Canopy architecture, carbon gain and grain properties of native Australian rices: effects of elevated atmospheric carbon dioxide

by

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Statement of Originality

I certify that, to the best of my knowledge, the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes.

I certify that the intellectual content of this thesis is the product of my original work and that all the assistance received in preparing this thesis and sources has been acknowledged.

Sayedur Rahman

15th December 2021

Dedication

This work is sincerely dedicated to

Those who devoted their lives to end

World Hunger

Acknowledgement

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I, **Sayedur Rahman** (lead author), in discussion with my principal supervisor Thomas H. Roberts, and auxiliary supervisors, Les Copeland, and Brian J. Atwell, designed and executed the experiments, gathered data, processed, and analysed the data; prepared the original draft of all articles. The contribution of authors other than myself are as follows:

Chapter 2. Thomas H. Roberts: Writing–review and editing; Supervision; Project funding and administration. Brian J. Atwell: Conceptualization; Recourses; Writing–review and editing; Supervision; and Project administration. Remko A. Duursma: Methodology; software; Writing–editing. Md. A. Muktadir: Helped with Digitization; Writing–editing.

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Sayedur Rahman

Attestation of Authorship Attribution Statement

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Thomas H. Roberts

Principal Supervisor

Journal articles

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Abbreviations

AC	Amylose content
A_C	Crown surface area
aCO_2	Ambient carbon dioxide
A_L	Leaf area (estimated)
Al	Aleurone layer
AM	Amylose
A_{max}	Light-saturated photosynthesis
A_N	Net photosynthetic rate
ANOVAR	Analysis of variance for repetitive measures
atm-CO_2	Atmospheric carbon dioxide
AP	Amylopectin
BD	Breakdown viscosity
Bd	Bulk density
Ch	Channels
cP	Centipoise
CSG	Compound starch granules
D	Dents

dH_2O	Distilled water
eCO_2	Elevated carbon dioxide
E	Endosperm cells
FA	Fatty acids
FACE	Free-air carbon dioxide enrichment
FV	Final viscosity or Cool paste viscosity
HSD	Honestly significant difference
LA	Leaf area
LAI	Leaf area index
LRC	Light response curve
LW	Length-to-width ratio
MC	Moisture content
OTC	Open top chamber
PAR (I)	Photosynthetically active radiation
PB	Protein bodies
PC	Protein content
Pc	Pericarp
Phi (φ)	Quantum yield

PT	Pasting temperature
Pt	Peak time
PV	Peak viscosity
RC	Relative change
R_d	Dark respiration rate
REML	Restricted or residual maximum likelihood
RVA	Rapid Visco Analyser
SB	Setback viscosity
SEM	Scanning Electron Microscope
SG	Starch granules
SLM	Specific leaf mass
Sl	Seed length
Sw	Seed width
Theta (Θ)	Curvature factor
TGA	Thermogravimetric Analyser
TSW	Thousand seed weight
TV	Trough or hot paste viscosity

Abstract

The genus *Oryza* consists of ~26 species but only two species, the Asian rice *O. sativa* and the African rice *O. glaberrima*, are domesticated. Although *O. sativa* has vast genetic diversity, far more genetic diversity resides within wild *Oryza* species. These wild relatives will be increasingly used in breeding programs over the coming decades. However, the effects of rising atmospheric CO_2 concentration on photosynthesis, light interception, canopy architecture, and grain properties of wild species are unknown. Two accessions of the Australian wild rice, *O. meridionalis* (Cape York and Howard Springs) were grown in ambient (aCO_2, 400 ppm) and elevated CO_2 (eCO_2,700 ppm) with *O. sativa* cv. Doongara in glasshouses and compared for photosynthesis, light interception, biomass, and carbon (C) gain. For grain quality and endosperm morphology characterisation, an accession of *O. australiensis* was included.

Photosynthesis (A_{max}) was enhanced at eCO_2 by 20–50% across the rice genotypes, doubling the number of tillers and leaves, and biomass. Light interception ($\overline{STAR}$) was lower in eCO_2 than in aCO_2 due to denser canopies and lower leaf dispersion. Plant biomass and C gain were higher in eCO_2 than in aCO_2, despite lower light interception. The wild rices had higher crown density and lower light interception than the domesticated rice in both CO_2 treatments. The ranking of the genotypes did not change at eCO_2 even when C gain was simulated at Summer Solstice and Equinox; however, at the Summer Solstice, C gain was greater than at the Equinox due to superior light capture.

Grain physicochemical analysis showed that seed length, seed width, and 1000-seed weight was higher in eCO_2 than in aCO_2. Protein content decreased at eCO_2 by 23% in Doongara and 15% in Howard Springs. Peak and final viscosity increased in the wild rices at eCO_2. However, in Doongara, only peak viscosity increased and final viscosity decreased. The use of 1% $AgNO_3$ inactivated α-amylase activity of the rice flour during pasting. SEM images showed that aleurone cell length and width were higher in Howard Springs, but the area and length of starch granules were larger in Cape York in eCO_2 than in aCO_2. Responses of rice endosperm morphology to eCO_2 were dependent on genotype.

In conclusion, photosynthesis, light interception, total biomass, canopy architecture, physicochemical properties and endosperm are differently affected by eCO_2 in the Australian wild rices compared with Asian rice.

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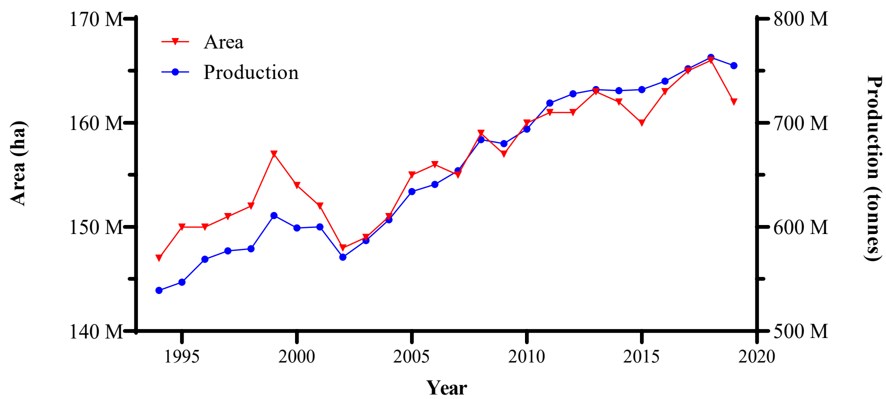
Chapter 1

Literature Review

1.1. *Rice in world agriculture*

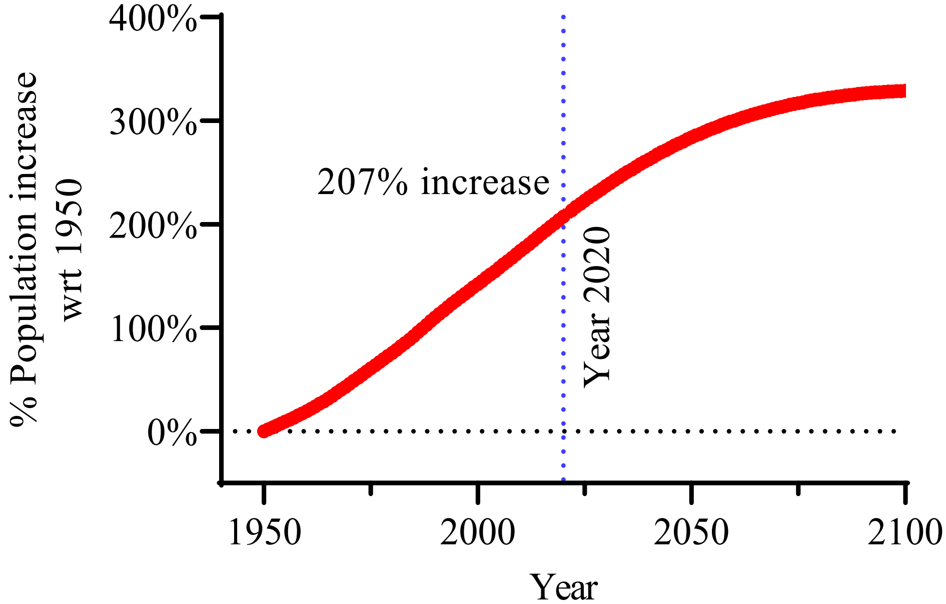
Rice is the most important cereal crop for humankind, consumed by more than half of the world's population for whom it meets more than 20% of daily calorie requirements (Brar and Khush, 2018) and up to 76% of the calorie intake of the South East Asian population (Fitzgerald et al., 2009). Asia produces and consumes ~90% of the world's rice. Approximately 162 million hectares, equivalent to almost ~12% of the total world's arable land, were under paddy cultivation in 2019 and produced ~750 million tonnes of paddy rice (FAO, 2021). The world's total area under rice cultivation and production is presented in Fig. 1.1.



Source: http://www.fao.org/faostat/

Fig. 1.1. World's total area under rice cultivation and paddy production, 1994–2019. Note that the left y-axis begins at an area of 140 M ha and the right y-axis at 500 M tonnes.

The world's population is expected to grow to 9.7 billion by 2050 and 10.9 billion by 2100, approximately a threefold increase relative to 1950 [Fig. 1.2, (United Nations, 2019)]; hence global rice production must increase by 20–30% over the next 20 years from the present level to meet food demands (Brar and Khush, 2018; Wei and Huang, 2019). As the availability of additional arable land becomes limiting, increasing yield is a crucial factor to ensure food security and curb poverty. Theoretically, rice still has untapped yield potential, with many ways to raise grain yield and quality. Management practices to improve rice yield include building irrigation works, better water management, improving soil conditions and adoption of new cultural practices. At the biological level, yield improvement could be achieved through breeding to optimise canopy architecture, as well as resistance to abiotic and biotic stresses, while breeding for nutritional quality is also possible (Huang et al., 2013; Yuan, 2014). Breeding can include conventional techniques and advanced breeding techniques such as the use of CRISPR-Cas9 gene-editing technology (Jaganathan et al., 2018; Ma et al., 2016).



(Source: DESA, United Nations, 2019)

Fig. 1.2. Changes in world population (%) 1950–2020, and projections for 2020–2100 (medium variant) relative to the 1950 population (2.5 billion). The intersection of the blue dotted line and the red line represents the percentage population increase in 2020 compared to 1950.

1.2. *Climate change, atmospheric CO_2 concentrations and atmospheric heating*

Rising food demand is a global challenge made more acute by uncertainties around the interaction with levels of atmospheric CO_2 (atm-CO_2). Current atm-CO_2 levels of 416 ppm (December 9, 2021, https://keelingcurve.ucsd.edu/) represent an increase of more than 20% in the past 40 years alone (Fig. 1.3). According to the Scripps Institution of Oceanography, under Future Emissions Scenario No. 1 (RCP8.5), atm-CO_2 could rise to 720 ppm by the end of the 21st century (http://scrippsco2.ucsd.edu). Direct effects of atm-CO_2 fertilisation on photosynthesis might accelerate yield and mitigate drought stress by raising water use efficiency in C_3 species such as rice. However, secondary effects of increased atm-CO_2 include unstable weather systems that cause heatwaves, drought, and tidal surges.

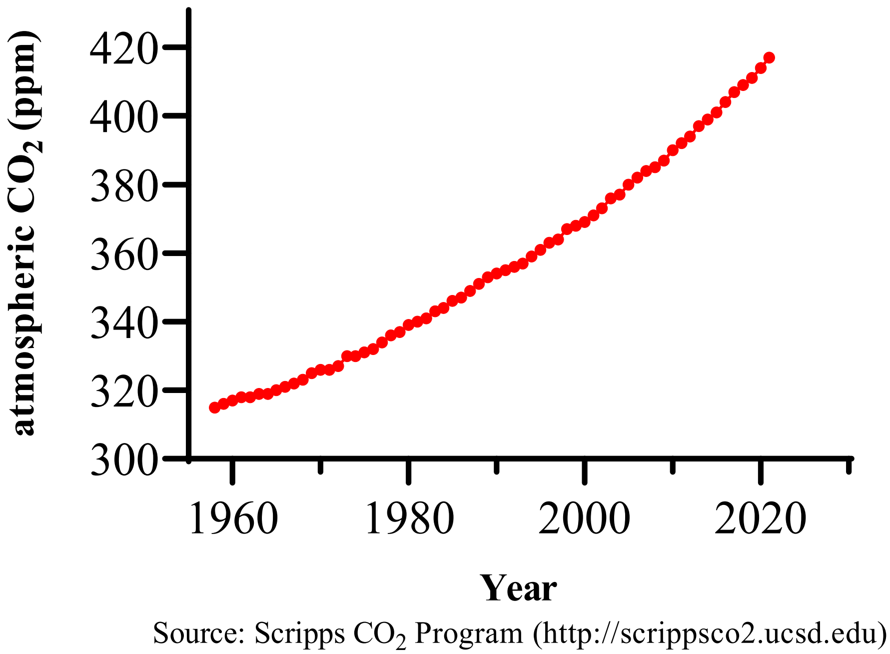
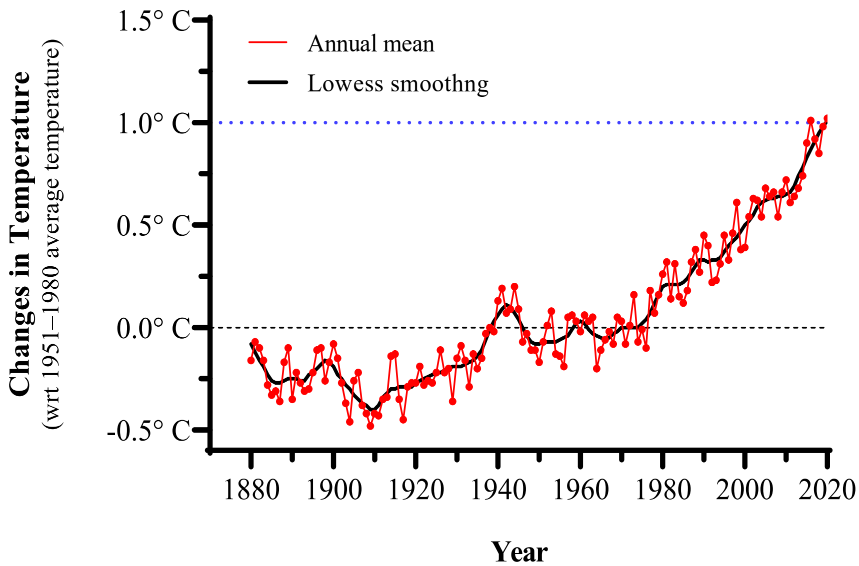


Fig. 1.3. World's yearly average atm-CO_2 concentrations, 1958–2021.

Depending on greenhouse gas emissions, the global average temperature will rise by 3.3−5.7 °C (SSP5-8.5) by the end of this century, with an average increase of 0.4 °C per decade (IPCC, 2021). The global average surface temperature (May 2021) has increased by 0.81 °C from the average of the 20th century (NOAA, 2021). The changes in global surface temperature relative to average temperatures of 1951−1980 (14.0 °C) are shown in Fig. 1.4.



Source: NASA/GISS, https://climate.nasa.gov/

Fig. 1.4. Changes in global surface temperature, 1880–2020, relative to the 1951–1980 average temperature of 14 °C. Lowess smoothing means Locally Weighted Scatterplot Smoothing.

1.3. *Domesticated rice and its wild relatives with special reference to Australian species*

The *Oryza* genus is composed of two domesticated (*O. sativa* and *O. glaberrima*) and at least 24 wild species with 11 distinct genome types, AA, BB, CC, EE, FF, GG, BBCC, CCDD, HHJJ, HHKK, and KKLL (Ammiraju et al., 2007; Atwell et al., 2014; Brar and Khush, 2018; Henry, 2019; Stein et al., 2018; Vaughan et al., 2003), consisting of 17 diploids and seven tetraploids (Piegu et al., 2006), with the AA, CC and EE genome types (all diploids) found in Australia (Atwell et al., 2014; Henry, 2019; Jacquemin et al., 2013). *O. sativa* is believed to have been domesticated from *O. rufipogon* and *O. nivara* (Crawford and Shen, 1998; Kovach et al., 2007; Li et al., 2006).

In Australia, the AA genome species have been classified as *O. meridionalis* or *O. rufipogon* (Henry, 2019). The genome sizes of the AA genomes, both in *O. sativa* (indica and japonica) (397 Mb) and *O. meridionalis* (435 Mb), are smaller than the EE genomes, including *O. australiensis* (965 Mb) (Ammiraju et al., 2008; Jacquemin et al., 2013). The *O. australiensis* genome size has doubled due to accumulated retrotransposon copies through its lineage over million years (Henry et al., 2010; Piegu et al., 2006). *O. australiensis* and *O. meridionalis* are endemic to Australia (Brar and Khush, 2018) but Moner and Henry (2018) reported an *O. meridionalis* population found in New Guinea. The plants of these two wild species are resistant to pests and diseases, and tolerant to drought and heat (Brar and Khush, 2018; Moner and Henry, 2018; Scafaro et al., 2016), while starch content was high in amylose and had high gelatinization temperatures (Tikapunya et al., 2017).

There are distinct taxonomical differences between wild and domesticated rice. The domesticated rices have erect tillers, more grains, and higher grain weight, while wild rices have prostrate tillers, coloured grain, fewer grains on the panicle and smaller seed weights. *O. australiensis* has long open panicles (up to 50 cm) and short awns compared with *O. meridionalis* (Henry, 2018), while the latter species has closed panicles (up to 30 cm) with long awns (up to 150 mm), small anthers, large lemmas and stem elongation ability (Brar and Khush, 2018; Moner and Henry, 2018; Sotowa et al., 2013; Sweeney and McCouch, 2007). The seed husk/hull of *O. sativa* and *O. meridionalis* is straw-coloured while it is black in *O. australiensis.* The differences in morphological characters between wild and domesticated rice are shown in Table 1.1.

Table 1.1. Morphological characteristics of wild and domesticated rice

Character	Wild Rice	Domesticated Rice
Awns	Medium/Long	Very small
Shattering	Severe	Minimal
Pericarp	Pigmented	White/Pigmented
Dormancy	High	Low
Seed Hulls	Black/Straw-coloured	Straw-coloured
Reproduction	Outcrossing (10–50%)	Inbreeding
Grain size	Small/Medium	Variable sizes (small to large)
Panicle structure	Open/closed, few secondary branches	Densely packed

Source: Modified from Brar and Khush (2018)

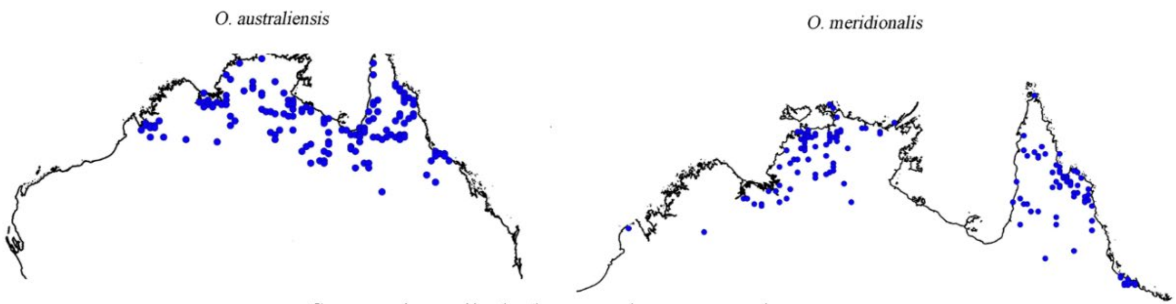
Worldwide cultivation of *O. sativa* extends between 35 °S (New South Wales, Australia, and Argentina) to 53 °N (Northern China) and in over 120 countries (Wei and Huang, 2019) from sea level to an altitude of ~4,000 m (Poudel and Kotani, 2013). Wild rice species grow in Asia, South America, Australasia and Africa (Atwell et al., 2014; Brar and Khush, 2018; Vaughan et al., 2003) in the natural habitats of savanna, savanna woodland, flood plain, seasonally flooded land, stagnant water and deep water conditions (Atwell et al., 2014; Henry, 2019; Henry et al., 2010). Temperature and water availability are the most important factors affecting the growth of cultivated rice. To grow a successful crop, the optimum daytime temperature must fall in the range of 25–30 °C, with an abundant supply of moisture in the soil naturally or through irrigation.

Oryza sativa sp. japonica was domesticated in Asia ~10,000 years ago, whereas *O. sativa* sp. indica was domesticated in India ~3,000 later. *O. glaberrima* was domesticated in Africa ~ 3,500–3000 years ago (Brar and Khush, 1997; Chang, 1976; Crawford and Shen, 1998; Gross and Zhao, 2014; Linares, 2002; Londo et al., 2006; Wei and Huang, 2019). However, the key domestication trait, non-shattering panicles, was only fixed genetically over the subsequent 1,000 years (Gross and Zhao, 2014). Three genes, *sh4* (Vaughan et al., 2008a), *Rc* (Sweeney et al., 2006) and *Wx* (Hirano et al., 1998), were identified as domestication genes for shattering, seed colour and waxiness of rice, respectively. In addition to these genes, *PROG1* was identified as a domestication gene responsible for prostrate growth (Jin et al., 2008; Tan et al., 2008). Furthermore, the gene *MOC1,* responsible for controlling tiller number (Li et al., 2003), and *LA1* and *TAC1*, controlling tiller angle (Li et al., 2007; Yu et al., 2007) were identified, and are important for canopy architecture and light interception.

Although rice preferentially grows in full sun, some species are adapted to shaded or woodland conditions (Vaughan et al., 2008a; Zhao et al., 2008). *O. australiensis* and *O. meridionalis* grow in northern Australia, from Queensland and the Northern Territory to Western Australia in seasonally flooded areas where there is heavy rainfall during the monsoon followed by drought. These species can be found in extensive grasslands where there is little or no competition (Henry, 2019; Henry et al., 2010). *O. australiensis* is a perennial grass and grows in seasonally dry embankments of lagoons in Queensland, the Northern Territory and Western Australia. Plants form rhizomes to survive in the dry season, although they can also reproduce from seeds. *O.*

meridionalis grows annually in wetter areas and survives by seeds that germinate after the dry season (Henry et al., 2010); however, this species was recently identified as having a perennial form as well (Moner and Henry, 2018; Sotowa et al., 2013).

The distribution of *O. australiensis* and *O. meridionalis* is shown in Fig. 1.5. Both these Australian wild rices have a reputation for tolerance to heat and drought, as they grow under erratic rainfall regimes (Gouda et al., 2012; Sundaramoorthi et al., 2009). Furthermore, *O. australiensis* is also resistant to brown planthopper (Jena et al., 2006; Multani et al., 1994), bacterial blight (Brar and Khush, 1997) and blast (Jeung et al., 2007; Suh et al., 2009).



Source: *https://avh.ala.org.au/occurrences/*

Fig. 1.5. Distribution of wild rice populations endemic to Australia, *O. australiensis* and *O. meridionalis*.

1.4. *Genetic diversity in* Oryza

Genetic diversity is the basis for plant breeding to develop improved varieties. The development of modern rice varieties depends on the continued availability of genetic diversity (Huang et al., 2013). Loss of genetic diversity has occurred among common cultivars due to genetic erosion over long periods of continuous selection pressure for desired traits. Maintenance of broad genetic diversity is important in developing superior genotypes with desired traits; e.g., higher grain yield, biomass, harvest index, photosynthesis, better light interception and improved canopy, better responses to biotic and abiotic stresses, better grain quality and high nutritional value.

Londo et al. (2006) found 22–28% total haplotype diversity (using *p-VATPase*, *VATPase B-subunit*, *SAM*, S-adenosyl methionine synthetase) in cultivated rice (*O. sativa*) as compared with

82–88% in the wild progenitor *O. rufipogon*. Thailand and Laos populations of *O. rufipogon* showed 73% genetic diversity (Prathepha, 2012). In a previous study, genome re-sequencing showed Australian wild rice *O. meridionalis* has 2.5 times more single nucleotide polymorphism (SNPs) than *O. sativa* ssp. indica and japonica (Krishnan et al., 2014). A total phenotypic variability of 55% for grain zinc was reported in *O. meridionalis*, explained by four quantitative trait loci (QTLs) detected: *qGZn9*, *qGZn10* and *qGZn2-1* and *qGZn2-2*. Of these, *qGZn9* on chromosome 9 explained only 22% of the phenotypic variability for higher Zn concentration in *O. meridionalis* (Ishikawa et al., 2017), indicating Zn concentration in this species is controlled by multiple genes.

Giuliani et al. (2013) suggested thicker leaves have lower transpiration rates and higher transpiration efficiency to conserve water. They also suggested thicker leaf mesophyll cell walls are beneficial for drought tolerance. As *O. australiensis* and *O. meridionalis* grow in the hotter and drier regions of Australia with low moisture availability, they are more likely to have drought tolerance traits with higher water use efficiency. Scafaro et al. (2011) reported thicker mesophyll cell walls and lower mesophyll conductance in both *O. meridionalis* and *O. australiensis* compared to *O. sativa*, potentially adapting these species to low availability of moisture, and underlining the fact that wild genotypes are a source of diversity.

Wei and Huang (2019) reported that *O. glaberrima*, African rice, has less genetic diversity than *O. sativa*. Li et al. (2011) reported 70% less genetic diversity in *O. glaberrima* than its progenitor, *O. barthii*, but nucleotide diversity was low in both species. Ge et al. (1999) reported 61.8% total genetic diversity in *O. rufipogon* between populations from two countries (China and Brazil) while there was only 14.9% and 23.3% between populations and within regions, respectively.

Since the domestication of *O. sativa*, introgression of genes from its wild relative *O. rufipogon* has been an ongoing process through variable degrees of natural out-crossing (Vaughan et al., 2008b). There are over 120,000 accessions of *O. sativa* in existence today. However, this species still has a much narrower genetic diversity than its wild relatives. The Australian wild populations contribute to this genetic diversity and can be used in breeding programs as a source of novel genes; e.g., for disease resistance, drought tolerance, insect resistance and unique starch properties (Brar and Khush, 2018; Henry, 2019).

1.5. *Photosynthesis, light interception, plant growth and elevated atm-CO_2 effects*

The principal substrate for photosynthesis is CO_2, and elevated atm-CO_2 (eCO_2) enhances photosynthesis, crop growth, canopy development, biomass production, grain yield and harvest index (Ainsworth, 2008; Ainsworth and Long, 2021; Gao et al., 2021; Hu et al., 2021; Li et al., 2017; Lv et al., 2020; Madan et al., 2012; Sakai et al., 2019; Usui et al., 2016; Wang et al., 2020; Ziska and Teramura, 1992a; Ziska and Teramura, 1992b). Elevated CO_2 stimulates photosynthesis, increases light use efficiency, reduces stomatal conductance, improves water use efficiency, and increases nutrient use efficiency (Ainsworth and Long, 2005; Kang et al., 2021; Sakai et al., 2019; Wang et al., 2020).

The primary effects of eCO_2 on rice plants include higher photosynthetic rates, a decrease in stomatal conductance and transpiration, and increased water use efficiency (Balbinot et al., 2021; Kang et al., 2021; Wang et al., 2015; Wang et al., 2020). Elevated CO_2 enhanced leaf photosynthetic rates in rice by 50%, which in turn increased total plant biomass by 30% (Ziska and Teramura, 1992b). The enhancement in photosynthetic rate did not decline even after three months of exposure to high CO_2 (660 ppm). The average increase in leaf photosynthesis was 31–43% and a decrease of 22–36% in stomatal conductance was reported in rice plants (Ainsworth et al., 2003; Ainsworth and Rogers, 2007; Yoshimoto et al., 2005). Leaf photosynthesis at tillering and at the grain filling stage was increased by 48–77% and 20–33%, respectively, at eCO_2 and it was argued that the differences in enhancements (photosynthesis) could be due to leaf senescence within the canopy at the grain filling stage and reduced radiation use efficiency (Lin et al., 1999; Shimono et al., 2009).

Yeo et al. (1994) reported higher rates of photosynthesis per unit leaf area (p = 0.001) in *O. australiensis* than *O. sativa*, despite millennia of selection for grain productivity in the latter. The lowest rates of CO_2 assimilation were observed in *O. rufipogon*, a progenitor of *O. sativa*. Moreover, Yeo et al. (1994) concluded that CO_2 assimilation is higher in sun-adapted diploid species of rice than in tetraploids that are adapted to shade. However, growth conditions are probably important in these interspecific comparisons, as indicated by Zhao et al. (2010), who reported photosynthetic rate per unit leaf area was higher in *O. australiensis* and *O. rufipogon* than *O. sativa*. Furthermore, in well-controlled glasshouse conditions, Taylor (2014) found leaf-level photosynthesis in Australian wild species to be lower or similar to that of cultivated species.

Optimising interactions between photosynthetic sources (mature leaves) and sinks (developing leaves, roots and reproductive organs) throughout the phenological cycle is critical for crop improvement. Under future atm-CO_2 scenarios, faster carbon assimilation in C_3 crops demands greater sink capacity. However, sinks and sources interact: responses of crops to eCO_2 reflect intrinsic sink capacity in a relationship known as *adaptive plasticity*. For example, enhanced branching is often reported in eCO_2, while weaker sinks with strong assimilation rates can lead to acclimation of photosynthesis in rice under eCO_2 (Fabre et al., 2020). Acclimation of photosynthesis in rice at eCO_2 was reported due to sink pruning, which was explained as sink limitation feeding back on photosynthetic rates (Fabre et al., 2019). Hence, to benefit fully from eCO_2, substantial enhancement of vegetative sink vigour, inflorescence size or number, and root sinks will be required (Dingkuhn et al., 2020). This optimisation is particularly important as modern crop cultivars are developed with radically altered morphology. For example, reduced tillering and smaller root systems require a revision of the source–sink relationship, whereas wild crop relatives and old cultivars have greater phenotypic plasticity and respond more strongly to eCO_2 than contemporary cultivars (Dingkuhn et al., 2020).

In the best illustration of this principle, Zhu et al. (2014) compared two rice cultivars under eCO_2 for physiological and yield responses. One of the cultivars showed photosynthetic acclimation to eCO_2, while the other cultivar showed stimulation of photosynthesis up to grain maturity due to adaptive plasticity of sink capacity. It was therefore suggested that triose phosphate utilization was involved in the regulation of photosynthesis in rice under eCO_2. In another free-air CO_2 enrichment (FACE) experiment, Zhu et al. (2015) compared a hybrid indica rice with a japonica cultivar. The hybrid indica rice avoided photosynthetic acclimation at eCO_2 due to stronger sink generation and N uptake capacity, which helped to maintain the C/N balance, which in turn produced higher grain yield than the japonica cultivar (approximately +13%). For details, please see review by Dingkuhn et al. (2020).

Canopy net photosynthesis in rice was found to increase by 24% at eCO_2 under standard N (9 g N m^{-2}) but a greater enhancement of net photosynthesis (31%) occurred at higher availability of N (15 g N m^{-2}) (Anten et al., 2004). Maximum photosynthesis (A_{max}) and A_{max} per unit leaf N were found to be 35% higher at eCO_2 in a free-air CO_2 enrichment (FACE) experiment than at aCO_2 (Aben et al., 1999; Ainsworth, 2008; Anten et al., 2004; Nakano et al., 1997). In open top chamber (OTC) experiments, light-saturated photosynthesis (A_{sat}) was found to be significantly higher in eCO_2 compared to FACE experiments (Ainsworth, 2008). In rice, Wang et al. (2016) reported a significant CO_2 × growth temperature interaction for leaf photosynthetic rate. Under eCO_2, stimulation of single leaf photosynthetic rate was usually lower at elevated growth temperature. However, under normal growth temperatures (29°/21°C day/night), leaf photosynthetic rate was greater in eCO_2. Rice plant growth in eCO_2 also showed a negative response to high growth temperature compared to that of plants grown under aCO_2 (Wang et al., 2016). The quantum yield increased but a slight decrease in dark respiration (R_d) was reported by many authors (Anten et al., 2004; Long, 1991). Under eCO_2, photosynthesis and quantum yield increased; at higher quantum yield with a lower compensation point, more leaves can be maintained at a lower light level, which would increase leaf area index (*LAI*) (Anten et al., 2004). A lack of response to eCO_2 was found when rice was grown at high temperature or in low N-supply (Ainsworth, 2008). It was argued that prolonged acclimation of photosynthesis might occur at eCO_2 due to reduced N-supply, which can limit total dry matter and leaf area increases (Ainsworth et al., 2003; Suter et al., 2001). When rice plants were grown at medium-P conditions (60, 120 mg kg^{-1} of soil) under eCO_2, the grain mass and the grain number increased more compared to plants grown in high-P conditions (Seneweera et al., 1996; Seneweera and Conroy, 1997b).

FACE experiments are considered to measure plant responses to eCO_2 in a natural environment. However, fluctuations of CO_2 level in the field are difficult to control. Responses of rice yield, above ground biomass and light-saturated photosynthesis to eCO_2 in FACE experiments were 67%, 67% and > 50%, respectively, of the values obtained in OTC experiments. Because CO_2 fluctuates in the field due to air turbulence and leaf oscillation, photosynthesis and plant growth responses to eCO_2 are likely to be underestimated (Allen et al., 2020). The authors estimated a correction factor of 1.5 to be applied to FACE results. For details please see review by Allen et al. (2020).

Considerable variations (increase) in photosynthesis (0–77%) under eCO_2 have been reported by several authors regardless of plant growing conditions (Ainsworth, 2008; Fabre et al., 2020; Kang et al., 2021; Lin et al., 1999; Sakai et al., 2019; Yeo et al., 1994; Zhao et al., 2008). Photosynthesis in rice was found to be higher at the vegetative phase (tillering) than the reproductive phase (grain filling) at eCO_2 (Lin et al., 1999; Shimono et al., 2009). However, rates of CO_2 assimilation are not the only driver for growth and yield. The photosynthetic rate of the crop canopy depends on many factors, such as weather, temperature, leaf age and plant development (Burgess et al., 2017). Moreover, photosynthetic rate is greatly dependent on light intensity and, in turn, the light intensity within crop canopies. Light interception is highly dependent on canopy architecture, including tiller number, tiller angle, leaf angle, leaf size and shape, leaf number and leaf arrangement.

The amount of light intercepted by the canopy is predominantly determined by the *LAI*. This parameter was expected to increase at eCO_2 as photosynthesis and quantum yield increased, giving a lower light compensation point, and maintaining more leaves at low light levels in the canopy. In some cases, *LAI* increases at eCO_2, while in others there were negative or no effects, as mentioned by Ainsworth (2008). There is evidence that *LAI* strongly correlates with N availability and controls leaf area growth at eCO_2 (Anten et al., 1995), or even in plants grown at the same level of atm-CO_2 (Hirose et al., 1996). It was argued that *LAI* would increase at eCO_2 due to increased N uptake from accelerated root growth of stands of plants (Hirose et al., 1996). Several researchers found increases in *LAI* at eCO_2 with constant levels of N-supply (Hartz-Rubin and DeLucia, 2001), but eCO_2 will have an adverse effect at low N-supply (Kim et al., 2001). In a rice paddy, N uptake was stimulated by eCO_2 and *LAI* at the early stages of growth (Anten et al., 2004; Kim et al., 2001; Kim et al., 2003). It was argued that the increase in *LAI* at the early stage of crop growth was stimulated by N availability and subsequently decreased with the depletion of N available in the field. Moreover, Sakai et al. (2006) reported a strong positive correlation between *LAI* and N uptake at eCO_2 when ample N was applied. Anten et al. (2004) concluded that neither *LAI* nor leaf mass was significantly enhanced by eCO_2; rather, N availability was the crucial factor.

Song et al. (2013) suggested that stem height, leaf angle and leaf width could be manipulated in rice to enhance canopy photosynthesis. A study using five genotypes from a multi-parent advanced generation of *O. sativa* inter-crosses revealed strong variation in light interception efficiency

(Burgess et al., 2017), thought to be genetically determined. The Australian wild species exhibit phenotypic variation in canopy architecture that could be utilised to redesign the canopy architecture of cultivated rice for better light interception and yield.

In a meta-analysis, the average yield of rice increased by 23% as CO_2 was elevated from 365 to 627 ppm (Ainsworth, 2008). Dry matter production also increased by 17% for above-ground and 30% for below-ground at eCO_2 compared with aCO_2 (Ainsworth and Long, 2005; De Graaff et al., 2006). Plant biomass and yield are driven by net photosynthesis if other physiological factors such as respiration are unaltered, as demonstrated in experiments where eCO_2 consistently stimulates carbon assimilation and enhances plant growth and yield in cultivated rice (Long et al., 2006a; Lv et al., 2020; Usui et al., 2016; Wang et al., 2015; Yang et al., 2009; Ziska et al., 1996). Murata (1981) also reported a high correlation ($r = 0.778$) between leaf photosynthesis and crop growth rates in many C_3 crops. Elevated atm-CO_2 enhances crop biomass (Madan et al., 2012; Ziska et al., 1996), tiller production (Yang et al., 2009), individual grain mass (Yang et al., 2009) and panicle and spikelet density (Usui et al., 2016) in *O. sativa*.

After many decades of research on photosynthetic efficiency, there is now a growing appreciation of the significance of modifying whole-canopy architecture to optimise light interception (Burgess et al., 2021; Chang et al., 2019; Horton, 2000; Jiao et al., 2010). While light interception is reduced in plants as leaf area increases due to self-shading by overlapping leaves (Delagrange et al., 2006; Niinemets et al., 2005), alteration of leaf shape and angle, as well as tiller orientation, could increase light interception (Niinemets, 2010) and improve yields of annual crops (Burgess et al., 2017).

1.6. *The potential use of wild species in rice canopy improvement*

Global commercial rice production is dominated by a single species, *O. sativa*, although *O. glaberrima* is also cultivated in Africa. However, far greater genetic diversity resides in the other ~24 *Oryza* species (Ammiraju et al., 2007; Atwell et al., 2014; Brar and Khush, 2018; Henry, 2019; Stein et al., 2018; Vaughan et al., 2003). This genotypic range offers unexplored opportunities for crop improvement and the modification of leaf canopies to optimise light capture as atm-CO_2 continues to rise. Superior light capture by the canopy and metabolism of carbon resources to grains is likely to be a major pathway to improved yields. The Australian wild species have the potential to contribute to the generation of new phenotypes in elite rice varieties.

1.7. *Effects of elevated atm-CO_2 on rice grain quality and their importance*

As discussed earlier, eCO_2 leads to several physiological changes in rice plants, such as rates of photosynthesis, canopy architecture and light interception (Fabre et al., 2020; Wang et al., 2011), which are anticipated to improve crop biomass and yield in rice (Madan et al., 2012; Sakai et al., 2019; Wang et al., 2020). However, these changes are also expected to affect the physicochemical properties of rice grains including endosperm morphology and flour pasting properties (Chaturvedi et al., 2017; Jing et al., 2021; Jing et al., 2016b).

Rice quality depends on consumer preferences and must be fit for purpose. It involves producers (farmers), brokers, processors, buyers/sellers (retailers) and consumers (Fig. 1.6). Rice grain quality is a complex property; in the narrow sense, it refers to cooking and eating quality (palatability). Broadly, the physical and chemical properties of rice seeds are the determinants of grain quality, which comprise four major categories: milling and processing quality, cooking and eating quality, appearance, and nutritional quality (Fig. 1.6) (Fitzgerald et al., 2009; Yang and Wang, 2019).

Many studies have been conducted on various aspects of the quality of cultivated rice; e.g., physical and chemical properties, cooking parameters, textural properties, and flour pasting properties (Seneweera, 2011; Seneweera et al., 1996; Usui et al., 2016; Wang et al., 2011; Ward et al., 2019; Ward, 2007; Zhao et al., 2021; Zhou et al., 2020), as well as antioxidant properties of rice grain and rice bran (Goufo et al., 2014c; Goufo and Trindade, 2014; Nam et al., 2006).

Nowadays, plant breeders and scientists are paying more attention to improve rice quality for marketing and industrial purposes, including nutritional values (Bergman, 2019; Chen et al., 2012; Ujiie et al., 2019; Zhou et al., 2020). For instance, soft and sticky rice is preferred in East and South East Asia, while non-sticky rice is preferred in South Asia (Indian subcontinent). Nutritional value is crucial for developing countries where rice is consumed as a principal source of nutrients and energy supply (Chen et al., 2012; Ujiie et al., 2019). The key factors affecting the cooking process and eating quality and nutritive quality will be discussed in the following sections.

Fig. 1.6. Steps and factors involved in rice marketing, from production to end-users.

1.7.1. Physical properties and milling and appearance quality

Physical characteristics of rice grain include size and shape, uniformity, colour, and the presence of damaged (broken) or imperfect grains in the sample. Physical evaluation of grains is performed mainly on the basis of grain length, translucency and chalkiness (Chen et al., 2012; Wang et al., 2011; Zhou et al., 2019). Appearance is primarily focused on the whiteness of the grain (Juliano, 1993), which is influenced by genetic and environmental factors (Wang et al., 2011). The appearance of rice grain was found to deteriorate greatly under eCO_2 compared to aCO_2; for example, chalkiness was increased due to eCO_2 (Terao et al., 2005; Usui et al., 2016; Wang et al., 2011; Yang et al., 2007).

Increases in the whiteness of the grain colour of brown rice (unpolished) were reported at eCO_2, which would achieve higher market value and are considered to be a beneficial effect of eCO_2 (Goufo et al., 2014a; Usui et al., 2016). Seed colour and lipoxygenase activity are negatively correlated (Borrelli et al., 1999); Goufo et al. (2014a) argued that rice grown under eCO_2 had a higher concentration of fatty acids with lower lipoxygenase activity, in turn improving the whiteness of the grain. However, Terao et al. (2005) suggested that the increase in the whiteness of the grain was because of the decrease in protein content (PC) at eCO_2 (indicated by a strong negative correlation, r = -0.874, between PC and whiteness of the grain).

Chalkiness, indicated by an opaque appearance of grain, is an undesired characteristic for the market. In cultivated rice, eCO_2 in most cases increased the chalkiness of the grain (Usui et al., 2016; Wang et al., 2011; Ward, 2007) and has been negatively associated with head rice yield; i.e., the mass percentage of milled rice kernels that are considered 'whole', normally including rice grains with ≥ 75% of their original length after milling (Badi and Osamu, 2013; Cooper and Siebenmorgen, 2007). Chalky grains are prone to milling damage because the starch granules are loosely packed in grains with substantial airspaces. The increased chalkiness might be due to the low starch granule density of grains, making them prone to milling damage such as decreases in head rice percentages. The average reduction in head rice and milled rice percentages at eCO_2 were 8.03% and 5.23%, respectively (Jing et al., 2016a). Others reported higher reductions in head rice percentages at eCO_2, ranging from 12 to 21%, (Biswal and Swain, 2019; Satapathy et al., 2014).

Broken rice caused by chalkiness could be reduced by applying a higher dose of N fertilizer during the late growth stage to grain formation at eCO_2. This would increase the N content of the grains and the hardiness of the seeds, in turn reducing milling damage. A possible explanation for an increase in damaged grain might be the increased canopy temperature at eCO_2 and grain filling (Usui et al., 2014; Yoshimoto et al., 2005). However, several other studies reported conflicting results (Madan et al., 2012; Ward, 2007; Xu et al., 2008). In FACE experiments in China, there was no effect of N × CO_2 interaction obtained for milled rice and head rice percentage (Yang et al., 2007).

Milling alters grain appearance by increasing the whiteness, making the grain attractive to customers at the cost of protein, vitamins and minerals. Higher PC in the rice kernel prevents milling damage (Leesawatwong et al., 2004; Leesawatwong et al., 2005). Mirroring this, lower PC in grains at eCO_2 makes them vulnerable during milling (Goufo et al., 2014a). Madan et al. (2012) reported grain width increase at eCO_2, resulting in improved milling quality and decreased broken grain percentages. The increase in grain width at eCO_2 could be due to the higher supply of photosynthetic assimilate to the panicle. Despite a significant increase in length (1.65%) and width (1.86%), the milling percentage was reduced by eCO_2 (Jing et al., 2016a), which could be due to genotype and environmental interactions. Milling helps in cooking by decreasing gelatinization temperature as well as improving flavour, resilience, chewiness and stickiness of cooked rice (Muramatsu et al., 2006).

Cooking is the dominant processing method that affects rice-eating quality. Eating quality is extremely important because it can be a driving force in the market price. The cooking process and eating quality are influenced by certain physiochemical characteristics such as degree of swelling, cooking time, water absorption and volume expansion, resilience, softness, AC, gel consistency, gelatinization temperature and the starch pasting properties of cooked rice (Wang et al., 2011). Starch and storage protein in the endosperm are the main components of the rice grain that determine cooking quality (Chen et al., 2012). However, only small or no changes in cooking and eating quality have been observed due to eCO_2 (Wang et al., 2011).

1.7.2. Gelatinization temperature, gel consistency and pasting properties

Gelatinization temperature and gel consistency are two important parameters determining rice cooking quality. Gelatinization temperature determines the cooking time; i.e., the temperature at which 90% of the starch granules have swelled irreversibly in hot water (Allahgholipour et al., 2006; Faruq et al., 2004; Kiani et al., 2008). Gel consistency measures the stickiness and tenderness of the cooled paste of cooked rice flour and is used for evaluating the cooked rice texture. A higher value for gel consistency is preferred because it indicates softer consistency and a higher degree of tenderness of the cooked kernel (Allahgholipour et al., 2006; Kiani et al., 2008).

Pasting properties are important for the uses of rice in the food and beverage industry (Kasem et al., 2009), and are measured using a rapid visco analyser (RVA). The three most important parameters in rice paste viscosity are the peak viscosity after gelatinization (PV), the viscosity at the end of a 95 °C holding period (TV, trough viscosity) and the final viscosity (FV) at the end of the test (Fig. 1.7). PV minus TV gives the breakdown (BD) value, while FV minus TV gives the setback (SB) value. The BD value is indicative of the fragility of the starch granules and is a good index for studying the susceptibility of starch to disintegration (Juliano, 1985), whereas the SB value indicates the degree of retrogradation or hardening of starch upon cooling (Zulueta et al., 2000).

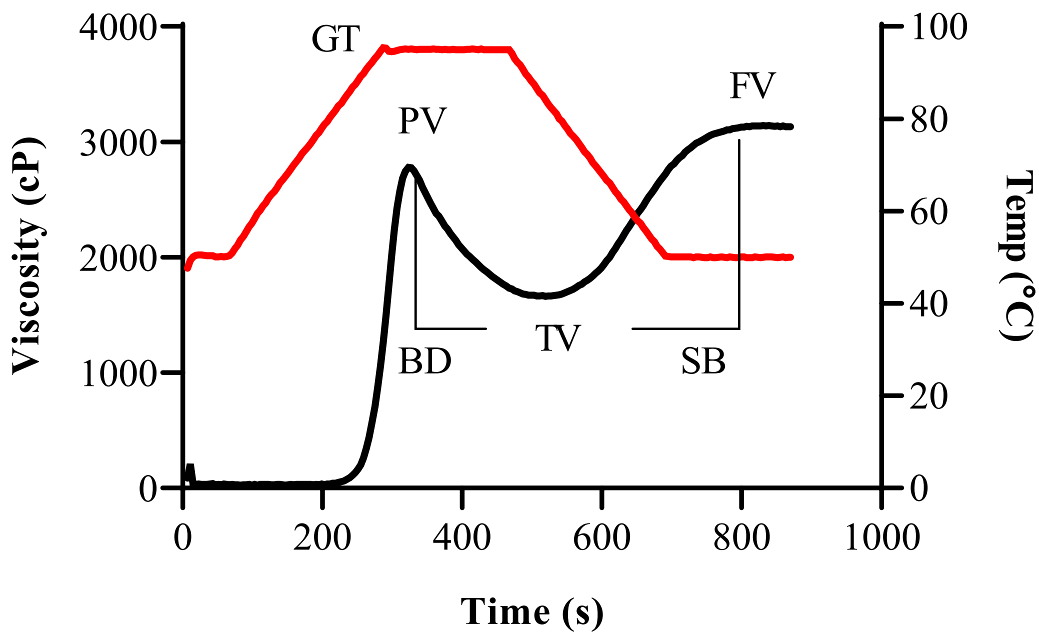


Fig. 1.7. A typical RVA viscogram of rice flour (*O. sativa*) showing gelatinization temperature (GT), and peak (PV), trough (TV), and final (FV) viscosity. Derivative parameters, breakdown (BD=PV-TV) and setback (SB=FV-TV) are calculated from RVA viscograms. Source: Rahman et al. (2021a).

Different studies have reported contradictory effects of plant growth at eCO_2 on starch gelatinization temperature, including no change (Ward, 2007; Xu et al., 2008), decrease (Uprety et al., 2010; Zhang et al., 1998) and increase (Dong et al., 2002; Yang et al., 2007). Elevated atm-

CO_2 did not change gel consistency (Dong et al., 2002; Yang et al., 2007; Zhang et al., 1998). The thermal and pasting properties, chemical composition and cooking properties of wild rices (*O. australiensis, O. alta, O. meridionalis, O. officinalis, O. rufipogon, O. spontanea, O. latifolia*) appear to be more similar to those of *O. sativa* indica varieties compared to japonica varieties (Kasem, 2012).

Negative relationships between PC and PV were also observed (Seneweera et al., 1996; Terao et al., 2005). A higher PV and BD with a decreased SB is associated with softness and palatability in cooked rice (Terao et al., 2005; Xu et al., 2008; Yang et al., 2007). RVA-derived SB has been reported to be both increased (Seneweera et al., 1996) or decreased (Terao et al., 2005) at eCO_2, both results being due to changes in PV rather than FV. Ward (2007) observed no effect of atm-CO_2 on SB and there was no correlation between N content and PV.

1.7.3. Amylose content

Amylose (AM) and amylopectin (AP) are the key components of starch, determining the processing, eating and cooking quality of rice (Bao, 2019; Bergman, 2019). Most investigations into the effect of eCO_2 on rice grain quality have focused on waxy rice. Based on amylose content (AC), rice is classified as waxy (0–8%), low (8–16%), intermediate (16–24%), and high (> 24%), (Allahgholipour et al., 2006). In a previous study, AC was found to be higher in Australian wild rices, *O. australiensis* and *O. meridionalis*, compared to domesticated rice (Tikapunya et al., 2017). A higher AC was reported in rice under eCO_2, increasing the firmness of the cooked rice (Seneweera et al., 1996; Seneweera and Conroy, 1997b; Uprety et al., 2010). However, other groups have reported either decreases (Goufo et al., 2014b; Yang et al., 2007) or no changes in AC at elevated CO_2 (Terao et al., 2005; Ward, 2007; Xu et al., 2008; Zhang et al., 1998; Ziska et al., 1997), indicating varietal dependence rather than eCO_2 effect alone. There is no report available on the effect of eCO_2 in any wild rices.

1.7.4. Crude protein content

Besides starch, other factors such as the protein content (PC) contribute to the sensory and functional characteristics of rice products. In cultivated rice, the PC falls in the range of 5–11%

(Ward et al., 2019). The AC is primarily responsible for the firmness of the cooked rice (Seneweera et al., 1996); however, the surface hardness of cooked rice is determined by PC as demonstrated by Okadome et al. (1999). The cooking and eating quality in rice is negatively correlated with PC (Goufo et al., 2014a). However, Martin and Fitzgerald (2002) demonstrated that PC influences the viscosity of rice flour through binding water and a network linked by disulfide bonds.

The PC of wild rice (*O. australiensis, O. meridionalis, O, alta, O, nivara, O. rufipogon, O. officinalis, O. spontanea, O. latifolia*) was found to be higher than cultivated rice (*O. sativa* cv. Doongara*,* cv. Amaroo*,* and cv. Shimizu) (Kasem, 2012). Wild species also had higher concentrations of a range of minerals than the cultivated species (Kasem, 2012). From the above findings, it is possible to conclude that grains of Australian native rices may be higher in nutritional value compared to domesticated rice.

The total PC of the grains was found to be lower when rice plants (*O. sativa*) were grown at eCO_2 (Chen et al., 2015; Taub et al., 2008; Terao et al., 2005; Ujiie et al., 2019; Uprety et al., 2010; Wang et al., 2011; Yang and Wang, 2019; Ziska et al., 1997). No significant effect of eCO_2 on milled grain PC was observed, even when plants were grown with a high N supply (350 kg N ha^{-1}) (Yang et al., 2007). It was concluded that grain protein content would be unaltered due to climate change (Ward, 2007). Wild rice is not only high in protein but also a good source of antioxidants, vitamins and minerals (Bergman et al., 2011; Umar et al., 2013).

1.7.5. Mineral content

In addition to starch and proteins, minerals are important for human health, especially for low-income countries, where people consume rice as a principal source of nutrient supply (Ujiie et al., 2019; Yang and Wang, 2019). Rice contains a wide range of micronutrients; for example, iron (Fe), zinc (Zn), copper (Cu), manganese (Mn) and selenium (Se), which are essential for human health (Chen et al., 2012; Norton, 2019; White and Broadley, 2009). Mineral content in rice grains is correlated with PC and free amino acids as well as cooking traits. Gel consistency was found to be highly correlated with K, Cu, and Mn contents in the grains, and AC with Na, K, Mn, Cu, and Mn (Chen et al., 2012). Grain minerals are shown to have negative relationships with chalkiness and AC (Chaturvedi et al., 2017).

A significant variation in mineral content was reported among species, both japonica and indica, and cultivars of domesticated rice (Huang et al., 2016). There is no published report available on the effect of eCO_2 on minerals in Australian wild rices. Many researchers reported increases in calcium (Ca), iron (Fe), and zinc (Zn) in milled rice due to eCO_2 (Goufo et al., 2014a; Lieffering et al., 2004); however, the bioavailability of Fe was higher while Ca and Zn were unaffected (Goufo et al., 2014a). In contrast, Fe and Zn content decreased in brown and milled rice grains at eCO_2 (Chaturvedi et al., 2017; Jena et al., 2018; Myers et al., 2014; Ujiie et al., 2019; Zhu et al., 2018), which could be due to genetic differences for the rice samples used in these studies. Likewise, Yang et al. (2007) reported a decrease in copper (Cu) content by eCO_2. Boron (B) content in rice grains was also found to be greater at eCO_2 (Lieffering et al., 2004; Myers et al., 2014). The grain levels of some other macro and microelements, including phosphorus (P), potassium (K), magnesium (Mn), and manganese (Mn) were positively affected by eCO_2 (Lieffering et al., 2004).

Many other reports have shown both positive and negative effects of eCO_2 on rice grain minerals (Seneweera et al., 1996; Seneweera and Conroy, 1997b; Wang et al., 2011), while a decrease in all elements measured (P, Ca, S, Mg, Fe, Zn, Mn, and Cu) was obtained in wheat and a subset of these in rice grains due to eCO_2 (Loladze, 2002, 2014). A decrease in elemental concentration in rice, even by a few percentages, could have major negative effects on the health and economy of Asia, where people consume rice is as a staple food for total energy and nutrient supply (Loladze, 2002; Norton, 2019).

1.7.6. Endosperm morphology

The morphology of the endosperm (aleurone and starch granules) of cereal grains influences the behaviour of rice grains and flour in processing operations. Endosperm components and composition play an important role in determining the nutritional value of rice. Microscopic investigations of endosperm morphology using light, scanning and transmission electron microscopy have enabled a detailed understanding of the structural and chemical properties of cereal grains (Dang and Copeland, 2003; Dang and Copeland, 2004b).

In the production of polished rice, the pericarp, aleurone layer, and germ are discarded as bran, which is ~5–7% of the whole grain. The pericarp and aleurone layer contain a significant proportion of nutrients and vitamins in cereal grains; e.g., ~3% Fe, ~2% Zn, ~3% fibre (Juliano, 1993). Variation in morphology of the pericarp and aleurone layer affects the nutritional value of the grains for end-users (Shapter et al., 2009). Polyphenols or tannins are at higher levels in grains with a thicker pericarp and have both nutritional and antinutritional effects on human health (Shapter et al., 2009). In rice, the thickness of the aleurone layer varies from one to several layers depending on species, cultivar, and the viewing angle (Kasem et al., 2011; Shapter et al., 2009).

The morphology and structure of starch granules play an important role in the eating and cooking quality of rice (Jing et al., 2021), as well as the pasting behaviour of starch and rice flour (Jing et al., 2016b). For example, in potato starch, smaller starch granules (20.3–23.4 µm) had higher peak viscosity-temperature and lower PV and BD compared to larger granules (39.9–43.7 µm) (Noda et al., 2005); however, in wheat and barley starch, smaller starch granules had higher gelatinization temperature (Chiotelli and Le Meste, 2002; Myllärinen et al., 1998). It was suggested that smaller starch granules can be used in making laundry stiffening agents, cosmetic ingredients, fine printing papers and textile and photographic industries, while larger starch granules can be used in making noodles (Lindeboom et al., 2004; Low et al., 2020; Wani et al., 2012). So far, the only report published on the effects of eCO_2 on rice endosperm showed an increase in the proportion of large starch granules (> 5 µm in diameter), which would affect the flour pasting properties of rice as described earlier (Jing et al., 2016b). There is no published report available on the eCO_2 effect on aleurone layer and starch granules morphology in any wild rice species.

1.8. *Conclusion*

Of the ~24 wild rice species globally, two species, *O. australiensis* and *O. meridionalis* are endemic to Australia and grow in Queensland, the Northern Territory and Western Australia. The Asian domesticated rice, *O. sativa*, dominates world rice cultivation and has greater genetic diversity than the African rice (*O. glaberrima*), albeit far less than found among the wild relatives of *Oryza*. The genetic diversity narrowed in *O. sativa* over thousands of years of selection, during which higher yield was pursued rather than other important traits.

The concentration of atm-CO_2 has increased more than 20% over the last 40 years alone. The primary effect of eCO_2 on rice plants includes higher photosynthetic rates (Kang et al., 2021; Sakai et al., 2019) since the current level of atm-CO_2 is limiting (Long et al., 2006b). In C_3 plants eCO_2 is expected to increase the photosynthetic rate of CO_2 uptake through carboxylation (Fabre et al., 2020; Long, 1991; Weber and Bar-Even, 2019). Increases in atm-CO_2 stimulate ribulose 1,5 bisphosphate carboxylase/oxygenase (Rubisco) and inhibit carbon loss via photorespiration (Long et al., 2006b; Ziska et al., 1996). Crop yield and plant biomass increase at eCO_2 due to increases in crop growth, tiller number, *LAI*, panicle size, and grain mass is well established (Ainsworth, 2008; Baker et al., 1990; Gao et al., 2021; Hu et al., 2021; Wang et al., 2015; Wang et al., 2020). However, vigorous growth could increase self-shading caused by denser canopies, leading to variable responses of crop yield; for example, in a previous study with *O. sativa*, elevated CO_2 increased yield by > 35% in one experiment but in another experiment yield increased as little as 3% (Sakai et al., 2019; Yang et al., 2009). Furthermore, wild species of *Oryza* showed variation in growth rates to eCO_2, though leaf photosynthetic rates were similar across genotypes (Taylor, 2014). The photosynthetic rate of the crop canopy depends on many factors, such as weather, temperature, leaf age and plant development; moreover, the photosynthetic rate is greatly dependent on light intensity and, in turn, the light intensity within crop canopies (Burgess et al., 2017; Kang et al., 2021). Light interception is highly dependent on canopy architecture, including tiller number, tiller angle, leaf angle, size and shape, leaf number and leaf arrangement.

The two Australian wild rices, *O. australiensis* and *O. meridionalis*, have erect and spread leaf canopies. The phenotypic variability in the canopy architecture of these genotypes provides new territory to investigate the full range of canopy phenotypes within the genus *Oryza*, defining light capture and photosynthetic parameters, which would help redesign canopy architecture for higher photosynthesis with better light capture efficiency, and finally higher yield.

Elevated atm-CO_2 will not only affect canopy architecture parameters but will also affect grain nutritional quality. Increases in photosynthesis mean higher accumulation of photosynthates, which will be translocated to the caryopsis resulting in higher grain mass; however, reduction of the grain quality is widely reported. Grain quality includes appearance and processing quality, eating quality, nutritional quality and physicochemical properties.

The milling process and appearance of rice grain are highly influenced by atmospheric CO_2 enrichment. Elevated CO_2 increases the chalkiness of the grain. Chalkiness is caused by softer grain due to incomplete grain filling at eCO_2. Chalky grains are prone to milling damage. The increase in the whiteness of brown rice caused by eCO_2 might be due to the increased fatty acid content or increased carbohydrate content in seeds, diluting the PC and other material involved in the whiteness of the grain. The chalkiness of the grains had both increased and decreased responses to eCO_2 in *O. sativa*, indicating genotype/varietal dependence.

There is a lot of variation among wild species in starch and AC. The Australian wild rice species, *O. meridionalis* and *O. australiensis*, are higher in AC compared to domesticated rice (Henry, 2019; Tikapunya et al., 2017). However, *O. officinalis*, O. *granulata*, and *O. rufipogon* had lower AC than *O. sativa* (Cheng et al., 2005), which could increase softness and taste of these wild rices (Cheng et al., 2012). Elevation of atm-CO_2 increased AC in *O. sativa*, resulting in increased firmness of cooked rice (Seneweera et al., 1996). However, in other instances, amylose content decreased at eCO_2. Cooking properties and eating quality displayed variable responses (increases, decreases or no change) to eCO_2. Pasting properties also showed variable responses in *O. sativa* indicating varietal differences as well as interaction effects with eCO_2.

Protein content is higher in wild rice than in cultivated rice. Elevated CO_2 caused deterioration of protein content (~8−27%) in *O. sativa*, which will improve the cooking and eating quality of rice (Goufo et al., 2014a; Yang and Wang, 2019). Amino acid composition is also an important factor determining the nutritional quality of rice. In two separate studies, the concentration of 19 and 15 amino acids decreased by 30–40% (total) due to increase atm-CO_2 (Goufo et al., 2014a; Zhang et al., 1998). There are no corresponding reports available for the wild relatives of *Oryza.* Increases in total saturated fatty acid and decreases in unsaturated fatty acids would be expected to be due to atm-CO_2 enrichment. Oil content was slightly reduced in rice bran at eCO_2 (Goufo et al., 2014a).

From this literature review, it can be concluded that, in rice, eCO_2 will increase photosynthesis resulting in increased plant biomass, grain mass and grain yield. The physiological changes in rice plants due to eCO_2 as well as genotype × CO_2 interaction could also have a variable effect on grain quality and nutrition. Most of the research on eCO_2 effects has concentrated on cultivated species.

The Australian wild rices, *O. australiensis* and *O. meridionalis*, avoided the impact of domestication of rice in Asia, possess novel alleles, and can be exploited as a source of the primary gene pool for desired traits such as erect habit, disease resistance, grain size and unique starch properties (Henry, 2019). These wild species can be used as sources of desired genes to be integrated into cultivated rice through conventional breeding via interspecific hybridization or other more advanced breeding techniques (Brar and Khush, 2018; Henry, 2019).

1.9. *Aims of the PhD project*

Prior to this PhD project, there was little or no research available on the impact of eCO_2 on photosynthesis, light interception, canopy architecture, and grain quality including endosperm morphology of the Australian wild rices. These topics are linked because, as atm-CO_2 rises, there is concern that the protein content of grains will decrease, forced by the increase in the ratio between plant-available C and N. Filling the information gap above is the main motivation for this research project, which is divided into two phases. The first phase is on the effect of eCO_2 on photosynthesis, light interception, carbon gain, and canopy architecture, whereas the second phase is on the effects of eCO_2 on the physical and chemical properties of rice grain, flour pasting properties and endosperm morphology. The outcome of this research will provide information on the impact of eCO_2 on Australian wild rice species, informing the utilisation of these valuable genotypes in the development of new elite rice varieties.

This PhD project aimed to: -

i. develop an understanding of light interception and CO_2 assimilation and its relation to canopy structure and daily carbon gain at eCO_2 in two accessions of *O. meridionalis* (Cape York and Howard Springs) vs. *O. sativa* (cv. Doongara)–***Chapter 2***

ii. compare the effects of eCO_2 on grain physicochemical properties and flour pasting properties of *O. australiensis*, *O. meridionalis* (acc. Cape York and Howard Springs), and *O. sativa* (cv. Doongara)

   a. optimize and validate a methodology to inactivate α-amylase activity in the determination of rice flour pasting properties–***Chapter 3***

b. evaluate the effect of eCO_2 on physicochemical properties of rice grains including flour pasting properties–***Chapter 4***
c. determine the effect of eCO_2 on endosperm morphology–***Chapter 5***

1.10. *Hypotheses*

The general hypotheses tested in this project are: -

1. The Australian wild rices will be affected differently by eCO_2 compared to domesticated rice for photosynthesis, light interception, and canopy architecture. Related specific hypotheses are given in ***Chapter 2***.
2. The eCO_2-mediated changes in photosynthesis, light interception, and canopy architecture (above) will differentially affect the grain physicochemical properties, flour pasting properties and endosperm morphology of the wild and domesticated rices. Related specific hypotheses are given in ***Chapters 3–5***.

Chapter 2

Canopy architecture and light interception of the Australian wild rice, *Oryza meridionalis*

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Leaf canopy architecture determines light interception and carbon gain in wild and domesticated* Oryza *species

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2.1. Introduction

Rice is a critically important food crop, with ever rising productivity essential to sustain human consumption (Khush, 2005). As demand for food grows, factors that compromise vegetative growth and yield will become a pressing concern. Rising atmospheric carbon dioxide concentrations generally lead to enhanced photosynthesis and changes to the whole-plant carbon economy but the degree of growth stimulation depends on how increased leaf growth feeds back on canopy architecture. With an average temperature rise of 0.4 °C per decade predicted (IPCC, 2021), optimal leaf display for maximum carbon gain, heat loss and water use efficiency will require breeding for novel canopy phenotypes. Therefore, while there is a prospect of substantial yield gains in C_3 species such as rice (Ainsworth, 2008; Ainsworth and Long, 2021; DaMatta et al., 2010; Hu et al., 2021; Sakai et al., 2019; Usui et al., 2016; Wang et al., 2020; Yang et al., 2009), defining the most efficient leaf architecture will be essential.

Commercial rice production is dominated by a single species (*Oryza sativa*) albeit one with vast genetic variation (Hu et al., 2021; Khush, 1997; Sakai et al., 2019). However, far greater genetic variation resides in the other ~24 *Oryza* species (Brar and Khush, 2018; Henry, 2019). The genotypic diversity of these wild rice species offers unexplored opportunities for crop improvement, including new options for canopy design.

Net photosynthesis is a strong driver of biomass accumulation and yield (Fabre et al., 2020; Long et al., 2006b; Wang et al., 2020; Yeo et al., 1994). This is demonstrated in experiments where elevated atmospheric CO_2 (eCO_2) consistently stimulates CO_2 assimilation and enhances total yield, plant growth and carbon gain in cultivated rice (Ainsworth and Long, 2021; Hu et al., 2021; Kang et al., 2021; Usui et al., 2016; Wang et al., 2020; Yang et al., 2009; Ziska et al., 1996). However, more vigorous growth raises the possibility of self-shading as leaf canopies become denser. This might partly explain yield variations in FACE (free-air CO_2 enrichment) experiments on *O. sativa* genotypes, where eCO_2 enhanced yield by more than 35% in highly responsive varieties but by as little as 4% in other genotypes (Ainsworth and Long, 2021; Hu et al., 2021; Sakai et al., 2019; Yang et al., 2009). Furthermore, by comparing two wild rice species with *O. sativa*, wide variations in growth rate were observed, even though the response of leaf-level photosynthesis to eCO_2 was very similar across all genotypes (Taylor, 2014). One explanation is that the contrasting leaf display in canopies of each species influenced light capture. This illustrates the importance of factors beyond leaf-level photosynthesis as determinants of yield. Here, I show that eCO_2 levels offer a tool to stimulate leaf growth (in two rice species) and thereby a means to investigate the constraints imposed by canopy density. Moreover, this experiment offered an opportunity to test the impact of rising atmospheric CO_2 (atm-CO_2) concentrations in these divergent species and whether light interception always predicts whole-canopy carbon gain.

There is a growing appreciation of the practical opportunities to modify whole-canopy architecture to optimise light interception (Burgess et al., 2021; Chang et al., 2019; Horton, 2000; Jiao et al., 2010), spurred on by the discovery of genes that confer changes in leaf (Zheng et al., 2008), panicle (Zhu et al., 2010a) and root (Topp et al., 2013; Uga et al., 2013) architecture. For example, structural changes to leaf and tiller orientation could increase light capture (Niinemets, 2010) and ultimately improve yields of annual crops (Burgess et al., 2017). A recent report using five contrasting rice genotypes from a multi-parent advanced generation of *O. sativa* intercrosses (Burgess et al., 2017) revealed strong variation in light interception efficiency; such variation is thus thought to be genetically determined, even between genotypes of *O. sativa*. Phenotypic variation in the canopy architecture of wild Australian *Oryza* species provides promising new territory to investigate the full range of canopy phenotypes within the genus *Oryza*, leading to this first attempt at defining the light capture characteristics of a wild *Oryza* relative. Specifically, I compared the splayed leaf canopies of the wild species *O. meridionalis* with the erect compact canopies of *O. sativa*. To gain further insights, I compared two morphologically contrasting accessions of *O. meridionalis* from geographically distinct regions of the savannah and humid tropics of Australia, each exhibiting high photosynthetic rates and rapid growth during the monsoon (Atwell et al., 2014) but with distinct canopy shape.

The three hypotheses tested in this study arose from the following questions : (i) Can light capture, calculated from digitized images of individual plants, explain the variation in canopy size?; (ii) Is light capture compromised in the dense canopies that develop in eCO_2 levels because of increased self-shading, and can enhanced photosynthetic rates overcome this? and (iii) Does the incidence of light simulated for two seasons (the Summer Solstice and Equinox) affect the estimated carbon gain differentially across species and CO_2 treatments? To address these questions, I digitized the canopies of individual plants and analyzed them using the YplantQMC model to deduce light capture for plants grown at ambient and elevated atm-CO_2 levels. Together with light-response curves, these digitized canopies enabled theoretical estimates of total daily carbon gain by whole plants.

2.2. Material and Methods

Oryza sativa cv. Doongara and two accessions of *O. meridionalis* (acc. Cape York and Howard Springs) were used in this experiment. The *O. meridionalis* accessions were collected from a region with higher temperatures and more erratic monsoon (Cape York) *vs* the more humid tropical climate of Darwin (Howard Springs). Seeds were germinated following the protocol in Taylor (2014) with minor modifications as follows: seeds were surface sterilized by soaking in water (30 min), 50% commercial bleach (available chlorine = 1.4% w/v) for 10 min, rinsed with water (4 × 1 min) and germinated in Petri dishes at 36 °C for 48 h (acc. Cape York and Howard Springs) or 28 °C (cv. Doongara). Doongara was used as control in this experiment because it was developed as an elite variety for Australian conditions. It is a long-grained indica type that bears some phenotypic resemblance to the wild relatives.

Germinated seeds were sown into a finely sieved clay loam previously used for rice cultivation (Scafaro et al., 2010). The soil was fertilized with Nutricote Standard Blue 140 day (total N 16.0, nitrate 6.8, ammonia 9.2; total P 4.4, water-soluble P 2.2; total K 8.3, all % w/w) (Yates Australia, Clayton, VIC, Australia) at a rate of 50 g per 10 kg soil. Plants were grown in 22 × 22 cm 10-L pots, each containing ~8 kg of soil and were watered daily to soil saturation. The large pots were used to prevent root restriction. Plants were fertilized weekly for the first month with 5 g L^{-1} Aquasol (Yates Australia, Clayton, VIC, Australia) then fortnightly at twice this rate. At each event, 200 mL of nutrient solution was added to individual pots. Replicate pots were square, allowing them to be butted together and arranged into a single block for each genotype (20 plants m^{-2}), with boundary pots to avoid edge effects. The experiment was conducted in glasshouses with 28/22 °C (12-h day/night cycle) from mid-spring to late-summer (September 2014 to February

2015) using Daikin reverse cycle unit with a 1 °C tolerance (Daikin Australia Pty Ltd. Chipping Norton NSW). Relative humidity was between 50 and 70%.

Plants were grown in a combination of natural and supplementary lighting provided by 400 W Phillips Contempra high pressure sodium lamps (20 lamps mounted at height 2.4 m in 27 m^2 glasshouses), adding 400 µmol m^{-2} s^{-1} to the natural photosynthetically active radiation. Lights switched on automatically throughout a 12-h day whenever the ambient light level fell below 400 µmol m^{-2} s^{-1}. Furthermore, the experiment was conducted in glasshouses at ambient (aCO_2, 400 ppm) or elevated (eCO_2, 700 ppm) levels of atm-CO_2. Elevated atm-CO_2 levels were held within a range of ± 50 ppm from 15 d after sowing using solenoids attached to an external gas line and monitored continuously (The Canary Company Pty Ltd NSW gas detection and monitoring systems). Food-grade CO_2 gas was supplied from 'Gasmatic' bulk cylinders (BOC Limited, North Ryde, NSW). Four replicates of each genotype were grown for each genotype and CO_2 treatment. Microclimatic effects were avoided by rotating plants within the glasshouse fortnightly until digitization of the canopy.

Numbers of tillers and leaves were counted for individual plants at the time of digitization (100–115 d after sowing, DAS). After digitization, the plants were harvested and leaf area was recorded using an LI-3100 Area Meter (LI-COR, Lincoln, Nebraska, USA). Shoot fresh and dry biomass were measured, and leaf area to shoot dry biomass ratio (specific leaf mass, *SLM*) was calculated.

2.2.1. Effect of elevated CO_2 on CO_2 assimilation and canopy architecture

2.2.1.1. Photosynthesis and related gas exchange variables

A portable photosynthetic system, LiCor-6400XT (LI-COR, Lincoln, Nebraska, USA), was used to measure CO_2 assimilation rates. Block temperature was set to 28 °C, relative humidity to 50–70% and chamber CO_2 concentration to 400 µmol mol $(air)^{-1}$ and 700 µmol mol $(air)^{-1}$ to match the growth conditions at ambient and elevated CO_2 concentrations, respectively. CO_2 assimilation rates were measured on fully expanded healthy leaves (3rd youngest leaf) between 9.30 am and 3.00 pm from 100–115-d-old plants at 16 levels of photosynthetically active radiation (*PAR*) in four replicates. The auto program function was used to set *PAR* at 0, 10, 20, 40, 60, 120, 250, 500, 750, 1000, 1250, 1500, 1750, 2000, 2100, and 2200 µmol m^{-2} s^{-1} (upward direction). Stabilization time between *PAR* treatments was six minutes and matching CO_2 concentration at ± 50 µmol (CO_2) mol (air)$^{-1}$ in the infrared gas analyzer (IRGA) between the sample and reference value, which allowed them to be matched before every change in *PAR*. GENSTAT (VSN International) was used to calculate analysis of variance for repetitive measures (ANOVAR) to compare the CO_2 assimilation rates (Potvin et al., 1990).

Light-saturated assimilation rate (A_{max}), dark respiration rate (R_d), quantum yield (φ) and theta (Θ) (curvature factor) were estimated by fitting a non-rectangular hyperbola equation (Prioul and Chartier, 1977) (Eq. 1).

$A_N = [[\varphi \times I + A_{max} - [(\varphi \times I + A_{max})^2 - 4\Theta \times \varphi \times I \times A_{max}]^{0.5}] / 2\Theta] - R_d$ (Eq. 1)

where A_N = net photosynthetic rate [µmol (CO_2) m^{-2} s^{-1}]; φ = quantum yield [µmol (CO_2) µmol^{-1} (photon)]; I = PAR or photosynthetic photon flux density [µmol (photon) m^{-2} s^{-1}], A_{max} = asymptotic estimate of the light-saturated photosynthetic rate [µmol (CO_2) m^{-2} s^{-1}]; Θ = convexity of the curve (dimensionless); R_d = dark respiration rate [µmol (CO_2) m^{-2} s^{-1}].

A_{max}, R_d, Θ and φ were calculated for every plant sampled using the non-linear function in MATLAB (MathWorks) and analyzed statistically with JMP Pro software. All these parameters were bootstrapped (Christie, 2004) to draw a light response curve (*LRC*) in MATLAB, which was then used in YplantQMC (Duursma and Cieslak, 2013) for plant simulation and daily carbon gain, as described in the next section.

2.2.1.2. Three-dimensional (3D) digitization and canopy architecture

2.2.1.2.1. Digitization of the canopy

Plants were digitized 100–115 d after sowing using a FASTRAK® 3D-digitizer (Polhemus, Colchester, VT, USA) and Floradig software (Hanan and Room, 2002; Hanan and Wang, 2004) between 24 December 2014 and 15 January 2015, following the protocol as described by Pearcy et al. (2011). Four points on each leaf and the location of the leaf on the subtending stem were recorded with a stylus, which translated the data into 3D coordinates by interaction with an adjacent magnetic digitizer. An individual leaf of each species was then digitized using 25 reference points to generate a reproducible leaf shape.

2.2.1.2.2. Construction of a 3D plant model

Using these reference points established for each leaf, the whole-canopy leaf display (single plant) could be reconstructed into a 3D architecture using the plant model YplantQMC (Duursma and Cieslak, 2013) to reconstruct the 3D display of leaf surfaces. YplantQMC uses a QuasiMC ray-tracer (Cieslak et al., 2008), which estimates the quanta and quality (spectral composition) of light reaching each leaf. The ray-tracer does this by simulating the path of photons from a light source towards the virtual plant canopy, considering the scattering of radiation due to reflection and transmission of light by the leaves. For this study, a reflection value of 0.10 and transmittance 0.05 was used. Using the 3D digitized canopy, displayed leaf area was calculated for 160 sectors of the hemisphere, corresponding to eight classes of azimuth and 20 solar elevation classes (Pearcy and Yang, 1996). From these data, a hemispherically averaged STAR (i.e., $\overline{STAR}$) was calculated by weighting each estimate of displayed leaf area by the relative area of each sector. An estimate of light capture by the canopy throughout a day of prescribed length, and therefore season, could then be combined with photosynthetic measurements using the Y-plant model (Pearcy and Yang, 1996). Thereby, total carbon gain was estimated by integrating rates of net photosynthesis over the diurnal cycle for individual leaves. The light response curves calculated above were applied to all leaves in the canopy, notwithstanding their age, position in the canopy or prior light exposure. While variation in A_{max} could be expected in different leaves, this could not be accounted for in such dense canopies and considering that any vertical gradient in A_{max} in the canopy would be similar for all mature rice plants studied. Net photosynthesis was estimated from species- and treatment-specific light response curves and the estimated diurnal courses of photosynthetic photon flux density ($PPFD$) for each leaf — see Pearcy et al. (2011) for details.

The plant canopy generally refers to the aboveground plant biomass, which consists of leaves, branches, stems, fruits and flowers (Campbell and Norman, 2012). ‘Canopy’ can refer to the extent of the shape of the entire leaf display of an individual plant or a group of plants. The plant crown refers to the architecture of the canopy, taking into the account variables used to analyse light penetration/ interception/ efficiency. Simply, the plant crown can be defined by the number, size and shape of individual leaves and how leaves are oriented in the crown to cause them to overlap ('self-shade') when viewed from a given direction (Duursma et al., 2012). The ratio of projected to total leaf area, also known as the silhouette to total area ratio ($STAR$) [Oker-Blom and Smolander (1998), as cited in Farque et al. (2001)] was extended by Duursma et al. (2012) to reflect the interception of diffuse light, ($\overline{STAR}$; i.e. $STAR$ averaged over all viewing angles), as occurs in the natural environment.

2.2.1.2.3. Determination of performance parameters of the canopy

Using YplantQMC, $\overline{STAR}$ was calculated, which quantifies light capture, and relates directly to the amount of direct and diffuse sunlight intercepted. $\overline{STAR}$ of a three-dimentional leaf canopy of plants varies between 0 and 0.5, depending on how many leaves overlap in the crown (Delagrange et al., 2006; Duursma et al., 2012; Farque et al., 2001; Guzmán, 2015). The amount of diffuse light intercepted by the plants relates directly to $\overline{STAR}$, which in turn affects CO_2 assimilation rate (Ackerly and Bazzaz, 1995; Roderick et al., 2001). Direct light interception also depends on the sunlit leaf area fraction (Campbell and Norman, 2012) and is possibly correlated with $\overline{STAR}$. As an estimate of self-shading, $\overline{STAR}$ can be used to predict whole-plant carbon gain (Falster and Westoby, 2003). Duursma et al. (2012) ascribed 85% of the variation in $\overline{STAR}$ to leaf dispersion and crown density, indicating that these variables should be targeted to explain the variation in light interception.

Light capture of the crown depends on leaf size, leaf angle, crown density and leaf dispersion (Farque et al., 2001). Crown surface area (Ac) with respect to light interception is defined by its three-dimensional convex hull, which contains all the leaves (Duursma and Mäkelä, 2007). The A_C was calculated using YplantQMC, where the *quickhull* algorithm (Barber et al., 1996) in the *geometry* package in R was used to find the convex hull (i.e. a 3D convex hull wrapped around the leaf cloud), and its surface area, spanning the canopy. All coordinates of the leaf edges were used to determine the convex hull (R. C. Team, 2014), which can be defined simplistically as the total surface area of a sheet wrapped around the crown. Crown surface was calculated as the area of the three-dimensional shape constructed by joining all distal points of the plant crown.

Crown density was determined by the ratio of total leaf area (A_L) to crown size (crown surface area, A_C). Both A_C and A_L are important factors determining light capture of plants. Plants with high crown density (A_L/A_C) generally capture less light *in proportion to* the size of their canopy because of self-shading (Duursma et al., 2012; Guzmán, 2015).

Leaf dispersion (β) plays an important role in light interception. Plants with more clumped leaves also have more self-shading and are less efficient at intercepting light. Leaf dispersion was calculated as the ratio of the observed average distance to the five nearest leaves to the expected average distance to the five nearest leaves for a random distribution. Leaf dispersion is positively correlated with $\overline{STAR}$ (Duursma et al., 2012; Guzmán, 2015); thus, plants with more dispersed leaves are more efficient in light interception. Leaves are considered to be randomly dispersed if $\beta = 1$, more clumped if $\beta < 1$, and more regular if $\beta > 1$.

The values obtained for A_{max}, R_d, Θ and φ were used to simulate photosynthetic performance, including the daily C gain.

2.2.2. Statistical analysis

JMP Pro (statistical software from SAS, Lane Cove, NSW, Australia) was used for statistical analysis unless otherwise stated. The random-effect model, REML (Restricted or Residual Maximum Likelihood) approach was used to analyze the data, where replication was treated as a random factor. Means were compared using Tukey's HSD. Regression analysis was performed at 95% confidence interval. For correlations, the Pearson r test ($p = 0.05$) was performed to determine whether two variables are correlated. Graph Pad Prism was used to generate graphs.

2.3. Results

2.3.1. Photosynthesis

Rates of CO_2 assimilation were measured 100–115 d after sowing in the late vegetative/early reproductive stage. Plants grown at eCO_2 (700 ppm) had higher CO_2 assimilation rates (df = 1, *p*

= 0.0001) when compared with plants grown at aCO_2 (400 ppm) (Table 2.1). For both species, eCO_2 raised A_{max} by 30% (Table 2.1). Genotypic and CO_2-induced differences in A_{max}, as well as an interaction between these factors in A_{max} and R_d, were significant (Table 2.1; Fig. 2.1). Significant genotypic differences in A_{max} were observed only at eCO_2, with Doongara and Cape York grown at eCO_2 having a higher A_{max} (42.1 and 37.1 µmol m^{-2} s^{-1}, respectively) than Howard Springs (31.2 µmol m^{-2} s^{-1}) (Table 2.1).

Table 2.1. A_{max}, R_d, theta (Θ) and phi (φ) calculated from CO_2 assimilation rates at 16 levels of *PAR* (0–2200 µmol m^{-2} s^{-1}) using a non-rectangular hyperbola equation of *O. meridionalis* (acc. Cape York and Howard Springs) and *O. sativa* cv. Doongara. Plants were grown at ambient (400 ppm) and elevated CO_2 (700 ppm) with supplementary lights to maintain light levels above 400 µmol m^{-2} s^{-1} during the day. Light-saturated CO_2 assimilation rate was measured on the third youngest fully expanded leaves of 100–115-d-old plants.

Source (Genotype × CO_2)	**CO_2 concentration**	**A_{max}**		**R_d**		**theta (Θ)**	**phi (φ)**	
O. meridionalis								
acc. Cape York	ambient CO_2	29.5	c	0.85	b	0.163	0.067	c
acc. Howard Springs	(400 ppm, aCO_2)	26.1	c	1.01	ab	0.243	0.091	ab
O. sativa								
cv. Doongara		27.9	c	1.24	a	0.108	0.085	bc
O. meridionalis								
acc. Cape York	elevated CO_2	37.1	ab	1.22	a	0.121	0.089	ab
acc. Howard Springs	(700 ppm, eCO_2)	31.2	bc	1.10	ab	0.189	0.109	a
O. sativa								
cv. Doongara		42.1	a	0.97	ab	0.058	0.091	ab
p, df = 2 (Genotype)		0.01		ns		ns	0.001	
p, df = 1 (CO_2)		0.0001		ns		ns	0.001	
p, df = 2 (Genotype × CO_2)		0.05		0.02		ns	ns	

Comparisons not sharing the same letter are significantly different, ns = not significant
(Note: A_{max} = light-saturated photosynthesis, R_d = Dark respiration, theta (Θ) = curvature factor and phi (φ) = quantum yield).

Cape York was the only genotype in which R_d increased at eCO_2 (Table 2.1). There were highly significant differences in quantum yield (φ) between genotypes and CO_2 treatments (both at p = 0.001), with Cape York having a notably low φ at aCO_2 (Table 2.1); there was no explanation for these differences but a connection between canopy shape and φ has recently been reported (Burgess et al., 2017).

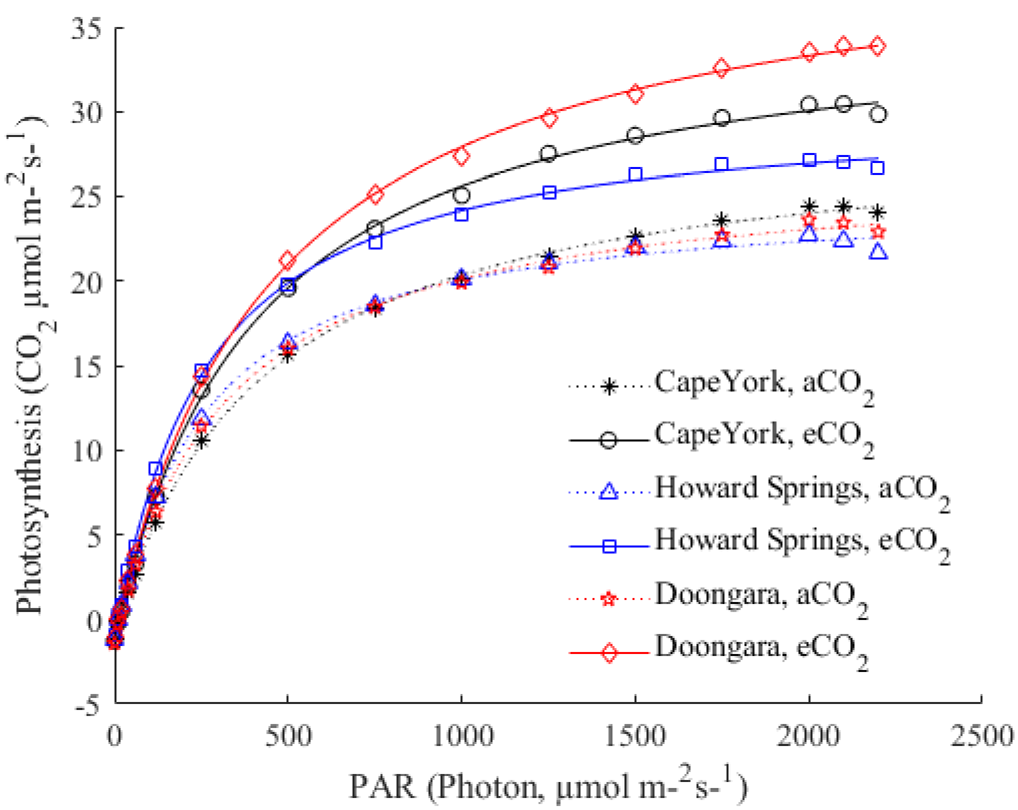


Fig. 2.1. Light response curves (*LRC*) for different rice genotypes were measured at two levels of CO_2. Estimation of A_{max} was performed as described in Eq. 1 and used in combination with light interception data to calculate total daily C gain for different genotypes under two levels of CO_2.

2.3.2. Growth, architecture, and light interception

Individual plants were digitized, and three-dimensional images were generated (Fig. 2.2) to study the effect of genotype and CO_2 concentration on morphology, light capture and simulated total carbon gain.

Tiller number, leaf number, leaf area and fresh biomass were approximately doubled when plants were grown at eCO_2 (Table 2.2). There was no interaction between genotype and CO_2 treatment for any of the growth variables, indicating a uniform physiological response to higher CO_2 concentrations. Genotypic variations in *SLM* were significant but eCO_2 had no significant effect on *SLM*. There was a significant interaction between genotype and CO_2 treatment for *SLM*, with Doongara the only genotype to respond positively in eCO_2 (Fig. 2.3).

Significant interactions between genotype and CO_2 were observed for two measures of canopy structure (Table 2.3), namely whole-plant leaf area (*LA*), and crown density (A_L/A_C) derived from YplantQMC. The average *LA* was significantly greater in wild rice genotypes than in cultivated rice (Table 2.3). The *LA* of all three genotypes increased by at least two-fold when plants were grown in eCO_2, with Howard Springs having a final leaf area of 4.23 m^2 per plant (Table 2.3). As well as *LA*, eCO_2 significantly enhanced A_L/A_C, because crown surface area (A_C) was unaffected by CO_2 (Fig. 2.2, B). Crown density was significantly higher in Howard Springs at eCO_2 (0.41 m^2 m^{-2}) than in the other genotypes (Table 2.3). The lowest A_L/A_C was recorded in 'Cape York' (0.12 m^2 m^{-2}) grown at aCO_2, significantly lower than A_L/A_C in any of the three genotypes when grown at eCO_2. The mean leaf angle was estimated for the three genotypes from digitized canopies, with leaves of Doongara displaced 14° from the vertical, while the wild species were up to 17° from the vertical (df = 2, $p = 0.024$) (Fig. 2.4).

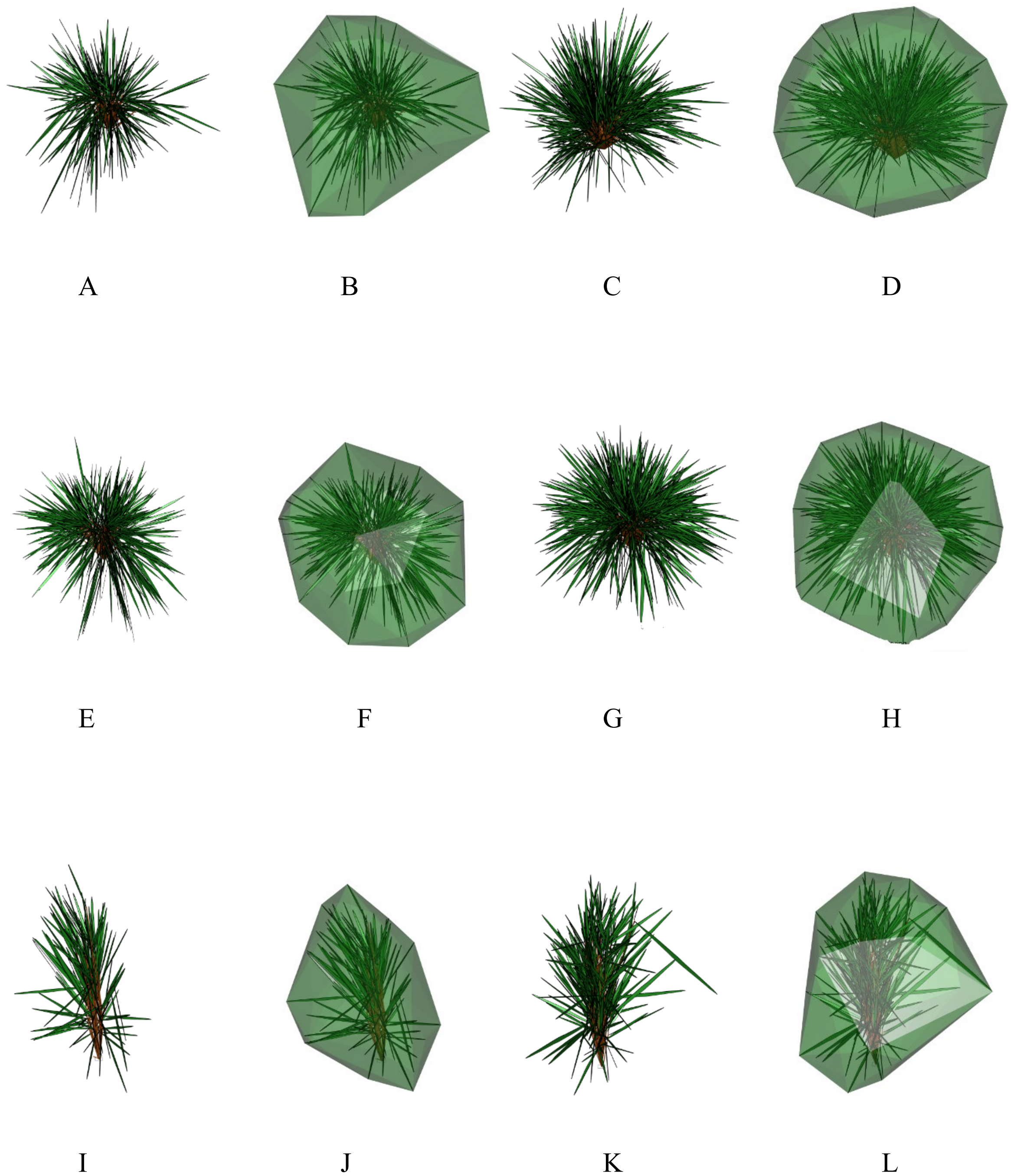


Fig. 2.2. Three-dimensional images were generated from digitized plants of *O. meridionalis* (acc. Cape York and Howard Springs) and *O. sativa* (cv. Doongara). A, C, E, G, I, K, 3-D images; B, D, F, H, L, Crown hull.

Table 2.2. Morphological characters of *O. meridionalis* (acc. Cape York and Howard Springs) and *O. sativa* cv. Doongara were measured after harvesting 100- to 115-d-old plants. Plants were grown in ambient (400 ppm) or elevated (700 ppm) levels of CO_2 as described in Table 2.1.

Source	Tillers		Leaf number		Leaf area (m^2)		Fresh biomass (g)	
Genotype								
acc. Cape York	120	a	464	a	1.15	a	803	ab
acc. Howard Springs	126	a	529	a	1.39	a	978	a
cv. Doongara	44	b	207	b	0.48	b	673	b
CO_2 Treatment								
ambient CO_2 (400 ppm)	64	b	245	b	0.69	b	559	b
elevated CO_2 (700 ppm)	130	a	555	a	1.32	a	1076	a
p, df = 2 (Genotype)	<0.0001		<0.0001		<0.0001		0.0032	
p, df = 1 (CO_2)	<0.0001		<0.0001		<0.0001		<0.0001	
p, df = 2 (Genotype × CO_2)	ns		ns		ns		ns	

Levels not connected by same letter are significantly different, ns = not significant

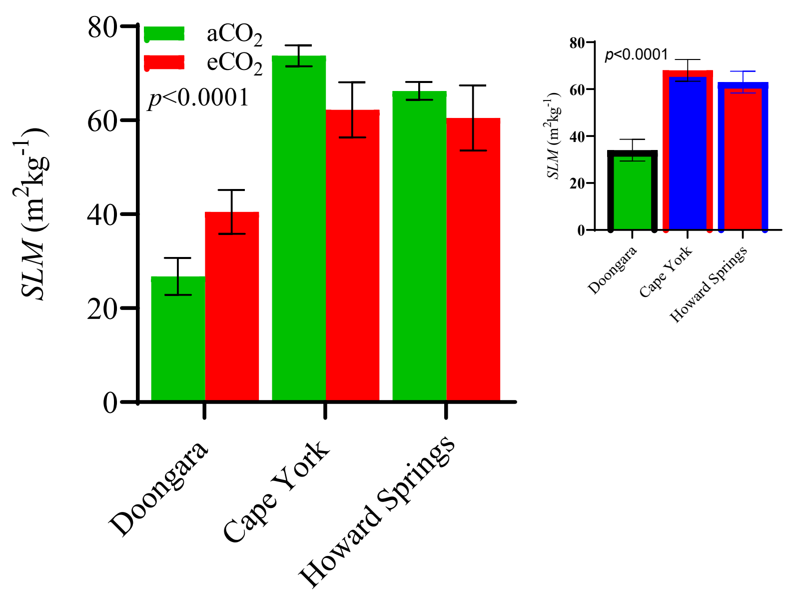


Fig. 2.3. Genotype × CO_2 interaction for Specific leaf mass (*SLM*) showing the differential interactions in *O. sativa* cv. Doongara, and two accessions of *O. meridionalis*, acc. Cape York, and Howard Springs (Mean ± SE). The embedded figure shows the genotypic variations on *SLM* between wild and domesticated rice.

$\overline{STAR}$ was significantly lower for Howard Springs than for Cape York and Doongara (Table 2.3). For all genotypes combined, plants grown in eCO_2 also had significantly lower $\overline{STAR}$ (0.27 m^2 m^{-2}) than those grown in aCO_2 (0.35 m^2 m^{-2}). Genotype and CO_2 levels did not interact significantly in their effect on $\overline{STAR}$ (Table 2.3). Larger, denser leaf canopies are reported for both species growing in eCO_2 compared with aCO_2 (Fig. 2.2), along with more self-shading and lower $\overline{STAR}$ (Fig. 2.5). Doongara and Cape York were more efficient at intercepting light than Howard Springs ($p = 0.0025$; Table 2.3).

Crown density responded differentially to CO_2 treatments in wild and cultivated genotypes ($p = 0.033$ for genotype × CO_2 interaction), with wild genotypes having higher A_L/A_C (denser leaf canopies) than the cultivated genotype (Table 2.3). Because Howard Springs had the highest A_L/A_C, it had the lowest $\overline{STAR}$ both at ambient and elevated CO_2, whereas Cape York had the highest $\overline{STAR}$ along with the lowest A_L/A_C at aCO_2 levels, indicating more efficient light interception per unit leaf area than Howard Springs. Regression analysis between $\overline{STAR}$ and A_L/A_C showed a strong negative association ($r = -0.96$, df = 22, $p < 0.0001$) (Fig. 2.5), illustrating that, plants with higher $\overline{STAR}$ and lower A_L/A_C had more efficient light interception. Furthermore, a significant negative relationship ($r = -0.83$) was observed between the number of leaves and $\overline{STAR}$ (Table 2.4), reflecting less efficient light interception by plants with more leaves (Fig. 2.6). In general, wild rice accessions grown at eCO_2 had more leaves and lower $\overline{STAR}$, while *O. sativa* had fewer leaves with higher $\overline{STAR}$ (Tables 2.2 & 2.3), showing that it was more efficient at intercepting light.

Leaf size showed a weak positive association ($r = 0.47$) with *LA* (Table 2.4). *LA* for the total canopy varied significantly between genotypes, while eCO_2 had its dominant effect through leaf number affecting *LA* (Table 2.2). Tiller number also significantly varied with genotype and CO_2 level (Table 2.2), being substantially lower in Doongara partly because tiller production had slowed dramatically by the time of digitization while tiller production in the wild accessions continued unabated. Thus, the increase in tiller number in plants grown at eCO_2 accounted for the increase in leaf number, greater leaf area and total aboveground biomass in those plants.

Crown density (leaf area per unit crown volume) correlated inversely (*r* = -0.91) with distance to the five nearest leaves. It followed that leaf number also correlated negatively with leaf dispersion (as measured by the distance to the nearest five leaves) (*r* = -0.76; Table 2.4), indicating that genotypes with more leaves had more clumped canopies. Plants grown in eCO_2 had higher crown densities than under aCO_2 (Table 2.3) and a smaller minimum distance to the five nearest leaves (Fig. 2.7). For example, Howard Springs grown in eCO_2 had the highest crown density because more leaves were accommodated in the same crown surface (Table 2.3) and leaves were consistently less dispersed (denser crowns) in Howard Springs compared with the other two genotypes (Table 2.3). $\overline{STAR}$ was positively correlated with leaf dispersion (*r* = 0.72), with a 25% increase in dispersion more than doubling $\overline{STAR}$ and thus, the efficiency of light interception (Table 2.4, Fig. 2.8). All correlation coefficients between different morphological data are presented in Table 2.4.

Table 2.3. Simulated variables describing the canopy architecture of *O. meridionalis* (acc. Cape York and Howard Springs) and *O. sativa* cv. Doongara. From these variables, C gain was calculated for plants notionally grown at the latitude of Sydney during summer. Plants were grown at either ambient (400 ppm) or elevated levels (700 ppm) of CO_2 throughout the entire experiment. Digitized plants at 100- to 115-d old were used for simulation. The growing conditions for the plants are described in Table 2.1.

Genotype	**CO_2 concentration**	**Crown surface (m^2) A_C**		**Simulated leaf area (m^2) A_L**		**Crown density ($m^2 m^{-2}$) A_L/A_C**		**Leaf dispersion (β)**		**$\overline{STAR}$ (m^2 m^{-2})**		**C gain (mol d^{-1})**	
O. meridionalis													
acc. Cape York	ambient CO_2	7.57	b	0.97	c	0.12	c	0.62	b	0.38	a	0.74	de
acc. Howard Springs	(aCO_2)	10.67	a	2.13	b	0.20	bc	0.58	bc	0.32	ab	1.58	bc
O. sativa	400 ppm												
cv. Doongara		3.13	c	0.52	c	0.17	bc	0.67	a	0.36	a	0.40	e
O. meridionalis													
acc. Cape York	elevated CO_2	9.17	ab	2.30	b	0.26	b	0.60	bc	0.29	bc	1.94	b
acc. Howard Springs	(eCO_2)	10.24	ab	4.23	a	0.41	a	0.57	c	0.23	c	3.35	a
O. sativa	700 ppm												
cv. Doongara		4.57	c	1.13	c	0.25	b	0.61	bc	0.29	bc	1.03	cd
p, df = 2 (Genotype)		<0.0001		<0.0001		0.0002		<0.0001		0.0025		<0.0001	
p, df = 1 (CO_2)		ns		<0.0001		<0.0001		0.002		<0.0001		<0.0001	
p, df = 2 (Genotype × CO_2)		ns		0.006		0.033		0.044		ns		0.001	

Levels not connected by same letter are significantly different, ns = not significant

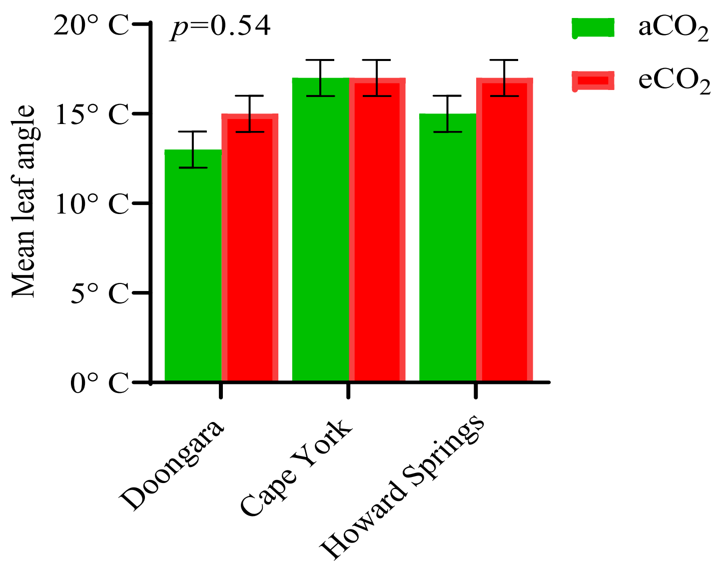


Fig. 2.4. Mean leaf angle from the vertical showing differences in domesticated and wild genotypes at ambient and elevated atm-CO_2 (Mean ± SE). aCO_2 represents ambient CO_2 (400 ppm) and eCO_2, represents elevated CO_2 (700 ppm).

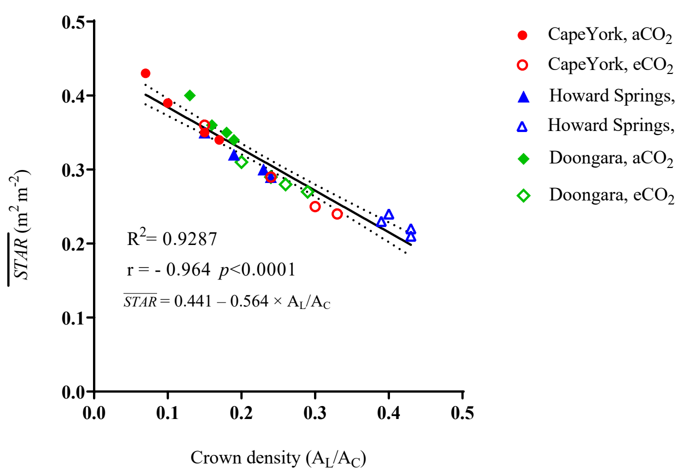


Fig. 2.5. Measured $\overline{STAR}$ (silhouette to total area ratio, averaged over all viewing angles) as a function of crown density (ratio of leaf area to crown surface area, A_L/A_C) of *O. meridionalis* (acc. Cape York and Howard Springs) and *O. sativa* cv. Doongara, illustrating the effects of CO_2 on the crown structure and the effectiveness of light capture of the genotypes. Dotted lines are 95% confidence intervals, solid black line a linear regression.

Table 2.4. Correlation values showing character association among different morphological characters simulated from digitized plants of wild and cultivated rice grown under elevated and ambient CO_2.

	C gain	Dry Biomass	Tiller Number	Leaf Number	Leaf size	Leaf Area	Leaf dispersion	Crown surface area
Tillers Number	0.81	0.83						
Leaf Number	0.91	0.91	0.94					
Leaf size	0.43	0.25	0.04	0.07				
Leaf Area	0.99	0.86	0.80	0.89	0.47			
Leaf dispersion	-0.77	-0.72	-0.77	-0.76	-0.34	-0.78		
Crown surface area	0.71	0.53	0.64	0.62	0.53	0.73	-0.76	
Crown density	0.89	0.88	0.68	0.82	0.31	0.88	-0.64	0.36
$\overline{STAR}$	-0.85	-0.89	-0.70	-0.83	-0.30	-0.84	0.72	-0.39

Values in red are not significant. Values in blue are significant at p = 0.05. All remaining values are significant at p = 0.01.

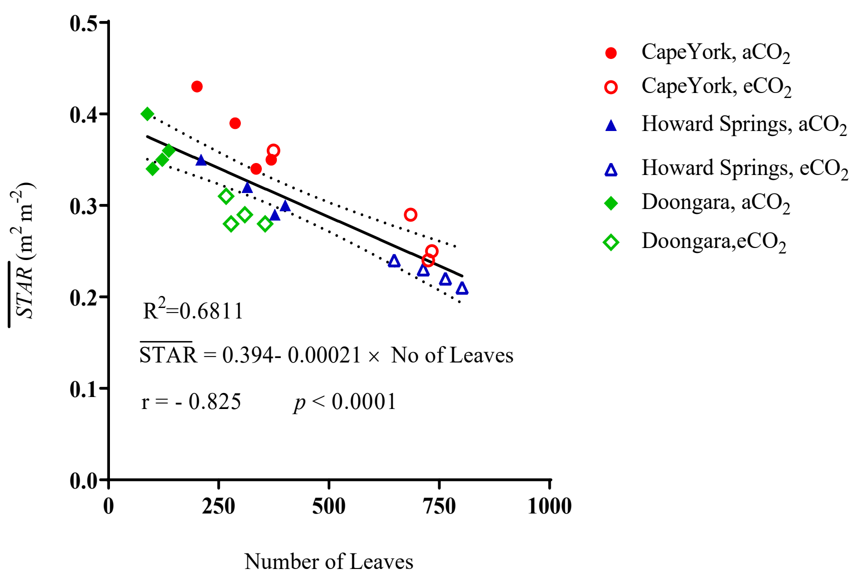


Fig. 2.6. $\overline{STAR}$ as a function of number of leaves for canopies of *O. meridionalis* (acc. Cape York and Howard Springs) and *O. sativa* cv. Doongara at two levels of CO_2, ambient (400 ppm) and elevated (700 ppm). Dotted lines are 95% confidence intervals, solid black line a linear regression.

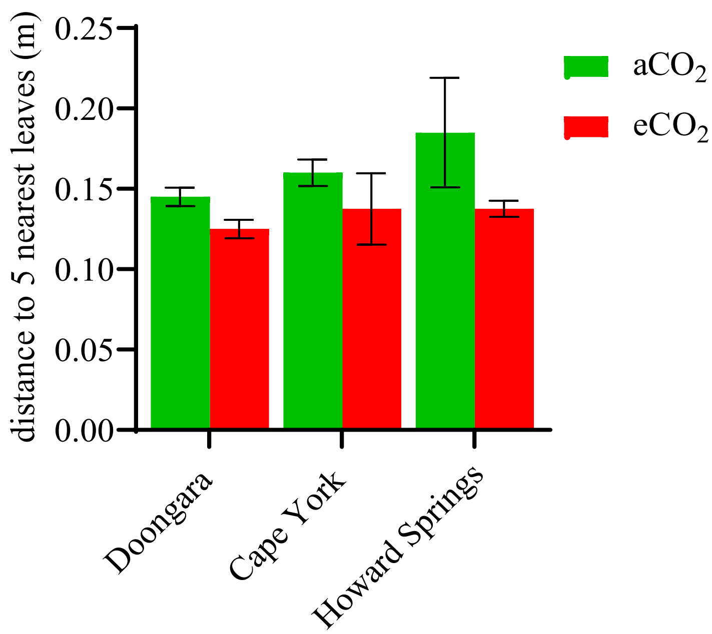


Fig. 2.7. Distance to five nearest leaves measured for *O. meridionalis* (acc. Cape York and Howard Springs) and *O. sativa* (cv. Doongara) illustrating the effect of elevated CO_2 (Mean ± SE).

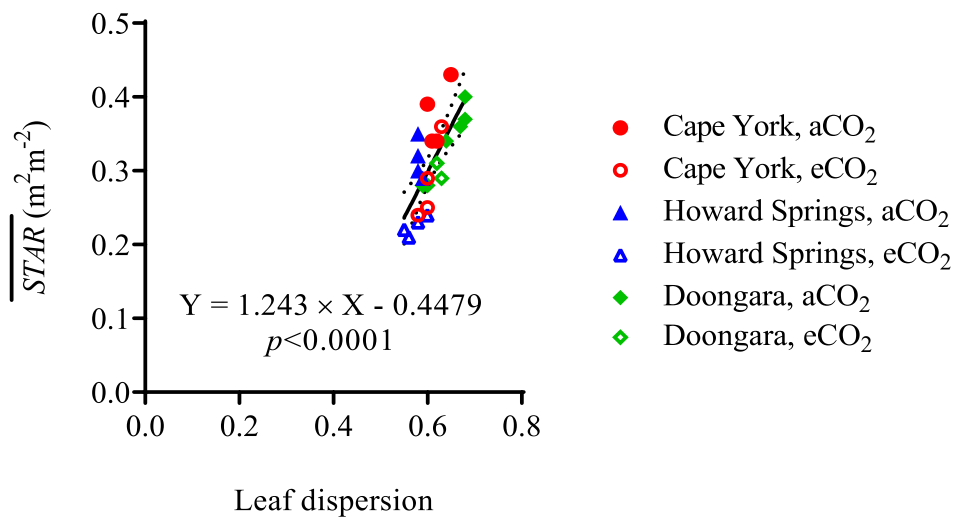


Fig. 2.8. Effects of CO_2 on rice genotypes compared using as a function of leaf dispersion. The growing conditions and simulation are described in Table 2.4. Dotted lines are 95% confidence intervals, solid black line a linear regression.

2.3.3. Calculating carbon gain from light interception and gas exchange

Preliminary experiments conducted with 12 plants sown in 50-kg tubs established that both *O. meridionalis* ecotypes had 2–3 fold larger final biomass, larger leaf area index and denser canopies than *O. sativa* (Table 2.5). Based on these findings from high-density planting in tubs, single plants were grown in pots arranged to produce a closed canopy, thereby preventing belowground competition. These plants were used to calculate theoretical carbon gain using light interception data.

Although *O. sativa* intercepted light as efficiently as the Cape York, as indicated by $\overline{STAR}$, both *O. meridionalis* accessions had significantly more simulated C gain than cultivated rice because of larger leaf areas (Table 2.3). The dominant influence of leaf area on C gain caused $\overline{STAR}$ (light interception) to correlate negatively with C gain (r = -0.85) (Table 2.4). For instance, Howard Springs had the lowest $\overline{STAR}$ and highest simulated (and actual) C gain (Tables 2.2 and 2.3).

Oryza meridionalis accessions had significantly higher C gain than Doongara and C gain per plant approximately doubled at eCO_2 across all three genotypes (Table 2.3). Regression analysis showed that simulated C gain appeared to be relatively overestimated in the wild rice accessions when compared with the actual biomass, especially for Howard Springs (Fig. 2.9B).

Crown surface area (A_C) had a strong positive relationship with dry shoot biomass (r = 0.53), while it had a weak negative relationship with $\overline{STAR}$ (r = -0.39) (Table 2.4), indicating that A_C only partially determines the total interception of light by plants.

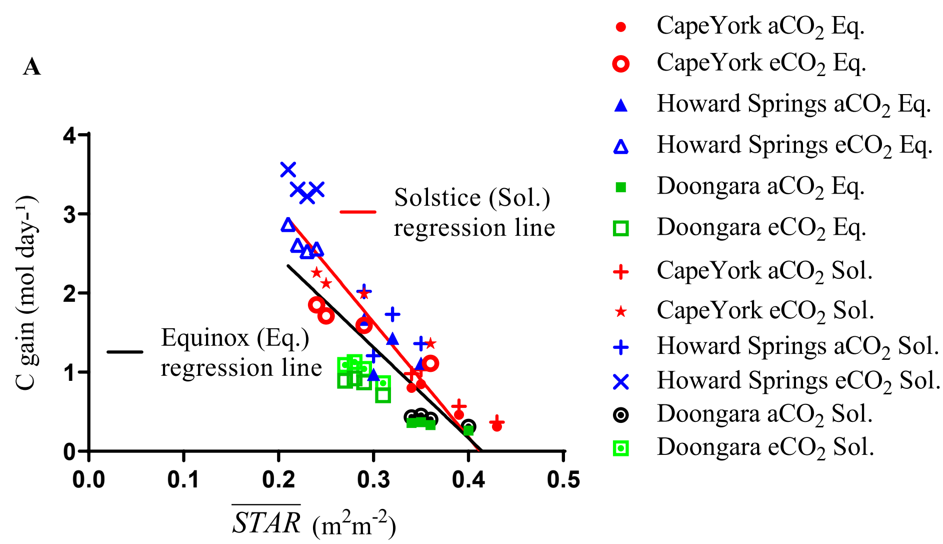
There was a strong inverse relationship between $\overline{STAR}$ and C gain (r = -0.85) across all plants (Fig. 2.9A). The path of the sun was simulated according to two settings of the azimuth, the southern Summer Solstice (December) and the Equinox (March or September). By applying these seasonal light simulations, two values for calculated C gain were derived for each value of $\overline{STAR}$, with C gain during the Summer Solstice always higher than for the Equinox. This in turn gave rise to two regressions, one for plants notionally receiving full summer illumination (red line) and the other for plants illuminated by equinoxial light (black line). These regressions were highly significant.

Fig. 2.9B relates these two seasonal simulations of C gain to *harvested biomass*, showing that simulated C gain predicted biomass with high accuracy and that the path of the sun and hence the incidence of light affected C gain.

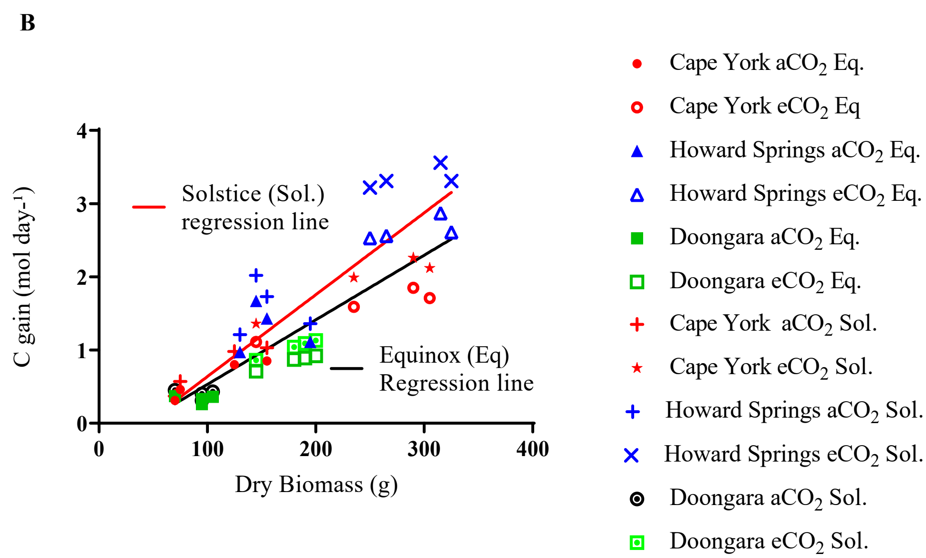
Table 2.5. Tiller number, aboveground fresh biomass, and leaf area (per plant), and specific leaf mass (*SLM*) and leaf area index estimated by the ratio of leaf area to ground area (*LAI*) in *O. meridionalis* (acc. Cape York and Howard Springs) and *O. sativa* cv. Doongara. Plants were grown in adjacent tubs (50 kg) forming a closed community in glasshouse conditions. Temperature was controlled at 28°C Day and 22°C night and plants were harvested at 115–120 days.

Genotype	**Tillers**		**Leaf Area (cm^2)**		**Fresh Biomass (g)**		***SLM* ($cm^2\ g^{-1}$)**		***LAI* ($m^2\ m^{-2}$)**	
O. meridionalis										
acc. Cape York	35	a	3415	a	307	a	46	a	18.6	a
acc. Howard Springs	35	a	3249	a	241	b	39	a	17.7	a
O. sativa										
cv. Doongara	19	b	1287	b	135	c	24	b	7.0	b
p, df = 2	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	

Levels not connected by same letter are significantly different



Equinox Y = -11.46*X + 4.753 ***
Solstice Y = -14.53*X + 5.99 ***



Equinox Y = 0.00883*X - 0.3524 ***
Solstice Y = 0.01118*X - 0.4803 ***

Fig. 2.9 A. Regression of total daily simulated carbon gain (mol day^{-1}) with variation in (m^2 m^{-2}) for four replicates of each of the three rice genotypes (*O. meridionalis* and *O. sativa*) at two CO_2 levels (400 denoted 'a' and 700 ppm denoted 'e'). The analysis generated regressions corresponding to the sun following the path of the Summer Solstice or the Equinox. **Fig. 2.9B. The same carbon gain data were then correlated with plant biomass.** The analysis represented in Fig. 2.9A was repeated, with the Sun set to follow the path of the Summer Solstice and Equinox. Correlations for the two respective regressions appear beneath each figure.

2.4. Discussion

This study was aimed at testing the impact of canopy architecture on light capture by contrasting the highly compact leaf canopy of domesticated rice (*O. sativa*) with the expansive leaf canopies of two ecotypes of *O. meridionalis*, a wild rice species adapted to tropical northern Australia. Individual plants were digitized immediately after being removed from the closed canopies that they grew in, thereby addressing the impact of canopy architecture on competition for light. Shading from neighbouring plants while plants were growing at high densities could not be taken into consideration because only individual plants could be digitized; however, the dominant influence of architecture on light interception and carbon gain was from the upper canopy during the midday hours, where light was unattenuated and at its highest intensity.

Canopy dynamics were also investigated by comparing plants grown to maturity at ambient and elevated CO_2 levels, with eCO_2 markedly increasing canopy density. This provided insights into future CO_2 regimes under which C_3 species are predicted to respond strongly. Finally, the robustness of these simulations under two seasonal conditions (high summer and the Equinox) was tested by comparing light capture and simulated C gain. These findings were compared with actual biomass yield for all genotypes.

Hypothesis 1: *Total leaf area and efficiency of light interception explain the genotypic variation in aboveground biomass*

Leaf canopies of the two *O. meridionalis* ecotypes were much denser and heavier than those of *O. sativa*. For example, they produced 200–250% more tillers, leaves, leaf area and *SLM* than *O. sativa* ($p < 0.0001$). These traits are directly associated with greater light harvesting in plants (Duursma et al., 2012). Destructive measurements reported in Table 2.2 suggest that there are genotypic differences in canopy density and, consequently efficiency of light interception and total light capture. Digitized images corroborated these destructive measurements of the canopy but provided further information, in particular, crown surface area and, when corrected for total leaf area, crown density. The denser canopies of *O. meridionalis* Howard Springs revealed a crown surface area and simulated leaf area 2–4 times greater than *O. sativa*, with *O. meridionalis* Cape York intermediate. However, the ratio between these variables (expressed as crown density) was less divergent among the three genotypes, being just 45% higher in Howard Springs than the other genotypes. Duursma et al. (2012) reported that crown density and leaf dispersion explain 85% of the variation in $\overline{STAR}$ but, across the 124 highly contrasting species examined, crown density varied by more than an order of magnitude (Duursma et al., 2012; Guzmán, 2015). In this study, $\overline{STAR}$ was also negatively correlated with crown density and positively with leaf dispersion (Figs. 2.5 and 2.8). However, larger rice leaf canopies occupied larger crown volumes (as estimated by crown surface area), partially optimizing crown density and diminishing the impact of less efficient light interception on total light capture by the canopy. For example, an eight-fold increase in leaf number between the smallest and largest canopy reported for the two rice species and CO_2 levels was associated with only a 25% decrease in leaf dispersion, again implying an ordered internal architecture within the canopies. However, these relatively small but significant changes in leaf dispersion coincided with much larger variation in $\overline{STAR}$. Thus, the eightfold increase in leaf number caused $\overline{STAR}$ to halve. This is reflected in the significant interaction between $\overline{STAR}$ and genotype (p = 0.003) or CO_2 treatment (p = 0.0001). In addition to the strong influence of leaf dispersion on $\overline{STAR}$, a negative relationship was obtained between leaf number and $\overline{STAR}$ as observed by Duursma et al. (2012), Guzmán (2015) and others (Delagrange et al., 2006; Farque et al., 2001; Lusk et al., 2011; Niinemets et al., 2005; Sinoquet et al., 2007), who also found negative relationships between $\overline{STAR}$ or related measures with plant size, tree size and total leaf area (Guzmán, 2015).

Genotypic contrasts in light interception canopies might be expected to influence growth rates and final shoot biomass. This hypothesis was tested by simulating daily rates of carbon gain and comparing these rates with measured final shoot biomass. Light response curves and light interception as measured by $\overline{STAR}$ were required to translate assimilation rates at the leaf level to whole-shoot carbon gain. Theoretically, plants with high $\overline{STAR}$ should intercept more light per unit leaf area than those with low $\overline{STAR}$ (Duursma et al., 2012), leading to higher carbon assimilation rates per unit leaf area. Cost-benefit analysis of plant architecture suggests sacrificing efficient light capture as a result of increasing crown density (lower $\overline{STAR}$) but increasing canopy size is a successful architectural strategy (Duursma et al., 2010; Mäkelä and Sievänen, 1992).

Similarly, in the rice species studied here, increasing leaf area, even at the cost of less efficient light capture, is a major driver of total biomass accumulation and carbon gain. I postulate that this strategy is typical in a fast-growing annual plant such as most rice species, where little structural biomass is needed to support a large, dense leaf canopy. In this study, leaf area and $\overline{STAR}$ were negatively correlated, indicating that plants with the greatest leaf area gained carbon fastest, notwithstanding less efficient light interception. A similar observation was made with maize hybrids, where leaf area strongly predominantly determined yield (Huang et al., 2017). Paradoxically, C gain was reduced in all three genotypes even when $\overline{STAR}$ was high. This was especially obvious in *O. sativa*, which gained less carbon than $\overline{STAR}$ would predict based on the behaviour of the *O. meridionalis* genotypes. Nonetheless, this study established overall that the product of leaf area, light interception and light response of CO_2 assimilation produced estimates of carbon gain that correlated strongly with actual biomass increase. Therefore, Hypothesis 1 is supported; i.e., total light capture predicts biomass accumulation for morphologically diverse canopies of wild and cultivated rice.

Hypothesis 2: *Light interception efficiency is diminished in denser leaf canopies formed in an elevated CO_2 atmosphere*

There was a significant reduction in $\overline{STAR}$ in plants grown at eCO_2, reflecting a doubling of the number of leaves and tillers, and therefore total leaf area. Such strong responses to atm-CO_2 are widely reported for *O. sativa* (Ziska et al., 1996) but not previously measured in a wild *Oryza* species. Interestingly, crown surface area (A_C) was not significantly affected by CO_2 enrichment, resulting in substantially denser crowns when plants were grown in eCO_2. However, crown density did not affect the relative ranking of $\overline{STAR}$ for each genotype when plants were grown in elevated *vs* ambient CO_2. Furthermore, leaves were less dispersed in eCO_2 than in aCO_2, with leaf dispersion varying significantly with genotype ($p < 0.0001$), CO_2 ($p = 0.0004$) and revealing an interaction between genotype and CO_2 ($p = 0.0343$): *O. meridionalis* Howard Springs had the most densely clumped leaves in both elevated and ambient CO_2. Crown density, leaf dispersion and total leaf area all contributed to a marked change in modelled carbon gain in eCO_2. However, the high productivity of *O. meridionalis* ecotypes in ambient conditions was conserved in eCO_2

treatments, with the ranking of the three genotypes remaining the same (Howard Springs > Cape York > Doongara). This indicates that intrinsic features of canopy architecture will not be corrupted by rising CO_2 levels, making wild relatives a valuable resource for canopy re-design. Hypothesis 2 is supported; i.e., the efficiency of light capture is diminished when plants are grown in eCO_2 as a result of denser leaf canopies and reduced leaf dispersion. However, increased total leaf area enabled canopies of all three genotypes to capture more light and grow larger at eCO_2 in spite of less efficient light capture.

Hypothesis 3: *Light interception, simulated carbon gain and harvested biomass are correlated, with seasonal changes in light regime not affecting relative ranking*

Having established that C gain was negatively correlated with $\overline{STAR}$ (see Hypothesis 1) because of the advantage conferred by large canopies, the *ranking* of the three genotypes was tested at two CO_2 levels to see whether it was preserved with respect to C gain and actual shoot biomass of plants as azimuth varied. It was hypothesized that the efficiency of these morphologically diverse canopies to harvest light would not be qualitatively affected by season. The angle of incident light was notionally varied by changing the azimuth in YplantQMC to test the ranking of theoretical C gain for the three genotypes and two CO_2 levels in this experiment. The slopes of the regression for each season shown in Fig. 2.9A were 14.53 and -11.46 at the Summer Solstice and Equinox, respectively, showing clearly that season affected the potential C gain of the plants but not the ranking of the genotypes and treatments; plants growing in summer gained more carbon in the model. As C gain was simulated, it was necessary to establish that this was a good predictor of actual biomass, which is generally demonstrated in Fig. 2.9B. However, the predicted C gain for Howard Springs plants appeared to be higher than the biomass would predict (Fig.2.9B), indicating that YplantQMC overestimated the actual growth rate in this accession. Acclimation of photosynthetic rates through the Howard Springs canopy requires more detailed analysis. In a study of understory species (Pearcy and Yang, 1996), light capture and simulated C gain was affected in a species-specific fashion by the angle of incident sunlight. Hypothesis 3 is generally supported; i.e., light interception, simulated carbon gain and harvested biomass are correlated, regardless of whether the direction of illumination is modelled at the Summer Solstice or Equinox.

2.5. Conclusion

This study shows several tight relationships between canopy architecture, simulated light capture and actual final biomass of cultivated and wild rice ecotypes. These relationships were quantitatively changed when the plants were supplied eCO_2 levels or simulated to grow in different seasons but the qualitative relationships as seen in rankings did not change. Wild rice species and ecotypes offer novel candidates for canopy re-design via breeding. While factors such as nutrition and water supply limit plant performance in many ecosystems, the productivity of wild *Oryza* species is a direct function of canopy dynamics, reflecting the rapid growth habit required for survival in their natural savannah habitats.

Chapter 3

Effects of silver nitrate treatment on the pasting properties of rice flour

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Optimal silver nitrate concentration to inhibit α-amylase activity during pasting of rice flour

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3.1. Introduction

Rice (*Oryza sativa*) is eaten in an unprocessed form after cooking and can also be an ingredient in puddings, pasta, noodles, cookies, crackers, snacks and other foods (Bhattacharya et al., 1999; Choi, 2012; Garcia et al., 2011; Moin et al., 2017; Puri et al., 2014; Siegel and Lineback, 1976; Vongsawasdi et al., 2009); thus the physicochemical properties of rice flour need to be accurately accessed by food processors.

Pasting properties obtained from Rapid Visco Analyser (RVA) pasting curves can be used to compare the processing, cooking, and eating qualities of rice varieties with a similar amylose content (AC) (Allahgholipour et al., 2006; Dang and Copeland, 2004a). The primary RVA parameters known as peak viscosity (PV), trough or hot paste viscosity (TV), final viscosity or cool paste viscosity (FV), as well as the derived parameters, breakdown viscosity (BD) and setback viscosity (SB), are important for processing, cooking and eating qualities (Juliano, 1985). Knowledge of pasting properties (PV, TV and FV) is critical for processing quality cereal products, including those based on rice. Such information can assist processors to optimise ingredient concentrations and temperature pressure shear limits to produce desired products (Dang and Copeland, 2004a).

The presence of α-amylase activity in grains influences the pasting properties of the corresponding flour and isolated starch. Silver nitrate ($AgNO_3$) can be used to inhibit α-amylase in wheat flour and thus enable determination of the pasting properties without interference of this enzyme activity; thus, for example, a higher PV is obtained for wheat flour in $AgNO_3$ solution than in water (Abdel-Aal et al., 2002; Purna et al., 2015). Other workers have reported increased viscosity of wheat flour in $AgNO_3$ (Bhattacharya and Corke, 1996; Hutchinson, 1966; Meredith, 1970) using a Brabender amylograph or RVA. Many other studies reported an increase in both PV and FV in wheat flour with $AgNO_3$ and concluded that the increase in viscosity was due to inhibition of endogenous α-amylase activity (Batey et al., 1997; Frauenlob et al., 2018; Olaerts et al., 2016).

For rice flour, endogenous α-amylase activity has been shown to decrease the PV obtained using an RVA. In an experiment conducted with three rice varieties, the PV increased in all varieties in 0.5 mM $AgNO_3$ solution (compared to dH_2O) (Zhu et al., 2010b). Others also reported increased

PV in rice flour in $AgNO_3$ solution (Ding et al., 2015; Huang and Lai, 2014). In contrast, when rice starch was analysed, pasting properties were relatively unaffected by $AgNO_3$ (Huang and Lai, 2014); these authors explained that most of the impurities (including α-amylase) must have been removed from the starch during its isolation. In barley flour and malt, a lower PV due to endogenous amylase activity was reported, and when $AgNO_3$ solution was used to inhibit the amylase activity, a higher PV was obtained (Cozzolino et al., 2016; Fox et al., 2014; Holmes, 1995; Zhou and Mendham, 2005). In sweet potato flour, a higher PV was reported using 0.05 mM $AgNO_3$, indicating inhibition of α-amylase activity (Collado and Corke, 1999).

Different studies have used a concentration of $AgNO_3$, often anecdotally based, varying from 0.1 mM to 0.1 M to inhibit amylase activity in starch, flour, and malt. Indeed, little is known (for any cereal) about the optimal concentration of $AgNO_3$ required to inhibit α-amylase activity during the characterisation of pasting properties using an RVA. This study aimed to address this issue using rice flour from contrasting origins. This knowledge will enable the accurate determination of the pasting properties of rice flour, which will be valuable for the food processing industry.

3.2. Materials and Methods

3.2.1. Materials

The first experiment was conducted with cv. Doongara, grown under elevated CO_2 (700 ppm) in glasshouses at Macquarie University, Sydney, Australia in 2015. For the second experiment, rice samples were purchased from the local market. The rice samples used were cv. Doongara white (polished, AC, 24%), Doongara brown (AC, 22%), long-grain brown (AC, 21%) and medium-grain brown (AC, 13%) (unknown cultivars). Silver nitrate (S8157-25g) was from Sigma-Aldrich (Sydney).

3.2.2. Methods

3.2.2.1. Sample preparation

Rice grains were ground with a coffee grinder (three times for 10 s with 10-s intervals between grinds) and sieved through a 450-μm sieve. Different concentrations of $AgNO_3$ solutions—0.001, 0.005, 0.01, 0.02, 0.05, 0.06 (1% w/v), 0.1 M—were tested to optimise the concentration of $AgNO_3$ required to inhibit α-amylase activity in the RVA. $AgNO_3$ solutions were prepared freshly with dH_2O. For the control, dH_2O was used instead of $AgNO_3$ solution.

3.2.2.2. Pasting properties using an RVA

A Rapid Visco Analyser (RVA-4SA, Newport Scientific) was used to determine pasting properties. Freshly prepared rice flour, 3.5 ± 0.01 g (12% moisture basis), was placed in an aluminium canister and weighed to 28.0 ± 0.01 g with $AgNO_3$ solution (or the dH_2O control). The flour was mixed with the $AgNO_3$ solution/dH_2O by stirring the paddle vigorously. The canister then was inserted into the RVA, which was run for a 14-min cycle following the method of Tong et al. (2014) with slight modifications. The heating and cooling cycle consisted of: (1) holding at 50 °C for 1 min; (2) heating to 95 °C in 3.75 min; (3) holding at 95 °C for 3 min; (4) cooling to 50 °C in 3.75 min and (5) holding at 50 °C for 2.5 min. The paddle speed was 960 rpm for the first 10 s to ensure dispersion of the sample and then slowed down to 160 rpm for the remainder of the test. The RVA runs were performed in duplicate.

The pasting properties recorded were: (1) peak viscosity (PV) (i.e., maximum viscosity between heating and holding cycle); (2) trough or hot paste viscosity (TV) (i.e., minimum viscosity after the peak); (3) final viscosity or cool paste viscosity (FV) (i.e., paste viscosity after cooling); (4) breakdown viscosity (BD) as PV minus TV and (5) setback viscosity (SB) as FV minus TV. The viscosity values were measured in centipoise (cP). Peak time (Pt) in minutes and pasting temperature (PT) (°C) were also recorded.

3.2.2.3. Statistical analysis

Statistical analysis was performed with JMP Pro 14 (statistical software from SAS, Lane Cove, NSW, Australia). The random-effect model (REML) was used to analyse the data where replication was treated as a random factor. The mean comparison was done using Tukey's HSD test. GraphPad Prism-8 was used to generate graphs.

3.3. Results

3.3.1. Pasting properties of cv. Doongara in RVA with $AgNO_3$ solution

Pasting properties (Pt, PT, PV, TV, FV, BD, SB) were assessed using RVA curves (Table 3.1). Highly significant differences were obtained for all the RVA parameters ($p < 0.0001$, df = 7) except PT ($p = 0.0025$, df = 7) (Table 3.1). The PV was numerically highest, 3364 cP, when 0.06 M $AgNO_3$ (1%) solution was used instead of dH_2O in the RVA and was not significantly different to the PV values obtained using 0.05 M (3351 cP) or 0.1 M (3327 cP) $AgNO_3$. However, these values differed significantly from the rest of the treatments including the control (dH_2O) (Table 3.1).

The lowest hot paste or trough viscosity (TV) was 1451 cP obtained in 0.1 M $AgNO_3$, followed by 1621 cP in 1% $AgNO_3$ solution (Table 3.1). The highest TV value, 2110 cP, was obtained using 0.005 M $AgNO_3$ solution, and differed significantly from the rest of the treatments (Table 3.1).

The maximum cool paste viscosity or final viscosity (FV) was obtained with 0.005 M $AgNO_3$ (3955 cP), which did not differ significantly from 0.001 M $AgNO_3$ (3856 cP) and control (3810 cP) (Table 3.1). The lowest FV (2734 cP) was obtained using 0.1 M $AgNO_3$ solution, which was significantly lower than any other treatments (Table 3.1).

Peak time (Pt) was lowest in 0.1 M $AgNO_3$ (5.2 min) followed by 1% (5.3 min) and 0.05 M $AgNO_3$ (5.4 min) and gradually increased to 6.0 min in the control. The differences between the control and these three treatments were small (≈1 min) but significant (Table 3.1). Pasting temperature (PT) values showed no significant differences among these three treatments (0.1 M, 1% and 0.05 M $AgNO_3$) but they varied significantly from control and the other treatments. The PT began to decrease significantly at 0.02 M $AgNO_3$ (Table 3.1).

3.3.2. Pasting properties of four rice samples in 1% $AgNO_3$ solution

The effect of 1% $AgNO_3$ solution on the pasting properties of various types of rice (unknown variety, purchased from the market) was tested using the RVA. All the properties assessed showed significant variations among the rices and between the 1% $AgNO_3$ and the control (Table 3.2). When 1% $AgNO_3$ was used in place of dH_2O the average PV and BD increased significantly, while the average TV, FV, and SB values decreased significantly. Significant variations were observed among the rice samples tested for all the pasting properties assessed (Table 3.2). Medium-grain brown rice had the highest PV and BD but lowest FV, and SB differed significantly from the other types of rice. The average Pt and PT also showed significant variations among cultivars. The lowest average PT recorded was 83 °C for medium-grain brown rice (Table 3.2).

The interaction between cultivars and treatments (1% $AgNO_3$ and dH_2O) showed a high level of significance for all parameters except PT (Table 3.2). The PV increased significantly in all rice samples when 1% $AgNO_3$ solution was used instead of dH_2O (Table 3.2). The highest PV measured was 4901 cP in medium-grain brown rice when 1% $AgNO_3$ solution was used instead of dH_2O (3779 cP). Doongara white rice (polished) also had a high PV in $AgNO_3$ solution compared with the control. Long-grain brown rice and Doongara brown rice did not differ significantly when PV was measured in dH_2O; however, they differed significantly in 1% $AgNO_3$ solution.

For Doongara white rice, the FV was lower in 1% $AgNO_3$ compared to the control (Fig. 3.1). The FV decreased when 1% $AgNO_3$ solution was used regardless of the rice tested. The TV was lowest in medium-grain brown when the analysis (RVA) was performed in 1% $AgNO_3$ and was significantly different from the control (Table 3.2). Similar results were obtained for other varieties.

Table 3.1. Effect of silver nitrate concentration on pasting properties of brown rice flour (cv. Doongara grown at elevated CO_2).

$AgNO_3$ Solution	PV (cP)	TV (cP)	BD (PV-TV) (cp)	FV (cP)	SB (FV-TV) (cP)	Pt (min)	PT (°C)
0.1 M	3327 a	1451 f	1876 a	2734 f	1283 d	5.2 d	86 c
1% (0.06 M)	3364 a	1621 e	1743 ab	3035 e	1415 c	5.3 cd	86 c
0.05 M	3351 a	1710 d	1641 b	3250 d	1540 b	5.4 c	86 c
0.02 M	2748 b	1837 c	912 c	3659 c	1822 a	5.6 b	88 b
0.01 M	2621 bc	1929 b	692 d	3791 bc	1862 a	5.7 b	90 a
0.005 M	2515 cd	2110 a	406 e	3955 a	1845 a	5.9 a	90 a
0.001 M	2310 de	1992 b	319 ef	3856 ab	1865 a	6.0 a	91 a
dH_2O (Control)	2195 e	1976 b	219 f	3810 abc	1834 a	6.0 a	90 a
p (df=7)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0025

Levels not connected by the same letter are significantly different.
Peak viscosity (PV), hot paste viscosity or trough (TV), final viscosity or cool paste viscosity (FV), breakdown viscosity (BD), and setback viscosity (SB) were measured in centipoise (cP). Peak time (Pt) in minutes and pasting temperature (PT) in °C were also recorded.

Table 3.2. Effects of 1% silver nitrate on pasting properties of different types of rice purchased from the market as determined using a rapid visco analyser (RVA).

Genotype × Treatment Genotype	Treatment	PV (cP)		TV (cP)		BD (PV-TV) (cP)		FV (cP)		SB (FV-TV) (cP)		Pt (min)		PT (°C)	
Doongara white	dH_2O	2955	e	2595	a	360	e	5213	a	2619	a	5.6	c	88	bc
Doongara brown	dH_2O	1947	f	1925	c	22	f	4040	c	2115	b	6.7	a	90	ab
Long grain brown	dH_2O	2043	f	1932	c	111	f	4558	b	2626	a	6.0	b	91	a
Medium grain brown	dH_2O	3779	c	2268	b	1511	c	4051	c	1783	d	5.6	c	86	d
Doongara white	1% $AgNO_3$	4042	b	1864	c	2178	b	3825	d	1962	c	5.2	d	86	d
Doongara brown	1% $AgNO_3$	3149	d	1701	d	1448	cd	3275	e	1574	f	5.3	d	86	d
Long grain brown	1% $AgNO_3$	2903	e	1553	ef	1351	d	3234	e	1682	e	5.3	d	87	cd
Medium grain brown	1% $AgNO_3$	4901	a	1535	e	3366	a	2848	f	1313	g	5.2	d	80	e
Genotype × treatment	*p (df= 3)*	0.0016		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		0.0187	
Genotype	*p(df=3)*	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
Treatment	*p(df=1)*	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	

Levels not connected by same letter are significantly different
Peak viscosity (PV), hot paste viscosity or trough (TV), final viscosity or cool paste viscosity (FV), breakdown viscosity (BD), and setback viscosity (SB) were measured in centipoise (cP). Peak time (Pt) in minutes and pasting temperature (PT) in °C were also recorded.

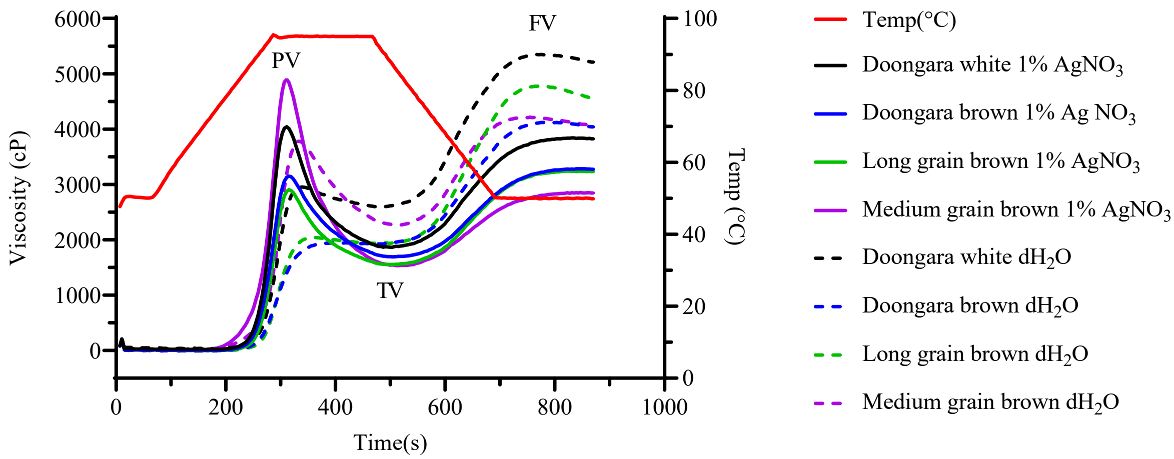

Fig. 3.1. RVA properties for different types of rice (purchased from the market) evaluated with 1% $AgNO_3$ solution and compared with dH_2O. Peak viscosity (PV), trough or hot paste viscosity (TV) and final viscosity or cool paste viscosity (FV) are shown.

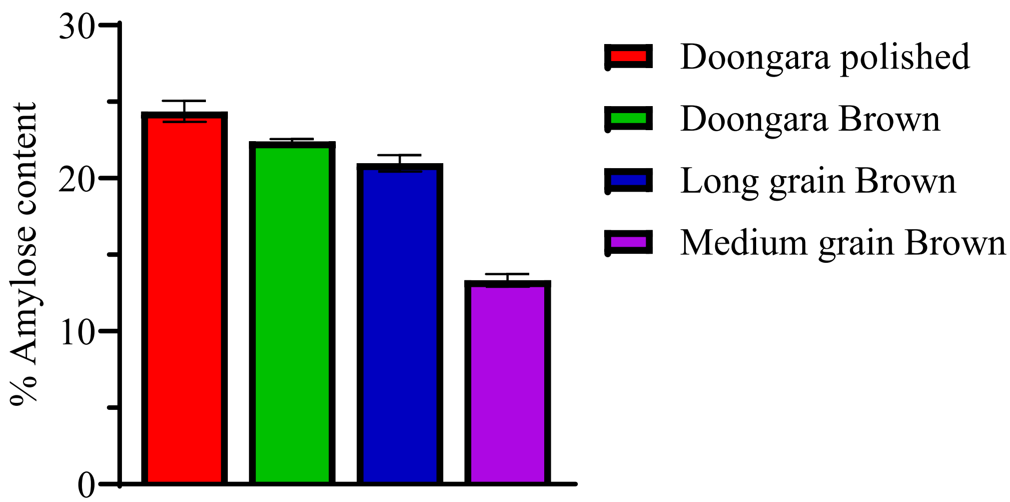

Fig. 3.2. Apparent amylose content of rices (purchased from the market) used in this experiment (Mean ± SE). Amylose content was determined following Mohammadkhani et al. (1998).

3.4. Discussion

3.4.1. Pasting properties of flour from cv. Doongara (grown at elevated CO_2) in an RVA with $AgNO_3$ solutions

Rice products are predominantly made from rice flour rather than rice starch (Huang and Lai, 2014). Pasting properties are important features for food processing to determine the application value of flour and the quality of end-use products (Bao et al., 2006). Starch is the main component of rice flour; however, lipids, proteins, and non-starch polysaccharides affect pasting properties and processed products (Huang and Lai, 2014).

Endogenous amylase activity affects the pasting properties of rice flour and therefore its impact on rice physical properties is routinely subjected to quality control testing prior to industrial-scale processing. The goal of this study was to establish the optimal concentration of $AgNO_3$ required to inhibit the endogenous α-amylase activity in rice flour during gelatinisation and pasting. The effect of $AgNO_3$ on pasting properties showed significant variation among treatments (Table 3.1). The peak viscosity value began to increase significantly at 0.005 M $AgNO_3$ when compared with dH_2O (Table 3.1). The highest PV was obtained in 1% $AgNO_3$, indicating inhibition of α-amylase activity in the rice flour during gelatinisation in the RVA. Cozzolino et al. (2016) and Zhou and Mendham (2005) used 0.1 M $AgNO_3$ to inhibit enzyme activity in barley flour and malt and obtained higher peak viscosity, supporting findings of this study. Huang and Lai (2014) used 0.0025 M $AgNO_3$ to inactivate α-amylase activity in rice flour during gelatinisation, while Ding et al. (2015) and Zhu et al. (2010b) used 0.005 M $AgNO_3$ solution in an RVA. The variation in results (inhibition of α-amylase activity by $AgNO_3$) is possibly due to the variation in genetic material and the AC of the rice samples used.

The FV values in this experiment were lower in 0.1 M $AgNO_3$ solution compared with all other treatments, results not supported by some studies (Batey et al., 1997; Ding et al., 2015; Olaerts et al., 2016). AC is the major determinant of FV; however, genetic differences between their material and mine, including the levels of lipids and proteins in the flour and their interaction with starches during gelatinisation, may also contribute to these differences. The PT decreased significantly when $AgNO_3$ solutions were used instead of dH_2O.

3.4.2. Pasting properties of four rice varieties in 1% $AgNO_3$ solution

This study compared pasting properties in 1% $AgNO_3$ solution versus dH_2O with four rice samples purchased from the market. The AC was lowest in medium-grain brown rice (≈12–13%), while the other varieties had higher AC (21–24%) (Fig. 3.2). The intention here is not to compare the variations in AC among the rice samples; however, it assisted me in explaining the results obtained. The PV and BD increased significantly in all the varieties, while the FV and SB decreased significantly when 1% $AgNO_3$ was used instead of H_2O in RVA (Table 3.2). These results were consistent with this experiment with varying concentrations of $AgNO_3$ (Table 3.1).

The variation in the viscosity values among the rice samples was possibly due to variation in AC, protein, lipids and phenolics. Comparing Doongara brown and Doongara white (polished) rice, the viscosity values were significantly different both in dH_2O and 1% $AgNO_3$, with the values always higher in the Doongara white rice. The aleurone layer and germ, which are removed during polishing, contain phenolics, lipids and protein which could interact with the starch and reduce the viscosity and increase PT.

BD was lowest in Doongara brown and long-grain brown rice. When PV, TV, BD and FV were measured in 1% $AgNO_3$, the BD and FV values were not significantly different between these samples, but BD and FV values were significantly higher than in dH_2O. The results suggested that using 1% $AgNO_3$ would inhibit α-amylase activity in rice flour with high AC and more accurately assess pasting properties (Cozzolino et al., 2016; Fox et al., 2014; Zhou and Mendham, 2005). Other studies support an increase in PV using $AgNO_3$ solution, but the concentration used was too low to fully inhibit α-amylase activity during gelatinisation of rice flour with higher AC (Ding et al., 2015; Huang and Lai, 2014; Zhu et al., 2010b).

3.5. Conclusion

Pasting properties of rice flour with medium to high AC content assessed in an RVA showed significant variation ($p < 0.0001$) with $AgNO_3$ concentration (Table 3.2). The PV increased significantly using 1% $AgNO_3$ instead of dH_2O. However, PV was not significantly different between 0.1 M and 0.05 M $AgNO_3$ solutions (Table 3.1). The PT decreased by 4 °C in all these treatments when compared with dH_2O. The results indicate that 1% $AgNO_3$ can be used to inhibit α-amylase activity during pasting of rice flour. Further experiments were conducted using 1% $AgNO_3$ solution to test the inhibition of enzyme activity in four rice samples with medium to high AC. The results demonstrated that using 1% $AgNO_3$ solution instead of dH_2O inhibits the α-amylase activity in rice flour during gelatinisation in the RVA and were used in the subsequent study (*Chapter 4*) to determine pasting properties.

Chapter 4

Grain properties of Australian wild rices and the effects of elevated atmospheric CO_2

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Elevated CO_2 differentially affects the properties of grain from wild and domesticated rice

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4.1. Introduction

Of the ~26 known species in the genus *Oryza*, only two species, namely the Asian rice *O. sativa* and the African rice *O. glaberrima*, have been domesticated (Brar and Khush, 2018; Henry, 2019; Stein et al., 2018). The wild species represent most of the genetic diversity within the *Oryza* genus, and thus accessions of these rices represent a valuable source of germplasm for the breeding of new elite genotypes for resistance to biotic and abiotic stresses. Responses of grain quality of the wild rices to increasing atmospheric CO_2 concentrations (atm-CO_2) need to be investigated to provide important information for breeders intending to utilise wild species in their improvement programs.

Under Future Emissions Scenario no. 1 (RCP8.5), as described by the Scripps Institution of Oceanography, atm-CO_2 could rise to 720 ppm by the end of the 21st century from the current value of 416 ppm (December 9, 2021; https://keelingcurve.ucsd.edu/). Rising atm-CO_2 will have a substantial bearing on crop production through the carbon fertilization effect. Elevated atm-CO_2 (eCO_2) leads to several physiological responses in crop plants, including changes to rates of photosynthesis, water use efficiency and carbon gain, canopy architecture and nutrient uptake and translocation. Such responses are expected to improve crop biomass in many cases, resulting in higher biomass and yield (Ainsworth and Long, 2021; Madan et al., 2012; Rahman et al., 2018; Ziska et al., 1997; Ziska and Teramura, 1992b). However, these changes are also expected to affect the physicochemical properties of seeds/grains (Jing et al., 2016a), which will impact the quality of food and beverage end-products.

Rice grain quality is important because rice is the major source of energy and nutrient supply for more than half of the world's population (Goufo et al., 2014a). The quality traits of rice are complex because they involve physical properties, cooking and sensory properties, and nutritional value (Fitzgerald et al., 2009). The chemical composition of the grain, including the amylose content of the starch and the protein content, influences the cooking and eating quality of rice (Allahgholipour et al., 2006; Jing et al., 2016a; Seneweera et al., 1996).

Many studies on domesticated rice (indica and/or japonica varieties) have shown increases in seed length (Sl) and/or seed width (Sw) due to eCO_2 (Jing et al., 2016a; Madan et al., 2012). The increase in Sw improved milling quality by decreasing the percentage of broken rice as wider grains can better withstand the frictional force of milling (Madan et al., 2012). Other studies reported eCO_2-induced increases in 1000-seed weight (Usui et al., 2014) and in single grain weight (Yang et al., 2009).

Apparent amylose content (AC) is a crucial factor in determining the processing, eating and cooking quality of rice (Yang et al., 2007). Thus, most research on the effects of eCO_2 on rice grain quality has focussed on waxy cultivars in comparison with their non-waxy counterparts. Rice is classified into groups depending on AC, namely waxy (0–8% AC), low (8.1–16%), intermediate (16.1–24%) and high AC > 24% (Allahgholipour et al., 2006). A higher AC due to eCO_2 increased the firmness of cooked rice (Chaturvedi et al., 2017; Idso, 2016; Seneweera et al., 1996; Uprety et al., 2010). Mirroring this, a lower AC at eCO_2 is reflected in a softer cooked grain (Goufo et al., 2014b; Jena et al., 2018; Yang et al., 2007). However, in several studies, AC was found to be unaffected by eCO_2 (Liu et al., 2017; Terao et al., 2005; Ward, 2007).

A decrease in nitrogen (N), and therefore, protein content (PC) of rice grain due to eCO_2 has been reported in many studies on domesticated rice, indica and japonica varieties (Jena et al., 2018; Jing et al., 2016a; Seneweera et al., 1996; Taub et al., 2008; Yang and Wang, 2019). The average decline in N due to eCO_2 ranged from 10 to 24% (Jing et al., 2016a; Terao et al., 2005; Ujiie et al., 2019; Zhang et al., 2015). The decreases in N/PC were explained as a dilution effect (i.e., increased abundance of carbohydrate diluting the N in the grain) caused by CO_2 elevation (Gifford et al., 2000; Jing et al., 2016a; Taub et al., 2008). Some researchers have argued that enhanced carbon assimilation and lower N uptake and translocation from leaf to grain at eCO_2 caused a decline in grain N/PC (Ujiie et al., 2019) because 40–50% of grain N is translocated from leaves. Lower grain N could be due to a decrease in leaf N concentration, in turn a result of a lower abundance of photosynthetic enzymes, mostly Rubisco (normally ~40% of total leaf protein), due to photosynthetic acclimation at elevated CO_2 (Lieffering et al., 2004). During the exposure of a crop to eCO_2, nitrate assimilation diminishes, and grain is depleted of organic N, diminishing nutritional quality (Taub et al., 2008). However, decreased PC at eCO_2 could conceivably enhance cooking and eating qualities of rice, as has been demonstrated through Rapid Visco Analyser (RVA) data from an OTC experiment (Goufo et al., 2014a; Jing et al., 2016a).

Values of the RVA parameters peak viscosity (PV), trough viscosity (TV), final viscosity (FV) and the derived parameters breakdown (BD) (= PV - TV) and setback (SB) (= FV - TV) are important in predicting eating, cooking and processing qualities of rice (Allahgholipour et al., 2006). PV and/or FV increased significantly when rice was grown under eCO_2 in OTC or free-air CO_2 enrichment (FACE) experiments (Goufo et al., 2014a; Jing et al., 2016a; Terao et al., 2005; Yang et al., 2007). A higher PV indicates starch gelatinizes more easily. In rice at eCO_2, the increased PV was explained by lower PC (Terao et al., 2005). In contrast, another study reported a decrease in PV at eCO_2, which was associated with increased firmness in cooked rice (Seneweera et al., 1996). A decrease in gelatinization temperature was also reported due to eCO_2 (Jing et al., 2016a). The presence of α-amylase activity in grains would decrease PV. Addition of silver nitrate inactivates α-amylase activity in waxy rice flours, resulting in increased PV values (Huang and Lai, 2014). In the previous study (*Chapter 3*), the use of 1% $AgNO_3$ solution instead of dH_2O in the RVA increased PV of rice substantially, indicating inactivation of α-amylase activity (Rahman et al., 2021b).

As discussed above, numerous studies on the effects of eCO_2 on the properties of rice grain from indica or japonica varieties have been conducted with plants grown in glasshouses, OTC or FACE experiments. However, little is known about the effects of eCO_2 on the properties of grains from wild species of rice compared with those of domesticated rice. It is important to fill this knowledge gap because the germplasm of wild *Oryza* species increasingly will be exploited in breeding programs targeting biotic and abiotic stress resistance in rice (Atwell et al., 2014). In the present study, it was hypothesised that the grain properties of wild rices will respond differently to eCO_2 from those of domesticated rice because selection has substantially altered starch and protein levels in domesticated rice over millennia, making their grain distinct from their wild ancestors. To my knowledge, no experiments have been conducted on the effect of eCO_2 on grain quality of any wild *Oryza* species.

To test the hypothesis that, "the grain properties of wild rices will respond differently to eCO_2 from those of domesticated rice", the effects of eCO_2 on the physicochemical properties of grain harvested from two wild Australian *Oryza* species (*O. australiensis* and *O. meridionalis*) were compared to those for grain from a long-grained Australian cultivar (*O. sativa* cv. Doongara). The effect of eCO_2 was determined on seed morphology, amylose content (AC), estimated protein content (PC) and pasting properties of flour from whole grain.

4.2. Material and Methods

4.2.1. Rice germplasm, plant growth and seed collection

Properties of grain from four rice genotypes, *O. australiensis*, *O. meridionalis* (acc. Cape York and Howard Springs) and *O. sativa* cv. Doongara, grown in aCO_2 and eCO_2 were examined (Fig. 4.1). *O. meridionalis* acc. Cape York was a gift from Dr Lindsay Campbell, University of Sydney, whereas the remaining accessions were sourced from the Australian Grains Genebank, Horsham, Victoria. Rice plants were grown in glasshouses as described in *Chapter 2* (Rahman et al., 2018). Water was applied directly to the soil, a finely sieved clay loam, to prevent pre-harvest sprouting that might have occurred with overhead irrigation.

Due to the indeterminate nature of the wild rices, seeds were collected by hand from panicles over four weeks. For each of the rice genotype/CO_2 treatment combinations, rice grains were collected from ten plants and pooled for analysis (individual seeds cannot be used to generate the data reported here). Technical replication was performed as stated for the individual analyses below.

4.2.2. Seed weight, size, and density

Thousand seed weight (TSW) was determined by weighing 100 seeds (± 0.001 g) selected randomly from the bulked seeds. To determine seed dimensions, ten seeds were randomly selected, and seed length (Sl) and seed width (Sw) measured using digital callipers (± 0.001 mm). Bulk density (Bd) in g/cm^3 was calculated following Dhananchezhiyan et al. (2013). All the procedures above were performed in quadruplicate.

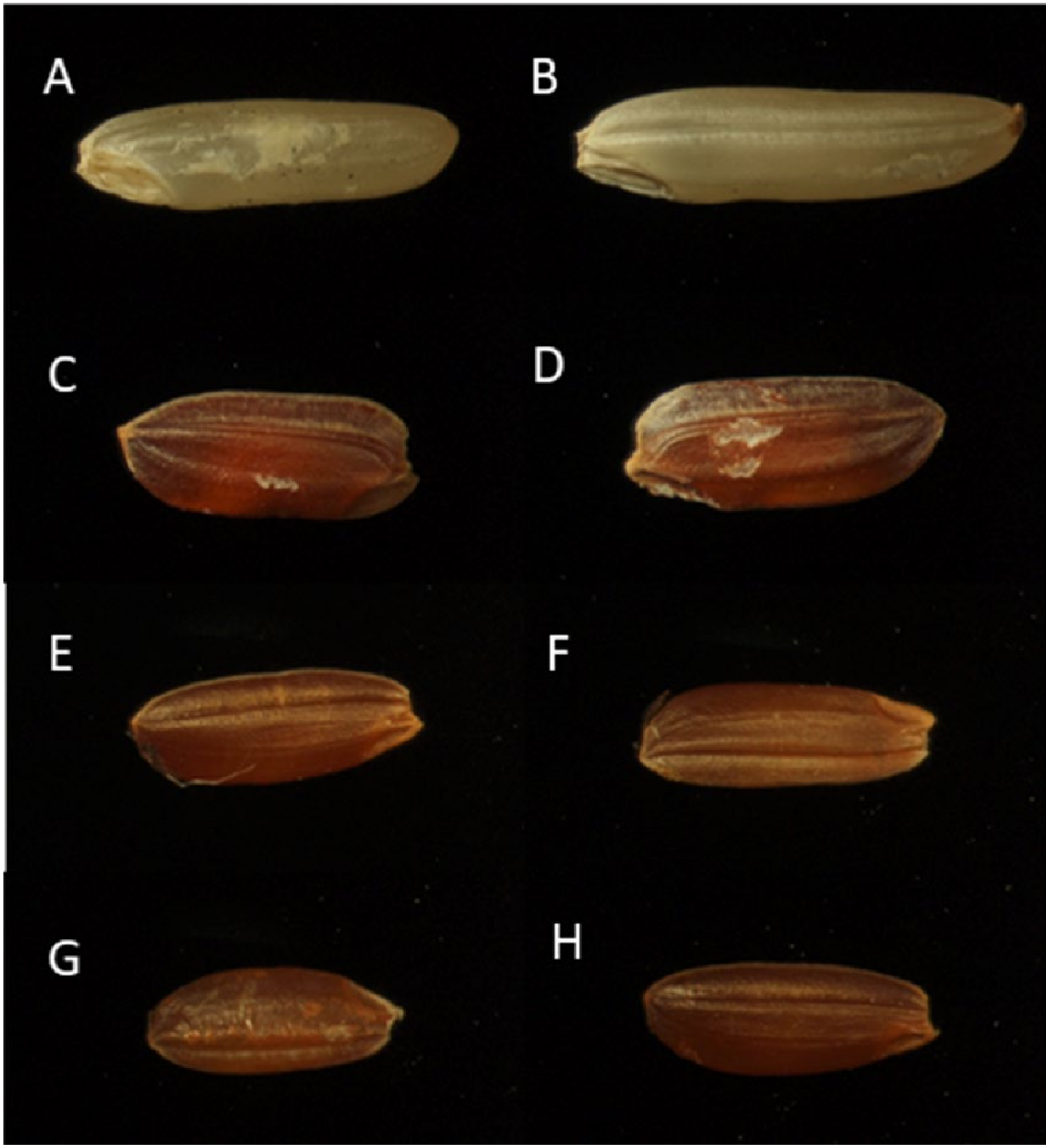


Fig. 4.1. Photographs of representative grains (with seedcoat removed) from rice plants grown in glasshouses at ambient (A, C, E, G) and elevated atm-CO_2 (700 ppm) (B, D, F, H). A, B, *O. sativa* cv. Doongara; C, D, *O. australiensis*; E, F, *O. meridionalis* acc. Cape York; G, H, *O. meridionalis* acc. Howard Springs. Average grain sizes are given in Table 4.1.

4.2.3. Moisture content (%)

Moisture content (MC) was measured using a Discovery Series Thermogravimetric analyser (TGA, TA Instruments, New Castle, DE, USA). Three to four seeds were placed on a clean platinum pan (100 µL) and inserted into the TGA. The initial weight of the seeds was recorded automatically. The N_2 flow rate was set at 20 mL/min. The temperature was ramped up to 150 °C @ 20 °C per min and isothermal conditions were maintained for 60 min. The final weight was recorded and the MC calculated as a total weight loss percentage. MC was determined in duplicate.

4.2.4. Carbon (C), nitrogen (N), C:N ratio and protein content (PC)

Seeds were placed in a metal crucible, weighed, and placed on the carousel of a Vario MAX CNS macro element analyser (Elementar Analysensysteme GmbH, Hanau, Germany). Protein content was calculated from the total N content using the conversion factor of 5.75 for rice (Fujihara et al., 2008). Measurements were performed in duplicate.

4.2.5. Starch extraction

Starch was extracted following Mohammadkhani et al. (1998) with slight modifications. Twelve seeds were placed in a 1.5-mL Eppendorf tube and soaked overnight in 0.2 M ammonia. The ammonia was decanted, and three ammonia-soaked seeds were placed into a 1.5-mL Eppendorf tube and 0.5 mL of 0.5 M NaCl was added. The seeds were ground with an Eppendorf pestle and the cell debris allowed to settle for 30 s. The supernatant containing suspended starch was transferred through a 150-µm sieve to a 1.5-mL Eppendorf tube. The procedure was repeated, and the starch suspensions were combined and centrifuged at 12,000 g for 1 min. The pelleted starch was resuspended in 1 mL of 0.1 M acetic acid, vortexed and centrifuged as above. The supernatant was discarded, and the pellet washed with ethanol and then with acetone as above. The process was repeated to extract the starch from all twelve seeds. The starch pellet was left to dry overnight and stored in a desiccator over silica gel.

4.2.6. Amylose assay

Apparent amylose content (AC) was determined using a modified method derived from Mohammadkhani et al. (1999). Starch (5 ± 0.1 mg) was weighed in a 5-mL test tube in duplicate, and 10 µL/mg of 95% ethanol (EtOH) and 90 µL/mg of 1 M NaOH were added to the sample. The sample was heated for 15 min in a hot water bath (95 °C) with occasional shaking to gelatinize the starch. The solution was cooled, and the volume brought up to 2.5 mL with dH_2O to a final starch concentration of 2 mg/mL. A 100-µL aliquot of the 2 mg/mL starch solution was transferred to a 5-mL test tube; 20 µL of 1 M acetic acid, 40 µL of iodine solution (0.2 g I_2 and 2.0 g KI in 100 mL aqueous solution) and dH_2O were added to the solution to make the final volume to 2 mL. The solution was mixed and left at room temperature for 20 min to develop a blue colour. A range of amylose/amylopectin solutions (100:0, 75:25, 50:50, 25:75 and 0:100 %) were prepared similarly as standards (2 mg starch/mL) using potato amylose (Sigma-Aldrich, Castle Hill, NSW, Australia) and maize amylopectin (Sigma-Aldrich, Castle Hill, NSW, Australia). An aliquot (200 µL) of test solution or standard was transferred into a 96-well ELISA plate and absorbance measured at A620 nm in a microplate reader (Synergy H1, BioTek Instruments, VT, USA) against water as the blank.

4.2.7. Pasting properties using a Rapid Visco Analyser (RVA)

Sufficient rice grains to produce about 10 g of flour were ground in a domestic coffee grinder (three times for 10 s with 10-s intervals) and sieved through a 450-µm sieve. Pasting properties of rice flour were determined using an RVA-4SA (Perten Instruments Australia Pty Ltd, Macquarie Park, NSW, Australia). Freshly prepared rice flour (3.5 ± 0.01 g) was weighed in an RVA canister and a freshly prepared 1% (w/v) $AgNO_3$ solution added to bring the total weight to 28 g (Rahman et al., 2021b). The flour was mixed by stirring the paddle vigorously. The canister was inserted into the instrument, which was run for a 14-min cycle as described in *Chapter 3* (Rahman et al., 2021b). All RVA tests were run in duplicate. The pasting parameters were recorded in centipoise (cP) and quantified using the software provided with the instrument.

4.2.8. Statistical analysis

Statistical analysis was performed with JMP Pro 14 (statistical software from SAS, Lane Cove, NSW, Australia). A random-effect model (REML) was used to analyse the data where replication was treated as a random factor. The means were separated using Tukey's HSD test. Graphs were generated using Graph Pad Prism-8.

4.3. Results

4.3.1. Effect of eCO_2 on physical properties of rice grains

Seed dimensions were affected by eCO_2 in a germplasm-specific manner. Elevated CO_2 increased seed length (Sl) in the Doongara cultivar and in the *O. meridionalis* accession Howard Springs ($p < 0.0001$) but reduced it in the Cape York accession ($p < 0.0001$; Table 4.1). In the Howard Springs samples, Sw was increased by eCO_2 ($p = 0.0041$), whereas it was unchanged in the Cape York, Doongara and *O. australiensis* samples. Length-to-width ratio (LW) was lower in the Cape York and Howard Springs samples ($p < 0.001$) at eCO_2 than at aCO_2 (Table 4.1).

Thousand seed weight (TSW) was greater ($p < 0.05$) across all four samples when plants were grown under eCO_2 compared with aCO_2 (Table 4.1). The domesticated rice (cv. Doongara) had heavier seeds than the wild relatives. Among the wild rices, *O. australiensis* had greater TSW than the two *O. meridionalis* accessions (Table 4.1). The effect of eCO_2 treatment on bulk density (Bd) was significant ($p < 0.001$) only for *O. australiensis*, which had greater Bd at eCO_2 than at aCO_2 ($p < 0.001$; Table 4.1).

Table 4.1. Seed length (Sl), seed width (Sw), seed length to width ratio (LW), 1000 seed weight (TSW) and bulk density (Bd) of *O. australiensis*, *O. meridionalis* (acc. Cape York and Howard Springs) and *O. sativa* cv. Doongara grains from plants grown in glasshouses at ambient (400 ppm) or elevated (700 ppm) CO_2. Supplementary lighting was used to maintain light levels above 400 μmol m^{-2} s^{-1} during the day. The temperature was maintained 28/22 °C in a 12-h day/night cycle. Moisture content of the harvested grains ranged from 9.1 to 11.0%.

Genotype	CO_2 Treatment		Sl (mm)		Sw (mm)		LW		TSW (g)		Bd (g/mL)	
O. sativa												
cv. Doongara	ambient CO_2 (aCO_2) 400 ppm		7.02	b	1.96	b	3.58	a	20.25	b	0.798	bc
O. australiensis			5.68	c	2.51	a	2.26	d	13.03	d	0.763	d
O. meridionalis												
acc. Cape York			5.36	d	1.92	b	2.80	b	8.69	f	0.810	ab
, acc. Howard Springs			5.00	f	1.74	c	2.87	b	7.24	h	0.818	a
O. sativa												
cv. Doongara	elevated CO_2 (eCO_2) 700 ppm		7.29	a	2.01	b	3.63	a	21.70	a	0.790	c
O. australiensis			5.68	c	2.60	a	2.19	d	14.10	c	0.795	bc
O. meridionalis												
acc. Cape York			5.24	e	1.98	b	2.65	c	10.11	e	0.813	ab
acc. Howard Springs			5.22	e	1.95	b	2.68	c	8.09	g	0.820	a
Genotype × CO_2 interaction		*p*	<0.0001		0.0041		0.0005		<0.0467		0.0006	
Genotype		*p*	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
CO_2 Treatment		*p*	<0.0001		<0.0001		<0.0001		<0.0001		0.0181	

Values not connected by the same letter are significantly different.

4.3.2. Effect of eCO_2 on composition of rice grains

4.3.2.1. Carbon (C), nitrogen (N), CN ratio and protein content (PC)

Elevated CO_2 as a function of genotype did not show significant changes in C, N and C:N, although N and C:N showed significant changes with CO_2 and genotype alone (Table 4.2). The average PC (calculated from N using conversion factor 5.75) was higher ($p < 0.01$) in aCO_2 (10.7%) than in eCO_2 (9.5%). The domesticated rice cv. Doongara had lower average PC (8.6%) than the wild genotypes (10.5–11.0%). PC did not differ significantly among the wild genotypes. The relative changes (RC) in N (and therefore PC) for eCO_2 compared to aCO_2 were substantial in Doongara (RC = -23.6%) and in the rice from Howard Springs (RC = -15.4%) (Table 4.2).

4.3.2.2. Apparent amylose content (AC)

Preliminary experiments were conducted on starch extraction methods and amylose assay methods to select the best methods for this study (Tables 4.3a and 4.3b). Starch extraction was carried out for five different types of rice following Mohammadkhani et al. (1998) and Mohammadkhani et al. (1999) and the apparent amylose content (AC) determined at A620 nm using a UV-Vis spectrophotometer (UVmini-1240), following Mohammadkhani et al. (1998). AC was higher from the starch extracted using Mohammadkhani et al. (1998) and was selected for future experiments (Table 4.3a).

Table 4.2. Effect of elevated atm-CO_2 on % carbon (C), % nitrogen (N), C:N ratio, AC (%) in wild and domesticated rices. C:N ratio was determined from the C and N value. The relative change (RC (%)) was calculated as a percent increase or decrease in eCO_2 (700 ppm) over aCO_2 (400 ppm). Plant growth conditions are described in Table 4.1.

Genotype	CO_2 Treatment	C (%)	%RC	N (%)	%RC	C:N ratio	%RC	AC (%)	%RC
O. sativa									
cv. Doongara	ambient CO_2	39.6		1.69 ab		23.4 b		22.3 b	
O. australiensis	(aCO_2) 400 ppm	39.8		1.95 a		20.4 b		26.3 a	
O. meridionalis									
acc. Cape York		39.4		1.85 a		21.3 b		28.1 a	
acc. Howard Springs		39.4		1.97 a		20.0 b		28.4 a	
O. sativa									
cv. Doongara	elevated CO_2	36.3	-8.3	1.29 b	-23.6	28.2 a	20.5	21.8 b	-2.2
O. australiensis	(eCO_2) 700 ppm	39.7	-0.3	1.86 a	-4.6	21.3 b	4.4	27.8 a	5.7
O. meridionalis									
acc. Cape York		39.3	-0.3	1.79 a	-3.2	22.0 b	3.3	27.0 a	-3.9
acc. Howard Springs		39.3	-0.3	1.67 ab	-15.4	23.6 b	18.0	27.7 a	-2.5
Genotype × CO_2 interaction	*p*	0.541		0.218		0.070		0.7174	
Genotype	*p*	0.551		0.007		0.001		0.0049	
CO_2 Treatment	*p*	0.357		0.008		0.002		0.8305	

Values not connected by the same letter are significantly different.

Table 4.3. Selection of starch extraction method and amylose content determination. a. Starch was extracted from five types of rice according to Mohammadkhani et al. (1998) and Mohammadkhani et al. (1999) and the AC was assayed following Mohammadkhani et al. (1998). Absorbance was recorded at A620 nm using a spectrophotometer. **b.** Starch was extracted from Doongara rice grown under aCO_2 following Mohammadkhani et al. (1998). AC was determined using four different methods and the values compared. Absorbance was recorded at A620 nm using a 96-well microplate reader.

a. Genotype × Starch extraction method

Genotype	AC (%)		Starch extraction method	AC (%)	
Doongara polished	24.8	b			
Doongara whole seed	22.8	c	Mohammadkhani et al. (1998)	23.54	a
Long grain whole seed	22.8	c	Mohammadkhani et al. (1999)	22.44	b
Medium grain whole seed	13.0	d			
Cape York composite seed	31.7	a			
p	<0.0001		*p*	0.0002	

Starch extraction method	Genotype	AC (%)
Mohammadkhani et al. (1998)	Doongara polished	25.2
	Doongara whole seed	23.9
	Long grain whole seed	23.0
	Medium grain whole seed	13.3
	Cape York composite seed	32.3
Mohammadkhani et al. (1999)	Doongara polished	24.3
	Doongara whole seed	21.7
	Long grain whole seed	22.5
	Medium grain whole seed	12.8
	Cape York composite seed	31.0
	p	0.0754

Values not connected by the same letter are significantly different

Table 4.3. ***(cont'd)***

b. Amylose determination by different assay methods. Starch extracted from cv. Doongara grown in aCO_2 following Mohammadkhani et al. (1998).

Assay method		AC (%)
Juliano, B. O. (1971)		23.5
Modified-1: 100 µL 2 mg/mL starch solution + 20 µL 1 N AA + 40 µL iodine solution + 1.84 mL H_2O		23.9
Modified 2: 100 µL 2 mg/mL starch solution + 30 µL 1 N AA + 40 µL iodine solution + 1.83 mL H_2O		23.0
Mohammadkhani et al. (1999)		22.2
**An aliquot (200 µL) solution was dispensed in each well and absorbance was recorded at 620 nm.	*p*	0.19

In another experiment, starch was extracted from Doongara grown under aCO_2 following Mohammadkhani et al. (1998) and AC was assayed following methods described in Juliano (1971), Mohammadkhani et al. (1999), and two modifications (Modified 1 and Modified 2–Table 4.3b). The absorbance was measured at A620 nm using a 96-well plate reader and AC was calculated (Table 4.3b). There was no significant difference obtained among the methods; however, since the AC in the Modified 1 method was numerically slightly higher (Table 4.3b), Modified 1 method was used to determine the CO_2 effects on AC in the cultivated and wild genotypes. The absorbance values for the standard solutions, 2 mg/mL and 1 mg/mL, were measured at A620 nm in a 96-well plate reader. The absorbance readings from the 96-well plate reader and spectrophotometer (1 mg/mL standard) were compared, which showed that the A620 value for a 2 mg/mL solution in the 96-well plate reader was close to that for a 1 mg/mL solution in the spectrophotometer (Fig. 4.2). When the absorbance of 2 mg/mL standard solution in the 96-well plate reader was plotted against 1 mg/mL standard solution in the spectrophotometer, the R^2 (0.99) for regression was highly significant (Fig. 4.3), confirming the validity of the plate reader method.

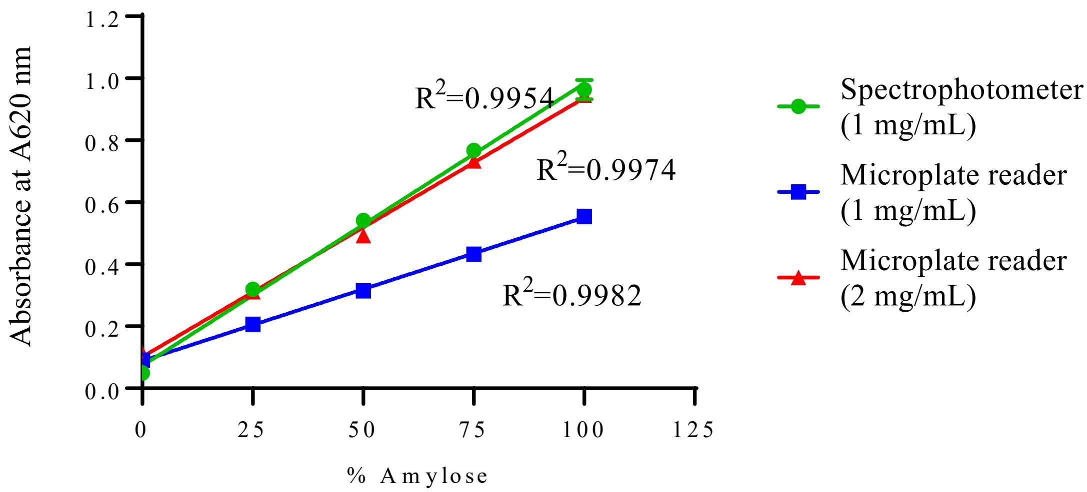


Fig. 4.2. Absorbance measured at A620 nm for amylose/amylopectin standards in a 96-well microplate reader. Standard solutions of 1 mg/mL and 2 mg/mL were used in the microplate reader and a 1 mg/mL solution in the spectrophotometer. The X-axis represents the standard concentrations as percentage amylose.

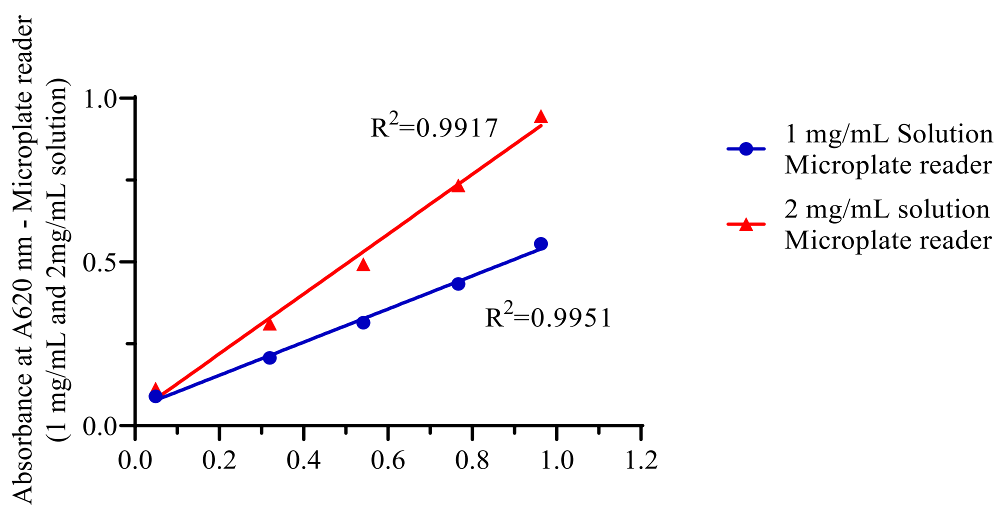


Fig. 4.3. The absorbance of standards of 1 mg/mL solution measured at A620 nm both in a spectrophotometer and a 96-well microplate reader. For Modified Method 1 (2 mg/mL solution), the absorbance of the standards was measured at A620 in a 96-well plate reader. The absorbance readings from the 96-well plate reader for both 1 mg/mL and 2 mg/mL method were plotted against absorbance for the 1 mg/mL method in spectrophotometer.

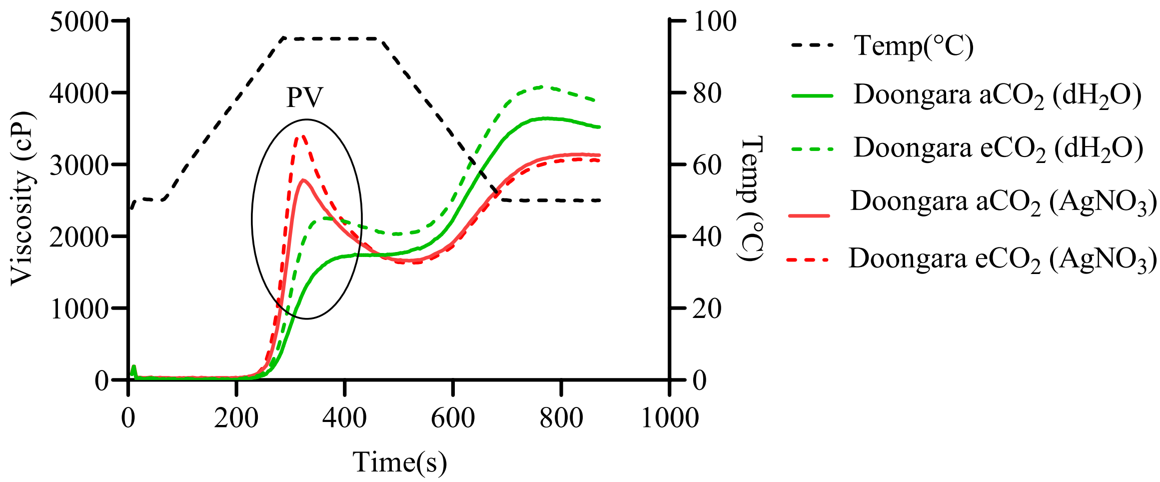
In this study, AC ranged from 21.8 to 28.4% among the eight germplasm/atm CO_2 combinations but was unaltered for any of the rices by eCO_2. Doongara had the lowest AC, which was significantly different from all three wild accessions. There were no significant differences in AC among the wild rices (Table 4.2).

4.3.2.3. Pasting properties of rice flour using an RVA

Pasting properties were determined in 1% $AgNO_3$ solution instead of dH_2O to avoid interference from α-amylase activity that may have been present in the rice flours (Fig. 4.4 A–C, Table 4.4). *Oryza australiensis* was excluded from this experiment due to insufficient sample. The effects of genotype, CO_2 and genotype × CO_2 interaction were highly significant ($p < 0.0001$) for PV and FV. Values for both these RVA parameters were significantly higher in flours from the eCO_2 than aCO_2 samples. Doongara had the highest PV and lowest FV both in ambient and elevated CO_2. FV was higher in Howard Springs than in Doongara and Cape York in both aCO_2 and eCO_2. The most noteworthy effect of eCO_2 was in flour from the Howard Springs accession of *O. meridionalis*. Growth in eCO_2 resulted in PV being increased by 30% and pasting time decreasing from about 7 to 5.7 min (Table 4.4).

The RVA pasting viscosities were also measured in dH_2O rather than 1% $AgNO_3$ solution. Significantly lower PV was recorded in dH_2O for all genotypes grown in ambient or elevated CO_2, showing that α-amylase activity was present in all of the rice flours (Table 4.5, Fig. 4.4A-C).

A.



B.

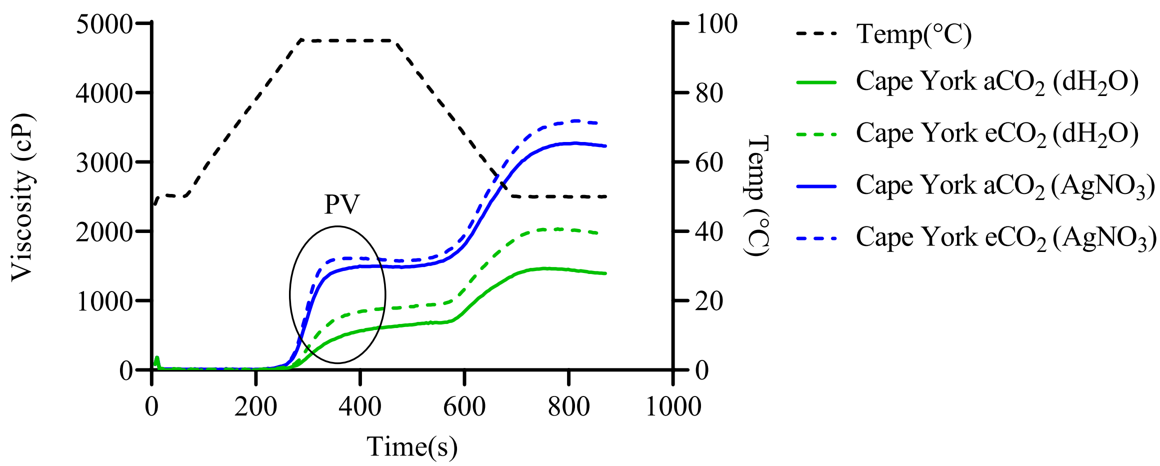


Fig. 4.4. RVA viscograms for rice grown under ambient or elevated CO_2, in the presence of 1% $AgNO_3$ and dH_2O. A. *O. sativa* cv. Doongara. B. *O. meridionalis* acc. Cape York. C. *O. meridionalis* acc. Howard Springs. Maximum PV was observed within 5–7 minutes (Pt) when gelatinisation took place. In the first instance, the PV increased due to eCO_2, which is the difference between the solid and broken lines, while in the second instance, the increase was due to α-amylase inactivation.

Fig. 4.4. (*cont'd*)

C.

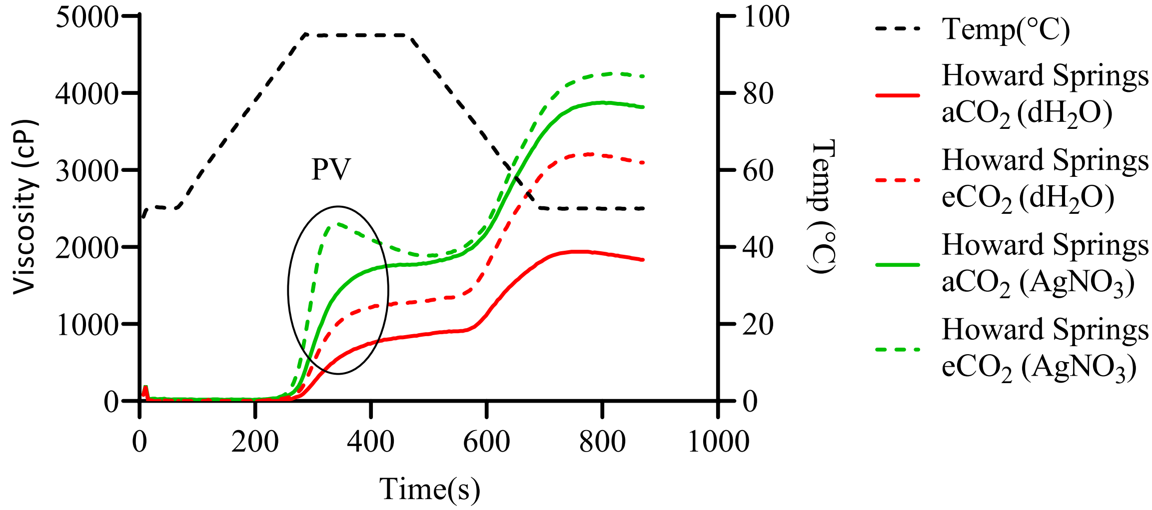


Fig. 4.4. (*cont'd*) RVA viscograms for rice grown under ambient or elevated CO_2, in the presence of 1% $AgNO_3$ and dH_2O. A. *O. sativa* cv. Doongara. B. *O. meridionalis* acc. Cape York. C. *O. meridionalis* acc. Howard Springs. Maximum PV was observed within 5–7 minutes (Pt) when gelatinisation took place. In the first instance, the PV increased due to eCO_2, which is the difference between the solid and broken lines, while in the second instance, the increase was due to α-amylase inactivation.

Table 4.4. Pasting properties of wild and domesticated rices determined in an RVA using 1% $AgNO_3$. Peak viscosity (PV), hot paste viscosity or trough viscosity (TV), final viscosity (FV), peak time (Pt) and pasting temperature (PT) were measured. Viscosity values are given as cP. Plants were grown in glasshouses under aCO_2 (400 ppm) and eCO_2 (700 ppm). Plant growth conditions are described in Table 4.1.

Genotype	CO_2 Treatment	PV (cP)		TV (cP)		BD (PV-TV) (cP)		FV (cP)		SB (FV-TV) (cP)		Pt (min)		PT (°C)	
O. sativa															
cv. Doongara	ambient CO_2 (aCO_2) 400 ppm	2780	b	1663	bc	1118	b	3133	e	1470	d	5.4	c	88	cd
O meridionalis															
acc. Cape York		1498	f	1489	c	9	d	3232	d	1743	c	6.8	a	91	ab
acc. Howard Springs		1739	d	1748	ab	-9	d	3820	b	2072	b	7.0	a	92	a
O. sativa															
cv. Doongara	elevated CO_2 (eCO_2) 700 ppm	3402	a	1629	bc	1773	a	3057	f	1428	d	5.3	c	87	d
O meridionalis															
acc. Cape York		1620	e	1573	bc	47	d	3546	c	1973	b	6.3	ab	91	ab
acc. Howard Springs		2301	c	1886	a	415	c	4217	a	2331	a	5.7	bc	90	bc
Genotype × CO_2	*p*	<0.0001		0.1094		<0.0004		<0.0001		0.0104		0.0158		0.0483	
Genotype	*p*	<0.0001		0.0009		<0.0001		<0.0001		<0.0001		0.0007		0.0003	
CO_2 Treatment	*p*	<0.0001		0.0659		<0.0001		<0.0001		0.0025		0.0027		0.0069	

Values not connected by the same letter are significantly different.

Table 4.5. Pasting properties of wild and domesticated rices determined in an RVA using dH_2O. Peak viscosity (PV), hot paste viscosity or trough viscosity (TV), final viscosity (FV), peak time (Pt) and pasting temperature (PT) were measured. Viscosity values are given as cP. Plants were grown in glasshouses under aCO_2 (400 ppm) and eCO_2 (700 ppm). Plant growth conditions are described in Table 4.1.

Genotype	CO_2 Treatment	PV (cP)		TV (cP)		BD (PV-TV) (cP)		FV (cP)		SB (FV-TV) (cP)		Pt (min)		PT (°C)	
O. sativa															
cv. Doongara	ambient CO_2 (aCO_2) 400 ppm	1746	b	1730	b	17	b	3524	ab	1794	a	6.98	a	90.60	cd
O. australiensis		867	d	861	d	6	bc	1903	c	1042	bc	6.98	a	91.88	bc
O. meridionalis															
acc. Cape York		589	e	595	e	-6	cd	1394	d	800	c	6.98	a	94.95	a
acc. Howard Springs		786	d	797	d	-11	cd	1837	c	1041	bc	6.98	a	95.40	a
O. sativa															
cv. Doongara	elevated CO_2 (eCO_2) 700 ppm	2250	a	2033	a	217	a	3877	a	1844	a	6.05	b	88.75	d
O. australiensis		875	d	884	d	-10	cd	1992	c	1108	b	6.95	a	93.08	abc
O. meridionalis															
acc. Cape York		863	d	870	d	-7	cd	1960	c	1090	b	6.95	a	93.15	abc
acc. Howard Springs		1244	c	1257	c	-13	d	3097	b	1841	a	6.98	a	93.83	ab
Genotype × CO_2	*p*	0.0003		0.0012		<0.0001		0.0005		0.0003		<0.0001		0.0339	
Genotype	*p*	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
CO_2 Treatments	*p*	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		0.0209	

Values not connected by the same letter are significantly different.

4.4. Discussion

The present study provided evidence that, for specific aspects of grain morphology and pasting properties, accessions of Australian wild rices *O. australiensis* and *O. meridionalis* respond differently to eCO_2 than domesticated rice. These results are discussed in more detail below.

Grain dimensions are important for the yield and nutritional value of flour, largely because of the contrast between the nutrient-rich outer seed layers and the endosperm, which is milled to become flour. In both *O. sativa* cv. Doongara and *O. meridionalis* acc. Howard Springs, Sl increased significantly at eCO_2, similar to the findings of Jing et al. (2016a) for the japonica cultivar Wuyunjing 23. However, *O. meridionalis* acc. Cape York had a lower Sl under eCO_2, mirroring results of Usui et al. (2016) for another japonica cultivar, Koshihikari. Thus, it appears that responses of Sl to eCO_2 vary between genotypes in both domesticated and wild rices. Sw increased significantly at eCO_2 in the Howard Springs accession, consistent with the results of Madan et al. (2012) for two varieties of *O. sativa* and also a hybrid (Jing et al., 2016a). The increase in Sw was proposed to be due to the higher supply of substrate to the panicle under eCO_2 and is expected to improve milling quality (Madan et al., 2012). In contrast to the other rice genotypes tested in this study, *O. australiensis* did not show any changes in Sl or Sw at eCO_2 compared with aCO_2.

TSW increased due to eCO_2 across all four rice types, consistent with the results of Usui et al. (2014) for seven heat-tolerant cultivars and five standard cultivars grown in Japan. The results of this study are also consistent with other studies that reported eCO_2-induced increases in single grain weight (Yang et al., 2009), which suggested that higher grain weights at eCO_2 were due to the enhanced remobilization of prestored carbon reserves to the grain. The increased TSW at eCO_2 is likely to be at least partly due to the greater Sl and Sw, but might also reflect a higher proportion of starch in the seeds, as starch has a density of 1.5–1.6 g/cc^3 (Dengate et al., 1978), significantly greater than that of structural polysaccharides and proteins (Fischer et al., 2004).

As well as these physical characteristics, amylose content (AC) is a crucial factor in determining the processing, eating and cooking quality of rice (Bao, 2019; Patindol et al., 2015; Yang et al., 2007). However, AC was not significantly affected by eCO_2, as reported previously by Terao et al. (2005) for *O. sativa* cv. Akitakomachi. However, protein content (PC) is also an important determinant of milling quality and is critical for the nutrition of people whose diets are dominated by rice. During the exposure of plants to eCO_2, nitrate assimilation is suppressed and grain can be depleted in organic N, diminishing the protein content of the grain (Taub et al., 2008). Estimated PC (from N content) decreased in all genotypes in the current experiment due to eCO_2, consistent with previous reports (Goufo et al., 2014b; Jing et al., 2016a; Seneweera et al., 1996; Taub et al., 2008; Ujiie et al., 2019; Zhu et al., 2018). The decrease in PC was smallest in *O. australiensis* and Cape York, whereas Doongara had the largest decrease in PC due to eCO_2. Apart from increased production of carbohydrate simply diluting the nitrogen (Taub et al., 2008), the lower PC could have been due to reduced translocation of N from leaf blade to grains under eCO_2 (Jena et al., 2018; Ujiie et al., 2019). Reductions in grain N in polished rice grains and in brown rice of 13.5% and 11.5%, respectively, due to eCO_2 have been reported (Ujiie et al., 2019). A close correlation between the PC values of milled rice and brown rice was obtained in FACE experiments, suggesting the decrease in PC was not only in the aleurone layer (removed by polishing) but also the endosperm (Terao et al., 2005).

Grain C was not significantly changed at eCO_2 in this study, in contrast to the significant increase in grain C observed in rice plants grown under a less elevated CO_2 concentration in a FACE experiment (Zhang et al., 2015). While C:N was not affected in the wild rice species at eCO_2, Doongara seeds had significantly lower C:N, possibly due to a greater sensitivity of N assimilation to eCO_2 in the higher yielding commercial rice under the mechanism proposed previously (Bloom et al., 2010). Application of a higher dose of N at 25 g/m^2 rather than the standard 8–9 g/m^2 increases rice yield and improved grain N (Yang et al., 2007). These genetically determined differences in C:N response to eCO_2 might provide the basis of a useful trait for breeders aiming to ensure more nutritious grain as CO_2 emissions accelerate. A possible explanation is that the wild rices were less affected than the domesticated genotype because they had very low harvest indices (Atwell et al., 2014) and an indeterminate phase of grain filling, thus a greater capacity to remobilize N from their large vegetative biomass during grain filling.

The values of AC and PC reported are predictive of milling properties whereas RVA parameters (PV, TV, FV) and the derived parameters breakdown (BD) (= PV - TV) and setback (SB) (= FV - TV) are useful in predicting eating, cooking and processing qualities of rice (Allahgholipour et al., 2006). PV is the point at which the number of swollen but still intact granules is at a maximum relative to granules that have ruptured. BD values are indicative of the fragility of the starch granules and are good indices for the susceptibility of starch to disintegration (Juliano, 1985), whereas SB is a measure of the initial stages of retrogradation or hardening of starch upon cooling (Zulueta et al., 2000). Increased PV and BD are reflected in more easily gelatinized starch in cooked rice, which enhances palatability (Juliano, 1985). A lower SB is important for ready-to-eat rice products because it reduces syneresis (the extraction or expulsion of a liquid from a gel) during freeze/thaw cycles (Goufo et al., 2014a). A high BD value is good for palatability whereas a low SB value is related to softness after cooking (Zulueta et al., 2000). PV and BD are higher in waxy rice whereas SB increases with increased AC in rice (Allahgholipour et al., 2006).

In the present study, increases in PV due to eCO_2 were observed in all three rice genotypes tested (Table 4.4). Even though the PV in the Cape York and Howard Springs accessions increased after growth in eCO_2, it was significantly lower than in cv. Doongara in both aCO_2 and eCO_2. Yamashita and Fujimoto (1974) suggested that reduced values for PV and BD are associated with higher PC in rice grains, consistent with the findings of this study. However, Seneweera et al. (1996) reported increases in AC and SB, and decreases in PV due to eCO_2. In this study, AC was unaltered at eCO_2, but PC was slightly decreased, which may have caused the increase in PV. The lower PV, TV and FV obtained by Seneweera et al. (1996) could be due to different levels of pentosans, which bind water strongly (more than protein), and the way flour was prepared may also confound the results. Cooking and eating qualities of rice grain are enhanced by decreased PC, as demonstrated through RVA data from an OTC experiment (Goufo et al., 2014a).

The presence of α-amylase activity in flour reduces the viscosity of flour pastes. In the previous RVA study (*Chapter 3*), PV values were lower when water was used instead of 1% $AgNO_3$ in the RVA, showing that α-amylase activity in the rice flour was inhibited by the $AgNO_3$ (Rahman et al., 2021b). In the present study, dH_2O instead of 1% $AgNO_3$ in the RVA decreased PV significantly, indicating inactivation of α-amylase activity contributed to differences in PV. Overall, flour viscosity after growth in eCO_2 increased in the wild rices as well as domesticated rice.

4.5. Conclusion

This study has shown that specific grain morphology and pasting properties of the Australian wild rices (*O. australiensis* and *O. meridionalis*) and cultivated rice are likely to be differently affected by elevated atmospheric CO_2 concentrations. For example, Sl increased in Doongara while it decreased in Cape York and was unaltered in *O. australiensis* at eCO_2, whereas Sw was unaltered in Doongara but increased in Howard Springs. Furthermore, eCO_2 increased Bd in *O. australiensis* but not in Doongara. Although the decline observed in total N content due to eCO_2 was not statistically significant, the consistent decrease among all four genotypes indicated the commonly observed trend of high CO_2 diluting grain N. The effect was most pronounced in the cultivated genotype, Doongara. Pasting properties of all four rice flours were differently affected by eCO_2, whereas FV showed increases in wild rices but decreased in Doongara at eCO_2. Future research should aim to determine the effects of eCO_2 on the molecular structure and functional properties of starch from the wild and domesticated rices. The different effects of eCO_2 between wild and domesticated rices should be considered in the breeding of new elite cultivars by harnessing favourable traits from wild rice relatives.

Chapter 5

Endosperm morphology of Australian wild rices and the effects of elevated atmospheric CO_2

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Impact of elevated atmospheric CO_2 on aleurone cells and starch granule morphology in domesticated and wild rices

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5.1. Introduction

A detailed understanding of the responses of cereal crops to elevated levels of atmospheric CO_2 (eCO_2) that are likely to be encountered later this century is required to predict changes in yield and grain quality. This information should be acquired not only for current elite cultivars but for germplasm that will be used in breeding programs for cereal improvement.

In rice, two species, *Oryza sativa* (Asian rice) and *O. glaberrima* (African rice), have been domesticated, but there are at least another 24 wild species representing a wide range of genetic diversity within the genus (Brar and Khush, 2018; Henry, 2019; Stein et al., 2018). Accessions of these wild relatives represent valuable sources of germplasm for breeding of new elite rice genotypes for resistance to biotic and abiotic stresses (Atwell et al., 2014; Brar and Khush, 2018; Henry, 2019). The influence of eCO_2 on aspects of rice grain quality has been established in *O. sativa* (DaMatta et al., 2010; Jing et al., 2021; Jing et al., 2016b; Madan et al., 2012; Yang et al., 2007), but because of genetic differences between the domesticated and wild rices, while these results cannot be extrapolated to wild rice species. The effects of eCO_2 on grain properties are also reported in other major cereals, including wheat and barley (Myers et al., 2014; Senghor et al., 2017; Wang and Liu, 2021). A common finding is that relative protein content and mineral content are lower when plants are grown in eCO_2 compared to aCO_2.

The current paper represents the third of three studies on the effects of eCO_2 on accessions of wild rice species from northern Australia. In the first study, the differences in shoot architecture between two accessions of *O. meridionalis* and a long-grain Australian cultivar (*O. sativa* cv. Doongara) and how canopies were affected by eCO_2 were examined (Rahman et al., 2018; Chapter 2). Among the results, all genotypes showed enhanced growth at eCO_2 through higher carbon assimilation rates, despite reduced efficiency of light interception caused by higher crown densities and lower leaf dispersion. The second study was on the effects of eCO_2 on properties of brown rice grain and flour of accessions of *O. meridionalis* and *O. australiensis* (with a Doongara control). The seed length and width, 1000-seed weight, as well as the peak viscosity and breakdown viscosity of flour pastes were higher when plants were grown in eCO_2 than in aCO_2 (Rahman et al., 2021a; Chapter 4). The larger grain size observed in eCO_2 compared to aCO_2 was probably caused by a greater transfer of photosynthetic assimilate from leaf to caryopsis associated with increased rates of photosynthesis observed in the first paper (Rahman et al., 2018). However, consistent interactions between CO_2 supply during plant development and genotype led to the hypothesis that endosperm morphology might also respond differentially to eCO_2 among wild and domesticated rices. Hence the current study is focused on the effects of eCO_2 on the size and shape of aleurone cells and starch granules across genotypes. Few reports are available on the qualitative effects of eCO_2 on endosperm morphology in *O. sativa* (Jing et al., 2021; Jing et al., 2016b), although more starch accumulates in grains from plants grown in eCO_2 than in aCO_2 (Madan et al., 2012; Rahman et al., 2021a; Yang et al., 2009).

The novelty of the current study is the focus on the effects of eCO_2 on the size and shape of aleurone cells and starch granules across genotypes consisting of Australian wild rices and a commercial rice cultivar. Aleurone structure was investigated because it is critical for the process of seed germination and crop establishment while granule morphology might explain the contrasts in flour pasting properties that was reported previously in Chapter 4. To test the hypothesis that endosperm morphology responds differently to eCO_2 among wild and domesticated rices, SEM images of aleurone cells and starch granules were compared from accessions of two wild species (*O. australiensis* and *O. meridionalis*) and *O. sativa* cv. Doongara, grown under ambient and elevated atm-CO_2.

5.2. Material and Methods

5.2.1. Plant materials and growing conditions

Four rice genotypes, *O. australiensis*, *O. meridionalis* (acc. Cape York and Howard Springs), and *O. sativa* (cv. Doongara), were grown in ambient (aCO_2, 400 ppm) and elevated CO_2 (eCO_2, 700 ppm) in glasshouses at Macquarie University, Sydney, Australia. The Cape York grain sample was donated by Dr Lindsay Campbell, University of Sydney, Australia. The other genotypes were sourced from the Australian Grains Genebank, Horsham, Victoria. The plant growth conditions and handpicking of seeds from the indeterminate panicles of the wild rices were as described in Chapters 2 and 4 (Rahman et al., 2021a; Rahman et al., 2018).

5.2.2. *Scanning electron microscopy (SEM)*

For each of the eight sample types (four rice genotypes × two CO_2 treatments), five grains were selected randomly, dehusked manually and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 24 h at ~5 °C. The fixed grains were rinsed with dH_2O and dehydrated with a standard series of ethanol washes (Kasem et al., 2011). The grains were dried using a Leica EM CPD 300 critical point drier (Leica Microsystems GmbH, Wetzlar, Germany) and carefully snapped in half transversely using two pairs of forceps. Halved grains were mounted on aluminium stubs using double-sided carbon tape and silver paint, with the fractured surface facing up, and spattered with 20 nm gold using a CCU-010 HV vacuum coating system under a vacuum pressure of $5.0e^{+}$ Pa and current of 30.0 mA (Safematic GmbH, Zizers, Switzerland). Images were recorded using a JCM-6000 NeoScope Bench Top SEM (JEOL Australasia Pty Ltd, Frenchs Forest, NSW, Australia) under high vacuum with an accelerated voltage of 10 kV.

5.2.3. *Image analysis*

Six fields of view were selected from each surface exposed by transverse sectioning of the grain and imaged at different magnifications (×40, ×750, ×1500 and ×4000). For each rice genotype/CO_2 treatment, 8–10 images were used to generate data. The lengths and widths of a total of 150 aleurone cells (×270–400 magnification), and the area and length of 100 starch granules (×4000), were measured using FIJI ImageJ software (https://imagej.net/software/fiji/). Cell area was measured by manually placing 6–10 points around the cell perimeter. Cell dimensions were measured by placing a point on opposite ends of the cell (largest and smallest distance for length and width, respectively), which were connected automatically, and the distances calculated by ImageJ (User Guide ImageJ-NIH). The images were processed using Photoshop, Adobe Creative Cloud (Adobe Inc, https://adobe.com/au). Histograms were generated using GraphPad Prism 9.

5.2.4. *Statistical analysis*

Statistical analysis was performed using JMP Pro 15 (SAS, Lane Cove, NSW, Australia). General linear models were used to analyse the data. The means were compared using Tukey's HSD.

5.3. Results

5.3.1. Variation in pericarp and aleurone thickness

The pericarp was slightly thicker in the wild genotypes than in the cultivated genotype Doongara (Fig.5.1). The aleurone varied in thickness from one to several cells, depending on the genotype and viewing direction (Table 5.1, Figs. 5.1–5.4). Aleurone layers that were multiple cell thick were predominantly on the dorsal side of the grain, where *O. sativa* cv. Doongara had three to five cell layers, whereas *O. australiensis* and *O. meridionalis* (accessions Cape York and Howard Springs) had three to four cell layers (Table 5.1). Ventral and lateral sides had mostly single-celled aleurone layers for all genotypes (Table 5.1, Figs. 5.1–5.4) with occasionally double layers (Fig. 5.1). The aleurone cells were irregular in shape on the dorsal side, but rectangular or elongate on the ventral and lateral side (and Figs. 5.1–5.4).

5.3.2. Effect of elevated atm-CO_2 on aleurone cell size

Significant differences ($p < 0.0001$) were obtained among genotypes for length, width, and length-to-width ratio of the aleurone cells (Table 5.2). The aleurone cells were larger in Doongara than in the other genotypes ($p < 0.0001$). The interaction effect of genotype and CO_2 was significant for length ($p = 0.01$) and width ($p < 0.0001$). However, eCO_2 alone did not have a significant effect on aleurone cell length and width or length-to-width ratio (Table 5.2).

Doongara (*O. sativa*) had aleurone cell lengths of 37.1 ± 0.7 and 35.2 ± 0.7 µm in aCO_2 and eCO_2, respectively, which were greater than in the wild genotypes ($p < 0.0001$). The aleurone cell lengths of the *O. meridionalis* accessions from both Cape York and Howard Springs were not different from each other but were smaller than those of *O. australiensis* in both aCO_2 and eCO_2 ($p = 0.01$) (Table 5.2). Growth in eCO_2 decreased aleurone cell length in Doongara and increased it in *O. meridionalis* Howard Springs and made no differences in *O. australiensis* and *O. meridionalis* Cape York (Table 5.2).

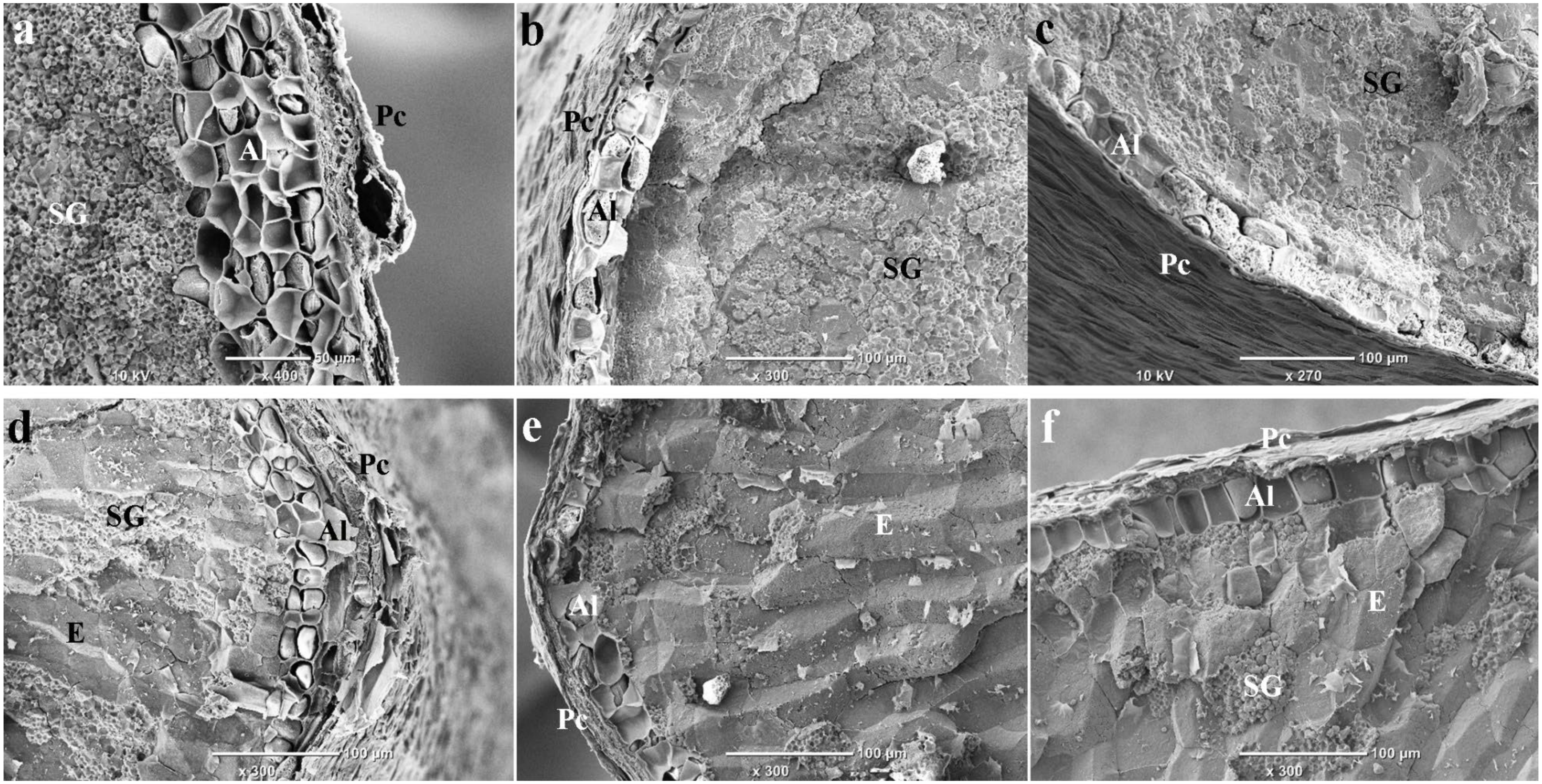

Fig. 5.1. Representative SEM images of the aleurone and the pericarp of cultivated rice *O. sativa* cv. Doongara (a-c), and the wild rice *O. australiensis* (d-f), dorsal, ventral and lateral views, respectively, grown at eCO_2 (700 ppm). Al, aleurone cell; E, endosperm cell; Pc, pericarp; SG, starch granules.

Table 5.1. Number of aleurone cell layers in cultivated and wild rice genotypes under elevated atm-CO_2 (700 ppm) compared with ambient atm-CO_2 (400 ppm).

Genotype	Treatment	Dorsal view	Ventral view	Lateral view
O. sativa	ambient CO_2			
cv. Doongara	(aCO_2), 400 ppm	3−5	1	1
O. australiensis		3−4	1	1
O. meridionalis				
acc. Cape York		2−3	1	1
acc. Howard Springs		2−3	1	1
O. sativa	elevated CO_2			
cv. Doongara	(eCO_2), 700 ppm	4−5	1−2	1−2
O. australiensis		3−4	1−2	1
O. meridionalis				
acc. Cape York		3−4	1	1
acc. Howard Springs		3	1	1

Genotype × CO_2 interaction showed that eCO_2 was associated with a greater aleurone cell width in *O. meridionalis* Howard Springs, but a smaller width in Doongara, as compared to aCO_2 (Table 5.2). The Doongara and *O. australiensis* aleurone cell widths were significantly different from those for the *O. meridionalis* accessions in both CO_2 treatments. No differences in aleurone cell width were observed between Doongara and *O. australiensis* and between *O. meridionalis* Cape York and Howard Springs in either CO_2 treatment (Table 5.2).

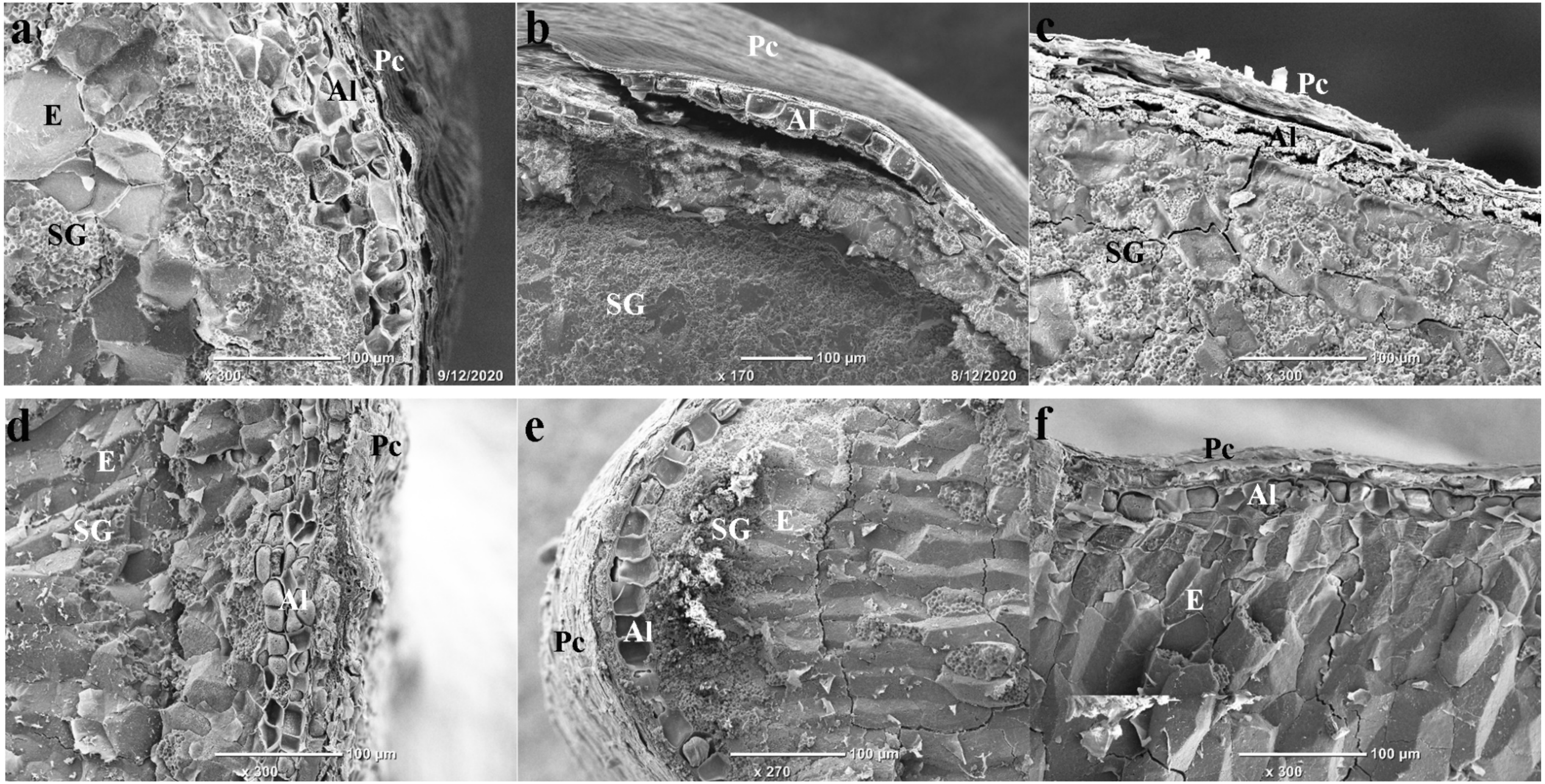

Fig. 5.2. Representative SEM images of the aleurone and the pericarp of cultivated rice, *O. sativa* cv. Doongara (a-c), and wild rice, *O. australiensis* (d-f), dorsal, ventral and lateral views, respectively, grown at aCO_2 (400 ppm). Al, aleurone cell; E, endosperm cell; Pc, pericarp; SG, starch granules.

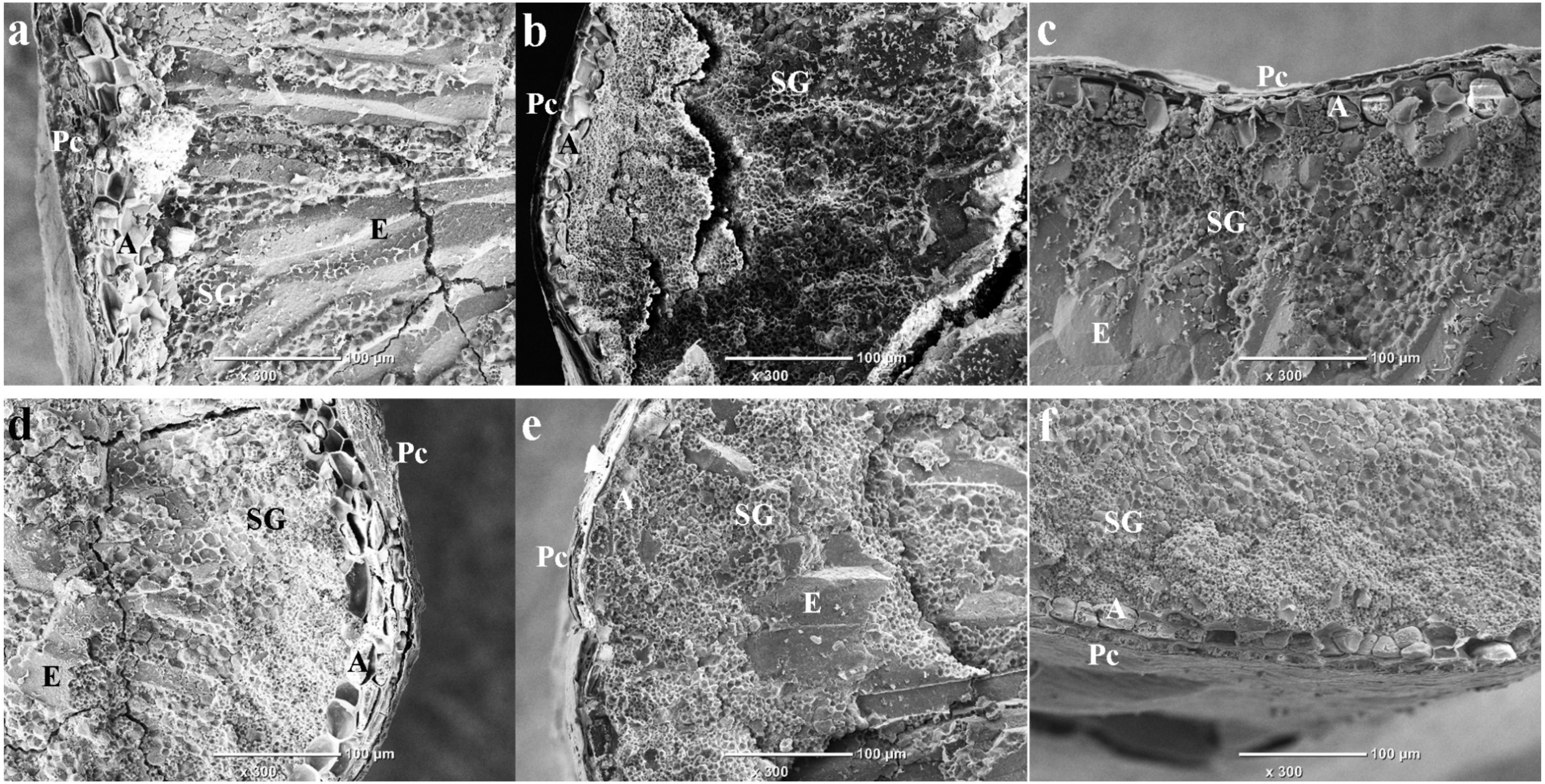

Fig. 5.3. Representative SEM images of the aleurone and the pericarp of wild rice (*O. meridionalis*) accessions, Cape York (a-c), and Howard Springs (d-f), dorsal, ventral and lateral view respectively, grown at ambient CO_2 (400 ppm, aCO_2). Al, aleurone cell; E, endosperm cell; Pc, preicarp; SG, starch granules.

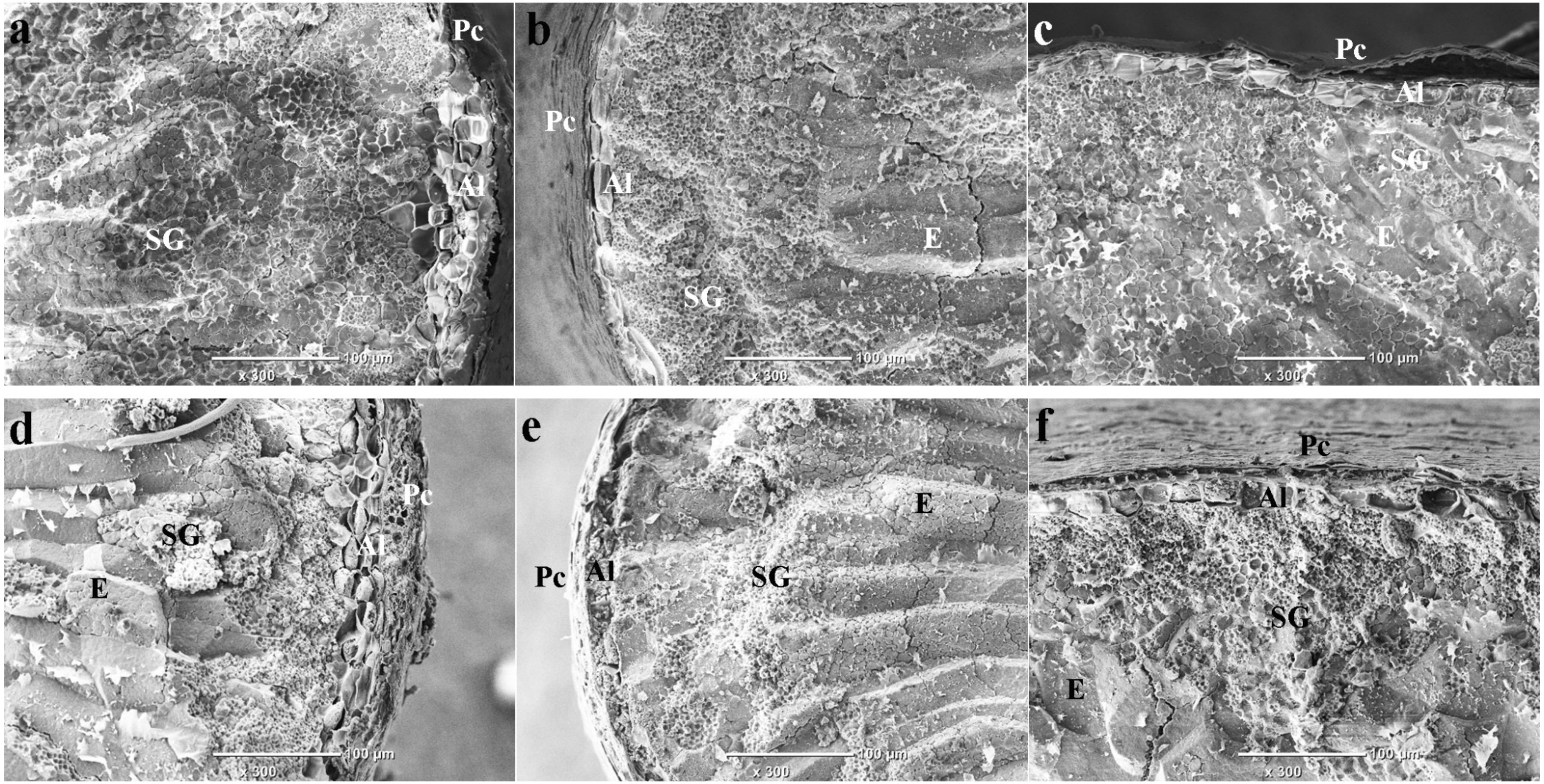

Fig. 5.4. Representative SEM images of the aleurone and the pericarp of wild rice (*O. meridional*) accessions, Cape York (a-c), and Howard Springs (d-f), dorsal, ventral and lateral view respectively, grown at elevated CO_2 (700 ppm, eCO_2). Al, aleurone cell; E, endosperm cell; Pc, preicarp; SG, starch granules.

Table 5.2. Variation in aleurone and starch granule size in cultivated and wild rice genotypes under elevated atm-CO_2 (700 ppm) compared with ambient atm-CO_2 (400 ppm).

Genotype (G)	Treatment (CO_2)	Aleurone cells					Starch granules					
		Length (μm) ± SE		Width (μm) ± SE		Length to Width ratio ± SE	Area (μm^2) ± SE		Length (μm) ± SE		Area to Length ratio (μm) ± SE	
O. sativa cv. Doongara	ambient CO_2 (aCO_2) 400 ppm)	37.1 ± 0.74	a	20.3 ± 0.53	a	1.94 ± 0.04	14.4 ± 0.57	a	4.7 ± 0.12	a	2.9 ± 0.06	a
O. australiensis		31.6 ± 0.63	b	19.9 ± 0.48	a	1.65 ± 0.03	9.3 ± 0.33	c	3.8 ± 0.07	c	2.4 ± 0.04	c
O. meridionalis												
acc. Cape York		27.3 ± 0.48	c	15.9 ± 0.30	cd	1.76 ± 0.03	9.6 ± 0.29	c	3.7 ± 0.06	c	2.5 ± 0.05	bc
acc. Howard Springs		26.3 ± 0.52	c	14.4 ± 0.30	d	1.88 ± 0.04	11.6 ± 0.40	b	4.2 ± 0.08	b	2.7 ± 0.06	b
O. sativa cv. Doongara	elevated CO_2 (eCO_2) 700 ppm	35.2 ± 0.68	a	18.1 ± 0.37	b	2.01 ± 0.04	11.8 ± 0.51	b	4.3 ± 0.10	b	2.7 ± 0.06	b
O. australiensis		31.3 ± 0.61	b	19.3 ± 0.42	ab	1.67 ± 0.03	9.3 ± 0.29	c	3.8 ± 0.07	c	2.4 ± 0.05	c
O. meridionalis												
acc. Cape York		27.1 ± 0.51	c	15.8 ± 0.38	cd	1.80 ± 0.04	10.8 ± 0.33	bc	4.1 ± 0.08	b	2.6 ± 0.05	bc
acc. Howard Springs		28.3 ± 0.51	c	16.2 ± 0.27	c	1.78 ± 0.03	10.5 ± 0.33	bc	3.9 ± 0.06	bc	2.6 ± 0.05	bc
G × CO_2	*p*	0.01		<0.0001		0.08	<0.0001		<0.0001		0.01	
G	*p*	<0.0001		<0.0001		<0.0001	<0.0001		<0.0001		<0.0001	
CO_2	*p*	0.82		0.338		0.791	0.02		0.127		0.028	

Levels not connected by same letter are significantly different.

5.3.3. Starch granule morphology

Representative SEM images of transverse sections of grains of the four rice genotypes at aCO_2 and eCO_2 are shown in Figs. 5.5–5.9. The fractured surface presented some surfaces that appeared smooth while others were rough. The smooth surfaces are likely to arise when fracturing of rice grains occurs between endosperm cells, whereas rough surfaces are probably due to fracturing within endosperm cells, resulting in exposure of starch granules (Figs. 5.5–5.8). The starch granules in Doongara (*O. sativa*) were, on average, larger than in the wild genotypes (*O. australiensis* and *O. meridionalis*) (Table 5.2 and Figs. 5.5–5.9). The shape of the granules was mostly polyhedral (Fig. 5.9). The granules in Doongara and *O. australiensis* appeared smoother than those from *O. meridionalis* Cape York and Howard Springs (Figs. 5.7 and 5.8). Ovoid starch granules were also observed in *O. australiensis* (Figs. 5.6 and 5.9, b). Structures that had the appearance of protein bodies were observed, as were pores and dimples on the starch granules that may have been left after the dislodgment of protein bodies (Fig. 5.9).

5.3.4. Effect of elevated atm-CO_2 on starch granule size

Variations in starch granule area ($p < 0.0001$), length ($p < 0.0001$) and area-to-length ratio ($p = 0.01$) were shown to be related to genotype × CO_2 interaction (Table 5.2).

For ~90% of the starch granules analysed for each sample, the observed granule area in Doongara was 5–23 and 6–19 μm^2, and the length was 4–7 and 3–6 μm, in aCO_2 and eCO_2, respectively (Fig. 5.10). In the wild rices, the granule area was 5–19 and 5–17 μm^2, and the length was 3–6 and 3–5 μm, in aCO_2 and eCO_2, respectively (Figs. 5.11–5.13).

Genotype × CO_2 interaction analysis revealed that the average starch granule area in Doongara was smaller in eCO_2 than in aCO_2 (Table 5.2). In aCO_2 Howard Springs had a greater granule area than the other *O. meridionalis* accession (Cape York) and *O. australiensis*. There were no significant effects of eCO_2 on granule area in *O. australiensis* and Cape York (Table 5.2). Doongara had a lower granule length at eCO_2 compared to aCO_2 (Table 5.2). The area-to-length ratio was also lower in Doongara at eCO_2 compared to aCO_2 (Table 5.2).

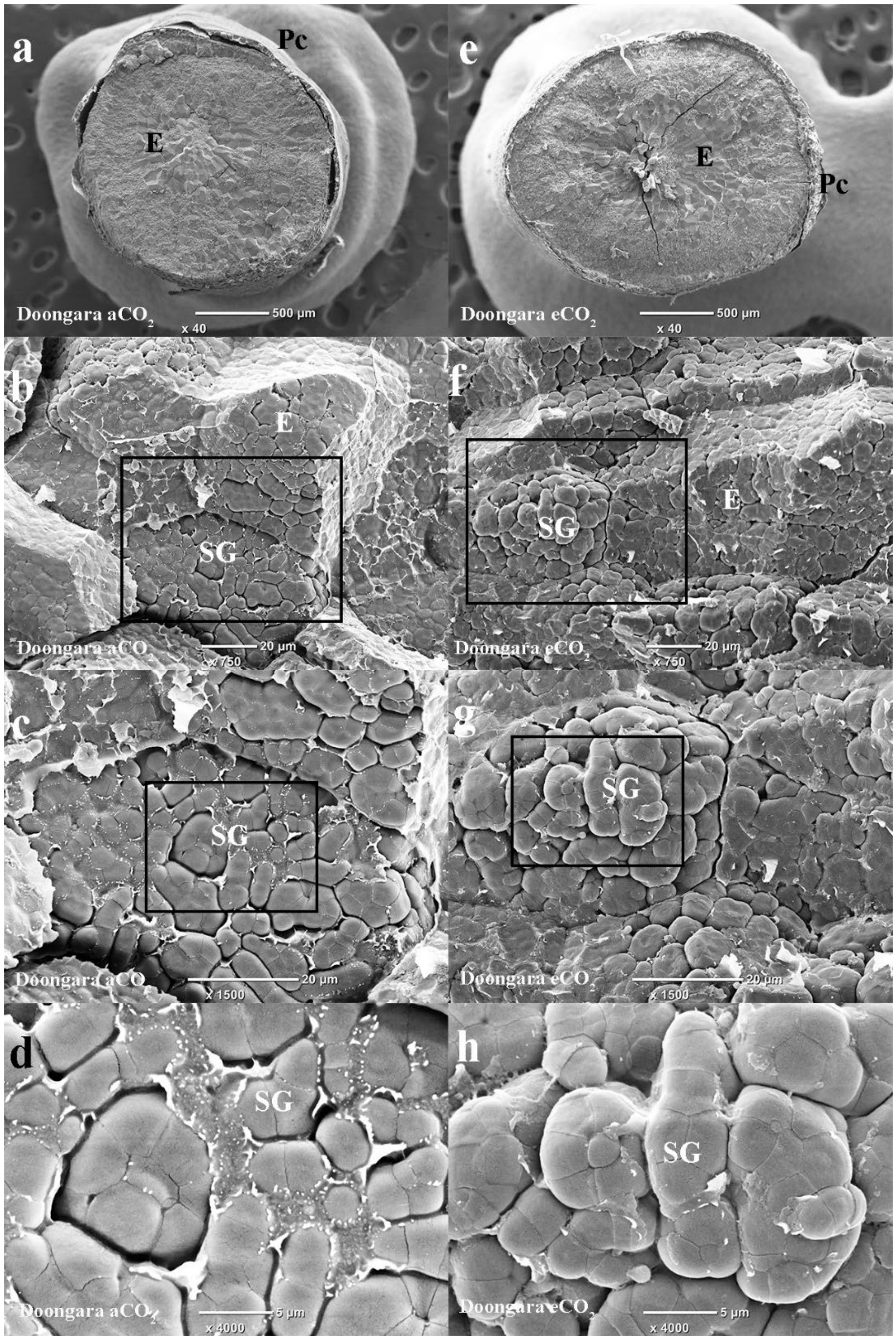

Fig. 5.5. SEM images of the fracture plane of *O. sativa* cv. Doongara grown under ambient (a–d) and elevated CO_2 (e–h) at different magnifications (a and e, ×40; b and f, ×750; c and g, ×1500; and d and h, ×4,000). The boxed region was enlarged into the subsequent images. E, endosperm cell; Pc, pericarp; and SG, starch granules.

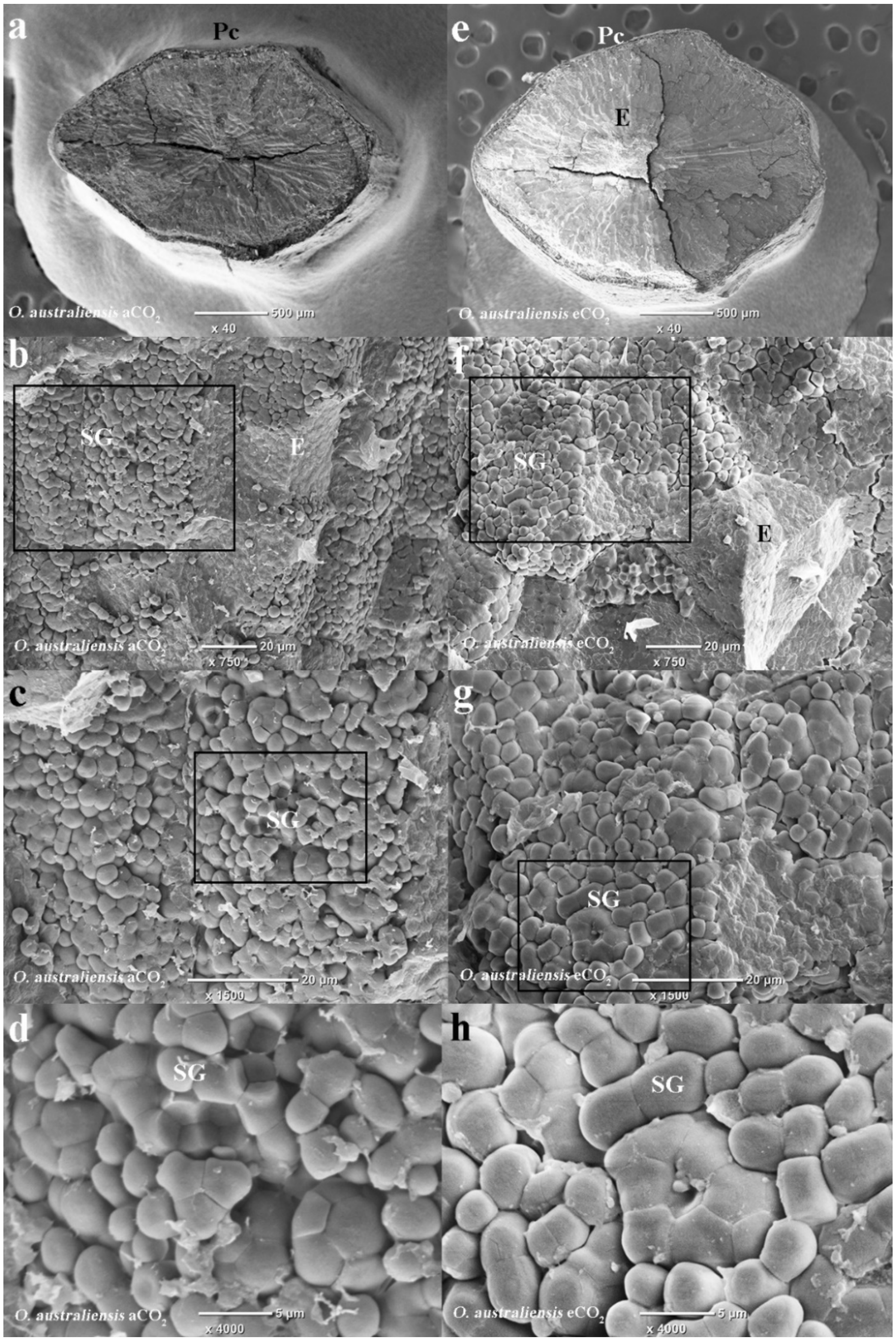

Fig. 5.6. SEM images of the fracture plane of *O. australiensis* grown under ambient (a–d) and elevated CO_2 (e–h) at different magnifications (a and e, ×40; b and f, ×750; c and g, ×1500; and d and h, ×4,000). The boxed region was enlarged into the subsequent images. E, endosperm cell; Pc, pericarp; and SG, starch granules.

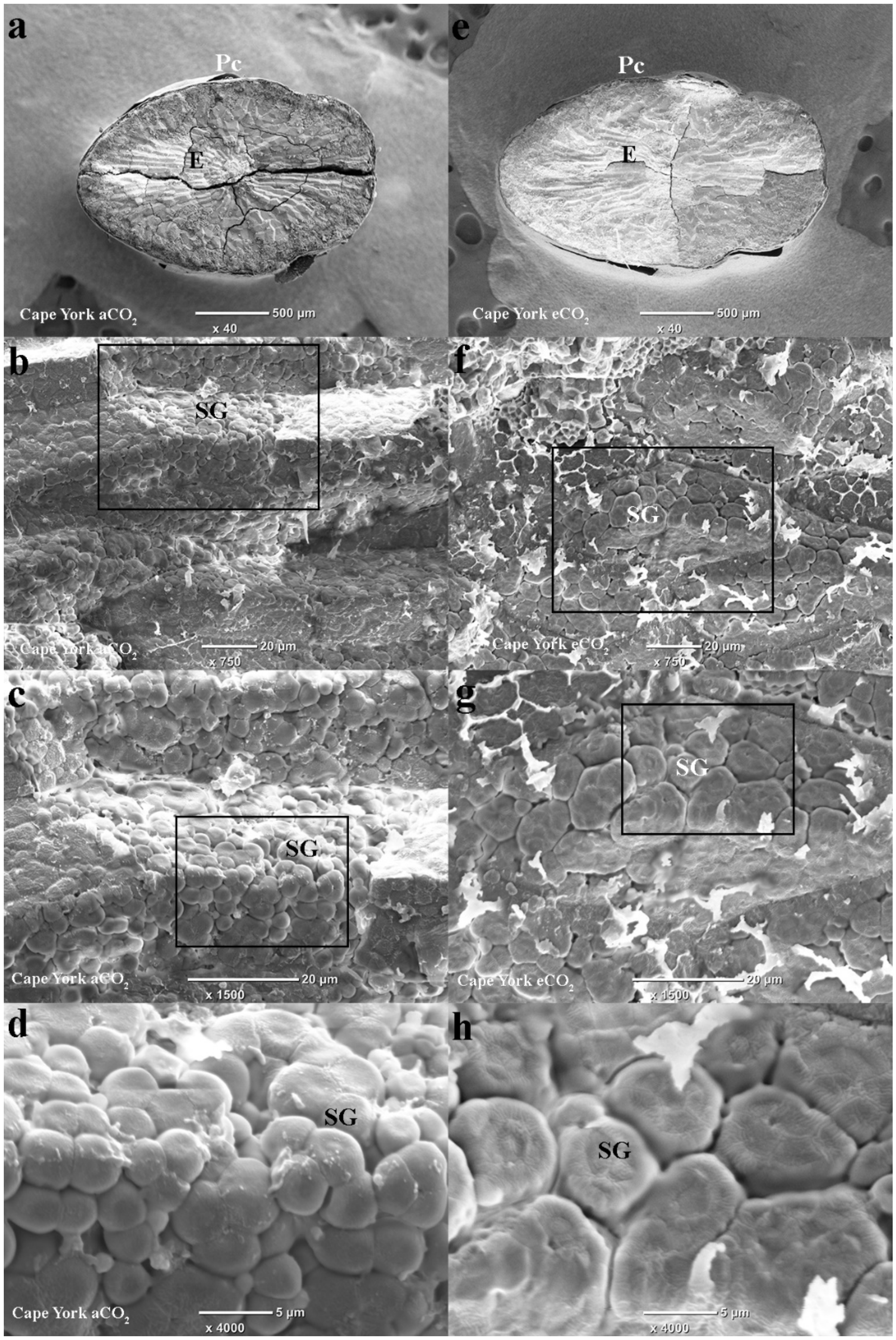

Fig. 5.7. SEM images of the fracture plane of *O. meridionalis* acc. Cape York grown under ambient (a–d) and elevated CO_2 (e–h) at different magnifications (a and e, ×40; b and f, ×750; c and g, ×1500; and d and h, ×4,000). The boxed region was enlarged into the subsequent images. E, endosperm cell; Pc, pericarp; and SG, starch granules.

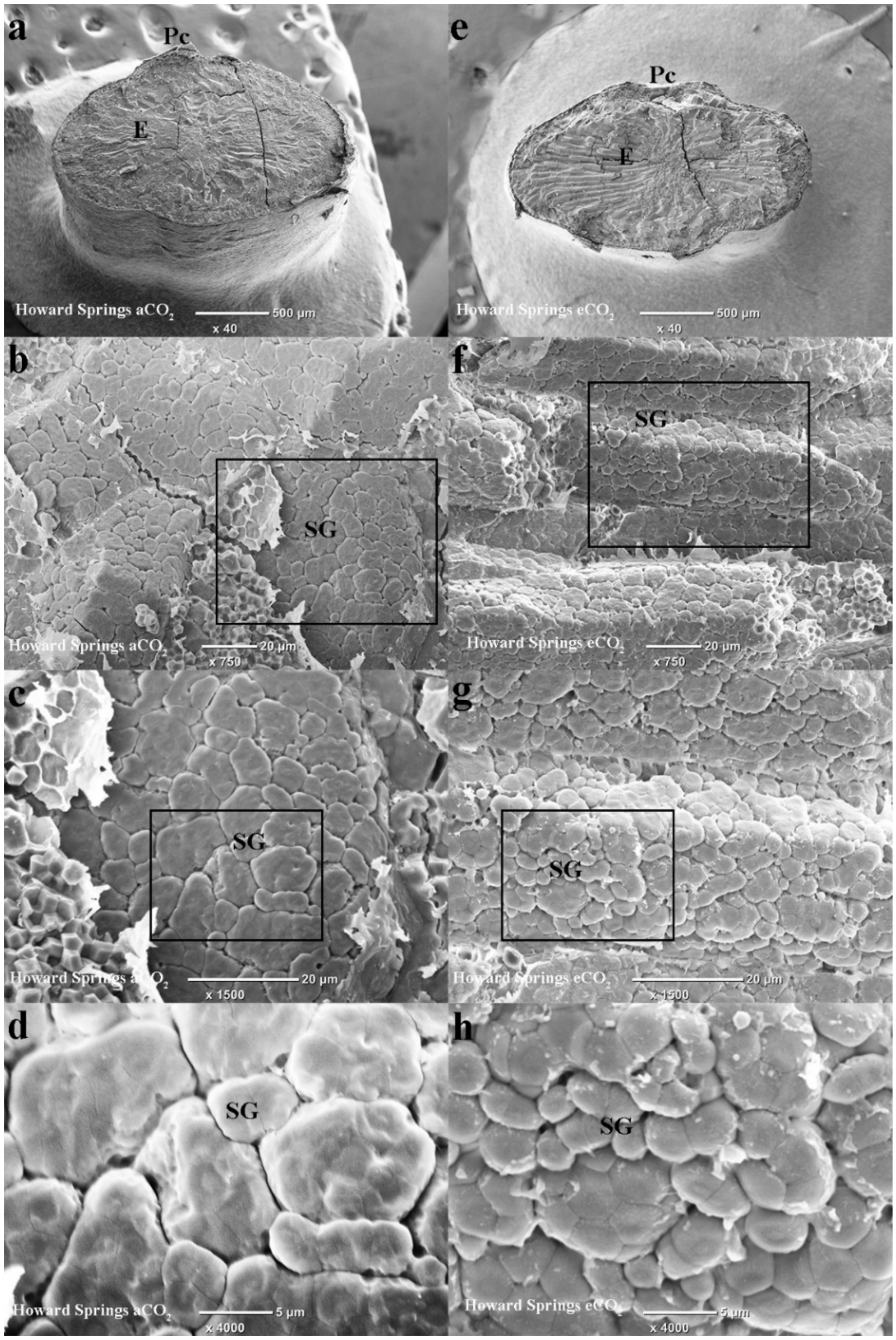

Fig. 5.8. SEM images of the fracture plane of *O. meridionalis* acc. Howard Springs grown under ambient (a–d) and elevated CO_2 (e–h) at different magnifications (a and e, at ×40; b and f, ×750; c and g, ×1500; and d and h, ×4,000). The boxed region was enlarged into the subsequent images. E, endosperm cell; Pc, pericarp; and SG, starch granules**.**

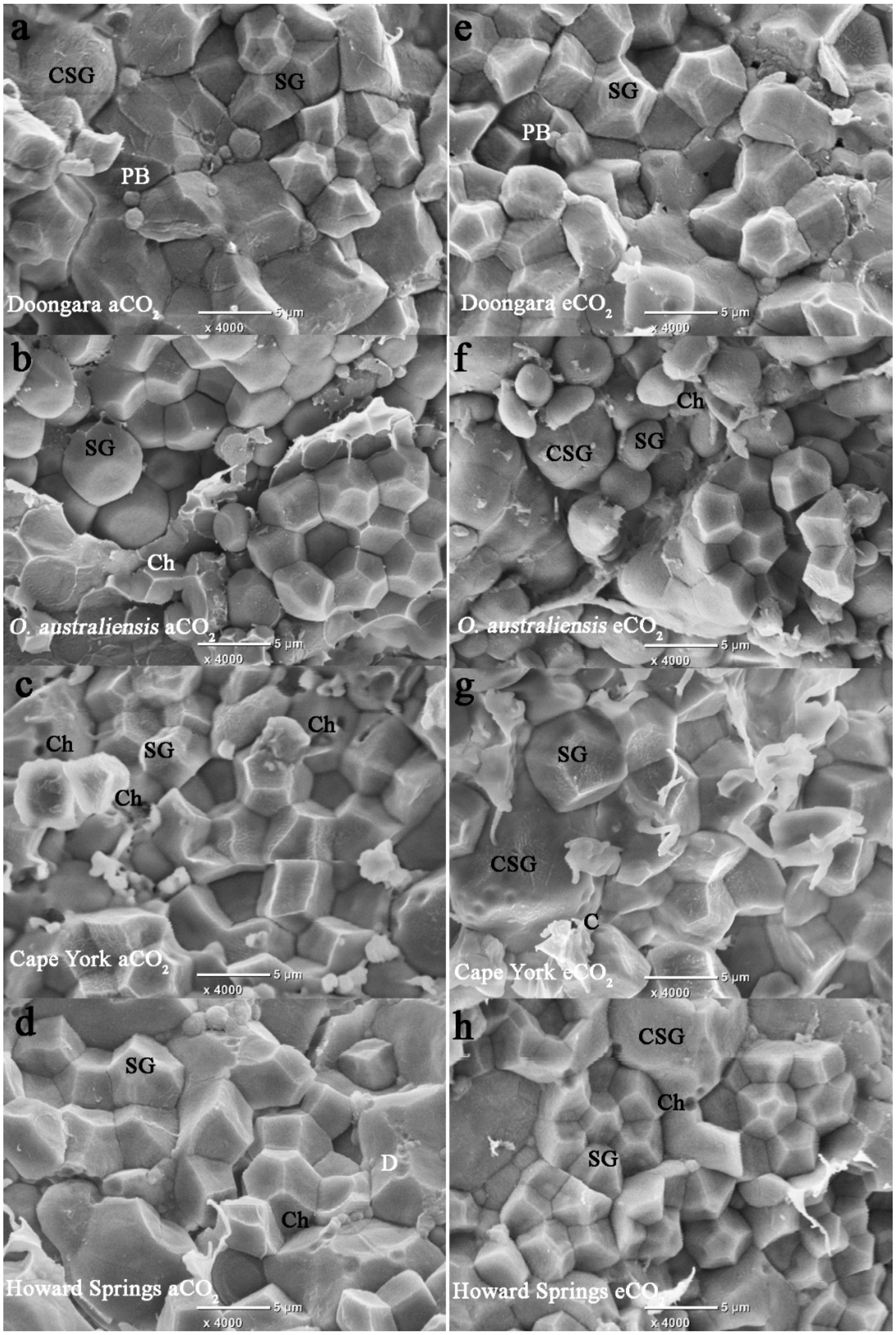

Fig. 5.9. Representative SEM images of fracture plane of *O. sativa* cv. Doongara (a and e), *O. australiensis* (b and f), *O. meridionalis* accessions Cape York (c and g) and Howard Springs (d and h) at ambient and elevated CO_2 at ×4K magnification showing starch granules where the fracture plane occurred through the endosperm. Ch, channels; CSG, compound starch granule; D, dent; SG, starch granule; PB, protein body.

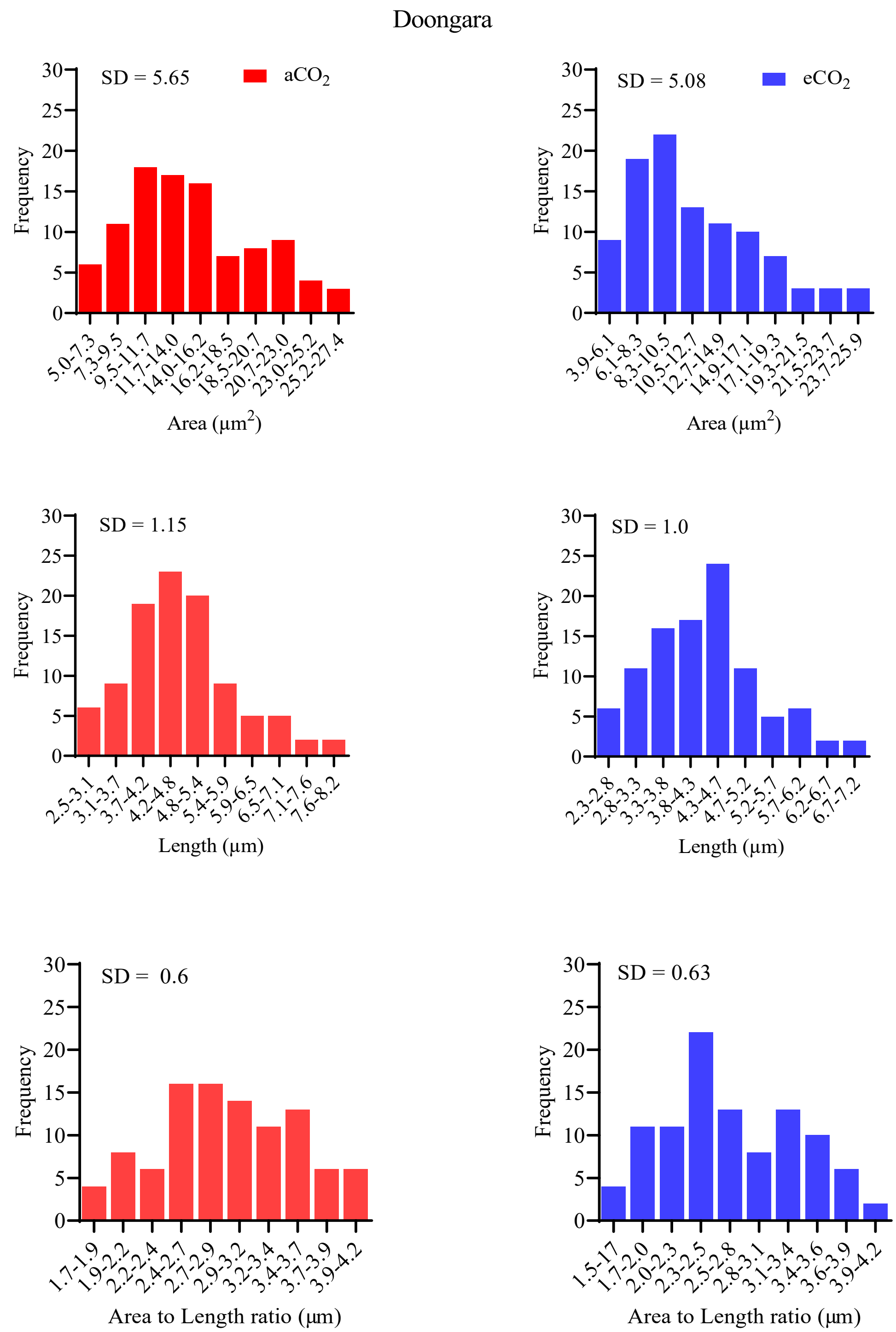

Fig. 5.10. Histogram showing the distribution of starch granule size, area (μm^2), length (μm), and area-to-length ratio (μm), in the endosperm of *O. sativa* cv. Doongara seeds at aCO_2 and eCO_2. SD, standard deviation.

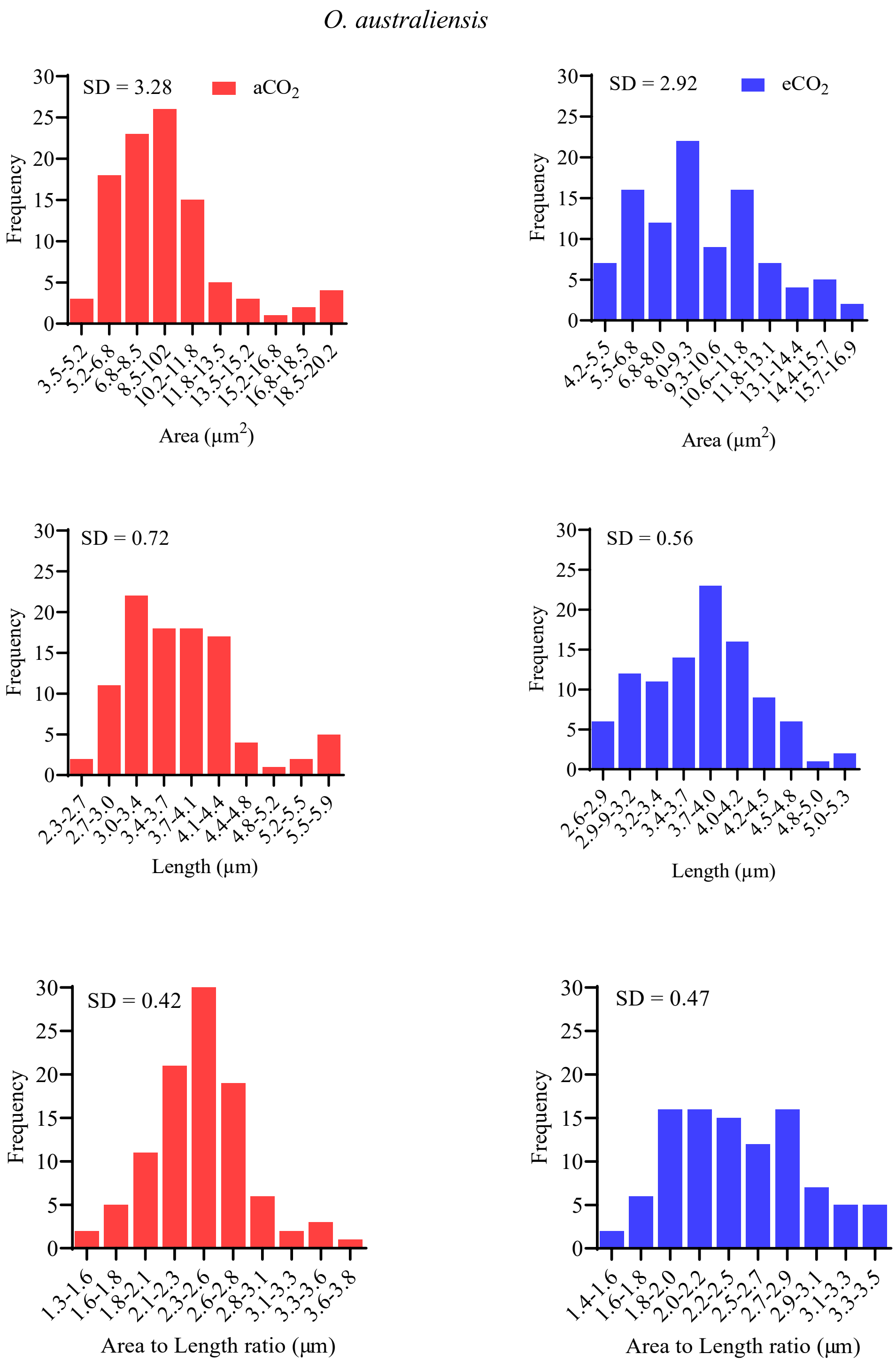

Fig. 5.11. Histogram showing the distribution of starch granule size, area (μm^2), length (µm), and area-to-length ratio (µm), in the endosperm of *O. australiensis* seeds at aCO_2 and eCO_2. SD, standard deviation.

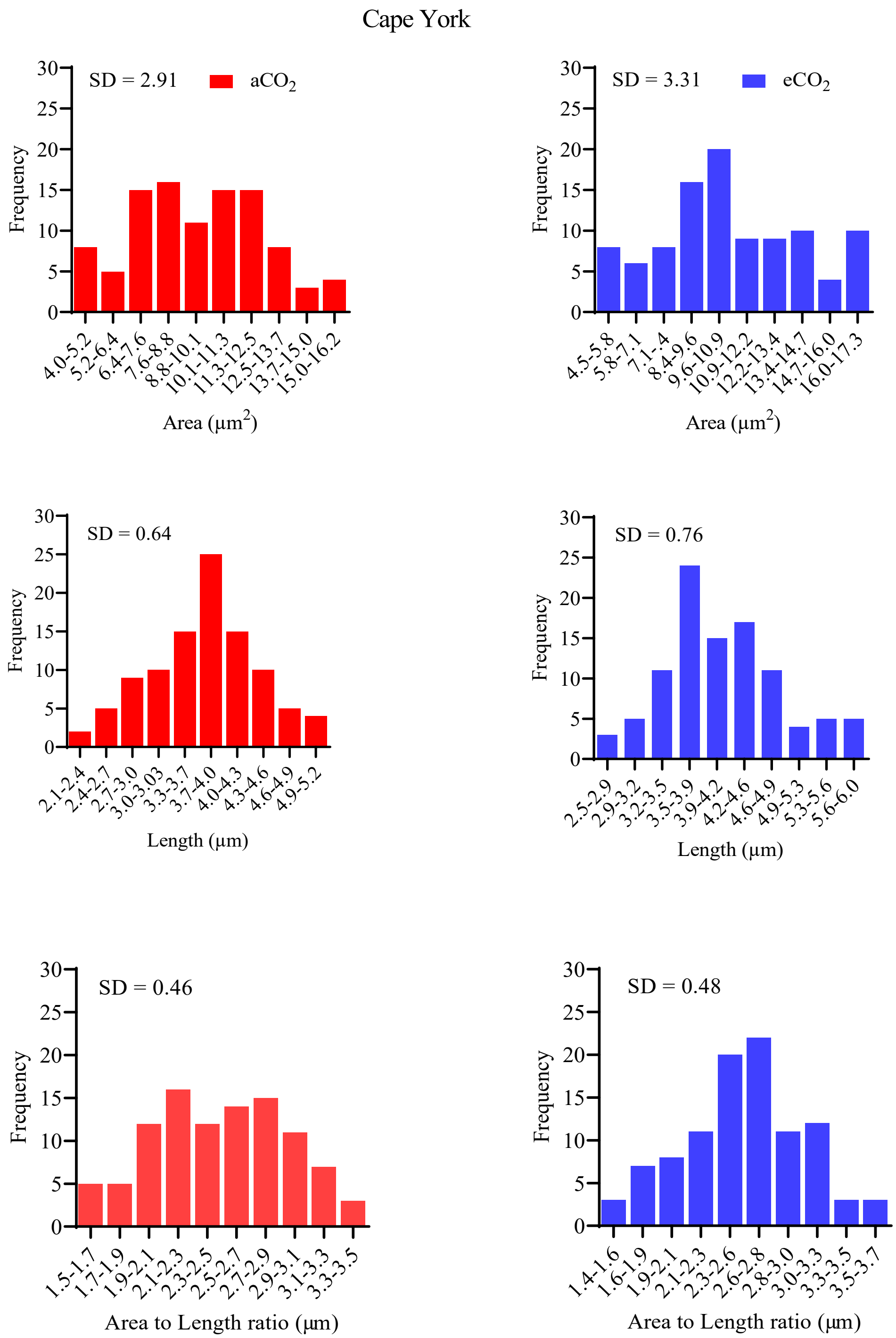

Fig. 5.12. Histogram showing the distribution of starch granule size, area (µm^2), length (µm), and area-to-length ratio (µm), in the endosperm of *O. meridionalis* acc. Cape York seeds at aCO_2 and eCO_2. SD, standard deviation.

Howard Springs

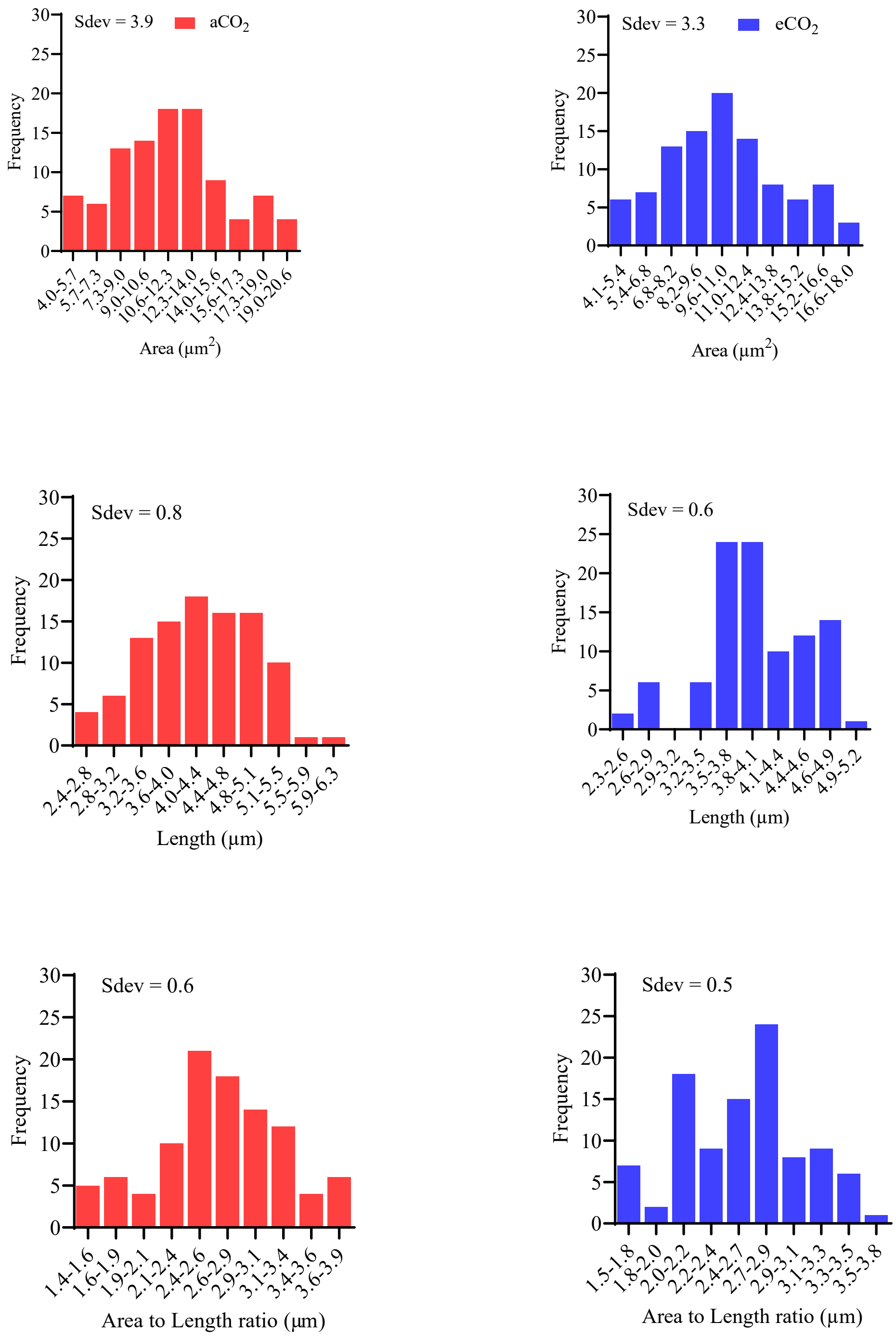

Fig. 5.13. Histogram showing the distribution of starch granule size, area (μm^2), length (µm), and area-to-length ratio (µm), in the endosperm of *O. meridionalis* acc. Howard Springs seeds at aCO_2 and eCO_2. SD, standard deviation.

5.4. Discussion

The present study has shown that responses of rice endosperm morphology to eCO_2 among two Australian wild species and one domesticated cultivar are dependent on genotype, and genotype × CO_2 interaction, thus supporting the hypothesis that "the endosperm morphology might also respond differentially to eCO_2 among wild and domesticated rices". Several studies had shown differences between and within cultivated and wild rice species in pericarp, aleurone and starch granule morphology (Kasem et al., 2011; Shapter et al., 2009; Shapter et al., 2008) but none to my knowledge has examined the effects of rising CO_2 levels on these traits.

5.4.1. Variation in pericarp and aleurone layer thickness and effect of elevated atm-CO_2

In the *Oryza* genus, the thickness of the aleurone varies from one to several cell layers depending on the position (dorsal, ventral, or lateral side), species and genotypes. The dorsal side has been shown in some studies to have more aleurone cell layers than the ventral or lateral side (Kasem et al., 2011; Shapter et al., 2009). *Oryza sativa* cv. Nipponbare and cv. TeQing have five and three dorsal aleurone cell layers, respectively, whereas *O. meridionalis* and *O. australiensis* have four and three dorsal aleurone cell layers, respectively (Kasem et al., 2011).

Genotype × CO_2 interaction showed that aleurone cell width was lower in Doongara but greater in Howard Springs in eCO_2 compared to aCO_2 ($p < 0.0001$), although there was no effect of CO_2 alone (Table 5.2). However, the thickness of the aleurone layer (in numbers of cells) was similar for the domesticated and wild species. The smaller aleurone cells in Doongara at eCO_2 might be due to an increase in cell number in the aleurone tissue. In Howard Springs, eCO_2 had the opposite effect on aleurone cell size. Pritchard et al. (1999) reported that, in leaves, eCO_2 was associated with increased cell expansion rather than cell division. The results in this study demonstrate genotype × CO_2 interaction effects, showing differences in responses of eCO_2 in wild rice and domesticated rice.

5.4.2. Variation in starch granule size and effect of elevated atm-CO_2

Starch is stored in the form of granules in the endosperm and is the principal component of rice grains, accounting for 90 to 95% of the dry weight of white rice (Fitzgerald et al., 2009; Zhou et al., 2002). Granule size and shape vary between and within species and have an impact on the physicochemical properties of the starch, including industrial applications (da Silva et al., 1997; Lindeboom et al., 2004; Wani et al., 2012).

Rice starch granules are small, irregular, and polyhedral. The average starch granule size in cultivated and wild rice falls in the size range of 2–10 µm in diameter (Dang and Copeland, 2004b; Kasem et al., 2011; Shapter et al., 2008). Starch granule size affects the gelatinization and pasting behaviour of rice and determines eating quality and uses in the food processing industry (Lindeboom et al., 2004; Low et al., 2020).

As CO_2 is the principal substrate for photosynthesis, increases in atm-CO_2 concentration will lead to enhanced photosynthesis, carbon gain and plant growth, which would improve crop biomass and individual grain mass and yield in rice (Madan et al., 2012; Rahman et al., 2021a; Rahman et al., 2018; Yang et al., 2009; Ziska and Teramura, 1992b). This in turn may have led to increased cell sizes in wild rices (Pritchard et al., 1999). However, in domesticated rice, cell sizes decreased, possibly due to increases in cell numbers (Tester et al., 1995).

Starch synthesis is genetically controlled, but environmental factors also influence starch accumulation in the rice endosperm (Larkin et al., 2003; Tikapunya et al., 2017). The greater starch granule size in Cape York and the lower granule size in Doongara under eCO_2 compared to aCO_2 suggests differential responses among genotypes. Statistical analysis indicated this effect was due to an interaction of genotype × CO_2 rather than genotype or CO_2 alone ($p < 0.0001$). The environmental conditions during endosperm cell division and amyloplast formation could affect starch granule number and dimensions (Tester et al., 1995). Jing et al. (2021) reported increased surface area and proportion of large starch granules in rice due to eCO_2. Others observed larger granule diameters (> 5 µm) in rice (Jing et al., 2016b), and a 5% increase in < 10 µm type B starch granule in wheat (Rogers et al., 1998) at eCO_2, which are consistent with the findings of this study.

In the current study, the average granule size (area and length) was greater in the cultivated rice Doongara than in the wild rices. Other researchers also reported similar starch granule sizes of 2–7 µm in domesticated and wild rice (Kasem et al., 2011; Shapter et al., 2008). Dang and Copeland (2004b) reported starch granules of varying sizes (4–11 µm in Doongara). The differences in starch granule size might be associated with the relative seed sizes of the different genotypes (Rahman et al., 2021a).

The differences in starch granules between wild and domesticated rices and the different physicochemical properties of the grain/flour I observed in my previous paper (Rahman et al., 2021a) support the suggestion that native genotypes may be suited to different uses in food processing and industrial applications (Tikapunya et al., 2017).

Previously, increases were observed in a range of morphological and physiological characters tested across genotypes in eCO_2 compared to aCO_2 (Rahman et al., 2018); however, the degree of variation associated with the eCO_2 treatment differed between wild and domesticated rices, suggesting genotype × environment interaction effects rather than uniformly (genetically) fixed traits. The increase in photosynthesis with eCO_2 (Rahman et al., 2018) enhanced total plant biomass and increased seed size (presumably due to higher translocation of assimilates; Rahman et al., 2021a), which in turn, increased starch granule size and aleurone layer cell size in wild rices, as shown in the present study. However, in Doongara, both starch granules and aleurone cell sizes decreased in eCO_2, which may at least partly be attributed to domesticated rice having been bred for yield and seed size and may have lost some of its ability to respond to eCO_2 compared to the wild rices. The wild rice genotypes evolved in radically different environments, with *O. australiensis* characteristically a perennial rice from locations with ephemeral water supply and requiring drought tolerance, whereas Howard Springs (*O. meridionalis*) is found in an 1800-mm per annum rainfall zone.

5.5. Conclusion

Australian wild rices are genetically distinct from domesticated rice (Henry, 2019; Tikapunya et al., 2017), and some of these genetic differences might account for the different responses of endosperm morphology to eCO_2. Domesticated rice has been bred for higher yield and greater starch accumulation in the grain under ambient CO_2 conditions. Elevated CO_2 led to an increase in the size of starch granules in the *O. meridionalis* Cape York accession, whereas the opposite effect was observed with *O. sativa* cv. Doongara. Similarly, the size of aleurone cells decreased in Doongara but increased in *O. meridionalis* Howard Springs in eCO_2. However, *O. australiensis* did not show any response in the size of aleurone cells or starch granules with eCO_2 treatment. These observations suggest that the morphological changes in aleurone cells and starch granules are due to genotype × CO_2 interactions rather than genotype or CO_2 alone. It can be concluded from this study that, among wild species of rice, there may be accessions with genetically determined responses to eCO_2 that present valuable traits for breeding programs.

Chapter 6

General discussion, conclusion, and future directions

6.1. General discussion

Rice grain globally is largely harvested from *Oryza sativa*, a species bred for high overall biomass production and harvest indices that often exceed 50%. High individual grain mass and improved grain quality have been priorities of breeding programs over many decades, principally at the International Rice Research Institute (IRRI), Philippines and its counterparts in different countries (Japan, China, Africa etc.). These days private seed companies, such as RiceTec (Texas, USA), are also involved in hybrid rice seed production for greater yield. Rising atmospheric carbon dioxide (atm-CO_2) concentrations generally lead to enhanced photosynthesis and changes to the whole-plant carbon economy in C_3 plants (including rice), with high-intensity cultivation making rice especially responsive to increasing atm-CO_2 (Ainsworth, 2008; Hasegawa et al., 2013; Kang et al., 2021; Ziska et al., 2012). The degree of growth stimulation depends on the leaf-level stimulation of assimilation rates, as well as canopy light interception as determined by foliage architecture.

Net photosynthesis (CO_2 assimilation rates minus dark respiration) is a strong driver of biomass production. Along with many additional processes such as carbohydrate transport and nitrogen metabolism, photosynthesis also influences yield and grain quality. For example, in rice, elevated atm-CO_2 (eCO_2) increased plant biomass by 14–70% (Madan et al., 2012; Senghor et al., 2017; Wang et al., 2015; Wang et al., 2020; Wu et al., 2018; Ziska et al., 1996) and yield by 3–58% (Ainsworth, 2008; Ainsworth and Long, 2021; Baker et al., 1990; Fabre et al., 2020; Gao et al., 2021; Hu et al., 2021; Lv et al., 2020; Sakai et al., 2019; Seneweera et al., 1996; Yang et al., 2006; Yang et al., 2009). However, eCO_2 lowers the grain quality as a trade-off; for example, decreases of 5–17% in protein content (PC), 2–23% iron (Fe), and 3–15% zinc (Zn) in rice grains were observed when plants were grown in ~600 atm-CO_2 compared to ~400 ppm atm-CO_2 (Myers et al., 2014; Seneweera and Conroy, 1997a; Ujiie et al., 2019; Zhu et al., 2018). The physiological and biochemical changes in plants in response to eCO_2 will also affect caryopsis development and thereby affect endosperm morphology in domesticated rice (Jing et al., 2021; Jing et al., 2016b).

The two wild rice species endemic to northern Australia, *O. australiensis* (EE) and *O. meridionalis* (AA), grow in the natural habitat where there is a wet season followed by a very dry and hot season with high levels of radiation (Atwell et al., 2014; Henry, 2018; Henry et al., 2010; Scafaro et al., 2016). These wild rices have distinct canopy shapes and exhibit high photosynthetic rates and rapid growth during the monsoon (Atwell et al., 2014; Rahman et al., 2018). Moreover, these native species have thicker leaves than domesticated rice, which relate to transpiration efficiency and drought tolerance (Giuliani et al., 2013; Scafaro et al., 2016).

Because of the genetic differences between wild and domesticated rice, results from many experiments on domesticated rice (*O. sativa*) cannot be extrapolated directly to wild rice species. The objective of this thesis was to integrate the diverse phenotypic responses to eCO_2 in wild relatives when compared with a cultivated rice, first testing the differential impacts on CO_2 assimilation and light interception efficiency, then proceeding to an investigation of grain properties, including endosperm morphology.

The effects of eCO_2 on photosynthesis, light interception, biomass production, yield and grain quality (including flour pasting properties) in domesticated rice are well documented (Ainsworth and Long, 2021; Gao et al., 2021; Hu et al., 2021; Myers et al., 2014; Sakai et al., 2019; Seneweera et al., 1996; Seneweera and Conroy, 1997a; Senghor et al., 2017; Wang et al., 2020), although limited reports are available on the effects on endosperm morphology (Jing et al., 2021; Jing et al., 2016b). However, no studies have emerged of canopy architecture parameters and grain properties in any wild *Oryza* species—whether at current atmospheric or elevated CO_2—least of all on the Australian native rice species.

To complete this PhD project, four studies were conducted with the two principal Australian native rice species, *O. australiensis* and *O. meridionalis*, which occur either solely or predominantly in Australia. Their responses to eCO_2 in experiments were compared with those of *O. sativa*. The **first study** (*Chapter 2*) dealt with whole-plant phenotypic responses to eCO_2, including leaf-level CO_2 assimilation rates (net photosynthesis) and light interception efficiency of canopies. These data were used to estimate carbon gain and theoretical biomass gain for two contrasting accessions of *O. meridionalis* (acc. Cape York and Howard Springs) when compared with an Australian cultivar of *O. sativa* (cv. Doongara, long grain). The **second study** (*Chapter 3*) focussed on the rheological properties of whole-grain rice flour as affected by a range of concentrations of $AgNO_3$

solution, which is known to inactivate α-amylase activity. The pasting properties of the rice flour were determined using a rapid visco analyser (RVA). The optimised and validated data obtained were used in the **third study** (*Chapter 4*), showing the effects of eCO_2 on rice grain physicochemical properties including flour pasting properties of wild and domesticated rice. The **fourth study** (*Chapter 5*) showed the eCO_2 effects on grain endosperm morphology of the same genotypes used in the pasting analysis.

Chapter 2 showed significant variation among the key parameters that define canopies, both between domestic and wild rice species but also within the two accessions of *O. meridionalis* that were investigated. Broadly, this supports the hypothesis that wild species would respond differently to eCO_2 from domesticated rice. While they are distinct species, it should be emphasised that *O. meridionalis*, like *O. sativa*, is within the AA clade (Brar and Khush, 2018; Henry, 2019; Stein et al., 2018; Vaughan et al., 2003).

In C_3 plants under optimal conditions of water and nutrient supply, eCO_2 increases the photosynthetic rate by accelerating carboxylation (Fabre et al., 2020; Long, 1991; Long et al., 2004; Long et al., 2006b; Weber and Bar-Even, 2019) and driving the accumulation of biomass. Increases in atm-CO_2 provide more substrate for Rubisco (ribulose 1,5-bisphosphate (RubP) carboxylase-oxygenase), while also minimising futile carbon loss via photorespiration. Since ambient CO_2 concentrations are sub-optimal for Rubisco, elevating CO_2 levels to 700 ppm as in the experiments reported here would be expected to enhance photosynthesis substantially (30–60%) in rice (Ainsworth, 2008; Fabre et al., 2020; Sakai et al., 2019; Vu et al., 1997; Ziska and Teramura, 1992b). In wild rice species, both A_{max} (light-saturated leaf photosynthesis) and R_d (dark respiration), increased in eCO_2 in this study; however, in *O. sativa*, A_{max} increased but R_d decreased. Both increases (Baker et al., 2000; Baker et al., 1992; Fukayama et al., 2011) and decreases (Noguchi et al., 2018; Wang et al., 2020; Ziska and Teramura, 1992b) in dark respiration have been observed in rice plants growing in steady-state eCO_2, consistent with these contrary trends. However, that two closely related species should respond in a contrasting manner at the level of mitochondrial respiration is surprising.

Apart from the direct effects of eCO_2 on the assimilation of carbon, the other vital resource driving the energetics of photosynthesis is light, and hence the capacity of leaves and leaf canopies to intercept it (Burgess et al., 2017; Kang et al., 2021). Dense leaf canopies, particularly of annual grasses such as rice, are naturally susceptible to self-shading. Thus, light interception is strongly influenced by leaf angle, size and shape, leaf number, leaf orientation, leaf arrangements, and the direction of incoming light throughout the day, as described by azimuth (Burgess et al., 2017; Yoshida, 1972). Average tiller number, leaf area and leaf number, LAI and fresh biomass were approximately doubled in eCO_2, resulting in a dense canopy and clumped leaves (Rahman et al., 2018). Light interception efficiency as measured by $\overline{STAR}$ decreased at eCO_2 across genotypes because of increased self-shading in these denser canopies and clumping leaves, as reflected in the leaf dispersion variable. The crown surface area (A_C) increased in eCO_2 in Doongara and in one accession of *O. meridionalis* (Cape York) while it decreased in the other (Howard Springs), indicating that wild rices will respond differently to eCO_2.

To support the general hypothesis that domesticated and wild rice species will respond differently to eCO_2, three more specific hypotheses were tested in this chapter (*Chapter 2*).

Hypothesis 1: *"Total leaf area and efficiency of light interception explain the genotypic variation in above-ground biomass"*. Results of destructive measurements (Table 2.2) suggest genotypic differences in canopy density and light interception efficiency, leading to differences in light capture of the canopy. The total leaf area in the wild rice species studied is higher in both CO_2 treatments than in cultivated rice (Table 2.2 and 2.5), which in turn led to higher leaf area index (*LAI*) (Wang et al., 2015; Wang et al., 2020), a measure of light interception by the canopy (Ainsworth, 2008). Digitised images corroborated these destructive measurements of the canopy (Fig. 2.2 and Table 2.3). In this study, wild rice had denser canopies than cultivated rice in both aCO_2 and eCO_2. $\overline{STAR}$ was negatively correlated with crown density but positively correlated with leaf dispersion, indicating that the wild rice species were less efficient in light interception than cultivated rice; however, total light capture was compensated by larger leaf canopies. Leaf area and carbon gain were negatively correlated with $\overline{STAR}$, indicating that plants with the greatest leaf area gained carbon fastest, though they were less efficient in light interception. Nonetheless, this study established that the product of leaf area, light interception and light response of CO_2

assimilation produced estimates of carbon gain that were correlated strongly with actual biomass increase. Therefore, the hypothesis is supported; i.e., total light capture predicts biomass accumulation for morphologically diverse canopies of wild and cultivated rice.

Hypothesis 2: *"Light interception efficiency is diminished in denser leaf canopies formed in an elevated atmosphere"*. The reduction in $\overline{STAR}$ in plants at eCO_2 is reflected by doubling the number of tillers and leaves, and therefore total leaf area. Although such strong responses to eCO_2 are widely reported for domesticated rice, they have never been observed in wild rice relatives. The crown surface area (A_C) was unchanged in eCO_2, resulting in more leaf matter compressed into a similar crown volume, thus denser canopies. Moreover, leaves were less dispersed (more clumped) in eCO_2 than in aCO_2. In particular, *O. meridionalis* Howard Springs had the densest canopies (A_L/A_C = 0.40 and 0.20), with clumped leaves (β = 0.57 and 0.58), in eCO_2 and aCO_2, respectively (Table 2.3). Crown density, leaf dispersion and total leaf area all contributed to a marked change in modelled carbon gain.

Notwithstanding the eCO_2 effects described above, the productivity of accessions of *O. meridionalis* did not change the ranking when compared with *O. sativa* in eCO_2 from aCO_2 (Howard Springs > Cape York > Doongara). This indicates that features of canopy architecture will not be altered by eCO_2; thus, wild rice species are a valuable source for canopy redesign because they developed mechanisms against the high light intensity and hotter and drier climate where they evolved. In the future climate scenario, these wild rices are expected to survive better than domesticated rice because of their faster growth habit, maintaining greater canopy structure despite lower light interception capacity and producing greater biomass. Consistent with this, over many decades IRRI (Philippines) has developed high-yielding dwarf rice varieties with erect leaves and fewer tillers for increased penetration of light to the deeper canopies and maximum net photosynthesis. Furthermore, steeper leaf angles would improve the plant daily carbon gain through higher light interception and absorption at a low solar angle, and erect leaves are associated with better crop health reducing the risk of diseases and overheating in the canopy (Burgess et al., 2017).

Hypothesis 2 is supported; i.e., the efficiency of light capture is diminished when plants are grown in eCO_2 as a result of denser leaf canopies and reduced leaf dispersion. However, increased total leaf area enabled the canopies of all three genotypes to capture more light and grow larger at eCO_2 despite less efficient light capture.

Hypothesis 3: *"Light interception, simulated carbon gain and harvested biomass are correlated, with seasonal changes in light regime not affecting relative ranking".* The first hypothesis established that C gain is negatively correlated with $\overline{STAR}$ because of the large canopies; the question arose as to whether the ranking of the three genotypes will be preserved for C gain and actual shoot biomass as the azimuth (Summer Solstice and Equinox) changes. In this experiment, the C gain was simulated for the three genotypes at varying azimuth using YplantQMC in both CO_2 levels. The C gain at the Summer Solstice was higher than at the Equinox for all three genotypes and did not change the ranking of the genotypes (Fig. 2.9A). As C gain was simulated, it was necessary to establish that this was a good predictor of actual biomass. The predicted C gain showed a positive correlation with actual biomass (Fig. 2.9B). However, in Howard Springs, YplantQMC predicted a higher C gain than the actual biomass would have been predicted. The third hypothesis is generally supported; i.e., light interception, simulated carbon gain and harvested biomass are correlated, regardless of whether the direction of illumination is modelled at the Summer Solstice or Equinox.

Chapter 3, the second study, illustrated the effects of $AgNO_3$ solution to inactivate α-amylase activity in rice flour during pasting. Rice products (processed foods) are predominantly made from rice flour, and pasting properties are important features in food processing to determine the application suitability of the flour and the quality of the end-use products. Endogenous amylase activity affects the pasting properties of rice flour, and therefore its impact on rice physical properties is routinely subjected to quality testing before industrial-scale processing. This study aimed to optimise the $AgNO_3$ concentration required to inhibit endogenous α-amylase activity in rice flour during measurement of gelatinization and pasting. The results showed that using 1% $AgNO_3$ would inhibit α-amylase activity in rice flour with high AC (amylose content) and more accurately assess the pasting properties in an RVA (Rahman et al., 2021b), and thus 1% $AgNO_3$ solution was used in the subsequent study.

Chapter 4, the third study, demonstrated the effects of eCO_2 on the physicochemical properties of grains including flour pasting properties of wild rice species of *O. australiensis* and the genotypes used in the first study (*Chapter 2*). This study showed that grain physicochemical properties and pasting properties of rice flour of accessions of *O. australiensis* and *O. meridionalis* responded differently to eCO_2 versus aCO_2 than domesticated rice. Seed length (Sl) increased in Doongara and Howard Springs (Jing et al., 2016a) but decreased in Cape York (Usui et al., 2016) at eCO_2. Seed width (Sw) increased in Howard Springs at eCO_2, possibly due to a higher supply of carbon assimilate to panicles at eCO_2, which would improve milling quality (Madan et al., 2012). 1000-seed weight increased at eCO_2 across all four genotypes (Ainsworth and Long, 2021; Gao et al., 2021; Hu et al., 2021; Li et al., 2017), possibly due to enhanced remobilization of pre-stored carbon reserves to the grain Yang et al. (2009).

Apart from these physical characteristics, AC and protein content (PC) are crucial factors in determining the milling, processing, eating, and cooking quality of rice, and for human nutrition. AC was not affected by eCO_2; however, PC decreased across all four genotypes. The decrease in PC in grains at eCO_2 could be due to the higher production of carbohydrates at eCO_2 diluting the N concentration in the grains (Gifford et al., 2000; Taub et al., 2008) or to higher translocation of carbohydrate to the panicle and reduced translocation of N from leaf blade to grains (Ujiie et al., 2019). The domesticated rice, cv. Doongara, had a larger decrease in PC compared to the wild rice accessions, possibly because domesticated rice was bred for high yield and lost its ability to respond to future climatic conditions.

In this study, 1% $AgNO_3$ solution was used to inhibit α-amylase activity in rice flour during pasting in the RVA. PV was more substantially increased in both CO_2 treatments with 1% $AgNO_3$ than with dH_2O (Fig. 4.3), indicating inhibition of α-amylase activity in the rice flour during gelatinization in the RVA (Rahman et al., 2021b). PV and FV increased in all wild genotypes at eCO_2 but, in the domesticated genotype, only PV was increased at eCO_2 compared to aCO_2. When dH_2O was used, both PV and FV increased in all genotypes (Fig. 4.3). The decrease in PC and unaltered AC due to eCO_2 in this study would have caused the increase in PV and FV. Overall, flour viscosity increased in wild and domesticated rice at eCO_2 compared to aCO_2. Apart from AC and PC, starch granule sizes affect the gelatinization and pasting behaviour of rice, which determines the eating quality and uses in the food processing industry (da Silva et al., 1997; Lindeboom et al., 2004; Low et al., 2020).

Chapter 5, the fourth study, describes the effect of eCO_2 on the endosperm morphology of *O. australiensis*, *O. meridionalis* and *O. sativa* used in the previous study (*Chapter 4*). This study showed that responses of endosperm morphology are dependent on genotype, and genotype × CO_2 interaction, thus supporting hypothesis that endosperm morphology might also respond differentially to eCO_2 among wild and domesticated rices.

Aleurone cell width showed variable responses to eCO_2. Aleurone cell width was higher at eCO_2 in the *O. meridionalis* accession Howard Springs but lower in *O. sativa* cv. Doongara. Starch granule area and length also showed variable responses to eCO_2. Starch granule area and length were higher at eCO_2 in the *O. meridionalis* accession Cape York but lower in *O. sativa* cv. Doongara. Although these characters are genetically controlled, the variable responses might be due to genotype × CO_2 interaction rather than genotype alone. The decrease in cell sizes at eCO_2 in *O. sativa* cv. Doongara could be due to higher cell division (Tester et al., 1995). However, increases in cell sizes in *O. meridionalis* accessions Cape York and Howard Springs could be due to cell expansion (Pritchard et al., 1999). These findings show that domesticated rice will respond differently than wild rice species to eCO_2.

As CO_2 is the principal substrate for photosynthesis, increases in atm-CO_2 concentration will lead to enhanced photosynthesis, carbon gain and plant growth, which will increase crop biomass and individual grain mass and yield in rice (Ainsworth and Long, 2021; Fabre et al., 2020; Hu et al., 2021; Madan et al., 2012; Rahman et al., 2021a; Rahman et al., 2018; Sakai et al., 2019; Wang et al., 2020; Yang et al., 2009; Ziska and Teramura, 1992b). This will be associated with increased cell sizes in wild rice species (Pritchard et al., 1999). However, in domesticated rice, cell sizes decreased, possibly due to increased cell numbers (Tester et al., 1995).

6.2. Conclusions

Wild species are a valuable source of genes for resistance to many biotic and abiotic stresses and can be used in developing new elite genotypes via conventional breeding or new advanced breeding technologies. Many studies have shown the effect of eCO_2 on photosynthesis, light interception, plant biomass and yield in domesticated rice; however, wild species have never been examined in this way. The novelty of this PhD study is that two wild species endemic to Australia were compared with a local cultivar grown in aCO_2 and eCO_2.

The first study in this thesis shows enhanced carbon assimilation rates across genotypes at eCO_2, resulting in a greater number of tillers and leaves and increased leaf area per plant, which in turn produced greater plant biomass and C gain. These variations are dependent on genotype and CO_2 treatment rather than genotype × CO_2 interactions (Tables 2.1 and 2.2). Light interception efficiency ($\overline{STAR}$) was negatively affected by eCO_2 across all genotypes caused by denser canopies (Table 2.3) and increased self-shading; however, wild genotypes were affected more than domesticated genotypes. Interestingly, one of the wild accessions, *O. meridionalis* acc. Howard Springs, maintained more tillers and leaves, without changing its surface area (A_C) at eCO_2, by increasing compactness of the canopies, as indicated by leaf dispersion (Tables 2.2 and 2.3). However, for R_d wild species responded differently to eCO_2 than domesticated rice. Furthermore, this study also showed that growing season (Summer Solstice and Equinox) affected light capture, plant biomass and C gain quantitatively in both CO_2 treatments without influencing the relative ranking of the genotypes (Fig. 2.9). From these results, it can be concluded that photosynthetic characteristics, light interception, and canopy architecture responses to eCO_2 are uniform (genetically controlled); however, differential responses were also observed among the wild rices.

The Australian wild rices naturally grow in poor soil conditions (low soil nutrients) with low moisture availability during summer. During the course of evolution, they developed mechanisms to grow rapidly during the monsoon and maintain their canopies during summer. When these genotypes were grown in optimal growth conditions in glasshouses at eCO_2, they were able to produce more shoot biomass and C gain despite lower photosynthetic rate and light interception compared to domesticated rice. These genotypes have erect leaves on the top and prostrate (droopy) leaves on the lower level of the canopy. For better light interception, erect leaves on the top and prostrate leaves at the lower level of the canopy is preferred. Wild rices may never be used to improve yield directly, but they can be used to improve the canopy architecture of domesticated rice by altering leaf angle and leaf dispersion to achieve higher light interception and thus yield indirectly. Thus, wild rice species and ecotypes offer novel candidates for canopy re-design via breeding.

The second study showed that using 1% $AgNO_3$ solution inactivated endogenous α-amylase activity of rice flour, and therefore, 1% $AgNO_3$ solution was used to accurately determine pasting properties in the subsequent study. It was established in the first study that eCO_2 enhances photosynthesis, resulting in a higher accumulation of biomass despite lower light interception efficiency and higher crown density. Numerous studies have shown that eCO_2 not only affects photosynthesis, plant architecture, biomass production and yield, but also affects the grain quality of cultivated rice (*O. sativa*), led to the hypothesis in the third study that "*Grain properties of wild rice species will respond differentially to eCO_2 from domesticated rice*". Selection has substantially altered starch and protein levels in domesticated rice over millennia, making their grain distinct from those of their wild ancestors. This study showed grain physicochemical properties of wild rice species and domesticated rice were affected differentially by eCO_2. For example, Sl increased in Doongara but decreased in Cape York at eCO_2, whereas Sw was unaltered in Doongara but increased in Howard Springs. Furthermore, eCO_2 increased Bd in *O. australiensis* but not in Doongara. The protein content decrease is more pronounced in domesticated rice than cultivated rice. The differential effect of eCO_2 between wild and cultivated rice should be considered in future breeding programs.

The larger grain size in eCO_2 compared to aCO_2 observed in the third study was probably caused by a greater transfer of photosynthetic assimilates from leaf to caryopsis associated with increased rates of photosynthesis observed in the first study. However, consistent interactions between CO_2 supply during plant development and genotype led to the hypothesis in the fourth study that *"Endosperm morphology might also respond differentially to eCO_2 among wild and domesticated rices"*. In the fourth study, the aleurone layer cells and starch granules of *O. sativa* were smaller at eCO_2 than aCO_2. However, one accession of *O. meridionalis* showed larger aleurone layer cells while the other accession showed larger starch granules at eCO_2. This study showed that the morphological changes in aleurone cells and starch granules are due to genotype × CO_2 interactions rather than genotype or CO_2 alone. It can be concluded that, among wild species of rice, there may be accessions with genetically determined responses to eCO_2 that present valuable traits for breeding programs.

It can be concluded from this PhD thesis that eCO_2 affects photosynthesis, light interception, canopy architecture, biomass production and C gain uniformly (genetically controlled) across all genotypes tested. However, eCO_2 affected R_d differently in wild rice than in domesticated rice. The actual biomass production is positively correlated with simulated C gain. Crown density, above-ground biomass and C gain were greater at eCO_2 despite lower light interception. Furthermore, changing the light regime affects genotypes quantitatively but not the relative ranking of genotypes. The grain morphology and pasting properties are genetically controlled and showed uniform responses to eCO_2; however, seed length and FV showed differential responses to eCO_2. Reduction in PC at eCO_2 was obtained across all genotypes but domesticated rice was affected more than wild rices. There are genetic variations in endosperm morphology between wild and domesticated rices and their responses to eCO_2. At eCO_2, aleurone cell sizes increased in one wild rice accession of *O. meridionalis* but decreased in *O. sativa*, while starch granule sizes increased in the other accession of *O. meridionalis* but decreased in *O. sativa*. It can be concluded that the endosperm morphology responds differentially to eCO_2, both between and within species.

The above information can be used to i) redesign the canopy architecture for better light interception and productivity and ii) improve grain quality using wild *Oryza* genotypes via breeding.

6.3. Future directions

Further studies should be aimed at determining the effect of eCO_2 and genotype × CO_2 interactions on Australian wild rice species on the:

1. ***Molecular structure and functional properties of the grain starch***

   Starch is composed of amylose and amylopectin. Changes in the amylose-to-amylopectin ratio, glucan chain-length distribution, degree of crystallinity, or granule size distribution can alter starch functional and pasting properties (Copeland et al., 2009). Starches with similar amylose content may have different functional properties (Kowittaya and Lumdubwong, 2014) due to the differences in molecular fine structure, especially amylose fine structure, which determines gelatinization and pasting properties of rice starch (Tao et al., 2019). Furthermore, the viscosity of starch depends on the number of long amylose chains and the size of whole amylopectin molecules (Tao et al., 2019). Environmental stress can alter starch biosynthesis in the cereal endosperm and genotypic variation appears to be the primary contributor for response to environmental stress—see review (Beckles and Thitisaksakul, 2014). Variation in soil moisture and nutrient availability, as well as temperature, affect starch functionality (Beckles and Thitisaksakul, 2014); for example, application of a higher rate of N application affected amylopectin chain length and the ratio of short to long amylopectin branches, gelatinization, hydrolysis and pasting properties (Teng et al., 2021; Yang et al., 2016). Because of genetic variation in starch molecular structure and its functional properties (Zhao et al., 2021), and differential responses of eCO_2 on pasting properties and endosperm morphology of wild and cultivated rice (Rahman et al., 2021a, 2022), eCO_2 might also affect wild rice differentially. There is no report available on the effect of eCO_2 on molecular structure and functional properties of the grain starch of any wild or domesticated rice; this topic should be pursued in future.

2. ***Elemental composition of the grain***

Proteins and minerals are important for human health. Declines in protein and mineral (Zn and Fe) content due to eCO_2, which have been reported widely in domesticated rice and other cereals (Chaturvedi et al., 2017; Loladze, 2014; Myers et al., 2014; Seneweera et al., 1996; Seneweera and Conroy, 1997a; Ujiie et al., 2019; Zhu et al., 2018), could have a negative impact on human health, especially where rice is consumed as the main source of nutrient supply (Loladze, 2002, 2014; Norton, 2019). However, some reports have shown increases in Zn and Fe in domesticated rice due to eCO_2 (Goufo et al., 2014a; Lieffering et al., 2004). The above findings indicate the differential effect of eCO_2 on minerals in domesticated rice. However, none has investigated the effect of eCO_2 on the mineral content of wild rice species. Due to the genetic variation among wild and domesticated rice, the mineral content of grains from wild rice might respond differently to eCO_2 compared to domesticated rice; this should be pursued in the future.

Chapter 7

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