁻²)	<i>O. australiensis</i>	<i>O. meridionalis</i> KR	<i>O. sativa</i>	Layer
Top	36	1324 (18%)	437 (2%)	1881 (15%)	b
Middle	36	3165 (43%)	7356 (40%)	5765 (46%)	a
Lowest	36	2802 (38%)	10568 (58%)	4875 (39%)	a
Total leaf area		7291 C	18361 A	12521 B	
Top	72	1332 (11%)	3527 (13%)	1531 (9%)	b
Middle	72	5876 (48%)	11023 (41%)	7898 (48%)	a
Lowest	72	5055 (41%)	12522 (46%)	7081 (43%)	a
Total leaf area		12262 C	27071 A	16509 B	

Leaf area cm² (% total plant leaf area), Variables not sharing a letter are significantly different (Tukey test, lower case = position in canopy compared, upper case = rice accession compared, $p < 0.05$). 81 DAS.

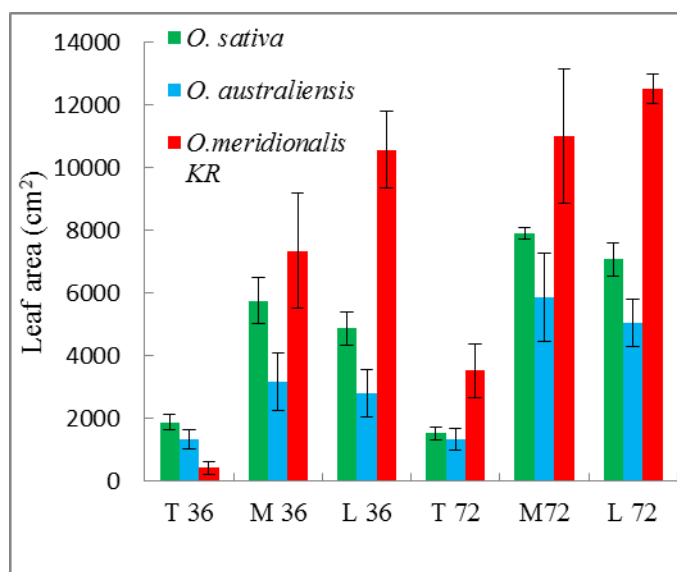


Figure 4.6 Leaf area (cm²) in top (T), middle (M) and lowest (L) thirds of the canopy as reported above in Table 4.2. Low density planting (36 plants m⁻²) on the left; high density planting (72 plants m⁻²) on the right.

4.3.4 Leaf Area Index

Leaf area index (*LAI*) was assessed as described in Section 4.2.4. The principal findings are that in spite of three disparate methods being used to determine canopy density, *O. meridionalis* canopies were always denser than those of the other two species, due to the proliferation of leaves at the base of the canopy (Figure 4.7). Secondly, *LAI* increased with planting density, but not in proportion to the number of plants in the sward. Figure 4.8 shows canopies in hemispherical and overhead photographs in tubs, with those of *O. meridionalis* conspicuously the densest. Tubs with double the planting density had between one-third and two-thirds more leaf area of those in low-density plantings.

Table 4.3 Leaf Area Index (LAI) estimated by three methods

Rice accession (plants m ⁻²)	LAI by direct measurement	% increase (72 vs 36 plants m ⁻²)	LAI (by GLA)	LAI (by Plant Canopy Analyzer)
<i>O. australiensis</i> (36)	3.35 (0.9)		1.50 (0.1)	1.43 (0.1)
<i>O. australiensis</i> (72)	5.62 (1.1)	68	2.3 (0.2)	2.53 (0.1)
<i>O. sativa</i> (36)	5.74 (0.7)		2.1 (0.1)	2.49 (0.3)
<i>O. sativa</i> (72)	7.56 (0.4)	31	2.6 (0.1)	3.52 (0.1)
<i>O. meridionalis</i> (36)	8.41 (1.5)		3.0 (0.4)	3.94 (0.3)
<i>O. meridionalis</i> (72)	12.40 (1.2)	47	3.3 (0.5)	6.10 (0.1)

Means (± SEM)

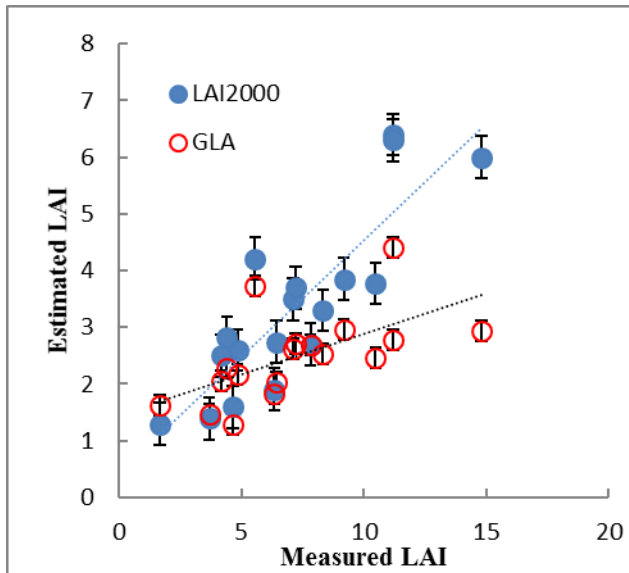


Figure 4.7 LAI from measured leaf area (leaf area meter) and LAI estimated by LAI2000 (solid circles) and Gap Light Analysis (open circles). Error bars ± SEM. Regression equations were:

GLA: $LAI = 0.1443 (\text{measured LAI}) + 1.14442$, $R^2 = 0.38$

LAI2000: $LAI = 0.4416 (\text{measured LAI}) + 0.4416$, $R^2 = 0.73$

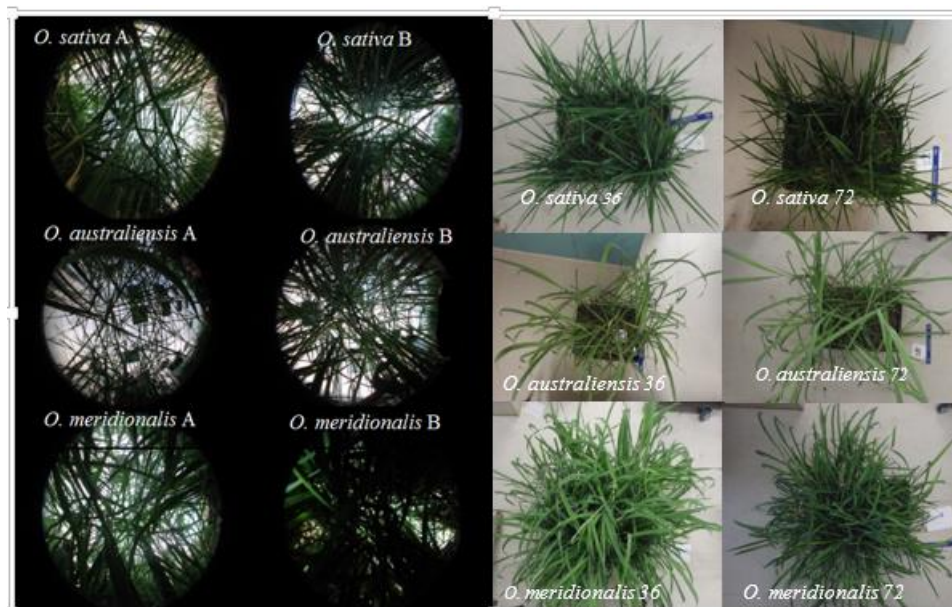


Figure 4.8 Hemispherical photographs of canopies of rice taken 90 DAS for *O. sativa*, *O. australiensis* and *O. meridionalis* sown at two densities (36 plants m⁻² (A) and 72 plants m⁻² (B)). The plants that gave rise to the canopies photographed in the left panel are shown in the right panel. Note that the hemispherical images include some unwanted shadows such as light fittings which were removed as described in 4.2.4 before light gap analysis.

4.3.5 Species morphological contrasts

Photographs of the shoots of each species at panicle emergence showed contrasting morphologies (Figure 4.9), while at stages up to early tillering, leaf orientation of the three species was very similar. Broadly speaking, *O. australiensis* was about 2 m tall at maturity with a loose open canopy comprising few tillers (Table 2.6) and long arching, broad leaves that formed into a parabolic strap-like conformation. *Oryza australiensis* continued to produce tillers and shoots from rhizomes (see inset photograph) when the other species had stopped growing. *Oryza sativa* had straight leaves in dense canopies with many erect tillers and leaves (Chapter 2, Table 2.7). *Oryza meridionalis* had relatively straight leaves that became less vertical and more curved as plants aged, although less so than leaves of *O. australiensis*.

Tillers often fell to ground-level in mature plants of the wild species, especially *O. australiensis*, while *O. sativa* maintained an erect canopies until senescent. New tillers of *O. meridionalis* were also erect while the outermost and oldest tillers were progressively more splayed.

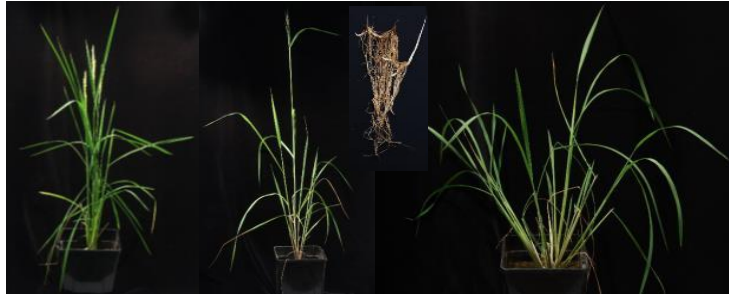


Figure 4.9 The three *Oryza* species used in this study (from left to right) - *O. sativa*, *O. australiensis* and *O. meridionalis* - taken at the stage the panicle emerged. Note also the inset showing a root system of *O. australiensis* and associated rhizome and emergent shoot (arrowed).

4.3.6 Digitised plant images

Digitized images were taken from the Floradig program. They show a stylised representation of the original plant (Figure 4.10). Yplant prepares slightly different images from those shown in Floradig (Figure 4.11), reflecting projected leaf area more accurately than culm and tillers areas, which are represented by lines only. Yplant uses a standard leaf silhouette which is applied to each plant with leaf length determined by the digitized coordinates. (Leaf file in Appendix 4.3).

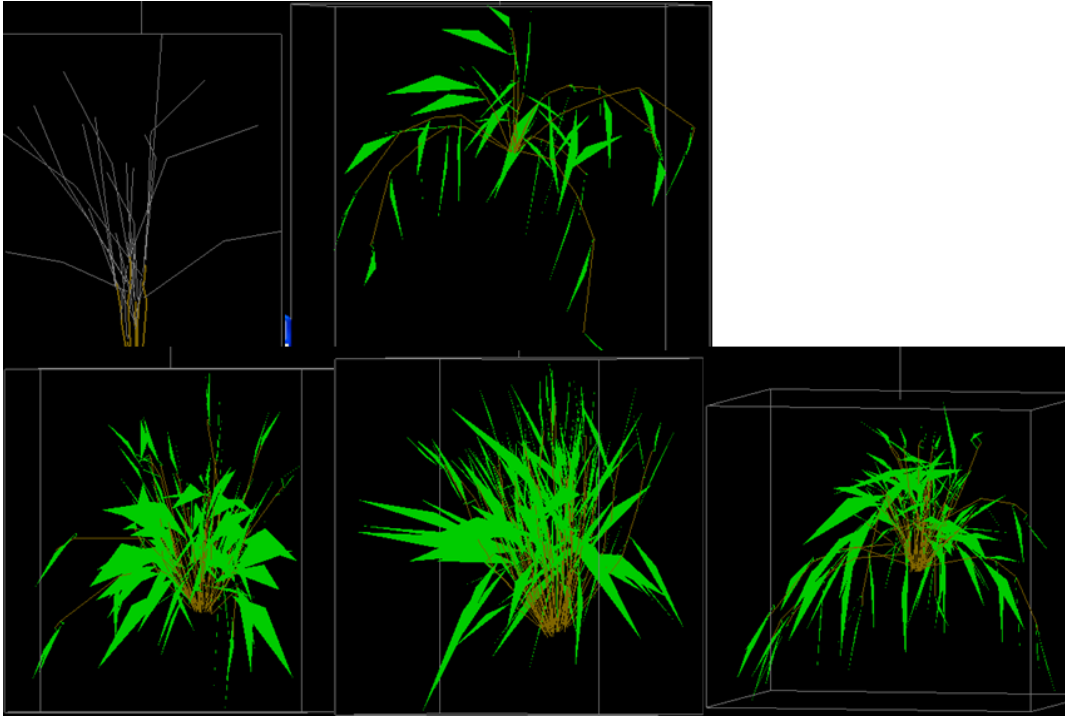


Figure 4.10 Floradig images of *O. sativa* 63 DAS, *O. australiensis* 119 DAS, *O. meridionalis* Cape York 119 DAS, *O. meridionalis* Keep River 119 DAS and *O. meridionalis* Howard Springs 119 DAS. Leaves and tillers are shown as lines only for *O. sativa*, leaves are shown as triangles in the other images but in Yplant all leaf images are scaled versions of a standard leaf, with digital coordinates given in Appendix 4.3.

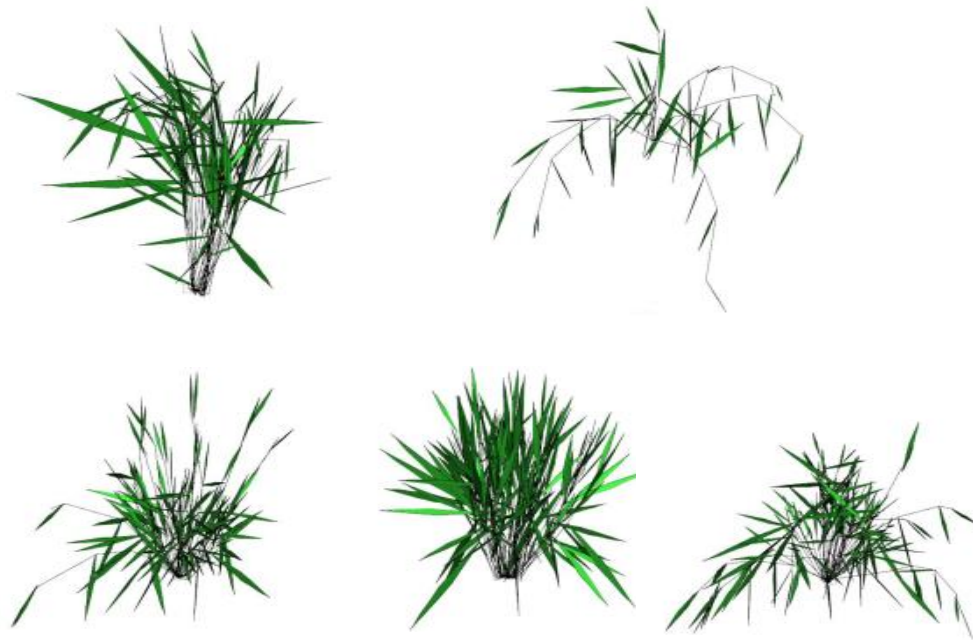


Figure 4.11 Yplant QMC (Pearcy et al. 2010) images of *Oryza*. Top row (l-r): *O. sativa* 86 DAS, *O. australiensis* 89 DAS. Bottom row (l-r): *O. meridionalis* CY 89 DAS, *O. DAS*, *O. meridionalis* HS 93 DAS, converted from the Floradig format for the five *Oryza* species/accessions shown in Figure 4.10. See also Appendix 4.4 for images of the same plants *meridionalis* KR 86 prepared with Floradig and Yplant.

4.3.7 Quantifying light interception by digitised canopies

(a) Projected areas

The area of leaves perpendicular to an incoming ray differed with sun elevation angles; the area is maximal for horizontal rays and minimal for vertical rays. Figure 4.12 shows displayed leaf area (*DAP*) - the leaf area not shaded by other leaves of the same plant, projected leaf area (*PAP*) - the projected area of leaves, and the ratio *DAP:PAP*, which is one measure of light interception efficiency. Leaves were generally closer to vertical than horizontal and therefore projections were maximal when viewed from a horizontal perspective. *Oryza meridionalis* had the largest projected areas (Figure 4.12) but *O. australiensis* had the highest ratio of *DAP* to *PAP*, even at high sun elevation angles, unlike the *DAP/TLA* ratio where *O. meridionalis* had the highest

DAP/*TLA* ratios at high sun elevation angles (see below). The distribution of *DAP* and *PAP* were both highly negatively skewed even when vegetative stage plants were excluded (Appendix Figure 4.5) The distribution of *DAP*:*TLA* ratios and *STAR* measurements is shown in Appendix Figure 4.6.

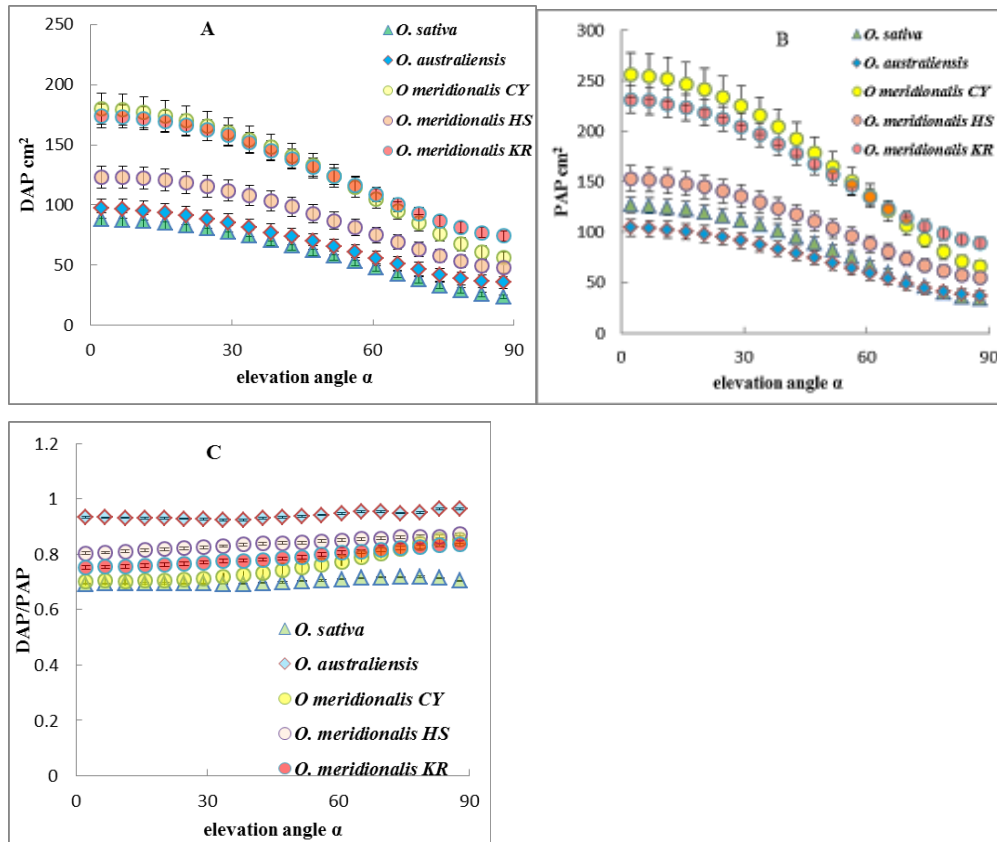


Figure 4.12 (A) Unshaded leaf area perpendicular to an incoming light ray at elevation α (*DAP*); **(B)** Leaf area perpendicular to an incoming light ray including shaded and unshaded leaves (*PAP*) and the elevation angle of the incoming ray, α . **(C)** Ratio *DAP*:*PAP* versus α . *DAP* = *PAP* – shaded area.

(b) Light interception efficiency- effect of solar elevation

The strongest influence on *LIE* was the angle of incoming light above the horizon, α . For both *DAP*:*TLA* and *STAR* (the alternative integrated measure of light interception), the association of

LIE and α was significant (Appendix 4.7 and 4.8). LIE was maximal for small α , that is when light was horizontal or close to horizontal, and minimal when α was high, vertical or close to vertical. This association was found for all species tested at all developmental stages. $\overline{STAR}$ is averaged over the whole hemisphere but for comparison with $DAP:TLA$, $\overline{STAR}$ (segmented)- $\overline{STAR}$ (is averaged over the whole hemisphere but for this comparison, STAR was averaged over six 15° segments) $\alpha = 0^\circ$ to $\alpha = 90^\circ$. Figures 4.13A&B show the relationship between α and LIE, as assessed by $DAP:TLA$ and $\overline{STAR}$.

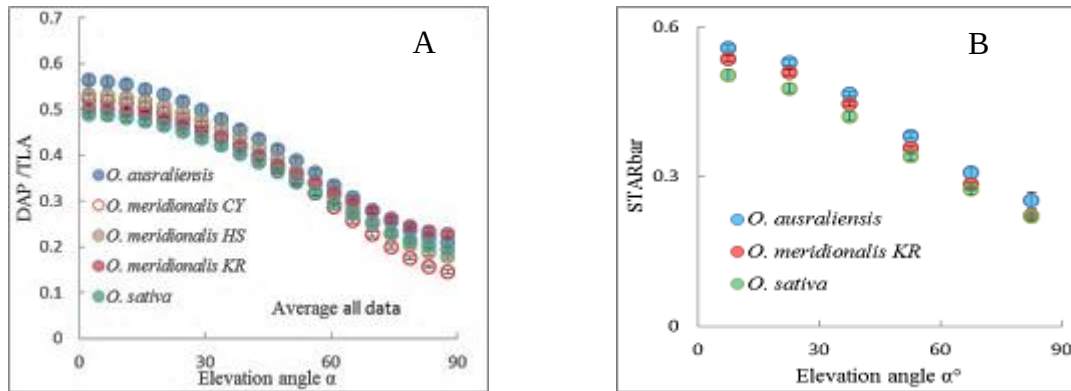


Figure 4.13 (A) $DAP:TLA$ ($\pm$ SEM) and **(B)** $\overline{STAR}$ ($\pm$ SEM) versus α . The effect of α on $DAP:TLA$ and $\overline{STAR}$ (segmented) was similar for all *Oryza* accessions tested.

(c) Light Interception Efficiency- effect of *Oryza* accession

LIE assessed by $\overline{STAR}$ shows no significant difference between accessions of vegetative stage *Oryza* as shown in Figure 4.14A, although slight differences are seen in older, reproductive stage, plants; LIE is highest in *O. australiensis* and least in *O. sativa* as shown in Figure 4.14B. These differences do not reach statistical significance (Appendix 4.9).

LIE assessed by the ratio of *DAP:TLA* varied between rice accessions, more obviously in reproductive stage plants than vegetative stage plants, as shown Figure 4.14C&D where differences reached significance. *Oryza australiensis* had the highest *DAP:TLA* ratio though most sun elevation angles (2.25° to 74.25°) but *O. meridionalis* had the highest *DAP:TLA* ratios at high sun elevation angles (78.75° to 87.75°) (Appendix notes 4.6 & 4.7). Overall *O. australiensis* was most efficient (Tukey A) and *O. sativa* least efficient (Tukey C) with *O. meridionalis* intermediate (Tukey B).

When averaged over all elevation angles, *DAP:TLA* for *O. australiensis* was highest, *O. meridionalis* was intermediate and *O. sativa* was lowest (Table 4.4) and all differences were significant (Appendix 4.7, Tukey test). Similarly, *STAR* results were in the same order - *O. australiensis* was highest, *O. meridionalis* was intermediate and *O. sativa* lowest (Table 4.4; see Appendix 4.7).

Table 4.4 Average *DAP:TLA* ratios and *STAR* values.

		<i>O. sativa</i>	<i>O. australiensis</i>	<i>O. meridionalis</i>
<i>DAP:TLA</i>	Vegetative	0.33 (0.003)	0.40 (0.004)	0.35 (0.002)
<i>DAP:TLA</i>	Reproductive	0.41 (0.004)	0.42 (0.005)	0.41 (0.003)
<i>STAR</i>	Vegetative	0.39 (0.01)	0.46 (0.01)	0.42 (0.01)
<i>STAR</i>	Reproductive	0.46 (0.01)	0.48 (0.001)	0.47 (0.005)

SEM in brackets.

Oryza australiensis and *O. meridionalis* grow at latitudes 10° to 16°S where average maximum sun elevation angles during the growing season are 85° to 91° and *DAP:TLA* ratios at the average sun elevation are 0.18 (reproductive phase) to 0.19 (vegetative phase). By contrast, *O. sativa* in Australia is cultivated at about 34°S, with average maximum sun elevation 68.5°; at that latitude, the *DAP:TLA* ratio is also approximately 0.19 (in the reproductive phase of development).

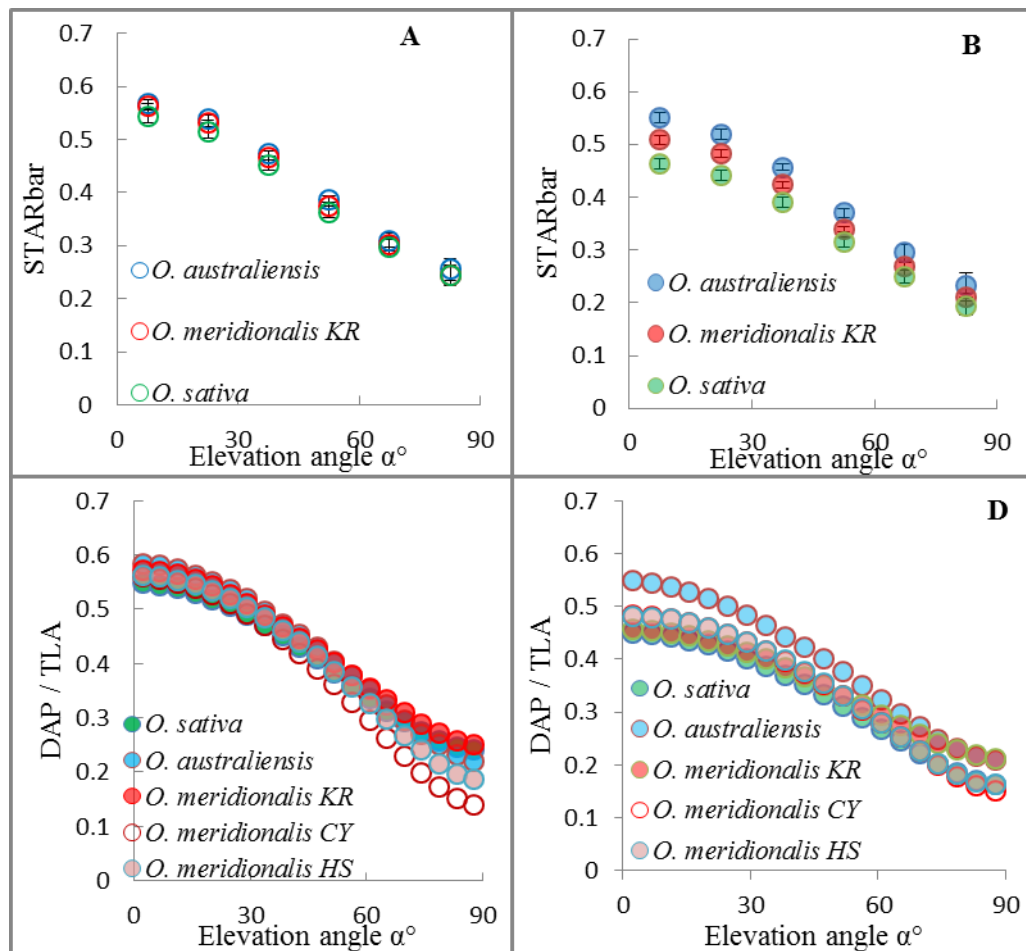


Figure 4.14 (A) $\overline{STAR}$ values in accessions indistinguishable in vegetative phase plants while $\overline{STAR}$ values in accessions diverged in the reproductive phase (B); DAP:TLA for plants in the vegetative (C) and reproductive (D) phases. Ratios were highest in *O. australiensis*, except at high α where *O. meridionalis* KR was greater.

(d) Light interception efficiency- effect of developmental stage

Younger plants had higher *LIE* than older plants, when assessed by DAP:TLA and $\overline{STAR}$. The same effect was noted in all rice accessions combined (Figures 4.15 and Figure 4.16). Vegetative plants had significantly higher $\overline{STAR}$ than reproductive stage plants ($DF_{1,105}$, $F = 44$, $p < 0.0005$).

Mean $DAP:TLA$ was higher in vegetative stage plants than in reproductive stage plants ($DF_{1,1750}$, $F = 508$, $p < 0.0005$).

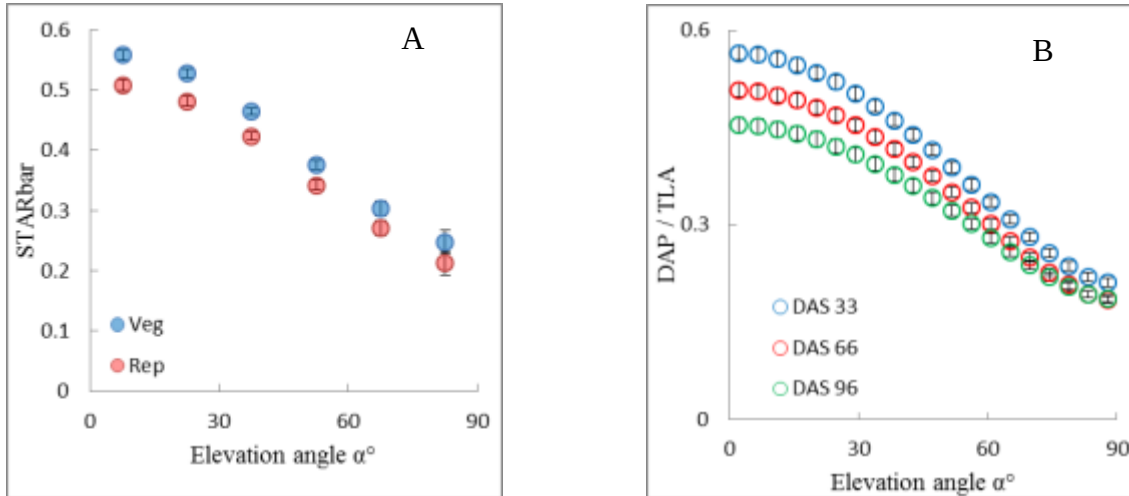


Figure 4.15 (A) $\overline{STARbar}$ and **(B)** $DAP:TLA$ plotted against elevation angle at three developmental stages (33, 66 and 96 DAS). Plants were vegetative until 45 DAS.

(e) Light Interception efficiency- effect of other factors

Growing plants, as expected, showed correlations between leaf number and leaf area with DAS but negative correlations with measures of light interception efficiency (Table 4.4). $DAP:TLA$ and $\overline{STARbar}$ both correlated negatively with DAS, leaf number, leaf area and positively with each other and dispersion. A plot for $\ln(\text{leaf number})$ vs DAS is shown in the Appendix 4.3 showing similar rates of increase in leaf numbers in the species/accessions with time, although leaf numbers in *O. meridionalis* were higher than those in *O. sativa* and *O. australiensis* which had fewer leaves up to 120 DAS. The figure shows similar slopes for the rice accessions of *O. meridionalis*.

Table 4.4 Correlation matrix for *DAS*, number of leaves, total leaf area, mean individual leaf area, leaf dispersion and *DAP:TLA*.

	<i>DAS</i>	Leaf No	Leaf Area	Mean Leaf size	<i>STAR</i>	Dispersion
Leaf No	0.6 ***					
Leaf Area	0.3 ***	0.5 ***				
Mean Leaf size	0.3 **	0.12 ns	0.7 ***			
<i>STAR</i>	-0.3 **	-0.4 ***	-0.3 **	-0.1 ns		
Dispersion	-0.3 **	-0.18 ns	0.7 ***	-0.2 *	0.5 ***	
<i>DAP:TLA</i>	-0.3 **	-0.3 **	-0.3 ***	-0.1 NS	0.8 ***	0.4 ***

p *** < 0.001; ** < 0.01; * < 0.05

(f) Light interception efficiency - comparison of methods

It was expected that average *DAP:TLA* and *STAR* should give similar results as both are averaged from the same measurements with the observations being weighted in *STAR* and unweighted in *DAP:TLA*. Figure 4.16 shows that that results were qualitatively similar. The *STAR* estimates were *O. australiensis* (0.47), *O. meridionalis* (0.45) and *O. sativa* (0.43) (each species significantly different) and vegetative stage (0.47) and reproductive stages (0.42) (stages of development are significantly different). Further data can be seen in Appendix 4.9. Average *DAP:TLA* showed that ratios for *O. meridionalis* were not significantly different from the other two species but *O. australiensis* did have a ratio of 0.40 which exceeded that of *O. sativa* 0.37. As was found with *STAR*, *DLA:TLA*, was higher for vegetative (0.41) than reproductive plants (0.36). (Table 4.5) (Appendix 4.19 show the two methods results for *DAP:TLA* and *STAR*. *STAR* and *DAP:TLA* were correlated (Figure 4.17).

Table 4.5 $\overline{STAR}$ and Average DAP/TLA

	$\overline{STAR}$	
	Reproductive	Vegetative
<i>O. sativa</i> b	0.41(0.01)	0.46 (0.01)
<i>O. australiensis</i> a	0.45 (0.01)	0.48 (0.02)
<i>O. meridionalis</i> KR ab	0.41 (0.01)	0.48(0.01)
<i>O. meridionalis</i> CY ab	0.42 (0.01)	0.46(0.01)
<i>O. meridionalis</i> HS ab	0.44 (0.01)	0.47(0.01)
Tukey	B	A
	Average DAP/TLA	
	Reproductive	Veg
<i>O. sativa</i> c	0.33(0.003)	0.41(0.004)
<i>O. australiensis</i> a	0.40(0.003)	0.42(0.005)
<i>O. meridionalis</i> KR b	0.35(0.002)	0.43(0.004)
<i>O. meridionalis</i> CY c	0.35(0.004)	0.38(0.004)
<i>O. meridionalis</i> HS c	0.34(0.004)	0.40(0.004)
Tukey	B	A

Mean (SEM) Variables not sharing a letter are significantly different (Tukey test).

$\overline{STAR}$ and $DAP:TLA$ were correlated. Regressing $\overline{STAR}$ on $DAP:TLA$ gave the equation

$\overline{STAR} = 0.205 + 0.638 DAP:TLA$; $R^2 = 0.68$ (correlation 0.82). Results are shown in Figure 4.17.

Mean (SEM) Variables not sharing a letter are significantly different (Tukey test).

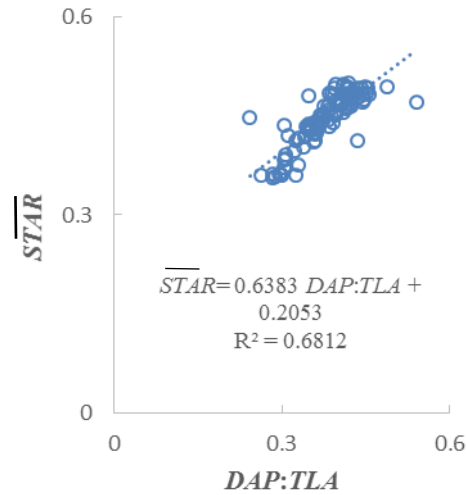


Figure 4.16 $\overline{STAR}$ v $DAP:TLA$ for 104 plants for which both measures were available.

4.3.8 [CO₂] effect

The effect of CO₂ was inconsistent. When the effect of ambient and high [CO₂] levels on *LIE* were tested, an effect was noted with two-way ANOVAR between [CO₂] and elevation angle but not when comparison was made without including elevation. Inspection of Figure 4.17 shows a ‘crossover’ which was reflected in significant interaction between elevation and [CO₂] for $DAP:TLA$. Although the difference attributable to [CO₂] reached statistical significance it was extremely small. (Appendix 4.10.

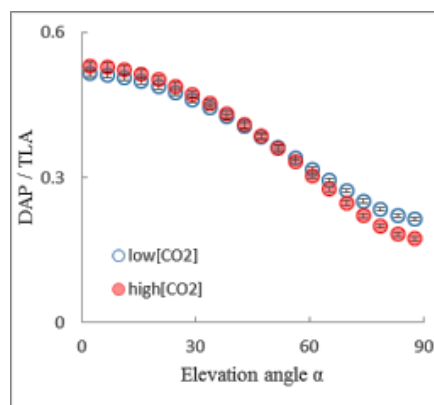


Figure 4.17 $DAP:TLA$ ratio of plants grown in 390 ppm [CO₂] and 700 ppm [CO₂].

4.3.9 Azimuth and elevation angle

There was no association between *DAP:TLA* and azimuth ($DF_{7,3192}$, $F = 0.74$, $p = 0.638$). Pots were moved at random so it would not be expected that an azimuth effect could be detected. This may not apply to plants in the wild. Elevation angle had significant effect ($DF_{19,3040}$, $F = 239.27$, $p < 0.001$) but not azimuth angles ($DF_{7,3040}$, $F = 1.76$, $p = 0.091$) and the interaction term was not significant ($DF_{133, 3040}$, $F = 0.08$, $p = 1.0$).

4.3.10 Leaf Dispersion

There was no significant difference between the species in a measure of leaf dispersion ($DF_{2,99}$, $F = 0.89$, $p = 0.415$), although *O. australiensis*, *O. meridionalis* and *O. sativa* varied a little -1.2, 1.0 and 0.9 respectively, where 1 = random, > 1 is regular, and < 1 is clumped. Vegetative stage (dispersion 1.3) was significantly more regular and less clumped than reproductive-stage plants (dispersion 0.8) ($DF_{1,98}$, $F = 27.5$, $p < 0.0005$). Leaf dispersion was negatively related to *DAS*. Species, total leaf area, developmental stage, elevation angle α and [CO₂] were all significant in determining *DAP:TLA* (MANOVA) (Appendix 4.11 and 4.12).

4.3.11 CO₂ assimilation modeled by Yplant QMC

Light response curves were shown in Figure 3.6. From measurements of CO₂ assimilation at eight photosynthetically active radiation (*PAR*) levels a maximum rate of photosynthesis ($A_{\max}$) was calculated using a non-rectangular hyperbola. Table 4.5 shows the rate of photosynthesis measured and the $A_{\max}$ computed from the light response curve, the $A_{\max}$ being substantially higher than the highest level of CO₂ assimilation measured.

Figure 4.18A shows computed photosynthesis in whole plants using the digitised plant model in Yplant in the vegetative and reproductive phases of growth. This model makes allowance for plant self shading. When allowing for shading, model plants have much lower rates of CO₂ assimilation than isolated leaves measured in the LiCor 6400 (shown previously in Chapter 3, Figures 3.4, 3.5 and 3.6). Plants in the reproductive phase have lower levels of CO₂ estimated by Yplant than plants in the vegetative phase; the two distributions overlap by only one case. Rates

in whole plants are lower because the shadows on the leaves mean the photosynthesis level on a plant is only 20% to 60% of the level of photosynthesis the plant could produce as *DAP:TLA* ranges from 0.2 to 0.6.

Table 4.5 Highest measured and calculated $A_{\max}$ ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in an individual leaf.

Variable	Calculated $A_{\max}$		Measured CO ₂ uptake	
	Vegetative	Reproductive	Vegetative	Reproductive
Average all accessions	46.5 (1)	25.0 (2)	36.2 (1)	19.6 (1.4)
<i>O. sativa</i>	45.9 (2)	19.0 (3)	35.9 (1.3)	15.5 (2.0)
<i>O. australiensis</i>	44.3 (1)	34.4 (2)	34.6 (1.1)	25.9 (1.5)
<i>O. meridionalis</i> KR	49.2 (1)	21.2(1)	37.9 (0.7)	17.3 (0.7)

Mean ($\pm$ SEM).

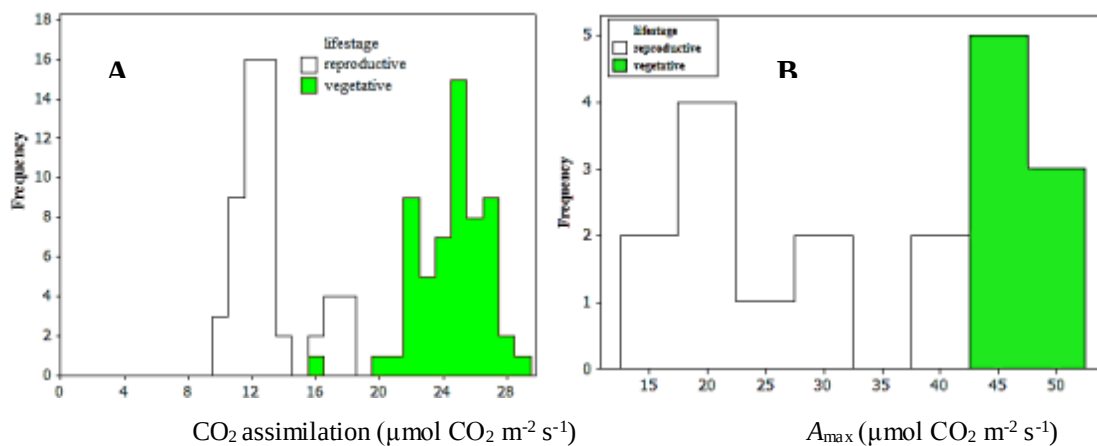


Figure 4.18 (A) CO₂ assimilation rate ($\mu\text{mol m}^{-2} \text{ s}^{-1}$) for whole plants modeled in Yplant QMC, reproductive (unfilled) and vegetative (green-filled) growth phases **(B)** Calculated maximum photosynthesis, $A_{\max}$, ($\mu\text{mol m}^{-2} \text{ s}^{-1}$) in plants at vegetative and reproductive stages of development.

4.4 Discussion

- Photographic and digitised images confirmed the different canopy shapes of the three species; *O. australiensis* very tall with sparse, with very curved leaves and an open canopy with near vertical tillers; *O. meridionalis* was shorter, with the biggest percentage of the leaf area low in the canopy, very widely angled tillers and leaves and very dense canopy. *Oryza sativa* is typical of modern cultivated rice with near vertical tillers and leaves and relatively straight leaves. Digitised data used to produce images of *O. sativa* by Watanabe et al. (2005) and Zheng et al. (2008) resulted in images like those found in this study. No published digitised images of *O. australiensis* and *O. meridionalis* were found.
- Within the *Oryza* canopy, leaf area was mainly in the lowest third of the vertical height of the canopy but there were differences between the accessions, with *O. meridionalis* having a smaller percentage of its leaf area in the top third of the canopy. *Oryza australiensis* had a slightly greater percentage of leaf area in the top third of the canopy than *O. sativa*. The *O. meridionalis* canopy was very dense, with the highest *LAI*, while the *O. australiensis* canopy was most open with the lowest *LAI* and highest leaf dispersion, while *O. sativa* had intermediate *LAI*. *LAI* values of 5 to 9 were reported for accessions of *O. sativa* by Yoshida (1981), about 5 by Sudhir-Yadav et al. (2011), 5 to 8, with maximum at about 75 *DAS* by Ahmed et al. (2009). *LAI* of 6 to 8 for *O. sativa* and 8 - 12 for *O. meridionalis* and 3 - 6 for *O. australiensis*. No previous reports of *LAI* were found for *O. australiensis* and *O. meridionalis*. Sinclair and Sheehey (1999) postulate a *LAI* > 7 is needed for maximum grain production.
- *LIE* was maximal at elevation angles near horizontal, as estimated by *DAP:TLA* ratio and by STAR. Graphing *DAP:TLA* ratio against α (Figures 4.17 - 4.19) produced curves very similar to those produced by Zheng et al. (2008) for projection efficiency. Rice accessions differed little in *LIE*, although usually *O. australiensis* had the greatest *LIE* until the sun was notionally overhead, when *O. meridionalis* KR was most efficient as light penetrated its widely splayed leaf canopy. The cultivated *Oryza* species, *O. sativa*, never had the most efficient *LIE* as assessed by the *DAP:TLA* ratio. *Oryza sativa* growing at a latitude of 34°S has a maximum sun elevation of 55° to 81° (average 68.5°) when the *DAP:TLA* ratios are 0.26 (for $\alpha = 55^\circ$) and 0.18 (for $\alpha = 81^\circ$) respectively. The wild *Oryza* species at latitudes grew at approximately 16° S and had a

maximum sun elevation of 74° to 97.5°, average 86° , with *DAP:TLA* ratios 0.2 (at 74°) to 0.18 (at 97.5°) . That is, when the latitudes at which the three species currently grow is considered, the *DAP:TLA* ratios were very close for the domesticated and wild rice species (about 0.19) nor did *STAR* estimates distinguish between the rice accessions. Hence, there was no apparent advantage to the wild *Oryza* species. However, this is an artificial distinction in that rice could be cultivated commercially in the Australian tropics in the future and wild species might even be domesticated in southern Australia. The striking differences in canopy morphology belie the very subtle contrasts in *LIE*. However, *LIE* varied qualitatively with sun elevation among species and even accessions, making the latitude at which any species are cultivated critical to performance and productivity of future rice cropping systems.

- There were some differences between *DAP:TLA* ratios and *STAR* estimates but they were not qualitatively significant. Both approaches showed the angle of elevation of the sun had a strong effect on *LIE*. Other factors associated with plant age, namely developmental stage, number of leaves and leaf area all affected *LIE* similarly whether measured by *DAP:TLA* ratios or *STAR*. Neither approach accurately records curved leaves. In direct sunlight this does not matter as the projection of leaf area at right angles to the incident radiation is the measure of interest. If light was mostly diffuse, taking account of curved leaves would matter more so a method capable of estimating leaf area of curved leaves, as used by Song et al. (2013), would be advantageous. *STAR* and *DAP:TLA* ratio have been used in several studies previously but no reports of the two methods being used on the same plants have been reported previously to my knowledge. Both measures showed the angle of elevation of the sun had a strong effect on *LIE*. Consistent with previous studies, *LIE* declines with leaf number and leaf size, as clumping and self-shading increase. Other studies suggest a variety of canopy structures can be effective in the same light environment (Duursma et al. 2012).

- Plant age or developmental stage was important for *LIE*, with vegetative stage plants most efficient at light interception. However, it is also at this stage that cereals accumulate carbohydrates that are vital for later grain filling. Thus, an emphasis on light interception during early development should be the focus of future analyses of genetic variation in canopy function. At the reproductive stage, resources are directed towards grain-filling from stored pools as *de*

novo photosynthates play a declining role in plant performance. The higher LIE in younger plants is because they have fewer leaves and less clumping and lower crown density, a finding noted in other plants (Duursma et al. 2012).

- The effect of elevated atmospheric [CO₂] on *DAP:TLA* ratios was subtle and not always significant. In a summary of all species over all stages of development, there appeared to be better light interception by plants at high [CO₂] when the sun was at low elevations but as the sun rose overhead, interception was inferior in high [CO₂] conditions. The implication is that deeper analysis into effects of elevated CO₂ is required, not just at the level of leaf CO₂ assimilation but also with respect to canopy shape and light capture.

Chapter 5 – General Discussion

While the growth trajectories of two Australian wild rice species, *O. australiensis* and *O. meridionalis*, were similar to the growth trajectory of *O. sativa*, when assessed on a plant dry weight or leaf area basis *O. meridionalis* plants had much greater leaf area biomass than the other species. Vegetative growth was vigorous in the wild *Oryza* species, in *O. meridionalis* through vigorous tillering and in *O. australiensis*, through production of large leaves on fewer tillers. Invariably, *O. sativa* had superior grain production and harvest index. However, canopy shape did differ between the species, with *O. sativa* being erect and compact, *O. australiensis* being tall with large looping leaves, and *O. meridionalis* having various degrees of angled, spreading tillers across the three accessions studied.

All *Oryza* species had high CO₂ assimilation rates; the differences between the species sometimes reached statistical significance. Vegetative stage plants had higher photosynthesis levels than reproductive stage plants. The apparently superior performance of *O. australiensis* in the reproductive phase may be because not all parts of the plant were at the same developmental stage, some tillers still being in the vegetative stage of development. This general decrease in assimilation as plants matured is typical of cereals, which begin to mobilise carbohydrates stored during the vegetative growth into grain.

The accelerated biomass accumulation in an atmosphere of increased [CO₂] is consistent with the results of several other studies on rice (Baker et al. 1993; Ziska et al. 1996; Ainsworth and Long 2004; Korner 2006; Norby and Zak 2011; Shimono and Okada (2013)), although the magnitude of the effect found here is lower than some previous reports. However future climate change is likely to include increased temperatures, particularly night temperatures, which will militate against increased grain yield (Peng et al., 2004) and possibly increase harvest losses due to biotic and abiotic factors (Ziska and Bunce, 2007). The net effect is uncertain but climate change is unlikely to produce significant improvement in rice crop yields unless CO₂ enrichment is the dominant factor. The core question in this thesis is whether the variation in canopy shape

amongst species and accessions of wild rice have significant effects on light interception and thereby, the opportunity to take advantage of CO₂ fertilisation.

LIE is the ratio of mean light intercepted by leaves to light intercepted by a horizontal surface of equal area (Delagrange et al., 2006). When LAI is greater than one all the ground surface will be covered so LIE will be the same as the leaf area displayed to incoming light, (E_d), the ratio, DAP/TLA (displayed leaf area/total leaf area). Although *O. sativa*, *O. australiensis* and *O. meridionalis* differ in canopy architecture the effect of these differences on light interception efficiency is surprisingly small. E_d (DAP/TLA) for *O. sativa* growing in the Australian Riverina at 34°S, where average maximum sun elevation is 68°, would be 0.19, while for *O. australiensis* and *O. meridionalis*, growing at 16°S in the tropical north, where average sun elevation is 88.5°, would be 0.18 to 0.20. Possibly the plants are naturally selected for an optimum *LIE* that balances highest photosynthetic performance yet avoids photoinhibition from excessive radiation absorption.

Pearcy et al. (2004) note canopy architecture affects *LIE* but Delagrange et al., (2006), and Valladares et al. (2002) found surprisingly little difference in *LIE* in plants growing in the same light environment. The *Oryza* species studied, *O. sativa*, *O. australiensis* and *O. meridionalis*, had average values of E_d around 0.38 (average of all sun elevation values, higher than the average values at the latitudes at which the plants grow, noted above), whereas the low-light environment plants reported by Valladares et al. (2006) averaged 0.68 (with small error terms), suggesting that *Oryza* species have not evolved to intercept light with maximum efficiency, consistent with their growth in an unshaded high-light environment. Thus, modification of the canopy of *O. sativa* to be more sprawling or with larger leaves to closer resemble the wild *Oryza* species would not be likely to be advantageous for improved light capture. This has become a practical possibility with genes coding for basic canopy architecture now having been cloned (Li et al., 2006).

It may be that in the high sunlight environments in which wild *Oryza* plants grow have selected for canopies that avoid photoinhibition, thus lower E_d . Demmig-Adams et al. (2012) note that photoprotective measures are evident at *PAR* levels of about 500 - 800 $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$,

well below clear sky levels of 2000 - 2200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, which are likely to be found in Australian rice-growing areas. As only about 20% of the available leaf area is exposed to sunlight, and the area exposed changes as the sun moves across the sky changing both elevation and azimuth, in both the tropical and Riverina sites, the full intensity of maximum irradiation should be experienced for only a short period at maximum sun elevation and for longer periods at lower elevation angles where the percentage of exposed leaves rises to 60%. It would be of interest to compare the architecture of the same species growing in the field at different latitudes. *Oryza australiensis* and *O. meridionalis* have quite different canopy structure yet normally grow in close proximity, suggesting neither species enjoys a distinct physiological advantage. This is consistent with the conclusion of Valladares et al. (2002) noted above that plants growing in the same light environment have similar LIE despite having different plant architecture.

Other architectural differences were noted in the canopy. Leaf area was distributed slightly differently in the canopies; greater leaf area was present low in the canopy of *O. meridionalis* than the other species, which would be expected to produce more self-shading. Leaf area index was high in *O. meridionalis*, higher than *O. sativa* which in this case was a little lower than the LAI reported by Yoshida (1981) for *O. sativa*. However, the wild relatives generally grow in dense swards in nature and, in combination with high LAI and leaf area in the lower canopy, *O. meridionalis* might be considered highly adapted to a very photo-oxidative environment. Leaf dispersion fell in a narrow range, 0.9 to 1.2, whereas Duursma et al. (2012) found a range of 0.5 to 1.5. Comparison to one of the many shade-tolerant *Oryza* species (Atwell et al. 2014) may show a greater range of dispersion.

Carbon budgets of individual shoots could be calculated applying light-response curves for assimilation to the information of light interception that the digital imaging yielded. Because of self-shading, modelled whole-plant photosynthesis was much less than the high instantaneous leaf-level photosynthesis rates observed in plants by gas exchange. The potential maximum leaf area exposed to light was 20% to 60% of the plant total leaf area.

There are other potential benefits in exploring the physiology of wild *Oryza*. Heat and drought tolerance are two potentially useful properties that could be explored (Atwell et al., 2014). C₄

metabolism enhancing photosynthetic capacity does not seem promising as photosynthesis is already good, but water saving may be a worthwhile gain. The introduction of genes enhancing these desirable characteristics may not be straightforward; apart from the technical problems, there is significant opposition to genetically modified plant crops (Anderson, 2010; Eggert and Greaker, 2011).

Average world rice yields are well below those achieved in the best managed rice production areas. Attention to the economic and political factors that impede wider adoption of known effective production techniques may produce a greater improvement in average rice yields than would improving the physiological efficiency of rice plants with new strains of rice. Gains in yield from improving all rice production to best practice could be 50% or more whereas gains from physiological manipulation of the rice plant are likely to be much less. Attempts to improve yield by biological research are more likely to advance scientific knowledge than to make a major contribution to feeding the people of the world in the short term, although long term gains are possible if political and economic constraints can be overcome.

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Appendices

Chapter 1 Notes

No notes

Chapter 2 Notes

2.1 Leaf area in plants grown in field simulation

General Linear Model: Leaf Area/Plant versus Rice Accessions 3, number of plants/pot

Factor Type Levels Values

ACC3 fixed 3 *O. australiensis*, *O. sativa*, *O. meridionalis* KR

Australian Wild Rice: Growth, Canopy Structure and Atmospheric CO₂ Effects

numpl fixed 2 8, 16

Analysis of Variance for LA/P, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
ACC3	2	3459211	3459211	1729605	18.74	0.000 (4.9347E-05)
ACC3*numpl	2	323965	323965	161982	1.76	0.214
numpl	1	887336	887336	887336	9.61	0.009
Error	12	1107452	1107452	92288		
Total	17	5777964				

S = 303.789 R-Sq = 80.83% R-Sq(adj) = 72.85%

Grouping Information Using Tukey Method and 95.0% Confidence

ACC3	N	Mean	Grouping
O. meridionalis KR	6	1937.2	A
<i>O. sativa</i>	6	1329.7	B
<i>O. australiensis</i>	6	866.6	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

numpl	N	Mean	Grouping
8	9	1599.9	A
16	9	1155.8	B

Means that do not share a letter are significantly different.

2.2 Plant height, rice accession and CO₂ effect.

General Linear Model: Ht(cm) versus Variety, CO₂ level

Factor Type Levels Values

Var fixed 5 A, CY, HS, KR, S

CO₂ level fixed 2 1, 2

Analysis of Variance for Ht(cm), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Var	4	2607.3	2697.8	674.5	4.21	0.003

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Var*CO ₂ level	4	1263.0	983.7	245.9	1.54	0.192
CO ₂ level	1	3641.8	3641.8	3641.8	22.76	0.000 (1.52061E-06)
Error	238	38085.9	38085.9	160.0		
Total	247	45598.1				

S = 12.6501 R-Sq = 16.47% R-Sq(adj) = 13.32%

Grouping Information Using Tukey Method and 95.0% Confidence

CO₂level N Mean Grouping

1 126 34.4 A

2 122 26.7 B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Var N Mean Grouping

A 49 35.2 A

S 50 33.9 A B

CY 41 28.4 A B

KR 50 27.8 B

HS 58 27.5 B

Means that do not share a letter are significantly different.

Table 2.3 Harvest Index

Species	Grain/shoot weight 2010 (SEM)	Grain/shoot weight 2012 (SEM)
<i>O. australiensis</i>	0.12 (0.07) C	.07 (0.01) C
<i>O. sativa</i>	0.34 (0.05) A	0.49 (0.02) A
<i>O. meridionalis</i> <i>KR</i>	0.19 (0.02) B	0.13 (0.02) B

Means that do not share a letter are significantly different.

2.4 Leaf and tiller zenith angles

One-way ANOVA: Leaf zenith angle versus *Oryza* Variety

Source	DF	SS	MS	F	P
Variety	4	60942	15236	15.36	0.000 (6.95255E 12)
Error	862	855083	992		
Total	866	916025			

S = 31.50 R-Sq = 6.65% R-Sq(adj) = 6.22%

Grouping Information Using Tukey Method

Variety	N	Mean	Grouping
<i>O. meridionalis</i> CY	163	53.60	A
<i>O. meridionalis</i> KR	200	47.24	A
<i>O. australiensis</i>	89	43.02	A B
<i>O. meridionalis</i> HS	226	38.09	B
<i>O. sativa</i>	189	29.39	C

Means that do not share a letter are significantly different.

One-way ANOVA: Tiller zenith angle versus *Oryza* Accession

Source	DF	SS	MS	F	P
Acc	4	37416	9354	19.59	0.000 (2.30539E-14)
Error	351	167628	478		
Total	355	205043			

S = 21.85 R-Sq = 18.25% R-Sq(adj) = 17.32%

Grouping Information Using Tukey Method

Acc	N	Mean	Grouping
<i>O. meridionalis</i> CY	63	36.90	A
<i>O. meridionalis</i> KR	78	35.78	A
<i>O. meridionalis</i> HS	100	29.95	A
<i>O. australiensis</i>	35	15.37	B
<i>O. sativa</i>	80	11.34	B

Means that do not share a letter are significantly different.

2.5 Specific leaf area

General Linear Model: SLA versus Rice, CO₂ at 92 DAS

Factor Type Levels Values

Rice fixed 5 1, 2, 3, 4, 5

CO₂ fixed 2 1, 2

Analysis of Variance for sla, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Rice	4	7845	5454	1364	0.49	0.740
Rice*CO ₂	4	9466	7481	1870	0.68	0.611
CO ₂	1	27557	27557	27557	9.99	0.003
Error	46	126884	126884	2758		
Total	55	171751				

S = 52.5199 R-Sq = 26.12% R-Sq(adj) = 11.67%

Grouping Information Using Tukey Method and 95.0% Confidence

Rice	N	Mean	Grouping
<i>O. australiensis</i>	13	246.1	A
<i>O. meridionalis</i> CY	11	243.7	A
<i>O. meridionalis</i> KR	8	232.9	A
<i>O. meridionalis</i> HS	12	231.6	A
<i>O. sativa</i>	12	219.3	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

CO ₂	N	Mean	Grouping
1 low	31	257.9	A
2 high	25	211.6	B

Australian Wild Rice: Growth, Canopy Structure and Atmospheric CO₂ Effects

General Linear Model: SLA 2 versus Species, [CO₂] at 41 DAS

Factor Type Levels Values

Species fixed 3 O. a, O. m, O. s

[CO₂] fixed 2 A, C

Analysis of Variance for SLA 2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Species	2	1229	1258	629	0.58	0.565
Species*[CO ₂]	2	5394	3196	1598	1.47	0.242
[CO ₂]	1	675	675	675	0.62	0.435
Error	42	45672	45672	1087		
Total	47	52971				

S = 32.9763 R-Sq = 13.78% R-Sq(adj) = 3.51%

Grouping Information Using Tukey Method and 95.0% Confidence

Species	N	Mean	Grouping
<i>O. australiensis</i>	9	147.6	A
<i>O. sativa</i>	9	145.4	A
<i>O. meridionalis</i> KR	30	136.0	A

[CO₂] N Mean Grouping

low 23 147.4 A

high 25 138.6 A

Means that do not share a letter are significantly different.

General Linear Model: sla versus Rice, CO₂

Factor Type Levels Values

Rice fixed 5 1, 2, 3, 4, 5

CO₂ fixed 2 1, 2

Analysis of Variance for sla, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Rice	4	7845	5454	1364	0.49	0.740
Rice*CO ₂	4	9466	7481	1870	0.68	0.611

Australian Wild Rice: Growth, Canopy Structure and Atmospheric CO₂ Effects

CO ₂	1	27557	27557	27557	9.99	0.003
Error	46	126884	126884	2758		
Total	55	171751				

S = 52.5199 R-Sq = 26.12% R-Sq(adj) = 11.67%

Grouping Information Using Tukey Method and 95.0% Confidence

Rice	N	Mean	Grouping
<i>O. australiensis</i>	13	246.1	A
<i>O. meridionalis</i> CY	11	243.7	A
<i>O. meridionalis</i> KR	8	232.9	A
<i>O. meridionalis</i> HS	12	231.6	A
<i>O. sativa</i>	12	219.3	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

CO ₂	N	Mean	Grouping
1 [CO ₂]390 ppm	31	257.9	A
2 [CO ₂] 700 ppm	25	211.6	B

Means that do not share a letter are significantly different.

2.6

Variable	Comparison	DAS	DF	F	P	O. s	O. a	KR	CY	HS	[CO ₂]C	[CO ₂]A
Leaf Area	Accession	18-41	2, 168	2.2	0.114	39 A	34 A	33 A			-	-
	CO ₂	18-41	1, 168	10.48	0.001	-	-	-			40 A	23 B
	Accession CO ₂	18-41	2, 168	0.22	0.803	-	-	-			-	-
	Accession	90	4, 42	1.97	0.116	1400a	1635a	1369a	1490a	1054a		
	CO ₂	90	1, 42	7.49	.009						1597 A	1182 B
	Accession CO ₂	90	4, 42	0.6	.668							
Tillers	Accession	18-41	2, 168	13.58	<0.0005	3.3A	1.4A	3.1A			-	-
	CO ₂	18-41	1, 168	7.31	0.008						3.0 A	2.2 B
	Accession CO ₂	18-41	2, 168	0.77	0.463	-	-	-			-	-
	Accession	90	2, 61	18.28	<0.0005	6.8 B	2.7 C	9.6A			-	-
	Accession CO ₂	90	2, 61	0.38	0.683	-	-	-			-	-
	CO ₂	90	1, 61	1.96	0.166	-	-	-				
Shoot	Accession	18-41	2, 168	3.46	0.034	0.54 A	0.26B	0.43 AB				
weight	Accession CO ₂	18-41	2, 168	0.13	0.882	-	-	-			-	-
	CO ₂	18-41	1, 168	12.45	0.001	-	-	-			0.55A	0.27B
Shoot	Accession	90	4, 55	4.79	0.002	5.8ab	3.8b	10.1a	7.9ab	7.9a		
Weight	Accession CO ₂	90	4, 55	0.36	0.8							
	CO ₂	90	1, 55	0	0.0						7.1a	7.1a
Root	Accession	18-41	2, 161	7.29	<0.0005	0.31A	0.13 B	0.21 AB			-	-
weight	Accession CO ₂	18-41	2, 161	0.24	0.916	-	-	-			-	-
	CO ₂	18-41	1, 161	12.84	<0.0005	-	-	-			.29A	.13B
	Accession	90	4, 51	0.15	0.96	3.3a	3.2a	3.7a	4.1a	3.1a		
	Accession*CO ₂	90	4, 51	0.9	0.47							
	CO ₂	90	1, 51	0.22	0.64						3.3a	3.7a
Height	Species	18-41	2, 168	10.06	<0.0005	36.82A	39.15A	29.3 B				
	Species CO ₂	18-41	2, 168	1.1	0.34	-	-	-			-	-
	CO ₂	18-41	1, 168	13.2	<0.0005	-	-	-			29.1 A	31.0 B
	CO ₂	90	33	T=0.76	0.45	-	-	-			714	759

Shaded rows show results for plants in the vegetative growth phase, unshaded rows are for reproductive growth stage plants. Variables not sharing a letter are significantly different

(Tukey); comparisons are across rows. O. s- *O. sativa*, O. a- *O. australiensis*, Y- *O. meridionalis* Cape York, HS-*O. meridionalis* Howard Springs, KR *O. meridionalis* Keep River.

Chapter 3 Notes

3.1 Data transformation

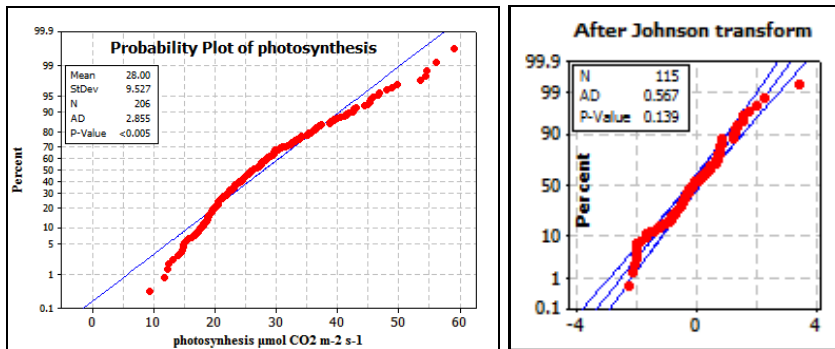


Figure 3.2 Probability plots of raw photosynthesis data and Johnson transformation of the same data showing the Johnson transformation is not significantly different from a normal distribution (Minitab).

3.2 General Linear Model: Photo versus var, A or C

Factor Type Levels Values

var fixed 5 1(*O. australiensis*), 2(*O. sativa*), 3(*O. meridionalis* CY), 4(*O. meridionalis* HS), 5(*O. meridionalis* KR)

A or C fixed 2 A (CO₂ 390ppm), C(CO₂ 700ppm)

Analysis of Variance for Photo, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
var	4	304.89	296.96	74.24	2.01	0.097
var*A or C	4	56.19	38.68	9.67	0.26	0.902
A or C	1	935.98	935.98	935.98	25.33	0.000
Error	129	4766.71	4766.71	36.95		
Total	138	6063.77				

S = 6.07876 R-Sq = 21.39% R-Sq(adj) = 15.91%

grouping Information Using Tukey Method and 95.0% Confidence

var N Mean Grouping

1	29	30.8	A
4	27	28.3	A
3	28	28.2	A
5	25	27.6	A
2	30	26.4	A

Means that do not share a letter are significantly different.

A or C N Mean Grouping

C	70	30.9	A
A	69	25.7	B

3.3 Experiment 1 *Oryza* accessions grown in 390 and 700 ppm CO₂.

Oryza accession and CO₂	Mmol CO₂ m⁻²s⁻¹
<i>O. sativa</i>	26.7 (0.9) a
<i>O. australiensis</i>	27.3 (0.9) a
<i>O. meridionalis</i> KR	26.7 (0.8) a
<i>O. meridionalis</i> CY	28.4 (1.0) a
<i>O. meridionalis</i> HS	28.5 (1.0) a
All accessions 390 ppm CO ₂	26 (0.6) B
All accessions 700 ppm CO ₂	31 (0.9) A

Variables not sharing a letter are significantly different (Tukey test). Lower case- *Orza* accessions compared, Upper case- [CO₂] in which plants were grown compared.

3.4 Developmental stage and CO₂ assimilation

General Linear Model: meanA versus lifestage_1, accssn

Factor	Type	Levels	Values
lifestage	fixed	2	reproductive, vegetative
accssn	fixed	5	a, cy, hs, kr, sat

Analysis of Variance for meanA, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P	P exact
Lifestage	1	3810.36	3215.00	3215.00	1114.69	0.000	(4.37794E-59)
lifestage*accssn	4	155.88	176.77	44.19	15.32	0.000	(5.91628E-10)
accssn	4	85.64	85.64	21.41	7.42	0.000	(3.55648E-05)
Error	105	302.84	302.84	2.88			
Total	114	4354.73					

S = 1.69829 R-Sq = 93.05% R-Sq(adj) = 92.45%

Grouping Information Using Tukey Method and 95.0% Confidence

Lifestage	N	Mean	Grouping
vegetative	59	24.64	A
reproductive	56	13.46	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

accssionn	N	Mean	Grouping
<i>O. australiensis</i>	21	20.16	A
<i>O. meridionalis</i> KR	25	19.54	A
<i>O. meridionalis</i> HS	23	19.27	A
<i>O. meridionalis</i> CY	17	18.63	A B
<i>O. sativa</i>	29	17.66	B

Means that do not share a letter are significantly different.

General Linear Model: Photosynthesis versus [CO₂]. Accession. (Experiment 2)

Factor	Type	Levels	Values
[CO ₂].	fixed	2	A (390 ppm), C (700 ppm)
Acc.	fixed	5	<i>O. australiensis</i> , <i>O. sativa</i> , CY, HS, KR

Analysis of Variance for Pn, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
[CO ₂].	1	1060.32	983.40	983.40	16.20	0.000
[CO ₂].*Acc.	4	581.27	807.27	201.82	3.32	0.012
Acc.	4	792.25	792.25	198.06	3.26	0.013
Error	188	11414.69	11414.69	60.72		
Total	197	13848.53				

S = 7.79207 R-Sq = 17.57% R-Sq(adj) = 13.63%

Grouping Information Using Tukey Method and 95.0% Confidence

[CO ₂].	N	Mean	Grouping
C	76	29.88	A

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A 122 25.21 B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Acc.	N	Mean	Grouping
<i>O. meridionalis</i> HS	42	30.01	A
<i>O. meridionalis</i> CY	39	29.49	A
<i>O. sativa</i>	34	27.47	A B
<i>O. meridionalis</i> KR	48	26.48	A B
<i>O. australiensis</i>	35	24.28	B

Means that do not share a letter are significantly different .

General Linear Model: Pn. versus ambient CO₂ in which plants were grown, Test CO₂

Factor Type Levels Values

Growth CO₂ fixed 2 A, C

TestCO₂ fixed 2 400 ppm, 700 ppm

Analysis of Variance for Pn., using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
G CO ₂	1	1214.3	150.0	150.0	4.21	0.041
G *T	1	483.4	379.5	379.5	10.64	0.001
TeastCO ₂	1	3424.7	3424.7	3424.7	95.99	0.000
Error	281	10025.0	10025.0	35.7		
Total	284	15147.3				

S = 5.97295 R-Sq = 33.82% R-Sq(adj) = 33.11%

Grouping Information Using Tukey Method and 95.0% Confidence

G CO ₂	N	Mean	Grouping
C 390 ppm	125	32.30	A
A 700 ppm	160	30.40	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

TeastCO ₂	N	Mean	Grouping
700 ppm	51	35.88	A
400 ppm	234	26.82	B

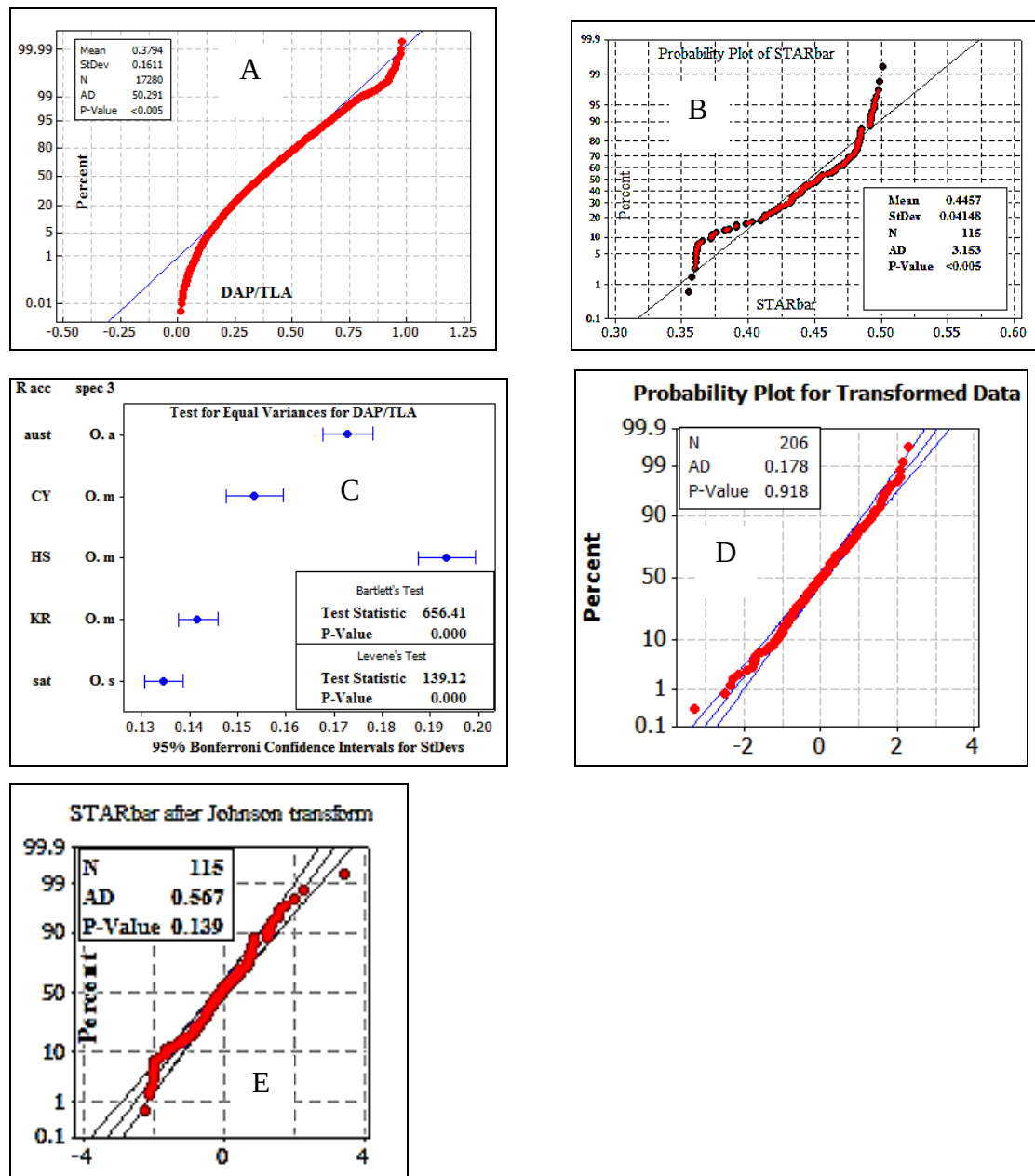
Means that do not share a letter are significantly different.

Chapter 4 Notes

4.1 Tests of normality and equal variance of *DAP:TLA* and *STAR* data.

Test	Variable	Test statistic	P	Conclusion
Anderson Darling	<i>DAP/TLA</i>	50.3	<0.005	Distribution not normal
Anderson Darling	<i>STAR</i>	3.153	<0.005	Distribution not normal
Levene	<i>DAP:TLA</i> Rice Accessions	139.12	<0.0005	Variances are significantly different
Levene	Elevation α	52.6	<0.0005	Variances are significantly different
Levene	Veg or Rep <i>STAR</i>	381.34	<0.0005	Variances are significantly different
Levene	Rice species	7.82	.001	Variances are significantly different
Levene	Veg or rep	8.5	0.004	Variances are significantly different

Australian Wild Rice: Growth, Canopy Structure and Atmospheric CO₂ Effects



4.1 **A** Non- normal distribution of *DAP:TLA* data. **B** Non- normal distribution of *STAR* data. **C** Levene test showing unequal variance of *DAP:TLA* data for *O. australiensis* (aust), *O. meridionalis* CY (CY), *O. meridionalis* HS (HS) and *O. meridionalis* KR (KR). **D** Normal distribution of *DAP:TLA* data after Johnson transformation. **E** Normal distribution of *STAR* data after Johnson transformation.

4.2 Leaf area in tub crop simulation

General Linear Model: Leaf Area versus Accession, Canopy level

Factor Type Levels Values

ACC2 fixed 3 *O. australiensis*, *O. sativa*, *O. meridionalis* KR

level fixed 3 low, mid, top

Analysis of Variance for cm sq, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P	P exact
ACC2	2	171431660	171431660	85715830	22.88	0.000	(6.91789E-08)
ACC2*level	4	81674015	81674015	20418504	5.45	0.001	
level	2	341321625	341321625	170660813	45.56	0.000	(5.05043E-12)
Error	45	168558970	168558970	3745755			
Total	53	762986270					

S = 1935.40 R-Sq = 77.91% R-Sq(adj) = 73.98%

ACC2 N Mean Grouping

O. meridionalis KR 18 7572.2 A

O. sativa 18 4838.4 B

O. australiensis 18 3258.9 C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

level N Mean Grouping

low 18 7150.3 A

mid 18 6847.2 A

top 18 1672.0 B

4.3 Leaf model for Yplant

Caleafnew

leaf 1

25

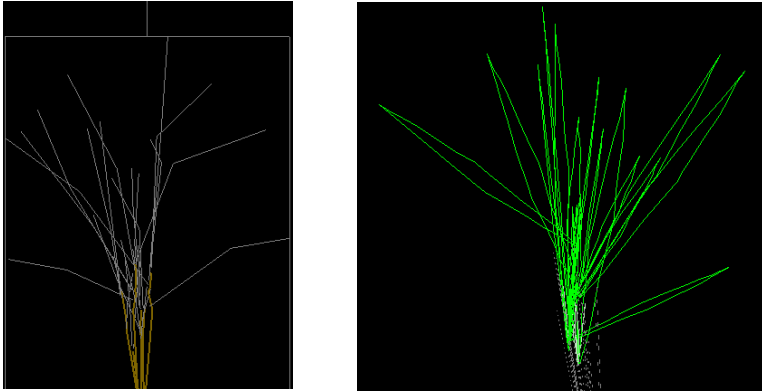
0 0

1.1 0.7

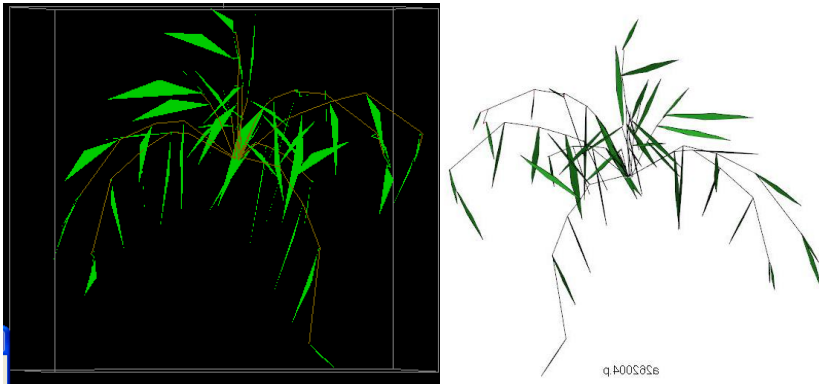
Australian Wild Rice: Growth, Canopy Structure and Atmospheric CO₂ Effects

3.95	26.8
4.95	72.2
5.3	85.5
7.95	115.6
8.5	125.9
7.45	137.2
6.4	147.9
5.15	166.6
4.5	191.9
2.8	207.9
0.35	220
0	234.8
-1.45	208.4
-2.85	193.3
-4.15	175
-6.45	145.2
-7.75	113.5
-8.6	102.6
-6.05	76
-4.7	53.8
-3.8	38
-2.05	27.2
-1.35	13
4.5	
0.1	
0.055	
0.85	
0.85	
0.1	

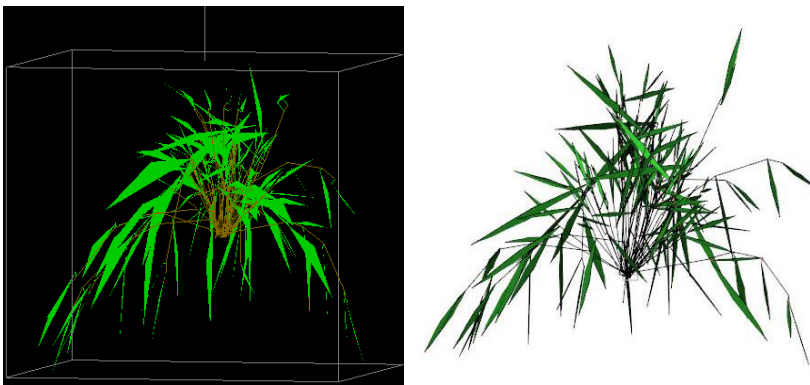
4.4 Floradig and Yplant images compared



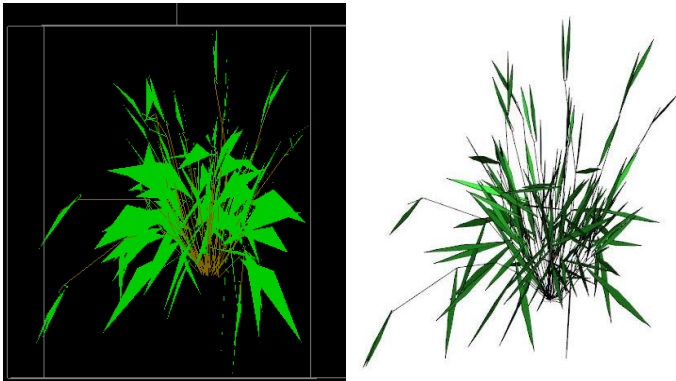
Floradig image on left, Yplant image on right for *O. sativa* as610105 using an old leaf model giving a linear leaf in Floradig.



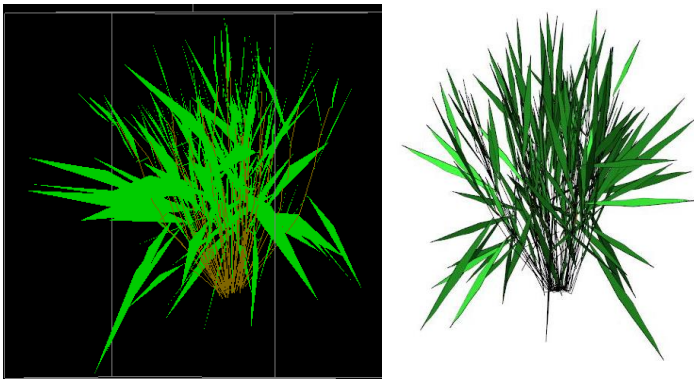
Floradig image on left, Yplant image on right for *O. australiensis* A262004 using new leaf model giving a triangular leaf in Floradig.



O. meridionalis Howard Springs, Floradig image on left, plant image on right.



O. meridionalis Cape York, Floradig image on left, Yplant on right.



O. meridionalis Keep River, Floradig image on left, Yplant on right.

4.5 One-way ANOVA: leaf angle versus variety

Source	DF	SS	MS	F	P	P exact
Variety	4	60942	15236	15.36	0.000	6.95255E-12
Error	862	855083	992			
Total	866	916025				

S = 31.50 R-Sq = 6.65% R-Sq(adj) = 6.22%

Grouping Information Using Tukey Method

Variety	N	Mean	Grouping
<i>O. meridionalis</i> CY	163	53.60	A
<i>O. meridionalis</i> KR	200	47.24	A
<i>O. australiensis</i>	89	43.02	A B
<i>O. meridionalis</i> HS	226	38.09	B
<i>O. sativa</i>	189	2 9.39	C

Means that do not share a letter are significantly different.

One-way ANOVA: Tiller Angle versus Accession

Source	DF	SS	MS	F	P	P exact
Acc	4	37416	9354	19.59	0.000	2.30539E-14
Error	351	167628	478			
Total	355	205043				

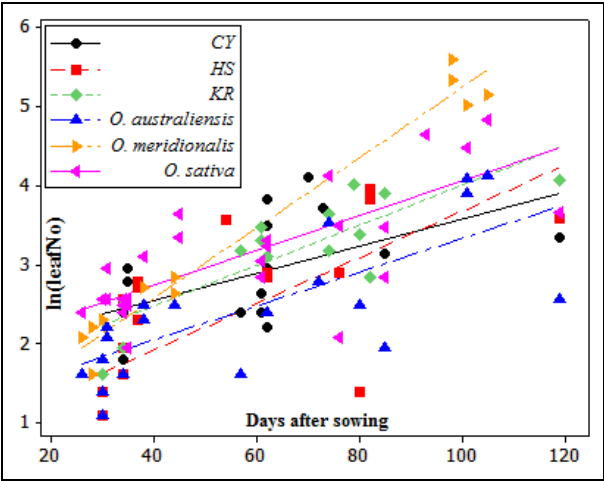
S = 21.85 R-Sq = 18.25% R-Sq(adj) = 17.32%

Grouping Information Using Tukey Method

Acc	N	Mean	Grouping
<i>O. meridionalis</i> CY	63	36.90	A
<i>O. meridionalis</i> KR	78	35.78	A
<i>O. meridionalis</i> HS	100	29.95	A
<i>O. australiensis</i>	35	15.37	B
<i>O. sativa</i>	80	1.34	B

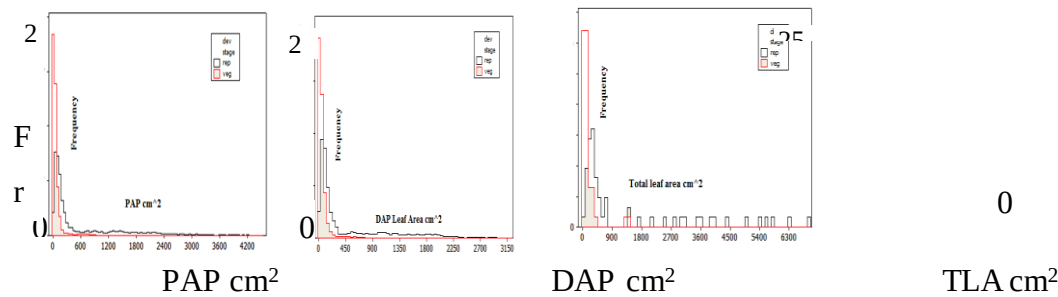
Means that do not share a letter are significantly different.

4.6 Leaf development over 120 DAS



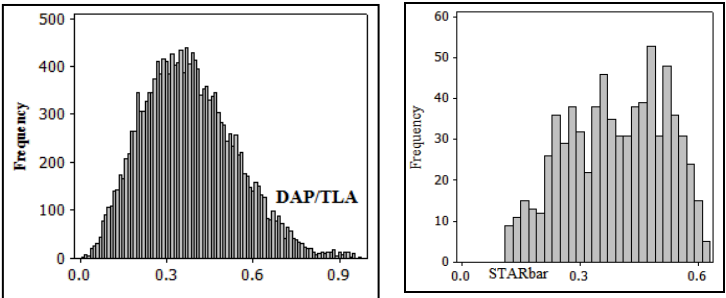
Natural log(ln) of leaf number and DAS. *O. australiensis* blue triangle, *O. meridionalis* CY = cy black disc, *O. meridionalis* HS = hs red square, *O. meridionalis* KR = kr green diamond; *O. sativa* = s blue arrowhead.

4.7 PAP, DAP and TLA distribution



Histograms of PAP, DAP and TLA for all plants 30 to 120 DAS, vegetative stage plants shaded.

4.8 Histograms of $\overline{DAP:TLA}$ and $\overline{STAR}$



Ratio $\overline{DAP:TLA}$ and $\overline{STAR}$ for the average of all the *Oryza* accessions.

Table 4.9 Tukey tests for elevation v *DAP:TLA*

α	<i>O. australiensis</i>	<i>O. meridionalis</i> <i>KR</i>	<i>O. sativa</i>	<i>O. meridionalis</i> <i>CY</i>	<i>O. meridionalis</i> <i>HS</i>
2.25	0.56 a	0.50 bc	0.49 c	0.52 abc	0.54 ab
6.75	0.56 a	0.50 bc	0.49 c	0.51 abc	0.53 ab
11.25	0.55 a	0.50 bc	0.48 c	0.51 abc	0.53 ab
15.75	0.54 a	0.49 bc	0.48 c	0.50 abc	0.52 ab
20.25	0.53 a	0.48 bc	0.46 c	0.49 abc	0.51 ab
24.75	0.52 a	0.47 bc	0.45 c	0.47 abc	0.50 ab
29.25	0.50 a	0.45 bc	0.44 c	0.46 abc	0.48 ab
33.75	0.48 a	0.44 bc	0.42 c	0.44 abc	0.46 ab
38.25	0.46 a	0.42 bc	0.40 c	0.42 abc	0.44 ab
42.75	0.44 a	0.40 b	0.39 b	0.39 b	0.42 ab
47.25	0.41 a	0.38 b	0.36 b	0.37 b	0.39 ab
51.75	0.39 a	0.36 b	0.34 b	0.34 b	0.37 ab
56.25	0.36 a	0.34 ab	0.32 b	0.32 b	0.34 ab
60.75	0.34 a	0.32 ab	0.30 bc	0.29 c	0.31 abc
65.25	0.31 a	0.30 ab	0.27 bc	0.26 c	0.28 abc
69.75	0.28 a	0.28 ab	0.25 b	0.23 b	0.25 b
74.25	0.26 a	0.26 ab	0.23 b	0.20 c	0.23 b
78.75	0.24 ab	0.25 a	0.21 bc	0.18 d	0.21 c
83.25	0.22 ab	0.23 a	0.20 bc	0.16 d	0.19 c
87.75	0.21ab	0.23 a	0.19 bc	0.15 d	0.18 c

Variables not sharing a letter are significantly different.

Tukey tests for elevation v *STAR*(segmented)

α	<i>O. australiensis</i>	<i>O. meridionalis</i>	<i>O. sativa</i>
0-15	a	a	b
15-30	a	a	a
30-45	a	a	a
45-60	a	a	a

60-75	a	a	a
75-90	a	a	a

Variables not sharing a letter are significantly different.

4.10 Elevation & species

General Linear Model: *DAP:TLA* versus species, Elevation

Factor Type Levels Values

spec 3 fixed 3 O. a, O. m, O. s

Elev fixed 20 2.25, 6.75, 11.25, 15.75, 20.25, 24.75, 29.25, 33.75,
38.25, 42.75, 47.25, 51.75, 56.25, 60.75, 65.25, 69.75,
74.25, 78.75, 83.25, 87.75

Analysis of Variance for d/t, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
spec 3	2	4.6467	4.6467	2.3233	175.80	0.000
spec 3*Elev	38	40.2265	0.6266	0.0165	1.25	0.141
Elev	19	176.1624	176.1624	9.2717	701.56	0.000
Error	17220	227.5755	227.5755	0.0132		
Total	17279	448.6110				

S = 0.114960 R-Sq = 49.27% R-Sq(adj) = 49.10%

Grouping Information Using Tukey Method and 95.0% Confidence

spec 3	N	Mean	Grouping
<i>O. australiensis</i>	3680	0.4081	A
<i>O. meridionalis</i> KR	9760	0.3763	B
<i>O. sativa</i>	3840	0.3596	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Elev N Mean Grouping

2.25	864	0.5239	A
6.75	864	0.5211	A
11.25	864	0.5153	A B
15.75	864	0.5069	A B
20.25	864	0.4960	B C
24.75	864	0.4827	C D
29.25	864	0.4668	D E
33.75	864	0.4487	E F
38.25	864	0.4289	F G
42.75	864	0.4088	G

Australian Wild Rice: Growth, Canopy Structure and Atmospheric CO₂ Effects

47.25	864	0.3869	H
51.75	864	0.3633	I
56.25	864	0.3388	J
60.75	864	0.3135	K
65.25	864	0.2885	L
69.75	864	0.2640	M
74.25	864	0.2415	N
78.75	864	0.2228	N O
83.25	864	0.2085	O P
87.75	864	0.2000	P

Means that do not share a letter are significantly different.

General Linear Model: **STAR** (segmented) versus Elevation, Species

Factor	Type	Levels	Values
Elevation	fixed	6	7.5, 22.5, 37.5, 52.5, 67.5, 82.5
Species	fixed	3	<i>O. australiensis</i> , <i>O. meridionalis</i> , <i>O. sativa</i>

Analysis of Variance for STAR, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Elevation	5	9.62899	7.34123	1.46825	534.97	0.000
Elevation*Species	10	0.01560	0.01560	0.00156	0.57	0.840
Species	2	0.14899	0.14899	0.07449	27.14	0.000
Error	756	2.07486	2.07486	0.00274		
Total	773	11.86843				

S = 0.0523881 R-Sq = 82.52% R-Sq(adj) = 82.12%

Grouping Information Using Tukey Method and 95.0% Confidence

Elevation	N	Mean	Grouping
7.5	129	0.5323	A
22.5	129	0.5045	B
37.5	129	0.4436	C
52.5	129	0.3597	D
67.5	129	0.2888	E
82.5	129	0.2318	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Species	N	Mean	Grouping
<i>O. australiensis</i>	156	0.4157	A
<i>O. meridionalis</i> KR	456	0.3923	B
<i>O. sativa</i>	162	0.3724	C

Means that do not share a letter are significantly different.

4.11 Developmental stage and Ea.

General Linear Model: *DAP:TLA* versus species, developmental stage

Factor	Type	Levels	Values
spec 3	fixed	3	<i>O. australiensis</i> , <i>O. meridionalis</i> , <i>O. sativa</i>
dev stage	fixed	2	reproductive, vegetative

Analysis of Variance for d/t, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
spec 3	2	4.6467	4.1151	2.0575	82.97	0.000
spec 3*dev stage	2	4.4983	1.2755	0.6378	25.72	0.000
dev stage	1	11.0960	11.0960	11.0960	447.45	0.000
Error	17274	428.3701	428.3701	0.0248		
Total	17279	448.6110				

S = 0.157476 R-Sq = 4.51% R-Sq(adj) = 4.48%

Grouping Information Using Tukey Method and 95.0% Confidence

spec 3	N	Mean	Grouping
O. a	3680	0.4100	A
O. m	9760	0.3758	B
O. s	3840	0.3663	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

dev stage	N	Mean	Grouping
veg	8160	0.4123	A
rep	9120	0.3558	B

Means that do not share a letter are significantly different.

General Linear Model: **STAR** versus species, lifestage

Factor	Type	Levels	Values
species	fixed	3	<i>O. australiensis</i> , <i>O. meridionalis</i> , <i>O. sativa</i>
lifestage	fixed	2	reproductive, vegetative

Analysis of Variance for STARbar, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
species	2	0.027544	0.022378	0.011189	11.98	0.000
species*lifestage	2	0.020828	0.005173	0.002586	2.77	0.067
lifestage	1	0.046016	0.046016	0.046016	49.28	0.000
Error	109	0.101777	0.101777	0.000934		
Total	114	0.196165				

S = 0.0305571 R-Sq = 48.12% R-Sq(adj) = 45.74%

Grouping Information Using Tukey Method and 95.0% Confidence

species	N	Mean	Grouping
<i>O. australiensis</i>	24	0.4681	A
<i>O. meridionalis</i>	65	0.4455	B
<i>O. sativa</i>	26	0.4254	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

lifestage	N	Mean	Grouping
vegetative	59	0.4686	A
reproductive	56	0.4241	B

Means that do not share a letter are significantly different .

4.12 Average *DAP:TLA*

General Linear Model: Average *DAP:TLA* versus species, Vegetative or Reproductive Stage

Factor	Type	Levels	Values
sp	fixed	3	<i>O. australiensis</i> , <i>O. meridionalis</i> , <i>O. sativa</i>
V or R	fixed	2	reproductive, vegetative

Analysis of Variance for Av d/t, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
sp	2	0.023193	0.016671	0.008335	4.86	0.010
sp*V or R	2	0.022597	0.003059	0.001529	0.89	0.413
V or R	1	0.059162	0.059162	0.059162	34.50	0.000
Error	109	0.186904	0.186904	0.001715		
Total	114	0.291856				

S = 0.0414091 R-Sq = 35.96% R-Sq(adj) = 33.02%

Grouping Information Using Tukey Method and 95.0% Confidence

sp	N	Mean	Grouping
<i>O. australiensis</i>	22	0.4041	A
<i>O. meridionalis</i> KR	64	0.3798	A B
<i>O. sativa</i>	29	0.3675	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

V or R	N	Mean	Grouping
veg	60	0.4091	A
rep	55	0.3585	B

4.13 Effect of [CO₂]

General Linear Model: *DAP:TLA* versus CO₂, Elevation

Factor	Type	Levels	Values
CO ₂	fixed	2	390ppm, 700ppm
Elev	fixed	20	2.25, 6.75, 11.25, 15.75, 20.25, 24.75, 29.25, 33.75, 38.25, 42.75, 47.25, 51.75, 56.25, 60.75, 65.25, 69.75, 74.25, 78.75, 83.25, 87.75

Analysis of Variance for d/t, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
CO ₂	1	0.0903	0.0903	0.0903	6.73	0.009

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CO ₂ *Elev	19	2.2734	1.5889	0.0836	6.24	0.000
Elev	19	215.0778	215.1	11.3199	844.21	0.000
Error	17240	231.1696	231.1696	0.0134		
Total	17279	448.6110				

S = 0.115797 R-Sq = 48.47% R-Sq(adj) = 48.35%

Grouping Information Using Tukey Method and 95.0% Confidence

CO ₂	N	Mean	Grouping
390 ppm	10240	0.3813	A
700 ppm	7040	0.3766	B

Means that do not share a letter are significantly different.

4.14 Leaf Dispersion MANOVA

General Linear Model: d/t versus species 3, CO₂, Elevation, TLA 3, dev stage

MANOVA for species 3

s = 1 m = 0.0 n = 8625.0

Criterion	Test	DF		P
	Statistic	F	Num Denom	
Wilks'	0.97989	176.995	2 17252	0.000
Lawley-Hotelling	0.02052	176.995	2 17252	0.000
Pillai's	0.02011	176.995	2 17252	0.000
Roy's	0.02052			

MANOVA for CO₂

s = 1 m = -0.5 n = 8625.0

Criterion	Test	DF		P
	Statistic	F	Num Denom	
Wilks'	0.99808	33.207	1 17252	0.000
Lawley-Hotelling	0.00192	33.207	1 17252	0.000
Pillai's	0.00192	33.207	1 17252	0.000
Roy's	0.00192			

MANOVA for Elev

s = 1 m = 8.5 n = 8625.0

Criterion	Test	DF		P
	Statistic	F	Num Denom	
Wilks'	0.49604	922.510	19 17252	0.000
Lawley-Hotelling	1.01598	922.510	19 17252	0.000
Pillai's	0.50396	922.510	19 17252	0.000
Roy's	1.01598			

MANOVA for TLA 3

s = 1 m = 1.0 n = 8625.0

Criterion	Test	DF			
	Statistic	F	Num	Denom	P
Wilks'	0.99437	24.428	4	17252	0.000
Lawley-Hotelling	0.00566	24.428	4	17252	0.000
Pillai's	0.00563	24.428	4	17252	0.000
Roy's	0.00566				

MANOVA for dev stage

s = 1 m = -0.5 n = 8625.0

Criterion	Test	DF			
	Statistic	F	Num	Denom	P
Wilks'	0.97392	462.030	1	17252	0.000
Lawley-Hotelling	0.02678	462.030	1	17252	0.000
Pillai's	0.02608	462.030	1	17252	0.000
Roy's	0.02678				

4.16 Leaf Dispersion Regression

Regression equation

dispersion = 1.61 - 0.00826 DAS - 0.00151 meanleafsize - 0.00108 nleavesp

Predictor	Coef	SE Coef	T	P
Constant	1.6055	0.1117	14.38	0.000
DAS	-0.008258	0.002064	-4.00	0.000
Meanleafsize	-0.001509	0.002246	-0.67	0.503
nleavesp	-0.001078	0.001256	-0.86	0.393

S = 0.433190 R-Sq = 27.1% R-Sq(adj) = 24.9%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	6.8324	2.2775	12.14	0.000
Residual Error	98	18.3901	0.1877		
Total	101	25.2225			

4.14 Digitization equipment set up.

Digitization

The Polhemus digitizer is connected to a computer, a stationary transmitter and a moveable probe. To take a reading, the tip of the probe is held next to the structure and the recording button is pressed. The Floradig program requires one point for each node and, in this case, four points for each leaf. The transmitter must be positioned so the plant does not overlap it, but the

transmitter is as close as possible to the plant. Cables connect the parts of the equipment. Each point is recorded as a distance on the x, y and z axis of a space with the transmitter as the origin.

