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International Agricultural Research

Applying photosynthesis research to improvement of food crops

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Applying photosynthesis research to improvement of food crops

Proceedings of a workshop held at the Australian National University, Canberra, Australian Capital Territory, Australia, 2–4 September 2009

Editors: Jill E. Gready, Simon A. Dwyer and John R. Evans

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2013

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Gready J.E., Dwyer S.A. and Evans J.R. (eds) 2013. Applying photosynthesis research to improvement of food crops. Proceedings of a workshop held at the Australian National University, Canberra, ACT, Australia, 2–4 September 2009. ACIAR Proceedings 140. Australian Centre for International Agricultural Research: Canberra. 145 pp.

ACIAR Proceedings – ISSN 1038-6920 (print), ISSN 1447-0837 (online)

ISBN 978 1 922137 81 4 (print)
ISBN 978 1 922137 80 7 (online)

Technical editing by Twofoot Consulting Group Pty Ltd, Australia
Design by Peter Nolan, Canberra, Australia
Printing by Paragon, Canberra, Australia

Cover: Viridiana Silva Perez (PhD student) measures CO_2–water vapour gas exchange and chlorophyll fluorescence in wheat genotypes at the CIMMYT Wheat Yield Consortium field trial in Ciudad Obregón, Mexico. (Photo: V.S. Perez)

Foreword

The challenges of world hunger and poverty continue to increase. Cereal production will need to double by 2050 to meet the demands of a higher global population, growing consumption of meat and other animal products, increasing animal feed requirements, and the increase in industrial use of these crops.

Expanding food crop production to meet the demands for food, feed and industrial use of staples will require increased productivity per unit of land, as well as more efficient use of water, fertiliser and labour inputs, all of which will become increasingly limited in availability and cost. In recent decades a large part of the increase in food crop yields has come from breeding for disease resistance or tolerance to environmental stress, as well as from improved crop management. However, it is now recognised that one next major change in crop productivity needed to meet global demands will come from increasing the photosynthetic efficiency of crop plants, better using the non-limiting inputs of sunlight and carbon dioxide from the air.

A key factor limiting the photosynthetic efficiency of crops is the efficiency of the enzyme Rubisco, which is involved with converting carbon dioxide (CO_2) into energy-rich organic compounds. However, Rubisco also fixes oxygen in a competing reaction that wastes carbon and energy, and this competing reaction increases at higher temperatures—a factor of growing concern with projected global warming. So, increasing the photosynthetic capacity of crops will require targeted improvements in Rubisco activity as well as other steps in carbon fixation, or in ways to increase the CO_2 concentration in the leaf. These improvements may have further major benefits, such as more efficient use of water and nitrogen, and resilience under adverse seasonal growing conditions.

To assist planning of food-security initiatives based on improving photosynthesis, the Australian Centre for International Agricultural Research (ACIAR) sponsored a three-day workshop at the Australian National University (ANU) in Canberra, Australia. Workshop participants addressed potential strategies for developing and applying research on photosynthesis to improving food crops, and considered pathways for translating the research results into crop breeding. The workshop proposed an international photosynthesis research initiative for increasing food crop productivity, which has been subsequently established with the support of the ANU.

Nick Austin
Chief Executive Officer
ACIAR

Contents

Abbreviations

All abbreviations are expanded at first mention in each paper with the exception of ATP, NADPH/$NADP^+$/NADH/NAD and Rubisco.

2-PG	2-phosphoglycolate
3-PGA	3-phosphoglycerate
A	CO_2 assimilation rate
A_{max}	maximum CO_2 assimilation rate
ABA	abscisic acid
ADP	adenosine-5'-diphosphate
ANU	Australian National University
APIC	Association for Potato Intergenebank Collaboration
ARS	Agricultural Research Service (US)
ATP	adenosine-5'-triphosphate
AVDRC	World Vegetable Centre
AWCC	Australian Winter Cereals Collection
BBSRC	Biotechnology and Biological Sciences Research Council (UK)
BMGF	Bill and Melinda Gates Foundation
BRDO	Biotechnology Research and Development Office, Pathumthani, Thailand
CA	carbonic anhydrase
C_a	ambient pCO_2 around the leaf
CA1P	2-carboxy-D-arabinitol 1-phosphate
CAAS	Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences, Beijing, China
C_c	chloroplastic pCO_2
CCM	carbon concentrating mechanism
C_i	inorganic carbon
C_i	intercellular pCO_2
CIAT	International Center for Tropical Agriculture (CIAT/CGIAR)
CIMMYT	*Centro Internacional de Mejoramiento de Maís y Trigo* (International Maize and Wheat Improvement Center, El Batán, Mexico)
CIP	International Potato Center (Lima, Perú)
FACE	free air CO_2 enrichment
FBPase	fructose-1,6-bisphosphatase
FIGS	Focused Identification of Germplasm Strategy
GIS	geographic information system

GM	genetically modified/genetic modification
GMO	genetically modified organism/organisms
GRDC	Grains Research and Development Corporation (Australia)
GRIN	Germplasm Resources Information Network
g_s	stomatal conductance to water vapour
HI	harvest index
ICARDA	International Center for Agricultural Research in the Dry Areas (Syria)
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IITA	International Institute of Tropical Agriculture
INGER	International Network for the Genetic Evaluation of Rice
INRA	*Institut National de la Recherche Agronomique* (France)
IP	intellectual property
IPK	Leibniz Institute of Plant Genetics and Crop Plant Research (Gatersleben, Germany)
IRRI	International Rice Research Institute
IRRI GRC	T.T. Chang Genetic Resources Center, International Rice Research Institute
j	energy content of plant biomass
J_{max}	maximum rate of chloroplast electron transport
k_{cat}	catalytic turnover rate (k_{cat}) for Rubisco carboxylation under CO_2-saturated conditions
K_C	Michaelis–Menten constant (K_m) of Rubisco for CO_2
K_O	Michaelis–Menten constant of Rubisco for O_2
k_{ocat}	catalytic turnover rate of Rubisco oxygenation
LAI	leaf area index
LMR	leaf mass ratio
ME	malic enzyme
NAD-ME	NAD-malic enzyme
NAD^+/NADH	oxidised/reduced form of nicotinamide adenine dinucleotide
NADP-ME	NADP-malic enzyme
$NADP^+$/NADPH	oxidised/reduced form of nicotinamide adenine dinucleotide phosphate
NAR	net assimilation rate of leaves
NBPGR	National Bureau of Plant Genetic Resources (New Delhi, India)
NCGRP	National Center for Genetic Resources Preservation (Fort Collins, USA)
NCRPIS	North Central Regional Plant Introduction Station (Ames, Iowa, USA)
NIAS gene bank	National Institute of Agrobiological Sciences (Tsukuba, Japan)
NPGS	National Plant Germplasm System
PAR	photosynthetically active radiation (400–700 nm)
PBR	plant breeder rights
PCO	photosynthetic carbon oxidation
pCO_2	partial pressure of CO_2
PCR	photosynthetic carbon reduction, also referred to as the Calvin cycle
PDBP	pentadiulose-1,5-bisphosphate

PEP	phosphoenol pyruvate
PEPC	PEP carboxylase
P_i	inorganic phosphate
P_{max}	maximum photosynthetic capacity in air
PSII	photosystem II
PVP	plant variety protection
QTL	quantitative trait locus
RA	Rubisco activase
R_d	mitochondrial respiration rate (measured in the dark)
RDA Genebank	Rural Development Association (Suwon, Republic of Korea)
RGR	relative growth rate
Rubisco	ribulose 1,5-bisphosphate carboxylase oxygenase (EC 4.1.1.39)
RuBP	ribulose-1,5-bisphosphate
RUE	radiation-use efficiency (also ε) – efficiency with which intercepted radiation is converted into biomass
SBPase	sedoheptulose-1,7-bisphosphatase
$S_{C/O}$	CO_2/O_2 specificity factor of Rubisco
SGRP	System-wide Genetic Resources Programme
SLA	specific leaf area
SMTA	Standard Material Transfer Agreement
USDA	US Department of Agriculture
V_{Cmax}, V_{Omax}	maximum rates of carboxylation and oxygenation by Rubisco
WARDA	Africa Rice Center (Benin)
WHO	World Health Organization
XuBP	xylulose-1,5-bisphosphate
YP	yield potential
$\Gamma*$	CO_2 compensation point of Rubisco (dependent on temperature and O_2 partial pressure)
ε_i	efficiency with which incident solar radiation is intercepted by a crop
θ	empirical curvature factor for describing the response of CO_2 assimilation rate to irradiance

Rubisco primer

Jill E. Gready[1] and Spencer M. Whitney[2]

Ribulose 1,5-bisphosphate carboxylase-oxygenase (EC 4.1.1.39), named Rubisco, provides the main route for accumulation of organic carbon in the biosphere. As part of the Calvin cycle (also known as the photosynthetic carbon reduction cycle), Rubisco catalyses a sequence of reactions during photosynthesis that results in carbon assimilation through fixation of atmospheric carbon dioxide (CO_2), a process that underpins all life. Despite this critical role, Rubisco has the paradoxical distinction of being one of the most inefficient enzymes, fixing only a few CO_2 molecules per second. This is partly a consequence of the rapid fall in atmospheric CO_2 partial pressure (pCO_2) over geological timescales since Rubisco first appeared. Furthermore, Rubisco fails to differentiate efficiently between CO_2 and O_2, whose atmospheric concentration has risen rapidly (from near zero) over the same evolutionary timescale.

Several hypotheses have been advanced to explain the apparent failure of the evolutionary machinery to re-tune the catalytic efficiency and CO_2/O_2 selectivity of Rubisco under changing environmental conditions, including limitations imposed by the complexity of the catalytic mechanism. Rubisco catalyses a series of steps. The chemistry takes place within a highly conserved active-site region in which the same atoms of reactant, intermediates and products are all coordinated to an Mg^{2+} complex. It is thought that this conserved complexity has restricted the 'residue space' that Rubisco can sample evolutionarily by mutation while maintaining sufficient activity in all its catalytic steps to constitute a viable enzyme. Whatever the reason—or, most likely, reasons—for Rubisco's slow evolution, the consequence is that Rubisco catalyses carboxylation and oxygenation of ribulose-1,5-bisphosphate (RuBP) in a ratio of only about 3:1 in many plants, with the oxygenation products resulting in the loss of fixed carbon. The net outcome is that the oxygenation reaction reduces the photosynthetic efficiency of Rubisco by up to 50%. To sustain sufficient rates of carbon assimilation, Nature's solution in most photosynthetic organisms is to synthesise large amounts of Rubisco, making it the most abundant protein in plants (and on Earth)—up to 50% of leaf protein. Alternatively, more complex mechanisms have been evolved that concentrate CO_2 around Rubisco and, hence, effectively reduce the extent of the wasteful oxygenation reaction (see Furbank et al. 2013).

Crystallographic, mutagenesis, kinetic and computational studies on Rubisco over three decades have revealed much about its structure and catalytic mechanism, including the role played by several active-site residues. Form I Rubiscos of higher plants consist of a hexadecamer of eight large and eight small subunits (L_8S_8), with the large subunits arranged as a core of four L_2 dimers centred on a fourfold axis. The active site is very well conserved in structure and sequence: it is at the interface of the two large subunits in an L_2 dimer, each dimer containing two active sites.

A notable feature in plants and green algae is that the large subunit of Rubisco is encoded by a chloroplast gene (*rbc*L), but the small subunit is coded by a family of nuclear genes (*rbc*S). Evidence suggests the *rbc*L gene is undergoing positive selection in land plants. Recent studies indicate that Rubisco evolutionary selection can respond by adaptation to different environmental conditions.

Reference

Furbank R.T., von Caemmerer S. and Price G.D. 2013. CO_2-concentrating mechanisms in crop plant so increase yield. In 'Applying photosynthesis research to improvement of food crops', ed. by J.E. Gready, S.A. Dwyer and J.R Evans. ACIAR Proceedings No. 140, 130–137. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

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Introduction

Jill E. Gready[1], Simon A. Dwyer[2] and John R. Evans[3]

Summary

The need to feed many more people in coming decades with increasingly stretched resources is recognised at the highest international levels. The urgency of this problem has placed campaigns against global poverty and global warming at political centre stage.

Food security challenge

The green revolution of the mid 20th century delivered high-yielding crop varieties together with improved farming practices, particularly the use of fertilisers, and prevented starvation for hundreds of millions of people. However, ongoing improvements based largely on refinements to green revolution methods are not likely to be sufficient to feed the forecast increase in world population. Since the mid 1990s the rate of increase in global productivity of all major food crops (maize, wheat, rice, soybean, and roots and tubers) has been declining. This decline is particularly serious for rice (van Nguyen and Ferrero 2006) and wheat (Dixon et al. 2009), which together with maize provide more than 50% of the food energy for the developing world. The global average annual yield increase of wheat has decreased from more than 2.5% during 1960–90 to less than half the rate since then, with the annual yield for rice decreasing to less than 1% in the same period.

Solutions to the food security problem need to account for other global factors such as climate change and environmental sustainability. Nelson et al. (2009) suggest that the net impacts of climate change on agriculture and human wellbeing will be negative. Impacts will be particularly severe in Sub-Saharan Africa and South Asia, the regions of the developing world expected to experience highest population growth. All irrigated food crops in South Asia will experience large yield declines, most seriously for wheat by 20–30%, with maize yields in Sub-Saharan Africa also expected to be much lower (Nelson et al. 2009). Although there are various causes of yield decline, including the lack of inputs and inadequate agronomy, the lack of improved germplasm plays a role and ultimately the yield potential ceiling will become a key constraint.

Declines in global productivity growth have occurred during a period of global underinvestment in agricultural research. CGIAR shows that funding for agricultural research has not increased in real terms until recently. International (Nelson et al. 2009) and national bodies (The Royal Society 2009) agree that a large increase in investment in research, development and delivery is necessary to produce the second major change in crop productivity needed to assure food security. Among the research priorities, germplasm improvement will be critical in the short and medium term and increased yield potential of major food crops for the medium and long term.

Research into photosynthesis

The major focus of crop productivity research in recent decades has been on improving drought and

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salt tolerance, water and fertiliser use efficiency, and disease and pest control. Even without the slow-down in the yearly gains accruing from these strategies, by themselves they are likely to be insufficient to meet future food security demands. Therefore it is time to diversify investment into new approaches that include improved photosynthesis.

The Royal Society of London (2009) argues that the solutions require technologies and approaches underpinned by good science, and recommends funding long-term, high-risk approaches to high-return targets in genetic improvement of crops, including genetically modified (GM) crops, with improved photosynthetic efficiency or nitrogen fixation. Over the past 50 years we have learned much about the processes involved in primary carbon fixation by plants, but this knowledge has not been a driver for increased crop productivity. Developing the knowledge base of plant photosynthesis and associated technologies for application to crops provides a route to the next green revolution. This will be driven by increases in photosynthetic efficiency that will lead to greater biomass and yield, water and nutrient use efficiency, and increased crop resilience. Well-targeted research into photosynthetic efficiency, combined with a technology delivery pathway, is expected to deliver greater plant biomass and crop yield, improved nitrogen-use efficiency, lower water use and higher resilience of crops under adverse seasonal growth conditions.

Research spearheaded by Australian researchers provides the basis for development of technology leading to improved plant photosynthesis and new hope for sustainable development. Australia is well positioned to play a lead role in the next green revolution through implementation of this cutting-edge research into food crops.

Australian agricultural aid organisations, including the Australian Centre for International Agriculture Research (ACIAR) and the Crawford Fund (Persley and Blight 2008) have been at the forefront in promoting discussion about the food security crisis. Hence ACIAR sponsored a workshop on research into photosynthesis in major food crops. Held at the Australian National University, Canberra, in September 2009, the workshop brought together Australian and international leaders in the fields of photosynthesis and associated crop development, and managers of international and national agriculture and their investors. Workshop participants were tasked with assessing the options for applying photosynthesis research to improving food crops, and drafting a strategy to bring this to fruition. The workshop papers are presented in this volume. To maintain currency with recent research, each paper includes additional references that have been published since the workshop was held.

Workshop focus

The workshop focused on three questions:

1. Can increased photosynthetic efficiency lead to higher yielding or more resource-efficient crops?
2. What is the potential for realising these gains in five of the world's major food crops: rice, wheat, maize, potato and legumes?
3. What are the available technologies that could be applied to achieve this?

References

Dixon J., Braun H-J., Kosina P. and Crouch J. 2009. Wheat facts and futures 2009. CIMMYT: Mexico.

Nelson G.C., Rosegrant M.W., Koo J., Robertson R., Sulser T., Zhu T., et al. 2009. Climate change: impact on agriculture and costs of adaptation. International Food Policy Research Institute: Washington, DC.

Persley G.J. and Blight D.G. (eds) 2008. A food secure world: how Australia can help: report of the Crawford Fund World Food Crisis Task Force. Australian Academy of Technological Sciences and Engineering: Melbourne.

The Royal Society 2009. Reaping the benefits: science and the sustainable intensification of global agriculture. The Royal Society: London.

van Nguyen N. and Ferrero A. 2006. Meeting the global challenges of global rice production. Paddy and Water Environment 4, 1–9.

Can improved photosynthesis lead to better crop yields?

Photosynthesis research and its application to yield potential

Robert T. Furbank[1]

Introduction

Over the past decade, annual gains in yield from cereal breeding programs have reached a plateau. From 1997 to 2006, annual increases in cereal yields from conventional breeding have dropped to less than one-third of the annual gains seen between 1960 and 1988 (FAOSTAT 2008). This yield stagnation has been exacerbated by population pressure, competition for agricultural land from urbanisation and biofuel feedstocks, increasing fuel and fertiliser costs and the uncertainty of climate change. These effects have now resulted in a crisis in global food production. World stocks of cereal grains are the lowest seen in the past 45 years (FAO 2008). While there has recently been a focus on the global crisis in rice production, trends in production of wheat, maize and other non-grain crops have been similar over the same period.

There is a clear need for a transformational advance in cereal and other crop yields above the incremental annual increases afforded by current plant breeding technologies. To address this problem it is necessary to understand the underlying cause of this stagnation in yield progress. Evidence is mounting that yield potential in a number of crops may now be limited by the capacity for the plant to fix sufficient carbon during its life cycle, provide carbon at key points in plant development and translate this carbon into harvestable grain. In part, this transition to a possible yield limit from carbon fixation has resulted from spectacular increases in harvest index (the proportion of plant biomass harvestable as grain).

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These increases have been made possible by the introduction of the gibberellic acid (GA)-insensitive dwarfing genes during the green revolution and increasing grain yield driven by selection for grain number (Fischer et al. 1998; Evans and Fischer 1999; Sheehy et al. 2001; Peng et al. 2008).

In rice it is most apparent that 'source capacity' or 'radiation-use efficiency' is limiting realisation of high yield in the new rice types (see Sheehy et al. 2001; Peng et al. 2008). This is evident both from theoretical calculations and from observations that the proportion of spikelets that are fertile, set grain and fill to harvestable grain has declined in recent rice breeding, despite the number of spikelets increasing (see Sheehy et al. 2001). For many other crops, there is evidence that increases in yield are correlated with increases in sink capacity, afforded by high potential grain number.

Photosynthetic capacity has closely tracked this trend (e.g. in wheat, see Fischer et al. 1998). Options for increasing crop photosynthesis are to increase photosynthetic efficiency per unit leaf area, elevate photosynthetic capacity without increasing pre-anthesis structural biomass, or at least balance this biomass with grain biomass, and not at the expense of harvest index.

Increasing leaf area per reproductive sink is not a preferred solution, as the green revolution gains of elevated harvest index could be lost and light penetration and radiation-use efficiency compromised. An approach of increasing photosynthetic capacity and hence protein nitrogen per unit leaf area would increase source strength but may be limited by the future availability and cost of nitrogen fertiliser.

Photosynthetic mechanism

Photosynthesis is the process where the chloroplast thylakoids of the leaf and other photosynthetic structures harvest light. The resultant chemical energy (ATP and NADPH) is used to fix atmospheric CO_2. In C_3 photosynthesis, CO_2 is fixed directly via Rubisco. In C_4 photosynthesis, CO_2 is fixed indirectly after primary fixation by phosphoenol pyruvate (PEP) carboxylase and subsequent re-release in adjacent cells not in direct communication with the atmosphere where CO_2 is concentrated.

These two photosynthetic pathways are ubiquitous among major crops. Most crops (rice, wheat, grain legumes, canola and all root crops) use C_3 photosynthesis, fixing CO_2 directly from the air. C_4 crops are in the minority, predominantly represented in world agricultural systems by maize, sorghum and sugarcane. Figure 1 illustrates simply C_3 and C_4 photosynthesis, indicating that the C_4 pathway overlays a 'CO_2 supercharger' on the basic C_3 photosynthetic process, which is also driven by light-derived NADPH and ATP. C_4 photosynthesis is more efficient and has better nutrient use; elevated CO_2 at the site of Rubisco means this enzyme operates at its maximum rate and photorespiration (the wasteful by-product of the Rubisco reaction) is reduced to zero. As the efficiency of Rubisco is increased, less is required, so less nitrogen is required per unit leaf area. These specific issues will be dealt with in other papers in these proceedings.

Improving leaf photosynthetic efficiency and capacity

To improve flux through photosynthesis and increase efficiency it is necessary to understand the major points controlling photosynthetic flux or biochemical 'bottlenecks'. A great deal of modelling has been done, and targeted transgenic plants have been used to obtain this information for both C_3 and C_4 plants (see von Caemmerer 2000). Figure 2, adapted from von Caemmerer (2000), summarises what is known about the relationship between leaf photosynthetic biochemistry and the rate of CO_2 fixation. By examining the response of photosynthesis to ambient CO_2 levels under saturating light, we can divide the 'bottlenecks' in photosynthesis into three groups:

1. limitation by Rubisco levels and kinetics
2. limitation by 'regeneration'—the rate of recycling of the sugar phosphate acceptor for Rubisco, ribulose-1,5-bisphosphate (RuBP)
3. phosphate regeneration (or sugar synthesis and export limitation).

Under low irradiance, efficiency of light harvesting may become limiting. This is relevant to canopies and is dealt with by Badger (2013).

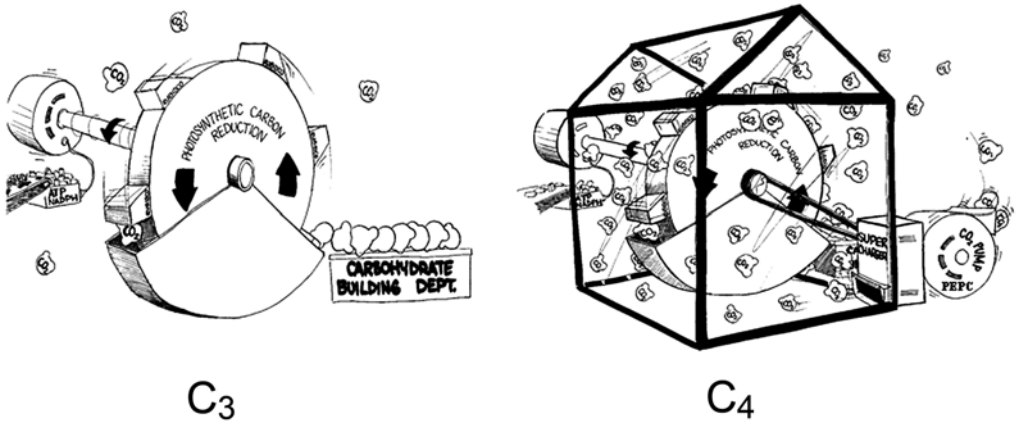

Figure 1. C_3 and C_4 photosynthesis. In C_3 photosynthesis, light-driven ATP and NADPH production fuel Rubisco-catalysed CO_2 fixation (the teeth on the wheel). Resultant sugar phosphates are recycled in the photosynthetic carbon reduction cycle and some are bled off to make stored or translocated carbohydrate. In C_4 plants, light energy is used to drive a biochemical CO_2 pump, which can elevate CO_2 up to tenfold atmospheric levels.

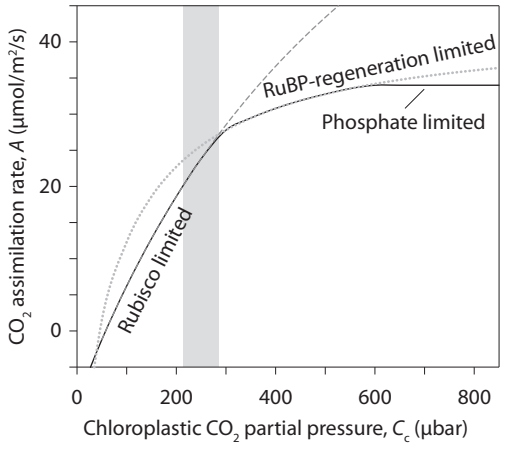

Figure 2. Limiting reactions in photosynthesis. Modelled CO_2 assimilation rate by a C_3 leaf (adapted from von Caemmerer 2000) showing the three major limitations to photosynthetic flux. The shaded area represents likely chloroplastic CO_2 partial pressures relevant to leaves now and in the near future. Dashed line is Rubisco limited, dotted line is RuBP-regeneration limited and the solid line is the achieved rate of CO_2 assimilation.

Figure 2 shows that at air levels of CO_2, Rubisco is predicted to exert the most control over photosynthetic flux. Experiments with transgenic plants containing reduced Rubisco levels (Hudson et al. 1992) confirmed this finding, as these workers estimated that, at high light, approximately 80% of the control over flux resides with Rubisco. Thus, Rubisco is an obvious target for manipulating C_3 photosynthesis, by manipulating either kinetic properties or amounts. This is discussed further below. Intriguingly, Rubisco also appears to have a high degree of control over photosynthesis in C_4 plants despite the high CO_2 concentration around Rubisco in these species (Furbank et al. 1996).

Targets for improving photosynthesis

At higher light intensities, modification of Rubisco's kinetic properties to improve catalytic competence, or in particular to decrease wasteful oxygenation and photorespiration, would be predicted to have a major effect on photosynthetic efficiency and flux. This result could also be achieved by introducing a CO_2-concentrating mechanism to C_3 plants that mimics either algal mechanisms or C_4 mechanisms (Furbank et al. 2013; Gready et al. 2013).

In many cases, the predicted benefit is difficult to model because of unknown parameters. A theoretical consideration of the consequences of photorespiration is presented in Zhu et al. (2008). This analysis calculates the efficiency of light conversion to carbohydrate in air for a C_3 plant and for a C_4 plant where photorespiration is assumed to be negligible. A 50% greater conversion efficiency in C_4 plants (3.7% vs. 2.4% of total solar radiation for C_4 and C_3, respectively) occurs mostly because photorespiration is absent, despite the additional energetic cost of the CO_2 pump.

Increasing photosynthetic efficiency though introducing a CO_2 concentrating mechanism to C_3 crop plants or through alteration of Rubisco's kinetic properties will be examined in detail in later papers. However, the value of these approaches can be estimated based on current knowledge of the energetic cost of photorespiration. Modelling of the consequences of these modifications is difficult to quantify because of unknowns (see Zhu et al. 2008; Reynolds et al. 2009). The details of these proposed modifications are dealt with by Furbank et al. (2013) but Table 1 shows that modification of the CO_2 environment around Rubisco and modification of Rubisco's properties offer the largest potential benefits.

Figure 2 shows that under well-watered conditions and present-day atmospheric CO_2 levels, and certainly CO_2 levels predicted with climate change, RuBP regeneration is likely to impose a considerable proportion of control over photosynthetic flux. A limitation in regeneration of RuBP, the acceptor of CO_2 in the Rubisco reaction, can be caused by either a deficiency in ATP or NADPH production through the light reactions, or a biochemical limitation to flux through the photosynthetic carbon reduction (PCR) cycle by individual rate-limiting enzymes. The limitations to biochemical flux through the PCR cycle have been studied in depth to show the enzyme sedoheptulose-1,7-biphosphatase (SBPase) has a high degree of control over this portion of photosynthesis. SBPase has now been overexpressed by a number of laboratories with increases in photosynthesis at high light in air of around 20% (Lawson et al. 2006 and references therein). This approach to boosting photosynthesis has not been tested in field trials of commercial crop species, but theoretically it should

be useful in combination with the other approaches in Table 1.

Mining genotypic variation in photosynthesis and 'whole of plant' approach

In cereals and probably other crop species, there is considerable genotypic variation in photosynthetic capacity (P_{max}), the maximum photosynthetic rate in air of leaf material at a given phenological stage under well-watered conditions (for wheat, see Fischer et al. 1998). Little work has been done to breed directly for photosynthetic rate of major source tissues. However, in wheat, yield progress has correlated well with this measurement (Fischer et al. 1998).

The difficulty in genotypic screens for photosynthetic capacity is that a whole of life cycle estimate is required for most crop species because a large proportion of harvestable carbon comes from remobilisation of carbon fixed pre-flowering (for wheat, see Gebbing and Schnyder 1999). Provision of sufficient carbon at key stages in the crop life cycle can have profound impacts on yield through early establishment of leaf area, establishment of the reproductive structure, pollen and ovule fertility and in late grain-filling through delayed senescence profiles (see Figure 3). Also, plant organs other than leaves can be important in providing carbon. In wheat, up to 28% of grain carbon can be derived from ear photosynthesis. In legumes, pod photosynthesis plays a key role in grain-filling and in *Brassica* grown at low nitrogen, leaves are completely senescent so pod photosynthesis predominates as the source of carbon during grain-filling (see King et al. 1998; Furbank et al. 2004). From this perspective, a transgenic approach to improving photosynthesis in a constitutive manner rather than a leaf-specific approach would be of greatest benefit.

The challenge for plant phenomics is to provide high-throughput tools to assay the whole plant, whole of life cycle photosynthesis. This way we can select germplasm with the best chance of translating photosynthetic performance into yield. While direct measurement of CO_2 assimilation is slow using traditional gas analysis techniques, the initial slope of the response of CO_2 assimilation to CO_2 concentration is a rich source of information on Rubisco kinetic properties and photosynthetic efficiency when coupled to modelling (von Caemmerer 2000). Infra-red thermography offers rapid measurement of plant water loss or conductance, a surrogate for CO_2

Table 1. Estimates of the likely benefits to photosynthetic rate per unit of light energy absorbed under optimal conditions by the leaf, for a variety of transgenic targets in photosynthesis

Target	Theoretical benefit (30°C, air)	Unknowns
Full C_4 pathway	~30–50%	Unknown CO_2 diffusion characteristics of C_3 bundle sheath cells Do we need kranz anatomy?
Single cell C_4	Only at low CO_2	Unknown CO_2 diffusion Characteristics of chloroplast envelope
Glycolate recycling	13%	Unknown CO_2 diffusion characteristics of chloroplast envelope or redox effects
HCO_3^- pump in chloroplast envelope	30–50%	Unknown CO_2 diffusion Unknown characteristics of chloroplast envelope Is Na gradient needed?
Rubisco with improved K_C/K_O, same k_{cat}	5–60%	Requires cereal plastid transformation
Rubisco with higher k_{cat}	17–30%	Requires cereal plastid transformation
SBPase overexpression	20%	Will it hold up in a canopy?

Data are based on calculations (Long et al. 2006; Zhu et al. 2008; Reynolds et al. 2009) and modelling (von Caemmerer 2000; von Caemmerer et al. 2007). Two options are shown in this table for introducing a modified C_4 pathway. Data are shown from work of Kebeish et al. (2007) for introducing a mechanism to improve recycling of photorespiratory products such as glycolate by introduction of bacterial enzymes.

K_O = Michaelis–Menten constant for oxygenation by Rubisco

K_C = Michaelis–Menten constant for carboxylation by Rubisco

k_{cat} = maximum catalytic CO_2 turnover rate of Rubisco

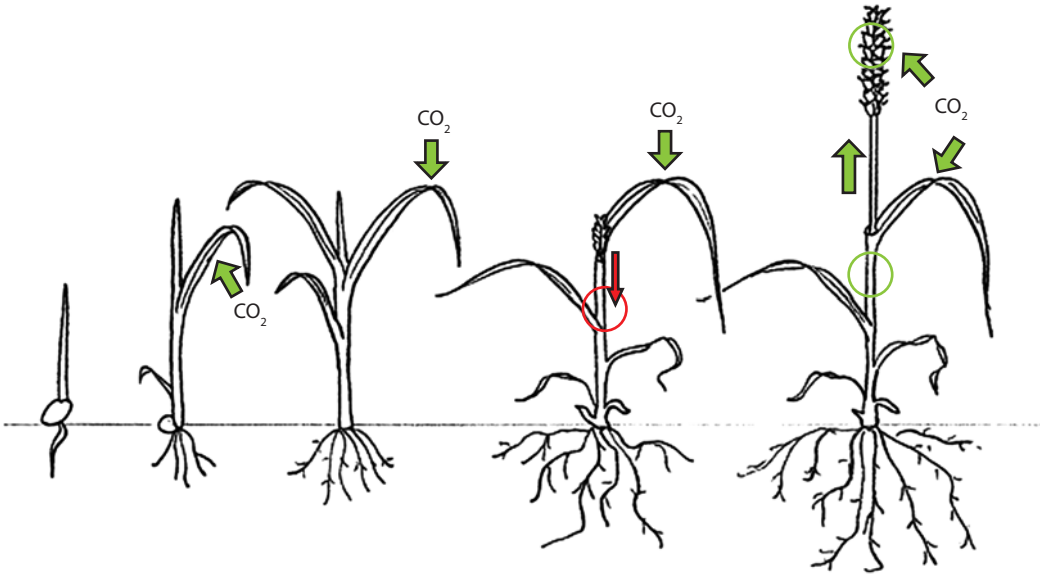

Figure 3. Stages of development in a cereal crop are shown where provision of sufficient photosynthate can have major effects on yield potential. Photosynthetic activity of the first source leaves can drive early canopy closure, and carbon fixed pre-anthesis can be stored in stems (red arrow and circle) and later remobilised (green circle). Persistent photosynthetic leaf area late in grain-filling (or 'staygreen') can 'finish' the crop.

assimilation and photosynthetic capacity under well-watered conditions. This method is applicable to field and controlled environment screens (see Fischer et al. 1998). Chlorophyll fluorescence is also a potential high-throughput screening tool for electron transport capacity as a surrogate for photosynthetic capacity. Coupled with imaging, it provides an opportunity to examine organ and tissue level contributions (see Furbank et al. 2009).

Balanced portfolio of approaches

An important consideration in a portfolio of projects to increase photosynthetic capacity and efficiency in crop plants is the time frame over which basic strategic work will translate into new genotypes. For the Rubisco–CO_2 concentrating transgenic approaches, a realistic time frame for a commercial cultivar might be 10–20 years after validation in model species. For a phenomic screen, material for first crosses could be available in 2–3 years. It is likely that a balance of transgenic and non-transgenic approaches will be needed to avert the world food security crisis.

References

Badger M.R. 2013. Role of plant leaf development in optimising photosynthetic efficiency, capacity, growth and yield. In 'Applying photosynthesis research to improvement of food crops', ed. by J.E. Gready, S.A. Dwyer and J.R Evans. ACIAR Proceedings No. 140, 20–26. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Evans L.T. and Fischer R.A. 1999. Yield potential: its definition, measurement, and significance. Crop Science 39, 1544–1551.

FAO 2008. United Nations Food and Agriculture Organization International Commodity Database. At <www.fao.org/economic/est/statistical-data/est-cpd/en>, accessed 13 August 2013.

FAOSTAT 2008. United Nations Food and Agriculture Organization Statistical Database. At <faostat.fao.org>, accessed 13 August 2013.

Fischer R.A., Rees D., Sayre K.D., Lu Z-M., Condon A.G. and Saavedra A.L. 1998. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate and cooler canopies. Crop Science 38, 1467–1475.

Furbank R.T., Chitty J.A., von Caemmerer S. and Jenkins C.L.D. 1996. Antisense RNA inhibition of *RbcS* gene expression in the C_4 plant *Flaveria bidentis*. Plant Physiology 111, 725–734.

Furbank R.T., von Caemmerer S. and Price G.D. 2013. CO_2-concentrating mechanism in crop plants to increase yield. In 'Applying photosynthesis research to improvement of food crops', ed. by J.E. Gready, S.A. Dwyer and J.R Evans. ACIAR Proceedings No. 140, 130–137. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Furbank R.T., von Caemmerer S., Sheehy J. and Edwards G.E. 2009. C_4 rice: a challenge for plant phenomics. Functional Plant Biology 36, 845–856

Furbank R.T., White R., Palta J.A. and Turner N.J. 2004. Internal recycling of respiratory CO_2 in pods of chickpea (*Cicer arietinum* L.): the role of pod wall, seed coat, and embryo. Journal of Experimental Botany 55, 1687–1696.

Gebbing T. and Schnyder H. 1999. Pre-anthesis reserve utilization for protein and carbohydrate synthesis in grains of wheat. Plant Physiology 12, 871–878.

Gready J.E., Kannappan B., Agrawa A., Street K., Stalker D.M. and Whitney S.M. 2013. Status of options for improving photosynthetic capacity through promotion of Rubisco performance—Rubisco natural diversity and re-engineering, and other parts of C_3 pathways. In 'Applying photosynthesis research to improvement of food crops', ed. by J.E. Gready, S.A. Dwyer and J.R Evans. ACIAR Proceedings No. 140, 96–111. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Hudson G.S., Evans, J.R., von Caemmerer S., Arvidsson R.B.C. and Andrews T.J. 1992. Reduction of ribulose 1,5-bisphosphate carboxylase oxygenase content by antisense RNA reduced photosynthesis in tobacco plants. Plant Physiology 98, 294–302.

Kebeish R., Niessen M., Thiruveedhi K., Bari R., Hirsch H.J., Rosenkranz R. et al. 2007. Chloroplastic photorespiratory bypass increases photosynthesis and biomass production in *Arabidopsis thaliana*. Nature Biotechnology 25, 593–599.

King S.P., Badger M.R. and Furbank R.T. 1998. CO_2 refixation characteristics of developing canola seeds and silique wall. Australian Journal of Plant Physiology 25, 377–386.

Lawson T., Bryant B., Lefebvre S., Lloyd J.C. and Raines C.A. 2006. Decreased SBPase activity alters growth and development in transgenic tobacco plants. Plant, Cell and Environment 29, 48–58.

Long S.P., Zhu X-G., Naidu S.L. and Ort D.R. 2006. Can improvement in photosynthesis increase crop yields? Plant, Cell and Environment 29, 315–330.

Peng S., Khush G.S., Virk P., Tang Q. and Zou Y. 2008. Progress in ideotype breeding to increase rice yield potential. Field Crops Research 108, 32–38.

Reynolds M., Foulkes M.J., Slafer G.A., Berry P., Parry M.A.J., Snape J.W. and Angus W.J. 2009. Raising yield potential in wheat. Journal of Experimental Botany 60, 1899–1918.

Sheehy J.E., Dionora M.J.A. and Mitchell P.L. 2001. Spikelet numbers, sink size and potential yield in rice. Field Crops Research 71, 77–85.

von Caemmerer S. 2000. Biochemical models of leaf photosynthesis, Vol. 2. CSIRO Publishing: Melbourne.

von Caemmerer S., Evans J.R., Cousins A.B., Badger M.R. and Furbank R.T. 2007. C_4 photosynthesis and CO_2 diffusion. In 'Reconfiguring the rice plant's photosynthetic pathway', ed. by J.E. Sheehy, P.L. Mitchell and B. Hardy. IRRI: Los Baños, Philippines.

Zhu X-G., Long S.P. and Ort D.R. 2008. What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? Current Opinion in Biotechnology 19, 153–159.

Additional references

Ainsworth E.A. and Ort D.R. 2010. How do we improve crop production in a warming world? Plant Physiology 154, 526–530.

Gregory P.J. and George T.S. 2011. Feeding nine billion: the challenge to sustainable crop production. Journal of Experimental Botany 62, 5233–5239.

Kirschbaum M.U.F. 2011. Does enhanced photosynthesis enhance growth? Lessons learned from CO_2 enrichment studies. Plant Physiology 155, 117–124.

von Caemmerer S. and Evans J.R. 2010. Enhancing C_3 photosynthesis. Plant Physiology 154, 589–592.

Role of plant leaf development in optimising photosynthetic efficiency, capacity, growth and yield

Murray R. Badger[1]

Summary

- This paper focuses on the links between leaf development and leaf photosynthetic properties in optimising the potential for plant growth and yield.
- The following leaf development issues are identified as important research questions for maximising light energy conversion by plants:
  - understanding the determinants of high and low specific leaf area (SLA) leaf development strategies and the links to having low and high photosynthetic capacity leaves
  - understanding the genetic determinants and variability of sun and shade leaf phenotypes is of primary importance, including issues relating to chloroplast-level acclimation and leaf-level acclimation, including cell and leaf morphology.
- If photosynthetic capacity per unit leaf area is linked to SLA, then the question arises as to what are the effects of breeding for high SLA early in canopy development for fast canopy development. The question of later potential consequences of limiting photosynthetic capacity development in the top layers of the canopy also arises.

Introduction

The capture of light by a plant or crop and the efficiency of its conversion to plant biomass are central to plant growth and crop yield. Influencing factors can be considered at two levels.

At the first, most basic, level, the intrinsic operation of photosynthetic processes is important, including the nature of light capture and energy conversion by photosynthetic reaction centres, and the conversion of this energy into accumulated biomass. Much of this operation is based on processes operating within the chloroplast. Second, the way in which leaves, including chloroplasts, develop and are used by a plant to optimise light capture and energy conversion is important. There has been considerable recent interest in ways to alter aspects of photosynthesis to improve the yield potential of crop species (see Long et al. 2006; Parry et al. 2007; Zhu et al. 2008; Murchie et al. 2009; Reynolds et al. 2009). This paper specifically considers the factors associated with leaf development that influence plant growth and yield.

Photosynthetic capacity vs. efficiency

Improvements in photosynthesis may contribute to biomass accumulation and yield in two ways:

1. Increasing the photosynthetic capacity of leaves, which directly involves an increase in the potential rate of photosynthesis per unit leaf area. It implies that there are changes to potential

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photosynthetic rates when photosynthesis is not limited by another factor such as light or nitrogen.

2. Changing the 'photosynthetic efficiency of leaves per unit leaf area', which implies that other inputs limit CO_2 fixation potential and that efficiency improvements to photosynthesis will achieve more carbon gain per unit limiting resource. Photosynthetic efficiency chiefly relates to:
   - radiation-use efficiency (RUE), which is mainly influenced by the quantum yield of CO_2 fixation
   - nitrogen-use efficiency
   - water-use efficiency.

Linking photosynthesis to plant growth and yield

There are two important relationships linking leaf photosynthesis to plant growth and crop yield.

At a single plant level, the relative growth rate of a plant can be described by equation (1):

$$RGR = SLA \times LMR \times NAR \quad (1)$$

where the relative growth rate (RGR) (g/g/day) represents the rate of biomass accumulation per unit biomass. SLA is the specific leaf area (m^2/g). The leaf mass ratio (LMR) (m^2/g) is the fraction of total biomass allocated to leaves and NAR is the net assimilation rate of leaves (g/m^2/day). NAR includes the gains through photosynthesis and the losses through respiration, including leaves and other parts of the plant. This simple equation implies that the growth rate of a plant has two main components that can be varied, NAR and SLA. Most immediately obvious is that NAR is mainly varied; a chief component of this is varying the rate of photosynthesis per unit leaf area. It is in this component that light interception has direct effects on photosynthesis. The second component is less obvious in that the plant may alter its SLA by making thinner or thicker leaves to vary the total amount of biomass invested in producing leaves. In this way, the rate of carbon gain can be increased by making extra leaf area with new carbon rather than changing the photosynthetic capacity per unit leaf area.

Research into differences between fast and slow growing plant species and the acclimation of species to different light regimes and elevated CO_2 has found that growth rate and light acclimation are significant and controlled by variation in SLA (Poorter and Evans 1998; Poorter and De Jong 1999; Evans and Poorter 2001). However, it is important to note that, within a species, there is a significant relationship between photosynthetic capacity and SLA. Increased SLA is associated with thinner leaves with reduced photosynthetic capacity per unit leaf area, and vice versa.

From a crop perspective, rather than describing the growth of an individual plant, it is more appropriate to develop equations that describe economic yield based on the growth and function of the crop canopy per unit ground area, rather than per unit leaf area. From this perspective, crop yield is frequently described as a function of the extent to which light per unit ground area is converted to economically important biomass (equation (2)):

$$\text{Crop yield} = LI \times RUE \times HI \quad (2)$$

where crop yield is the biomass of economically important product such as wheat grain (energy equivalents/m^2/crop cycle duration). *LI* is the amount of light intercepted by photosynthetically active leaves (energy equivalents/m^2/crop cycle duration). Radiation-use efficiency (*RUE*) is the fraction of intercepted light energy converted into accumulated biomass energy. Harvest index (*HI*) is the fraction of final biomass that is partitioned into economic yield.

LI is influenced by the development of the crop canopy such as speed of canopy development and closure, longevity and architecture. *RUE* is determined by the combined photosynthetic rates of all leaves within the canopy over the life cycle, minus crop respiratory losses. *HI* is determined by aspects of plant development, which influences the production of differently sized yield structures such as seeds and ears.

Determinants of net photosynthesis at a leaf level

The process of photosynthesis determines the *RUE* of crop yield and the NAR of plant growth equations. Treating the leaf as a homogenous entity (which it is not), net photosynthesis per unit leaf area can be expressed by equation (3):

$$\text{Net photosynthesis} = (LI/\text{chlorophyll} \times \text{energy capture efficiency} \times CO_2 \text{ uptake efficiency} \times \text{chlorophyll/unit leaf area}) - \text{respiration/unit area} \quad (3)$$

where *LI*/chlorophyll is the average light intercepted by a chloroplast per unit leaf area, energy

capture efficiency is the fraction of intercepted light converted into chemical energy available for CO_2 fixation, CO_2 uptake efficiency is the fraction of chemical energy that is converted to biomass, and chlorophyll/unit leaf area is the density of chloroplasts per unit leaf area. Respiration per unit leaf area contributed to energy loss.

Light-limited vs. light-saturated leaf photosynthesis

The light level received by the leaf or chloroplast determines the nature of factors that limit the various light and energy conversion efficiencies. This affects net photosynthesis.

Photosynthesis can be partitioned into two primary response regions based on the light intensity absorbed by leaves and chloroplasts (Figure 1). These are light-limited and light-saturated regions of photosynthesis. The response of photosynthesis to light is somewhat hyperbolic, with photosynthesis saturating at high light intensities. When light is low, photosynthesis responds linearly to intercepted light, and net photosynthesis is limited primarily by factors that affect the intrinsic quantum yield of photosynthesis. However, when light is high, photosynthesis ceases to respond to increased light and is limited by the capacity of CO_2-fixing reactions to capture the energy produced by harvesting light. With this information, we can assess how various aspects of chloroplast, leaf and canopy development influence growth and light use efficiency depending on the light environment being experienced by leaves. Table 1 summarises chloroplast factors that influence energy conversion efficiencies in equation (3).

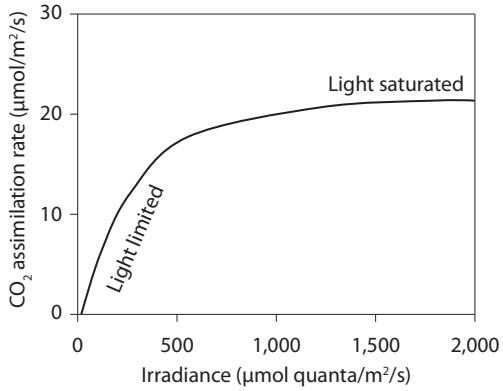

Figure 1. Light response curve for photosynthesis, showing the light-limited and light-saturated parts of the photosynthetic response curve

Light-limited region

Equation (3) shows light energy conversion efficiency in the light-limited region is limited by factors that influence the primary quantum yield of CO_2 fixation of photosynthesis. For C_3 plants, factors are mainly the biochemical inefficiencies of Rubisco and the energy demands of photorespiration. For C_4 photosynthesis, the extra energy demand associated with CO_2 concentration in the bundle sheath is most important. For both C_3 and C_4 plants, primary inefficiencies inherent in the reactions of thylakoid photochemistry and electron transport are similar.

Light-saturated region

In the light-saturated region, mainly factors that influence the capacity of chloroplast stromal reactions to fix CO_2 limit energy conversion efficiency. This can be associated with the potential rate of CO_2 fixation by Rubisco. This rate is determined by the amount present and kinetic properties of Rubisco, and the capacity to regenerate ribulose-1,5-bisphosphate (RuBP), which is a substrate for the Rubisco reaction. Fructose-1,6-bisphosphatase (FBPase) and sedoheptulose-1,7-bisphosphatase (SBPase) have been identified as limiting this reaction (Harrison et al. 2001; Raines 2003; Lefebvre et al. 2005).

Table 1. Factors affecting light conversion efficiency in the chloroplast in light-limited and light-saturated regions of photosynthesis

	Factors affecting light energy conversion					
Light region	Photochemistry efficiency	Rubisco efficiency	Rubisco capacity	RuBP regeneration	CO_2 elevation mechanisms	Nitrogen redistribution
Light limited	yes	yes[a]	no	no	yes	yes
Light saturated	yes	no	yes	yes	yes	yes

[a] Depends on whether the photosynthetic process is primarily C_3 or C_4

Nitrogen redistribution

Under light-limiting and light-saturating conditions, changing the allocation of nitrogen between components of the light or the dark reactions of photosynthesis helps optimise the plant's capacity to fix CO_2 (Evans and Poorter 2001; Tazoe et al. 2006; Zhu et al. 2007). At limiting light, nitrogen is allocated to light harvesting complexes to help capture the maximum amount of available light, while at high light there is a shift towards increasing the abundance of proteins of the Calvin cycle in the stroma. Thus, appropriate nitrogen redistribution can optimise photosynthesis under all light conditions.

Light acclimation in leaf development

How leaves acclimate to different growth light intensities provides a valuable example for how factors that affect chloroplast and leaf development can vary leaf photosynthetic capacity and efficiency. Dicot and monocot plant studies show that there are two related parts to leaf acclimation to light: changes to leaf structure and the properties of individual chloroplasts. These changes are shown in Figure 2.

Chloroplast-level acclimation includes changes to contents of thylakoids, proteins, pigments and Calvin cycle enzymes on a per-chloroplast basis. Individual chloroplast properties are adjusted depending on the position of the chloroplast in the light profile of the leaf (sun chloroplasts at the top and shade chloroplasts at the bottom).

Leaf development factors include changes in the anatomy of a leaf, similar to the development of low- and high-SLA leaves. In general a 'sun leaf' develops thicker leaves with lower SLA, with more or larger cells across the transverse profile of the leaf (Evans and Poorter 2001; Terashima et al. 2005, 2006). Total numbers of chloroplasts and total chlorophyll, stromal protein and Rubisco content per unit leaf area increase strongly with acclimation to higher light. Various studies have identified that this SLA response is the most important component of photosynthetic light acclimation.

These two levels of leaf acclimation appear to be differently regulated. Chloroplast acclimation is most likely controlled by signals originating in the chloroplast, such as redox control and carbohydrate production. Leaf development changes can be regulated at various stages in leaf expansion with the potential involvement of systemic hormone signals and carbohydrate supply (Murchie et al. 2005; Walters 2005). For leaf development, the role of signals affecting either periclinal or anticlinal cell division is important but the nature of the signals is unknown (Terashima et al. 2005, 2006).

Optimising photosynthesis in crop development

Crop development represents a time course where leaves are exposed to various combinations of light-limited and light-saturated photosynthesis conditions. This is illustrated in Figure 3.

During the canopy development part of the crop growth cycle (Region 1), the light that leaves receive is determined by the growth of isolated individual plants whose leaves gradually coalesce to form a closed canopy. In Region 1, most leaves receive

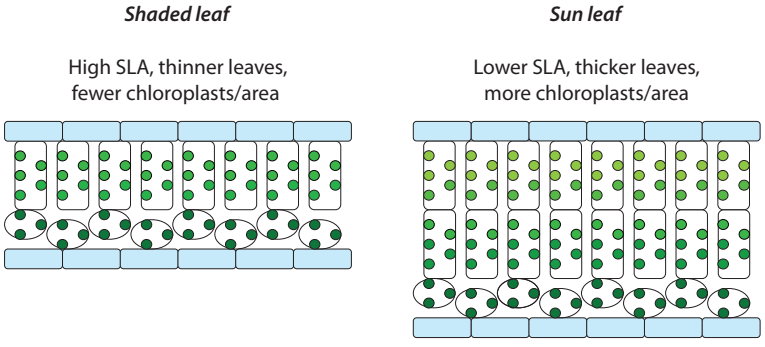

Figure 2. Characteristics of leaves developed under shaded or sunlit conditions

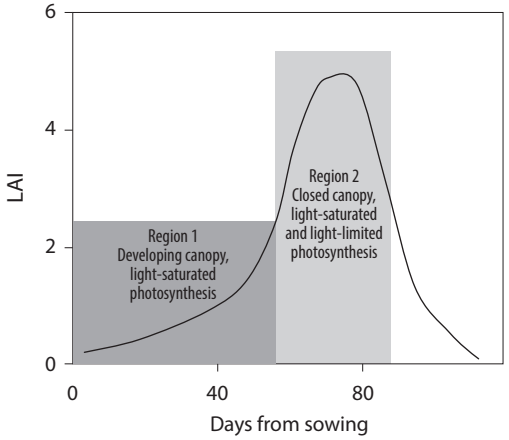

Figure 3. Development of leaf area index (LAI) in a crop canopy during the growing season. Developing (Region 1) and closed canopy (Region 2) regions are indicated by differently shaded areas.

unshaded light so photosynthesis will generally respond in the light-saturated part of the light response curve (Figure 1). However, in Region 2, above an LAI of 2–3, leaves at the top of the canopy receive high light while those at the bottom are increasingly shaded (Wall and Kanemasu 1990). Table 1 outlines factors relevant to defining the light conversion efficiency of photosynthesis in the two regions.

Region 1 will generally be dominated by photosynthetic efficiency factors operating at light saturation (Table 1). In addition, plants can rapidly develop leaves to quickly obtain the maximum amount of light intercepted per unit ground area. Light that is not absorbed by leaves is not available for biomass production. Thus, strategies to develop high-SLA (thinner) leaves, which occupy more area, may be more advantageous (Rebetzke et al. 2004).

In Region 2, optimising the development of 'sun'-type leaves at the top of the canopy with a transition to shade-adapted leaves at the bottom will be the most effective strategy. Additionally, there is a great advantage to manipulating canopy architecture through leaf size and angle to optimise the penetration of light down through the canopy.

There are a number of ways in which leaf and chloroplast development may be involved in maximising energy conversion efficiency during the development of the canopy.

Light-saturated—Region 1

Two conflicting driving forces in Region 1 may operate to maximise light conversion efficiency per unit ground area.

A sun leaf strategy with low SLA, high numbers of chloroplasts and high photosynthetic capacity per unit leaf area would be optimal to maximise efficiency per unit leaf area. Conversely, to maximise efficiency per unit ground area, high-SLA leaf development is a strong driver. However, high-SLA leaves will likely have reduced photosynthetic capacity and reduced light conversion efficiency per unit leaf area. These two factors are somewhat opposed in trying to achieve maximum light conversion efficiency during the open canopy part of crop development.

Light-limited—Region 2

Region 2 is dominated by achieving maximum energy efficiency per unit leaf area rather than ground area.

Leaves at the top of the canopy will maximise their sun-type physiology to get the best efficiency under high light intensities. The per-chloroplast and per-leaf area Rubisco capacity, RuBP regeneration capacity and the possibility of CO_2 elevation all influence conversion efficiency.

At the bottom of the canopy, the ability to optimise shade leaf performance is paramount. Leaves that had previously been developed at high light will need to be remodelled, including redistributing protein nitrogen to newly developing sun leaves at the top of the canopy.

Plant leaf architecture is significant for promoting the more equal distribution of light from the canopy top to the bottom. Developmental factors relating to leaf angle and leaf size are important. Therefore, smaller, erect leaves with low SLA and high photosynthetic capacity are best for the top of the canopy; more horizontal, higher SLA leaves may be best at lower levels.

Photosynthetic leaf acclimation in C_3 vs. C_4 plants

In considering maximal energy conversion during crop canopy development, whether the photosynthetic pathway is C_3 or C_4 becomes an interesting issue. C_3 leaves generally show considerable light acclimation potential as they develop leaves with different photosynthetic capacities and leaf structures. However, C_4 leaves appear to show much less flexible

leaf development. A C_4 leaf shows proscribed spacing of mesophyll and bundle sheath cells for the efficient operation of C_4 photosynthesis. It appears that there is a restricted ability to alter this anatomy during leaf development (Sage and McKown 2006; Tazoe et al. 2006). C_4 leaves have characteristics that promote maximum energy efficiencies in light-saturated photosynthesis. However, these characteristics are not optimal in shaded, closed-canopy situations, where leaf-level acclimation is reduced and there are reduced light-limited quantum yields for C_4 photosynthetic CO_2 fixation.

International research in photosynthetic leaf development

The following issues in leaf development are important research questions for maximising light energy conversion in crops.

Understanding the genetic determinants and variability of sun and shade leaf phenotypes is of primary importance. This includes issues relating to chloroplast- and leaf-level acclimation, including cell and leaf morphology.

It is important to understand the determinants of high- and low-SLA leaf development strategies and how these relate to leaves with low and high photosynthetic capacities.

If photosynthetic capacity per unit leaf area is linked with SLA, then the question arises as to what are the effects of breeding for high SLA early in canopy development for fast canopy development. The question of later potential consequences of limiting photosynthetic capacity development in the top layers of the canopy also arises.

There has been considerable research into the light acclimation of plants and the effects of leaf area development on growth rate (Poorter and Evans 1998; Poorter and De Jong 1999; Evans and Poorter 2001; Bailey et al. 2004; Murchie et al. 2005; Terashima et al. 2005, 2006; Walters 2005). Although we know a lot about what happens and what the relationships are, we have very limited understanding of the molecular genetic effects on SLA and the sun and shade leaf phenotype at the leaf or chloroplast levels. The following questions are of primary importance:

- What are the nuclear to chloroplast interaction signals that control the development of the individual chloroplast?
- What determines chloroplast size and number per cell?
- What determines the development of thicker sun-type high photosynthetic capacity leaves, or their thinner shade equivalent?

Studies of chloroplast and leaf development have been slow to yield relevant answers. A large body of research relates to the signalling between chloroplasts and the nucleus, but little of it answers these issues. Rather, current research focuses on chloroplast biogenesis, not fine-tuning for light. However, a foundation for future research is starting to form. Much of the leaf development research has been at an ecophysiological level, yielding valuable information about natural variation in leaf parameters. However, there has been a dearth of molecular genetic research linking leaf development to photosynthetic acclimation and capacity. A current European Union (EU)-funded research project is being undertaken by the Arabidopsis GROwth Network integrating OMICS technologies (AGRON-OMICS) consortium (at <agron-omics.eu/>). The project addresses identifying the molecular determinants of leaf growth and development. Whether any parts of this project emphasise issues of leaf photosynthetic acclimation is unclear, but this type of research shows a promising direction.

References

Bailey S., Horton P. and Walters R.G. 2004. Acclimation of *Arabidopsis thaliana* to the light environment: the relationship between photosynthetic function and chloroplast composition. Planta 218, 793–802.

Evans J.R. and Poorter H. 2001. Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. Plant Cell and Environment 24, 755–767.

Harrison E.P., Olcer H., Lloyd J.C., Long S.P. and Raines C.A. 2001. Small decreases in SBPase cause a linear decline in the apparent RuBP regeneration rate, but do not affect Rubisco carboxylation capacity. Journal of Experimental Botany 52, 1779–1784.

Lefebvre S., Lawson T., Zakhleniuk O.V., Lloyd J.C., Raines C.A. and Fryer M. 2005. Increased sedoheptulose-1,7-bisphosphatase activity in transgenic tobacco plants stimulates photosynthesis and growth from an early stage in development. Plant Physiology 138, 451–460.

Long S.P., Zhu X.G., Naidu S.L. and Ort D.R. 2006. Can improvement in photosynthesis increase crop yields? Plant Cell and Environment 29, 315–330.

Murchie E.H., Hubbart S., Peng S. and Horton P. 2005. Acclimation of photosynthesis to high irradiance in rice: gene expression and interactions with leaf development. Journal of Experimental Botany 56, 449–460.

Murchie E.H., Pinto M. and Horton P. 2009. Agriculture and the new challenges for photosynthesis research. New Phytologist 181, 532–552.

Parry M.A.J., Madgwick P.J., Carvalho J.F.C. and Andralojc P.J. (2007) Prospects for increasing photosynthesis by overcoming the limitations of Rubisco. Journal of Agricultural Science 145, 31–43.

Poorter H., and De Jong R. 1999. A comparison of specific leaf area, chemical composition and leaf construction costs of field plants from 15 habitats differing in productivity. New Phytologist 143, 163–176.

Poorter H. and Evans J.R. 1998. Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. Oecologia 116, 26–37.

Raines C.A. 2003. The Calvin cycle revisited. Photosynthesis Research 75, 1–10.

Rebetzke G.J., Botwright T.L., Moore C.S., Richards R.A. and Condon A.G. 2004. Genotypic variation in specific leaf area for genetic improvement of early vigour in wheat. Field Crops Research 88, 179–189.

Reynolds M., Foulkes M.J., Slafer G.A., Berry P., Parry M.A.J., Snape J.W. and Angus W.J. 2009. Raising yield potential in wheat. Journal of Experimental Botany 60, 1899–1918.

Sage R.F. and McKown A.D. 2006. Is C_4 photosynthesis less phenotypically plastic than C_3 photosynthesis? Journal of Experimental Botany 57, 303–317.

Tazoe Y., Noguchi K. and Terashima I. 2006. Effects of growth light and nitrogen nutrition on the organization of the photosynthetic apparatus in leaves of a C_4 plant, *Amaranthus cruentus*. Plant Cell and Environment 29, 691–700.

Terashima I., Araya T., Miyazawa S., Sone K. and Yano S. 2005. Construction and maintenance of the optimal photosynthetic systems of the leaf, herbaceous plant and tree: an eco-developmental treatise. Annals of Botany 95, 507–519.

Terashima I., Hanba Y.T., Tazoe Y., Vyas P. and Yano S 2006. Irradiance and phenotype: comparative eco-development of sun and shade leaves in relation to photosynthetic CO_2 diffusion. Journal of Experimental Botany 57, 343–354.

Wall G.W. and Kanemasu E.T. 1990. Carbon dioxide exchange rates in wheat canopies. 1. Influence of canopy geometry on trends in leaf-area index, light interception and instantaneous exchange rates. Agricultural and Forest Meteorology 49, 81–102.

Walters R.G. 2005. Towards an understanding of photosynthetic acclimation. Journal of Experimental Botany 56, 435–447.

Zhu X.G., de Sturler E. and Long SP 2007. Optimizing the distribution of resources between enzymes of carbon metabolism can dramatically increase photosynthetic rate: a numerical simulation using an evolutionary algorithm. Plant Physiology 145, 513–526.

Zhu X.G., Long S.P. and Ort D.R. 2008. What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? Current Opinion in Biotechnology 19, 153–159.

Additional references

Ainsworth E.A., Yendrek C.R., Skoneczka J.A. and Long S.P. 2012. Accelerating yield potential in soybean: potential targets for biotechnological improvement. Plant Cell and Environment 35, 38–52.

Lawson T., Kramer D.M. and Raines C.A. 2012. Improving yield by exploiting mechanisms underlying natural variation of photosynthesis. Current Opinion in Biotechnology 23, 215–220.

Long S.P. and Ort D.R. 2010. More than taking the heat: crops and global change. Current Opinion in Plant Biology 13, 241–248.

Reynolds M., Foulkes J., Furbank R., Griffiths S., King J., Murchie E. et al. 2012. Achieving yield gains in wheat. Plant, Cell and Environment 35, 1799–1823.

Zhu X.G., Long S.P. and Ort D.R. 2010. Improving photosynthetic efficiency for greater yield. Annual Review of Plant Biology 61, 235–261.

Improved photosynthetic efficiency is necessary to increase potential crop yield

John R. Evans[1]

Summary

- Yield gains have been achieved primarily from altering harvest index. As this avenue is almost exhausted, to achieve further gains will require increases to biomass production, which requires increased photosynthetic efficiency.
- The most likely candidate for increasing photosynthetic efficiency is to reduce photorespiration.
- Growth under elevated atmospheric CO_2 suppresses photorespiration and demonstrates that crops can yield more.
- Photorespiration is being reduced as atmospheric CO_2 concentrations rise, but it could be achieved by improving the specificity factor of Rubisco, or by concentrating CO_2 around Rubisco.
- Potential yield increases of up to 45% are theoretically possible from improved photosynthetic efficiency in C_3 crops.

Introduction

This paper considers the evidence that the conversion of sunlight to biomass has not been improved by breeding efforts. To date, there have been huge improvements in crop yield, which are continuing. These improvements have been associated with maximising the length of the growing season through earlier germination and delayed canopy senescence; altered canopy architecture, which allows more leaf area per unit ground area; shorter stature, which allows the use of nitrogen fertiliser and increases harvest index (the ratio of yield to above-ground biomass); and weed, pest and disease control. There is no evidence that photosynthetic efficiency has been improved over the centuries of selection for yield. However, as farm yield climbs towards potential yield (that possible under optimal conditions), it is necessary to consider a new approach if further yield improvement is to be sustained.

This paper is concerned with the limits to potential growth and does not consider the many other factors that reduce farm yield below potential yield. Clearly these factors will need to be addressed if improved potential yield is to translate into wide-scale production. Evidence from crops grown under enriched atmospheric CO_2 proves that increased CO_2 assimilation achieved by suppression of photorespiration translates into increased biomass production and yield. Therefore, engineering-improved photosynthesis initially will not be limited by sink capacity. Rates of CO_2 assimilation could be improved by altering the specificity factor of Rubisco and the catalytic rate of Rubisco, conferring advantages at low and high irradiance, respectively.

Light interception

Crop yield is the mass of seed (or tuber) produced per unit land area per cropping cycle. The length of the cropping cycle varies with location and climate.

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Generally one crop is produced per year, but in some tropical regions up to three crops per year have been achieved. The cropping cycle consists of three phases: canopy establishment from germination to canopy closure, full light interception and canopy decline (Reynolds et al. 2000). The interception of sunlight sets the upper limit for biomass production. Increases in light interception have been achieved through earlier sowing and germination, earlier canopy closure and/or delayed leaf senescence. Climate generally limits the extent of the growing season because of low or high temperatures and the timing of rainfall. However, recent gains in light interception have been achieved for maize (Hammer et al. 2009) and further gains may still be possible.

Radiation-use efficiency

Having intercepted the sunlight, the efficiency with which crops convert it to fixed carbon and subsequently biomass determines crop growth rate. Solar conversion efficiency can be analysed upwards from a consideration of the photochemistry and biochemistry, or from field measurements of light interception and destructive harvests. The latter approach led Monteith (1978) to propose the concept of radiation-use efficiency (RUE), the biomass produced per unit of solar radiation intercepted (g/MJ). It is unlikely that radiation interception always limits growth, for example under conditions of water or nutrient stress. However, any stress that restricts leaf area growth will necessarily reduce light interception. Consequently, although light may not be the main limitation, the efficiency of its conversion to biomass still sets an upper bound to production. Measurements have been made for a range of crops in various locations (Kiniry et al. 1989; Sinclair and Muchow 1999; Evans and von Caemmerer 2000; Sheehy et al. 2000, 2007). The technique is time-consuming and difficult (Gower et al. 1989) and sufficient variation exists among measurements to limit quantitative comparisons. However, values obtained are generally greater for C_4 than C_3 crops.

This conclusion is consistent with expectations based on analysing the process of photosynthesis. The rate of CO_2 assimilation by a leaf is curvilinearly related to irradiance. Two parameters that capture the essence of this response are the maximum photon yield at limiting irradiance (C fixed per photosynthetically active photon absorbed) and the photosynthetic capacity at saturating irradiance.

When expressed on an absorbed light basis, photon yield does not vary among diverse unstressed C_3 species (Björkman and Demmig 1987; Evans 1987). However, because of photorespiration, photon yield depends on the concentration of CO_2 and temperature. This is related to the kinetic properties of the specificity factor of Rubisco ($\Gamma*$) (Farquhar et al. 1980) (Figure 1). Carboxylation increases hyperbolically with increase in CO_2 concentration in 21% oxygen. Since $\Gamma*$ increases curvilinearly with temperature, at a given CO_2 concentration carboxylation decreases with increasing temperature. Theoretical curves illustrate the dependence in Figure 1a based on the model of Farquhar et al. (1980). The dependence of a wheat canopy CO_2 assimilation rate on ambient CO_2 concentration can be predicted from $\Gamma*$ under non-saturating irradiance (Figure 1b). For C_4 leaves, additional photons are required for the C_4 cycle. The number depends on the amount of leakage from the CO_2 pump. Variation in photon yield between C_4 types was thought to indicate variation in leakiness (Furbank et al. 1990), but this proposition is still unresolved (von Caemmerer et al. 2007). The CO_2 pump renders photon yield independent of leaf temperature, which results in C_4 photon yields exceeding those of C_3 leaves above about 25°C.

Photosynthetic capacity varies among species and throughout the life of a leaf. It reflects the amount of photosynthetic protein per unit leaf area. The content and activities of the many photosynthetic proteins vary in a coordinated way such that they all increase when a plant is well supplied with nitrogen and decrease together as the leaf ages (Makino et al. 1983). Under saturating light the rate of CO_2 assimilation reflects Rubisco carboxylase activity, the primary CO_2-fixing enzyme that typically accounts for 20–25% of leaf nitrogen in C_3 crop leaves (von Caemmerer and Farquhar 1981; Evans 1986). In a crop canopy, the combination of leaves increases photosynthetic capacity per unit ground area. Consequently, canopy photosynthesis shows less saturation in response to increasing irradiance than does a single leaf (Figure 2). The suppression of photorespiration in C_4 leaves generally results in their photosynthetic capacity exceeding that of C_3 leaves. Consequently C_4 canopy photosynthesis is more linearly related to irradiance than C_3 canopies.

The daily integral of photosynthesis combines periods of low irradiance, which are determined by maximum photon yield, with periods during the middle of the day with high irradiance where the rate

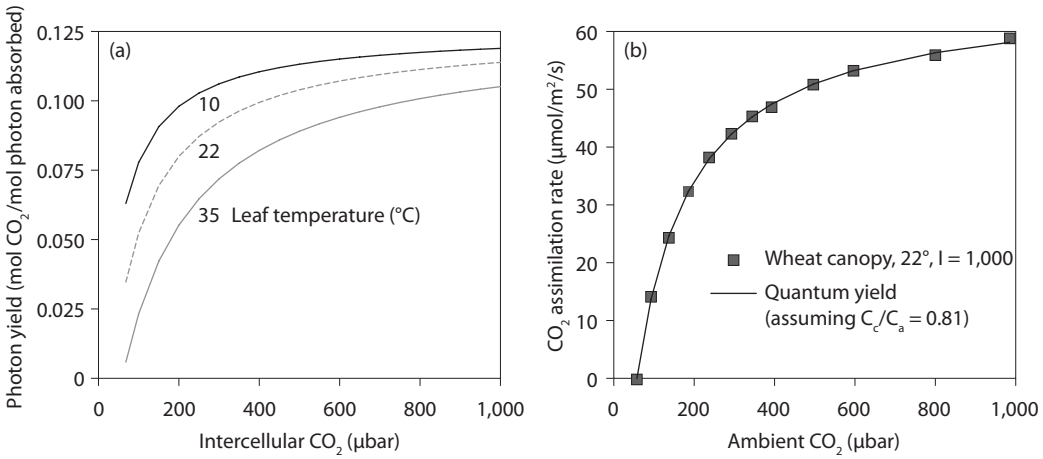

Figure 1. Dependence of photon yield on CO_2 and temperature. (a) Photon yield, ϕ_a, as a function of chloroplast CO_2 partial pressure, C_c, for three leaf temperatures. Curve is the function $\phi = (C_c - \Gamma*)/2((4\ C_c + 8\Gamma*)$ (Farquhar and von Caemmerer 1982) where $\Gamma*$ is the CO_2 compensation point in the absence of day respiration, $\Gamma* = 0.5 \times O\ V_{Omax}\ K_C/(V_{Cmax}\ K_O)$, V_{Cmax} and V_{Omax} are the maximum rates of carboxylation and oxygenation, respectively, K_C and K_O are the Michaelis–Menten constants for CO_2 and O_2, respectively, and O is the oxygen concentration. The temperature dependence of $\Gamma*$ is taken from Brooks and Farquhar (1985). (b) The CO_2 assimilation rate for a wheat canopy with a leaf area index of 6.3 (Evans and Farquhar 1991). The model curve is given by $A = J(C_c - \Gamma*)/(4\ C_c - 8\Gamma*) - R$ where the rate of electron transport, J = 350 µmol $e^-/m^2/s$, $C_c = 0.81 \times C_a$, where C_a is ambient CO_2 partial pressure (pCO_2) around the leaf, $\Gamma*$ = 31.6 µbar and the rate of day respiration, R = 12.1 µmol/m^2/s.

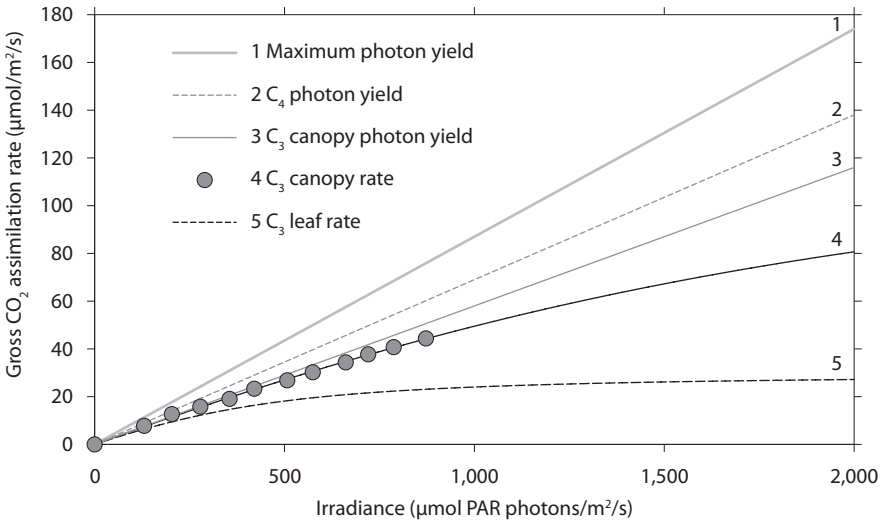

Figure 2. Canopy gross CO_2 assimilation rate as a function of irradiance. Data measured for a wheat crop (Evans and Farquhar 1991) with leaf area index (LAI) of 7.1, leaf temperature 22°C and C_a 340 µbar. Photon yields are maximum 0.088, C_4 0.069 and C_3 0.058. Maximum rate of CO_2 assimilation, A_{max}, for canopy is 135, for leaf 30, empirical curvature factor, θ, 0.7 (typical value). PAR = photosynthetically active radiation.

depends on the photosynthetic capacity of the canopy. A crude comparison between C_3 and C_4 daily canopy photosynthesis has been made in Table 1 (using Figure 2). The impact of improving photon yield or photosynthetic capacity differs depending on the leaf temperature. At 22°C, where C_3 photon yields are closer to those of C_4 leaves, increases to both photon yield and photosynthetic capacity are required for C_3 canopies to achieve daily C_4 photosynthesis. At 30°C, improving photon yield is more important because photorespiratory losses are greater although photosynthetic capacity increases with temperature. If C_3 performance could be improved to that of C_4, the potential for improvement is between 34 and 45%. Ideally, a more comprehensive analysis would use a crop growth model that is driven by diurnal photosynthesis calculations. This is a complex task requiring detailed climate data and assumptions about stomatal behaviour, respiration and carbon allocation.

To convert from daily photosynthesis to biomass, it is necessary to subtract the CO_2 evolved through respiration associated with maintenance and growth (Masle and Farquhar 1988; Poorter et al. 1990). Although this CO_2 can account for 30–50% of daily carbon gained by photosynthesis, efforts to find variation in this efficiency have so far been unsuccessful. The combination of greater maximum photon yield and photosynthetic capacity of C_4 leaves and crop canopies, particularly at higher temperatures, results in greater ratios of daily carbon fixed per light absorbed. It follows that if the conversion efficiency from fixed carbon to biomass is similar for C_3 and C_4 species, then biomass production per unit intercepted solar radiation will be greater in C_4 than C_3 crops. The greater RUE measured in C_4 compared to C_3 crops confirms the expectation based on photosynthetic properties of leaves.

Previous gains in yield potential

Selection for yield has resulted in continuous improvements. Gains have been achieved by altering crop architecture. Reduction in crop height confers resistance to lodging, which enables greater input of nitrogen fertiliser. More erect leaves help light to penetrate into the canopy and allow a higher leaf area index (LAI) (leaf area per unit ground area) to form because lower leaves are kept for longer before senescing. Potential yield, which is the yield under unstressed conditions with high fertility, weed, pest and disease control, has doubled over the last century for wheat (Austin et al. 1989; Fischer and Edmeades 2010) and maize (Russell 1984 cited in Evans 1993; Hammer et al. 2009). For wheat, the gain in yield can be attributed almost entirely to changing the harvest index (Austin et al. 1989; Shearman et al. 2005) (Figure 3a). By contrast, there is no evidence that above-ground biomass has been increased (Figure 3b). For maize, improved yield is associated with increased plant densities and partly to earlier sowing (Fischer and Edmeades 2010) and delayed canopy senescence (Hammer et al. 2009). Both of these effectively increase light interception. Since there must be a limit to how far harvest index (HI) can be increased, and for light interception during the growing season, further yield gains require another approach. There is no evidence that conversion efficiency of intercepted solar radiation into biomass has been altered through breeding for any major crop. Consequently, if yield gains are to be maintained into the future, a new approach of increasing photosynthetic efficiency must be used.

Evidence that increased photosynthesis translates into increased yield

Crop growth rate can be increased in two ways. The first way is to increase the photosynthetic capacity of the crop canopy. This has been achieved by breeding varieties capable of using high fertiliser inputs without lodging. This enables an increased LAI and greater nitrogen contents per unit leaf area,

Table 1. Potential increase to C_3 canopy photosynthesis from achieving a C_4 irradiance response by increasing photon yield, photosynthetic capacity, or both

Leaf temperature (°C)	Photon yield[a] (%)	Photosynthetic capacity (%)	Daily integral (%)
22	19	26	34
30	44	12	45

[a] Increasing photon yield is shown in Figure 2.

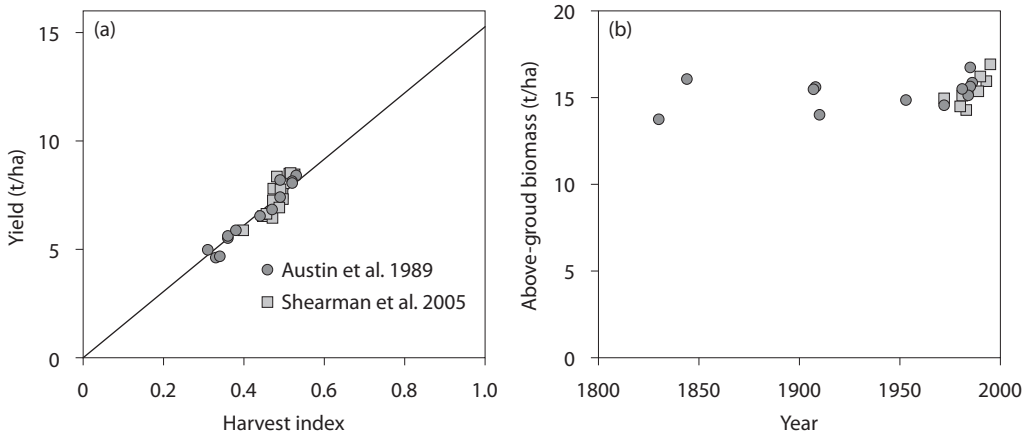

Figure 3. UK wheat potential yield for varieties released between 1830 and 1985. (a) Grain yield (adjusted to 0% moisture content) vs. harvest index. Data from Austin et al. (1989) are the mean of 3 years but data from Shearman et al. (2005) are from each of 2 years, normalised by adjusting biomass using the two common cultivars, Maris Huntsman and Norman. (b) Above-ground biomass vs. year of cultivar release.

which reflect greater content of proteins associated with photosynthesis. Increased chlorophyll content increases leaf absorptance and together with greater LAI it increases light interception. Higher contents of Rubisco and other photosynthetic proteins associated with electron transport, photophosphorylation and carbon metabolism confer greater photosynthetic capacity per unit leaf area and ground area, thereby increasing photosynthetic efficiency at high irradiance. By definition, this option is not available under optimal growing conditions. It partly accounts for the gap between farm yield and potential yield. Since biomass production under optimal conditions has not increased despite a century of intense selection in either wheat or maize, crops face a major constraint set by their metabolism.

Second, crop growth rate can be increased by enriching the atmosphere with CO_2. Extensive research has investigated plant responses to elevated CO_2 in controlled environments and crops grown under free air CO_2 enrichment (FACE) (Figure 4). For C_3 crops, elevated CO_2 increases the rates of CO_2 assimilation by leaves, consistent with the progressive suppression of photorespiration. Rates of CO_2 assimilation under high irradiance increased by about 40% in soybean (Ainsworth et al. 2002) and rice (Ainsworth 2008), or by 25–45% across C_3 plants (Kimball et al. 2002). Fewer measurements exist for canopy photosynthesis, but these confirm that the increases observed at the leaf level also applied at the canopy level.

The effects of CO_2 enrichment lead to increases in above-ground production of 20% in C_3 grasses and 24–37% in legumes, but a decrease in potato (Kimball et al. 2002; Ainsworth and Long 2005). CO_2 enrichment resulted in increases in crop yield of 12–23% for C_3 grasses, 10–23% for rice, 24% for soybean and 28% for potato. The size of biomass increase is consistent with that expected from improved photon yield following canopy closure rather than that associated with faster canopy closure during exponential early growth. The general ability of C_3 crops to use increased CO_2 assimilation and translate this into greater biomass and yield demonstrates that even existing elite cultivars have sufficient sink capacity to exploit an increase in photosynthetic efficiency. Were photosynthetic efficiency increased by more than 20%, then it is likely that additional adjustments would have to be made to maintain high HI and grain protein content.

Candidates for improving photosynthetic efficiency

Potential photosynthetic efficiency could be improved by reducing photorespiration in C_3 leaves and increasing the specific activity of Rubisco.

As mentioned, maximum photon yield for CO_2 assimilation in C_3 leaves depends on CO_2 concentration and leaf temperature. Improving the specificity factor of Rubisco would reduce the energy a crop spends on photorespiration (Figure 5). Achieving the relative photon yield of about 0.78 for C_4 photosynthesis would require a decrease in $\Gamma*$ to 20 (equivalent to CO_2/O_2 specificity factor, $S_{C/O} = 200$). This value is far greater than any value yet reported. The increase in photorespiration as temperature increases could also be reduced if the temperature dependence of the specificity factor could be altered.

Increasing the specific activity of Rubisco (k_{cat}, the catalytic turnover number in units of carboxylations per active site per second) affects photosynthetic efficiency. If k_{cat} could be improved without detriment to the affinity of Rubisco for CO_2, a given rate of CO_2 assimilation could be achieved with less Rubisco protein. Since Rubisco is the most abundant soluble protein in a leaf, the amount of nitrogen needed to form new leaf area would be less, thereby allowing more rapid leaf area development. Alternatively, the rate of CO_2 assimilation would be greater for a given leaf nitrogen content. This increase in k_{cat} could benefit both C_3 and C_4 leaves under high irradiance, although different Rubiscos (i.e. types of Rubisco with different kinetics) would be needed, because the Rubiscos in C_3 and C_4 plants require different affinities for CO_2. However, increasing CO_2 assimilation rate for a given nitrogen content could result in changes to the nitrogen concentration of the biomass, which in turn could affect grain protein content.

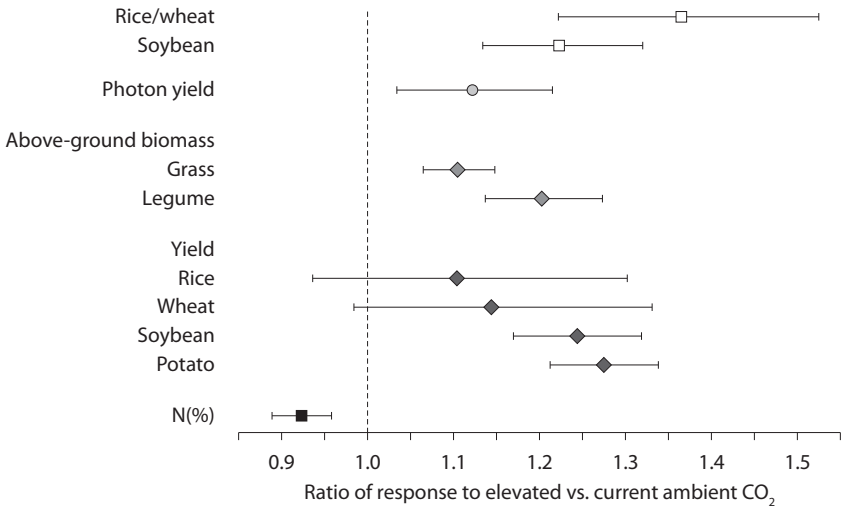

Figure 4. Average ± SE responses to growth under elevated atmospheric CO_2 (550 µL/L) relative to that under current ambient CO_2. Rate of CO_2 assimilation, A, photon yield, above-ground biomass, yield and nitrogen concentration are shown. Data are drawn from reviews (Ainsworth et al. 2002; Kimball et al. 2002; Ainsworth and Long 2005; Ainsworth 2008).

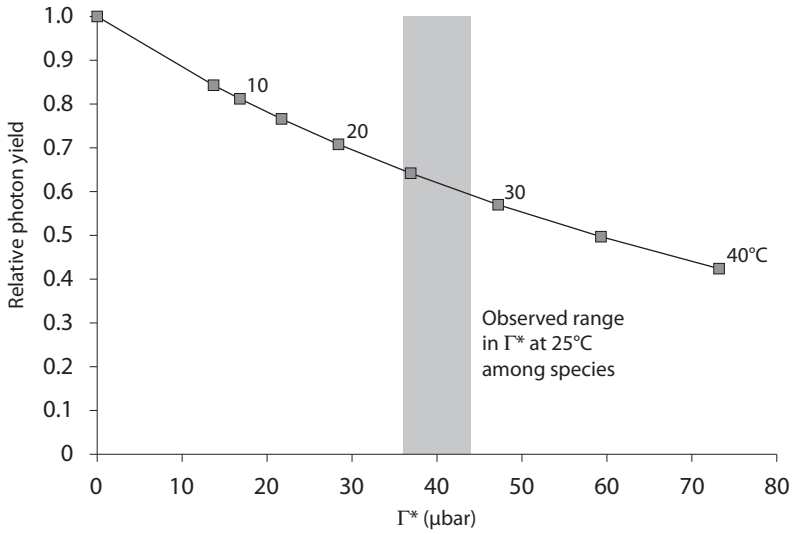

Figure 5. Relative quantum yield for a C_3 leaf as a function of the CO_2 compensation point in the absence of mitochondrial respiration, Γ∗. Chloroplast CO_2 partial pressure is assumed to be 235 μbar. The temperature response function of Γ∗ was measured with *Spinacia oleracea* (Brooks and Farquhar 1985) and the points indicate 5°C increments. The striped bar illustrates the narrow range in Γ∗ that has been found for diverse terrestrial plants, including C_3 and C_4 species (Kent and Tomany 1995; Evans and Loreto 2000; Galmes et al. 2005). To convert between Γ∗ and specificity factor, $S_{C/O}$, divide 3,961 by Γ∗ or $S_{C/O}$ (valid for 25°C, see von Caemmerer et al. 1994); for example, Γ∗ 40 μbar is equivalent to $S_{C/O}$ of 99.

References

Ainsworth E.A. 2008. Rice production in a changing climate: a meta-analysis of responses to elevated carbon dioxide and elevated ozone concentration. Global Change Biology 14, 1642–1650.

Ainsworth E.A., Davey P.A., Bernacchi C.J., Dermody O.C., Heaton E.A., Moore D.J. et al. 2002. A meta-analysis of elevated [CO_2] effects on soybean (*Glycine max*) physiology, growth and yield. Global Change Biology 8, 695–709.

Ainsworth E.A. and Long S.P. 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy. New Phytologist 165, 351–371.

Austin R.B., Ford M.A. and Morgan C.L. 1989. Genetic improvement in the yield of winter wheat: a further evaluation. Journal of Agricultural Science 112, 295–301.

Björkman O. and Demmig B. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. Planta 170, 489–504.

Brooks A. and Farquhar G.D. 1985. Effect of temperature on the CO_2/O_2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. Planta 165, 397–406.

Evans J.R. 1986. The relationship between carbon dioxide-limited photosynthetic rate and ribulose-1,5-bisphosphate-carboxylase content in two nuclear-cytoplasm substitution lines of wheat, and the coordination of ribulose-bisphosphate-carboxylation and electron-transport capacities. Planta 167, 351–358.

Evans J.R. 1987. The dependence of quantum yield on wavelength and growth irradiance. Australian Journal of Plant Physiology 14, 69–79.

Evans J.R. and Farquhar G.D. 1991. Modelling canopy photosynthesis from the biochemistry of the C_3 chloroplast. Pp. 1–15 in 'Modelling crop photosynthesis—from biochemistry to canopy', Ed. by K.J. Boote and R.S.

Loomis. Crop Science Society of America: Madison, USA.

Evans J.R. and Loreto F. 2000. Acquisition and diffusion of CO_2 in higher plant leaves. Pp. 321–351 in 'Photosynthesis: physiology and metabolism', ed. by R.C. Leegood, T.D. Sharkey and S. von Caemmerer. Kluwer: Dordrecht.

Evans J.R. and von Caemmerer S. 2000. Would C_4 rice produce more biomass than C_3 rice? Pp. 53–71 in 'Redesigning rice photosynthesis to increase yield', ed. by J.E. Sheehy, P.L. Mitchell and B. Hardy. Elsevier Science: Amsterdam.

Evans L.T. 1993. Crop evolution, adaptation and yield. Cambridge University Press: Cambridge.

Farquhar G.D. and von Caemmerer S. 1982. Modelling of photosynthetic responses to environmental conditions. Pp. 549–587 in 'Physiological plant ecology II. Water relations and carbon assimilation plant physiology', ed. by O.L. Lange, P.S. Nobel, C.B. Osmond and H. Ziegler. Springer Verlag: Berlin.

Farquhar G.D., von Caemmerer S. and Berry J.A. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149, 78–90.

Fischer R. and Edmeades G.O. 2010. Breeding and cereal yield progress. Crop Science, 50, S85–S98.

Furbank R.T., Jenkins C.L.D. and Hatch M.D. 1990. C_4 photosynthesis—quantum requirement, C_4 acid overcycling and Q-cycle involvement. Australian Journal of Plant Physiology 17, 1–7.

Galmes J., Flexas J., Keys A.J., Cifre J., Mitchell R.A.C., Madgwick P.J. et al. 2005. Rubisco specificity factor tends to be larger in plant species from drier habitats and in species with persistent leaves. Plant Cell and Environment 28, 571–579.

Gower S.T., Grier C.C. and Vogt K.A. 1989. Aboveground production and N and P use by *Larix occidentalis* and *Pinus contorta* in the Washington Cascades, USA. Tree Physiology 5, 1–11.

Hammer G.L., Dong Z.S., McLean G., Doherty A., Messina C., Schusler J. et al. 2009. Can changes in canopy and/or root system architecture explain historical maize yield trends in the US corn belt? Crop Science 49, 299–312.

Kent S.S. and Tomany M.J. 1995. The differential of the ribulose 1,5-bisphosphate carboxylase oxygenase specificity factor among higher-plants and the potential for biomass enhancement. Plant Physiology and Biochemistry 33, 71–80.

Kimball B.A., Kobayashi K. and Bindi M. 2002. Responses of agricultural crops to free-air CO_2 enrichment. Advances in Agronomy 77, 293–368.

Kiniry J.R., Jones C.A., O'Toole J.C., Blanchet R., Cabelguenne M. and Spanel D.A. 1989. Radiation-use efficiency in biomass accumulation prior to grain-filling for five grain-crop species. Field Crops Research 20, 51–64.

Makino A., Mae T. and Ohira K. 1983. Photosynthesis and ribulose 1,5-bisphosphate carboxylase in rice leaves—changes in photosynthesis and enzymes involved in carbon assimilation from leaf development through senescence. Plant Physiology 73, 1002–1007.

Masle J. and Farquhar G.D. 1988. Effects of soil strength on the relation of water-use efficiency and growth to carbon isotope discrimination in wheat seedlings. Plant Physiology 86, 32–38.

Monteith J.L. 1978. Reassessment of maximum growth rates for C_3 and C_4 crops. Experimental Agriculture 14, 1–5.

Poorter H., Remkes C. and Lambers H. 1990. Carbon and nitrogen economy of 24 wild species differing in relative growth rate. Plant Physiology 94, 621–627.

Reynolds M.P., van Ginkel M. and Ribaut J.M. 2000. Avenues for genetic modification of radiation use efficiency in wheat. Journal of Experimental Botany 51, 459–473.

Russell W. 1984. Agronomic performance of maize cultivars representing different eras of breeding. Maydica 29, 375–390.

Shearman V.J., Sylvester-Bradley R., Scott R.K. and Foulkes M.J. 2005. Physiological processes associated with wheat yield progress in the UK. Crop Science 45, 175–185.

Sheehy J.E., Ferrer A.B., Mitchell P.L., Elmido-Mabilangan A., Pablico P. and Dionora M.J.A. 2007. How the rice crop works and why it needs a new engine. Pp. 3–26 in 'Charting new pathways to C_4 rice', Ed. by J.E. Sheehy, P.L. Mitchell and B. Hardy. International Rice Research Institute: Los Baños, Philippines.

Sheehy J.E., Mitchell P.L. and Hardy B. 2000. Redesigning rice photosynthesis to increase yield. Elsevier Science B.V.: Amsterdam.

Sinclair T.R. and Muchow R.C. 1999. Radiation use efficiency. Advances in Agronomy 65, 215–265.

von Caemmerer S., Evans J., Cousins A., Badger M. and Furbank R. 2007. C_4 photosynthesis and CO_2 diffusion. Pp. 95–115 in 'Charting new pathways to C_4 rice', ed. by J.E. Sheehy, P.L. Mitchell and B. Hardy. International Rice Research Institute: Los Baños, Philippines.

von Caemmerer S., Evans J.R., Hudson G.S. and Andrews T.J. 1994. The kinetics of ribulose-1,5-bisphosphate carboxylase/oxygenase in vivo inferred from measurements of photosynthesis in leaves of transgenic tobacco. Planta 195, 88–97.

von Caemmerer S. and Farquhar G.D. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153, 376–387.

Additional references

Evans J.R. and von Caemmerer S. 2011. Enhancing photosynthesis. Plant Physiology 155, 19.

von Caemmerer S. and Evans J.R. 2010. Enhancing C_3 photosynthesis. Plant Physiology 154, 589–592.

Potential for yield improvement in some major crop groups

Potential for enhanced photosynthetic efficiency to increase yield potential in C_3 crops by the addition of C_4 photosynthetic pathways

Paul Quick, John Sheehy and Anaida Ferrer[1]

Summary

- Predicted increases in food demands, competition for water and increasing use of agricultural products for biofuels require a major increase in the yield potential of crops.
- Increased photosynthetic efficiency through the introduction of the C_4 photosynthetic pathway is one mechanism by which this can be achieved. This paper outlines the potential for increased photosynthesis to increase yield potential, then describes a recently funded project to introduce C_4 photosynthesis into rice.

Current and emerging challenges

In a symposium like this one, it is important to show that an investment in improving canopy photosynthesis in agricultural crops would significantly improve yields rather than discover that other factors limit productivity. Furthermore, it is useful to demonstrate that such improvements would not only help solve current humanitarian problems but also prevent future ones emerging. Both of these issues are addressed in this paper together with a brief description of the C_4 Rice Project.

Agriculture is the indispensable base of human society. Solar radiation, water, temperature, crop management and agricultural research determine the nature and productivity of agriculture. Rice, wheat, maize, millet and sorghum provide 70% of the calories and up to 90% of all protein consumed by the world's population. About half the world's population has rice as the staple cereal and almost all of the 600 million tonnes of rice produced each year is consumed directly by humans.

The surface of the Earth is 71% water and 29% land. A little over one-third of the land is suitable for agriculture; the rest is ice, desert, forest or mountains unsuitable for farming. Currently only 10% of the surface of the Earth has topographical and climatic conditions suitable for producing food. In 1843, when the first agricultural research station was founded at Rothamsted in the UK, there was 4.0 ha of farmland available per person for food production. By 2050 there will be 0.6 ha available. Today, 75% of the world's 6.6 billion people live in the developing world where most of the world's existing poverty is concentrated. Currently, one billion people live on less than a dollar a day and spend half their income on food; 854 million people are hungry and each day about 25,000 people die from hunger-related causes. Over the next half-century, the predicted 3 billion (50%) increase in the number of humans on the planet threatens the ability of agricultural technologies to produce sufficient food to meet the demand.

Over the past half-century, there has been a linear relationship between the population in Asia and rice production. The population in Asia, where 60% of the

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world's population lives, will increase by 1.4 billion over the next half century; hence, rice production must increase to keep pace (Figure 1). Each hectare of land used for rice production in Asia currently provides food for 27 people, but by 2050 that land will have to support at least 43 people if we are to avoid loss of forests and wetlands.

The green revolution more than doubled food supply in Asia in 25 years, with an increase of only 4% in net cropped area (Rosegrant and Hazell 2000; Lipton 2007). Since the early 1980s the increase in productivity of green revolution rice has been slowing (Figure 2). The elite rice cultivars, which dominate the food supplies of millions of people in Asia on lower incomes, have approached a yield barrier (Kropff et al. 1994; Sheehy 2001; Sheehy et al. 2007b). The gains made from the green revolution technologies (focused on canopy architecture and crop nutrition) have been fully exploited (Dawe 2007).

Ninety-seven per cent of the water on Earth is sea water. Of the 3% of fresh water, 2% is locked up in ice at the poles. There is rising competition for the remaining 1%, not only from agriculture and human consumption, but also for industry. There is a biophysical relationship between the biomass of crops and their water consumption. Given the food demands of the current 6 billion people on the planet, it is not surprising that agriculture accounts for about 70% of all fresh water used. Increased competition for water will threaten the productivity of intensive agriculture. Furthermore, the increasing demand for biofuels will result in competition between grain for fuel and grain for food, resulting in price increases (Cassman and Liska 2007).

Research institutes like the International Rice Research Institute (IRRI) operate on a strategic timescale of about 50 years. It is not easy to predict the effects of climate change on rice production over the next 50 years. Calculations made by Sheehy et al. (2007a) suggest that the beneficial effects of increasing CO_2 on rice yields would likely be offset by the negative effects of increasing temperatures. However, an increase in the frequency of weather-related disasters, driven by climate change, could seriously damage future rice production.

Not surprisingly, scientists have taken an interest in improving the efficiency and productivity of agricultural systems, but conventional approaches are likely exhausted.

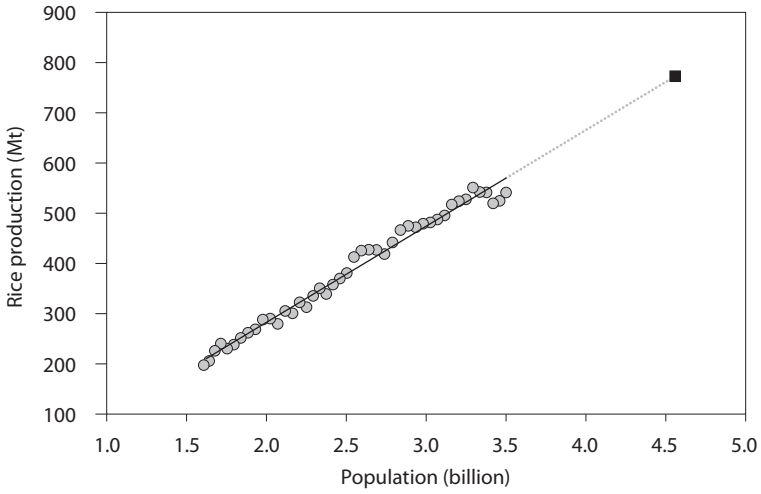

Figure 1. Rice production and the population for Asia for 1961–2004. To indicate the trend, the solid line is the regression of production on population ($y = 191.1x - 98.3$; $P < 0.001$; $r^2 = 0.98$) and the dotted line is an extrapolation to the Asian population predicted for 2050 (square).

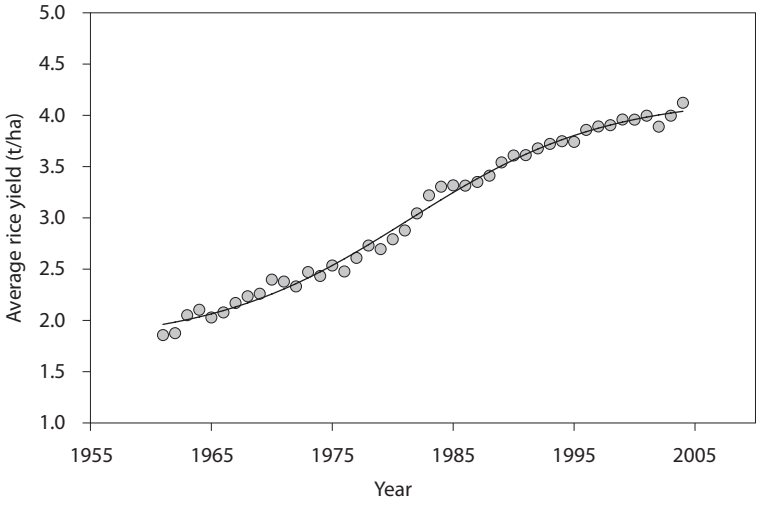

Figure 2. Average yield of paddy rice for Asia between 1961 and 2004. The curve is $y = 1.78 + 2.40/(1 + e^{(-(x - 1.981)/8.12)})$ with $r^2 = 0.99$ (data from D. Dawe, pers. comm.).

Yield, photosynthesis and radiation-use efficiency

For scientists, rice production has to be about converting the maximum fraction of solar energy into the maximum amount of chemical energy in grain in the shortest possible time; that conversion should be achieved using the smallest amount of land, water and fertiliser. However, crop plants are complex organisms that integrate processes from the molecular to the community level in continuously varying weather. Both roots and shoots exchange matter and energy with their environments and are responsible for the net capture of resources. In grain crops, the outcome is a harvestable yield.

The phenotype of a given genotype can vary markedly according to its interaction with the environment (Miflin 2000). Genetic complexity underlies that plasticity. In addition, the 'same' crop is grown in geographically different regions with different climates and weather conditions, and on different soil types with different histories of management. Given these factors, precise repeatability, in the usual scientific sense, is the exception rather than the rule for field experiments. As a result of this imprecision and the absence of universally acceptable theoretical models of crop growth, disagreements about what specifically determine biomass and grain yield are commonplace.

Whenever the issue of yield increases is discussed, at some point the relative importance of source strength vs. sink capacity arises. Sheehy et al. (2001) showed that the sink capacity in rice was much higher than that actually used, even at high yield, suggesting that the yield barrier was the consequence of source limitations. Experiments in which increased concentrations of CO_2 were made available to rice resulted in increased yields (Yoshida 1973; Ziska et al. 1997), suggesting that improvements in photosynthesis might have a role to play in increasing yield. What is the link between photosynthesis and yield and how can it be quantified?

The law of mass conservation can be used to link growth rate, crop photosynthesis, respiration and the loss of biomass by detachment. The product of the integral of crop growth rate and harvest index gives grain yield; note that grain yields for rice are quoted at 14% moisture content. To make progress and derive a simple equation linking yield and canopy gross photosynthesis, Sheehy (2000) assumed a

constant root weight ratio and units of CH_2O, so that the grain yield could be described by equation (1):

$$Y = HI\left\{(1-\beta)\int_{t_i}^{t_f}\left[P_g(t) - R(t) - D(t)\right]dt\right\} \quad (1)$$

where Y is grain yield, HI is harvest index (unless otherwise stated, calculated as the fraction of above-ground dry weight that is grain weight), β is the root weight ratio, P_g is the rate of canopy gross photosynthesis, R is the rate of shoot and root respiration and D is the loss of dry matter through detachment. A time step of a day is usually used for time, t, so that t_i could be the day of germination or some other suitable starting time and t_f, the day of harvest.

Equation (1) shows the link between photosynthesis and yield is complex and mediated by several factors. The most obvious are differences in crop duration ($t_f - t_i$) and a difference in the partitioning of assimilates to roots (β). HI can be affected by many factors including differences in susceptibility to thermally induced sterility (Satake and Yoshida 1978; Horie 1993) or differences in the ability to partition nitrogen to the grain (Sinclair 1998; Sinclair and Sheehy 1999). Nevertheless, it would be expected that yield improvements in modern cultivars were accompanied by improvements in canopy photosynthesis (Robson 1982; Long 1999a,b).

It is convenient to define radiation-use efficiency (RUE) (Monteith 1977). In this paper, RUE is the slope of the linear relationship between shoot dry weight and accumulated intercepted photosynthetically active radiation. Consequently, for the same growing season, an increase in the yield of biomass must be accompanied by a proportionate increase in the value of the RUE. The value for any day (Sheehy 2000) can be calculated by equation (2):

$$RUE = \frac{dWs/dt}{I_{int}} \quad (2)$$

where dWs/dt is the daily growth rate of the shoots, I_{int} is the total amount of photosynthetically active radiation (PAR) intercepted by the crop for the same day. By substituting for crop growth rate in equation (2), RUE can be written as equation (3):

$$RUE = \frac{(1-\beta)\left(P_g(t) - R(t) - D(t)\right)}{I_{int}} \quad (3)$$

Equation (3) shows that RUE is strongly influenced by canopy photosynthesis.

Radiation-use efficiency and yield

The maximum yields and RUEs of rice and maize growing unrestricted by water and nutrients in the dry season in the tropics were measured concurrently (Sheehy et al. 2007a). The RUEs of maize and rice, measured in units of grams of dry weight per megajoule of intercepted PAR (g/MJ DW), were 4.4 g/MJ DW and 2.9 g/MJ DW, respectively; the ratio of the values was 1.52. At 14% moisture content, the grain yield for maize was 13.9 t/ha and for rice, 8.3 t/ha. Maize, the C_4 crop, out-yielded the C_3 crop by about 67%. This result is consistent with the results published by Monteith (1978) for a comparison of the yields of a number of C_3 and C_4 crops growing over a range of crop durations (Figure 3). Monteith's (1978) results suggest that C_4 crops could produce about 66% more biomass than C_3 crops in the hot developing countries of the world. If we convert the photosynthetic system of rice from the C_3 to the C_4 form, maximum yields should increase by about 5 t/ha. A huge added benefit of the C_4 system in rice would be the doubled water-use efficiency that accompanies the trait (Loomis and Connor 1992). The benefits for economically disadvantaged people of such an improvement in the face of increasing world populations and decreasing natural resources would be immense.

Conclusions

In well-managed crops, in which the fraction of grain per unit of biomass has been maximised, future yield improvements must be accompanied by increases in RUE. Mitchell et al.'s (1998) data suggest that RUEs for C_4 crops were 50% greater than for C_3 crops. The simple model in that review led to the suggestion that rice photosynthesis would have to be converted from the C_3 to the C_4 photosynthetic pathway to achieve yield increases of 50%. Sheehy et al. (2007a) went some way to confirming this conclusion when they reported that the difference in the yields of rice and maize crops grown without limitations of water or nutrients at IRRI was 5.6 t/ha. Furthermore, although C_4 plants display plasticity (Sage and McKown 2006), their C_4 nature is not lost during plastic responses to the environment. The attraction of the full C_4 system is not only high productivity and yield, but also better use of water and nitrogen (Loomis and Connor 1992). No known non-C_4 solution offers this complete package of benefits.

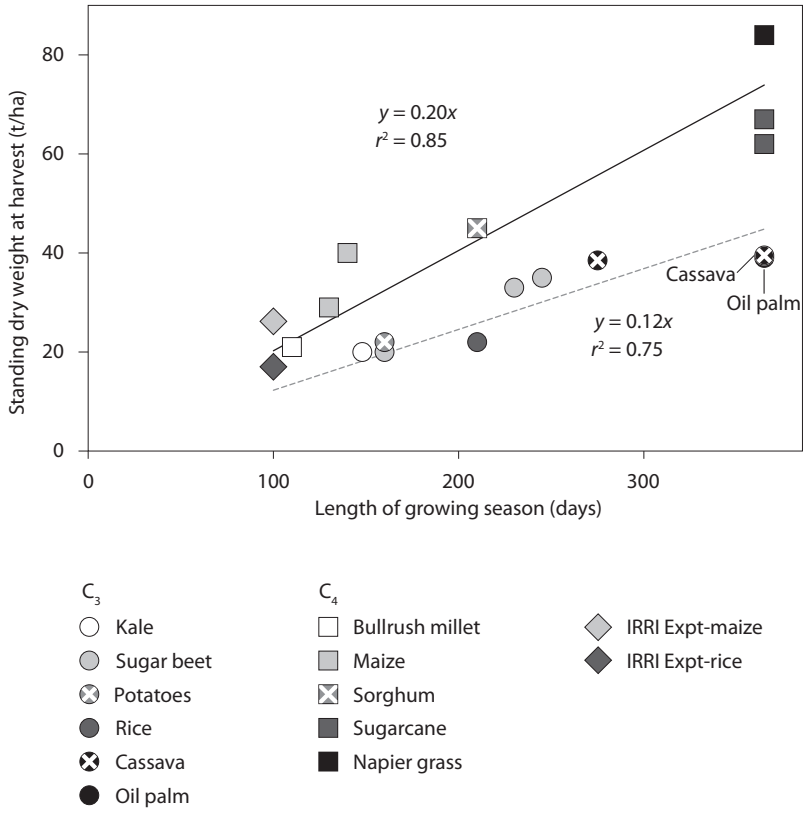

Figure 3. Final dry weights of crops in C_3 (dashed line) and C_4 (solid line) groups correlated with the length of growing season (Monteith 1978)

Bill and Melinda Gates Foundation C_4 Rice Project

In October 2008, IRRI was delighted to learn that the Bill and Melinda Gates Foundation would support the C_4 Rice Project. The project is a large collaborative program involving twelve organisations worldwide. The members of the C_4 consortium were confident that we had the scientific ability to deliver our objectives. However, we needed to invest time in learning how to manage the project and our interactions so as to produce the most synergy.

The project can be divided into three phases over an estimated 15 years of coordinated research carried out at IRRI and the laboratories of the C_4 Rice Consortium (Table 1).

Phase I is largely about proving the concept and assembling the components required to construct C_4 rice. The major themes are: (1) discovering the genes responsible for kranz anatomy; (2) assembling the molecular tools that will enable cell specific expression of transgenes; and (3) conducting research to ensure there is sufficient understanding of the factors required to maximise the efficiency of C_4 rice.

Phase II is about constructing prototype C_4 rice types using the genes, tools and understanding generated in phase I.

Phase III is about optimising C_4 rice in locally adapted cultivars and delivering its benefits to consumers.

Table 1. The C_4 consortium comprises five research sub-groups (SG 1–5), each responsible for a task, and coordinated by a team leader (indicated in italics).

SG 1	SG 2	SG 3	SG 4	SG 5
Wild relatives of rice, 'revertants' in maize and sorghum	Molecular phenotyping	Molecular engineering	IR64 mutants and activation-tagged lines	Informatics and systems biology
P. Quick J. Sheehy J. Dionora R. Sage	*R. Furbank* S. von Caemmerer G. Edwards R. Leegood J. Burnell	*J. Hibberd* J. Langdale P. Westhoff I. Slamet-Loedin A. Kohli	*H. Leung* E. Murchie G. An S-M. Yu C. Hsing	*R. Bruskiewich* T. Brutnell C. Myers X. Zhu T. Nelson

Mission

The C_4 Rice Project is a consortium of scientists aiming to discover the cassette of plant genes responsible for the greatest known efficiency of solar energy conversion in plant photosynthesis. The cassette not only increases yields, but also enables crops to improve their efficiency of nitrogen fertiliser use and double their water-use efficiency. We wish to install the cassette and functionalise it in prototypes of crops grown for food in the developing world to help eliminate hunger and poverty.

Goals

The goals of the C_4 Rice Project are to:

- use discoveries to create prototypes of crop plants with improved photosynthesis to improve yield and resource-use efficiency in a sustainable manner by plant breeders in the developing world
- create synergies between leading research teams worldwide who are involved in photosynthesis research so as to accelerate the discovery of genes that can alleviate hunger in the developing world.

Objectives

The objectives of the C_4 Rice Project are to:

- discover the genes (known as C_4 genes) responsible for high solar energy conversion in leaf photosynthesis
- generate a model rice plant with increased photosynthetic efficiency by installing the cassette of genes responsible for expressing the C_4 pathway of photosynthesis
- introduce C_4 photosynthesis into widely used rice cultivars and to test for yield, water use and nitrogen fertiliser requirements under a range of agronomic conditions
- produce a toolkit for introducing the cassette of genes responsible for expressing C_4 photosynthesis into other important crop species growing in the tropics.

Strategies

The strategies of the C_4 Rice Project are to:

- engage dynamic, multidisciplinary teams that contain molecular biologists, geneticists, physiologists, biochemists and mathematicians; these teams must be continually renewed and strengthened so as to remain at the cutting edge of science
- develop project management architecture and interfaces among the various teams that maximise the probability of success; a small team will be responsible for managing the project in a cost-effective manner to meet evolving needs, and for disseminating information about budgets, time lines and reporting procedures
- develop a global, dynamic and integrated research agenda for installing a maize-like photosynthesis engine (C_4) in rice, wheat and legumes.

Work in progress

The Bill and Melinda Gates Foundation C_4 Rice Project commenced in April 2009. Since work is ongoing and will be published in the scientific literature, we will not present it in this paper.

References

Cassman K.G. and Liska A.J. 2007. Food and fuel for all: realistic or foolish? Biofuels, Bioproducts and Biorefining 1, 18–23.

Dawe, D. 2007. Agricultural research, poverty alleviation, and key trends in Asia's rice economy. Pp. 37–53 in 'Charting new pathways to C_4 rice', ed. by J.E. Sheehy,

P.L. Mitchell and B. Hardy. International Rice Research Institute: Los Baños, Philippines.

Horie T. 1993. Predicting the effects of climatic variation and elevated CO_2 on rice yield in Japan. Journal of Agricultural Meteorology 48, 567–574.

Kropff M.J., Cassman K.G., Peng S., Matthews R.B. and Setter T.L. 1994. Quantitative understanding of yield potential. Pp. 21–38 in 'Breaking the yield barrier. Proceedings of a workshop on rice yield potential in favorable environments', ed. by K.G. Cassman. International Rice Research Institute: Los Baños, Philippines.

Lipton M. 2007. Plant breeding and poverty: can transgenic seeds replicate the 'green revolution' as a source of gains for the poor? Journal of Development Studies 43, 31–62.

Loomis R.S. and Connor D.J. 1992. Crop ecology: Productivity and management in agricultural systems. Cambridge University Press: Cambridge.

Long S.P. 1999a. Understanding the impacts of rising CO_2: the contribution of environmental physiology. Pp. 263–282 in 'Physiological plant ecology', ed. by M.C. Press, J.D. Scholes and M.G. Barker. Blackwell Science Ltd: Oxford.

Long S.P. 1999b. Environmental responses. Pp. 215–249 in 'C_4 plant biology', ed. by R.F. Sage and R.K. Monson. Academic Press: San Diego.

Miflin, B. J. 2000. Crop improvement in the 21st century. Journal of Experimental Botany 51, 1–8.

Mitchell P.L., Sheehy J.E. and Woodward F.I. 1998. Potential yields and the efficiency of radiation-use in rice. Discussion Paper No. 32. International Rice Research Institute: Manila, Philippines.

Monteith J.L. 1977. Climate and the efficiency of crop production in Britain. Philosophical Transactions of the Royal Society B: Biological Sciences 281, 277–294.

Monteith J.L. 1978. Reassessment of maximum growth rates for C_3 and C_4 crops. Experimental Agriculture 14, 1–5.

Robson M.J. 1982. The growth and carbon economy of selection lines of *Lomium perenne* cv. s23 with differing rates of dark respiration. I. Grown as simulated swards during a regrowth period. Annals of Botany 49, 321–329.

Rosegrant M. and Hazell P. 2000. Transforming the rural Asian economy: the unfinished revolution. Oxford University Press: Hong Kong.

Sage R.F. and McKown A.D. 2006. Is C_4 photosynthesis less phenotypically plastic than C_3 photosynthesis? Journal of Experimental Botany 57, 303–317.

Satake T. and Yoshida S. 1978. High temperature-induced sterility in *Indica* rices at flowering. Japanese Journal of Crop Science 47, 6–17.

Sheehy J.E. 2000. Limits to yield for C_3 and C_4 rice: an agronomist view. Pp. 39–52 in 'Redesigning rice photosynthesis to increase yield, Studies in Plant Science 7', ed. by J.E. Sheehy, P.L. Mitchell and B. Hardy. Elsevier Science BV: Amsterdam.

Sheehy J.E. 2001. Will yield barriers limit future rice production? Pp. 416 in 'Crop science: progress and prospects. Proceedings of the 3rd International Crop Science Congress, August 2000', ed. by J. Nosberger, H.H. Geiger and P.C. Struik. CABI: Hamburg.

Sheehy J.E., Dionora M.J.A. and Mitchell P.L. 2001. Spikelet numbers, sink size and potential yield in rice. Field Crops Research 71, 77–85.

Sheehy J.E., Ferrer A.B., Mitchell P.L., Elmido-Mabilangan A., Pablico P. and Dionora M.J.A. 2007a. How the rice crop works and why it needs a new engine. Pp. 3–26 in 'Charting new pathways to C_4 rice', ed. by J.E. Sheehy, P.L. Mitchell and B. Hardy. International Rice Research Institute: Los Baños, Philippines.

Sheehy J.E., Mitchell P.L., Hardy B. (eds) 2007b. Charting new pathways to C_4 rice. International Rice Research Institute: Los Baños, Philippines.

Sinclair T.R. 1998. Historical changes in harvest index and crop nitrogen accumulation. Crop Science 38, 638–643.

Sinclair T.R. and Sheehy J.E. 1999. Erect leaves and photosynthesis in rice. Science 283, 145.5

Yoshida S. 1973. Effects of temperature on growth of the rice plant (*Oryza sativa* L.) in a controlled environment. Soil Science & Plant Nutrition 19, 299–310.

Ziska L.H., Namuco O., Moya T. and Quilang J. 1997. Growth and yield response of field-grown tropical rice to increasing carbon dioxide and air temperature. Agronomy Journal 89, 45–53.

Additional references

Kajala K., Covshoff S., Karki S., Woodfield H., Tolley B.J., Dionora M.J.A. et al. 2011. Strategies for engineering a two-celled C_4 photosynthetic pathway into rice. Journal of Experimental Botany 62, 3001–3010.

Langdale J.A. 2011. C_4 cycles: past, present, and future research on C_4 photosynthesis. The Plant Cell 23, 3879–3892.

Rizal G., Karki S., Thakur V., Chatterjee J., Coe R.A., Wanchana S. et al. 2012. Towards a C_4 rice. Asian Journal of Cell Biology 7, 13–31.

Sheehy J.E. and Mitchell P.L. 2011. Developments in rice research: visions and pragmatism. World Agriculture 2, 7–19.

von Caemmerer S., Quick W.P. and Furbank R.T. 2012. The development of C_4 rice: current progress and future challenges. Science 336, 1671–1672.

Status of photosynthetic and associated research in wheat and prospects for increasing photosynthetic efficiency and yield potential

Matthew P. Reynolds[1], Anthony G. Condon[2], Martin A.J. Parry[3] and Robert T. Furbank[2]

Summary

- This paper reviews recent research on the relationship between photosynthesis and yield of wheat. It evaluates prospects for substantial increases in yield potential of wheat through increases in leaf-level photosynthetic efficiency.
- There is substantial variation in rate of leaf photosynthesis among modern, high-yielding wheat varieties grown in favourable environments. This variation results from two factors: genotypic variation in leaf demand for CO_2 (i.e. photosynthetic capacity, which is the amount and activity of photosynthetic machinery) and variation in the supply of CO_2 to the leaf interior (largely determined by stomatal conductance).
- Several recent experiments have shown that it is already possible to select for high yield potential within wheat breeding populations by using measurements of leaf photosynthesis, stomatal conductance or related traits.
- It may be possible to raise the baseline for wheat yield potential higher, perhaps by 50% or more, by further improvements to photosynthesis, such as through exploiting natural variation in Rubisco's catalytic rate or, at the other extreme, engineering C_4 metabolism into wheat.
- If gains in photosynthetic efficiency are to be realised as substantial gains in yield potential in the field, there must be other, complementary changes to the wheat plant, just as the original green revolution relied on fundamental changes in plant architecture to complement pivotal changes in agronomy at that time.
- Spike fertility must be improved to allow full use of photosynthetic capacity throughout the crop life cycle.
- Greater radiation-use efficiency will increase total assimilates available for spike growth, thereby increasing the potential for grain number.
- Phenological patterns and stem growth need to be optimised to permit maximum partitioning of available assimilates to spikes.
- There is evidence for underused photosynthetic capacity during grain-filling in elite material, suggesting unnecessary floret abortion. A better understanding of the physiological and genetic bases for floret abortion may minimise floret abortion to achieve a better source–sink balance.
- Further trade-offs in terms of partitioning of assimilates to competing sinks during spike growth, to improve root anchorage and stem strength, may be necessary to minimise yield losses as a result of lodging.
- Breeding technologies that can be used to complement conventional approaches include wide crossing with members of the Triticae tribe to broaden the wheat gene pool and physiological and molecular breeding to strategically combine complementary traits and identify elite progeny more efficiently.

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Introduction

Since demand for wheat globally is predicted to increase at a faster rate (Rosegrant and Cline 2003) than the present annual genetic gains (Fischer 2007), it is clear that improvement in genetic yield potential (*YP*) will need to be accelerated. In addition, while climate change will lead to less predictability in terms of agricultural productivity, serious concerns about the environment highlight the need to develop cropping systems that use inputs more efficiently. Increasing the genetic *YP* of wheat cultivars is likely to have an impact in satisfying demand for wheat while mitigating environmental issues. It has been proven empirically that wheat yield potential is expressed across a wide range of environments without additional inputs of water or nitrogen (Calderini and Slafer 1998).

Yield potential can be expressed in its simplest form as a function of the light intercepted by the crop canopy (*LI*) and radiation-use efficiency (*RUE*), whose product is biomass, and the partitioning of biomass to yield, that is, harvest index (HI), as shown in equation (1):

$$YP = LI \times RUE \times HI \quad (1)$$

In the past 20 years or so, progress in a number of research areas has the potential to boost wheat *YP* through interventions that affect *LI*, *RUE* and *HI*. Some of these research areas are shown in Table 1. They include: (1) using physiological selection tools to identify high-yielding entries at early generations (Condon et al. 2008); (2) increasing the efficiency of carbon fixation in C_3 species (Long et al. 2006; Parry et al. 2007); (3) creating a substantial body of work pointing to the pivotal role of spike fertility in determining *YP* (Fischer 2007); (iv) creating the first comprehensive mechanistic model of physical processes that cause lodging in wheat (Berry et al. 2007); and (v) developing genetic tools that can take these physiological platforms closer to breeding application (Collins et al. 2008). Although not discussed here, improved crop management will also play a crucial role in stabilising and increasing crop yields (Hobbs 2007).

Genotypic variation in photosynthesis in modern wheats

At the leaf level, higher RUE effectively equates to a higher rate of CO_2 assimilation (*A*) per unit leaf area. It is well established that there is substantial genotypic variation in *A* within modern wheats, with values of *A* measured under favourable conditions and at ambient CO_2 levels ranging from 20 to about 30 µmol $CO_2/m^2/s$ (e.g. Condon et al. 1990; Morgan et al. 1991; Fischer et al. 1998). This large range results from genotypic variation in the combination of stomatal conductance and photosynthetic capacity, both of which vary by at least 50% among modern wheats.

Photosynthetic tools to identify high-yielding wheats and accelerate breeding progress

Given that wheats vary so substantially in their photosynthetic rate at the leaf level, what evidence is there that wheat yield will respond positively to a higher rate of photosynthesis? An obvious precedent is the yield response of wheat to the 'sledge-hammer' approach of doubling atmospheric CO_2, as in several climate-change studies. Invariably there has been a large yield response to these elevated CO_2 treatments, particularly if restricted water inputs have limited stomatal conductance (Ziska and Bunce 2007).

Table 1. Complementary strategies that increase wheat yield potential and some examples of interventions for each strategy

Strategy	Interventions
Increase photosynthetic capacity	Rubisco catalytic properties Canopy or spike photosynthesis Stomatal and mesophyll conductance C_4 metabolism
Maximise use of photosynthetic capacity	Partitioning to spike and grain Optimal phenological pattern Address conservative 'survival' traits
Prevent avoidable yield losses	Lodging resistance Biotic stress resistance Optimal crop management
Tap genetic resources more effectively	Explore Triticae tribe Wide crossing Transformation Strategic trait-based crossing Molecular breeding

Source: adapted from Reynolds et al. (2009), with permission

A revealing set of studies on *YP* progress and photosynthesis used a historic series of prominent International Maize and Wheat Improvement Centre (*Centro Internacional de Mejoramiento de Maís y Trigo*, CIMMYT) semi-dwarf wheats. These studies found that wheat YP trended upwards at about 0.9% per year with year of release from the early 1960s to the mid-1980s (Sayre et al. 1997). Coinciding with this *YP* trend, there were positive trends, with year of release, in rates of flag-leaf photosynthesis, stomatal conductance and photosynthetic capacity. Also, canopies of more recent wheats were cooler because of higher transpiration rates (Fischer et al. 1998). There was no clear trend in crop biomass, as *YP* trend was largely driven by increasing *HI*.

More recent studies at CIMMYT tested the idea that selecting for photosynthesis-related traits should help identify higher yielding lines in wheat breeding populations. Gutierrez-Rodriguez et al. (2000), in a study at a warm CIMMYT location, demonstrated genetic gains in yield in response to selection for flag-leaf photosynthesis rate in F_5 sister lines from the cross of Seri-M82 and Siete Cerros T66. Yield and biomass measured at $F_{5:7}$ were each positively correlated with *A*, stomatal conductance and intercellular CO_2 concentration measured at F_5. Measuring *A* on large numbers of lines using gas-exchange equipment is tedious. In subsequent studies in a high-yield environment at Obregon, Mexico, genetic gains in yield were determined using a larger number of lines from several crosses; different but potentially more-practical tools were used. These were leaf porosity (a rapid measure of stomatal conductance), canopy temperature (a canopy-scale measure of stomatal conductance) and carbon isotope discrimination (a canopy-scale measure of internal CO_2 concentration). All three traits showed useful associations with *YP* (Condon et al. 2008), with the first two cheap enough per plot to warrant serious consideration as screening tools for high *YP* in breeders' populations (Brennan et al. 2007).

It is unlikely that genetic differences in photosynthetic capacity alone would explain the gains in yield observed in these studies. The associations of yield with internal CO_2 concentration and carbon isotope discrimination suggest that stomatal conductance was a significant factor limiting photosynthesis of some lower yielding lines. Stomatal conductance of these lines may have been lower because of inherent anatomical differences. However, conductance may also have been lower due to enhanced stomatal response to incipient soil drying, vapour pressure deficit or even insufficient demand for photo-assimilates caused by low sink strength (Reynolds et al. 2000).

Routes to further improve *RUE*

Further gains in *YP* could be achieved through gains in *LI*, *RUE*, *HI* or combinations of these. This paper is concerned mainly with *RUE*, so there is only brief consideration here of *YP* gains through improved *LI* and *HI*.

Traits related to *LI* include better stand establishment to more quickly approach full *LI* and delayed canopy senescence to maintain *LI* longer into grain-filling. These traits show significant genetic variation in conventional gene-pools (Richards 2000). These traits are also highly amenable to visual selection, suggesting that they are probably not currently major bottlenecks for improving *YP*.

Genetic gains in wheat *YP* during the 20th century have been associated with increased *HI* not only as an immediate result of the introgression of *Rht* genes but also as a result of continued selection for yield in the post-green revolution period (Calderini et al. 1995). Despite a theoretical limit to *HI* of 62% in wheat (Austin 1980), comparisons of genetic progress in *HI* over time in spring wheat indicate no systematic progress since the mid-1980s from values of approximately 50% (Sayre et al. 1997).

Calculations of theoretical limits to *RUE* indicate that there is still considerable potential to increase the biomass of C_3 species (Long et al. 2006). Increases in above-ground biomass of wheat have been reported recently (Shearman et al. 2005), in some cases as a result of using exotic germplasm in breeding, including alien introgressions such as *7Ag.7AL* (Reynolds et al. 2001).

Long et al. (2006) and Murchie et al. (2009) reviewed various approaches for improving *RUE*, focusing largely on increasing leaf cellular-level photosynthesis. These leaf-cellular approaches have been recently reviewed elsewhere in the context of raising wheat *YP* (Reynolds et al. 2009). A summary is presented in Table 2. Most of the approaches listed in Table 2 are reviewed extensively in other papers at this symposium and so they are not considered in detail in this paper. Some of these approaches may have additive, or at least complementary, effects. For example, increased ribulose-1,5-bisphosphate (RuBP) regeneration is most likely to be effective when CO_2 supply to Rubisco is least limiting, which is when stomata are fully open. Alternatively, improved Rubisco

Table 2. Speculated increases in net photosynthesis by selected modifications to current C_3 crops such as wheat, possible time for adoption into breeding programs and likely need for GM technologies

Modification	Predicted increase (%)	Timescale (years)	Source of modification
Increased stomatal and mesophyll conductance	5	5	Non-GM and GM
Increased RuBP regeneration	10	5	GM
Optimised Rubisco regulation	10	10	Non-GM and GM
CO_2 pump	10	10	GM
Rubisco—increased specificity factor and increased k_{cat}	60	15	Non-GM and GM
CO_2 pump with kranz anatomy	50	20	GM
Rubisco without oxygenase and high k_{cat}	100	25	GM

Source: adapted from Long et al. (2006); Reynolds et al. (2009)
The predictions assume that water and nutrients are not limiting.
k_{cat} = catalytic turnover number of Rubisco in units of CO_2 per active site per second; GM = genetic modification

kinetics may be more influential when CO_2 supply to Rubisco is limited by low stomatal conductance.

At the canopy level, modification of leaf architecture may improve *RUE* by permitting a light distribution profile that reduces the number of leaves experiencing wasteful and potentially destructive supersaturated light levels, while increasing light penetration to canopy levels where photosynthesis responds linearly to light (Fischer 2007). Modelling suggests that there should still be scope for further optimising both light and nitrogen distribution in the wheat canopy (Long et al. 2006). Measurement of the relative contribution of spike photosynthesis (Tambussi et al. 2007) to overall canopy photosynthesis has never been seriously considered despite the large proportion of light that spikes intercept during grain-filling. However, recent comparative studies of the integrated contribution of spike photosynthesis to grain weight showed strong genetic effects (Reynolds et al., unpubl. data).

At the level of plant growth and development, a better balance between source and sink is expected to improve overall *RUE* (Slafer et al. 1996). An increasing body of evidence suggests sink strength, especially during grain-filling, is still a critical yield-limiting factor in wheat (Miralles and Slafer 2007) and that improving the balance between source and sink is a highly promising approach for raising *RUE*, biomass and yield (Reynolds et al. 2001, 2005; Shearman et al. 2005). Key to achieving progress in this complex area will be obtaining a better understanding of the genetic and molecular controls of how partitioning of assimilates at key developmental stages affects spike size and spike fertility, and hence, the determination of grain number and sink strength.

Partitioning of assimilates to reproductive growth and spike fertility

Increasing the relative partitioning of assimilates to the developing spike before anthesis might increase grain set (Bingham 1969). Spike index, defined as spike dry matter at anthesis divided by above-ground dry matter at anthesis, has been shown to be associated with yield improvement in comparisons of landmark wheat cultivars (Calderini et al. 1999). Work on the association between spike index and resources available during the spike-growth stage has also shown the critical importance of this period in determining final grain number (Fischer 1985; Abbate et al. 1995; Demotes-Mainard and Jeuffroy 2004). As yield is more dependent on grain number (specifically grains per square metre) than on the weight of the grains, raising spike dry matter at anthesis to boost grain number is an obvious target for genetic improvement.

The rapid spike-growth stage of wheat coincides with the middle of the stem elongation phase. Thus, spike growth and stem growth compete strongly for available assimilates. The higher *YP* of semi-dwarf wheats than tall wheats is in part due to less competition from the growing stems of the shorter, semi-dwarf wheats (Fischer 2007). There may be additional means of tipping the balance more in favour of spike growth. By examining the relationship between photoperiod and changes in relative duration of phenological phases, Slafer et al. (1996, 2001) proposed that increasing the relative duration of spike growth through manipulation of genetic

sensitivity to photoperiod is a means to achieve larger spike mass. Data of Gonzalez et al. (2005), who examined the fate of florets after a range of light and daylength manipulations of Ghiglione et al. (2008), gave this proposal strong support. Ghiglione et al. (2008) examined gene-expression changes associated with floret death, which was accentuated by accelerated plant development under long days. Another way to increase investment in spike growth is to increase pre-anthesis *RUE* and therefore biomass at anthesis, making more assimilates available to increase spike mass. Association between these traits has been shown in winter wheat cultivars (Shearman et al. 2005) and random inbred lines from spring bread wheat crosses (Reynolds et al. 2007a).

There is also potential to alter competition between the growing stem and growing spike by changing the dwarfing genes used to limit crop height. Two dwarfing genes are in common use in wheat: *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*). These genes were pivotal to the gains in wheat *YP* achieved in the original green revolution (Evans 1993). Several other available dwarfing genes have similar effects on final crop height. Some of these alternative genes have patterns of stem internode elongation that are different from *Rht-B1b* and *Rht-D1b* (Rebetzke et al. 2011) and these may promote greater assimilate partitioning to the growing spike.

New wheats that show better partitioning of assimilates between competing growth processes, and full use of the canopy's photosynthetic capacity, are possible. To develop these wheats, we need to understand the genetic basis of the physiological processes determining spike fertility, including how it interacts with: (1) crop phenology; (2) partitioning of assimilates between the growing spike and other competing sources; and (3) environmental constraints. The work becomes even more crucial to any new generations of wheat representing a step-change in photosynthetic capacity for the true yield potential of these wheats to be realised under agronomic conditions.

Underused photosynthetic capacity post-anthesis in modern wheats

Some recent studies have already provided evidence for underuse of crop photosynthetic capacity post-anthesis, apparently due to sub-optimal grain set. Direct evidence for conservative behaviour of modern wheat varieties in terms of grain set has come from observations of *7Ag.7DL* chromosomal substitution lines grown in a high-yield environment. In contrast to the six recurrent parents used, the presence of *7Ag.7DL* was associated with average increases of 12.8% in grains per spike, 7.5% in flag-leaf light-saturated photosynthetic rate and 9.2% in post-anthesis biomass accumulation. Yield and biomass increased by 13% and 10%, respectively (Reynolds et al. 2001). To test the hypothesis that increased sink strength could directly influence *RUE* during grain-filling, grains per spike were increased artificially with a 12-day light treatment during the boot stage in four of the highest yielding spring wheat cultivars available (Reynolds et al. 2005). Very much like the effect of *7Ag.7DL*, the light treatment increased sink strength by increasing spike index and grains per spike. The treatment also resulted in increased light-saturated rates of flag-leaf photosynthesis and *RUE* during grain-filling. Yield and biomass were increased by 20% and 18%, respectively (Table 3).

These observations raise the questions of why elite cultivars carry apparently excess photosynthetic capacity, and the corollary, why elite cultivars express sub-optimal spike fertility. The explanation is likely to be associated with the fact that wheat is self-pollinating, which depends on the production of viable seed for evolutionary survival. Grain number determination in primitive wheat (and its self-pollinating ancestors) would have evolved in response to two conflicting selection pressures: the need to produce enough seed to survive and the need to adjust seed number to ensure viability (Sadras 2007). However, while seed number is fixed around anthesis (Fischer 1985), seed viability is determined by subsequent unpredictable events including radiation levels, soil water status, competition from neighbouring plants for growth resources, as well as loss of photosynthetic tissue from foliar diseases, insects or herbivores. Therefore, plants would have been under selection pressure to evolve a relatively conservative strategy for determining seed number. This could explain why, under well-managed conditions, even modern wheat cultivars show an apparent excess photosynthetic capacity (Table 3). In other words, since throughout most of its domestication, wheat was cultivated under highly variable agronomic and environmental conditions, it is probable that this conservative tendency has become relatively genetically fixed, and may represent a bottleneck for achieving genetic gains in optimal environments.

Table 3. Effects of increasing sink, using a 12-day 'extra light' treatment during boot stage, averaged for four elite wheat cultivars over two cycles in north-west Mexico, 2001–02

	Effect of light treatment		Yield components				*RUE* effects during grain-filling	
	Biomass at anthesis (g/m^2)	Spike index (ratio)	Yield (g/m^2)	Grains per m^2	Biomass (g/m^2)	Grains per spike	g_s ($mmol/m^2/s$)	A_{max} ($\mu mol/m^2/s$)
Check	1,020	0.255	790	18,590	1,800	40.3	559	25.9
Extra sink	1,170	0.270	950	22,320	2,125	43.3	668	28.6
% effect	15	6	20	20	18	8	19	10.4
P main effect	0.01	0.05	0.01	0.01	0.01	0.03	0.01	0.01
P interaction	0.25	0.50	0.00	0.03	0.03	0.37	0.85	0.50

Source: adapted from Reynolds et al. (2005), with permission.
A_{max} = maximum CO_2 assimilation rate; g_s = stomatal conductance
The 'extra light' treatment was applied over 12 days before flowering in the mornings by bending the adjacent rows away from the central rows of treated plots, and in the evenings by restoring the adjacent rows upright. Treatment was stopped at anthesis. Three bread wheat cultivars were used: Siete Cerros 66, Baviacora 92 and Babax/Lr24//Babax, and the durum wheat Atil 2000 was used. No effects were found for kernel weight, harvest index, leaf chlorophyll or leaf internal CO_2 concentration.

Approaches for boosting grain set

A working hypothesis is that increased genetic capacity to set more grains per spike would result in more efficient use of photosynthetic capacity during grain-filling in favourable environments, giving a substantial boost to yield potential. There are two potentially complementary means to achieving this. The first involves an empirical approach to increasing the genetic variability of modern wheat varieties by introducing genes from wild ancestors. Interspecific hybridisation between the ancestral genomes of wheat occurred spontaneously an estimated 10,000 years ago, creating a genetic bottleneck that resulted in restricted genetic diversity in the bread wheat genome (Trethowan and Mujeeb-Kazi 2008). Wide crossing techniques have re-created this event and so-called 'resynthesised' or 'synthetic' wheats have already provided new sources of disease resistance (Villareal et al. 1994) and drought adaptive traits (Reynolds et al. 2007b). To increase spike fertility in bread wheat, donor genomes (AB-durum wheat and D-*Aegilops tauschii*) could be screened for favourable expression of the trait and the products of interspecific hybridisation used as genetic sources. The feasibility of this approach is supported by past success in transferring traits from the D genome to cultivated bread wheat (Reynolds et al. 2007b). Also, the alien introgression of *Lr19* for leaf rust into bread wheat from *Agropyron* (*7Ag.7DL*) resulted serendipitously in increased spike fertility, yield, biomass and *RUE*, as discussed above.

Second, mechanistic approaches can be applied to pinpoint the underlying physiological and genetic bases of variation in successful grain setting in potentially fertile florets, rather than abortion of such florets (and sometimes grains), as a means to engineering plants with a less conservative strategy. This strategy is better adapted to modern agronomy. It is well established in wheat that kernel set can be especially sensitive to environmental conditions such as moisture stress (Fischer 1980), light (Fischer 1985) and probably soil nitrogen levels (Abbate et al. 1997). Thus, signalling may be involved in response to these environmental factors, especially at the critical growth stage when final grain number is determined through abortion of potential florets (Fischer 1985). Signalling in plants is well established. For example, when plant roots detect the soil profile is drying, roots send signals to leaves, resulting in reduced transpiration rate mediated by reduced stomatal conductance. The signal appears to be complex but involves transport of abscisic acid (ABA) from roots to aerial parts (Davies et al. 2002). The important point is that these signals are pre-emptive; by relaying information about soil water status in advance of adverse effects on plant–water relationships, growth rate is reduced. Signalling may also be important in determining grain number under drought stress (Westgate et al. 1996). Recently, complex signalling altering the expression of key sugar-transport genes has been implicated in the pollen-abortion of wheat, which may be an important factor in lower grain number under drought stress (Ji et al. 2010). Since

the *7Ag.7DL* translocation in wheat is associated with reduced floral abortion in high-yield environments (Reynolds et al. 2001), it may be a suitable model for studying cues that determine final grain number.

Improved resistance to lodging

Any comprehensive strategy to improve wheat *YP* must consider the fact that heavier, more fertile spikes will increase yield losses associated with lodging unless traits associated with stem strength and root anchorage are simultaneously improved. Lodging is a persistent phenomenon in wheat. It reduces yield by up to 80% and reduces grain quality (Stapper and Fischer 1990; Easson et al. 1993). A validated model of lodging has identified the characters that determine stem and root lodging risk of wheat (Berry et al. 2003). This model indicates that plant breeders need to improve: (1) the spread of the root plate; (2) stem thickness; and (3) material strength of the stem wall, while minimising the width of the stem wall. We need to understand the genetic control of each of these traits, identify pleiotropic effects and develop methods to rapidly screen for them. For a given acceptable level of lodging risk, there is a limit on *HI*. The lodging model indicates an optimum crop height of 0.7 m (Berry et al. 2007). This height is shorter than many current high-yielding crops. It may be that crop height could be lowered to limit the potential for lodging but this may compromise the extent of biomass accumulation and achievable *YP*. Further, the anti-lodging characteristics specified above may compete for resources with developing spikes and grains, also limiting *YP*. Clearly, lowering the lodging risk is a complex issue that must be tackled if there are to be substantial gains in wheat *YP*.

Conclusions

This paper has outlined a comprehensive strategy for achieving a substantial increase in wheat *YP*. Different technologies and approaches will be required to most effectively apply the strategy in modern wheat breeding programs. These include improving the available level of genetic variation for traits of interest, developing tools to select for these traits more efficiently and applying modern breeding technologies to speed the release of new cultivars. Table 4 shows possible priorities for these approaches in terms applicable to the practicalities of breeding new wheat cultivars with very high *YP*.

In summary, there is substantial variation in leaf photosynthesis among modern wheats and within wheat breeding populations. Tools are available to more effectively exploit the already-available variation to accelerate gains in *YP*. Further improvement of photosynthetic capacity of wheat may be achieved through genetic modifications at the cellular and whole-plant levels. Increased spike fertility, achieved at least partially through better partitioning of assimilates to the growing spike, appears to be necessary to fully realise biomass gains before anthesis and photosynthetic potential during grain-filling. A better understanding of how crops respond to environmental cues such as photoperiod and availability of growth inputs may help us design crops that balance source and sink potential to maximise yield in favourable environments. However, trade-offs in partitioning additional biomass to root and stem will be necessary to construct lodging-proof crops. The wheat gene pool can be broadened using interspecific hybridisation with wild relatives or even transgenes from alien taxa. In addition to exploring genetic resources, we can use trait-based strategic crossing, physiological

Table 4. Prioritisation[a] of traits and technologies to accelerate gains in yield potential in a modern wheat breeding program

Trait	Source of genetic variation		Selection method		Breeding technology	
	Wide crosses	Transformation	Physiological trait breeding	Molecular markers	Doubled haploids	Hybrids
Greater biomass	1	1	2	2	2	2
Better partitioning	1	3	1	2	2	2
Better grain-set	2	3	1	1	2	3
Better lodging tolerance	3	2	1	1	1	2

Source: adapted from Reynolds et al. (2009), with permission

[a] 1 = high priority

and molecular markers for early generation selection, and doubled haploid breeding and hybrid wheats to accelerate rates of genetic gains.

Acknowledgements

We thank Tony Fischer for critical comments and suggestions. This paper, prepared for the ACIAR Workshop 'Applying photosynthesis research to crop yield', has drawn heavily on a recently prepared and published review on a similar topic (Reynolds et al. 2009).

References

Abbate P.E., Andrade F.H. and Culot J.P. 1995. The effects of radiation and nitrogen on number of grains in wheat. Journal of Agricultural Science 124, 351–360.

Abbate P.E., Andrade F.H., Culot J.P. and Bindraban P.S. 1997. Grain yield in wheat: effects of radiation during spike growth period. Field Crops Research 54, 245–257.

Austin R.B. 1980. Physiological limitations to cereals yields and ways of reducing them by breeding. Pp. 3–19 in 'Opportunities for increasing crop yields', ed. by R.G. Hurd, P.V. Biscoe and C. Dennis. Pitman: Boston.

Berry P.M., Sterling M., Baker C.J., Spink J. and Sparkes D.L. 2003. A calibrated model of wheat lodging compared with field measurements. Agricultural and Forest Meteorology 119, 167–180.

Berry P.M., Sylvester-Bradley R. and Berry S. 2007. Ideotype design for lodging-resistant wheat. Euphytica 154, 165–179.

Bingham J. 1969. The physiological determinants of grain yield in cereals. Agricultural Progress 44, 30–42.

Brennan J.P., Condon A.G., van Ginkel M. and Reynolds M.P. 2007. An economic assessment of the use of physiological selection for stomatal aperture-related traits in CIMMYT's wheat breeding program. Journal of Agricultural Science 145, 187–194.

Calderini D.F., Dreccer M.F. and Slafer G.A. 1995. Genetic improvement in wheat yield and associated traits. A re-examination of previous results and the latest trends. Plant Breeding 114, 108–112.

Calderini D.F., Reynolds M.P. and Slafer G.A. 1999. Genetic gains in wheat yield and associated physiological changes during the twentieth century. Pp. 351–377 in 'Wheat: ecology and physiology of yield determination', ed. by E.H. Satorre and G.A. Slafer. Food Product Press: New York.

Calderini D.F. and Slafer G.A. 1998 Changes in yield and yield stability in wheat during the 20th century. Field Crops Research 57, 335–347.

Collins N.C., Tardieu F. and Tuberosa R. 2008. Quantitative trait loci and crop performance under abiotic stress: where do we stand? Plant Physiology 147, 469–486.

Condon A.G., Farquhar G.D. and Richards R.A. 1990. Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat: leaf gas exchange and whole plant studies. Australian Journal of Plant Physiology 17, 9–22.

Condon A.G., Reynolds M.P., Brennan J., van Ginkel M., Trethowan R., Rebetzke G.J. et al. 2008. Stomatal aperture related traits and yield potential in bread wheat. Pp. 126–133 in 'International symposium on wheat yield potential: challenges to international wheat breeding', ed. by M.P. Reynolds, J. Pietragalla and H-J. Braun. CIMMYT: Mexico, D.F.

Davies W.J., Wilkinson S. and Loveys B. 2002. Stomatal control by chemical signalling and the exploitation of this mechanism to increase water use efficiency in agriculture. New Phytologist 153, 449–460.

Demotes-Mainard S. and Jeuffroy M.H. 2004. Effects of nitrogen and radiation on dry matter and nitrogen accumulation in the spike of winter wheat. Field Crops Research 87, 221–233.

Easson D.L., White E.M. and Pickles S.J. 1993. The effects of weather, seed rate and cultivar on lodging and yield in winter wheat. Journal of Agricultural Science, Cambridge 121, 145–156.

Evans L.T. 1993. Crop evolution, adaptation, and yield. Cambridge University Press: Cambridge, UK.

Fischer R.A. 1980. Influence of water stress on crop yield in semiarid regions. Pp. 323–339 in 'Adaptation of plants to water and high temperature stress', ed. by N.C. Turner and P.J. Kramer. Wiley Interscience: New York.

Fischer R.A. 1985. Number of kernels in wheat crops and the influence of solar radiation and temperature. Journal of Agricultural Science, UK 105, 447–461.

Fischer R.A. 2007. Understanding the physiological basis of yield potential in wheat. Journal of Agricultural Science 145, 99–113.

Fischer R.A., Rees D., Sayre K.D., Lu Z., Condon A.G. and Larque-Saavedra A. 1998. Wheat yield progress is associated with higher stomatal conductance, higher photosynthetic rate and cooler canopies. Crop Science 38, 1467–1475.

Ghiglione H.O., Gonzalez F.G., Serrago R., Maldonado S.B., Chilcott C., Cura J.A. et al. 2008. Autophagy regulated by day length determines the number of fertile florets of wheat. The Plant Journal 55, 1010–1024.

Gonzalez F.G., Slafer G.A. and Miralles D.J. 2005. Floret development and survival in wheat plants exposed to contrasting photoperiod and radiation environments during stem elongation. Functional Plant Biology 32, 189–197.

Gutierrez-Rodriguez M., Reynolds M.P. and Larque-Saavedra A. 2000. Photosynthesis of wheat in a warm, irrigated environment II. Traits associated with genetic gains in yield. Field Crops Research 66, 51–62.

Hobbs P.R. 2007. Conservation agriculture: what is it and why is it important for future sustainable food production? Journal of Agricultural Science 145, 127–137.

Ji X.M., Shiran B., Wan J.L., Lewis D.C., Jenkins C.L.D., Condon A.G. et al. 2010. Importance of pre-anthesis anther sink strength for maintenance of grain number during reproductive stage water stress in wheat. Plant, Cell & Environment 33, 926–942.

Long S.P., Zhu X-G., Naidu S.L. and Ort D.R. 2006. Can improvement in photosynthesis increase crop yields? Plant, Cell and Environment 29, 315–330.

Miralles D.J. and Slafer G.A. 2007. Sink limitation to yield in wheat: how could it be reduced? Journal of Agricultural Science 145, 139–149.

Morgan, J.A. and LeCain D.R. 1991. Leaf gas exchange and related traits among 15 winter wheat genotypes. Crop Science 31, 443–448.

Murchie E.H., Pinto M. and Horton P. 2009. Agriculture and the new challenges for photosynthesis research. New Phytologist 181, 532–552.

Parry M.A.J, Madgwick P.J., Carvalho J.F.C. and Andralojc P.J. 2007. Prospects for increasing photosynthesis by overcoming the limitations of Rubisco. Journal of Agricultural Science 145, 31–43.

Rebetzke G.J., Ellis M.H., Bonnett D.G., Condon A.G., Falk D. and Richards R.A. 2011. The *Rht13* dwarfing gene reduces peduncle length and plant height to increase grain number and yield of wheat. Field Crops Research 124, 323–331.

Reynolds M.P., Calderini D.F., Condon A.G. and Rajaram S. 2001. Physiological basis of yield gains in wheat associated with the *LR19* translocation from *Agropyron elongatum*. Euphytica 119, 137–141.

Reynolds M., Calderini D., Condon A. and Vargas M. 2007a. Association of source/sink traits with yield, biomass and radiation use efficiency among random sister lines from three wheat crosses in a high-yield environment. Journal of Agricultural Science 145, 30–16.

Reynolds M., Dreccer F. and Trethowan R. 2007b. Drought-adaptive traits derived from wheat wild relatives and landraces. Journal of Experimental Botany 58, 177–186.

Reynolds M.P., Foulkes M.J., Slafer G.A., Berry P., Parry M.A.J., Snape J.A. and Angus W.J. 2009. Raising yield potential in wheat. Journal of Experimental Botany 60, 1899–1918.

Reynolds M.P., Pellegrineschi A. and Skovmand B. 2005. Sink-limitation to yield and biomass: a summary of some investigations in spring wheat. Annals of Applied Biology 146, 239–259.

Reynolds M.P., Van Ginkel M. and Ribaut J.M. 2000. Avenues for genetic modification of radiation use efficiency in wheat. Journal of Experimental Botany 51, 459–473.

Richards R.A. 2000. Selectable traits to increase crop photosynthesis and yield of grain crops. Journal of Experimental Botany 51, 447–458.

Rosegrant M.W. and Cline S.A. 2003. Global food security: challenges and policies. Science 302, 1917–1919.

Sadras V.O. 2007. Evolutionary aspects of the trade-off between seed size and number in crops. Field Crops Research 100, 125–138.

Sayre K.D., Rajaram S. and Fischer R.A. 1997. Yield potential progress in short bread wheats in northwest Mexico. Crop Science 37, 36–42.

Shearman V.J., Sylvester-Bradley R., Scott R.K. and Foulkes M.J. 2005. Physiological processes associated with wheat yield progress in the UK. Crop Science 45, 175–185.

Slafer G.A., Abeledo L.G., Miralles D.J., Gonzalez F.G. and Whitechurch E.M. 2001. Photoperiod sensitivity during stem elongation as an avenue to raise potential yield in wheat. Euphytica 119, 191–197.

Slafer G.A., Calderini D.F. and Miralles D.J. 1996. Yield components and compensation in wheat: opportunities for further increasing yield potential. Pp. 101–134 in 'Increasing yield potential in wheat: breaking the barriers', ed. by M.P. Reynolds, S. Rajaram and A. McNab. CIMMYT: Mexico, D.F.

Stapper M. and Fischer R.A. 1990. Genotype, sowing date and plant spacing influence on high-yielding irrigated wheat in southern New South Wales. I. Potential yields and optimum flowering dates. Australian Journal of Agricultural Research 41, 1043–1056.

Tambussi E.A., Bort J., Guiamet J.J., Nogues S. and Araus J.L. 2007. The photosynthetic role of ears in C_3 cereals: metabolism, water use efficiency and contribution to grain yield. Critical Reviews in Plant Sciences 26, 1–16.

Trethowan R.M. and Mujeeb-Kazi A. 2008. Novel germplasm resources for improving environmental stress tolerance of hexaploid wheat. Crop Science 48, 1255–1265.

Villareal R.L., Mujeeb-Kazi A., Fuentesdavila G., Rajaram S. and Deltoro E. 1994. Resistance to karnal bunt (*Tilletia indica* Mitra) in synthetic hexaploid wheats derived from *Triticum turgidum* × *T. tauschii*. Plant Breeding 112, 63–69.

Westgate M.E., Passioura J.B. and Munns R. 1996. Water status and ABA content of floral organs in drought-stressed wheat. Australian Journal of Plant Physiology 23, 763–772.

Ziska L.H. and Bunce J.A. 2007. Predicting the impacts of changing CO_2 on crop yields: some thoughts on food. New Phytologist 175, 607–618.

Additional references

Parry M.A.J., Reynolds M.P., Salvucci M.E., Raines C., Andralojc P.J., Zhu X.G. et al. 2011. Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. Journal of Experimental Botany 62, 453–467.

Reynolds M., Foulkes J., Furbank R., Griffiths S., King J., Murchie E. et al. 2012. Achieving yield gains in wheat. Plant, Cell & Environment 35, 1799–1823.

Photosynthetic efficiency and its impact on yield in potato

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Summary

- Abiotic stress factors such as heat and drought are the main limitations for potato productivity in many regions of the world.
- These stress factors affect the plant in various ways, but have in common that they reduce net photosynthesis, although through different mechanisms. Therefore, developing plants with improved photosynthetic efficiency could contribute to increased potato yields in areas frequently exposed to drought spells, heat waves, cold stress or low light conditions.
- There is increasing demand for potato varieties with extremely short crop duration; however, crop yields correlate with net photosynthesis over time. Thus, short crop duration results in lower yields. Increasing daily net photosynthesis would have the potential to increase yields of potato with extreme short crop duration.
- In this paper we review the effects of abiotic stresses on net photosynthesis in potato and discuss approaches to increasing photosynthetic efficiency with the potential to generate the yield increases required to meet the increasing demand for potato.

Introduction

Potato is the third most important food crop in the world, with an annual production approaching 300 million tonnes. Potatoes are invaluable for the diets and livelihoods of millions of people worldwide. Potato provides more nutrients per hectare than any other crop for human consumption and grows even under unfavourable conditions. More than one-third of the global potato harvest comes from developing countries. Many potato production areas in developing countries are located in semi-arid areas, where drought spells and heat stress cause large harvest losses. In these regions, where the yields range around 30% of the global mean, increased photosynthetic efficiency under abiotic stress conditions could improve yield considerably.

Photosynthesis limitations with an impact on crop yield may appear at the level of ribulose bisphosphate carboxylase (Rubisco) activity, ribulose-1,5-bisphosphate (RuBP) regeneration in the Calvin cycle, the rate of triose phosphate use, and on reduced CO_2 diffusion to the site of photosynthesis. Yield potential depends on converting intercepted radiation into biomass. Consequently, increasing photosynthesis has the potential to increase crop yields. Like in other crops, photosynthetic efficiency of potato is likely to decrease in hot, dry, cold and low light environments. However, in any environment, the crop will be exposed at least temporarily to some kind of stress that can affect net photosynthesis.

Under hot and dry conditions, low vapour pressure increases transpiration. When water supply is insufficient, stomata close and mesophyll conductivity declines, limiting the CO_2 movement from the air through the intercellular air spaces to the mesophyll

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and the chloroplast stroma (Evans and Loreto 2000; Flexas and Medrano 2002). Low CO_2 levels in chloroplasts favour the oxygenation activity of Rubisco, leading to increased photorespiration and decreased net photosynthesis. In C_3 plants, about one-third of fixed carbon is lost by photorespiration (reviewed in Monteith 1977).

In plants, heat stress directly limits photosynthesis when the leaf temperature reaches levels above the photosynthetic thermal optimum. The thermal limitation of photosynthesis is typically a mixture of the effects of Rubisco, electron transport and pyrophosphate regeneration (reviewed in Sage and Kubien 2007). Rubisco is considered to be heat tolerant. Denaturation of this enzyme takes place well above 50°C (reviewed in Salvucci and Crafts-Brandner 2004). Therefore, heat is not likely to affect Rubisco activity directly, but oxidative stress that generally builds up under abiotic and biotic stress conditions may damage this enzyme and mark it for degradation. Rubisco activity is regulated through binding to the active site of metabolites such as RuBP, 2-carboxyarabinitol 1-phosphate (CA1P), xylulose-1,5-bisphosphate (XuBP) and pentadiulose-1,5-bisphosphate. Rubisco activity is also regulated by Rubisco activase, which removes inhibitors from the active site, thereby promoting carbamylation and full activity of the catalytic sites (Portis 1995; 2003). Deactivation of Rubisco activase leads to deactivation of Rubisco. Rubisco activase is a heat labile enzyme and its activity decreases at increased temperatures (Law and Crafts-Brandner 1999). Rubisco activase is regulated through the adenosine triphosphate – adenosine diphosphate (ATP/ADP) ratio in the chloroplast and by redox sensing. Consequently, Rubisco activase might not be the limiting factor for photosynthesis per se under any stress condition. Instead, it is regulated by the energy supply from thylakoids and may be inhibited by down-regulation of electron transport, which again is regulated by the capacity of carbon metabolism to consume ATP and the reduced form of nicotinamide adenine dinucleotide (NADH).

Under heat stress, the capacity for photosynthetic electron transport decreases, which leads to decreased regeneration of RuBP for the carboxylation reaction. Most probably, increased proton leakiness across the thylakoid membrane or altered interactions between membranes and the thylakoid protein complexes are the cause of this decline (reviewed in Sharkey and Schrader 2006). Cyclic electron flow around photosystem (PS) I is accelerated under stress conditions to dissipate excess excitation energy (Yang et al. 2006; Laisk et al. 2007).

There are links and feedback regulation between sugar partition and photosynthesis. Low sink demand decreases photosynthesis, in the short term by decreased pyrophosphate recycling to the chloroplast and increased sugar concentrations in source tissues, and in the long term by cytokinin signalling (reviewed in Paul and Foyer 2001).

Heat stress effects on carbon gain in potato

Potato evolved in cool tropical zones and heat affects potato yield in multiple ways. The maximum development rate for potato tubers has been reported to be in the range of 14 to 22°C (Driver and Hawkes 1943; Yamaguchi et al. 1964; Marinus and Bodlaender 1975). Temperatures above the optimum resulted in taller plants with high stem dry weight and smaller leaves (Prange et al. 1990; Lafta and Lorenzen 1995), accelerated leaf senescence and decreased photosynthetic capacity of the canopy. This results in reduced tuber production for most cultivars (Ben Khedher and Ewing 1985; Menzel 1985; Levy 1986). Heat affects tuber weights more than it affects the whole plant. Tuberisation is restricted under hot conditions, probably through hormonal effects (Menzel 1985; Prange et al. 1990).

The optimum temperature for photosynthesis in potato is around 20 to 24°C (Ku et al. 1977; Ghosh et al. 2000). Leaf photosynthetic rates decrease with increasing temperature (Leach et al. 1982; Wolf et al. 1990). For every 5°C rise in leaf temperature above the optimal temperature, a reduction of approximately 25% in the rate of photosynthesis is observed (Burton 1981). Hence, at temperatures above 30°C, net assimilation for potato approaches zero. Heat sensitivity of potato photosynthesis was attributed, along with accelerated senescence, chlorophyll loss and reduced stomatal conductance, with inhibition of dark reactions (Reynolds et al. 1990). Data on Rubisco activity under heat in potato are not available.

Decreased leaf photosynthesis rate at high temperature has been suggested to be largely due to reduced efficiency in PSII (Prange et al. 1990), but Havaux (1993; 1995) showed that PSII activity remains stable up to 38°C, and after acclimation even up to 40°C. Thus, it was concluded that PSII of potato is highly stress tolerant.

There are contrasting reports about the impact of photosynthetic limitations on potato yield. While Prange et al. (1990) and Timlin et al. (2006) found reduced photosynthetic activity in potato under heat stress, Lafta and Lorenzen (1995) reported that high temperatures (31°C day, 29°C night) reduced biomass accumulation, but did not affect the rate of photosynthesis of either heat-tolerant or heat-susceptible varieties. These contrasting observations might be caused by differences in the experimental settings or because different genotypes were investigated in these studies.

Assimilate partitioning to the tubers depends on sucrose translocation and its subsequent metabolism within starch biosynthetic and respiratory pathways (reviewed in Stitt et al. 2007), and is impaired at elevated temperatures. Increase of sucrose phosphate synthase activity in leaves and decrease in sucrose synthase activity in tubers leads to reduced sucrose transport to the tubers and sucrose accumulation in leaves (Lafta and Lorenzen 1995; Timlin et al. 2006). The shoot becomes an important sink for photosynthates instead of the tubers (Basu and Minhas 1991). Reduced tuber development under heat stress also results in a smaller sink for photosynthates and concomitant reduction in sink strength reduces photosynthesis (Basu et al. 1999). This suggests that heat stress has larger effects on tuberisation and translocation of sugars to tubers than on the production of sugars from photosynthesis. The effect on photosynthesis may be secondary and result from reduced sink strength under heat (Basu and Minhas 1991; Basu et al. 1999).

Under elevated temperatures respiration rates increase. High respiration rates may even result in a negative carbon balance on some days, especially toward the end of the season as the proportion of young leaves decreases. Winkler (1971) showed that dark respiration rates of potato leaves roughly doubled for each 10°C increase in temperature. Wivutvongvana (1979) compared heat-tolerant and heat-sensitive genotypes of the wild potato relatives *Solanum chacoense* and *S. acaule* growing under a non-tuberising long-day photoperiod. Wivutvongvana (1979) found that heat-sensitive clones had higher rates of dark respiration than heat-tolerant clones but that these did not differ in the rate of CO_2 uptake during photosynthesis. This finding suggests that tolerance to high temperatures may be associated more with differences in respiration than in photosynthesis.

Photosynthetic limitations

Photosynthesis in potato plants is limited under stress from cold, drought and low light.

Cold stress

At temperatures below the optimum of 20–24°C, the activity of photosynthetic enzymes decreases (Steffan and Palta 1996). Lowering the growth temperature of potato to 10°C caused a 25% reduction in net photosynthesis (Burton 1981).

Low temperatures reduce electron transport through PSII and from PSII to PSI because membrane viscosity increases and the biophysical properties of thylakoid lipids change. This results in inhibition of the Calvin cycle activity and decreased photosynthetic rates (reviewed in Huner et al. 1998; Ensminger et al. 2006). The rates of photochemical processes are adjusted to the decreased metabolic sink capacity for the consumption of photosynthates through a redox sensor within the photosynthetic electron transport chain and through pyrophosphate availability (Escoubas et al. 1995; Maxwell et al. 1995; Allen and Nilsson 1997; reviewed in Pfannschmidt 2003). Low-temperature stress inhibits sucrose synthesis in the cytosol of *Arabidopsis thaliana*, causing lower inorganic phosphate (P_i) cycling between the cytosol and the chloroplast (Hurry et al. 2000). Thus, the chloroplast becomes P_i-limited, impeding the synthesis of ATP, which is needed in the regeneration of RuBP.

Improvement of Rubisco would be of little help to increase yields in cold environments. However, improving electron transport capacity at low temperature could improve RuBP regeneration in the Calvin cycle. It would remain to increase sink strength under cold stress to mitigate the effect of reduced pyrophosphate recycling.

At sub-optimal temperatures and high irradiation the energy flow to the photosystem exceeds demand and leads to excess excitation energy in the chloroplasts. Accumulation of reactive oxygen species results, leading to injury from oxidative stress (reviewed in Krause and Weiss 1991). High excitation pressure under low temperatures can induce photoinhibition, a dissipation mechanism that might result in photodamage (Melis 1999). Fluorescence measurements showed that photosynthesis of a freezing-tolerant genotype was transiently reduced during frost, whereas in frost-sensitive clones photosynthesis was reduced more and irreversible damage

occurred (Seppänen and Coleman 2003). Several potato species, particularly *S. juzepczukii*, have high plasticity of the PSII thermotolerance. The photosynthetic apparatus of *S. juzepczukii* × *S. tuberosum* potato hybrids is adapted to the changing temperature conditions prevailing in the natural habitat of its wild progenitor, where night frosts are associated with warm and sunny days (Havaux 1995).

Drought stress

Maintaining photosynthetic activity under water stress is a key element of plant drought tolerance. Photosynthesis in water-stressed plants is restricted mainly when stomatal and mesophyll conductance is kept low to avoid excessive transpiration. This determines how far CO_2 remains available for the photosynthetic apparatus. Non-stomatal metabolic limitations, such as reduced RuBP regeneration and ATP synthesis, restrict carbon assimilation under drought (high stress levels). However, at high irradiances excess RuBP is present and CO_2 should remain the limiting factor for photosynthetic rate (reviewed in Parry et al. 2007). At low irradiance, RuBP might be limiting through inhibition of ATP synthesis, caused by progressive inactivation or loss of coupling factor resulting from increasing ionic concentration (reviewed by Lawlor 2002).

High O_2/CO_2 ratios at the site of carboxylation increase photorespiration, which can consume up to 50% of the CO_2 fixed under water stress conditions. Low CO_2 can also lead to lower Rubisco activity. Under conditions that favour the oxygenase reaction and photorespiration, more Rubisco inhibitor pentadiulose-1,5-bisphosphate (PDBP) is produced, resulting in a depression of the total Rubisco activity (Parry et al. 1993, 2002). PSII functioning and its regulation are not qualitatively changed during desiccation (Cornic and Freseau 2002).

In summary, reducing photorespiration, besides ensuring the maintenance of a large photosynthetically active leaf area, may mitigate yield losses of potato under drought. Photorespiration might be reduced by increasing the affinity of Rubisco to CO_2 and by lowering its oxygenase activity. Reducing the Rubisco oxygenase activity is associated with reduced turnover rate of this enzyme. However, this would hold true only under conditions where the plant achieves maximum catalytic rates. It may not apply when CO_2 availability is severely restricted, such as under drought stress. Thus, increased specificity may result in improved photosynthesis even if maximum catalytic rates are lowered (reviewed in Parry et al. 2005). Moreover, CO_2 concentration mechanisms as discussed below could contribute to reduce photorespiration. How far these approaches provide yield benefits for potato in drought-prone areas remains to be tested.

A candidate gene for reducing photorespiration would be the cyanobacterium *ictB* gene product. This protein is thought to be involved in concentrating CO_2 for utilisation by Rubisco (Bonfil et al. 1998). Transgenic plants containing *ictB* had faster photosynthetic rates and increased biomass accumulation than the wild types under CO_2-limiting concentrations (Lieman-Hurwitz et al. 2003). Thus, the expression of the *ictB* gene product in crop plants may increase photosynthetic carbon assimilation under drought when stomata are closed and low internal CO_2 results in increased photorespiration. A proof of concept for the practicability of this approach to increase yield in potato under water stress conditions has not yet been completed.

Low light

Potato grows best in cool climates with full sunlight. In some potato growing regions, prevailing foggy conditions cause low light stress for the plants. Under low light conditions, CO_2 assimilation does not limit the light reaction; thus, photosynthetic efficiency of a plant under such conditions will depend mainly on its ability to acclimate the light-harvesting apparatus to low light intensities.

The assimilation rate under low light is limited by the amount of RuBP (Farquhar et al. 1980; von Caemmerer 2000). The rate of RuBP regeneration is theoretically limited by the slowest step in the process, catalysed by sedoheptulose-1,7-bisphosphatase (SBPase) (Miyagawa et al. 2001; reviewed in Raines 2003; Lefebvre et al. 2005). The Rieske FeS protein of the cytochrome b_6/f protein complex may also limit RuBP regeneration by limiting the capacity of photosynthetic electron transport (Price et al. 1998).

Plants use light not only as an energy source for photosynthesis but also as an environmental signal (reviewed in Briggs and Christie 2002). Low irradiance delays tuberisation in vitro (Jackson 1999) and reduces tuberisation in the field.

An array of morphological, biochemical and molecular adaptation mechanisms supports plant growth and tuberisation at low light. Phototropins are plant-specific blue light receptors that mediate phototropism, chloroplast movements, stomatal

opening and leaf expansion under changing light conditions (Kagawa et al. 2001; Kinoshita et al. 2001; Briggs and Christie 2002; Takemiya et al. 2005). Simultaneously optimising chloroplast localisation and stomatal opening, and maximising effective leaf area under low light, contribute to increased photosynthetic rates and, consequently, accelerated growth.

Light quality gradients create an imbalance of excitation energy distribution between PSI and PSII. Under persisting low light conditions, the stoichiometry of the photosystems is readjusted by actively changing the relative number of the two photosystems in favour of the rate-limiting one (Melis 1991; Kim et al. 1993; Walters and Horton 1994; Murakami et al. 1997; reviewed in Allen 1995 and Anderson et al. 1995). This adjustment is a way to maximise the efficiency of using absorbed light energy under conditions when light is strongly limiting for growth.

Photosynthetic limitations under low light conditions also may appear on the Rubisco level. In the dark and at low irradiation, the Rubisco inhibitor 2-carboxyarabinitol-1-phosphate blocks Rubisco activity. Low ATP levels under light-limiting conditions may cause inefficient CA1P removal by Rubisco activase. This may result in reduced net photosynthesis (reviewed in Portis 2003).

Photosynthetic activity and crop duration

For tropical and subtropical environments, potato varieties with very short growth duration of less than 80 days are highly desirable to fit a potato crop between two cereal harvests. Crop yield tightly correlates with net photosynthesis over time; thus, shortening the crop duration inevitably will decrease yields. Increasing daily net photosynthesis may promote acceptable yields with a short-duration crop. Besides early emergence, rapid groundcover, very early tuberisation and high harvest index, high photosynthetic efficiency and low photorespiration would be desirable traits for an extreme early potato variety. However, apparently wasteful processes that reduce net photosynthesis and yield, such as photorespiration, the Mehler reaction and chlororespiration, reduce the exposure of the plant to oxidative stress. These processes are required to adjust photosynthesis to environmental constraints. Transgenic approaches that reduce photorespiration by concentrating CO_2 in chloroplasts, such as the coordinated expression of C_4 photosynthetic enzymes in C_3 plants, could contribute to reducing photorespiration without exposing the plant to greater oxidative stress.

The data available so far give no clear indication whether a C_4-like CO_2 concentration in the chloroplasts might be functional in a C_3 plant (reviewed in Peterhänsel et al. 2008). Leaf parenchyma of C_3 plants lacks the gastight bundle sheet cells. Therefore, there is the risk that CO_2 brought in by a CO_2 pump would diffuse out and instead of improving photosynthetic efficiency would just represent a waste of energy. Moreover, the installation of a C_4 pathway in a C_3 plant will require the coordinated expression of multiple enzymes in the chloroplasts and the avoidance of pleiotropic effects. C_4 cycle enzymes are present in C_3 plants, but their expression patterns and functional roles are different between C_3 and C_4 plants. Transgenically introduced C_4 cycle enzymes can interfere with the role of the native enzymes in C_3 plants and can cause various undesired effects. For example, potato overexpressing the C_4 enzymes phosphoenol pyruvate carboxylase (PEPC) and NADH-malic enzyme apparently had lower photorespiration rates, but plant growth was impaired (reviewed in Häusler et al. 2002). In tobacco, the expression of four C_4 cycle enzymes did not show a clear effect on biomass or growth and the effect on photosynthetic efficiency remains unclear.

An alternative path to CO_2 concentration in chloroplasts for decreasing carbon loss through photorespiration is to direct glycolate produced during photorespiration to peroxysomes rather than chloroplasts (Kebeish et al. 2007). This could be conferred by transgenic expression of a bacterial enzyme that converts glycolate to glyoxylate without producing H_2O_2 in the chloroplast. Subsequently, glyoxylate is converted to tartronic semi-aldehyde. This step releases CO_2, but as this reaction takes place in the chloroplast, the CO_2 could be used by Rubisco, in contrast with the CO_2 produced during photorespiration in mitochondria. Furthermore, this alternative pathway would save reduction equivalents, as no reduced nitrogen is consumed. Tartronic semi-aldehyde is further reduced to glycerate. This pathway has been successfully introduced into *A. thaliana*. The resulting plants show increased growth and biomass production, which can be correlated with reduced photorespirative flow, improved photosynthetic properties and increased leaf sugar contents (Peterhänsel et al. 2008).

Conclusions

There are scant data concerning photosynthetic limitations in potato. From the available information we assume that decline of net photosynthesis under abiotic stresses is caused by reduced sink strength, CO_2 limitation and increased respiration rates rather than by impairment of Rubisco activity or RuBP regeneration. However, there are indications that optimisation of Rubisco and Rubisco-related processes could contribute to maintaining yields under stressful conditions, but more research is required to better determine the contribution of photosynthetic limitation to yield drops. Improved heat tolerance of Rubisco activase and selection for improved tuberisation and reduced impairment of sugar partition could mitigate yield drops in hot environments. Under drought, optimised stomatal control and possibly CO_2 concentrating mechanisms at the site of photosynthesis could reduce photorespiration. Cold stress tolerance is present in potato germplasm and consists of the capacity to adjust photosynthesis to the energy demand of the plants, thereby avoiding oxidative stress and photoinhibition. Under low light conditions, Rubisco-associated processes are less important in yield development than adaptation of antenna complexes or coordinated activity between PSI and PSII.

Improved net photosynthesis and reduced photorespiration may contribute to the development of extreme early potato varieties with acceptable yields. Increasing Rubisco specificity to CO_2, which could lower photorespiration, did not show the desired results in model plants, as the overall turnover rate of this enzyme decreased with increased specificity. As an alternative, either CO_2 concentration at the carboxylation site using C_4 pathway enzymes, or channelling photorespiration products back to the chloroplast, are possible strategies to improve the photosynthetic efficiency of potato.

Proposed research

We propose the following research is needed:

- Evaluation of a biodiverse panel of potato genetic materials selected on adaptation or breeding and selection history. Material would include representatives of cultivated native potato groups, an array of wild potato species and bred materials. Materials would show tolerance to heat, drought and cold conditions for chlorophyll content, leaf area index, net assimilation rate, groundcover, early tuberisation and plant type.
- Validation of Rubisco activity limitation under stress in potato. In growth chamber experiments under a saturating CO_2 atmosphere, photosynthetic activity and biomass accumulation, tuberisation and tuber yield should be determined in a biodiverse panel of potato accessions to assess the share of Rubisco, electron transport of photosynthetic limitation and genotypic differences in photosynthetic efficiency under stress.
- Detailed quantification of the effects of stress on dry matter production and C partitioning. This information is important to develop simulation models for potato breeders and growers. Several potato models are available (Ingram and McCloud 1984; Hodges et al. 1992; Wolf and van Oijen 2003). The stress dependencies used in these models have largely been developed from field trial and greenhouse data. However, canopy-level carbon assimilation has not been sufficiently integrated into these models. Little is known about whole-canopy gas exchange rates in potato as a function of stress where CO_2 is not limiting. Leach et al. (1982) established a detailed carbon budget for potatoes, but did not investigate different stress levels.
- Follow up on past experiments introducing C_4 enzymes into C_3 plants or redirecting photorespiration products. Based on the results obtained in model plants and cereals, experimental plans for potato should be developed and tested.

References

Allen J.F. 1995. Thylakoid protein-phosphorylation, state-1-state-2 transitions, and photosystem stoichiometry adjustment—redox control at multiple levels of gene-expression. Physiologia Plantarum 93, 196–205.

Allen J.F. and Nilsson A. 1997. Redox signalling and the structural basis of regulation of photosynthesis by protein phosphorylation. Physiologia Plantarum 100, 863–868.

Anderson J.M., Chow W.S. and Park Y-I. 1995. The grand design of photosynthesis: acclimation of the photosynthetic apparatus to environmental cues. Photosynthesis Research 46, 129–139.

Basu P.S. and Minhas J.S. 1991. Heat tolerance and assimilate transport in different potato genotypes. Journal of Experimental Botany 42, 861–866.

Basu P.S., Sharma A., Garg I.D. and Sukumaran N.P. 1999. Tuber sink modifies photosynthetic response in potato under water stress. Environmental and Experimental Botany 42, 25–39.

Ben Khedher M. and Ewing E.E. 1985. Growth analysis of eleven potato cultivars grown in the greenhouse under long photoperiods with and without heat stress. American Potato Journal 62, 537–554.

Bonfil D.J., Ronen-Tarazi M., Sultemeyer D., Lieman-Hurwitz J., Schatz D. and Kaplan A. 1998. A putative HCO_3 transporter in the cyanobacterium *Synechococcus* sp. Strain PCC 7942. FEBS Letters 430, 236–240.

Briggs W.R. and Christie J.M. 2002. Phototropins 1 and 2: versatile plant blue-light receptors. Trends in Plant Science 7, 204–210.

Burton W.G. 1981. Challenges for stress physiology in potato. American Potato Journal 58, 3–14.

Cornic G. and Freseau C. 2002. Photosynthetic carbon reduction and carbon oxidation cycles are the main electron sinks for photosystem II activity during mild drought. Annals of Botany 89, 887–894.

Driver C.M. and Hawkes J.G. 1943. Photoperiodism in Potato. Technical Communication 10. Imperial Bureau of Plant Breeding and Genetics: Cambridge, UK.

Ensminger I., Busch F. and Huner N.P.A. 2006. Photostasis and cold acclimation: sensing low temperature through photosynthesis. Physiologia Plantarum 126, 28–44.

Escoubas J., Lomas M., LaRoche J. and Falkowski P.G. 1995. Light intensity regulation of cab gene transcription is signaled by the redox state of the plastoquinone pool. Proceedings of the National Academy of Sciences USA 92, 10237–10241.

Evans J.R. and Loreto F. 2000. Acquisition and diffusion of CO_2 in higher plant leaves. Pp. 321–351 in 'Photosynthesis: physiology and metabolism', ed. by R.C. Leegood, T.D. Sharkey and S. von Caemmerer. Kluwer Academic: Dordrecht.

Farquar G.D., von Caemmerer S. and Berry J.A. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149, 78–90.

Flexas J. and Medrano H. 2002. Drought-inhibition of photosynthesis in C_3 plants: stomatal and non-stomatal limitations revisited. Annals of Botany 89, 183–189.

Ghosh S.C., Asanuma K., Kusutani A. and Toyota M. 2000. Leaf gas exchange properties of potato under different temperature and soil moisture at different growth stages. Environment Control in Biology 38, 229–239.

Häusler R.E., Hirsch H.J., Kreuzaler F. and Peterhansel C. 2002. Over-expression of C_4-cycle enzymes in transgenic C_3 plants: a biotechnological approach to improve C_3 photosynthesis. Journal of Experimental Botany 53, 591–607.

Havaux M. 1993. Rapid photosynthetic adaptation to heat stress triggered in potato leaves by moderately elevated temperatures. Plant Cell and Environment 16, 461–467.

Havaux M. 1995. Temperature sensitivity of the photochemical function of photosynthesis in potato (*Solanum tuberosum*) and a cultivated Andean hybrid (*Solanum* × *juzepczukii*). Journal of Plant Physiology 146, 47–53.

Hodges T., Johnson S.L. and Johnson B.S. (1992.) SIMPOTATO: a highly modular program structure for an IBSNAT style crop simulation. Agronomy Journal 84, 911–915.

Huner N.P.A., Öquist G. and Sarhan F. 1998. Energy balance and acclimation to light and cold. Trends in Plant Science 3, 224–230.

Hurry V., Strand A., Furbank R. and Stitt M. 2000. The role of inorganic phosphate in the development of freezing tolerance and the acclimation of photosynthesis to low temperature is revealed by the *pho* mutants of *Arabidopsis thaliana*. Plant Journal 24, 383–396.

Ingram K.T. and McCloud D.E. 1984. Simulation of potato crop growth and development. Crop Science 24, 21–27.

Jackson S.D. 1999. Multiple signalling pathways control tuber induction in potato. Plant Physiology 119, 1–8.

Kagawa T., Sakai T., Suetsugu N., Oikawa K., Ishiguro S., Kato T. et al. 2001. Arabidopsis NPL1: A phototropin homolog controlling the chloroplast high-light avoidance response. Science 291, 2138–2141.

Kebeish R., Niessen M., Thiruveedhi K., Bari R., Hirsch H-J., Rosenkranz R. et al. 2007. Chloroplastic photorespiratory bypass increases photosynthesis and biomass production in *Arabidopsis thaliana*. Nature Biotechnology 25, 593–599.

Kim J.H., Glick R.E. and Melis A. 1993. Dynamics of photosystem stoichiometry adjustment by light quality in chloroplasts. Plant Physiology 102, 181–190.

Kinoshita T., Doi M., Suetsugu N., Kagawa T., Wada M., Shimazaki K. 2001. *Phot1* and *phot2* mediate blue light regulation of stomatal opening. Nature 414, 656–660.

Krause G.H. and Weis E. 1991. Chlorophyll fluorescence and photosynthesis: the basics. Annual Review of Plant Physiology and Plant Molecular Biology 42, 313–349.

Ku S.B., Edwards G.E. and Tanner C.B. 1977. Effects of light, carbon dioxide, and temperature on photosynthesis, oxygen inhibition of photosynthesis, and transpiration in *Solanum tuberosum*. Plant Physiology 59, 868–872.

Lafta A.M. and Lorenzen J.H. 1995. Effect of high temperature on plant growth and carbohydrate metabolism in potato. Plant Physiology 109, 637–643.

Laisk A., Eichelmann H., Oja V., Talts E. and Scheibe R. 2007. Rates and roles of cyclic and alternative electron flow in potato leaves. Plant and Cell Physiology 48, 1575–1588.

Law R.D. and Crafts-Brandner S.J. 1999. Inhibition and acclimation of photosynthesis to heat stress is closely correlated with activation of ribulose-1,5-bisphosphate carboxylase/oxygenase. Plant Physiology 120, 173–181.

Lawlor D.W. 2002. Limitation to photosynthesis to water-stressed leaves: stomata vs. metabolism and the role of ATP. Annals of Botany 89, 871–85.

Leach J.E., Parkinson K.J. and Woodhead T. 1982. Photosynthesis, respiration and evaporation of field-grown potato crop. Annals of Applied Biology 101, 377–390.

Lefebvre S., Lawson T., Fryer M., Zakhleniuk O.V., Lloyd J.C. and Raines C.A. 2005. Increased sedoheptulose-1,7-bisphosphatase activity in transgenic tobacco plants stimulates photosynthesis and growth from an early stage in development. Plant Physiology 138, 451–460.

Levy D. 1986. Genotypic variation in the response of potatoes (*Solanum tuberosum* L.) to high ambient temperatures and water deficit. Field Crops Research 15, 85–96.

Lieman-Hurwitz J., Rachmilevitch S., Mittler R., Marcus Y. and Kaplan A. 2003. Enhanced photosynthesis and growth of transgenic plants that express *ictB*, a gene involved in HCO_3 accumulation in cyanobacteria. Plant Biotechnology Journal 1, 43–50.

Marinus J. and Bodlaender K.B.A. 1975. Response of some potato varieties to temperature. Potato Research 18, 189–204.

Maxwell D.P., Laudenbach D.E. and Huner N.P.A. 1995. Redox regulation of light-harvesting complex II and *cab* mRNA abundance in *Dunaliella salina*. Plant Physiology 109, 787–795.

Melis A. 1991. Dynamics of photosynthetic membrane composition and function. Biochimica et Biophysica Acta 1058, 87–106.

Melis A. 1999. Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage in vivo? Trends in Plant Science 4, 130–135.

Menzel C.M. 1985. Tuberization in potato at high temperatures: interaction between temperature and irradiance. Annals of Botany 55, 35–39.

Miyagawa Y., Tamoi M. and Shigeoka S. 2001. Overexpression of a cyanobacterial fructose-1,6-sedoheptulose-1,7-bisphosphatase in tobacco enhances photosynthesis and growth. Nature Biotechnology 19, 965–969.

Monteith J.L. 1977. Climate and efficiency of crop production in Britain. Philosophical Transactions of the Royal Society of London 281, 277–294.

Murakami A., Fujita Y., Nemson J.A. and Melis A. 1997. Chromatic regulation in *Chlamydomonas reinhardtii*: time course of photosystem stoichiometry adjustment following a shift in growth light quality. Plant and Cell Physiology 38, 188–193.

Parry M.A.J., Andralojc P.J., Khan S., Lea P.J. and Keys A.J. 2002. Rubisco activity: effects of drought stress. Annals of Botany 89, 833–839.

Parry M.A.J, Delgado E., Vadell J., Keys A.J., Lawlor D.W. and Medrano H. 1993. Water-stress and the diurnal activity of ribulose-1,5-bisphosphate carboxylase in field-grown *Nicotiana tabacum* genotypes selected for survival at low CO_2 concentrations. Plant Physiology and Biochemistry 31, 113–120.

Parry M.A.J., Flexas J. and Medrano H. 2005. Prospects for crop production under drought: research priorities and future directions. Annals of Applied Biology 147, 211–218.

Parry M.A.J., Madgwick P.J., Carvalho J.F.C. and Andralojc P.J. 2007. Prospects for increasing photosynthesis by overcoming the limitations of Rubisco. Journal of Agricultural Sciences 145, 31–43.

Paul M.J. and Foyer C.H. 2001. Sink regulation of photosynthesis. Journal of Experimental Botany 52, 1383–1400.

Peterhänsel C., Hirsch H.J. and Kreuzaler F. 2008. Overexpression of C_4 pathway genes in the C_3 dicots potato, tobacco and *Arabidopsis*: experiences and future challenges. Pp. 163–174 in 'Charting new pathways to C_4 rice', ed. by J.E. Sheehy, P.L. Mitchel and B. Hardy. IRRI: Los Baños, Philippines.

Pfannschmidt T. 2003. Chloroplast redox signals: how photosynthesis controls its own genes. Trends in Plant Science 8, 33–41.

Portis A.R. 1995. The regulation of Rubisco by Rubisco activase. Journal of Experimental Botany 46, 1285–1291.

Portis A.R. 2003. Rubisco activase—Rubisco's catalytic chaperone. Photosynthesis Research 75, 11–27.

Prange R.K., McRae K.B., Midmore D.J. and Deng R. 1990. Reduction in potato growth at high temperature: role of photosynthesis and dark respiration. American Potato Journal 67, 357–369.

Price G.D., von Caemmerer S., Evans J.R., Siebke K., Anderson J.M. and Badger R.M. 1998. Photosynthesis is strongly reduced by antisense suppression of chloroplastic cytochrome *bf* complex in transgenic tobacco. Australian Journal of Plant Physiology 25, 445–452.

Raines C.A. 2003. The Calvin cycle revisited. Photosynthesis Research 75, 1–10.

Reynolds M.P., Ewing E.E. and Owens T.G. 1990. Photosynthesis at high temperature in tuber-bearing *Solanum* species. Plant Physiology 93, 791–797.

Sage R.F. and Kubien D.S. 2007. The temperature response of C_3 and C_4 photosynthesis. Plant Cell & Environment 30, 1086–1107.

Salvucci M.E. and Crafts-Brandner S.J. 2004. Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. Physiologia Plantarum 120, 179–186.

Seppänen M.M. and Coleman G.D. 2003. Characterization of genotypic variation in stress gene expression and photosynthetic parameters in potato. Plant Cell & Environment 26, 401–410.

Sharkey T.D. and Schrader S.M. 2006. High temperature stress. Pp. 101–129 in 'Physiology and molecular biology of stress tolerance in plants', ed. by K.V.M. Rao, A.S. Raghavendra and K.J. Reddy. Springer: Dordrecht.

Steffan K.L. and Palta J.P. 1996. Effect of light on photosynthetic capacity during cold acclimation in a cold-sensitive and a cold-tolerant potato species. Physiologia Plantarum 66, 353–359.

Stitt M., Gibon Y., Lunn J.E. and Piques M. 2007. Multilevel genomics analysis of carbon signalling during low carbon availability: coordinating the supply and utilisation of

carbon in a fluctuating environment. Functional Plant Biology 34, 526–549.

Takemiya A., Inoue S.I., Doi M., Kinoshita T. and Shimazaki K.I. 2005. Phototropins promote plant growth in response to blue light in low light environments. Plant Cell 17, 1120–1127.

Timlin D., Lutfor Rahman S.M., Baker J., Reddy V.R., Fleisher D. and Quebedeaux B. 2006. Whole plant photosynthesis, development, and carbon partitioning in potato as a function of temperature. Agronomy Journal 98, 1195–1203.

von Caemmerer S. 2000. Biochemical models of leaf photosynthesis. CSIRO Publishing: Melbourne.

Walters R.G. and Horton P. 1994. Acclimation of *Arabidopsis thaliana* to the light environment: changes in composition of the photosynthetic apparatus. Planta 195, 248–256.

Winkler E. 1971. Kartoffelbau in Tirol. II. Photosynthesevermoegen und respiration von verschiedenen kartoffelsorten. Potato Research 14, 1–18.

Wivutvongvana M. 1979. Physiological response of heat tolerant and heat sensitive potatoes (*Solanum* species). PhD dissertation, Cornell University, Ithaca, New York, USA.

Wolf J. and van Oijen M. 2003. Model simulation of effects of changes in climate and atmospheric CO_2 and O_3 on tuber yield potential of potato (cv. Bintje) in the European Union. Agriculture, Ecosystems & Environment 94, 141–157.

Wolf S., Olesinski A.A., Rudich J., Marani A. 1990. Effect of high temperature on photosynthesis in potatoes. Annals of Botany 65, 179–185.

Yamaguchi M., Timm H. and Spurr A.R. 1964. Effects of soil temperature on growth and nutrition of potato plants, and tuberization, composition and periderm structure of tubers. Proceedings of the American Society for Horticultural Sciences 84, 412–423.

Yang Y., Jiang D-A., Xu H-X., Yan C-Q. and Hao S-R. 2006. Cyclic electron flow around photosystem I is required for adaptation to salt stress in wild soybean species *Glycine curtiloba* ACC547. Biologia Plantarum 50, 586–590.

Additional references

Leakey A.D.B., Ainsworth E.A., Bernacchi C.J., Zhu X-G., Long S.P. and Ort D.R. 2012. Photosynthesis in a CO_2-rich atmosphere. Pp. 733–768 in 'Photosynthesis: plastid biology, energy conversion and carbon assimilation, Advances in Photosynthesis and Respiration Vol. 34', ed. by J.J. Eaton-Rye, B.C. Tripathy and T.D. Sharkey. Springer: Dordrecht, The Netherlands.

Gururani M.A., Upadhyaya C.P., Strasser R.J., Woong Y.J. and Park S.W. 2012. Physiological and biochemical responses of transgenic potato plants with altered expression of PSII manganese stabilizing protein. Plant Physiology and Biochemistry 58, 182–194.

Chen C-T. and Setter T.L. 2012. Response of potato dry matter assimilation and partitioning to elevated CO_2 at various stages of tuber initiation and growth. Environmental and Experimental Botany 80, 27–34.

Legume productivity and photosynthetic responses anticipated with climate change—insights from lupins

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Summary

- Legume crops, including narrow-leafed lupin, symbiotically fix nitrogen and provide high-protein vegetables and grains.
- Soybean, pea and narrow-leafed lupin have shown both nitrogen fixation and grain yield responses to higher levels of CO_2.
- Cowpea has genetic variation for heat tolerance at the floral bud and pod set growth stages, with heat stress tolerant vegetable and grain types bred and released as cultivars; this might be expected in other legumes.
- The rate of net photosynthesis in narrow-leafed lupin is higher than in wheat, but lupin is more sensitive to water deficits and shade.
- Pre-anthesis growth of lupin is slow in low-nitrogen soils because substantial amounts of the daily assimilated carbon are allocated to the nodulated roots to support nitrogen fixation.
- Nitrate supply to lupin does not improve pre-anthesis growth, but reduces nitrogen fixation.
- Elevated CO_2 increases biomass and the nitrogen fixed from the atmosphere in lupin under terminal drought.
- The carbon 'cost' of fixing N_2 in nodules is high but varies significantly among symbioses.
- Both plant and bacterial traits determine the 'cost'.
- Lupin nitrogen fixation declines during post-anthesis because there is competition for photosynthate, which is directed to branch growth and grain-filling.
- In lupin, translocation of assimilates, and particularly nitrogenous solutes, may limit grain yield and depress harvest index.
- Conservation of translocated carbon by refixing respired CO_2 within developing legume pods could equate to as much as 20% of grain yield.

Introduction

Legume species are unique among major crops in their capacity to fix nitrogen (N) from the air (as N_2) via symbiosis with rhizobial bacteria. Nitrogen fixation is driven by supply of photosynthates to root nodules containing rhizobia, from soil or inoculum sources of root infection (Sinclair 2004). This enables legumes to be self-sufficient in nitrogen requirements in the absence of severe abiotic stresses, and to produce high-protein grains for nutritious foods—a major protein source in developing countries. Higher temperatures, drier environments and increased atmospheric CO_2 are predicted with climate

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change in southern Australia and most temperate and subtropical zones. An increase in atmospheric CO_2 concentration results in increased photosynthesis and hence in nitrogen fixation (Luscher and Nosberger 1997; Cabrerizo et al. 2001; Rogers et al. 2006). Thus, legume crops may benefit from this aspect of global change, as well as from increasing nitrogen residues in the soil for following cereal crops.

There has been little investigation of photosynthesis in legume crops except for soybean; hence, whole-plant responses are reported. Genetic variation in whole-plant responses to stress and to higher atmospheric CO_2 concentrations may indicate underlying variation in photosynthetic and carbon-delivery systems. This may indicate potential direction for research on photosynthesis in legumes, with implications for adaptation to global change.

Response to atmospheric CO_2 concentration

Soybean cultivars released in Canada showed yield increases of 0.47% per year associated with date of release over 58 years. Cultivars also showed increases in harvest index, photosynthetic rate per leaf area (0.52% per year) and stomatal conductance (0.48% per year), but reduced leaf area index (Morrison et al. 1999). Their review of various studies reported genetic variation for single-leaf photosynthesis in soybean and leaf thickness association with increased area-based photosynthesis, but inconsistent associations between yield and photosynthesis.

Higher CO_2 concentration correlated with increase in photosynthesis by soybean cultivar Bragg, and associated increased leaf area index and canopy cover, but there was no response in Rubisco levels in a field study (Campbell et al. 1990). Free-air carbon dioxide enrichment (FACE) with soybean (from canopy closure to senescence) showed increased yield and decreased stomatal conductance associated with reduced evapotranspiration, which resulted in improved water-use efficiency (Bernachi et al. 2006).

In peas, plant growth and nodule biomass increased with CO_2 enrichment. Moreover, nodules of plants grown at increased CO_2 showed a higher sugar content and improved nodule carbon metabolism (Cabrerizo et al. 2001). Nitrogen fixation increased on a plant basis because of larger nodules, although specific nitrogen-fixing activity was not increased, possibly because both carbon and oxygen supply need to be non-limiting. Kimball et al. (2002) cite clover with a 38% dry weight growth response to higher CO_2 with FACE at low soil nitrogen. In contrast, grasses had only 4% dry weight growth response at low nitrogen. Thus legumes have an advantage, influenced by both interspecific and intraspecific variation for nitrogen fixation, in meeting an increased plant nitrogen demand at elevated CO_2.

A meta analysis review across 111 studies of soybean responses to increased CO_2 showed more than 50% increase in canopy photosynthesis associated with increased leaf-level CO_2 assimilation, decreased stomatal conductance and increased leaf area (Ainsworth et al. 2002). Gains in total dry weight and grain yield were lower. Photosynthesis was increased in soybean (cv. Bragg) at a range of temperatures with elevated CO_2, with a maximum at maximum/minimum daily temperatures of 32/22°C and a decline above 40/30°C. However, starch and sucrose responses were highest at 36/26 – 40/30°C (Vu et al. 2001). In the same study, Rubisco midday activity and protein content decreased only at temperatures above 40/30°C.

A molecular study of soybean response to FACE enhancement of CO_2 concentration found increased levels of 132 transcripts for cell growth and cell proliferation in growing leaves (Ainsworth et al. 2006). The study suggested that the 327 CO_2-responsive genes stimulated respiratory breakdown of carbohydrates, thus providing energy and precursors for leaf expansion and growth. The FACE approach is canvassed by Ainsworth et al. (2008) for screening quantitative trait locus (QTL) populations for mapping of markers for photosynthetic traits, searching for novel genes for responsiveness to increased CO_2, and identifying parents for breeding programs. The FACE approach has the potential to help identify photosynthetic variation in legumes.

Genetic variation

Genetic variation exists in legumes for key expressions interacting with photosynthesis: nitrogen fixation (Phillips 1980; Herridge et al. 1991), high temperature (e.g. cowpea in Ehlers et al. 2000, common bean in Agtunong et al. 1991) and drought (Sinclair et al. 2000). Austin (1989) reviewed genetic variation across species including peas and soybeans for photosynthesis at light saturation. Selection for increased maximum photosynthetic capacity (P_{max}) at light saturation in both maize and soybean was effective, but did not result in increased grain yield.

Wild wheats, with higher P_{max} than wheat cultivars, can provide novel genetic variation to breeders. Wild relatives of wheat and rice have higher chlorophyll *a* : *b* ratios with greater adaptation to high light. Additionally, staygreen expressions increased the photosynthetic rate per leaf area but were dependent on nitrogen supply.

Genetic variation in these key interacting expressions may be indicators of genetic variation in photosynthetic systems, and of targets for research. Because of the genetic bottleneck associated with domestication of most crops, there is a high likelihood that more extensive variation may be found in the germplasm of wild relatives (Austin 1989). These germplasms have yet to be screened for legume crops, and since many wild relatives still survive in stress-prone environments, further investigation is suggested.

High-temperature tolerance

Tolerance of reproductive heat stress at maximum/minimum daily temperatures of 41/24°C has been identified in cowpea *Vigna unguiculata* germplasm under both long (Patel and Hall 1990) and short days (Ehlers and Hall 1998). Agtunong et al. (1991) identified reproductive heat stress tolerance at 34/29°C in two Mexican cultivars of common bean *Phaseolus vulgaris*. Such genetic variation is likely to be found in various legume crops, especially those that evolved in high-temperature environments.

In cowpea, pod set is sensitive to heat in the last 6 hours of the night, to a greater extent under long days than short days (Mutters et al. 1992). Degeneration of tapetal cells associated with development of pollen grain causes low pod set under high night temperature (Ahmed et al. 1992). Thus, heat effects on reproduction may differ between long- and short-day screening, and genotypes vary in sensitivity at the floral bud stage and at the flowering and pod set stages. Of 268 accessions screened at 41/24°C maximum/minimum daily temperatures under long days, two with heat tolerance at both floral development and pod set were Prima and Tvu4552 from Nigeria. Under short days, the heat-tolerant accessions were TN88-63 from Niger and B89-600 from Senegal (Ehlers and Hall 1998).

Heat tolerance during floral bud development appears to be controlled by a single recessive gene, but possibly two genes during pod set (Marfo and Hall 1992; Hall 1993). An indirect method of screening for high-temperature tolerance is to select for low leaf electrolyte leakage (Ismail and Hall 1999). This method assisted in the breeding of a heat-tolerant cultivar (Ehlers et al. 2000; Thiaw and Hall 2004). A dwarfing gene was found to be linked to the gene for heat tolerance at the floral bud stage, resulting in higher harvest index under heat stress but not under normal conditions (Ismail and Hall 2000). In addition, an unlinked delayed leaf senescence trait can add to the expression of heat tolerance (Ismail and Hall 2000). Vegetable type cowpeas of dwarf bush habit, with heat tolerance at floral bud and pod set stages, have also been bred in India (Patel and Hall 1986). Further, Ahmed et al. (1993) showed that a heat-tolerant line of cowpea was more responsive to elevated CO_2 than a heat-sensitive line for pod production under high temperature.

Genetic variation for area-based photosynthesis in legumes has not had much attention except in cowpea and soybean. Some scope has been shown in common beans. Further research with other major pulses and their wild relatives could be very useful.

Lupin case study

Lupin, mainly narrow-leafed lupin *Lupinus angustifolius*, is the most important grain legume crop in Australia and a vital component of the sustainable farming system of the Mediterranean climatic region of southern Australia. Relative to wheat, lupin has an area-based photosynthetic rate that is 80% higher than that of wheat when soil water is adequate (Henson et al. 1988). At flowering of both lupin and wheat, canopy photosynthesis in lupin is higher than in wheat (J. Palta and C. Ludwig, unpublished data 1998). However, the rate of net photosynthesis in lupin is more sensitive to water deficits than the rate of net photosynthesis in wheat.

Pre-anthesis growth of lupin is slow, accounting for almost half the pre-anthesis growth of wheat, despite its much higher rate of net photosynthesis. This is because the lupin plant allocates 62% of the daily assimilated carbon to the roots at the five-leaf stage, and 51% at floral initiation (Palta et al. 2008). Wheat has a more abundant root system than lupin (Dracup et al. 1992), but lupin allocates more assimilated carbon to the roots. Wheat allocates 42% of its assimilated carbon to roots before tillering and 20% at floral initiation (Gregory and Atwell 1991; Palta and Gregory 1997). Root and nodule respiration in lupin uses 72–80% of the assimilated carbon

allocated to the roots (Palta et al. 2008). This high use of assimilated carbon in respiration of nodulated roots of lupin is mainly in support of nitrogen fixation. Nitrogen fixation is an expensive process. Nodulated roots of lupin used 5.0 – 6.5 g carbon (C) to fix 1 g of nitrogen (Pate and Herridge 1978). As reported for soybean (Arrese-Igor et al. 1997), nitrate supply to lupin did not show any improvement in pre-anthesis growth, but reduced nitrogen fixation through an inhibition in root nodule growth (J. Palta, unpublished data).

Measures are being sought to improve pre-anthesis growth in lupin because genetic variation exists in lupin for early growth. Selecting lupin cultivars with vigorous seedling growth is probably one option, since this has been shown to increase biomass accumulation and yield in wheat, particularly in drier environments and seasons (Rebetzke and Richards 1999; Richards and Lukacs 2002). A possible advantage of improving early growth in narrow-leafed lupin may be the provision of a carbon store for subsequent pod development and grain-filling during periods of water shortage after flowering (Palta et al. 2008). However, consideration should be given to preventing a reduction in the nitrogen-fixing capacity of narrow-leafed lupin while improving early growth.

Elevated CO_2 (700 µmol/mol) under adequate soil water increases pre-anthesis growth of lupin by only 5–7%, but increases the nitrogen fixed from the atmosphere by 22 to 27% (J. Palta and C. Ludwig, unpublished data). Exposure to elevated CO_2 and terminal drought after anthesis increases total biomass and grain yield in lupin by 42–45% and the nitrogen fixed from the atmosphere by 35–40% (J. Palta and C. Ludwig, unpublished data). Of the nitrogen remaining in the plant at maturity, 49% can be recovered in the grain, 40% as other above-ground residues and 10% in the roots (approximately totalling 100%).

Lupin is sensitive to changes in ambient temperature, particularly during pod filling. Ambient temperatures of more than 27°C can promote floret sterility and hence reduce grain yield (Biddiscombe 1975; Downes and Gladstones 1984). Episodes of 6 hours at 34, 36 or 38°C can reduce grain size by 12%. Average seed size decreases by 2% for each hour that the pods spend at temperatures greater than 35°C (Reader et al. 1997).

Lupin is also sensitive to end-of-season drought and terminal drought, which are common features of the lupin cropping regions of Australia. Terminal drought occurs when rainfall decreases and evaporation increases in the spring, when lupin enters its reproductive stage (Fitzpatrick 1970; Reader et al. 1995). Current assimilation in narrow-leafed lupin is very sensitive to water deficit (Turner and Henson 1989). Such sensitivity causes the end of vegetative growth of the apical branches and the end of reproductive growth (French and Turner 1991; Palta and Plaut 1999). This ending of both vegetative and reproductive growth causes most of the yield reduction and variability (Palta and Dracup 1994; Dracup et al. 1998; Palta and Plaut 1999). Yield under terminal drought is often reduced through pod and seed abortion (Palta and Ludwig 1996, 2000). Varietal selection has ensured early flowering in narrow-leafed lupin (Gladstones 1994), providing more time for pod filling before the severe effects of drought on carbon assimilation occur (Palta et al. 2004). Terminal drought escape is characteristic of modern narrow-leafed lupin cultivars such as Belara, Quinilock and Mandelup, with early flowering and podding and higher rates of seed-filling than in other cultivars.

Terminal drought escape may limit yield improvement because grain yield in narrow-leafed lupin is limited by photosynthetic carbon and there is little capacity to store and remobilise carbon to the grain (Palta and Ludwig 2000). Finishing the growing season early limits the time available for accumulating biomass, generating a tension between drought escape and maximising source potential. The yield of narrow-leafed lupin is source-limited, not sink-limited (Palta and Ludwig 2000). Increasing the number of pods (sink size) by applying cytokinin (Atkins and Pigeaire 1993; Palta and Ludwig 1996) does not necessarily increase the grain yield, because many pods fail to fill their seeds (Palta and Ludwig 1996). However, increasing the availability of carbon assimilates (source) by exposing plants with more pods (large sink) to elevated CO_2 during pod filling increases grain yield by 42% (Palta and Ludwig 2000). Pod filling is almost entirely dependent on current assimilation (Pate et al. 1980) rather than on pre-anthesis stored carbon (Palta and Ludwig 2000). Grain yield is limited by the availability of carbon assimilates after pod set (Palta and Ludwig 1996, 2000), which causes pod and seed abortion mainly because narrow-leafed lupin does not store or use enough reserves to support reproductive growth under shortages of carbon assimilates (J. Palta and C. Ludwig, unpublished data; Berger et al. 2008). This effect is apparent in some environments

and some seasons where lupin crops abort young pods (≤20 mm) after 2–3 successive overcast days (≤430 µmol/m²/s, photosynthetically active radiation) (Berger et al. 2008). The limited use of reserves may be due to an anatomical constriction between stems and pods (C. Atkins, pers. comm.). The failure to fill extra pods arises from a limited capacity to accumulate carbon in vegetative parts for remobilisation to the developing grain (Palta et al. 2000).

Photosynthesis in relation to carbon and nitrogen delivery

Legumes have the ability to form symbiotic relationships with soil-borne bacteria known collectively as 'rhizobia' and so can fix atmospheric nitrogen. These traits have special significance for photosynthesis. Nitrogenase activity in the root nodules that house the bacteria is a particularly energy-demanding reaction that derives reductant and ATP from the respiration of sugar translocated in phloem from the leaves. Nitrogen fixation is thus a significant sink for the allocation of photosynthate during vegetative growth, and more significantly, at a time when grain development and filling is in progress. Because grain protein synthesis depends on fixed nitrogen at a time when carbon allocation to the process in nodules may be restricted, there is a complex carbon–nitrogen interrelationship with regulation imposed by changes in translocation to these competing sinks for carbon.

Symbioses in different crop legumes have different demands on photosynthesis for fixing and assimilating nitrogen; this varies during vegetative and reproductive phases of growth (Atkins 1984). In white lupin *Lupinus albus*, for example, 4.5 g C is consumed in nodules prior to anthesis for every 1 g N fixed and exported in the transpiration stream to the leaves. This represents 21.8% of the net photosynthetic carbon fixation by the plant. After anthesis and during grain-filling, the amount of carbon used is increased slightly to 5 g/g N fixed, but the proportion of carbon allocated to the nodules falls to 10% of the plant's production. Measurements made with a different legume, cowpea *Vigna unguiculata*, indicate that only 2.3 g C is required per 1 g N fixed, accounting for just 10% of photosynthesis prior to anthesis, and 2 g C and 2.7%, respectively, during grain-filling.

Several factors together result in cowpea's ability to fix nitrogen at around half the 'cost' compared with lupin (reviewed in Atkins 1986). Lupin nodules (as well as those of pea, chickpea, faba bean and most temperate forage legumes) are indeterminate and have a persistent meristem, while those of cowpea (as well as those of soybean and common bean) are determinate. The indeterminate types form the amides glutamine and asparagine as translocated products of fixation. These amides require roughly twice as much carbon in their synthesis as the ureides allantoin and allantoic acid, which are formed as products of nitrogen assimilation in nodules of the determinate type. Measurements of CO_2 fixation by nodules show that these types of symbioses also vary in the levels and activity of phosphoenol pyruvate carboxylase (PEPC) so that cowpea nodules, for example, conserve more of the respired CO_2 than lupin nodules.

Symbioses also differ in their abilities to oxidise the hydrogen gas that is formed concomitantly with N_2 reduction and as an inevitable consequence of nitrogenase chemistry in the microsymbiont. Lupins, for example, form symbioses in Western Australia with strains of rhizobia that are hydrogen uptake negative (hup^-); that is, they do not reassimilate the H_2. In cowpea (and also soybean), both hup^- and hydrogen uptake positive (hup^+) strains of rhizobia form nodules. Measurements of the carbon economy of these two types of symbiosis in cowpea indicate that those with a hup^+ microsymbiont require 36% less carbon for the same amount of nitrogen fixed during vegetative growth and 16% less over the whole growth cycle (Rainbird et al. 1983). While H_2 evolution from nodules may appear to be a process that is wasteful of the plant's photosynthate resource, there is some evidence that H_2 production benefits soil fertility and may be a factor for the positive effects of legumes in crop rotations (Dong et al. 2003; Peoples et al. 2008). Unique H_2-oxidising bacterial isolates collected from the rhizosphere of legume nodules evolving H_2 stimulate growth of *Arabidopsis thaliana* in culture and offer a possibility for developing beneficial inocula (Maimaiti et al. 2007).

While many aspects that relate to the efficiency with which nodules function have been documented, there has been little research to exploit such traits in improving legume productivity.

Source–sink relationships

The processes that link photosynthesis in source organs (the leaves) with the demands of sink organs for carbon (roots and nodules, and fruits and seeds)

are those of transport and translocation in phloem. Similarly, distribution of the nitrogenous products formed in nodules depends on transport and translocation in both xylem and phloem. Both xylem and phloem translocation contribute to fruit and seed development, but it is the phloem where photosynthates (sugars) and nitrogenous solutes (amides or ureides) travel together to provide most of the nutrition during the critical period of grain-filling. While these simple statements hide a multitude of component processes that together are described loosely as the source–sink relationships of the plant, it is these relationships that determine yield and harvest index. In many crop legumes both yield and harvest index are low and variable. While the sites and mechanisms for regulating translocation are poorly understood it seems reasonable to ask whether translocation to the reproductive organs, especially during periods of grain-filling, limits yield and harvest index in legumes. A second, related, question is whether a translocation limitation applies to carbon, nitrogen, or both.

In lupin, as is the case in most grain legumes, most flowers abort and abscise post-anthesis. However, it has proved possible to greatly increase the number of flowers that set pods by applying cytokinin to the basal flower parts around anthesis (Atkins and Pigeaire 1993). This increased 'sink' for assimilates does not result in higher grain yield, as most of the extra pods do not fill their seeds. As mentioned, the onset of grain-filling requires significant translocated carbon and, probably as a consequence, nitrogenase activity and the availability of fixed nitrogen declines after anthesis. To overcome this problem, the lupin plants that had initially set many more pods were supplemented with fertiliser (urea) nitrogen during grain-filling (Ma et al. 1998). Despite increasing the total pool of assimilated nitrogen in the vegetation of the plant by up to 43%, sufficient to provide nitrogen for twice as many seeds than plants reliant on current nitrogen fixation alone, this potential was not translated into increased seed yield. Interestingly, cytokinin treatment also caused the raceme tissues bearing the developing pods to increase by as much as five times in dry weight and they accumulated up to 10 times the levels of nitrogen compared with racemes in non-fertilised plants. While these data do not address the question of improving phloem delivery of carbon as well as nitrogen, it is likely that translocation is limited close to the developing fruit, possibly at the pedicel.

A detailed structural analysis of the translocatory elements in the pedicel of lupin was made many years ago (Pate et al. 1978). The authors concluded that the number and dimensions of phloem elements were sufficient to account for the rates of specific mass transfer predicted by the rate of growth of the fruit. However, how these processes are regulated and whether genotypes can be developed with higher rates of phloem mass transfer might be a useful approach to realising yield gains unlocked by specific increases in the plant's net photosynthate supply.

Role of pods in legumes

Typically, legume seeds develop within a closed pod, which has restricted exchange of gases with the outer atmosphere and as a consequence CO_2 levels are as high as 1.5% (v/v) (Flinn et al. 1977). The pod walls are green and in species like peas both the outer tissue and inner epidermis can fix CO_2 at higher rates in the light than in the dark. A significant portion of the CO_2 released in respiration by the seed is 'refixed' in the inner epidermis. In pea, the amount of carbon conserved in this way is equivalent to a carbon content of 0.5 – 1 seed per pod and so could be equated with as much as 10–20% of grain yield (Flinn et al. 1977). In chickpea, the pod walls are considerably thinner but here stomatal frequency is low and ventilation reduced, permitting accumulation of respired CO_2 and its refixation by the pod mesocarp (Ma et al. 2001). Importantly, the data for chickpea indicate that refixation of respired carbon may be more significant for grain-filling under conditions of water stress. Pod and seed structural traits vary markedly among species and in lupin, for example, the cotyledons remain photosynthetically competent for most of the period of seed development. Cotyledons also refix respired CO_2 and contribute to grain-filling, according to ^{14}C-labelling studies (Atkins and Flinn 1978).

Proposed research in legumes

We propose the following research:

- screening of wild relatives for genetic variation in photosynthetic systems under high-temperature stress and under elevated atmospheric CO_2 concentrations (glasshouse initially, then at the FACE facility at Horsham)
- assessment of the impact of the interactive effect of CO_2 and temperature on high-yielding traits

- evaluation of the interactive effect of CO_2 and temperature on the source–sink relationships
- research to address whether translocation limitation applies to carbon, nitrogen, or both
- genetic studies with domesticated and wild germplasm for identification of molecular markers for carbon and nitrogen translocation and for photosynthetic mechanisms
- carbon availability and climate change effects on nitrogen fixation and seed-filling
- research to assess the role of photosynthesis by pods in conserving respired C and contributing to grain yield.

References

Agtunong T.P., Redden R., Mengge-Nang M.A., Searle C. and Fukai S. 1991. Genotypic variation in response to high temperature at flowering in bean (*Phaseolus vulgaris* L.) Australian Journal Experimental Agriculture 32, 1135–1140.

Ahmed F.E., Hall A.E. and DeMason D.A. 1992. Heat injury during floral development in cowpea (*Vigna unguiculata*, Fabaceae). American Journal of Botany 79, 784–791.

Ahmed F.E., Hall A.E. and Madore M.A. 1993. Interactive effects of high temperature and elevated carbon dioxide concentration on cowpea [*Vigna unguiculata* (L.) Walp.]. Plant, Cell and Environment 16, 835–842.

Ainsworth E.A., Davey P.A., Bernacchi C.J., Dermody O.C., Heaton E.A., Moore D.J., et al. 2002. A meta-analysis of elevated CO_2 effects on soybean (*Glycine max*) physiology, growth and yield. Global Change Biology 8, 695–709.

Ainsworth E.A., Rogers A., Vodkin L.O., Walter A. and Schurr U. 2006. The effects of elevated CO_2 concentration on soybean gene expression, an analysis of growing and mature leaves. Plant Physiology 142, 135–147.

Ainsworth E.A., Beier C., Calfapietra C., Ceulemans R., Durand-Tardif M., Farquhar G.D., et al. 2008. Next generation of elevated CO_2 experiments with crops: a critical investment for feeding the future world. Plant, Cell and Environment 31, 1317–1324.

Arrese-Igor C., Minchin F.R., Gordon A.J. and Nath A.K. 1997. Possible causes of the physiological decline in soybean nitrogen fixation in the presence of nitrate. Journal of Experimental Botany 309, 905–913.

Atkins C.A. 1984. Efficiencies and inefficiencies in the legume/*Rhizobium* symbiosis—a review. Plant and Soil 82, 273–284.

Atkins C.A. 1986. The legume: *Rhizobium* symbiosis—limitations to maximising nitrogen fixation. Outlook on Agriculture 15, 128–134.

Atkins C.A. and Flinn A.M. 1978. CO_2 fixation in the carbon economy of developing seeds of *Lupinus albus* L. Plant Physiology 62, 486–490.

Atkins C.A. and Pigeaire A. 1993. Application of cytokinins to flowers to increase pod set in *Lupinus angustifolius*. Australian Journal of Agricultural Research 44, 1799–1819.

Austin R.B. 1989. Genetic variation in photosynthesis. The Journal of Agricultural Science 112, 287–294.

Berger J.D., Buirchell B., Palta J.A., Ludwig C. and Shrestha D. 2008. G × E analysis of narrow-leafed lupin historical trials indicates little specific adaptation among Australian cultivars. Pp. 317–321 in 'Lupins for health and wealth', ed. by J.A. Palta and J.D. Berger. ILA Press: Canterbury.

Bernacchi C.J., Kimball B.A., Devin R., Quarles D.A., Long S.P. and Ort D.A. 2006. Decreases in stomatal conductance of soybean (*Glycine max*) under open-air elevation of CO_2 are closely coupled with decreases in ecosystem evapotranspiration. Plant Physiology 143, 134–144.

Biddiscombe E.F. 1975. Effect of moisture stress on flower drop and seed yield of narrow-leafed lupin (*Lupinus angustifolius* L. cv. Unicrop). Journal of the Australian Institute of Agricultural Science 46, 70–72.

Cabrerizo P.M., González E.M., Aparicio-Tejo P.-M. and Arrese-Igor C. 2001. Continuous CO_2 enrichment leads to increased nodule biomass, carbon availability to nodules and activity of carbon-metabolising enzymes but does not enhance specific nitrogen fixation in pea. Physiologia Plantarum 113, 33–40.

Campbell W.J., Allen I.H. Jr and Bowes G. 1990. Response of soybean canopy photosynthesis to CO_2 concentration, light, and temperature. Journal of Experimental Botany 41, 427–433.

Dong Z., Wu L., Kettlewell B., Caldwell C.D. and Layzell D.B. 2003. H_2 fertilization of soils—is this a benefit of legumes in rotation? Plant, Cell and Environment 26, 1875–1879.

Downes R.W. and Gladstones J.S. 1984. Physiology of growth and seed production in *Lupinus angustifolius* L. I. Effects on pod and seed set of controlled short duration high temperatures at flowering. Australian Journal of Agricultural Research 35, 493–499.

Dracup M., Belford R.K. and Gregory P.J. 1992. Constraints to root growth of wheat and lupin crops in duplex soils. Australian Journal of Experimental Agriculture 32, 947–961.

Dracup M., Reader M. and Palta J.A. 1998. Timing of terminal drought is an important cause of yield variability in lupin grown on duplex soils in southern Australia. Australian Journal of Agricultural Research 49, 799–810.

Ehlers J.D. and Hall A.E. 1998. Heat tolerance of contrasting cowpea lines in short and long days. Field Crops Research 55, 11–21.

Ehlers J.D., Hall A.E., Patel P.N., Roberts P.A. and Matthews W.C. 2000. Registration of 'Californian Blackeye 27' cowpea. Crop Science 40, 853–854.

Fitzpatrick E.A. 1970. The expectancy of deficient winter rainfall and the potential for severe drought in the southwest of Western Australia. 'Miscellaneous Publication

70/1'. Institute of Agriculture, Agronomy Department, University of Western Australia: Nedlands.

Flinn A.M., Atkins C.A. and Pate J.S. 1977. The significance of photosynthetic and respiratory exchanges in the carbon economy of the developing pea fruit. Plant Physiology 60, 412–418.

French R.J. and Turner N.C. 1991. Water deficits change dry matter partitioning and seed yield in narrow-leafed lupins (*Lupinus angustifolius* L.). Australian Journal of Agricultural Research 42, 471–484.

Henson, I.E., Jensen C.R. and Turner N.C. 1988. Leaf gas-exchange and water relations of lupins and wheat. II. Influence of leaf age, light environment at high soil water content. Australian Journal of Plant Physiology 16, 414–429.

Herridge D.F., Rose I.A., Holland J.F. and Redden R.J. 1991. Selection and breeding of legumes for enhanced nitrogen. Pp. 139–140 in 'Ninth Australian Nitrogen Fixation Conference', ed. by A.E. Richardson and M.B. Peoples. Australian Society for Nitrogen Fixation: Melbourne.

Gladstones J.S. 1994. An historical review of lupins in Australia. Pp. 1–38 in 'Proceedings of the First Australian Lupin Technical Symposium', ed. by M. Dracup and J.A. Palta. Western Australian Department of Agriculture: Perth.

Gregory P.J. and Atwell B.J. 1991. The fate of carbon in pulse-labelled crops of barley and wheat. Plant and Soil 136, 205–213.

Hall A.E. 1993. Physiology and breeding for heat tolerance in cowpea, and comparisons with other crops. Pp. 271–284 in 'Adaptation of food crops to temperature and water stress', ed. by C.G. Kuo. Asian Vegetable Research and Development Center: Taipei.

Ismail A.M. and Hall A.E. 1999. Reproductive-stage heat tolerance, leaf membrane thermostability and plant morphology in cowpea. Crop Science 39, 1762–1768.

Ismail A.M. and Hall A.E. 2000. Semidwarf and standard-height cowpea responses to row spacing in different environments. Crop Science 40, 1618–1623.

Kimball B.A., Kobayashi K. and Bindi M. 2002. Responses of agricultural crops to free-air CO_2 enrichment. Advances in Agronomy 77, 293–368.

Luschner A. and Nosberger J. 1997. Interspecific and intraspecific variability in the response of grasses and legumes to free-air carbon enrichment. Acta Oecologia 18, 269–275.

Ma Q., Behboudian M.H., Turner N.C. and Palta J.A. 2001. Gas exchange by pods and subtending leaves and internal recycling of CO_2 by pods of chickpea (*Cicer arietinum* L) subjected to water deficits. Journal of Experimental Botany 52, 123–131.

Ma Q., Longnecker N. and Atkins C.A. 1998. Exogenous cytokinin and nitrogen do not increase grain yield in narrow-leafed lupin. Crop Science 38, 717–721.

Maimaiti J., Zhang Y., Yang J., Cen Y-P., Layzell D.B. and Peoples M. 2007. Isolation and characterization of hydrogen-oxidizing bacteria induced following exposure of soil to hydrogen gas and their impact on plant growth. Environmental Microbiology 9, 435–444.

Marfo K.O. and Hall A.E. 1992. Inheritance of heat tolerance during pod set in cowpea. Crop Science 32, 912–918.

Morrison M.J., Voldeng H.D., Elroy R. and Cober E.R. 1999. Physiological changes from 58 years of genetic improvement of short-season soybean cultivars in Canada. Agronomy Journal 91, 685–689.

Mutters R.G. and Hall A.E. 1992. Reproductive responses of cowpea to high night temperatures during different night periods. Crop Science 32, 202–206.

Palta J.A., Berger J.D. and Ludwig C. 2008. The growth and yield of narrow leafed lupin: myths and realities. 12th International Lupin Conference, Canterbury, New Zealand, 1, pp. 20–25.

Palta J.A. and Dracup M. 1994. Water deficits in narrow-leafed lupin: Implications for seed yield. Pp. 288–291 in 'Proceedings of the first Australian Lupin Technical Symposium', ed. by M. Dracup and J.A. Palta. Western Australian Department of Agriculture: Perth.

Palta J.A., Dracup M. and Ludwig C. 2000. Retranslocation of C and N to lupin pods as influenced by terminal drought. P. 74 in 'Proceedings of plant form and function. Adaptations to stress symposium', ed. by T.L. Bell and A.J. Smit. University of Western Australia: Perth.

Palta J.A. and Gregory P.J. 1997. Drought affects the fluxes of carbon to roots and soil in ^{13}C, pulse-labelled plants of wheat. Soil Biology and Biochemistry 29, 1395–1403.

Palta J.A. and Ludwig C. 1996. Pod set and seed yield as affected by cytokinin application and terminal drought in narrow-leafed lupin. Australian Journal of Agricultural Research 48, 81–90.

Palta J.A. and Ludwig C. 2000. Elevated CO_2 during pod-filling increased seed yield but not harvest index in indeterminate narrow-leaf lupin. Australian Journal of Agricultural Research 51, 279–286.

Palta J.A. and Plaut Z. 1999. Yield and components of seed yield of narrow-leafed lupin (*Lupinus angustifolius* L.) subjected to transient water deficit. Australian Journal of Agricultural Research 50, 1225–1232.

Palta J.A., Turner N.C. and French R.J. 2004. The yield performance of lupin genotypes under terminal drought in a Mediterranean-type environment. Australian Journal of Agricultural Research 55, 449–459.

Pate J.S., Atkins C.A. and Perry M.W. 1980. Significance of photosynthate produced at different stages of growth as carbon source for fruit filling and seed reserve accumulation in *Lupinus angustifolius* L. Australian Journal of Plant Physiology 7, 283–297.

Pate J.S. and Herridge D.F. 1978. Partitioning and utilization of net photosynthate in a nodulated legume. Botany 29, 401–412.

Pate J.S., Kuo J. and Hocking P.J. 1978. Functioning of the conducting elements of phloem and xylem in the stalk

of the developing fruit of *Lupinus albus* L. Australian Journal of Plant Physiology 5, 321–326.

Patel P.N. and Hall A.E. 1986. Registration of snap-cowpea germplasms. Crop Science 26, 207–208.

Patel P.N. and Hall A.E. 1990. Genotypic variation and classification of cowpea for reproductive responses to high temperature and long photoperiods. Crop Science 30, 614–621.

Peoples M.B., McLenna P.D. and Brockwell J. 2008. Hydrogen emission from nodulated soybeans (*Glycine max* (L.) Merr.) and consequences for the productivity of subsequent maize (*Zea mays* L.) crop. Plant Soil 307, 67–82.

Phillips D.A. 1980. Efficiency of symbiotic nitrogen fixation in legumes. Annual Review of Plant Physiology 31, 29–49.

Rainbird R.M., Atkins C.A., Pate J.S. and Sanford P. 1983. The significance of hydrogen evolution in the carbon and nitrogen economy of nodulated cowpea. Plant Physiology 71, 122–127.

Reader M.A., Dracup M. and Atkins C.A. 1997. Transient high temperatures during seed growth in narrow-leafed lupin (*Lupinus angustifolius* L.) II. Injuriously high pod temperatures are likely in Western Australia. Australian Journal of Agricultural Research 48, 1179–1186.

Reader M.A., Dracup M. and Kirby E.J.M. 1995. Time to flowering in narrow-leafed lupin. Australian Journal of Agricultural Research 46, 1063–1077.

Rebetzke G.J. and Richards R.A. 1999. Genetic improvement of early vigour in wheat. Australian Journal of Agricultural Research 50, 291–301.

Richards R.A. and Lukacs Z. 2002. Seedling vigour in wheat—sources of variation for genetic and agronomic improvement. Australian Journal of Agricultural Research 53, 41–50.

Rogers A., Gibon Y., Stitt M., Morgan P.B., Bernacchi C.J., Ort D.R. and Long S.P. 2006. Increased carbon availability at elevated carbon dioxide concentration improves N assimilation in a legume. Plant, Cell and Environment 29, 1651–1658.

Sinclair T.R. 2004. Increasing yield potential of legumes—similarities and contrasts with cereals. 4th International Crop Science Conference Section 2.8.

Sinclair T.R., Purcell L.C., Vadez V., Serraj T., King C.A. and Nelson R. 2000. Identification of soybean genotypes with N_2 fixation tolerance to water deficits. Crop Science 40, 1803–1809.

Thiaw S. and Hall A.E. 2004. Comparison of selection for either leaf-electrolyte-leakage or pod set in enhancing heat tolerance and grain yield of cowpea. Field Crops Research 86, 239–253.

Turner, N.C. and Henson I.E. 1989. Comparative water relations and gas exchange of wheat and lupins in the field. Pp. 269–328 in 'Structural and functional responses to environment', ed. by K.H. Kreeb, H. Ritcher and T.M. Hinckly. SPB Academic Publishing: Gravenhage, Netherlands.

Vu J.C.V., Gesch R.W., Pennanen A.H., Allen L.H.J., Boote K.J. and Bowes G. 2001. Soybean photosynthesis, Rubisco, and carbohydrate enzymes function at supraoptimal temperatures in elevated CO_2. Journal of Plant Physiology 158, 295–307.

Additional references

Abdelhamid M.T., Palta J.A., Veneklaas E.J., Atkins C., Turner N.C. and Siddique K.H.M. 2011. Drying the surface soil reduces the nitrogen content of faba bean (*Vicia faba* L.) through a reduction in nitrogen fixation. Plant Soil 339, 351–362.

Gray B.S., Strellner R.S., Puthuval K.K., Shulman R., Siebers M.H., Rogers A. and Leakey A.D.B. 2012. Minirhizotron imaging reveals nodulation of field grown soybean is enhanced by free-air CO_2 enrichment only when combined with drought stress. Functional Plant Biology 40, 137–147.

Potential contribution of increased photosynthetic efficiency to increased yield potential of maize

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Summary

- Rapidly growing global demand for food and feed maize must be met by increased crop production per unit of land area under cultivation. This requires closing the gap between farm and potential yields, as well as continued improvement in potential crop yield.
- Over recent decades, conventional breeding has increased maize crop yield mainly by improving the crop's tolerance to intensification.
- Incremental increases in yield of newly released maize lines have been achieved by improving dry matter accumulation as a result of more erect canopy architecture and slower decline of photosynthetic rates during grain-filling.
- Breeding has increased maize crop yield without affecting harvest index, maximal photosynthetic capacity or potential yield under non-limiting resources.
- Recent maize yield increases resulting from higher biomass accumulation suggest that there is scope for achieving further yield increases by selectively improving maize photosynthetic capacity.
- Possible avenues for improving photosynthetic capacity in maize include: (1) breaking the leaf photosynthetic capacity – leaf size paradigm; (2) up-regulating the activity of sedoheptulose-1,7-bisphosphatase in the mesophyll and the capacity for electron transport in the bundle sheath; (3) improving Rubisco turnover rates of high-yielding maize crops; and (4) improving drought tolerance of maize crops.

Introduction

Increased demand for maize as livestock feed in the developing world and continued demand for food maize arising from population growth and poverty in the least-developed parts of the world are changing the global cereal demand. By 2020, global demand for maize is expected to increase by 50% relative to the 1995 level. In developing countries, demand for maize will surpass that for both wheat and rice. Given the growing environmental awareness and the limited availability of new arable lands, meeting the increased demand for maize must come through increases in maize production per unit area under cultivation (Pingali 2001).

Improved agronomic practices (e.g. fertilisation and irrigation) and improved yield can increase production. This paper, however, focuses on yield potential rather than agronomic practices to improve on-farm yield.

There remains significant scope for increasing maize yield by improving agronomic practices in the developed and developing parts of the world. For example, Cassman (1999) estimated that intensification of wheat, rice and maize has contributed 79–97% yield increase since 1967. The gap between yield potential and commercial on-farm yield in the USA is estimated at 50% (Lee and Tollenaar 2007). It is also important to note that of the 140 million hectares

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of global maize cropping area, 50% is under temperate maize production while the other 50% is tropical. Temperate maize production accounts for about 90% and 25% of total production in the developed and developing parts of the world, respectively (Pingali 2001). Hence, from a research perspective, there are two major types of maize crop: temperate and tropical. It follows that improving the yield of temperate and tropical maize is a double-task with common and distinct challenges. Given that this paper deals with yield potential, the available literature is heavily skewed toward temperate maize. Nevertheless, conclusions ought to apply to any maize crop.

Physiological basis of improving maize yield

Similar to other grain crops, maize yield depends on dry matter accumulation (source activity) and allocation to the grain (sink activity). For an increase in crop yield, source and sink activity must increase together and remain in balance (Tollenaar and Lee 2006). For the post-1930s maize hybrids bred in the USA, 50% of the dry matter is usually accumulated by flowering. In contrast to other crops such as wheat, all the dry matter allocated to the grain of maize is fixed during grain-filling; that is, no dry matter is remobilised from the stems during grain-filling (Lee and Tollenaar 2007). Using the example of these North American maize hybrids, Tollenaar and colleagues found that from 1930 to 2000, yield increased 2.13-fold. The incremental yield increases for the newly released hybrids were not related to yield potential per plant (determined under non-limiting resource availability), maximum potential photosynthesis, harvest index (proportion of dry matter allocated to the grain), plant growth rate at silking or dry matter accumulation up to silking (Tollenaar and Wu 1999; Tollenaar and Lee 2002; Lee and Tollenaar 2007). Increases in maize yield were mainly brought about by: (1) increases in dry matter accumulation caused by increases in leaf area index (LAI) and leaf erectness of the newer hybrids; and (2) the slower decline of photosynthetic rates during grain-filling (Lee and Tollenaar 2007). The latter phenotype is known as visual or functional 'staygreen'.

In summary, the North American hybrid breeding program has improved maize yield by increasing stress tolerance, particularly to intensification, in the newer cultivars. This is best shown by the similar yields given by new (2000s) and old (1930s) maize lines under conditions of low competition for resources (low planting density) (Tollenaar and Wu 1999; Lee and Tollenaar 2007; Hammer et al. 2009). Hence, decades of maize breeding have selected for neither greater yield potential nor maximal photosynthetic capacity, which indicates the difficulties in achieving this task. It follows that improving either yield potential or photosynthetic capacity can only be tackled by targeted genetic engineering. The good news is that, on the whole, yield increases in maize have come about as a result of increased biomass accumulation rather than harvest index. For modern maize crops, harvest indexes average 50%, which is close to the theoretical maximum (Sharma-Natu and Ghildiyal 2005). These observations suggest that improving whole-plant photosynthesis is the only remaining avenue that can lead to higher grain yield in maize.

C_4 photosynthesis in maize

Maize fixes atmospheric CO_2 using the C_4 photosynthetic pathway, using the NADP-malic enzyme (NADP-ME) C_4 acid decarboxylation subtype (Hatch 1987). The key feature of C_4 photosynthesis is the operation of a CO_2-concentrating mechanism in the leaves, which suppresses apparent photorespiration in air. For the NADP-ME type, two photosynthetic cycles (C_3 and C_4) operate across two photosynthetic cell types (mesophyll and bundle sheath), which are arranged in concentric layers around the vascular bundle. In maize, the bundle sheath cell wall is lined with a suberin lamella and the bundle sheath chloroplasts are arranged around the periphery of the bundle sheath ring where these cells contact the mesophyll (Hatch 1987). Bundle sheath chloroplasts also lack photosystem II (PSII) activity (Hatch 1987).

Atmospheric CO_2 diffuses through the stomata into the mesophyll where it is hydrated into bicarbonate, which reacts with phosphoenol pyruvate (PEP) with the aid of PEP carboxylase (PEPC) to produce oxaloacetate, a C_4 acid. Oxaloacetate is converted into malate, which diffuses into the bundle sheath chloroplasts where it is decarboxylated by NADP-ME, releasing CO_2 for fixation by Rubisco and the rest of the C_3 cycle. The C_3 product of the decarboxylation reaction, PEP, returns to the mesophyll, completing the C_3 cycle (Hatch 1987).

The C_4 cycle acts like a CO_2 concentrating mechanism for two main reasons. First, PEPC is faster than

Rubisco and insensitive to O_2. Second, the bundle sheath cell wall presents a significant gaseous diffusion barrier (Hatch 1987). Consequently, the high CO_2 concentration in the bundle sheath leads to the suppression of apparent photorespiration in air as well as the saturation of C_4 photosynthesis at a lower ambient CO_2 concentration than for C_3 plants. Photorespired CO_2 is released within the bundle sheath, and either is refixed or contributes to increasing bundle sheath CO_2 concentration, which in turn leads to reduced photorespiration. High bundle sheath CO_2 concentration gives rise to the characteristic photosynthesis–CO_2 response curve of C_4 leaves—high maximal photosynthetic rates and saturation at a relatively low intercellular CO_2 concentration. These constitute the basis for a number of advantages conferred by the C_4, relative to C_3, photosynthetic pathway. Chief of these are higher water and nitrogen-use efficiencies (Ghannoum et al. 2009).

In the NADP-ME type plants, the basic energy requirements for CO_2 fixation up to the level of triose phosphate are those of the C_3 cycle (i.e. two molecules of reduced nicotinamide adenine dinucleotide (NADPH) and three molecules of adenosine-5'-triphosphate (ATP)), in addition to the two ATP molecules required to regenerate PEP from pyruvate by the C_4 cycle. The NADPH used for the reduction of oxaloacetate to malate in the mesophyll is regenerated in the bundle sheath by NADP-ME and used in the C_3 cycle. However, there are additional energy costs associated with: (1) the overcycling of CO_2 caused by CO_2 leakage out of the bundle sheath (about 20% of photosynthetic rates; Henderson et al. 1992); and (2) the refixing of photorespired CO_2 within the bundle sheath. Consequently, the observed quantum requirement in maize is about 16.1 mol photons/mol CO_2 (Lee and Tollenaar 2007).

Improving photosynthesis to improve crop yield

Breeding programs managed to increase crop yield without increasing leaf photosynthetic capacity (Evans 1997; Richards 2000). Generally, there is no correlation between yield potential and leaf photosynthesis. Nevertheless, the yields of most C_3 crops increase in response to growth at elevated CO_2 concentration (Long et al. 2006). Maize yield is not affected by growth at elevated CO_2 concentration under non-limiting water availability (Leaky et al. 2006). These results suggest that the yield of modern grain C_3 crops is responsive to increases in leaf photosynthetic rates (Long et al. 2006). This is yet to be demonstrated for C_4 crops.

Increasing photosynthetic rates requires greater efficiencies in the processes of light capture, conversion into chemical energy, its utilisation in CO_2 fixation and carbohydrate synthesis and the utilisation of newly synthesised carbohydrate by developing sinks. Each of these processes is complex, yet they must be considered in their totality in order to achieve the desired outcome. Yield potential (*YP*) can be expressed as equations (1) and (2):

$$YP = HI \times PP \tag{1}$$

and

$$PP = \frac{S \times \varepsilon_i \times RUE}{j} \tag{2}$$

where *HI* is the harvest index, *PP* the primary productivity, *S* the annual integral of incident solar radiation, ε_i the efficiency of radiation interception, *RUE* the efficiency of converting absorbed radiation into biomass and *j* the energy content of plant biomass (Long et al. 2006).

A maize crop with a quantum requirement of 16.1 mol photons/mol CO_2 is operating at about 93% of its theoretical efficiency. When basic assumptions are made about leaf absorptance, the costs of dark respiration, nitrate reduction and the conversion of triose phosphate into sucrose, and taking into account that 50% of sunlight cannot be used in photosynthesis, then the maximal theoretical *RUE* for a C_4 crop is 0.06. The highest short-term *RUE* reported is 0.043, while the highest growing season *RUE* reported is 0.034 (Long et al. 2006). This implies that there is scope for improving field *RUE* by about 30% in maize. Can this gap be closed?

Scope for improving photosynthesis in maize

Several reviews have attempted to identify the key remaining opportunities for improving photosynthesis in C_3 crops such as rice and wheat (Evans 1997; Horton 2000; Richards 2000; Sharma-Natu and Ghildiyal 2005; Long et al. 2006). The following is an attempt to adapt the main findings of these reviews to C_4 photosynthesis. Improving photosynthesis is considered in terms of both photosynthetic rates and capacity because both are important in field situations. Note that any genetic manipulation in C_4 plants must take into account important considerations

such as targeted expression to the mesophyll or the bundle sheath cells with their common and distinct metabolic functions, and the potential for disturbing the intricate metabolic coordination between the C_3 and C_4 cycles, which underpins the efficiency of C_4 photosynthesis.

The seven main findings of the reviews are:

1. Whole-plant photosynthesis is the product of leaf photosynthesis per unit area and whole plant leaf area. For many crops, a negative relationship exists between photosynthetic capacity and leaf size (Evans 1997; Sharma-Natu and Ghildiyal 2005; Lee and Tollenaar 2007). The same trade-off is observed when photosynthetic capacity is increased by genetic manipulation (Pellny et al. 2004). Large leaves are important during crop establishment, while smaller leaves with high photosynthetic capacity are important after canopy closure, when competition for light becomes a critical determinant for photosynthesis (Lee and Tollenaar 2007). To increase photosynthetic rates in either situation will require breaking the leaf photosynthetic capacity – size paradigm (Evans 1997). It may be insightful to establish the relationship between leaf photosynthetic capacity and leaf size among maize cultivars with different release dates.
2. For modern maize crops, the erectophile phenotype of upper leaves has been selected during breeding programs in order to allow greater light penetration into the lower leaves in the canopy. Lower leaves operate at sub-saturating light; however, canopies where light is distributed more evenly among leaves may have greater photoassimilate production than a canopy of horizontal leaves, which become light saturated, especially at midday (Long et al. 2006; Lee and Tollenaar 2007). This balance may be upset by: (1) the limitation of C_4 photosynthesis at low light; (2) senescence of lower leaves caused by low light levels; and (3) poor low light acclimation in some C_4 plants, which is manifest by inefficient nitrogen allocation in the form of excess Rubisco and PEPC activity (Sage and McKown 2006). Hence, the highly erect stature of modern maize crops may not provide the best canopy architecture in terms of biomass accumulation (Horton 2000). Such information may be obtained by comparing leaf nitrogen and photosynthetic rates at different light penetration levels of field-grown maize cultivars with different degrees of leaf erectness.
3. A leaf's ability to recover from dynamic photoinhibition or exploit sun flecks may be related to biomass accumulation. Up-regulation of the xanthophyll cycle may increase the capacity for thermal dissipation of excess light and hence improve the recovery rate of photosynthesis after short-term photoinhibition. In C_4 plants, consideration should be given to which xanthophyll cycle (mesophyll, bundle sheath or both) is best up-regulated. C_4 plants have lower ability to exploit sun flecks than C_3 plants. Improving a C_4 leaf's ability to exploit sun flecks is problematic because it is related to the requirement to maintain high activation of C_3 and C_4 cycle enzymes and high metabolic gradients between the mesophyll and the bundle sheath. In maize, sun flecks cause a breakdown in metabolic coordination between the C_3 and C_4 cycles, such that CO_2 is cycled into the bundle sheath faster than the slowly recovering C_3 cycle enzymes can fix it (Sage and McKown 2006).
4. C_4 plants, like C_3 plants grown at elevated CO_2 concentration, require a large capacity for RuBP regeneration and chloroplast electron transport. In C_3 plants, two main limitations to RuBP regeneration capacity have been identified as the activities of sedoheptulose-1,7-bisphosphatase (SBPase) in the Calvin cycle and cytochrome b_6/f complex in the electron transport chain. Greater photosynthetic rates have been obtained by up-regulating SBPase in tobacco (Lefebvre et al. 2005). In NADP-ME C_4 plants such as maize, the energetic requirements of photosynthesis are met by inputs from both the mesophyll and bundle sheath photochemical and metabolic reactions. For example, in maize, more than 50% of phosphoglycerate produced in the bundle sheath is transported to the mesophyll for reduction to triose phosphate, which is then returned to the bundle sheath to regenerate RuBP. Part of the NADPH requirements of the C_3 cycles is met by the decarboxylation of malate in the bundle sheath chloroplast. These and other reactions necessitate large metabolic pools to be maintained in both the mesophyll and bundle sheath cells of C_4 leaves. These metabolites compete in various metabolic pathways and some act as effectors of key C_3 and C_4 cycle enzymes (Leegood and Walker 1999). Hence, increasing RuBP regeneration capacity in C_4 leaves may need steps beyond the simple up-regulation of

SBPase activity. Steps could involve modifications at the levels of mesophyll electron transport, mesophyll enzymes that complement the bundle sheath C_3 cycle and the metabolic exchange between the mesophyll and the bundle sheath. This task can be guided by careful biochemical investigation of the key steps that limit RuBP regeneration in maize leaves.

5. The bundle sheath cells of C_4 grasses possess different levels of photosystem (PS) II activity depending on their biochemical subtypes. About 5% and 25% of leaf PSII activity and amount, respectively, are found in the thylakoid membranes of two NAD-malic enzyme (NAD-ME) and two NADP-ME grasses (Ghannoum et al. 2005). Despite the significant levels of PSII activity found in the bundle sheath chloroplasts of NAD-ME grasses, there are no apparent differences in photosynthetic sensitivity to either CO_2 or O_2 oxygen between NADP-ME and NAD-ME type C_4 grasses when measured under ambient CO_2 concentrations and high light (Siebke et al. 2003). These findings suggest that up-regulation of PSII activity in the bundle sheath of maize leaves may stimulate electron transport without compromising photosynthetic rates under moderate to high light intensities.
6. As mentioned, the activity of cytochrome b_6/f can be limiting in RuBP regeneration during C_3 photosynthesis. For example, there is a linear relationship between the CO_2-saturated rate of oxygen evolution and the cytochrome b_6/f content in the leaves of peas and spinach (Evans 1988). C_4 photosynthesis has a greater ATP requirement, and potentially a greater demand for cyclic electron transport and therefore cytochrome b_6/f activity. Surprisingly, the content of cytochrome b_6/f does not appear to differ between C_3 and C_4 leaves, whether expressed on a leaf area or chlorophyll basis (Evans 1988; Ghannoum et al. 2005). This situation may be a consequence of the need to distribute the electron transport components between the mesophyll and the bundle sheath. Therefore, up-regulating cytochrome b_6/f activity may be an avenue worth pursuing in maize. This is supported by the fact that mesophyll chloroplasts of NAD-ME grasses contain 1.5–2 times more cytochrome b_6/f per chlorophyll molecule than their NADP-ME counterparts. Therefore, it seems that increased cytochrome b_6/f activity in C_4 grasses has come at the expense of greater nitrogen and chlorophyll investment in the thylakoids (Ghannoum et al. 2005). In summary, it appears that one way of up-regulating the RuBP regeneration capacity in maize is to transform the bundle sheath thylakoids from the agranal to granal arrangement. It is not certain whether this is a realistic possibility or there are maize mutants that can inform us if this is a positive or negative step for maize photosynthesis.
7. Rubisco kinetics are pinpointed as targets for genetic manipulations to improve photosynthetic rates. For C_3 photosynthesis, the emphasis is on reducing the enzyme's sensitivity to molecular O_2 and/or its maximum catalytic turnover rate (k_{cat}) (e.g. Long et al. 2006). For C_4 photosynthesis, the emphasis must be different because C_3 and C_4 Rubisco proteins have different kinetics. In brief, because it operates at high CO_2 concentrations, C_4 Rubisco has a relaxed affinity to CO_2 and a faster k_{cat} than C_3 plants (Ghannoum et al. 2005; Cousins et al. 2010). In particular, grasses of the NADP-ME family of Andropogoneae, to which maize belongs, have some of the highest Rubisco k_{cat} values recorded in higher plants (up to 6.4/s at 25°C) (Ghannoum et al. 2005). However, a modern maize variety has a k_{cat} of 4.1–4.4/s at 25°C (Cousins et al. 2010). This raises the questions of whether breeding programs have been selecting maize lines with lower k_{cat}, and how much k_{cat} varies among maize lines. The former question may be related to the moderately negative relationship between Rubisco k_{cat} and allocation of nitrogen to Rubisco (Figure 1). It is possible that breeders selecting maize leaves with higher leaf nitrogen as a marker for photosynthetic rates have also been selecting for Rubisco with lower k_{cat}. Note that increasing the expression of Rubisco proteins in C_4 leaves has limited scope because Rubisco concentration per chlorophyll molecule is similar for C_3 chloroplasts and C_4 bundle sheath chloroplasts (Ghannoum et al. 2005).

Improving drought tolerance to improve tropical maize yield

Unlike in the developed world, maize production in the developing world is mainly destined for human consumption. Therefore, continuous improvement of maize yield in the tropical regions is a matter of food security. Unlike their temperate counterparts, tropical

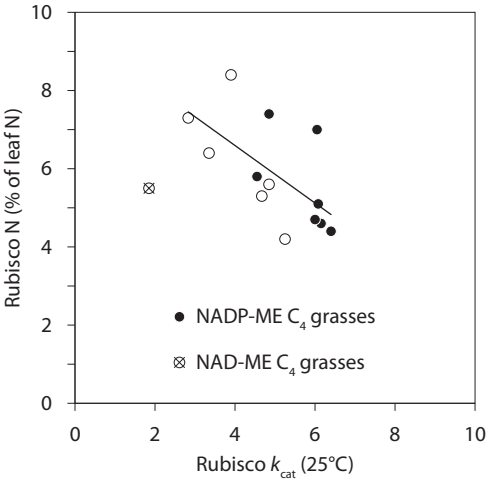

Figure 1. Relationship between nitrogen allocation to Rubisco and Rubisco turnover rate, k_{cat}, in NAD-ME and NADP-ME grasses. Crossed symbol was left out of regression; solid circles are NADP-ME and open circles are NAD-ME. Adapted from Ghannoum et al. (2005). The linear relationship is given by $y = -0.74x + 9.53$, with $r^2 = 0.40$.

maize lines have greater genetic diversity and have been bred under wide-ranging environments (Anami et al. 2009). The key environmental challenge for tropical maize is water stress. Despite the higher water-use efficiency of C_4 than C_3 photosynthesis, photosynthetic metabolism is equally sensitive to water stress in both photosynthetic pathways (Ghannoum 2009; Ghannoum et al. 2009). Therefore, attempts to improve drought tolerance in maize have been similar to those used with other crops (Anami et al. 2009). For example, yield has been improved in maize lines transformed with enzymes in the biosynthetic pathway of glycine betaine, a known metabolic osmoticum (Anami et al. 2009). Drought tolerance of maize grown in the field has also been improved by overexpressing one component of a transcription factor. Another broad avenue for improving stress tolerance of the maize crop to generic stress may be to improve the plant's energy homeostasis under water stress. This is mainly concerned with preventing the build-up of damaging reactive oxygen species under drought (Anami et al. 2009).

Conclusions

Improving photosynthetic capacity or rates in modern maize cultivars is very challenging. Maize belongs to a group of land plants where nature has already enacted an amazing level of photosynthetic optimisation. Nevertheless, a number of theoretical opportunities were identified. It is important to note that a more in-depth theoretical examination and some experimental investigations (some of which are suggested in the text) are required to critically appraise the way ahead for maize. Hence, this paper should be viewed as a discussion starter. The five main areas identified are:

1. breaking the leaf photosynthetic capacity – leaf size paradigm
2. re-examining whether the highly erect upper leaves offer the most productive canopy architecture for a C_4 crop
3. up-regulating electron transport capacity of the bundle sheath
4. searching for maize crops with higher Rubisco k_{cat}
5. improving the drought tolerance of tropical maize.

References

Anami S., De Block M., Machuka J. and Van Lijsebettens M. 2009. Molecular improvement of tropical maize for drought stress tolerance in sub-Saharan Africa. Critical Reviews in Plant Sciences 28, 16–35.

Cassman K.G. 1999. Ecological intensification of cereal production systems: yield potential, soil quality, and precision agriculture. Proceedings of the National Academy of Sciences USA 96, 5952–5959.

Cousins A.B., Ghannoum O., von Caemmerer S. and Badger M.R. 2010. Simultaneous determination of Rubisco carboxylase and oxygenase kinetic parameters in *Triticum aestivum* and *Zea mays* using membrane inlet mass spectrometry. Plant, Cell and Environment 33, 444–452.

Evans J.R. 1988. Acclimation by the thylakoid membranes to growth irradiance and the partitioning of nitrogen between soluble and thylakoid proteins. Australian Journal of Plant Physiology 15, 93–106.

Evans L.T. 1997. Adapting and improving crops: the endless task. Philosophical Transactions of the Royal Society London B 352, 901–906.

Ghannoum O. 2009. C_4 Photosynthesis and water stress. Annals of Botany 103, 635–644.

Ghannoum O., Evans J.R. and von Caemmerer S. 2009. Nitrogen and water use efficiency of C_4 plants. In 'C_4 photosynthesis and related CO_2 concentrating mechanisms', ed. by A.S. Raghavendra and R.F. Sage. Springer: Berlin.

Ghannoum O., Evans J.R., Chow W.S., Andrews T.J., Conroy J.P. and von Caemmerer S. 2005. Faster Rubisco is the key to superior nitrogen use efficiency in NADP-ME relative to NAD-ME C_4 grasses. Plant Physiology 137, 638–650.

Hammer G.L., Dong Z., McLean G., Doherty A., Messina C., Schussler J. et al. 2009. Can changes in canopy and/or root system architecture explain historical maize yield trends in the U.S. Corn Belt? Crop Science 49, 299–312.

Hatch M.D. 1987. C_4 photosynthesis: a unique blend of modified biochemistry, anatomy and ultrastructure. Biochimica et Biophysica Acta 895, 81–106.

Henderson S.A., von Caemmerer S. and Farquhar G.D. 1992. Short-term measurements of carbon isotope discrimination in several C_4 species. Australian Journal of Plant Physiology 19, 263–285.

Horton P. 2000. Prospects for crop improvement through genetic manipulation in photosynthesis: morphological and biochemical aspects of light capture. Journal of Experimental Botany 51, 475–485.

Leakey A.D.B., Uribelarrea M., Ainsworth E.A, Naidu S.L., Rogers A., Ort D.R. and Long S.P. 2006. Photosynthesis, productivity and yield of *Zea mays* are not affected by open-air elevation of CO_2 concentration in the absence of drought. Plant Physiology 140, 779–790.

Lee E.A. and Tollenaar M. 2007. Physiological basis of successful breeding strategies for maize grain yield. Crop Science 47, S202–S215.

Leegood R.C. and Walker R.P. 1999. Regulation of the C_4 pathway. Pp. 89–131 in 'The biology of C_4 plants', ed. by R.F. Sage and R.K. Monson. Academic Press: San Diego.

Lefebvre S., Lawson T., Zakhleniuk O.V., Lloyd J.C. and Raines C.A. 2005. Increased sedoheptulose-1,7-bisphosphatase activity in transgenic tobacco plants stimulates photosynthesis and growth from an early stage in development. Plant Physiology 138, 451–460.

Long S.P., Zhu X-G., Naidu S.L. and Ort D.R. 2006. Can improvement in photosynthesis increase crop yield? Plant, Cell and Environment 29, 315–330.

Pellny T., Ghannoum O., Conroy J.P., Schluepmann H., Smeekens S., Andralojc J. et al. 2004. Genetic modification of photosynthesis with *E. coli* genes for trehalose synthesis. Plant Biotechnology Journal 2, 71–82.

Pingali P.L. (ed.) 2001. CIMMYT 1999–2000 world maize facts and trends. Meeting world maize needs: Technological opportunities and priorities for the public sector. CIMMYT: Mexico, D.F.

Richards R.A. 2000. Selectable traits to increase crop photosynthesis and yield of grain crops. Journal of Experimental Botany 51, 447–458.

Sage R.F. and McKown A.D. 2006. Is C_4 photosynthesis less phenotypically plastic than C_3 photosynthesis? Journal of Experimental Botany 57, 303–317.

Sharma-Natu P. and Ghildiyal P.M. 2005. Potential targets for improving photosynthesis and crop yield. Current Science 88, 1918–1928.

Siebke K., Ghannoum O., Conroy J.P., Badger M.R. and von Caemmerer S. 2003. Photosynthetic oxygen exchange in C_4 grasses: the role of oxygen as electron acceptor. Plant, Cell and Environment 26, 1963–1972.

Tollenaar M. and Lee E.A. 2002. Yield potential, yield stability and stress tolerance in maize. Field Crops Research 75, 161–170.

Tollenaar M. and Lee E.A. 2006. Dissection of physiological processes underlying grain yield in maize by examining genetic improvement and heterosis. Maydica 51, 399–408.

Tollenaar M. and Wu J. 1999.Yield improvement in temperate maize is attributable to greater stress tolerance. Crop Science 39, 1604–1610.

Additional references

von Caemmerer, S., Quick, W.P. and Furbank, R.T. 2012. The development of C_4 rice: current progress and future challenges. Science 336, 1671–1672.

Whitney, S.M., Houtz, R.L. and Alonso, H. 2011. Advancing our understanding and capacity to engineer nature's CO_2-sequestering enzyme, Rubisco. Plant Physiology 155, 27–35.

Practical considerations in using photosynthesis research to create higher yielding crops

Intellectual property management—helping deliver improved photosynthesis technology to the world's farmers

John Thorne[1]

Summary

- Improving photosynthesis performance in economically important food crops will play a vital part of meeting future global food security requirements. An effective and appropriate intellectual property (IP) management strategy is an important part of a research and development program designed to deliver improved photosynthetic plant technology to farmers globally.
- An IP strategy will consist of a judicious mix of IP protection and appropriate licensing. IP protection can include patent protection for key core technologies and photosynthetic performance improved germplasm, as well as plant breeder rights for new plant varieties incorporating this new technology. Thoughtful licensing can be used to encourage collaboration and encourage technology delivery in major food crops.
- A quality intellectual property management strategy can help:
  - attract investment in a research, development and delivery program from the government, aid, philanthropic and private sectors
  - encourage mutually beneficial collaboration between the international agriculture organisations and private agricultural biotechnology companies
  - enable the delivery of the new technology to farmers in developed, transition and developing countries, including timely delivery of the new technology to resource-poor farmers.
- An effective IP management strategy will complement work undertaken by researchers by attracting funds for research and encouraging publication. It will ensure relevance by enabling delivery of the technology to farmers and, through the use of appropriate intellectual property expertise, not impinge unduly on the workloads of researchers.

Introduction

This paper discusses intellectual property issues related to research on:

- improvement of photosynthesis performance in plants
- translation of this research into significant improvement in plant performance including:
  - increased yield
  - improved efficiency in the use of sunlight, water and nutrients
- delivery of the technology developed to farmers (end users).

This new technology is delivered to farmers globally in the form of genetically improved propagating materials (seeds, tubers and cuttings). Maximisation of benefits globally from delivery of this new technology will require collaboration between organisations involved in research, development and delivery of new plant genetic technology.

There is a requirement to feed many more people over the coming decades with increasingly stretched land, water and nutrient resources. Therefore, there is an urgent need to increase food supply. As part of an overall research and delivery program to meet

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future food security needs, it will be necessary for interested parties to invest significant funds over an extended period in:

- research to create more efficient plant technology based on improved photosynthetic performance
- delivery of this improved plant technology in both developing and developed countries.

Delivery pathways

In coming years, the predicted growth in demand for economically important food crops is likely to exceed supply. Meeting anticipated demand will require delivery to all farmers in developing and developed countries. However, delivery pathways will vary:

- Developing countries—Genetically improved plant propagating materials can be delivered through
- CGIAR-related organisations, government and non-government agencies and commercial sales.
- Developed countries—Genetically improved plant propagating materials can be delivered only by commercialisation processes through commercial pathways; there is no significant alternative delivery pathway.
- Transition countries—Some countries may be considered in the process of transitioning from developing to developed status. Specific arrangements will need to be negotiated in these countries to ensure that genetically improved plant propagating materials are delivered efficiently to all farmers, in particular, farmers from lower socioeconomic areas.

Technology transfer methods

By their very nature, researchers are in the business of creating new intellectual property (research outputs). Various methods used to deliver new intellectual property to users include:

- peer-reviewed scientific publication
- education and technical publications aimed at students and end users
- extension, using resources of public and private organisations and companies
- commercial arrangements including patenting of key intellectual property sales, licensing or establishing start-up companies.

Stakeholders including researchers and research institutions, research investors (research institutions, governments, aid donors and philanthropic organisations) and end users (farmers) benefit from technology transfer in various ways. A brief summary of adoption pathways, research stakeholder benefits, methods for developing research IP and mechanisms for transfer to end users is tabulated in the appendix to this paper. This table does not specifically include benefits that may accrue to private sector companies as a result of the transfer of research outputs from research organisations under formal exploitation agreements, as these benefits are well understood.

Intellectual property protection

Registered IP is a subset of the IP spectrum. Plants with improved photosynthesis performance may be developed and delivered as:

- genetically modified plants
- conventionally bred plants using naturally occurring genetic diversity, some identified through biotechnology techniques.

Registered intellectual property protection may be available as outlined in Table 1.

Patents and plant breeder rights (PBR) are applied for and granted on a country-specific basis. Patent and PBR can be enforced by IP rights owners only in individual countries where patent rights are granted and still in force. The patenting system is expensive and patents are usually sought in developed countries where markets are large and rich enough for the patent owner to have the potential to make a sufficient return to justify the investment in research, development and commercialisation.

In countries that patents are not applied for, or applied for but not granted, any organisation or individual is free to use a particular technology assuming they can get access to the required material. However, farmers in developing countries will not be free to export commodities produced using patented technology to countries where patent protection is in force (until the patent has expired) unless a suitable licence agreement is negotiated with the patent owner.

IP may also be protected by keeping it confidential. For example, know-how and expertise (sometimes called tacit knowledge) surrounding the practice of a new technology is not written (or cannot easily be written) but may be passed on by direct training. Other key information that may be kept confidential could include databases and other proprietary information and techniques developed.

Table 1. Registered IP protection mechanisms for photosynthetically enhanced plants

Technology category	Broad description of IP	Potential IP protection
Core technology	Research results, methodology and know-how to enable the development of photosynthetically improved plant species (genetically modified or sourced from existing genetics) Development or identification of genetic modification technology to enable the transformation of plants—nuclear or plastid transformation technology	Patent protection on methodology Patent protection of transformation technology
Application technology	Development of photosynthetically improved germplasm for individual economically important plant species	Patent protection for species-specific germplasm showing improved performance
New plant varieties	Photosynthetically improved species-specific germplasm used in plant breeding programs to introduce improvements into elite plant varieties Multiple new varieties required to suit different geographic areas and climates	Plant breeder rights for new varieties that meet required criteria

It is important to note that time-limited (20 years maximum) patent rights are granted in return for publication of the technology concerned, as a minimum in the form of the patent specification. After the date of filing a patent application (the priority date), researchers are free to publish their research outcomes and, in this way, the patent system enables sharing of new research outputs with the community. Resulting peer-reviewed scientific publication often plays a vital role in acceptance and hence application of new technology.

Research, development and commercialisation time line and risks

Ultimately benefits arising from the application of the improved photosynthesis plant technology can be delivered to end users (farmers) only in seed or other propagating material that incorporates enhanced photosynthesis genetics. Research, development and delivery of plants with enhanced photosynthetic performance in both developing and developed countries is a high-risk activity requiring considerable investment of funds and resources over a lengthy period. A generic pathway for the development of new seed and other propagating material products is set out in Table 2.

Table 2 shows that the time line for research, development and commercialisation of genetically modified material varies widely between approximately 6 and 14 years. It is possible that the time line will be shorter for new plant varieties incorporating naturally occurring improved photosynthesis genetics, or new genetically modified plants developed through plastid transformation where the modified genetics are transmitted through the maternal line.

Technology development risks are high, with the highest risk at the start of research but reducing as new potential plant products move down the product development pipeline. Anecdotally, the cost of developing a new genetically modified plant for a major food crop from initial research (trait identification) to commercial launch of a new seed product may be about $50–100 million. Application of the improved photosynthesis technology in each individual economically important food crop will follow a research, development and commercialisation pathway similar to the generic pathway described.

The key message is that the release of a suite of new plant varieties with improved photosynthetic performance across a range of plant species across all appropriate world geographic regions will require serious investment partner organisations to invest very significant funds.

Table 2. Generic pathway and timeline for research, development and commercialisation of new plant varieties[a]

Research and development stage	Key product development activities	Typical duration range (months)	Average probability of success (%)
Discovery of gene or trait identification	Identification of valuable genes to improve plants through: • biotechnology • conventional breeding	24–48+	5
Proof of concept	Biotechnology traits: • test genes in plants and screen for desired performance Conventional breeding: • breed plants from parents with desired traits	12–24+	25
Early product development	Biotechnology traits: • lab and field tests of genes in plants to select commercial candidates and meet regulatory requirements Conventional breeding: • conduct field trials of plants with desired traits	12–24+	50
Advanced product development	Biotechnology traits: • demonstrate efficacy of biotechnology trait in elite germplasm and develop regulatory data as necessary Conventional breeding: • demonstrate performance of new variety developed	12–24+	75
Final product development and regulatory submission	For all new conventional and biotechnology variety products: • bulk up seed supplies for commercial release • develop commercial launch plans For biotechnology products: • complete regulatory requirements	12–36+	90

[a] Table content is based on information from a Monsanto website but is generic.

Delivery and uptake of new plant technologies by farmers

Philosophically, a technology delivery strategy designed to maximise benefits to farmers globally should include, but not be limited to:

- enabling the basic photosynthesis research to be applied over time to all major food crops across all global agroecological zones
- negotiating collaborative research, development and delivery arrangements with interested investors from international agriculture, philanthropic and commercial organisations
- managing IP arrangements to attract the investment funds required to enable technology delivery
- licensing the technologies developed to ensure delivery to farmers in developed, developing and transition countries
- delivering new technology to resource-poor farmers in developing and transition countries on a similar scale and time frame of delivery to commercial farmers in developed countries.

As stated, new genetic plant technologies are delivered to farmers in developed countries through large and small commercial companies. Well-developed support systems are provided by a mix of government agriculture, research, grower and commercial advice organisations.

However, it is less clear that new genetic based plant technologies provide benefits to resource-poor farmers in developing and transition countries. Some guidance can be provided from the uptake of *Bt* cotton in Argentina, China, Mexico and South Africa described by Pehu and Ragasa (2008). These authors report substantial benefits for resource-poor farmers. National average increase in cotton yield ranged

from 11% to 64% with associated pest management costs reduced by between 42% and 67%. These gains were more than sufficient to offset higher seed costs. However, these impressive national average increases hide marked variation, with impacts varying according to factors including impact year to year, institutional setting and agroecological zone.

Lessons that can be learned from the *Bacillus thuringiensis* (*Bt*) cotton initiative designed to improve the position of resource-poor farmers in the uptake of new genetic plant technologies include:

- Where possible, provide a competitive seed price through competition from multiple seed suppliers.
- In developing countries and regions, establish appropriate institutional and social support arrangements including a regulatory framework and suitable research capacity, and apply effective IP management.
- Ensure systems for supplying inputs, especially seed, as well as access to finance for resource-poor farmers are in place.

Where possible, these lessons should be kept in mind when negotiating research, development and delivery arrangements for genetically improved photosynthetic plant propagating materials.

Attracting investment for research, development and delivery

IP protection has a role in attracting investment. The research, development and delivery of a suite of new plant products with yield, sunlight, water and nutrient use advantages arising from photosynthetic improvements will require significant investment of funds and resources (expertise, facilities, plant germplasm, IP, marketing and distribution). Potential investors might include:

- aid donors
- governments and associated agencies
- international agriculture (including CGIAR organisations)
- philanthropic organisations
- private equity investors
- private sector life science companies
- public equity investment
- research institutions.

Each investor will have individual requirements that will need to be accommodated and it is likely that multiple investors will be required. Requirements will differ between public good investors and commercial investors. Provided that there is clarity around inputs and access to research results and materials and ownership of intellectual property and exploitation rights, it should be possible to ensure that germplasm containing improved photosynthetic performance genetic material (both genetically modified and naturally occurring) is delivered through new or improved plant varieties to farmers in developing countries (including resource-poor farmers) and developed countries.

Investment by public good investors and private sector investors need not be mutually exclusive. However, to assist in enabling co-investment by both categories of investors in new technology, it will be necessary to negotiate a series of investment and exploitation agreements probably over an extended period (rather than one multiparty agreement with investors at the commencement of a research program). However, it will be necessary to ensure that negotiated individual agreements are compatible with any earlier agreements.

Delivery in developed countries may be achieved more efficiently through building alliances with the major life science companies. In order to protect their investment and help ensure that they can achieve the required commercial return on investment, it will be necessary to protect key parts of the improved photosynthesis technology package with patents and/or PBR.

In summary, IP protection plays an essential role in attracting investment and providing the opportunity to deliver the technology via commercial pathways, particularly to farmers in developed countries. While PBR protection may be useful in developing countries, patents are likely to be less important in attracting investment in research and delivery of technology in these countries. A suite of patent-protected technologies may, however, be useful to help develop collaboration among research institutions, private life science companies and international agriculture organisations.

Collaboration among research institutions, international agriculture and the private sector

A well-targeted IP protection and access strategy to new agricultural biotechnology may assist resource-poor farmers access that technology. Such a strategy would provide a vital part of an overall framework to enable and promote global collaboration among research institutions, international agriculture

(including CGIAR organisations) and the private sector. Private sector companies undertake approximately half of the world's agricultural research and development, but technologies commercialised in developed countries are not usually delivered to farmers in developing countries.

Life science companies dominate the field of genetic modification of crops. These companies invest approximately nine times more funds and resources for the research, development and commercialisation of agriculture biotechnology than international agriculture including the CGIAR centres.

Private sector companies focus on the major crops produced in developed countries (e.g. maize, soybean and canola) because these markets have the potential to achieve a profitable return on investment commensurate with the risks. Sales are made to farmers in developed countries who can afford to pay for the technology and to commercially viable farmers in some developing countries. As a result, productivity and environmental benefits available from the application of agricultural biotechnology, for example for maize and soybean, to farmers in developed countries are not generally available to resource-poor farmers.

Because they cannot afford to pay, new biotechnology developments will bypass resource-poor farmers or take much longer to filter down than desirable. Therefore, positive collaborations are needed to provide mutual benefits over the longer term to resource-poor farmers, international agriculture and private sector companies.

While international agriculture does not invest in development of agricultural biotechnology nearly to the same extent as private sector companies, these organisations have resources that may be beneficial to the private sector companies, such as:

- extensive germplasm resources collected from many major food crops across the world
- facilities including biotechnology facilities distributed in many different geographic locations with widely variable climate, vegetation and soils
- plant breeding programs that can cross new genetically improved germplasm into elite regionally and locally important plant varieties, creating more productive plant varieties suited to regional and local use
- development of agricultural information packages to promote adoption and ensure best practice to maximise benefits
- distribution channels to enable the delivery of new genetically improved plant varieties to resource-poor farmers.

In the case of the proposed research, development and commercialisation for plant productivity through improvement in photosynthetic efficiency, the needs of farmers (increased yield and improved efficiency in the use of sunlight, water and nutrients) in both developed and developing countries are the same.

Around 2000, CGIAR partners collaborated with life science companies to develop, for example, transgenic potatoes (Pinstrup-Anderson and Cohen 2000) that are pest and disease resistant. By agreement, companies exclusively exploited the technology in specified developed countries and international agriculture had non-exclusive freedom to operate in most developing countries. No doubt there has been much subsequent collaboration between life science companies and international agriculture organisations since 2000 in many different forms.

It may be possible to establish collaboration in the research, development and commercialisation of improved photosynthetic plant biotechnology that is mutually beneficial to international agriculture and the private sector. While international agriculture is currently working to raise income levels for resource-poor farmers, this is also in the long-term interest of the private sector because it will expand the pool of future customers who can afford to buy private sector products. Mutual collaboration will also provide improved access and more efficient use of public and private sector agricultural biotechnology development resources.

IP protection can play an important role in enabling the development of mutually beneficial collaboration. Carefully targeted IP protection will:

- provide the private sector and research institutions with the confidence to invest significant funds in research and development of new biotechnology
- ensure the opportunity for the private sector to obtain a reasonable return on investments through protection of key markets for a finite period
- enable international agriculture to deliver new plant technology to resource-poor farmers.

Roles of collaborators and negotiation of agreements

It is not proposed that CGIAR partners and their research investors transform into semi-commercial or commercial organisations. Their role as public good research organisations working to benefit the world's

resource-poor farmers must be maintained. Mutually beneficial collaboration with the private sector that maximises the use of resources and delivery of new technology to all farmers, including resource-poor farmers, can be negotiated.

Collaborative agreements need to be negotiated in an ethical and business-minded manner. Agreements must clearly address all issues including mutual benefits, research roles and responsibilities, resource investment, IP and delivery rights and responsibilities. Public good research organisations probably do not need to establish expensive systems to manage IP issues but could draw on external professional expertise on as-needs basis.

Implementing an IP protection strategy

To assist in the future delivery (application), any research investment program designed to improve photosynthetic efficiency in plants should incorporate an IP strategy. This strategy must be based on needs of individual IP owners, the terms of any research collaboration or licensing agreements and a market assessment. IP protection should be sought mainly for key research outputs that have significant potential for delivery in global markets.

The strategy should include policy and implementation components for the specific research program. The policy component should state objectives and clearly set out responsibilities of the research institution and individual researchers with respect to the strategy. Implementing the strategy should include the following components:

- A consistent methodology for identifying research results worthy of IP protection prior to publication must be established.
- Professional assistance should be sought for implementing IP protection, including:
  - application, drafting and filing of patents and PBR
  - prosecution (application for a patent for novel material) and grant (where the patent office grants the patent).
- Maintenance of patents and PBR granted should be established.
- An IP watch and defence mechanism should be established.

The strategy should be designed to provide clarity to researchers and provide the key support they need to participate its implementation. A well-designed IP strategy actively supports publishing of research outputs. Scientific peer-reviewed publication actively supports delivery of new technology to the market. IP owners may decide that some information remains confidential. Information in this category may include know-how, databases and other propriety information.

While initial patent costs should be viewed as an investment by the research institutions, fundamentally patents support commercial activity and agreements need to be implemented that transfer IP protection costs to the private sector as part of the research collaboration and commercialisation agreements.

References

Pehu E. and Ragasa C. 2008. Agricultural biotechnology: transgenics in agriculture and their implications for developing countries. Background paper for the World Development Report 2008. World Bank: Washington, DC

Pinstrup-Anderson P. and Cohen M.J. 2000. Biotechnology and the CGIAR. in 'Proceedings of the Sustainable Agriculture in the Next Millennium conference, 28–31 May 2000'. At <research.cip.cgiar.org/confluence/download/attachments/3550/AY2.pdf>, accessed 23 September 2013.

Appendix—Research output for technology transfer spectrum

	Delivery pathway	Benefit mechanisms				Mechanism for developing and protecting project IP[a]	Mechanism for transfer to end user
		Return type	Research institution	Research investors	End users (farmers)		
Communication mechanisms	Scientific publication (peer reviewed)	Kudos Financial returns Other direct benefits	✓✓✓ × ✓✓✓	✓ × ✓✓ Adds to research knowledge base	× × ×	No development required beyond peer review of papers	Publication in peer-reviewed scientific journals Results available to other researchers
	Communication and education	Kudos Financial returns Other direct benefits	✓✓ × ×	✓✓✓ ✓ Return from sales of some publications ×	× × ✓✓✓ Farmer can directly apply project IP on-farm Contributes to productivity improvements	Project IP revised to a form that can be easily interpreted by farmers and other non-researchers Publications of various types cater for the needs of different end user types Websites developed or updated	Students taught new technology Publications printed and distributed or sold by: • research institutions • research investors • rural distribution networks • websites
	Extension	Kudos Direct financial returns Other direct benefits	✓✓ × ×	✓✓ × ✓✓✓	× ✓✓✓ ✓✓✓ Project IP available to the stakeholder through professional service providers	Project IP presented to professional advisers at seminars and through written material: • government extension officers • private consultants • supplier and service industry employees Professional educators and communicators prepare material Information, software and training packages prepared and distributed	Professional advisers and consultants • private • government • industry services

Continued ...

Appendix—Research output for technology transfer spectrum (continued)

	Delivery pathway	Benefit mechanisms				Mechanism for developing and protecting project IP[a]	Mechanism for transfer to end user
		Return type	Research institution	Research investors	End users (farmers)		
Commercial mechanisms	Franchising[b]	Kudos Direct financial returns Other direct benefits	✓ ✓ Franchise fees ✓	✓✓ ✓ Franchise fees ✓	× ✓✓✓ ✓✓✓ Project IP made available to farmers	Franchisor responsible for: • transforming project IP to a form suitable for franchisee • developing the franchised business system • further developments	Patent and PBR protection may be required Franchisee responsible direct to end users: • distribution of product • servicing of product
	Sale or assignment of project IP[c,d,e]	Kudos Direct financial returns Other direct benefits	✓ ✓✓ ××× Sale may hamper future research opportunities	✓ ✓✓ × No longer controls IP	× ✓✓✓ ✓✓✓ Project IP made commercially available to stakeholders by owner	Technology to be prepared in the format agreed between seller or purchaser—RI will usually have to undertake this activity Further development is the responsibility of the purchaser	Patent and PBR protection essential Purchaser responsible for: • translational research • development • manufacture • marketing • distribution • servicing
	Licensing[f]	Kudos Direct financial returns Other direct benefits	✓ ✓ Licence fee or royalty returns usually small in comparison to research investment ✓ Proof of adoption by industry	✓✓ ✓ Licence fee or royalty returns usually small in comparison to research investment ✓ Proof of adoption by industry	× ✓✓✓ ✓✓✓ Project IP made available commercially to stakeholders by licensee	Technology transferred to licensee in agreed form with assistance from RI Licensee undertakes commercial product development Often means the RI may undertake contracted R&D for the licensee	Patent and PBR protection essential Licensee responsible for: • manufacture • marketing • distribution • servicing

Continued ...

Appendix—Research output for technology transfer spectrum (continued)

	Delivery pathway	Benefit mechanisms				Mechanism for developing and protecting project IP[a]	Mechanism for transfer to end user
		Return type	Research institution	Research investors	End users (farmers)		
Commercial mechanisms	Start-up company	Kudos Financial returns Other direct benefits	✓✓✓ ✓✓✓ Successful spin-off could provide earlier and higher return on investment through sale of company shares in addition to royalties ✓✓✓ Employment opportunities for university graduates in spin-off companies	✓✓ ✓✓✓ Successful spin-off could provide a higher return on investment through sale of company shares in addition to royalties	× ✓✓✓ ✓✓✓ Project IP made available through the spin-off companies	Spin-off responsible for: • commercial development of the technology • organising the transfer of technical specialist from RI to company[g]	Patent and PBR protection essential Spin-off responsible for • manufacturing • marketing • distributing • servicing In the business phase the company grows using funding raised through: • venture capital funds • IPO or trade sale Return to investors generated from: • value of company shares • licence fees and royalties on sale of product

[a] Research output requires interpretation or development work to enable transfer to the end user.
[b] Franchising is very rarely used but may be worth considering for some information-based products.
[c] This option is rarely used by Australian research institutions.
[d] Industry may not prefer this because of higher up-front costs and risks.
[e] This is the monetary investment return through sale price.
[f] Licensing is overwhelming the main mechanism used for the commercial transfer of technology from research institutions.
[g] Funds may be provided initially through seed investment from research institutions or private investors, seed capital or venture capital in return for company shares.
PBR = plant breeders' rights
RI = research institution
R&D = research and development
IPO = initial public offering

Translational research—mobilising photosynthesis research to produce higher yielding crops

John Passioura[1]

Summary

- Success at scaling up from laboratory research to application in the field depends on being aware of the constraints and other complications that are likely to arise as scaling up proceeds. Such awareness comes best from dialogue with people familiar with the performance of crops in the field.
- While knowledge of the photosynthetic machinery in plants is immense at organisational levels from the gene, through organelles, cells and tissues to leaves, it is much sparser at the level of the canopy. Better understanding of carbon balance in canopies is essential to scaling up from the laboratory to the field.
- This paper is an abridged version of ideas expressed in Passioura (2010)

Introduction

'Translational research' is a term from the medical domain, and is predominantly concerned with human health. The health of crop plants is also a major concern. No new cultivar is likely to be released that is not resistant to the major diseases of that species. However, much agricultural research deals with trying to improve the performance of crops, essentially their yield and quality. In relation to the medical domain, making such improvements is more like finding ways of alleviating poverty than of improving health. As such, it requires a thorough understanding of how plants work at every level of organisation, from the gene, through enzymes, organelles, cells, tissues, organs and whole plants to the canopy—and, of great importance, beyond the canopy, to farmers' fields.

In trying to improve the performance of crops, the final arbiter is the farmer. New agronomic techniques or new cultivars will be adopted only if farmers find them effective. Many laboratory scientists who may have a great idea for improving crops find themselves eventually deeply disappointed. This is typically because they are unaware of constraints and essential requirements at higher levels of organisation than those where they are working (Passioura 1979, 1999). There are, of course, exceptions. These relate not to improving the intrinsic performance of crops, but to knocking out possible external impediments. Resistance to leaf-eating caterpillars (*Bt*) and herbicide resistance are clear examples in the genetic modification (GM) arena.

The history of crop improvement has shown that almost all of the success has come from cleverly focused empirical breeding and new agronomic techniques (often inspired by mechanistic ecophysiological understanding). We are now faced with a slowdown in the rate of genetic progress by empirical means, and we have the prospects before us of making use of our knowledge of the workings of plants to modify and incorporate specific beneficial traits into breeders' lines. Such traits can relate to any level of biological organisation. One of the most exciting is the prospect of improving the effectiveness of photosynthetic machinery, the central topic of this symposium.

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Dealing with roadblocks

Before looking at how we might best make use of fundamental knowledge to devise novel traits that could be incorporated into breeders' lines, it is worthwhile looking for, and learning from, common patterns of failure in the past. Promising ideas can head for oblivion as we scale up unless we are aware of complications, constraints and interactions at other levels of organisation than those we may be focusing on. Two examples from earlier research on salt tolerance follow:

1. There has been intense interest by practitioners of functional genomics in discovering genes that could help plants cope better with salt. Unfortunately, much of this research has, in the past, involved suddenly exposing plants to such high concentrations of salt that the cells of the roots plasmolyse. The membranes surrounding the cell contents are ripped off the cell walls as the contents shrink when the surrounding salt sucks out the water. This induces major trauma that never occurs in nature. Exploring gene expression in such circumstances is irrelevant to how plants behave in the field. The failure to understand cell biology relegated that approach to oblivion.
2. Similarly, a few decades ago there was much interest in trying to select salt tolerance at the cellular level in tissue culture. Cells that survived the challenge of salinity could be used to regenerate plantlets. These plantlets turned out to be no more salt tolerant than normal plants. This idea failed because its protagonists were unaware that: (1) the plant tissue most sensitive to salt was that of the expanding cells in the leaves; and (2) those cells contained almost no salt, and indeed were not directly exposed to salt.

These examples illustrate how easily mistakes can be made, and complete failure experienced, by practitioners who are not aware of what makes sense at the next larger scale than the one they are working on. This applies at any level of organisation—whether going from functional genomics to cell biology, cell biology to tissues or organs, organs to whole plants, whole plant to field plots, or field plots to paddocks.

Operationally (perhaps sociologically), the main source of this type of failure is lack of dialogue with people working at higher levels of plant organisation. To facilitate such dialogue is not trivial, because it requires learning other languages. A good example is given by the connection between the ideal gas laws, which we all experience when pumping up a tyre, and the kinetic theory of gases, which provides a molecular explanation of the gas laws. Two of the central ideas of the gas laws are pressure and temperature, but a molecule does not have a temperature or a pressure. It is analysis of momentum and kinetic energy of the molecules that provides the connection to temperature and pressure. Even then the gas laws cannot be derived unless two constraints are entrained—that there is a fixed number of molecules and that they are enclosed by an encompassing wall.

That said, it is evident from the discussion at this symposium that there is deep understanding among photosynthesis researchers about the interactions and significance of myriad processes in the complete sequence of scales from gene to leaf. These researchers understand what are the most likely prospects for substantially increasing photosynthetic performance.

The challenge ahead is not only to deepen that understanding further in order to elaborate the prospects. An equally important challenge is to generate equally profound knowledge about the behaviour of canopies, through time, in a highly variable environment, beset by physical and biological dangers, while heading towards a large yield of grain of good quality. It is still a long road ahead to convert the prospects of improving photosynthesis at levels of enzyme, chloroplast, leaf and plant into outstanding performance of cultivars in the field.

The journey may well take several decades. In the context of getting a radical new cultivar into the hands of farmers it is salutary to remember that it took 20 generations of breeding to produce, by incorporation of the simply inherited *Rht* genes, the first commercial cultivars of the semi-dwarf wheat that were the basis (together with semi-dwarf rice) of the green revolution.

Are we any better prepared now to make faster progress than that? Perhaps we are. But progress will be fastest if we are prepared in advance for possible roadblocks, especially those involving canopies and broadacre operations. At the level of the canopy, one example of interest is the effect of rising CO_2. What are the reasons for the substantially less-than-expected increase in grain yield from a crop fertilised with CO_2 in a free air CO_2 enrichment (FACE) experiment (Long et al. 2006)? Will similar effects be induced by more efficient photosynthetic machinery? If so, we must look out for them. This will require the eyes not only of photosynthesis researchers, but also

of crop physiologists and agronomists. Grain yield is the culmination of the season-long development and environmental experience of a crop, from sowing to harvest. Many things other than instantaneous carbon acquisition influence it. The involvement of experienced field scientists will help ensure that unpleasant surprises will be avoided. An opportunity is to become involved with the FACE facility at Horsham, where it may be possible to test ideas arising from crop physiology and agronomy (FACE undated).

Achieving success in farmers' fields

The eventual successful development and adoption of crops with more efficient photosynthetic machinery will depend on the experiences of crop physiologists, agronomists and breeders in the field before such crops are in the hands of farmers. This symposium is a little short of such people, but they are nevertheless available, both locally and globally.

It is therefore worth imagining some possible dialogues between farmers and field scientists. Suppose that these scientists are given transformed lines of crop plants to see how they perform. Farmers (or more importantly farm advisers, who distil the experience of perhaps 50 farmers) will be looking over their shoulders. What will they be thinking? What will the field scientists be thinking? What will they discuss?

Here are some guesses of mine, mostly in relation to dryland winter cereals:

- Given that much of the protein in the grain comes from Rubisco, will grain protein levels fall if a more efficient Rubisco results in lower concentrations of it in leaves?
- If the novel plants photosynthesise much faster, will the crops be too leafy, and possibly lodge or run out of water too fast?
- What will happen to a surplus supply of photosynthate? Will cereal plants produce too many tillers, or produce larger, deeper roots that will be able to get more water and nutrients? Will the roots increase the amount of exudates and thereby greatly alter the populations of soil organisms, populations that are known to affect yield substantially?
- Will plants with copious photosynthates be more susceptible to disease, or less so?
- Will the time of flowering be affected? If so, will our current understanding of flowering genes have to be revised?

Experienced crop physiologists, agronomists and breeders will have many additions to this list, and their expertise should be engaged right from the beginning if the research we have been discussing is to be translated most effectively.

Scaling up at this advanced level is exceedingly difficult. That is why the Grains Research and Development Corporation (GRDC) is looking to set up dedicated field phenotyping sites to explore and expedite the introduction of novel traits into advanced breeding lines. It would be nice to think that one of the eventual outputs of this symposium will be new material to be used at such sites.

Translational research? One of the favourite expressions in the business world is 'the value chain'. In agriculture it applies very much to what happens after the farm gate. In essence, what I have been arguing is that it applies just as importantly before the farm gate. Scaling up through successive levels of biological organisation is the value chain of agricultural research and development. Successful scaling up is what adds value.

References

FACE undated. AGFACE (Australian Grains Free Air CO2 Enrichment). At www.piccc.org.au/research/project/252, accessed 23 September 2013.

Long S.P., Ainsworth E.A., Leakey A.D.B., Nosberger J. and Ort D.R 2006. Food for thought: lower-than-expected crop yield stimulation with rising CO_2 concentrations. Science 312, 1918–1921.

Passioura J.B. 1979. Accountability, philosophy and plant physiology. Search (Sydney) 10, 347–350.

Passioura J.B. 1999. A plant science manifesto. Pp 5–7 in 'Plants in Action', ed. by B.J. Atwell, P.E. Kriedemann and C.G.N. Turnbull. Macmillan: Melbourne.

Passioura J.B. 2010. Scaling up: the essence of effective agricultural research. Functional Plant Biology 37, 585–591.

Biosafety and regulatory issues in GM

T.J. Higgins[1]

Summary

- Foods made from genetically modified organisms (GMOs) can contribute to enhancing human health and the environment.
- Although consumption of GMOs has not caused any known negative health effects, the degree of regulatory-testing scrutiny applied to GM plants is both unprecedented and highly complex.
- Any GM project working towards an agricultural outcome will need to take account of the regulatory codes from the beginning.

Introduction

Modern biotechnology is often used synonymously with genetically modified (GM) or genetically engineered plants, animals or microbes. The World Health Organization (WHO) concludes that foods made from genetically modified organisms (GMOs) can contribute to enhancing human health and the environment. It finds that GM tools can enhance crop production, food quality and the diversity of foods that can be grown in a given area. This in turn can lead to better health and nutrition, which can then help to raise health and living standards. However, the WHO also argues for continued safety assessments of GMOs before they are marketed, to reduce risks to both human health and the environment. Therefore, the potential environmental and human health effects of new GMOs need to be assessed case-by-case before they are grown and marketed. In this regard, WHO acknowledges that GMOs are examined more thoroughly than other foods for their potential health and environmental impacts and that the consumption of foods from GMOs has not caused any known negative health effects.

WHO recommends holistic evaluation and suggests that, in future, evaluations of GMOs should be widened to include social, cultural and ethical considerations. Currently, evaluations mostly focus on agronomic performance and on possible environmental and health effects.

Each country has different prevailing social and economic conditions, and people have different views of what they eat and what food means to them. All of these factors can affect how GM foods will be regarded from a safety point of view and emphasises the need for case-by-case evaluations.

Robust safety evaluations

Existing commercialised GM crops have undergone robust safety evaluations. The current evaluation process was conceived and formulated by international regulatory safety experts and scientific researchers representing numerous institutions and government bodies. The process continues to evolve as refinements to testing methodologies are introduced. Government regulatory bodies, technology developers, public sector researchers and the community at large participate in this debate to ensure transparency, ensure that the safety testing process is rigorous and foster confidence in the community that the food supply is safe.

The degree of regulatory-testing scrutiny applied to GM plants is unprecedented. We should be confident that as a result of this safety testing process, food derived from GM plants is as safe as food derived from traditional crops.

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Biosafety refers to measures put in place to prevent or mitigate potential risks to human health and the environment resulting from use of modern biotechnology for research or commercial purposes. There is not a 'single procedure' for conducting a safety assessment of a GM plant. The nature and scope of the scientific studies are decided on a case-by-case basis, depending on the plant, the GM trait and the anticipated use of the GM plant. The safety studies are conducted in a step-wise fashion, and may use 'decision trees'. The elements of the risk assessments are derived from scientific principles developed and evolved from expert consultations conducted by, among others:

- Codex Alimentarius Commission
- European Food Safety Authority
- Food and Agriculture Organization of the United Nations
- Food Standards Australia New Zealand
- International Life Sciences Institute
- Organisation for Economic Co-operation and Development
- United States Food and Drug Administration
- World Health Organization of the United Nations.

The factors assessed are:

- novel substance (e.g. protein) that is expressed
- source of the gene that expresses the novel substance
- nutritional composition of the GM plant and its products
- anticipated dietary intake and impact of postharvest processing on the novel substance
- characterisation of the DNA insert
- toxicity and allergenicity potential of the novel substance
- unintended effects
- mammalian toxicity
- allergenic potential
- nutritional composition
- unintended effects.

Mammalian toxicity and allergenic potential

The evaluation processes are especially stringent and include but are not limited to:

- evaluating the donor (source) of the introduced gene
- impact of digestive enzymes and pH on the novel protein
- estimation of potential dietary exposure to the protein
- presence of antigen-specific IgE in sera of hyperallergic individuals
- comparison of the amino acid sequence of the novel protein with known allergens and toxins.

Is there any significant amino acid homology between the novel protein and known toxins or known allergens?

Experts have established a set of criteria. Several public databases are available via the internet to conduct this analysis, including <ncbi.nlm.nih.gov> for toxins and <allermatch.org> for allergens.

Compositional analysis

Compositional analysis assesses whether the expression of the novel trait affects the nutritional value of the consumed portion of the plant. The following factors are considered:

- Levels of key nutrients and anti-nutrients are established that are characteristic of the crop and are significant contributors to the human or animal diet.
- The targeted nutrient levels in the GM crop are compared with a non-GM (conventional) counterpart and to historical values.
- Depending on crop, material analysed may be grain, leaf, whole plant, root tissue or silage.
- Material to be analysed is derived from crops grown under typical agricultural management practices.
- Analyte levels are compared to historical (literature-derived) values and material derived from isogenic control plantings.
- Specific analytes measured are typically those for which the crop is known to be a significant contributor to the diet. A select group of additional analytes is evaluated as a measure of unintended effects.

For example, in cereals the following characteristics or compounds are analysed:

acid detergent fibre	total carbohydrate
neutral detergent fibre	starch
amino acids	raffinose
fatty acids	phytic acid
folic acid	beta carotene
inositol	thiamine (vitamin B_1)
riboflavin (vitamin B_2)	niacin (vitamin B_3)
ascorbate (vitamin C)	tocopherols (vitamin E)
ferulic acid	coumaric acid

Environmental safety

Environmental assessments are a component of regulatory deliberations when an application for cultivation approval is under consideration. Environmental assessments typically look at hazards and their risks at two levels:

1. potential effect on a set of representative indicator organisms
2. potential effect on organisms that may be specific to the geography in question.

Examples of environmental effects are:

- effects on fish, birds, soil invertebrates (e.g. earthworms and Collembola), aquatic invertebrates (e.g. *Daphnia*), beneficial insects (e.g. ladybugs, parasitic wasps and lacewings)
- gene flow into wild and weedy relatives—this is especially important if wild relatives co-exist with the cultivated plants.

There are now 15 international legally binding instruments and non-binding codes of practice that address aspects of GMOs. This is an important and complex area that requires specialist skill and knowledge to be applied from the beginning of any project.

Further information

Safety data information and agency decision documents regarding GM plants are available online at the following websites. All accessed 23 September 2013:

<www.aphis.usda.gov/biotechnology/index.shtml>

<epa.gov/oppbppd1/biopesticides/pips/index.htm>

<cera-gmc.org/?action=gm_crop_database>

<efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_opinion_FINALGMplantsfornonfood-feedpurposes_en.pdf?ssbinary=true>.

Pathways to improved photosynthesis

Status of options for improving photosynthetic capacity through promotion of Rubisco performance—Rubisco natural diversity and re-engineering, and other parts of C_3 pathways

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Summary

The Rubisco literature is very large. Our focus here is a critical assessment of current Rubisco research that most directly impacts on crop photosynthesis research, and that provides direction to strategies and technologies for implementing into crops to improve productivity. Recent comprehensive reviews are given by Spreitzer and Salvucci (2002), Parry et al. (2003), Portis and Parry (2007) and Andersson and Backlund (2008).

Specific findings in this paper are as follows:

- We have examined the implications of Rubisco's slow and non-selective catalysis and its highly conserved sequence and structure. No useful mutants have been reported in the published literature so far from targeted mutagenesis, directed evolution or gene shuffling methods. However, we conclude that there is no evidence that substantially better Rubiscos cannot have evolved or be engineered by mutation.
- We have examined Rubisco's complex requirements for folding and assembly and other aspects of its regulation. We conclude that transforming foreign Rubiscos into crop plants is not promising. Thus, mutant Rubiscos should be carefully engineered so as not to introduce changes that hinder folding and assembly, post-translational modifications or binding of other proteins such as Rubisco activase.
- These examinations highlight a major problem with the reliability of kinetic parameters reported in the Rubisco literature, which makes it difficult to compare results from different studies. This makes assessment of progress difficult and is impeding advancement in the field.
- We have reviewed three types of indirect strategies for improving carbon assimilation in C_3 plants by enhancing Rubisco's capacity to carboxylate ribulose-1,5-bisphosphate (RuBP). These comprise improving CO_2 levels around Rubisco, increasing regeneration of RuBP by overexpression of the Calvin cycle enzyme sedoheptulose-1,7-bisphosphatase, and introducing more thermotolerant Rubisco activase. All three are novel engineering strategies that complement direct methods for improving Rubisco performance.
- To illustrate how better understanding of Rubisco kinetics might be effectively used to guide Rubisco re-engineering or search for naturally variant Rubiscos, we undertook some simple 'what if?' simulations of carbon assimilation, using measured and hypothetical Rubisco kinetic parameters. We compared the performance of wheat and rice Rubiscos, and tobacco Rubisco with a mutant tobacco Rubisco with red-algal-like kinetics. We considered conditions mimicking drought and normal water-use conditions, with ambient atmospheric CO_2 concentration at levels currently and projected for 2050. These *gedanken* experiments reinforced the need for measurements of complete and reliable sets of kinetic parameters for

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crop and model plant species; the currently available data limit application of these convenient simulation tools to assess potential carbon-accumulation gains in-planta from mutant or other novel Rubiscos.

- Our literature analysis provided strong empirical and theoretical evidence for the existence of variation in Rubisco properties within species of plants, and that this is correlated with environmental growing conditions, demonstrating a response to evolutionary selection. Recent evidence suggests greater scope for improved Rubiscos of higher plants from natural variation than previously thought.
- Following these analyses, we reviewed two novel recent approaches to developing improved Rubiscos for crops:
  1. identifying naturally occurring potentially superior Rubiscos from crop germplasm banks
  2. re-engineering Rubiscos, which is patented technology developed at the Australian National University (ANU). The technique uses an in silico phylogenetic grafting method to predict mutants with improved kinetic properties.

  Both strategies have rational defined workflows that can be iterated to identify sources of germplasm more enriched with better Rubiscos or provide guidance on how to refine mutants to improve their activity. The diversity approach has the advantage of not requiring a genetic transformation but, as it has not yet been tested for a crop species, the gains in Rubisco performance obtainable are uncertain. Initial results from the mutant approach have already shown large gains in activity but a disadvantage of the approach is the requirement for chloroplast transformation, which has not been achieved in monocots such as cereals.
- Our consideration of bottlenecks in chloroplast transformation technologies indicates several new possible solutions. Apart from the need for Rubisco transformation, the availability of efficient chloroplast transformation technologies would provide many benefits for other genes. Notable are higher biosafety if transgenes are contained within the plant plastid genome, and greater acceptance by regulatory bodies. Future crop development needs argue for an increased investment in these technologies.
- We note a recent increase in patent applications related to Rubisco, Rubisco activase and respiratory bypass technologies, including the ANU technology. This suggests greater commercial interest from agbiotech companies in photosynthesis research for crop development.

Implications of Rubisco's catalysis

Kinetic characterisation of Rubiscos from the major evolutionary branches of photosynthetic organisms provides typical ranges of values for each branch for the catalytic rate for carboxylation, k_{cat}, the Michaelis–Menten constant for carboxylation, K_C, the carboxylation efficiency, k_{cat}/K_C, and the specificity factor, $S_{C/O}$, which is a measure of the relative efficiency of carboxylation to oxygenation. Values are shown in Table 1 for some Form I Rubiscos. Table 1 also shows Form II Rubisco from anaerobic bacteria. A maximally efficient Rubisco would have high k_{cat}, k_{cat}/K_C and $S_{C/O}$ values and low K_C.

Jordan and Ogren (1981) made the early observation of an apparent inverse correlation between k_{cat} and $S_{C/O}$, which gained support as more data became available, especially for red algae in the late 1990s (Zhu et al. 2004). This has led to a general view in the literature, when assessing possibilities for improving Rubisco performance in crops, that only modest improvements in both k_{cat} and $S_{C/O}$ may be possible. Contrary to the title of work by Tcherkez et al. (2006) that Rubisco 'may be nearly perfectly optimized', they conclude there is room for improvements within the scatter of the experimental data points from a linear correlation. Galmés et al. (2005, 2007) recently showed significant scope for improvement of Rubiscos for higher plants, as has our Rubisco re-engineering technology.

It is apparent from Table 1 that although Rubisco generally shows low $S_{C/O}$, its value has increased over evolutionary timescales from the earliest anaerobic bacteria through cyanobacteria to green plants. The kinetic parameters for red algal Rubiscos (Form ID) are an interesting outlier. Groundbreaking work of Delwiche and Palmer (1996) demonstrated a separate phylogenetic origin of the cyanobacteria – green algae – green plant lineage (Forms IA and IB) from that of red algae and other Form IC and ID Rubiscos. They found the lineage arises from a complex history of horizontal gene transfers and duplications during the evolution of the plastid *rbc*L gene. The high $S_{C/O}$ value for red algal Rubisco led to considerable enthusiasm for improvement of crops by transplanting their genes into higher plants (Mann 1999), but efforts thus far have been fruitless.

Although the temperature dependence of Rubisco's kinetic parameters is particularly significant for its function in the plant—in contrast to enzymological characterisation of (homeothermic) mammals—and to the deliberations of this symposium, it has been little studied and remains a significant caveat in our understanding of Rubisco biology (Sage 2002).

Implications of Rubisco's sequence and structure

The protein sequence of the Rubisco large subunit (LSU; L) is highly conserved. Rubiscos of higher plants consist of a hexadecamer of eight LSUs and eight small subunits (SSU), with the LSUs arranged as a core of four L_2 dimers centred on a fourfold axis. The active site is very well conserved in both structure and sequence and is at the interface of the two LSUs in an L_2 dimer, each dimer containing two active sites.

The major regions for sequence variation in the LSU are in the N- and C-termini, dimer–dimer, intradimer, LSU–SSU and Rubisco–Rubisco activase interactions (Kapralov and Filatov 2007), and the Rubisco–RbcX protein interaction (Saschenbrecker et al. 2007). Mutational attempts, directed evolution (Mueller-Cajar and Whitney 2008) and gene shuffling to engineer catalytic improvements have had limited success, as only one cyanobacterial Rubisco-mutant derived from directed evolution showed modest improvement in overall catalytic prowess (Greene et al. 2007). Spreitzer et al. (2005) focused on mutation of intersubunit regions. Although they reported some kinetic variation there is no indication it is large enough to be useful in re-engineering Rubisco for improved efficiency in crops.

Pioneer – Du Pont's patented laboratory evolution strategy in *Chlamydomonas* may not be directly superimposable onto plant Rubiscos as they propose (Zhu et al. 2008). The kinetic parameters reported for their best derived tobacco transformant were not replicated by an independent test, as shown in Table 2. This example highlights a general problem in the Rubisco literature with reliability of reported kinetic parameters. Available kinetic measurements in the literature need to be treated with some caution, in particular as measurements of k_{cat} have approximately doubled over the decades with improvements

Table 1. Kinetic data for Rubiscos from various bacteria, algae and higher plants

	Rubisco CO_2–O_2 environment	$S_{C/O}$	k_{cat} (/s)	K_C (μM)	k_{cat}/K_C (/s mM)
Red algae					
Griffithsia monilis	CCM absent?	167	2.6	9.3	279
Galdieria sulfaria	CCM absent?	166	1.2	3.3	364
C_3 plants					
Tobacco	CCM absent	82	3.4	11	309
Spinach	CCM absent	82	3.7	14	264
Wheat	CCM absent	90	2.5	14	179
C_4 plants					
Maize	CCM present	79	4.4	34	129
Sorghum bicolor	CCM present	70	5.4	30	180
Green algae					
Chlamydomonas reinhardtii	CCM present	61	5.8	29	200
Euglena gracilis	CCM present	54			
Cyanobacteria					
Synechococcus 6301	CCM present	42	12	340	34
Anabaena variabilis	CCM present	43			
Bacteria					
Rhodospirillum rubrum	Anaerobic	12	7.3	80	91
Riftia pachyptila	Anaerobic	6			

Source: adapted from Tcherkez et al. (2006)
CCM = carbon dioxide concentrating mechanism; k_{cat} = catalytic rate for CO_2; K_C = Michaelis–Menten constant for carboxylation by Rubisco.

Table 2. Comparison of tobacco Rubisco catalytic properties at 25°C for wild-type and a mutant transformant as determined by ANU and Du Pont

Kinetic parameter	(unit)	Wild-type tobacco	Mutant A99T, D281S, D352G	Wild-type tobacco	Mutant T798 A99T, D281S, D352G
		(ANU)	(ANU)	(DuPont)	(DuPont)[a]
[b] $S_{C/O}$		79 ±3	78 ± 3 (–1%)[f]	78 ± 1	89 ± 3 (14%)
[c] K_C	(mM)	12.8 ± 0.7	13.8 ± 0.2 (8%)	12.6	7.6 (–40%)
[d] k_{cat}	(/s)	3.1 ± 0.2	3.5 ± 0.1 (14%)	1.41	1.74 (23%)
k_c/K_C	(/s mM)	242	253 (5%)	112	228 (104%)
[c] K_O	(mM)	221 ± 27	213 ± 7	–	–
[e] k_{ocat}	(/s)	[g](0.7)	[g](0.7)	–	–

[a] Mutant T798 reported by Caspar et al. (2008b)
[b] Measured from replicate assays ($n \geq 3$) by the method of Kane et al. (1994) using ion-exchange purified Rubisco (Sharwood et al. 2008) from wild-type and the mutant transplastomic line tob^{mut}44.4
[c] Measured using rapidly extracted leaf protein (Sharwood et al. 2008) from wild-type ($n = 2$) and the mutant tob^{mut}44.2 ($n = 1$) and tob^{mut}44.4 ($n = 1$) mutant transplastomic lines
[d] Measured using rapidly extracted leaf protein (Sharwood et al. 2008) from wild-type ($n = 2$) and the tob^{mut}44.2 ($n = 1$) and tob^{mut}44.4 ($n = 2$) transplastomic mutants
[e] Calculated using the equation $S_{C/O} = (k_{cat}/K_C)/(k_{ocat}/K_O)$
[f] Per cent changes compared with wild-type
[g] Single replicate with no error given
– = not available

in experimental design. In this paper we outline ANU's novel in silico phylogenetic grafting technology for designing improved Rubisco mutants of the host plant with minimal structural perturbations to the overall Rubisco LSU structure.

Folding and assembly of Rubisco

Folding and assembly of Form I Rubiscos of higher plants into the functional complex comprised of eight LSUs and eight SSUs is a complex process that is poorly understood. Folding and assembling of LSUs into L_2 dimers is likely followed by assembly into the $4 \times L_2$ core, and completed by adding eight SSUs. In higher plants, assembly requires specific chaperones such as BSDII (Brutnell et al. 1999; Wostrikoff and Stern 2007) and possibly RbcX (Saschenbreker et al. 2007). However, S.M. Whitney (unpublished data) suggests modifications to the putative C-terminal RbcX-binding motif in the tobacco large subunit have no influence on Rubisco expression. Likewise, deletion of RbcX in the cyanobacterium *Synechococcus* PCC7942 has no influence on Rubisco synthesis (Emlyn-Jones et al. 2006).

Chaperone incompatibilities also affect production of Form I Rubiscos in *E. coli*; they entirely prevent the folding and assembly of plant Rubiscos in this favoured expression host. This impeded engineering of higher plant Rubisco until the recent development of plastome transformation technologies that enable manipulation of Rubisco in-planta, most efficiently in tobacco (Whitney and Sharwood 2008). A recently developed method for production of chimeric Rubiscos by transforming a foreign plant *rbc*L gene into tobacco indicates that correct folding and assembly are achievable if the LSU of the foreign species is not 'too different' from tobacco in its capacity to fold with the tobacco SSU (Sharwood et al. 2008). Attempts at transforming red algal Rubisco into green plants has not been successful (Whitney et al. 2001). However, recent patent applications indicate work is ongoing (Bracher et al. 2008).

Other aspects of Rubisco regulation

In addition to understanding the complex catalytic chemistry and assembly requirements of Rubisco there is a need to take into account the roles of the co- and post-translational modifications that accompany Rubisco biogenesis and subsequent regulation by its helper protein Rubisco activase. In

all plants examined so far, translation of the LSU is initiated by a complex series of modifications and deletions at the N-terminus, resulting in an acetylated Pro-3 (Houtz and Portis 2003). An additional post-translation modification to the LSU in some plants is the trimethylation of the γ-amino group of Lys-14; the function and necessity of this change remains an enigma (Houtz and Portis 2003). Ongoing work (S.M. Whitney and R.L. Houtz, unpublished data) aims to establish the functional necessity of retaining the integrity of N-terminal LSU residues, particularly in regulating protein degradation.

The catalytic competency of Rubisco in higher plants also requires Rubisco activase (Salvucci 2013). This nucleus-encoded protein removes bound sugar phosphate inhibitors that can bind to the Rubisco catalytic sites and inhibit their activity. Exactly how RA interacts with, and promotes conformational change to, Rubisco remains uncertain. This is mainly because of the inability to obtain a crystal structure of RA. A model for Rubisco activation by RA has been slowly pieced together. The model identifies interacting domains and residues between both proteins (Portis et al. 2008) with the species-specificity of some RA–Rubisco interactions helping identify the residues in the plant Rubisco LSU that influence its capacity to interact with RA. Ongoing work (S.M. Whitney, unpublished data) is assessing how mutations in tobacco Rubisco influence its capacity for regulation by tobacco RA, and the resultant changes in photosynthesis and growth under varying temperatures.

This knowledge is important for improving our fundamental understanding of Rubisco structure–function biology. It is also crucial to defining which LSU residues are requisite for sufficiently sustaining these processes and thus, should be avoided in the ongoing development of in silico design technology for Rubisco-mutant phylogenetic grafting.

Indirect strategies for overcoming limitations to Rubisco activity

Strategies to improve carbon assimilation by increasing the capacity of Rubisco to carboxylate RuBP are being tested in model C_3 plants (reviewed in Raines 2006; Peterhansel et al. 2008). Six main strategies are indicated in Figure 1. Three strategies aim to increase CO_2 levels around Rubisco by introducing: (1) C_4 assimilation characteristics into C_3 cells (Furbank et al. 2013); (2) CO_2/HCO_3^- pumping proteins from cyanobacteria into chloroplast membranes (Lieman-Hurwitz et al. 2003; Price et al. 2008); or (3) new 'catabolic bypass' pathways into plastids. This third strategy bypasses the energy-expensive photorespiratory recycling of Rubisco's oxygenase product, 2-phosphoglycolate (2-PG), so releases CO_2 in the stroma rather than the mitochondria (Kebeish et al. 2007).

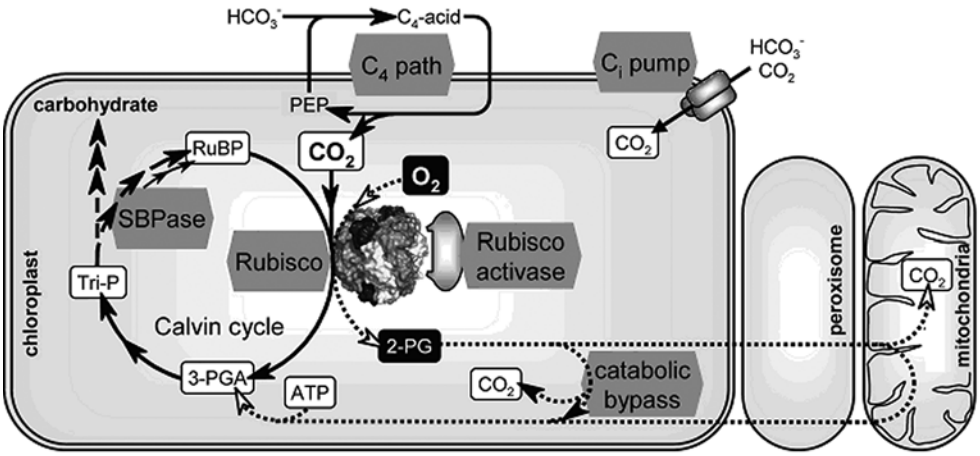

Figure 1. Idealised schematic summarising current strategies for improving carbon assimilation in C_3 plant chloroplasts. Dashed lines and white arrowheads indicate the photorespiratory pathway for recycling of Rubisco's oxygenase product 2-phosphoglycolate (2-PG) into 3-phosphoglycerate (3-PGA). Tri-P = triose phosphate.

The fourth strategy aims to increase the throughput of the Calvin cycle's RuBP regenerative phase by increasing the content of the enzyme sedoheptulose-1,7-bisphosphatase (SBPase). Under non-Rubisco limiting growth conditions (e.g. high illumination or/and high stromal pCO_2), photosynthesis and growth improve (Miyagawa et al. 2001; Lefebvre et al. 2005; Tamoi et al. 2006).

The fifth strategy is to increase photosynthetic carbon assimilation under temperature-limiting conditions by introducing a more thermotolerant Rubisco activase, sustaining Rubisco activity under moderately elevated temperatures (Kurek et al. 2007a; Kumar et al. 2009; Salvucci 2013). On the whole, this strategy poses novel engineering challenges. If successfully implemented, the fifth strategy may complement the technology that focuses directly on the sixth strategy—improving the catalytic prowess of Rubisco in plant chloroplasts. This is outlined in the next section.

Natural variation and response to evolutionary selection

Empirical and theoretical evidence from kinetic measurements and sequence analysis show Rubisco properties vary within plant species and are correlated with environment. The comprehensive sequence analysis of Kapralov and Filatov (2007) demonstrated that despite its high conservation, Rubisco evolves under positive selection in most lineages of land plants, and that even after billions of years of evolution, selection is still fine-tuning its performance.

Many species of C_3 plants in diverse habitats in the Balearic Islands show a wide range of growth limitations, particularly water availability and temperature (Galmés et al. 2005, 2007). Interestingly, the $S_{C/O}$ values correlated better with environmental pressure than phylogeny, with higher values characteristically found in species growing in dryer environments. Notably, they demonstrated differences in $S_{C/O}$ values among species from the same genus, suggesting differences will likely be detected in important crop genera such as *Triticum* (wheat). Studies of plants in other environments, including nutrient-poor environments (Mitchell et al. 2005), and of the Hawaiian genus *Schiedea* of vines and woody shrubs (Kapralov and Filatov 2006) and other photosynthetic organisms (extremophilic red algae; Ciniglia et al. 2004), have confirmed this variability trend. These results support the hypothesis that Rubisco properties in C_3 plants have been modified by selective pressures affecting carbon assimilation and plant growth. As noted earlier, the relationship between K_C and k_{cat} does not show a simple trade-off in a single direction (Galmés et al. 2007). The special case of variability in C_4 plants is more complex (Salvucci 2013); variability in k_{cat} of Rubisco of C_4 grasses has been shown with such plants, reducing their N-requirement by making less enzyme.

Improving crop Rubiscos

The following section presents selected topics in the area of improving Rubiscos for crops.

Correlation of carbon accumulation with Rubisco kinetics

The rate of CO_2 assimilation in C_3 plants reflects Rubisco's kinetic properties and content in the plant (Farquhar et al. 1980; von Caemmerer 2000). This correlation has been validated for mutant, foreign-transformant or differently expressed tobacco Rubiscos. It is thus possible to model CO_2 assimilation under variable growth conditions such as CO_2 concentration, water, nutrients, temperature and light intensity, to predict leaf photosynthetic performance for a range of plants using Rubisco kinetic data (von Caemmerer 2000). An analogous model to predict performance within the leaf canopy of the whole plant has also been reported (Zhu et al. 2004). By these means the growth performance of a plant from knowledge of its Rubisco kinetic parameters—as determined in vitro using isolated enzyme—can be simulated. Aspects of these models need refining to improve their predictive translatability from the leaf processes to the canopy level under different growth conditions.

In Figures 2–4 we show the results of some 'What if' simulations to illustrate the use of these models to forecast the effects of different growth conditions or Rubisco kinetic parameter values on the rate of carbon accumulation. These 'experiments' consider the effects of water limitation (lower effective pCO_2 in the chloroplast) and increased atmospheric pCO_2 (projected from climate change) for tobacco, the consequences of transplanting a red algal Rubisco into tobacco, and comparisons of wheat and rice.

In Figure 2, the boxed regions highlight assimilation rates with stomatal conductance values representing average water use and drought conditions for current ambient and future projected pCO_2 levels.

Ambient pCO_2 is projected to rise to 550 µbar by 2050 (Leakey 2009). It is clear that under such high pCO_2 levels photosynthetic CO_2-assimilation rates (A) become electron-transport limited. In addition, the drop in assimilation rate on changing the stomatal conductance from normal water-use conditions to drought conditions is slightly smaller (A_2'–A_1' compared with A_2–A_1). Thus, the influence of drought on plant assimilation rates would be reduced under future atmospheric conditions.

Figure 3 compares the CO_2 assimilation response to increase in intercellular CO_2 partial pressure (C_i) for wheat and rice using data from Makino et al. (1998) (full details in caption). Under current pCO_2, the assimilation rates would be Rubisco-limited for wheat and rice under moderate drought. However, under future CO_2 levels the assimilation rates for both crops are predicted to be electron-transport limited. The key difference in the kinetic parameters between the two Rubiscos is the lower k_{cat} for rice Rubisco compared to that of wheat Rubisco. However, the data available for modelling (Makino et al. 1998) are inconsistent with this same paper, which also reports that the leaf Rubisco content is 20% higher than in wheat. Applying this scaling shifts the rice plot (broken grey line) closer to the wheat plot and approximately overlapping it under the current range of CO_2 conditions. Increasing the rice k_{cat} value shifts

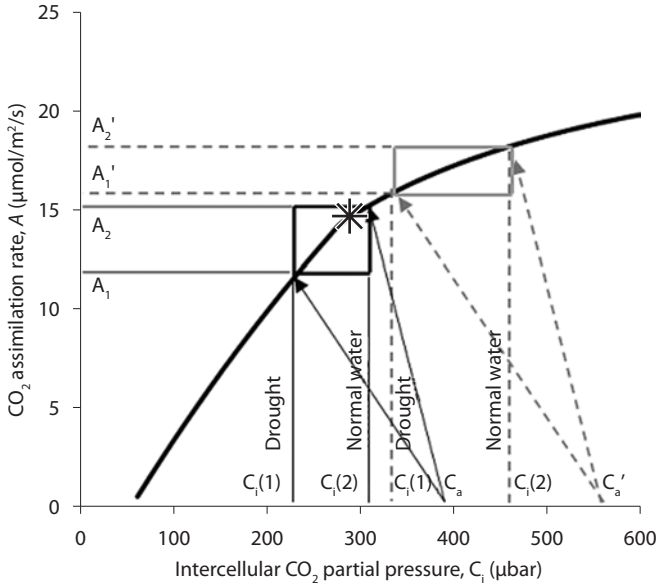

Figure 2. Leaf CO_2 assimilation rate as a function of intercellular CO_2 partial pressure for tobacco, modelled using Rubisco kinetic parameters from Andrews and Whitney (2003). K_C = 320 µbar, K_O = 234 mbar, $S_{C/O}$ = 82, k_{cat} = 3.4/s, average content of Rubisco in the leaf (E) = 20 µmol/m², V_{Cmax} (calculated as $k_{cat} \times E$) = 68 µmol/m²/s, maximum rate of chloroplast electron transport (J_{max}) = 120 µmol/m²/s, mitochondrial respiration rate (R_d) = 1 µmol/m²/s, T = 25°C and irradiance (PAR) (I) = 1000 µmol quanta/m²/s. C_a is the ambient pCO_2 in the current atmosphere (385 µbar) and C_a' is the projected pCO_2 (550 µbar) in 2050 (Leakey 2009). The arrows point to intercellular pCO_2 under stomatal conductance values representing average water use (C_i/C_a = 0.8; $C_i(1)$ and $C_i(1)$') and drought (C_i/C_a = 0.6; $C_i(2)$ and $C_i(2)$') conditions. A_1 and A_1' are the CO_2 assimilation rates under drought at current atmospheric pCO_2 and projected pCO_2 for the year 2050, while A_2 and A_2' are the CO_2 assimilation rates for normal water use at current pCO_2 and projected pCO_2 for the year 2050. In this plot, the stomatal conductance is assumed to be similar under current and 2050-projected levels of pCO_2 and hence the arrows leading from C_a and C_a' appear parallel. Asterisk denotes the crossover from Rubisco-limited to electron-transport limited assimilation.

the plot (broken black line) further upwards, predicting greater assimilation than wheat under the current range of CO_2 conditions but overlapping wheat under the range of CO_2 conditions projected for 2050. Both modified rice plots, however, predict that assimilation would still be electron-transport limited except under severe drought.

The $S_{C/O}$ parameters for wheat (120) and rice (128) in Makino et al. (1998) are significantly higher than typical values for C_3 plants, including wheat and rice, of 80–90 (Table 1). Therefore, we tested the consequences of using the higher values by modelling rice and wheat with $S_{C/O}$ = 85 and all other parameters unchanged (see Figure 3 caption). These plots (data not shown) do not show significant differences in the relative assimilation rates, but the crossover points for Rubisco-limited and electron-transport assimilation are shifted to the left.

The modelling in Figure 3 demonstrates that lack of complete and reliable sets of kinetic parameters for crop (and model plant species) limits application of this quick and convenient tool to assess potential carbon-accumulation gains in-planta from mutant or other novel Rubiscos. Researchers rarely measure complete sets of data, which would include oxygenase parameters. In general, incompleteness and unreliability of kinetic parameters reported in the Rubisco literature make it difficult to compare results from measurements by different researchers in different studies (compare Table 1). Apart from

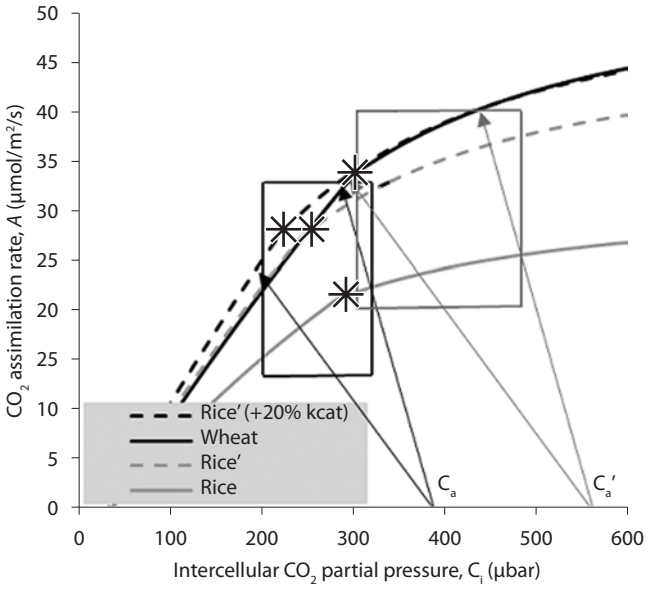

Figure 3. Leaf CO_2 assimilation rate as a function of pCO_2 for wheat (black solid line) and rice (grey solid line) modelled using kinetic parameters from Makino et al. (1988). For wheat, K_C = 335 µbar, K_O = 304 µbar, $S_{C/O}$ = 120, k_{cat} = 2.63 µmol/(mg enzyme)/min, average content of Rubisco in the leaf (E) = 3.77 g/m^2 and V_{Cmax} (calculated as $k_C \times E$) = 165 µmol/m^2/s; for rice, K_C = 239 µbar, K_O = 266 mbar, $S_{C/O}$ = 128, k_{cat} = 1.77 µmol/(mg enzyme)/min, E = 2.62 g/m^2, V_{Cmax} = 77 µmol/m^2/s, using T = 25°C, I = 1,000 µmol quanta/m^2/s and R_d = 1 µmol/m^2/s, and assuming $J_{max} = 2 \times V_{Cmax}$. C_a is the current atmospheric pCO_2 (385 µbar) and C_a' is the projected pCO_2 (550 µbar) in 2050 (Leakey 2009). Broken grey line models rice with Rubisco content 20% higher than wheat ($E = 1.2 \times 3.77$ g/m^2; Makino et al. 1998); broken black line models rice with both 20% increased Rubisco content and 20% increase in k_{cat}. Arrows point to intercellular pCO_2 under stomatal conductance values representing average water use and drought conditions as in Figure 2. Asterisks denote the crossover from Rubisco-limited to electron-transport limited assimilation.

limiting the usefulness of simulations, this makes assessing progress from mutational and other studies difficult and impedes progress in the field.

Figure 4 compares the CO_2 assimilation response to increase in C_i for tobacco and the red algae *Griffithsia monilis* Rubiscos. For *G. monilis*, *A* could be limited by Rubisco for C_i experienced by leaves even at the very high ambient pCO_2 projected for 2050. Rubiscos for *G. monilis* and other red algae have very high specificity for carboxylation over oxygenation (see Table 1). However, the striking difference between the kinetic parameters of tobacco and *G. monilis* is the unusually high K_O value for the latter, more than twice that for tobacco. The broken line in Figure 4 simulates the assimilation rate of tobacco Rubisco, with K_O and $S_{C/O}$ increased by 100%, while the maximum rates of carboxylation and oxygenation by Rubisco, respectively (V_{Cmax}, V_{Omax}), and K_C remain unaltered. A Rubisco with these characteristics is predicted to outperform *G. monilis*, and possibly other red algal Rubiscos, both at the current ambient pCO_2 and at the high pCO_2 projected for the future. Thus, it would be useful to better understand how the *G. monilis* Rubisco can retain its affinity for CO_2, while drastically losing its ability to bind O_2.

Naturally occurring superior Rubiscos in germplasm collections

Evidence for variation in Rubisco activity under different agro-climatic conditions, especially heat, water and nitrogen stress, suggests the possibility of mining germplasm collections (Street et al. 2013)

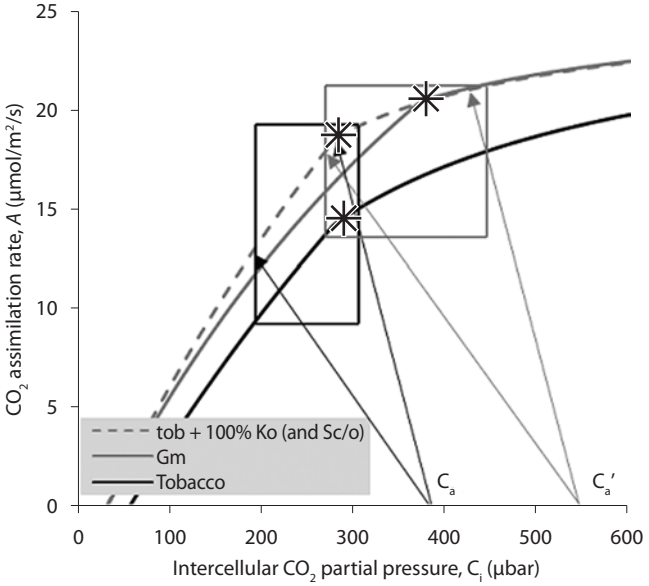

Figure 4. Leaf CO_2 assimilation rate modelled as a function of intercellular pCO_2 for tobacco (black solid line) and *Griffithsia monilis* (Gm, grey solid line) using kinetic data from Andrews and Whitney (2003). For tobacco, K_C = 320 µbar, K_O = 234 µbar, $S_{C/O}$ = 82, k_C = 3.4/s, average content of Rubisco in the leaf (E) = 20 µmol/m^2, V_{Cmax} (calculated as $k_C \times E$) = 68 µmol/m^2/s, J_{max} = 120 µmol/m^2/s; for Gm, K_C = 278 µbar, K_O = 563 mbar, $S_{C/O}$ = 167, k_C = 2.6/s, E = 20 µmol/m^2, V_{Cmax} = 52 µmol/m^2/s, J_{max} = 120 µmol/m2/s, and using T = 25°C, I = 1,000 µmol quanta/m^2/s and R_d = 1 µmol/m^2/s. Broken grey line shows the modelled assimilation rate for tobacco Rubisco with a 100% increase in K_O, and as a consequence of a 100% increase in $S_{C/O}$. C_a and C_a' are the current atmospheric pCO_2 level and that projected for 2050 (550 µbar) (Leakey 2009), respectively. Arrows point to intercellular pCO_2 under stomatal conductance values representing average water use and drought conditions as in Figure 2. Asterisks denote the crossover from Rubisco-limited to electron-transport limited assimilation.

for naturally occurring, better Rubiscos. We have developed a strategy to identify Rubisco variants by coupling the ANU in silico Rubisco technology with developments at the International Center for Agricultural Research in the Dry Areas (Syria) (ICARDA). The strategy uses geo-referencing samples in germplasm banks and the FIGS (focused identification of germplasm strategy) method (Kaur et al. 2007; Strelchenko et al. 2008; <www.icarda.org/tools/figs>.

Annotating the accessions with a geo-reference makes the germplasm bank data more useful (within a FIGS approach or otherwise), as relevant environmental features (climate, soil) can be mapped to the sample-collection site using tools such as the geographic information system (GIS). By obtaining climate data and mapping it as various layers of information (using GIS), it is possible to predict the selection pressures that have been applied to the genotype at particular collection sites. Thus, it is possible to exploit this genotype–environment interaction in reverse to identify accessions most likely to contain the genetic variation required to improve productivity in a specific, or target, environment.

In the first step of our strategy, a modified FIGS procedure and wheat phylogenies were used to select approximately 2,000 wheat samples for collections (landrace, wilds and primitives) in Australia (Australian Winter Cereals Collection, AWCC), ICARDA, the US Department of Agriculture (USDA) and several European germplasm banks. The procedure considered germplasm from diverse eco-ethno origins to reflect broad similarities in the environment and ethnological histories of wheat cultivation and evolution. Within these eco-ethno zones, agro-climatic variables used in the cluster analysis included altitude, yearly monthly average maximum temperature, seasonal temperature variation, annual precipitation, precipitation seasonality (CV) and aridity. As an example, Figure 5 shows how the genetic variation of the samples map to annual precipitation and annual mean temperature. The second component of the strategy involves using the ANU in silico technology in reverse to identify naturally occurring superior Rubiscos from LSU sequences obtained from seedlings of the selected samples.

This combined strategy provides a feasible approach to several otherwise intractable problems. Selecting a relatively small number of samples likely to be heavily enriched with naturally occurring superior Rubiscos from tens of thousands of possible samples, and predicting functionally significant Rubiscos from variation in LSU sequences, avoids large numbers of time-consuming Rubisco kinetic assays. The strategy has the potential to refine the sampling strategy if rich sources of naturally occurring superior Rubiscos are found. The strategy also has the potential to be replicated for other crops with geo-referenced collections.

ANU's in silico phylogenetic grafting technology for improved Rubisco mutants

A novel patented technology for prediction and re-engineering of Rubiscos with improved activity has been developed at ANU (Gready and Kannappan 2008a,b). It couples results of a novel chemical mechanism (Kannappan and Gready 2008) with publicly available databases for protein sequences, and phylogeny and structures, of Rubiscos. The method identifies regions of a Rubisco sequence that evolution has not fully sampled by mutational selection, and enables rational design of better Rubiscos starting from Nature's current solutions, as encoded in existing Rubiscos. The method identifies sets of amino-acid residue positions to mutate, what they should be mutated to, and what combinations of mutations should be made.

Using this in silico method, sets of Rubisco mutants with a variety of kinetic profiles of improved activity in key kinetic parameters (specificity, carboxylation rate k_{cat}, affinity for CO_2, (K_C)) have been predicted and successfully tested in two model photosynthetic organisms (a cyanobacterium and tobacco). Mutant tobacco lines with improvements in kinetic efficiency (k_{cat}/K_C) greater than approximately 30% have been produced. Simulations of the CO_2-assimilation rate (A) of the best tobacco mutant using the methods discussed with Figures 2–4 predict improvements of 15 and 23%, respectively, under effective drought conditions. Under drought, the reduced stomatal conductance lowers intracellular pCO_2 to about 230 µbar (von Caemmerer 2000) at normal and high irradiance (I = 1,000 and 1,600 µmol quanta/m^2/s). Large improvements in CO_2 assimilation are also predicted under effective nitrogen limitation (reduced Rubisco content). For example, at 30% average content of Rubisco in the leaf (E) (Figure 2 caption provides values of typical E), assimilation rates are increased by 33 and 31% under drought and average water-use conditions, respectively. That is, they show little dependence on water availability. On the other hand, for average leaf Rubisco content, the increase

in *A* is smaller and varies significantly with water availability, at 15% and 4% for drought and normal water-use conditions, respectively.

Relevant patent positions

There has been a recent increase in patent applications related to Rubisco, Rubisco activase and respiratory bypass technologies, including the ANU technology. Some of these technologies have been discussed elsewhere in this paper. Some relevant patent applications are given in the references. Much of this work has not been published in the conventional literature, highlighting the importance for crop-development researchers to access the patent literature.

Most of the dominant broad-spectrum chloroplast transformation patents are considered to be held by Daniell and McFadden (1999; 2003; 2004) rather than by Maliga et al. (1999; 2002). The solanaceous patent of Monsanto (Nehra et al. 2003) still covers potato.

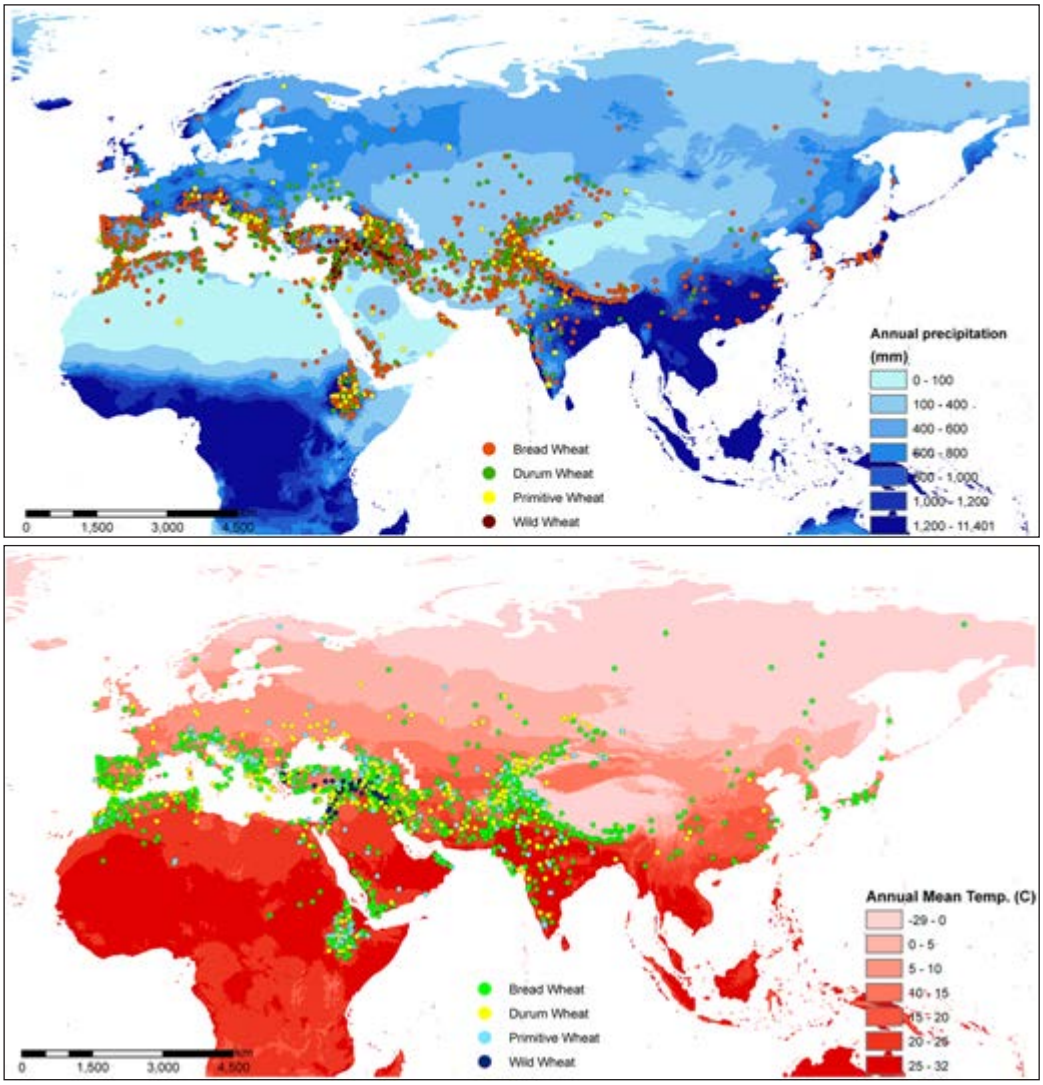

Figure 5. Geographical distribution of wheat accessions (*Triticum* spp. only) selected for the Rubisco sequencing project overlaid on GIS annual precipitation and mean temperature gradient maps

Technical and biosafety issues

Selected important technical and biosafety issues are covered in this section.

Chloroplast transformation (for Rubisco mutants)

Tobacco model

At least two transplastomic tobacco lines specifically tailored for engineering Rubisco have been constructed. In the master line generated in the Whitney laboratory, ^{cm}trL, the *rbc*L gene is replaced with a synthetic ^{cm}*rbc*M Rubisco gene (which codes for the L_2 Rubisco dimer from the bacterium *Rhodospirillum rubrum*). *Rhodospirillum rubrum* Rubisco shows less than 25% similarity with any other Form I *rbc*L gene. It is thus immune to unwanted recombination events with L_8S_8 Rubisco genes. The ^{cm}trL line enables Form I Rubisco synthesis to be rapidly identified and allows transformants producing little or no Rubisco, or catalytically inert Rubiscos, to be recovered (Whitney and Sharwood 2008). Another, but slow growing, tobacco *rbc*L-knockout (D*rbc*L) line has been generated at Icon Genetics (Halle, Germany). The variegated transformant is not fully segregated to homoplasmicity and produces leaves with both green wild-type and bleached D*rbc*L sectors. The D*rbc*L leaf sectors produce no Rubisco as the *rbc*L genes in their plastome copies have been replaced with the *GFP* gene (Casper et al. 2008a,b). The bleached-leaf sectors are excised and used for transforming in foreign or modified Rubisco *rbc*L genes.

Other plants

Since plastid transformation was successfully demonstrated in tobacco (Svab et al. 1990), more than 40 different foreign proteins have been expressed in transgenic chloroplasts (Grevich and Daniell 2005) generated by both biolistics and polyethylene glycol (PEG)-mediated methods. In recent years, plastid transformation of a wider number of species has been sporadically reported as using different DNA delivery techniques and different tissue culture methods (Bock 2007; Koop et al. 2007).

Published reports indicate that plastid transformation has been achieved for lettuce (Lelivelt et al. 2005), cauliflower (Nugent et al. 2006), potato (Sidorov et al. 1999; Nguyen et al. 2005), tomato (Ruf et al. 2001; Nugent et al. 2005), oilseed rape (Hou et al. 2003), *Lesquerella fendleri* (Skarjinskaia et al. 2003), petunia (Zubko et al. 2004), *Arabidopsis* (Sikdar et al. 1998), poplar (Okumura et al. 2006), cabbage (Liu et al. 2007) and sugar beet (De Marchis et al. 2009). These plants were transformed by particle bombardment using leaf, cotyledon or hypocotyl material. Plastid transformation has also been reported for carrot (Kumar et al. 2004a), cotton (Kumar et al. 2004b), soybean (Dufourmantel et al. 2004) and rice (Lee et al. 2006) by bombarding regenerating somatic embryonic non-green cells and tissues and regenerating subsequent plants.

The reality is that in most of the above transformation reports, very few transgenic plastid-transformed plants were generated and conversion to homoplasmy was not achieved. Therefore, plastid transformation in most of these systems is not routine, if even reproducible (Koop et al. 2007). Attaining homoplasmic transformants at any reasonable, reproducible frequency is still only efficient in tobacco (Maliga 2004).

Although the initial breakthrough in plastid transformation of higher plants is about 20 years old, many factors have limited its routine application in a wider variety of plant species or commercial crops. These factors include lack of plastid genome sequences for the development of species-specific plastid homology vectors, inadequate tissue culture and regeneration protocols (where the frequency of obtaining transgenic plants is quite low and/or too costly from an infrastructure perspective), absence and development of functional selectable markers specifically detailed for plastid transformation, inability to express transgenes effectively in non-green plastids (Daniell et al. 2005) and small surface area of chloroplasts (Bogorad 2000). These factors are major obstacles for routinely extending this technology to a wider variety of crop plants.

Continuing improvements in nuclear transformation and plant tissue culture technologies provide attractive options to removing bottleneck in establishing reproducible plastid transformation for the major crops, especially monocots. However, a major impetus in the field is now required. The RMIT University (Melbourne) group is piloting options including novel procedures for establishing monocot tissue culture lines, modified tissue culture methods and plastid number to increase the efficiency of the gene transfer targeting process. The group is also developing alternative selection systems and adapting an appropriate plant nuclear transformation method.

Benefits from chloroplast transgenics for crops

Apart from being able to perform in-depth studies on a statistically significant number of plastid-transformed plants containing modified Rubisco variants, there are benefits from having routine plastid transformation systems in the major crop species. These include the ability to:

- express foreign genes that are under the control of bacterial-type expression or control systems at extremely high levels
- express multiple genes coordinately and/or in the form of prokaryotic-type operons
- stack more beneficial transgenes onto nuclear transform plants
- compartmentalise genes and their corresponding gene products, where such proteins may be deleterious to normal plant growth and development when expressed by nuclear transformation.

Biosafety benefits can be derived where transgenes are localised within the plant plastid genome. This prevents outcrossing of plastid-borne transgenes though pollen transfer (exclusive maternal inheritance in certain plants). Plastid transformation would therefore provide a level of containment that is currently favoured by regulatory bodies for the release of certain genetically modified crops (Daniell 2007; Ruf et al. 2007; Svab and Maliga 2007).

Thus, apart from being needed for Rubisco *rbc*L transformation, the availability of efficient chloroplast transformation technologies in plants has other potential benefits. Future crop-development planning argues for an increased investment in these technologies.

References

Andersson I. and Backlund A. 2008. Structure and function of Rubisco. Plant Physiology and Biochemistry 46, 275–291.

Andrews T.J. and Whitney S.M. 2003. Manipulating ribulose bisphosphate carboxylase/oxygenase in the chloroplasts of higher plants. Archive of Biochemistry and Biophysics 414, 159–169.

Bock R. 2007. Plastid biotechnology: prospects for herbicide and insect resistance, metabolic engineering and molecular farming. Current Opinion in Biotechnology 18, 100–106.

Bogorad L. 2000. Engineering chloroplasts: an alternative site for foreign genes, proteins, reactions and products. Trends in Biotechnology 18, 257–263.

Bracher A., Hartl U., Hayer-Hartl M. and Saschenbrecker S. 2008. Means and methods for providing ribulose bisphosphate-carboxylase-oxygenase with improved properties. PCT EP2008/003475, filed 29 April 2008.

Brutnell T.P., Sawers R.J.H., Mant A. and Langdale J.A. 1999. Bundle Sheath Defective2, a novel protein required for post-translational regulation of the *rbc*L gene of maize. Plant Cell 11, 849–864.

Caspar T., Klein T.M., Qi M. and Yang J. 2008a. Expression of recombinant genes encoding Rubisco proteins in C_3 plants. US Appl. 20090172842. Filed 4 September 2008, published 2 July 2009.

Caspar T., Klein T.M., Qi M., Yang J. 2008b. Expression of Rubisco enzyme from a non-Rubisco locus. US Appl. 20090172832. Filed 4 September 2008, published 2 July 2009.

Ciniglia C., Yoon H.S., Pollio A., Pinto G. and Bhattacharya D. 2004 Hidden biodiversity of the extremophilic Cyanidiales red algae. Molecular Ecology 13, 1827–1838.

Daniell H. 2007. Transgene containment by maternal inheritance: effective or elusive? Proceedings of the National Academy of Sciences of the USA 104, 6879–6880.

Daniell H., Kumar S. and Dufourmantel N. 2005. Breakthrough in chloroplast genetic engineering of agronomically important crops. Trends in Biotechnology 23, 238–245.

Daniell H. and McFadden B.A. 1999. Genetic engineering of plant chloroplasts. US patent 5,932,479. Filed 3 August 1999.

Daniell H. and McFadden B.A. 2003. Genetic engineering of plant chloroplasts. US patent 6,642,053. Filed 4 November 2003.

Daniell H. and McFadden B.A. 2004. Genetic engineering of plant chloroplasts. US patent 6,680,426. Filed 20 January 2004.

De Marchis F., Wang Y., Stevanato P., Arcioni S. and Bellucci M. 2009. Genetic transformation of the sugar beet plastome. Transgenic Research 18, 17–30.

Delwiche C.F. and Palmer J.D. 1996. Rampant horizontal transfer and duplication of Rubisco genes in eubacteria and plastids. Molecular Biology and Evolution 13, 873–882.

Dufourmantel N., Pelissier B., Garcon F., Peltier G., Ferullo J.-M. and Tissot G. 2004. Generation of fertile transplastomic soybean. Plant Molecular Biology 55, 479–489.

Emlyn-Jones D., Woodger F.J., Price G.P. and Whitney S.M. 2006. RbcX can function as a Rubisco chaperonin, but is non-essential in Synechococcus PCC7942. Plant and Cell Physiology 47, 1630–1640.

Farquhar G.D., von Caemmerer S. and Berry J.A. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149, 78–90.

Furbank R.T., von Caemmerer S. and Price G.D. 2013. CO_2-concentrating mechanisms in crop plant so increase yield. In 'Applying photosynthesis research to

improvement of food crops', ed. by J.E. Gready, S.A. Dwyer and J.R Evans. ACIAR Proceedings No. 140, 130–137. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Galmés J., Andralojc P.J., Keys A.J., Medrano H., Flexas J. and Parry M.A.J. 2007. A search for plant superior Rubiscos: kinetic variability related to species from extreme environments. Poster, in 'Proceedings of 14th International Congress on Photosynthesis, Glasgow 22–27 July 2007'.

Galmés J., Flexas J., Keys A.J., Cifre J., Mitchell R.A.C., Madgwick P.J., Haslam R.P., Medrano H. and Parry M.A.J. 2005. Rubisco specificity factor tends to be larger in plant species from drier habitats and in species with persistent leaves. Plant Cell and Environment 28, 571–579.

Gready J.E. and Kannappan B. 2008a. Process for generation of protein and uses thereof. PCT Application No. WO2008/043147.

Gready J.E. and Kannappan B. 2008b. Process for generation of protein and uses thereof. US provisional patent application No. 61/045552.

Greene D.N., Whitney S.M. and Matsumura I. 2007. Artificially evolved *Synechococcus* PCC6301 Rubisco variants exhibit improvements in folding and catalytic efficiency. Biochemical Journal 404, 517–524.

Grevich J. and Daniell H. 2005. Chloroplast genetic engineering: recent advances and future perspectives. Critical Reviews in Plant Science 24, 83–107.

Hou B.K., Zhou Y.H., Wan L.H., Zhang Z.L., Shen G.F., Chen Z.H. and Hu Z.M. 2003. Chloroplast transformation in oilseed rape. Transgenic Research 12, 111–114.

Houtz R.L. and Portis A.R. Jr 2003. The life of ribulose 1,5-bisphosphate carboxylase/oxygenase- posttranslational facts and mysteries. Archive of Biochemistry and Biophysics 414, 150–158.

Jordan D.B. and Ogren W.L. 1981. Species variation in the specificity of ribulose bisphosphate carboxylase/oxygenase. Nature 291, 513–515.

Kane H.J., Viil J., Entsch B., Paul K., Morell M.K. and Andrews T.J. 1994. An improved method for measuring the CO_2/O_2 specificity of ribulose-bisphosphate carboxylase-oxygenase. Australian Journal of Plant Physiology 21, 449–461.

Kannappan B. and Gready J.E. 2008. Redefinition of Rubisco carboxylase reaction reveals origin of water for hydration and new roles for active-site residues. Journal of the American Chemical Society 130, 15063–15080.

Kapralov M.V. and Filatov D.A. 2006. Molecular adaptation during adaptive radiation in the Hawaiian endemic genus Schiedea. PLoS ONE 1, e8.

Kapralov M.V. and Filatov D.A. 2007. Widespread positive selection in the photosynthetic Rubisco enzyme. BMC Evolutionary Biology 7, 73.

Kaur N., Street K., Mackay M., Yahiaoui N. and Keller B. 2007. Molecular approaches for characterization and use of natural disease resistance in wheat. European Journal of Plant Pathology 121, 387–397.

Kebeish R., Niessen M., Thiruveedhi K., Bari R., Hirsch H.-J., Rosenkranz R., Stäbler N., Schönfeld B., Kreuzaler F. and Peterhänsel C. 2007. Chloroplastic photorespiratory bypass increases photosynthesis and biomass production in *Arabidopsis thaliana*. Nature Biotechnology 25, 593–599.

Koop H.-U., Herz S., Golds T.J. and Nickelsen J 2007. The genetic transformation of plastids. Topic in Current Genetics. doi: 10.1007/4735_2007_0225.

Kumar A., Li C. and Portis A.R. Jr. 2009. *Arabidopsis thaliana* expressing a thermostable chimeric Rubisco activase exhibits enhanced growth and higher rates of photosynthesis at moderately high temperatures. Photosynthesis Research 100, 143–153.

Kumar S., Dhingra A. and Daniell H. 2004a. Plastid-expressed betaine aldehyde dehydrogenase gene in carrot cultured cells, roots, and leaves confers enhanced salt tolerance. Plant Physiology 136, 2843–2854.

Kumar S., Dhingra A. and Daniell H. 2004b. Stable transformation of the cotton plastid genome and maternal inheritance of transgenes. Plant Molecular Biology 56, 203–216.

Kurek I., Chang T.K., Bertain S.M., Madrigal A., Liu L., Lassner M.W. and Zhu G. 2007a. Enhanced thermostability of *Arabidopsis* Rubisco activase improves photosynthesis and growth rates under moderate heat stress. Plant Cell 19, 3230–3241.

Kurek I., Liu L. and Zhu G. 2007b. Rubisco activase with increased thermostability and methods of use thereof. US Appl. No. 11/867,723. Filed 5 October 2007.

Leakey A.D.B. 2009. Rising atmospheric carbon dioxide concentration and the future of C_4 crops for food and fuel. Proceedings of the Royal Society. B, Biological Sciences 276, 2333–2343.

Lee S.M., Kang K., Chung H., Yoo S.H., Xu X.M., Lee S-B., Cheong J-J., Daniell H. and Kim M. 2006. Plastid transformation in the monocotyledonous cereal crop, rice (*Oryza sativa*) and transmission of transgenes to their progeny. Molecular Cell 21, 401–410.

Lefebvre S., Lawson T., Zakhleniuk O.V., Lloyd J.C., Raines C.A. and Fryer M. 2005. Increased sedoheptulose-1,7-bisphosphatase activity in transgenic tobacco plants stimulates photosynthesis and growth from an early stage in development. Plant Physiology 138, 451–460.

Lelivelt C.L.C., McCabe M.S., Newell C.A., de Snoo C.B., Dun K.M.P., Birch-Machin I., Gray J.C., Mills K.H.G. and Nugent J.M. 2005. Stable plastid transformation in lettuce (*Lactuca sativa* L.). Plant Molecular Biology 58, 763–774.

Lieman-Hurwitz J., Rachmilevitch S., Mittler R., Marcus Y. and Kaplan A. 2003. Enhanced photosynthesis and growth of transgenic plants that express *ictB*, a gene involved in HCO_3^- accumulation in cyanobacteria. Plant Biotechnology Journal 1, 43–50.

Liu C.W., Lin C.C., Chen J.J. and Tseng M.J. 2007. Stable chloroplast transformation in cabbage (*Brassica oleracea* L. var. *capitata* L.) by particle bombardment. Plant Cell Reports 26, 1733–1744.

Makino A., Mae T. and Ohira 1988. Differences between wheat and rice in the enzymic properties of ribulose-l,5-bisphosphate carboxylase/oxygenase and the relationship to photosynthetic gas exchange. Planta 174, 30–38.

Maliga P. 2004. Plastid transformation in higher plants. Annual Review of Plant Biology 55, 289–313.

Maliga P., Maliga Z.S., Staub J.M., Zoubenko O., Allison L.A., Carrer H. and Kanevski I.1999. DNA constructs for stably transforming plastids of multicellular plants and expressing foreign recombinant proteins. US patent 5,877,402. Filed 2 March 1999.

Maliga P., Maliga Z.S. and Staub J.M. 2002. DNA constructs for stably transforming plastids of multicellular plants and expressing foreign recombinant proteins. US patent 6,388,168. Filed 14 May 2002.

Mann C.C. 1999. Genetic engineers aim to soup up crop photosynthesis. Science 283, 314–316.

Mitchell R.A., Keys A.J., Madgwick P.J,. Parry M.A. and Lawlor D.W. 2005. Adaptation of photosynthesis in marama bean *Tylosema esculentum* (Burchell A. Schreiber) to a high temperature, high radiation, drought-prone environment. Plant Physiology and Biochemistry 43, 969–976.

Miyagawa Y., Tamoi M., Shigeoka S. 2001. Overexpression of a cyanobacterial fructose-1,6-/sedoheptulose-1,7-bisphosphatase in tobacco enhances photosynthesis and growth. Nature Biotechnology 19, 965–969.

Mueller-Cajar O. and Whitney S.M. 2008. Directing the evolution of Rubisco and Rubisco activase: first impressions of a new tool for photosynthesis research. Photosynthesis Research 98, 667–75.

Nehra N.S., Schaaf D.J., Sidorov V., Stalker D.M. and Ye G. 2003. Plastid transformation of solanaceous plants. US patent 6,541,682. Filed 1 April 2003.

Nguyen T.T., Nugent G., Cardi T. and Dix P.J. 2005. Generation of homoplasmic plastid transformants of a commercial cultivar of potato (*Solanum tuberosum* L.). Plant Science 168, 1495–1500.

Nugent G.D., Coyne S., Nguyen T.T., Kavanagh T.A. and Dix P.J. 2006. Nuclear and plastid transformation of *Brassica oleracea* var. *botrytis* (cauliflower) using PEG-mediated uptake of DNA into protoplasts. Plant Science 170, 135–142.

Nugent G.D., ten Have M., van der Gulik A., Dix P.J., Uijtewaal B.A. and Mordhorst A.P. 2005. Plastid transformants of tomato selected using mutations affecting ribosome structure. Plant Cell Reports 24, 341–349.

Okumura S., Sawada M., Park Y., Hayashi T., Shimamura M., Takase H. and Tomizawa K-I. 2006. Transformation of poplar (*Populus alba*) plastids and expression of foreign proteins in tree chloroplasts. Transgenic Research 15, 637–646.

Parry M.A., Andralojc P.J., Mitchell R.A., Madgwick P.J. and Keys A.J. 2003. Manipulation of Rubisco: the amount, activity, function and regulation. Journal of Experimental Botany 54, 1321–1333.

Peterhansel C., Niessen M. and Kebeish R.M. 2008. Metabolic engineering towards the enhancement of photosynthesis. Photochemistry and Photobiology 84, 1317–1323.

Portis A.R. Jr and Parry M.A.J. 2007. Discoveries in Rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase): a historical perspective. Photosynthesis Research 94, 121–143.

Portis A.R., Li C., Wang D. and Salvucci M.E. 2008. Regulation of Rubisco activase and its interaction with Rubisco. Journal of Experimental Botany 59, 1597–1604.

Price D., Badger M.R., Woodger F.J. and Long B.M. 2008. Advances in understanding the cyanobacterial CO_2-concentrating-mechanism (CCM): functional components, C_i transporters, diversity, genetic regulation and prospects for engineering into plants. Journal of Experimental Botany 59, 1441–1461.

Raines C.A. 2006. Transgenic approaches to manipulate the environmental responses of the C_3 carbon fixation cycle. Plant Cell and Environment 29, 331–339.

Ruf S., Hermann M., Berger I.J., Carrer H. and Bock R. 2001. Stable genetic transformation of tomato plastids and expression of a foreign protein in fruit. Nature Biotechnology 19, 870–875.

Ruf S., Karcher D. and Bock R. 2007. Determining the transgene containment level provided by chloroplast transformation. Proceedings of the National Academy of Sciences of the USA 104, 6998–7002.

Sage R.F. 2002. Variation in the k_{cat} of Rubisco in C_3 and C_4 plants and some implications for photosynthetic performance at high and low temperature. Journal of Experimental Botany 53, 609–620.

Saschenbrecker S., Bracher A., Rao K.V., Rao B.V., Hartl F.U. and Hayer-Hartl M. 2007. Structure and function of RbcX, an assembly chaperone for hexadecameric Rubisco. Cell 129, 1189–1200.

Sharwood R.E., von Caemmerer S., Maliga P. and Whitney S.M. 2008. The catalytic properties of hybrid Rubisco comprising tobacco small and sunflower large subunits mirror the kinetically equivalent source Rubiscos and can support tobacco growth. Plant Physiology 146, 83–96.

Sidorov V.A., Kasten D., Pang S-Z., Hajdukiewicz P.T.J., Staub J.M. and Nehra N.S. 1999. Stable chloroplast transformation in potato: use of green fluorescent protein as a plastid marker. Plant Journal 19, 209–216.

Sikdar S.R., Serino G., Chaudhuri S. and Maliga P. 1998 Plastid transformation in *Arabidopsis thaliana*. Plant Cell Reports 18, 20–24.

Skarjinskaia M., Svab Z. and Maliga P. 2003. Plastid transformation in *Lesquerella fendleri*, an oilseed Brassicaceae. Transgenic Research 12, 115–122.

Spreitzer R.J., Peddi S.R. and Satagopan S. 2005. Phylogenetic engineering at an interface between large and small subunits imparts land-plant kinetic properties to algal Rubisco. Proceedings of the National Academy of Sciences of the USA 102, 17225–17230.

Spreitzer R.J. and Salvucci M.E. 2002. Rubisco: structure, regulatory interactions, and possibilities for a better enzyme. Annual Review of Plant Biology 53, 449–475.

Salvucci M.E. 2013. Enhancing Rubisco activity at higher temperatures by re-engineering Rubisco activase. In 'Applying photosynthesis research to improvement of food crops', ed. by J.E. Gready, S.A. Dwyer and J.R Evans. ACIAR Proceedings No. 140, 138–146. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Street K., Hamilton R.S., Tay D., Taba S. and Mackay M. 2013. Mining germplasm for photosynthetic improvement—wheat, rice, potato, legumes and maize. In 'Applying photosynthesis research to improvement of food crops', ed. by J.E. Gready, S.A. Dwyer and J.R Evans. ACIAR Proceedings No. 140, 112–129. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Strelchenko P., Street K., Mitrofanova O., Hill H., Henry R. and Mackay M. 2008. Comparative assessment of wheat landraces from AWCC, ICARDA and VIR germplasm collections based on the analysis of SSR markers. Paper presented to 11th International Wheat Genetics Symposium, Brisbane, Qld, 24–29 August. Accessible at <epubs.scu.edu.au/cgi/viewcontent.cgi?article=1196&context=cpcg_pubs>.

Svab Z., Hajdukiewicz P. and Maliga P. 1990. Stable transformation of plastids in higher plants. Proceedings of the National Academy of Sciences of the USA 87, 8526–8530.

Svab Z., Maliga P. 2007. Exceptional transmission of plastids and mitochondria from the transplastomic pollen parent and its impact on transgene containment. Proceedings of the National Academy of Sciences of the USA 104, 7003–7008.

Tamoi M., Nagaoka M., Miyagawa Y. and Shigeoka S. 2006. Contribution of fructose-1,6-bisphosphatase and sedoheptulose-1,7-bisphosphatase to the photosynthetic rate and carbon flow in the Calvin cycle in transgenic plants. Plant and Cell Physiology 47, 380–390.

Tcherkez G.G., Farquhar G.D. and Andrews T.J. 2006. Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized. Proceedings of the National Academy of Sciences of the USA 103, 7246–7251.

von Caemmerer S. 2000. Biochemical models of leaf photosynthesis. CSIRO Publishing: Melbourne.

Whitney S.M., Baldet P., Hudson G.S. and Andrews T.J. 2001. Form I Rubiscos from non-green algae are expressed abundantly but not assembled in tobacco chloroplasts. Plant Journal 26, 535–547.

Whitney S.M. and Sharwood R.E. 2008. Construction of a tobacco master line to improve Rubisco engineering in chloroplasts. Journal of Experimental Botany 59, 1909–1921.

Wostrikoff K. and Stern D. 2007. Rubisco large-subunit translation is autoregulated in response to its assembly state in tobacco chloroplasts. Proceedings of the National Academy of Sciences of the USA 104, 6466–6471.

Zhu G., Kurek I. and Liu L. 2008. Methods of identifying and creating Rubisco large subunit variants with improved Rubisco activity, compositions and methods of use thereof. US Appl. 20080256666. Filed 14 April 2008, published 16 October 2008.

Zhu X.G., Portis A.R. and Long S.P. 2004. Would transformation of C_3 crop plants with foreign Rubisco increase productivity? A computational analysis extrapolating from kinetic properties to canopy photosynthesis. Plant Cell and Environment 27, 155–165.

Zubko M.K., Zubko E.I., van Zuilen K., Meyer P. and Day A. 2004. Stable transformation of petunia plastids. Transgenic Research 13, 523–530.

Mining germplasm banks for photosynthetic improvement—wheat, rice, potato, legumes and maize

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Summary

This paper discusses the availability and variability of gene pools for five key food crops—wheat, rice, potato, legumes and maize—to ascertain the potential for using naturally occurring variation as foundations for photosynthetic improvement.

- Up to 560,000 accessions of wheat are available worldwide. However, this number exaggerates the readily available genetic diversity from variability in ease of gene transfer, a high level of duplication between collections and poor documentation.
- The long history of domestication of rice and its wide distribution across Asia has lead to a genetically diverse crop with about 540,000 accessions held globally. Incidental hybridisation between wild and domestic species is so common that domestication is considered an ongoing process. Techniques are also available to create hybrids from lines with incompatible genomes.
- About 59,000 accessions of cultivated and wild potato germplasm exist in 23 potato gene banks worldwide. Of these, about 17,500 accessions are the 187 wild tuber-bearing species in *Solanum* section *Petota*. Most of these are conserved as botanical seed in 17 gene banks. There are 17,000 accessions of the seven cultivated species with ploidy from diploid to pentaploid in 17 gene banks conserved as individual clones, 11,000 accessions of modern cultivars in 19 gene banks, and 13,500 accessions of research and breeding lines in 18 gene banks. Two hundred years of modern breeding of potato has resulted in a great diversity of modern lines. Genotypic variation in photosynthetic traits is still to be assessed.
- National legume collections house a high percentage of indigenous species, making accession collections unique. The diversity of chickpea, faba bean, grasspea and lentil accessions could be further increased by including more wild species from targeted areas.
- An estimated 300,000 cultivated and wild maize accessions are held in collections worldwide. These have captured much of the maize diversity from the Americas and areas of secondary diversity (Old World). Three major gene banks respond internationally to seed requests. These are the International Maize and Wheat Improvement Center (CIMMYT) in Mexico; North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa, USA; and Maize Genetic Cooperation Stock Center in the University of Illinois, Urbana, Illinois, USA.

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The gene banks of CGIAR maintain collections of global importance, in terms of diversity and uniqueness, for the five crop groups. CGIAR distributes the germplasm free of charge on request by bona fide users.

- Availability of accurate and complete information about gene bank holdings is the key to using these gene banks. While most important gene banks have in-house information systems containing accession-level passport and characterisation data, most of this information remains difficult to access easily, with the notable exception of the collections maintained by the US Department of Agriculture (USDA).
- Given the large global collections of crops of interest, a rational strategy is needed to target manageable subsets of germplasm that are likely to contain sufficient Rubisco diversity. For accessions whose collection sites are geo-referenced, the Focused Identification of Germplasm Strategy (FIGS) would be a rational method.
- Although collections are imperfect and still improving, the currently available genetic diversity of these five crops is high, suggesting much potential for mining of germplasm for crop features that will lead to photosynthetic and yield improvements.

Wheat—genetic resources for improved photosynthesis

Gene pool

The gene pool potentially available to users includes modern and obsolete varieties, landraces, wild relatives and progenitors of cultivated forms, breeding lines and genetic stocks. This broad pool can be subdivided into primary, secondary and tertiary gene pools (Harlan and de Wet 1971). The primary gene pool consists of species among which gene transfer is considered easy and includes mostly cultivated and some wild forms. The secondary gene pool consists of wild species of *Triticum* from which gene transfer is possible but more difficult, while the tertiary gene pool comprises species from which gene transfer may be possible but with great difficulty, or not at all.

The cultivated and wild forms of *Triticum* species have three ploidy levels as described in Table 1.

The *Aegilops* genus, which makes up part of the tertiary gene pool, is closely related to *Triticum*, and various species, particularly carrying the D genome, have been widely used in wheat improvement programs. *Aegilops* consists of 11 diploid species and 12 polyploid species, including tetraploids and hexaploids.

Ex situ collections

The advisory group responsible for the Global Wheat Conservation Strategy (at <www.croptrust.org/documents/cropstrategies/Wheat%20Strategy.pdf>) identified wheat collections residing in 44 institutes worldwide, which conserve close to 560,000 accessions of wheat species. The advisory group identified 15 gene banks (Table 2) that are in theory accessible, of global or regional significance, and are well managed, maintain a link to users and are committed to long-term preservation of the collections they curate.

Some countries are still working through issues, mostly related to the interpretation of the access and benefit sharing components of the International Treaty on Genetic Resources for Food and Agriculture, which impede free and easy access to germplasm. For example, of the countries listed in Table 2, it is still difficult to access germplasm from Iran, India and Ethiopia.

Table 1. *Triticum* genus

Species	Subspecies	Chromosome no.	Nuclear genome
Triticum aestivum L.	5	$2n = 42$	ABD
Triticum turgidum L.	8	$2n = 28$	AB
Triticum zhukovskyi Menabde and Ericz.	0	$2n = 42$	AAG
Triticum timopheevii (Zhuk.) Zhuk.	2	$2n = 42$	AG
Triticum monococcum L.	2	$2n = 14$	A
Triticum urartu Tumanian ex Gandilyan	0	$2n = 14$	A

Table 2. Key global wheat collections

Country	Organisation	No. of accessions
International	CIMMYT, El Batán, Mexico	97,641
International	ICARDA, Aleppo, Syria	37,830
Australia	Australian Winter Cereals Collection, Tamworth	23,917
Bulgaria	Institute for Plant Genetic Resources 'K. Malkov', Sadovo	9,747
Canada	Plant Gene Resources of Canada, Saskatoon	5,052
Cyprus	CYPARI, Agricultural Research Institute, Nicosia	7,696
Czech Republic	Research Institute of Crop Production, Prague	11,018
Ethiopia	Plant Genetic Resources Centre, Institute of Biodiversity Conservation and Research, Addis Ababa	10,745
France	INRA Station d'Amelioration des Plantes, Clermont-Ferrand	15,850
Germany	Gene bank, IPK, Gatersleben	9,633
India	NBPGR, New Delhi	32,880
Iran	National Gene Bank of Iran, Genetic Resources Division, Karaj	12,169
Japan	Genetic Resources Management Section, NIAR (MAFF), Tsukuba	7,148
Japan	Plant Germplasm Institute, Graduate School of Agriculture, Kyoto University, Kyoto	4,378
Netherlands	Centre for Genetic Resources, Wageningen	5,529
Total	15 institutes	291,233

Source: Bioversity (2006)
CIMMYT = International Maize and Wheat Improvement Center; CYPARI = National Gene Bank; ICARDA = International Center for Agricultural Research in the Dry Areas; INRA = Institut National de la Recherche Agronomique; IPK = Institute for Plant Genetics and Crop Plant Research; NBPGR = National Bureau of Plant Genetic Resources; NIAR = National Institute of Agrobiological Resources; MAFF = Ministry of Agriculture, Forestry and Fisheries, Japan

Duplication

While more than 430,000 accessions sounds impressive, there is significant duplication among gene banks. For example, Table 3 shows an estimate of the duplication between the wheat collections of the USDA, the International Center for Agricultural Research in the Dry Areas (ICARDA) and the International Maize and Wheat Improvement Center (CIMMYT). Tracking down duplicate accessions relies on accurate documentation that retains the unique identifiers used by other gene banks and which may not always be retained when accessions move between gene banks.

Diversity within collections

The number of accessions held in a collection does not necessarily reflect the diversity captured because the approaches used by gene banks to assemble and curate their collections differ. For example, CIMMYT, one of the largest wheat collections in the world, has tended to focus on conserving breeding lines, genetic stocks and selections from landraces, which are more convenient to work with for breeding. ICARDA has focused on conserving landraces and wild material as populations; approximately 75% of the ICARDA wheat collections are maintained this way. The rationale for this approach is that it maximises the probability of retaining potentially useful alleles in the collection. Thus, the aim is to retain as much diversity as possible. Thus, CGIAR and the Food and Agricultural Organization of the United Nations (FAO) have prioritised landrace varieties for conservation in recent years because of the threat posed by displacement with improved modern cultivars. However, landraces are still poorly represented in world collections compared to modern and obsolete cultivars and advanced breeding material. Likewise, the wild relatives of wheat are also poorly represented in global wheat germplasm collections because, for example, the difficulty of deploying them in breeding programs and problems associated with seed production ex situ (Global Crop Diversity Trust 2006).

Table 3. Duplication in wheat collections between CIMMYT, ICARDA and USDA

Collections compared	Duplication (%)
CIMMYT–ICARDA	11
CIMMYT–USDA	29
ICARDA–USDA	18
ICARDA–USDA–CIMMYT	33

Source: T. Hazekamp (Bioversity International) (pers. comm.)

Rice—genetic resources for improved photosynthesis

Gene pool

The rice gene pool comprises the genus *Oryza*, a grass in the subfamily Bambusoideae, with two cultivated and 19–24 (depending on taxonomic preferences) diploid or tetraploid wild species (Table 4). Some of the wild species are invasive and weedy, classified as noxious and subject to strict biosafety regulations in many countries. As such, they are a usable component of the rice gene pool only for laboratories with adequate containment facilities.

Ten genomes are recognised within the genus: AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ and HHKK. Cultivated species have genome AA; subsequent letters indicate progressively more distant species.

Rice was first cultivated in Asia, probably more than 10,000 years ago, reaching the Middle East, Europe and Africa more than 2,000 years ago. The resulting domesticated species, *Oryza sativa*, is now distributed globally in all tropical and subtropical regions of the world. As a result of its long history of domestication and wide distribution, it is genetically highly diverse, and constitutes the bulk of the genetic resources conserved for rice research and improvement. Four to five major variety groups are recognised, although there are many intermediate forms:

- Indica—widespread, genetically diverse and primarily tropical. Sometimes it is wrongly referred to in the literature as a subspecies; however, no subspecies of *Oryza* is recognised by taxonomists. 'Molecular clocks' estimate a divergence date between Indica and Japonica long before agriculture started.
- Aus—somewhat related to Indica, with distribution restricted largely to Bangladesh and north and east India. It is genetically diverse and a source of many useful genes. It is named after the traditional 'aus' (summer rainfed) rice farming system in Bangladesh.
- Japonica—widespread but less diverse than Indica. It is now commonly divided into temperate and tropical Japonica, based on molecular data showing close similarity between 'Javanica' (now tropical Japonica) and 'Japonica' (now temperate Japonica).
- Aromatic—predominantly aromatic, Basmati-like varieties, somewhat related to Japonica, distributed from Iran to Pakistan and north-west India. Aroma is a highly prized trait found in all groups, not only this group.

A second species, *O. glaberrima*, was domesticated in western Africa, probably around 3,500 years ago. Its cultivation is still largely restricted to western Africa, although a hybrid between *O. glaberrima* and *O. sativa*, known as Nerica, is being widely promoted. With its relatively recent domestication history and narrow geographic distribution, the species contains relatively little genetic diversity.

Within the genus, each taxonomic series is a species complex of partially interfertile species. Thus the 4–6 wild species in series Sativae (with AA genome) can hybridise with the two cultivated species. Where two species of the same series occur together, for example where crops of *O. sativa* are grown close to wild populations of *O. nivara* or *O. rufipogon*, naturally occurring interspecific hybrid swarms are common, leading to many intermediate forms that are difficult to classify. There is therefore considerable gene flow among wild and cultivated species, so much so that domestication is considered an ongoing process. Extant populations of *O. nivara* and *O. rufipogon* are so influenced by domesticated genes that they should be considered cousins rather than ancestors of *O. sativa*. This ease of crossing and frequent recombination among chromosomes of AA genome species has been successfully exploited by rice breeders to introduce a wide range of traits from AA genome wild species, including cytoplasmic male sterility for hybrid rice, and biotic and abiotic stress tolerances (Brar and Khush 2002, 2006).

For wild species with genomes B to F, it is possible to generate hybrids with the cultivated species through embryo rescue. These hybrids are sterile and exhibit restricted recombination with cultivated

Table 4. Annotated taxonomy of the genus *Oryza*

Taxon	2*n*	Genome	Distribution	Notes
Section Oryza				
Series Sativae				
O. sativa	24	AA	Global	Cultivated rice, domesticated in Asia
O. glaberrima	24	AA	Western Africa	Cultivated rice, domesticated in western Africa
O. barthii	24	AA	Africa	Ancestor of *O. glaberrima*
O. glumaepatula	24	AA	South America	Considered by some as American race of *O. rufipogon*
O. longistaminata	24	AA	Africa	Perennial outbreeder
O. meridionalis	24	AA	Australia, Indonesia, Papua New Guinea	
O. nivara	24	AA	Asia	Considered by some as annual inbreeding race of *O. rufipogon*, based on DNA data
O. rufipogon	24	AA	Asia	Perennial outbreeder; together with *O. nivara*, ancestor of *O. sativa*
Series Latifoliae				
O. alta	48	CCDD	South America	
O. eichingeri	24	CC	Africa and Sri Lanka	Only species of *Oryza* found on more than one continent
O. grandiglumis	48	CCDD	South America	
O. latifolia	48	CCDD	South America	
O. minuta	48	BBCC	Southeast Asia–Pacific	
O. officinalis	24	CC	Asia	
O. malamphuzaensis	48	CCDD	Asia	Considered by some as a tetraploid race of *O. officinalis*
O. punctata	24	BB	Africa	
O. schweinfurthiana	48	BBCC	Africa	Considered by some as a tetraploid race of *O. punctata*
O. rhizomatis	24	CC	Sri Lanka	
Series Australienses				
O. australiensis	24	EE	Australian	
Section Brachyantha				
Series Brachyanthae				
O. brachyantha	24	FF	Africa	
Section Padia				
Series Meyerianae				
O. granulata	24	GG	South, South-East, East Asia and Pacific	Considered by some as a race of *O. meyeriana*
O. meyeriana	24	GG	South-East Asia–Pacific	
O. neocaledonica	24	GG	New Caledonia	
Series Ridleyanae				
O. longiglumis	48	HHJJ	Indonesia – Papua New Guinea	
O. ridleyi	48	HHJJ	South-East Asia–Pacific	
Series Schlechterianae				
O. schlechteri	48	HHKK	Indonesia – Papua New Guinea	

species (AA genome). However, a few genes have been successfully transferred from CC, BBCC, CCDD, EE and FF genomes into *O. sativa*. For wild species with genomes G to K, even where hybrids among cultivated species are produced, no crossing over is found between genomes. Thus, there has been no successful transfer of genes from these wild species into the cultivated species (Brar and Khush 2002, 2006).

Chloroplast sequencing of nine related genera in the tribe Oryzeae indicates that *Porteresia* and *Leersia* are the most closely related genera, forming a good monophyletic group with *Oryza* (Ge et al. 2002). The other genera fall into a separate monophyletic group; these include *Zizania*, available in shops under the name 'wild rice' but not considered part of the conventional rice gene pool.

Ex situ collections

A survey conducted in 2006 with assistance from the Global Crop Diversity Trust identified approximately 575,000 accessions held in three international and 42 national gene banks (Global Crop Diversity Trust 2010). Three-quarters of these are held by only eight gene banks (Table 5). Each holds more than 15,000 samples.

The largest collection, managed by the International Rice Research Institute, is also the most diverse, as it has a global mandate to conserve the diversity of the rice gene pool. It focuses on diverse traditional varieties and wild relatives, with relatively few genetic stocks, breeding lines and improved varieties. The collection has samples from 128 countries, with more than 103,000 accessions of *Oryza sativa* and nearly 5,000 accessions of wild rice, including at least one accession of each wild species.

The five largest national collections are held in Asia. Approximately 90% of accessions held in national gene banks in India, China and Thailand are of national origin. Only one of the national collections in Table 2 (India) is under the International Treaty on Plant Genetic Resources for Food and Agriculture (at <planttreaty.org>). Accessions held in these and the international collections should be accessible, at least to laboratories in member countries, with the Standard Material Transfer Agreement (SMTA) of the Treaty.

Breeding lines and genetic stocks are largely outside the scope of the rice gene banks, so are available mainly on an ad hoc basis from the scientists involved. However, the International Network for the Genetic Evaluation of Rice (INGER), coordinated by IRRI, provides for the short-term conservation and maintenance of sets of elite lines selected for particular objects, and makes them available for distribution with the SMTA.

Potato—genetic resources for improved photosynthesis

Gene pool and collections

The centre of diversity for *Solanum* section *Petota* species extends from the south-western USA to central Argentina and Chile. There are 187 species of wild tuber-bearing species in the world (Spooner and Salas 2006). The highest diversity is found in Peru (with 84 endemic species), Mexico (29), Bolivia

Table 5. Largest global rice collections

Country	Organisation	No. of accessions
International	IRRI GRC, Los Baños, Philippines	109,166
International	WARDA, Cotonou, Benin	19,066
India	NBPGR, New Delhi	79,930
China	Institute of Crop Germplasm Resources, CAAS, Beijing	79,783
Japan	NIAS Genebank, Tsukuba	44,224
Republic of Korea	RDA Genebank, Suwon	27,240
Thailand	BRDO, Pathumthani	25,493
USA	NCGRP, Fort Collins	18,824

BRDO = Biotechnology Research and Development Office; CAAS = Chinese Academy of Agricultural Sciences; IRRA GRC = T.T. Chang Genetic Resources Center, International Rice Research Institute; NBPGR = National Bureau of Plant Genetic Resources; NCGRP = National Center for Genetic Resources Preservation; NIAS = National Institute of Agrobiological Sciences; RDA = Rural Development Association; WARDA = Africa Rice Center

(16), Argentina (11), then Ecuador, Colombia and Chile. The wild species of section *Petota* have a range of ploidy levels from diploid ($2n = 24$) to hexaploid ($6n = 72$), including triploids, tetraploids and pentaploids. Most wild species are diploid and 36% of the species are entirely or partly polyploid (Hijmans et al. 2007).

The cultivated diploid and tetraploid species are easily intercrossable in breeding. The triploid and pentaploid species are also crossable because of the presence of unreduced gametes. The first domesticated potato is believed to be *Solanum stenotomum* from which the other six species were derived (Figure 1). There is evidence of frequent introgression from the wild species. In fact, two bitter cultivated species with high alkaloid contents, *S. ajanhuiri* and *S. juzepczukii*, are of hybrid origin between cultivated forms with *S. megistacrolobum* (2×) and *S. acaule* (4×), respectively. Many of the wild and cultivated species are cross-compatible. Thus, the primary gene pool of potato is relatively large compared with other crops.

Much of the *Solanum* section *Petota* biodiversity is conserved in gene banks. Wild species are typically maintained as botanical seed. The great diversity of cultivated landraces as well as thousands of cultivars, and breeding and genetic stocks from nearly 200 years of modern breeding, are maintained clonally as tubers, in vitro or as cryopreserved explants. The world's largest potato collection is held in trust by the International Potato Center (CIP) in Lima, Perú. CIP is member of the Association for Potato Intergenebank Collaboration (APIC), which in 2006 held 7,112 different accessions of 188 taxa (species, subspecies, varieties and forms) out of the 247 tuber-bearing wild potato taxa recognised (reviewed by Hawkes 1990; Huaman et al. 2000; Bradshaw et al. 2006).

More than 17,500 accessions of the wild species are conserved as seed populations in 17 gene banks worldwide (see Global Potato Conservation Strategy at <www.croptrust.org/documents/cropstrategies/Potato.pdf>). A comparison of the diversity between the gene banks has not been attempted and thus the representation of the whole gene pool of the 187 species is not clear. At the CIP gene bank, nearly 2,000 accessions of 141 species were held in trust under the international treaty for worldwide distribution. The CIP wild potato catalogue recently has been published in the World Catalogue of Cultivated Potato (Hils and Pieterse 2009).

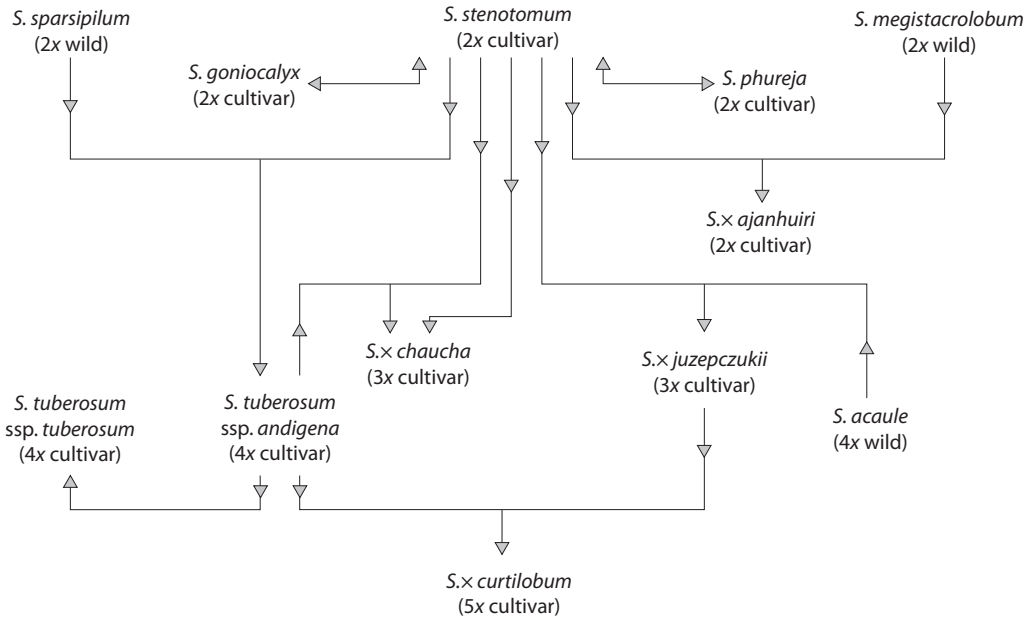

Figure 1. Evolutionary relationships of cultivated potato, genus *Solanum*. *S*× indicates hybrid species. Numbers in parentheses indicate ploidy level for each wild-type or cultivar.

Modern potato cultivars are tetraploids (*S. tuberosum* ssp. *tuberosum*) and in the Andes there are seven cultivated native species ranging from diploid to pentaploid. There are 17,000 accessions of native cultivars of the seven species conserved as individual genotypes (clones) and seed populations in 17 gene banks, 11,000 accessions of modern cultivars in 19 gene banks and 13,500 accessions of research and breeding lines in 18 gene banks according to the Global Potato Conservation Strategy (Table 6).

At the CIP gene bank, more than 17,000 accessions of native cultivars were collected from Venezuela in the north to Argentina and Chile in the south. After duplicates were identified using both morphological and isozyme studies, only 4,235 accessions of unique native cultivars are currently conserved clonally as tuber and in vitro collections. Some are also kept as a cryogenic collection. The duplicates were converted into true seed and maintained in a seed storage vault at –20°C. We estimate that these 4,235 accessions

Table 6. Global potato collections

Collection, country	Wild species		Native cultivars		Cultivars (old/new)	Other materials[a]	Total accessions
	No. species	Total	No. species	Total			
Latin America							
CIP, Perú	151	2,363	8	4,461	314	3,170	10,308
INTA, Argentina	30	1,460	2	551	0	0	2,011
CORPOICA, Colombia	17	108	5	915	36	100	1,159
PROINPA, Bolivia	35	500	7	1,400	7	300	2,207
UACH, Chile	6	183	2	331	83	1,500	2,097
INIAP, Ecuador	43	275	4	222	14	0	511
INIA, Perú	0	0	?	310	20	300	630
Subtotal		4,889		8,190	474	5,370	18,923
Europe							
VIR, Russian Federation	172 (192)	3,100	12?	3,400	2,100	200	8,800
IPK, Germany	132	1,349	7	1,711	1,989	845	5,894
CGN, Netherlands	125	1,961	4	740	0	15	2,716
INRA, France	25	600	3	250	1,000	4,600	6,450
Suceava, Romania	0	0	0	0	150	0	150
VSUZ, Slovakia	12	12			475	525	1,012
KIS, Slovenia	0	0	0	0	61	30	91
CPC, UK	83	912	4	692	0	0	1,604
PRI, Czech Republic	28	293	1	3	1,111	638	2,045
NGB, Sweden	0	0	0	0	57	7	64
CABTFE, Spain	0	0	3	116	0	0	116
Subtotal		8,227		6,912	6,943	6,860	28,942
North America							
USDA/ARS, USA	130	3,791	4	1,022	312	534	5,659
PGRC3, Canada	0	0	0	0	52	67	119
Subtotal		3,791		1,022	364	601	5,779
Asia							
CAAS, China	10	150	0	0	300	400	850
CPRI, India	134	395	2?	924	1,240	69	2,628
NIAS, Japan	35	127	1	25	1,660	31	1,843
Subtotal		672		949	3,200	500	5,321
Total		17,579		17,073	10,981	13,331	58,964

[a] Breeding lines, hybrids, etc.

represent about 80% of the total native potato gene pool in the Andes (Tables 7 and 8). The collection is now fully geo-coordinated. Geographical information system (GIS) maps have been produced and additional layers showing characteristics relating to photosynthetic activities once available can be used to identify subsamples for detailed evaluation. However, a taxon classified as distinct species, *S. hygrothermicum* by Ochoa (2003), and a subspecies of *S. phureja* by Hawkes (1990), is not represented in any of the potato collections. It may be of interest in relation to photosynthetic activities because of its adaptation to high temperature and humidity. This taxon has to be collected and conserved.

Table 7. Andean native potato collection (for genus *Solanum* by species[a]) conserved at the International Potato Center

Species	Ploidy	Total
S. ajanhuiri	2*x*	14
S. chaucha	3*x*	116
S. curtilobum	5*x*	6
S. juzepczukii	3*x*	36
S. phureja	2*x*	204
S. stenotomum	2*x*	397
S. tuberosum ssp. *andigenum*	4*x*	3,311
Unknown	–	151
Total		4,235

[a] Species held in trust under the International Treaty on Genetic Resources for Food and Agriculture

Table 8. Andean native potato collection (by country[a]) held at the International Potato Center

Country name of origin	Total
Argentina	206
Bolivia	541
Chile	143
Colombia	253
Ecuador	361
Peru	2,694
Venezuela	37
Grand Total	4,235

[a] Species held in trust under the International Treaty on Genetic Resources for Food and Agriculture

Genotypic diversity

Genotypic differences in photosynthetic efficiency in potato have not yet been assessed on a large scale. Midmore and Prange (1991) have assessed carbon assimilation in a set of potato cultivars, breeding lines and *Solanum* section *Petota* under non-tuberising conditions and elevated temperatures. They found great genotypic differences in biomass accumulation and photosynthesis parameters, indicating variation in heat tolerance and photosynthetic efficiency in potato germplasm.

The scarce available sequence information on Rubisco genes of potato suggests considerable sequence diversity in the chloroplast-coded large (*rbc*L) as well as in the nucleus coded small subunits (*rbc*S). Between the two publicly available *rbc*L sequences, 20 polymorphic sites have been identified in the 1,600 bp full-length sequences. Nine of these sites represent non-synonymous sequence changes leading to different amino acids at these positions (CIP, unpublished data). Fritz et al. (1993) showed that potato and tomato *rbc*S share more interspecific sequence identity than within one species. This suggests the presence of high diversity in Rubisco genes in potato. The wide range of different growth habitats of wild potato, reaching from the Andean highlands to desert and rainforest environments, suggests that great variation in all kinds of gene functions, including photosynthetic activity, can be found in the *Solanum* species. However, it has not yet clearly been established whether significant differences in Rubisco kinetics can be found among different potato cultivars.

Legumes—genetic resources for improved photosynthesis

This section gives an overview of the size and availability of cool-season food legume collections including lentil, chickpea, faba bean and grasspea. While the workshop focused on these species as a starting point, significant collections of other food legume species of global importance including cowpea, groundnut (including peanut), soybean, bean (*Phaseolus* spp.) and field peas should be considered as potential targets for research into manipulating photosynthetic mechanisms for higher yield. Table 9 summarises the ex situ global holdings of these crops.

Much of the information presented here was sourced from the crop conservation strategies

developed under the auspices of the Global Crop Diversity Trust and funded by the Grains Research and Development Corporation of Australia. Thus, the tables represent the most up-to-date survey of ex situ holdings of these crops at the time of writing.

Ex situ collections

Table 9 shows the total number of accessions held in ex situ collections globally. Tables 10 and 11 list the gene banks or countries, in order of their collection size, that hold most of the world's food legume genetic resources. The International Center for Agricultural Research in the Dry Areas (Syria) (ICARDA), and in the case of chickpea, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and ICARDA, hold significantly more accessions than the other gene banks listed in Table 10.

Table 9. Global ex situ holding of cool-season food legumes

Crop group	Cultivated species (food)	Species in gene pool	Gene banks	Total accessions
Chickpea (*Cicer* spp.)	*Cicer arietinum*	43	31	86,499
Lentil (*Lens* spp.)	*Lens culinaris*	7 taxa, 4 species	41	43,214
Faba bean	*Vicia faba*	1	32	37,980
Grasspea	*Lathyrus* spp.	160	29	21,227

Source: Global Conservation Strategies

Table 10. Global ex situ holdings of cool-season food legumes

Crop group	Major gene banks	Global accessions (%)	Landrace and wild accessions (%)	Total accessions
Chickpea	ICRISAT India ICARDA Australia USDA Iran Russian Federation (VIR) Pakistan Turkey	87	70	52,218
Lentil	ICARDA Australia (ATFCC) Iran USDA Russia (VIR) India	60	67	26,052
Faba bean	ICARDA China Australia Germany Italy Ecuador Russian Federation (VIR)	70	45	26,800
Grasspea (*Lathyrus* spp.)	ICARDA France India Bangladesh Chile	64	85	13,573

Source: Global Conservation Strategies
ATFCC = Australian Temperate Field Crops Collection; VIR = NI Vavilov Centre, Russian Federation; all other abbreviations given on pp 7–9

Table 11. Global ex situ holdings of other important food legume species

Crop group	Major gene banks	Global accessions (%)	Total accessions
Bean (*Phaseolus* spp.)	CIAT–CGIAR USA Mexico	31	268,400
Soybean (*Glycine* spp.)	USDA China AVRDC Ukraine	31	176,400
Cowpea (*Vigna* spp.)	IITA–CGIAR Philippines USDA AVRDC India Indonesia Brazil	62	85,600
Peanut (*Arachis* spp.)	ICRISAT–CGIAR USDA India	54	81,200
Field pea (*Pisum* spp.)	UK Italy Australia	20	75,300

Source: Commission on Genetic Resources for Food and Agriculture (1997)
AVDRC = World Vegetable Centre; CIAT = International Centre for Tropical Agriculture; ICRISAT = International Crops Research Institute for the Semi-Arid Tropics; IITA = International Institute of Tropical Agriculture; all other abbreviations given on pp 7–9

Duplication

National gene banks tend to house a high proportion of indigenous germplasm. Thus they can be considered to have a high degree of uniqueness, with the exception of Australia and the USDA, whose accessions are all introduced.

While accurate data on duplication are patchy and thus difficult to estimate accurately, it is generally accepted that a high level of duplication exists within the major gene banks, such as ICARDA, ICRISAT, USDA and the Australian gene bank. For example, 5,140 of the 10,520 food legume accessions held by the USDA are duplicated at ICARDA. Likewise, 18,990 of the 42,655 food legume accessions conserved at ICARDA were either created or collected uniquely by ICARDA; the remaining successions were donated by 134 institutes from around the world and are likely duplicates.

Gaps in collections

The major gaps in *Cicer* and *Lens* collections are the wild species. Berger et al. (2003) demonstrate that of the 572 wild *Cicer* accessions in the whole world collection, only 124 were collected from distinct wild populations. This low number indicates that the ex situ collection represents only a fraction of the potential diversity available in wild populations. Likewise in wild *Lens*, Ferguson et al. (1998) and Ferguson and Erskine (2001) conclude that germplasm from northern African countries and taxa from the new central and west Asian republics of the former Soviet Union are under-represented in the world collection. However, the overall collection priority for wild species of *Cicer* and *Lens* remains south-west Turkey.

For the cultivated legumes, geographic regions identified as under-represented in the global collection are detailed in the global crop conservation strategies (at <www.croptrust.org/content/crop-strategies>). Regions of particular significance are south-eastern Turkey, northern Iraq, north-western Iran, parts of Afghanistan, the Ethiopian highlands and the mountainous regions of the Central Asian republics.

Maize—genetic resources for improved photosynthesis

Classification

To access maize genetic resources from gene banks it is useful to understand the taxonomic structure and classification of the species. Maize is an outbreeding crop that includes maize, teosinte and the genus *Tripsacum*. The genus *Zea* (maize and teosinte) and *Tripsacum*, a sister genus of *Zea*, belong to subtribe Tripsacinae, tribe Andropogoneae, subfamily Panicoideae of the family Poaceae (Grass Phylogeny Working Group 2001).

Maize

Maize was domesticated at mid altitudes in south-western Mexico (Piperno et al. 2009). It has evolved and differentiated into more than 250 races of tropical lowland, mid-altitude and highland adaptation in Latin America (Taba 1997). Maize further formed subtropical and temperate races through migration and recombination during the last few thousands of years. Maize spread from the Americas into Africa, Asia and Europe in the late 15th to 16th centuries and thus has evolved in different climatic regimes of photoperiod, temperature and precipitation.

Maize landraces, containing the wealth of maize diversity, were collected in the Americas and in other continents by systematic national and international collection missions. Since the early 1940s, maize races have been studied Latin America, particularly Mexico, Central America and the Caribbean. They have also been studied in Asia and Europe, and have been preserved in national and international gene banks (North Central Regional Plant Introduction Station 2005; Taba et al. 2005).

Other categories of maize germplasm have been developed by breeding and pre-breeding. They include inbred lines, hybrids, synthetics, composites, breeding populations (cycles of selection), gene pools (cycles of selection) and genetic stocks. Recently expired plant variety protection (PVP) lines from the US PVP office were released to the active maize gene bank of the North Central Regional Plant Introduction Station in Ames, Iowa, USA (NCRPIS). A number of lines are now available to public users from the gene bank.

Teosinte

Wilkes (1967, 2004), Sanchez-Gonzalez and Ordaz (1987), Sanchez-Gonzalez et al. (1998) and Iltis and Benz (2000) described the geographic distribution of annual teosintes in Mexico, Guatemala and Nicaragua. In the phylogeny of the genus *Zea*, as shown below, section *Zea* includes maize and annual teosintes from Mexico and Guatamala, while section *Luxuriantes* includes annual teosintes from Guatemala and Nicaragua and perennial teosintes from Mexico. It recognises five species (four in section *Luxuriantes* and one in section *Zea*) and four subspecies in section *Zea* and three races in Mexican annual teosintes. Wilkes (1967, 2004) grouped maize and teosinte in separate species, *Zea mays* and *Zea Mexicana*, respectively. *Zea mays* L. subsp. *parviglumis* is phylogenetically close to maize (Matsuoka et al. 2002; Buckler and Stevens 2005). *Zea diploperennis* and *Z. perennis* are more distantly placed from maize among annual teosintes and close to *Tripsacum* in the summary on maize origins, domestication and selection (Buckler and Stevens 2005). As teosinte and maize can cross-fertilise in nature, although in some cases with difficulty, they form the primary maize gene pool.

Section *Zea* comprises:

- *Zea mays* L. subsp. *mexicana* (Schrader) Iltis, (n = 10, annual teosinte), Race Chalco, Race Central Plateau, Race Nobogame and Race Durango
- *Zea mays* L. subsp. *parviglumis* Iltis and Doebley (race Balsas, annual teosinte)
- *Zea mays* L. subsp. *huehuetenangensis* (Iltis and Doebley) (race Huehuetenango, annual teosinte)
- *Zea mays* L. subsp. *mays* (L.) Iltis and Doebley, (n = 10, maize).

Section *Luxuriates* comprises:

- *Zea diploperennis* Iltis, Doebley and Guzmán (n = 10, perennial diploid teosinte)
- *Zea perennis* (Hitch.) Reeves and Mangelsdorf (n = 20, perennial tetraploid teosinte)
- *Zea luxurians* (Durieu and Ascherson) Bird, (n = 10, race Guatemala, annual teosinte)
- *Zea nicaraguensis* Iltis and Benz, (n = 10, race Nicaragua, annual teosinte).

Tripsacum

Tripsacum is a perennial grass whose centres of diversity are Mexico and Guatemala. They are more distantly related to maize than teosintes and thus are

placed in the secondary gene pool of maize. Thus, some species can hybridise with maize, but with difficulty. There is a natural hybrid of Tripsacum and teosinte, *Tripsacum andersonii* Gray ($n = 64$), formed by *Zea luxurians* ($n = 10$) and *Tripsacum latifolium* ($3n = 54$, triploid form) (Berthaud et al. 1997). The genus *Tripsacum* has more than 13 species and is divided into sections *Tripsacum* and *Fasciculata* (Doebley 1983). Theses species occur from temperate North America (Massachusetts) to Paraguay in South America. Having the basic chromosome number of $n = 18$, it has diploid, triploid, tetraploid and pentaploid forms. Even one population of *Tripsacum pilosum* Scribner and Merrill can contain diploid, triploid and tetraploid forms (Berthaud et al. 1997).

In general it is difficult to obtain *Tripsacum* seeds in a large quantity as the inflorescences do not bear many seeds and disarticulate at maturity. There are insufficient herbarium materials, especially from South America (Global Crop Diversity Trust 2010).

Ex situ collections

The Global Crop Diversity Trust (unpublished data) estimates the global maize collection to be more than 300,000 accessions preserved in approximately 250 collections.

Access from ex situ gene banks

Three active gene banks respond internationally to seed requests: International Maize and Wheat Improvement Center (CIMMYT) in Mexico; North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa, USA; and Maize Genetic Cooperation Stock Center at the University of Illinois, Urbana, Illinois, USA. These gene banks maintain active maize germplasm collections to bona fide users on request. Other national and regional gene banks can also meet seed requests (Global Crop Diversity Trust 2010).

Primary maize diversity is from the Americas (New World), while secondary diversity is from Asia, Africa and Europe (Old World) (Global Crop Diversity Trust 2010). The three international maize gene banks contain accessions from the original centres of diversity (New World) and other continents (Old World). They also conserve newly developed germplasm produced in research, breeding and germplasm enhancement programs.

The CIMMYT Germplasm Bank currently holds more than 27,000 accessions, including landrace (23,987), CIMMYT maize lines (CML) (513 tropically adapted inbred lines), gene pools and populations (909), synthetics and composites (329), varieties (1097), teosinte (191) and *Tripsacum* (134) (Table 12). The collection information is available from the System-wide Genetic Resources Programme (SGRP) at <www.genesys-pgr.org/> or by contacting the Maize Genetic Resources Program at CIMMYT at <www.cimmyt.org/>.

NCRPIS distributes expired PVP lines with a small amount of seeds of each. It expects in the next 3 years that 135–140 more expired PVP lines released from the PVP office of the USDA Agricultural Research Service (ARS). In 2008, NCRPIS held 20,018 accessions in its active collection, 75.6% of which are backed up at the NCGRP base collection in Fort Collins, Colorado, USA (M. Millard, pers. comm.). The NCRPIS maize collection can be reached at <www.ars.usda.gov/Main/docs.htm?docid=8642>.

NCGRP not only holds the NCRPIS backup, but also holds other maize collections from the Maize Genetic Cooperation Stock Center (at <maizecoop.cropsci.uiuc.edu/>), PVP lines and the Crop Science-registered germplasm, totalling more than 24,000 accessions. Apart from these backup collections, NCGRP preserves duplicates of the CIMMYT maize collection and special collections of maize scientists (David Ellis, pers. comm.).

The NCGRP (at www.ars.usda.gov/Main/docs.htm?docid=17923) is a US-based collection that does not distribute seed accessions. Rather it serves as a backup for the NCGRP and other local and international collections.

The Maize Genetic Cooperation Stock Center holds about 8,000 accessions that can be accessed through the MaizeGDB database at <maizegdb.org/>. Small seed amounts of genetic stock are available from the centre.

MaizeGDB contains biological information about the crop plant *Zea mays* ssp. *mays*. It provides data on genetic, genomic, sequence, gene product, functional characterisation, literature, and personal and organisational contact information. The NCGRP, NCRPIS, Maize Genetic Cooperation and Stock Center are operated by USDA–ARS. The Germplasm Resources Information Network (GRIN) (at <www.ars-grin.gov/npgs/acc/acc_queries.html>) supports the National Plant Germplasm System (NPGS) in theUSA.

In summary, ex situ maize germplasm is available to scientists and breeders from the CIMMYT Maize Germplasm Bank, NCRPIS/NCGRP and the Maize Genetic Cooperation Stock Center collection

with appropriate documentation for seed exchange. *Tripsacum* is maintained in CIMMYT and USDA field gene banks at Miami, Florida, and Woodward, Oklahoma, USA (Global Crop Diversity Trust 2007).

Accessibility of genetic resources

Not all gene banks make their accessions freely available. For example, while India, Iran, Turkey, Italy, China and Russia are listed as major gene banks for important crop species, their collections are not freely accessible now. Access to their collections is either completely restricted to outside countries or provided within the context of bilateral projects where the flow of germplasm tends to be limited to carefully chosen accessions of specific interest. Access to the CGIAR gene banks, and Australian, USDA and European gene banks, is generally in accordance with the International Treaty on Plant Genetic Resources for Food and Agriculture, which has attempted to promote free access and benefit sharing of plant genetic resources. These gene banks will send out small quantities of seeds, free of charge, for research and breeding purposes on request.

Documentation

Various authors and surveys cite a lack of readily available information describing accessions held in collections as a major impediment to gene bank use. While most gene banks maintain their own information systems, which may or may not have a full complement of passport, characterisation and evaluation data, the information is not necessarily available in an easy-to-access format.

Various attempts to make information on components of the global collection available have been successful. However, considerable challenges remain. For example, the SINGER portal, designed to deliver information about the CGIAR gene bank holdings, still does not reflect the full suite of information available in internal databases. Likewise the EURISCO portal at <eurisco.ecpgr.org>, implemented by the European network of gene banks, does not publish various classes of information that would allow more rational choices to be made when selecting germplasm for specific purposes. The USDA's GRIN database provides very detailed information about its accessions including full passport data,

Table 12. *Tripsacum* species preserved in CIMMYT ex situ field gene bank

Code	Species	Clonal accessions	Chromosome no.	Ploidy
TAD	*T. andersonii* Gray	2	64	Hybrid
TAA	*T. australe* var. *australe* Cutler and Anderson	2	36	Diploid
TBV	*T. bravum* Gray	15	72	Tetraploid
TCD	*T. cundinamarca* de Wet and Timothy	4	36	Diploid
TDD	*T. dactiloides* (L.) L.	3	72	Tetraploid
TDH	*T. dactiloides* var. *hispidum* (Hitchc.) de Wet and Harlan	32	72	Tetraploid
TMR	*T. dactiloides* var. *meridionale* de Wet and Timothy	6	36	Diploid
TDM	*T. dactiloides* var. *mexicanum* de Wet and Timothy	23	72	Tetraploid
TIT	*T. intermedium* de Wet and Harlan	6	72	Tetraploid
TJL	*T. jalapense* de Wet and Brink	2	72	Tetraploid
TLC	*T. lanceolatum* Ruprecht ex Fournier	3	72	Tetraploid
TLT	*T. latifolium* Hitchc.	1	54	Triploid
TMZ	*T. maizar* Hernandez and Randolph	5	54	Triploid
TMN	*T. manisuroides* de Wet and Timothy	2	36	Diploid
TPR	*T. peruvianum* de Wet and Timothy	2	90	Pentaploid
TPL	*T. pilosum* Scribner and Merrill	6	36	Diploid
TZP	*T. zopilotense* Hernandez and Randolph	3	36	Diploid
	Not classified	17	72	Tetraploid
Total duplicates	134	134	–	–

Source: code from Berthaud et al. (1997)
– = not applicable

collection site data and evaluation data. GRIN is a good example of what is possible. However there are still limitations with downloading batch files of data without the help of the database curators. Thus, public access to the types of information needed to make rational choices when selecting germplasm for a specific purpose remains limited. Access usually takes place within informal networks between gene bank curators, documentation officers and scientists in the genetic resources user community.

However, the Global Information on Germplasm Accessions initiative, funded by the Global Crop Diversity Trust and implemented by Bioversity International, is currently developing tools and a portal designed to significantly improve the quality and access to global accession-level information.

Sampling strategies for large ex situ germplasm collections

More than 6 million plant germplasm accessions reside in 1,300 gene banks globally. Thus, a researcher seeking novel genes from a genetic resource collection needs to know how to choose a subset of germplasm to screen with a reasonable probability that it will contain the variation needed. Clearly it is beyond the resources of most research programs to screen all available gene bank accessions for the crop group of interest. Thus, to make mining gene banks for useful traits more efficient, a rational strategy is required that will increase the likelihood of finding a sought-after trait while reducing the amount of germplasm that needs to be screened.

The core collection strategy has received a lot of attention and resources, particularly by the genetic resource community. The idea was proposed as a way to work with fewer accessions that would represent, 'with a minimum of repetitiveness, the genetic diversity of a crop species and its relatives' (Frankel 1984). Many examples of methodologies to develop core collections (see van Hintum et al. 1995 for examples) tend towards limiting the size of the core collection to around 10% of the original collection size (Brown 1989a,b). However, the contribution of the core collection to usage has not been clearly demonstrated. The literature suggests much of the focus has been on methods (or sampling strategies) to establish core collections (Holbrook et al. 1993; Ortiz et al. 1998; Hu et al. 2000; Malosetti and Abadie 2001) and the analysis of the diversity held within core collections (Casler 1995; Tohme et al. 1996; Bartish et al. 2000; Fu et al. 2005). While numerous references mention use, the vast majority do so in terms of the opportunity to improve use (Diwan et al. 1994; Bisht et al. 1998), rather than demonstrate the identification of new sources of genetic variation through the core collection or modified versions of it.

While core collections aim to maximise the genetic diversity (of some larger collection) in a smaller number of accessions, Mackay (1995) asserted that breeders and pre-breeders usually seek only one or a few traits at a time when approaching a genetic resource collection. Mackay (1990) earlier outlined how smaller subsets of accessions could be designed to capture variation for specific traits. This proposal to develop trait-specific subsets to improve use was recognised by Rana and Kochhar (1996) and has subsequently been suggested for assessment (Allem 2001). Mackay's (1990) trait-specific subset proposal has recently been developed into a GIS-based approach, coined the Focused Identification of Germplasm Strategy (FIGS) (Street et al. 2008).

FIGS uses agroecological parameters to describe the sites from which landrace and wild germplasm were collected to predict in situ selection pressures. These pressures in turn indicate the adaptive traits likely within populations of material collected from a given site. The FIGS methodology has been developed and tested at the Australian Winter Cereals Collection, ICARDA and the NI Vavilov Research Institute of Plant Industry, St Petersburg, Russian Federation. FIGS proved to be more efficient at developing subsets of germplasm containing useful traits than selecting material at random from gene banks or by using a core collection approach. New sources of resistance in bread wheat were isolated from relatively small subsets of germplasm for Sunn pest (El Bouhssini et al. 2009), Russian wheat aphid (virulent Syrian biotype) (El Bouhssini et al. 2011), Hessian fly, powdery mildew (Kaur et al. 2008) and UG99 stem rust (K. Street, unpublished data). FIGS also proved more effective than a core collection for isolating salinity (I. A. Kosareva, pers. comm.) and drought tolerance in bread wheat (K. Street, unpublished data). Given there are indications of eco-geographic variation in photosynthetic activity (Galmes et al. 2005), a FIGS approach could be an effective way to subsample collections to search for variation in traits that underpin photosynthetic efficiency.

However, global collection site data remain patchy in databases, are retained in hard copy or are buried within collection mission reports. Thus, not

all globally available accessions held in gene banks would be candidates for a FIGS-based investigation. Despite this, enough geo-referenced accessions are available for the approach. For example, more than 16,000 unique geo-referenced wheat (bread, Durum and primitive) accessions are listed in the Australian Winter Cereals Collection, ICARDA, USDA and EURISCO gene banks.

In the absence of geo-referenced accessions, characterisation and evaluation data are more important as there is no other means on which to base a subsampling strategy, other than a random selection. However, characterisation, and in particular evaluation data, are more sparse in readily accessible databases than collection site geo-references. Further, when selecting germplasm to screen for photosynthetic activity, the value of many classes of characterisation data that are available would be of limited value because they are not necessarily correlated with traits specifically linked to superior photosynthetic activity. Finally, evaluation data specifically linked to photosynthetic activity are generally absent from gene bank databases. These data are likely to be produced by laboratories focused on photosynthetic research but for a very limited portion of the available gene pool.

References

Allem A.C. 2001. Managing genebanks: seed base collection examined. Genetic Resources and Crop Evolution 48, 321–328.

Bartish G.I., Jeppsson N., Bartish I.V. and Nybom H. 2000. Assessment of genetic diversity using RAPD analysis in a germplasm collection of sea buckthorn. Agricultural and Food Science in Finland 9, 279–289.

Berger J., Abbo S. and Turner N.C. 2003. Ecogeography of annual wild *Cicer* species: the poor state of the world collection. Crop Science 43, 1076–1090.

Berthaud J., Savidan Y., Barre M. and Leblanc O. 1997. *Tripsacum*. Pp. 227–233 in 'Biodiversity in trust', ed. by D. Fucillo, L. Sears and P. Stapleton. Cambridge University Press: Cambridge, UK.

Bioversity 2006. Bioversity directory of germplasm collections. Bioversity International: Rome.

Bisht I.S., Mahajan R.K. and Patel D.P. 1998. The use of characterisation data to establish the Indian mungbean core collection and assessment of genetic diversity. Genetic Resources and Crop Evolution 45, 127–133.

Bradshaw J.E., Bryan G.J. and Ramsay G. 2006. Genetic resources (including wild and cultivated *Solanum* species) and progress in their utilisation in potato breeding. Potato Research 49, 49–65.

Brar D.S. and Khush G.S. 2002. Transferring genes from wild species into rice. Pp. 197–297 in 'Quantitative genetics, genomics and plant breeding', ed. by M.S. Kang. CAB International: Wallingford.

Brar D.S. and Khush G.S. 2006. Cytogenetic manipulation and germplasm enhancement of rice (*Oryza sativa* L.). Pp. 115–158 in 'Genetic resources, chromosome engineering and crop improvement', ed. by R.J. Singh and P.P. Jauhar. CRC Press: Boca Raton, USA.

Brown A.H.D. 1989a. Core collections—a practical approach to genetic resources management. Genome 31, 818–824.

Brown, A.H.D. 1989b. The case for core collections. Pp. 136–156 in 'The use of plant genetic resources', ed. by A.H.D. Brown, O.H. Frankel, D.R. Marshall and J.T. Williams. Cambridge University Press: Cambridge.

Buckler E.S. and Stevens N.M. 2005. Maize origins, domestication and selection. Pp. 67–90 in 'Darwin's harvest', ed. by T.J. Motley, N. Zerega and H. Cross. Columbia University Press: New York.

Casler M.D. 1995. Patterns of variation in a collection of perennial ryegrass accessions. Crop Science 35, 1169–1177.

Commission on Genetic Resources for Food and Agriculture 1997. The state of the world's plant genetic resources, FAO: Rome.

Diwan N.G.R., Bauchan G.R. and McIntosh M.S. 1994. A core collection for the United States annual *Medicago* germplasm collection. Crop Science 34, 279–285.

Doebley J. 1983. The taxonomy and evolution of *Tripsacum* and *Teosinte*, the closest relatives of maize. Pp. 15–28 in 'Proceedings of the International Maize Virus Disease Colloquium and Workshop', ed. by D.T. Gorden, J.K. Knoke and L.R. Nault. Ohio Agricultural Research and Development Center: Wooster, Ohio.

El Bouhssini M., Street K., Amri A., Mackay M., Ogbonnaya F.C., Omran A., Abdalla O., Baum M., Dabbous A. and Rihawi F. 2011. Sources of resistance in bread wheat to Russian wheat aphid (*Diuraphis noxia*) in Syria identified using the Focused Identification of Germplasm Strategy (FIGS). Plant Breeding 130, 96–97.

El Bouhssini M., Street M.K., Joubi A., Ibrahim Z. and Rihawi F. 2009. Sources of wheat resistance to Sunn pest, *Eurygaster integriceps* Puton, in Syria. Genetic Resources and Crop Evolution 56, 1065–1069.

Ferguson M.E and Erskine W. 2001. Lentils (*Lens* L.). Pp. 125–131 in 'Plant genetic resources of legumes in the Mediterranean', ed. by N. Maxted and S.J. Bennett. Kluwer: Dordrecht, Netherlands.

Ferguson M.E., Ford-Lloyd B.V., Robertson L.D., Maxted N. and Newbury H.J. 1998. Mapping of geographical distribution of genetic variation in the genus *Lens* for enhanced conservation of plant genetic diversity. Molecular Ecology 7, 1743–1755.

Frankel O.H. 1984. Genetic perspectives of germplasm conservation. Pp. 161–170 in 'Genetic manipulation:

impact on man and society', ed. by W. Arber. Cambridge University Press: Cambridge, UK.

Fritz C.C., Wolter F.P., Schenkemeyer V., Herget T. and Schreier P.H. 1993. The gene family encoding the ribulose-(1,5)-bisphosphate carboxylase/oxygenase (Rubisco) small subunit of potato. Gene 137, 271–274.

Fu Y.B., Peterson G.W., Williams D., Richards K.W. and Fetch J.M. 2005. Patterns of AFLP variation in a core subset of cultivated hexaploid oat germplasm. Theoretical and Applied Genetics 111, 530–539.

Galmes J., Flexas, J., Keys A.J., Cifre J., Mitchell R.A.C., Madgwick P.J., Haslam R.P., Medrano H. and Parry M.A.J. 2005. Rubisco specificity factor tends to be larger in plant species from drier habitats and with persistent leaves. Plant Cell and Environment 28, 571–580.

Ge S., Li A., Lu B-R., Zhang S-Z. and Hong D-Y. 2002. A phylogeny of the rice tribe Oryzeae (Poaceae) based on *matK* sequence data. American Journal of Botany 89, 1967–1972.

Global Crop Diversity Trust 2010. Global strategy for the ex situ conservation of rice genetic resources. Global Crop Diversity Trust: Rome.

Grass Phylogeny Working Group 2001. Phylogeny and subfamilial classification of the grasses (Poaceae). Annals of the Missouri Botanical Garden 88, 373–457.

Harlan J.R. and de Wet J.M.J. 1971. Toward a rational classification of cultivated plants. Taxon 20, 509–517.

Hawkes J.G. 1990. The potato: evolution, biodiversity and genetic resources. Belhaven Press: London.

Hijmans R.J., Gavrilenko T., Stephenson S., Bamberg J., Salas A. and Spooner D.M. 2007. Geographical and environmental range expansion through polyploidy in wild potatoes (*Solanum* section *Petota*). Global Ecology and Biogeography 16, 485–495.

Hils U. and Pieterse L. 2009. World catalogue of potato varieties 2009/2010. AGRIMEDIA GMBH: Clenze, Germany.

Holbrook C.C., Anderson W.F. and Pittman R.N. 1993. Selection of a core collection from the U.S. germplasm collection of peanut. Crop Science 33, 859–861.

Hu J., Zhu J. and Xu H.M. 2000. Methods of constructing core collections by stepwise clustering with three sampling strategies based on the genotypic values of crops. Theoretical and Applied Genetics 101, 264–268.

Huaman Z., Hoekstra R. and Bamberg J.B. 2000. The inter-genebank potato database and the dimensions of available wild potato germplasm. American Journal of Potato Research 77, 353–362.

Iltis H.H. and Benz B.F. 2000. *Zea nicaraguensis* (Poaceae), a new teosinte from Pacific coastal Nicaragua. Novon 10, 382–390.

Kaur N., Street K., Mackay M., Yahiaoui M.N. and Keller B. 2008. Molecular approaches for characterization and use of natural disease resistance in wheat. European Journal of Plant Pathology 121, 387–397.

Mackay M.C. 1990. Strategic planning for effective evaluation of plant germplasm. Pp. 21–25 in 'Wheat genetic resources: meeting diverse needs', ed. by J.P. Srivastava. John Wiley & Sons: Chichester, UK.

Mackay M.C. 1995. One core collection or many? Pp. 199–212 in 'Core collections of plant genetic resources', ed. by T.J.L. van Hintum, A.H.D. Brown, C. Spillane and T. Hodgkin. John Wiley & Sons: Chichester UK.

Malosetti M. and Abadie T. 2001. Sampling strategy to develop a core collection of Uruguayan maize landraces based on morphological traits. Genetic Resources and Crop Evolution 48, 381–390.

Matsuoka Y.Y., Vigouroux M.M., Goodman J., Sanchez G., Buckler E. and Doebley J. 2002. A single domestication for maize shown by multilocus microsatellite genotyping. Proceedings of the National Academy of Sciences 99, 6080–6084.

Midmore D.J. and Prange R.K. 1991. Sources of heat tolerance amongst potato cultivars, breeding lines, and *Solanum* species. Euphytica 55, 235–245.

North Central Regional Plant Introduction Station 2005. Races of maize collection. Version 22. Digital compilation (CD-ROM) created by USDA-ARS, North Central Regional Plant Introduction Station, Iowa State University, Ames, Iowa.

Ochoa C.M. 2003. Las papas del Peru. CIP, UNALM, COSUDE: Lima, Peru.

Ortiz R., Ruiz-Tapia E.N. and Mujica-Sanchez A. 1998. Sampling strategy for a core collection of Peruvian quinoa germplasm. Theoretical and Applied Genetics 96, 475–483.

Piperno D.R., Ranere A.J., Holst I., Iriarte J. and Dickau R. 2009. Starch grain and phytolith evidence for early ninth millennium maize from the Central Balsa River Valley, Mexico. Proceedings of the National Academy of Sciences 106, 5019–5024.

Rana R.S. and Kochhar S. 1996. Core subsets of base collections and priorities of national programmes: Indian perspective. Genetic Resources and Crop Evolution 43, 423–428.

Sanchez-Gonzalez J.J., Kato-Yamakake T.A., Aguilar-Sanmiguel M.A., Hernandez-Casillas J.M., Lopez-Rodriguez A. and Ruiz-Corral J.A. 1998. Distribucion y caracterizacion del Teocintle. Libro Tecnico 2. Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias: Guadalajara, Mexico.

Sanchez-Gonzalez J.J. and Ordaz L. 1987. Systematic and eco-geographic studies on crop gene pools: 2. El Teocintle en Mexico. Distribución y Situación Actual de las Poblaciones. IBPGR: Rome.

Spooner D.M. and Salas A. 2006. Structure, biosystematics, and genetic resources. Pp. 1–39 in 'Handbook of potato production, improvement, and post-harvest management' ed. by J. Gopal and S.M. Paul Khurana. Haworth's Press, Inc: Binghampton, New York.

Street K., Mackay M., Mitrofanova O., Konopka J., El Bouhssini M., Kaul N. and Zuev E. 2008. Swimming in the genepool—a rational approach to exploiting large genetic resource collections. In 'The 11th International Wheat Genetics Symposium Proceedings', ed. by R. Appels, R. Eastwood, E. Lagudah, P. Langridge, M. Mackay, L. McIntyre and P. Sharp. Sydney University Press: Sydney.

Taba S. 1997. Maize. Pp. 213–226 in 'Biodiversity in trust', ed. by D. Fucillo, L. Sears and P. Stapleton. Cambridge University Press: Cambridge, UK.

Taba S., Shand H.L. and Eberhart S.A. 2005. The growth of CIMMYT's maize collection with the introduction of Latin American maize landrace accessions through the cooperative regeneration project. Pp. 1–8 in 'Latin American maize germplasm conservation: regeneration, *in situ* conservation, core subsets, and prebreeding: proceedings of a workshop held at CIMMYT, April 7–10, 2003', ed. by S. Taba. CIMMYT: Mexico.

Tohme J., Gonzalez O.D., Beebe S. and M.C. Duque M.C. 1996. AFLP analysis of gene pools of a wild bean core collection. Crop Science 36, 1375–1384.

van Hintum T.J.L., Brown A.H.D., Spillane C. and Hodgkin T. (eds) 1995. Core collections of plant genetic resources. John Wiley & Sons: Chichester UK.

Wilkes G. 1967. Teosinte: the closest relative of maize. Bussey Institute, Harvard University: Cambridge, Massachusetts.

Wilkes G. 2004. Corn, strange and marvelous: but is a definitive origin known? Pp. 3–63 in 'Corn: origin, history, technology and production', ed. by W. Smith, J. Betran and E.C.A. Runge. John Wiley & Sons Inc: Hoboken, New Jersey.

CO_2-concentrating mechanisms in crop plants to increase yield

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Summary

- Relative to C_4 plants (such as sugarcane and maize) key C_3 crop plants (such as wheat, rice and barley, etc.) are known to suffer from photorespiratory losses due to the oxygenase reaction of Rubisco. C_4 plants, on the other hand, are able to suppress photorespiration by actively maintaining an elevated CO_2 level around Rubisco.
- New approaches aimed at improving photosynthetic yield and water-use efficiency in C_3 crop plants include making use of components of the CO_2 concentrating mechanism (CCM) present in cyanobacteria and the CCMs present in single-cell C_4 plants. The objective here is to raise the CO_2 level within the chloroplasts of targeted C_3 plants by transfer of genes from cyanobacteria or single-cell C_4 plants.

Introduction

The clear challenge in global agriculture is to boost the yield from cereal, tuber and vegetable crops, not only by increasing the yield potential under optimal growth conditions, but also by improving yield realisation under marginal growth conditions where water and nitrogen deficiency put place severe limitations on yield. To meet this challenge, photosynthetic efficiency and capacity must ideally be increased per unit leaf or cultivated area, without elevating nitrogen use or compromising performance under stress conditions.

Most of the important grain crops (rice, wheat, barley, canola, soybean and pulses), tuber crops (potato, cassava, yams and sweetpotato) and vegetable crops (including tomato, carrots and cabbages) use C_3 photosynthesis, capturing CO_2 directly from the sub-stomatal air spaces of the leaf via Rubisco. The efficiency with which CO_2 is assimilated by these C_3 crop plants is severely compromised by photorespiratory activity (Zhu et al. 2008). In C_3 plants in air, almost one-third of the flux through Rubisco results in the incorporation of oxygen rather than CO_2. The subsequent processing and recycling of the product of this reaction, phosphoglycolate, requires both energy and the loss of CO_2. A group of plants termed C_4 plants has evolved a complex biochemical mechanism to concentrate CO_2 at the site of Rubisco. C_4 crop plants include maize, sorghum and sugarcane, but unfortunately the number of commercially cultivated C_4 species is small. In C_4 photosynthesis, photorespiration is eliminated and Rubisco operates at close to its theoretical maximum catalytic rate. The efficiency of converting total solar energy to grain in a C_4 plant is approximately 2.2%; in a C_3 cereal, it is only 1.4% (assuming a harvest index of 0.6 and the figures of Zhu et al. 2008). This 60% increase in photosynthetic efficiency, if translated into yield, would be compounded by the elevated nitrogen-use efficiency and water-use efficiency of C_4 crops, making the installation of C_4 photosynthesis, or a modified version, attractive for increasing rice yields (see papers in Sheehy et al. 2007).

Engineering biochemical C_4 pathways and appropriate morphological specialisation into C_3 plants is being seriously attempted (Bill and Melinda Gates

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Foundation C_4 Rice Project; Quick et al. 2013), but it entails a considerable amount of gene discovery, genetic engineering and analysis. Much of the biochemistry, physiology and molecular genetics of C_4 photosynthesis have been pioneered in Canberra—CSIRO, ANU and the Australian Plant Phenomics Facility are key members of the C_4 rice consortium. The C_4 pathway has evolved independently more than 60 times and there are various options for installation of a partial or 'intermediate' C_4 pathway in C_3 plants. These opportunities will be discussed below.

An alternative strategy to installing a C_4-like biochemical pump into C_3 plants to increase photosynthetic efficiency is to mimic the inorganic CO_2 concentrating mechanism (CCM) present in cyanobacteria and algae. This biophysical mechanism has the advantage that only a small subset of genes needs to be transferred to C_3 crop species and specialised anatomy and morphology may not be required. In addition, the energy costs of this mechanism may be inherently lower than that of the C_4 pathway. A plasma membrane or chloroplast envelope localised CCM has never been observed in a terrestrial plant. However, our knowledge of CCMs in cyanobacteria and micro-algae indicates there are clear opportunities to extend this approach based on CCMs that feature active accumulation of CO_2. These prospects are explored in the following section.

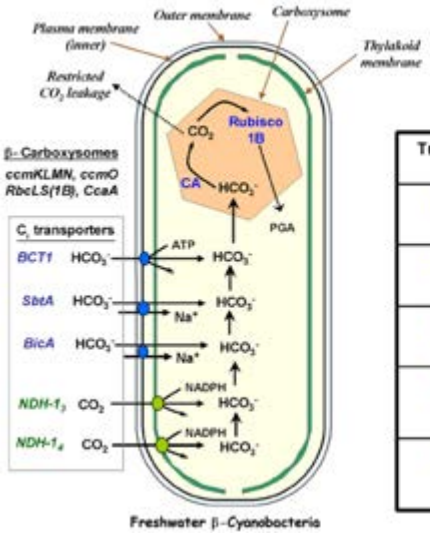
Installing algal-type CCM to C_3 crops

Cyanobacteria have evolved one of the most efficient CCMs known—a system that can concentrate CO_2 around the primary carboxylating enzyme, ribulose bisphosphate carboxylase-oxygenase (Rubisco) by a factor of up to 1,000. As a result, cyanobacterial CO_2 fixation has been able to retain a Rubisco with very high catalytic activity (which improves nitrogen-use efficiency) and the near saturation of the enzyme with substrate CO_2 has almost eliminated wasteful photorespiration. Some micro-algae have also evolved different CCMs that can accumulate CO_2 by 50–100 times, and these systems are also relevant to this paper. A key question arises—given that an early cyanobacterial progenitor is considered to have become the original endosymbiont for chloroplast evolution in land plants and algae, why do present-day land plants lack any apparent chloroplast-based CCM?

Cyanobacterial progenitors first appeared some 2.7 billion years ago, but it is almost certain that cyanobacteria have been subjected to periods of rapid evolutionary change since. In particular, the marked drop in CO_2 levels and rise in O_2 levels that occurred around 350 million years ago represent the most likely trigger that forced the evolution of adaptations to cope with photorespiration and low-efficiency carbon gain. These adaptations included transport mechanisms for active uptake of inorganic carbon (C_i) and subsequent localised elevation of CO_2 around Rubisco, and the partitioning of Rubisco into microcompartments known as carboxysomes (Price et al. 2008). This may have also been the stage when micro-algae developed CCMs. If, as seems likely, cyanobacteria did not evolve CCMs until 350 million years ago, it would be significant this event is well after the first terrestrial plants evolved from eukaryotic algae around 450 million years ago (Price et al. 2008). This probably explains why present-day crop plants lack any form of chloroplast-based CCM based on that present in modern cyanobacteria. This insight provides an opportunity for targeted genetic engineering to retrofit components of such a mechanism in crop plants. The prospects for expression of a cyanobacterial HCO_3^- transporter in the chloroplast of a C_3 model are particularly good and should be achievable within the next 2 years with sufficient funding.

Cyanobacterial CCM

The CCM in cyanobacteria is extraordinarily efficient, vastly improving photosynthetic performance and survival under limiting CO_2 concentrations. The CCM actively transports and accumulates inorganic carbon (C_i as HCO_3^- and CO_2) into the cell where the accumulated C_i pool is used to generate elevated CO_2 concentrations around Rubisco (Figure 1). In cyanobacteria, Rubisco is encapsulated in unique microcompartments (90–200 nm diameter) known as carboxysomes. These polyhedral bodies act as sites of CO_2 elevation as a result of the provision of carboxysome-located carbonic anhydrase (CA). The key to the efficiency of any CCM is its ability to minimise the loss of CO_2 from the elevation zone. In cyanobacteria, this is accomplished by a combination of accumulation of the ionic form of C_i (HCO_3^- is less membrane-permeable than CO_2), the complete elimination of CA activity from the general cytosol (which helps reduce CO_2 leakage), the special properties of the carboxysome protein shell (which retards CO_2 leakage) and the action of the CO_2 pumps in recycling CO_2 leakage from the carboxysome back into HCO_3^- pool (Price et al. 2008).



Transporter type	Genes	Mechanism	Affinity for substrate
BicA	*bicA*	Na^+-dependent HCO_3^- uptake	Low-medium
SbtA	*sbtA*	Na^+-dependent HCO_3^- uptake	High
BCT1	*cmpABCD*	Traffic ATPase, HCO_3^- uptake	High
NDH-1$_4$	*ndhF4D4ChpX, ndhAIGE, ndhB, ndhCJK, ndhH, ndhL*	NADPH-driven CO_2 uptake, conversion to HCO_3^-	Medium
NDH-1$_3$	*ndhF3D3ChpY, ndhAIGE, ndhB, ndhCJK, ndhH, ndhL*	NADPH-driven CO_2 uptake, conversion to HCO_3^-	High

Figure 1. Components of cyanobacterial CCMs, using up to five intake systems for inorganic carbon (C_i) and the polyhedral microcompartments, carboxysomes, that contain Rubisco and act as the site of CO_2 elevation

Engineering cyanobacterial CCM components into crop plants

The most immediate and achievable objective would be to place a cyanobacteria HCO_3^- transporter on the chloroplast inner envelope membrane. Price et al. (2008) are already well advanced towards achieving this goal. This approach is targeting a 5–10% improvement in photosynthetic CO_2 fixation efficiency. Supplementary enhancements also being considered include iRNA suppression of chloroplast aquaporins to reduce CO_2 leakage (Flexas et al. 2006) and addition of extra Na^+/H^+ antiporter activity (using a gene sourced from cyanobacteria) to improve the energisation capacity for Na^+-dependent HCO_3^- uptake (Woodger et al. 2007).

A longer term objective is to engineer a more potent form of the cyanobacterial CCM into the chloroplast. This would involve more advanced forms of engineering (more genes) and would target a greater than 15% improvement in photosynthetic CO_2 fixation efficiency. Briefly, the objective would be to add two transporters (possibly including a CO_2 uptake system) to the envelope, then compartmentalise Rubisco and CA into carboxysomes or pyrenoid-like structures. Other necessary improvements would include eliminating chloroplastic CA from the general stroma of the chloroplast, reducing aquaporin activity and adding extra Na^+/H^+ antiporter activity.

Adding HCO_3^- transporter to chloroplast envelope

The first and most obvious approach is to express a cyanobacterial HCO_3^- transporter in the C_3 chloroplast. Single-subunit HCO_3^- transporters such as BicA and SbtA are the best first candidates. However, within technical restraints the transfer of multisubunit transporters such as the BCT1 HCO_3^- transporter and NDH1-based CO_2 uptake systems could also be considered. In addition, the use of HCO_3^- transporters from micro-algae such as *Chlamydomonas* are also viable candidates (Spalding 2008).

It has been established that a CO_2 diffusion gradient exists between the sub-stomatal cavity of the leaf and the chloroplast. The magnitude of this gradient is about 35% below the CO_2 passive exchange equilibrium (Evans and von Caemmerer 1996) and dependent on chloroplastic CA activity. From a theoretical viewpoint, and depending on the diffusion constraints to CO_2 efflux, the engineering of an active cyanobacterial HCO_3^- pump within the chloroplast means it could operate at a higher CO_2 level, allowing the plant better potential to use less water and nitrogen for the same crop yield (Figure 2). Even a small increase in the C_i level in the chloroplast would be highly beneficial to the efficiency of CO_2 fixation, producing a consequent improvement in water-use efficiency. Such a situation is very similar to the

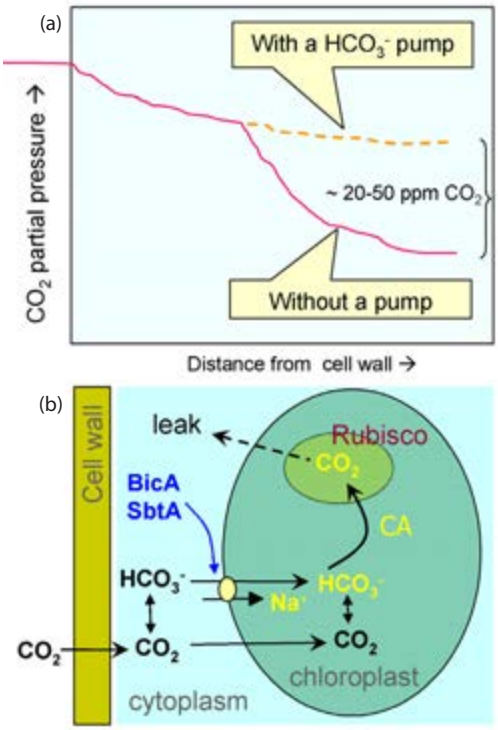


Figure 2. Modelling indicates that the draw-down diffusion gradient across the chloroplast could be rectified by adding a cyanobacterial HCO_3^- transporter, allowing CO_2 fixation to proceed at a higher average partial pressure. (a) CO_2 partial pressure vs distance from cell wall, (b) schematic of mechanism within the cell. Distance in (a) corresponds to the distance in (b).

concept of 'single-cell C_4-cycle', which as been modelled for C_3 transplantation (von Caemmerer 2003) and found to be theoretically capable of raising the steady-state CO_2 level within the chloroplast. More specific modelling data on the theoretical engineering of BicA into a chloroplast has confirmed that such an approach is useful (data not shown). One of the biggest uncertainties relates to the conductance of the envelope to CO_2 diffusion, with a range of estimates available (see section below on CO_2 diffusion properties of C_3 leaves). Aquaporins seem to play a role in CO_2 conductance. Thus, it might be useful to reduce aquaporin levels in the envelope by iRNA technology.

In terms of establishing active HCO_3^- uptake across the chloroplast envelope the question arises as to whether a Na^+-dependent HCO_3^- transporter could function in a chloroplast. The uptake affinities of SbtA (low flux rate) and BicA (high flux rate) for HCO_3^- in cyanobacteria are 5–15 and 40–100 µM, respectively (Price et al. 2008). There appears to be sufficient HCO_3^- substrate since at least 250 µM HCO_3^- is present in the cytosol of a leaf cell, maintained by cytosolic CA activity. Both SbtA and BicA require about 1 mM Na^+ for half maximal activity. The leaf cytosol possesses 1–3 mM Na^+ and recent proteomic analyses have revealed that the *Arabidopsis* chloroplast envelope possesses several potential Na^+-coupled transporters and Na^+/H^+ antiporters that are homologous to cyanobacterial forms (Price et al. 2008). Thus, it is possible that the chloroplast possesses an inwardly directed Na^+ gradient.

Work in Price's lab has focused on determining the membrane topology structure of BicA and SbtA as an initial step in identifying the most likely cytoplasmic regulatory domains in these transporters. These structures are now complete and represent the first topology maps for any members of the BicA (sulfate permease, SulP) or SbtA (major facilitator family, MFS) families. Conserved residues in the putative regulatory domains (probable protein kinase phosphorylation sites) are now being altered by site-directed mutagenesis to test the roles of putative residues in transporter activation using frog oocyte and cyanobacterial expression systems.

Installing C_4-like biochemical CO_2 pump in C_3 crops

Recently, the Bill and Melinda Gates Foundation funded a major initiative to discover the genes necessary to install a functional C_4 pathway in rice. This initiative focused on one of the three biochemical variants of the C_4 pathway (NADP-malic enzyme (ME) type) and will attempt to re-create kranz anatomy in rice. This is a cellular specialisation present in all commercial C_4 crop species that provides cellular separation of the CO_2 pump and the more C_3-like cells containing Rubisco (Figure 3; von Caemmerer and Furbank 2003; Furbank et al. 2009). In some cases this can also be achieved in terrestrial plants by spatial separation of the two types of chloroplast within a single cell (reviewed in Edwards et al. 2004). The necessity for a barrier to CO_2 diffusion out of the bundle sheath cells in the form of a specialised cell wall or suberised lamella has been a subject of discussion (von Caemmerer and Furbank 2003). It

remains unknown whether the efficient installation of the C_4 pathway into rice will require a modification to CO_2 diffusion properties (see von Caemmerer et al. 2007). This unknown concerning CO_2 diffusion also applies to the strategy of installing an algal-like CCM, described above.

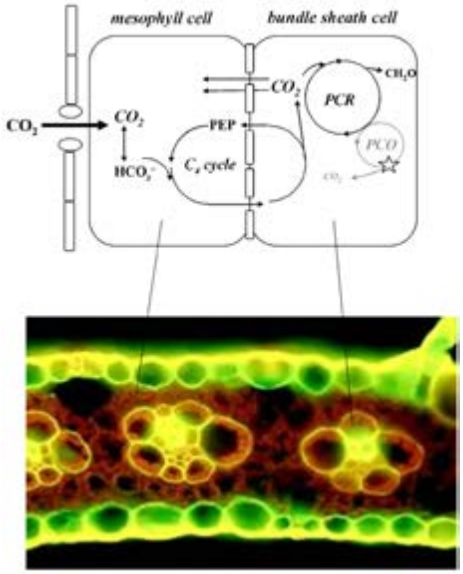


Figure 3. A simplified scheme of the C_4 pathway (adapted from von Caemmerer 2000) above a transverse section of a maize leaf viewed using fluorescence microscopy, to indicate cellular localisation of processes. CO_2 is converted to HCO_3^-, which is fixed by phosphoenolpyruvate (PEP) carboxylase to a C_4 acid product, transported to the bundle sheath cells and decarboxylated to a C_3 product, then recycled to the HCO_3^- acceptor phosphoenol pyruvate (PEP) in the mesophyll. CO_2 concentrations of up to 10 times ambient are built up in the bundle sheath cells by this biochemical pump, where Rubisco and the photosynthetic carbon reduction cycle (PCR) are located. Glycine decarboxylase, the step in photorespiration where CO_2 is released under low CO_2 conditions (indicated by a star), and the photosynthetic carbon oxidation cycle (PCO), are located exclusively in the bundle sheath compartment. This maximises refixation in the event of photorespiratory conditions.

It has been suggested that evolution of C_4 has occurred many times by a stepwise progression of structural and biochemical changes that were induced by CO_2-limiting conditions (Monson et al. 1984; Edwards and Ku 1987; Monson and Moore 1989; Rawsthorne and Bauwe 1998; Sage 2004). The occurrence of 'intermediates' between C_3 and C_4 plants has provided a basis for suggesting how C_4 may have evolved from C_3 to intermediates that reduce photorespiration without a C_4 cycle, to intermediates with a partially functioning C_4 cycle, to full development of C_4 (Figure 4).

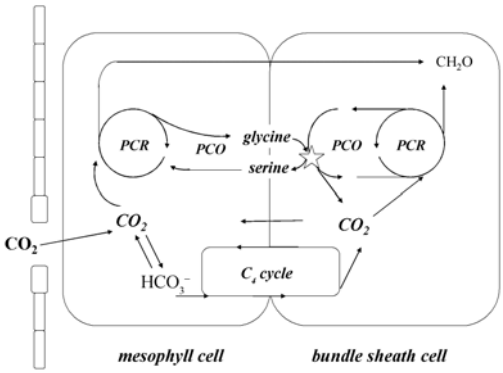


Figure 4. A simplified scheme of the C_3–C_4 intermediate photosynthetic pathway (adapted from von Caemmerer 2000). Both the intermediate pathway variants are shown. In both cases, glycine decarboxylation is localised solely to the bundle sheath compartment as in C_4 plants, but a partial C_4 mechanism may or may not be present. Rubisco and the photosynthetic carbon reduction cycle are present in both cell types. Abbreviations as in Figure 3.

In the first type of intermediate, normal C_3-type photosynthesis occurs in mesophyll cells; however, the release of CO_2 in photorespiration occurs in bundle sheath cells (by selective localisation of glycine decarboxylase in bundle sheath mitochondria), where it is partially recaptured by bundle sheath chloroplasts. This increases the efficiency of photosynthesis under limiting CO_2 concentration (von Caemmerer 1989). While the energetic benefit of a partial C_4 pathway or C_3–C_4 intermediacy has been extrapolated from gas exchange (see von Caemmerer

1989), it has yet to be clearly demonstrated through studies of physiological fitness or growth. Therefore, options exist for mimicking evolution of a C_4-like mechanism and testing the benefits in rice.

As discussed above, C_4 photosynthesis, as it currently exists in C_4 crop species, requires a complex mix of morphological and biochemical specialisation. What is not known is how much we can strip down the C_4 pathway before it becomes unviable and offers no advantage over C_3. What is a minimalist version of the C_4 system we can install in rice to gain the necessary benefits to photosynthesis and yield? Gene suppression experiments have been done in *Flaveria*, where the control of photosynthetic flux occurs in the C_4 pathway (Furbank et al. 1997; von Caemmerer and Furbank 1999). However, no experiments have been done specifically to reverse evolution and mimic C_3–C_4 metabolism in a compromised C_4 plant with incomplete biochemical and cellular specialisation and determine the physiological consequences.

Preliminary attempts to engineer C_4 photosynthetic mechanisms into C_3 plants have met with little success. This is not surprising because a functional mechanism theoretically requires appropriate levels of gene expression, location and regulation of a number of C_4 enzymes, metabolite transporters and appropriate leaf anatomy for spatial separation for C_4 function (Leegood 2002; Sheehy et al. 2007). We propose a stepwise introduction of a C_4-like biochemical pump at both the chloroplast and cellular levels using a high-throughput model cereal system as a test case for stripping down the C_4 mechanism.

Mimicking C_3–C_4 intermediacy

It is widely believed that the first step in reducing photorespiration in evolution of the C_4 mechanism was the movement of glycine decarboxylation, the step where CO_2 is released when dealing with the products of photorespiration, into bundle sheath cells adjacent to the vascular bundles (Sage 2004). This could be mimicked in a cereal or dicot crop, where chloroplast-containing bundle sheath cells are present. Mesophyll-specific promoters suitable for C_3 cereal transformation are already available to drive the gene suppression constructs necessary to achieve this end. In a readily transformable, short life cycle cereal such as *Brachypodium*, this approach could be readily validated and transferred to crop species.

Single-cell C_4 mechanism

A simple mechanism for concentrating CO_2 in the chloroplast has been proposed. It is based on the mechanism present in aquatic macrophytes, where C_4 acids are synthesised in the cytoplasm of the C_3 mesophyll cell, then transported into the chloroplast where they are decarboxylated, theoretically elevating the CO_2 concentration around Rubisco (see von Caemmerer et al. 2007). This approach has been attempted with some promise of success (Taniguchi et al. 2008). However, increased gene expression levels and modification of chloroplast diffusion properties may require substantial additional research. Once again, it is unknown whether a C_3 chloroplast will provide sufficient resistance to CO_2 diffusion for this approach to be energetically advantageous, although modelling suggests some advantages under sub-ambient CO_2 (see von Caemmerer 2003). All genes and targeting sequences for this approach are currently available.

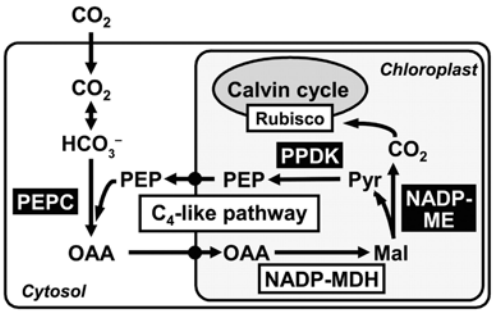


Figure 5. A single-cell C_4 approach as described in Taniguchi et al. (2008). Abbreviations as for Figure 3.

Full kranz C_4-like mechanism

This approach will not be reviewed here in depth but is described in Furbank et al. (2009) for rice. In brief, a mutant screening approach is being used to discover C_4 'revertants' where anatomical specialisation has been lost. Genes responsible for kranz anatomy will then be used to transform rice, in addition to the biochemical gene constructs required for C_4 enzyme expression. In parallel, mutants in rice that gain functions are to be isolated with more C_4-like

characteristics in which further components of a C_4-like system can be installed. This approach could also be used with dicot crops and in high-throughput model systems, using the gene constructs from the C_4 rice consortium if agreement was reached.

CO_2 diffusion properties of C_3 leaves

In all the approaches to concentrate CO_2 around Rubisco described above, the major research gap is knowledge of the diffusion properties of the chloroplast envelope and plasma membrane – cell wall (von Caemmerer 2003; von Caemmerer et al. 2007; Evans et al. 2009). This is the unknown factor. If CO_2 can freely pass across the compartment where it is being concentrated and back into the atmosphere, the cost of the CCM would likely be too high to provide an energetic benefit translatable to yield. Perhaps the first research priority should be to investigate these diffusion properties to provide proof of concept. Creation of the transgenic plants described above in model systems would provide these data. There is currently great interest in internal diffusive properties of leaves, with an issue of the *Journal of Experimental Botany* devoted to it. It has been suggested that aquaporins may be involved in modulating membrane permeability to CO_2 and this raises the question whether permeability could be manipulated by altering levels of these proteins (Uehlein et al. 2008). A second opportunity to reduce CO_2 permeability across the chloroplast envelope would be to reduce chloroplast surface area appressed to intercellular airspace. At present the chloroplast surface to leaf area ratio is approximately 15 to facilitate CO_2 diffusion to Rubisco (Evans et al. 2009).

References

Edwards G.E., Franceschi V.R. and Voznesenskaya E.V. 2004. Single-cell C_4 photosynthesis versus the dual-cell (Kranz) paradigm. Annual Review of Plant Biology 55, 173–196.

Edwards G.E. and Ku M.S.B. 1987. The biochemistry of C_3–C_4 intermediates. Pp. 275–325 in 'The biochemistry of plants', ed. by M.D. Hatch and N.K. Boardman. Academic Press, Inc: New York.

Evans J.R., Kaldenhoff R., Genty B. and Terashima I. 2009. Resistances along the CO_2 diffusion pathway inside leaves. Journal of Experimental Botany 60, 2235–2248.

Evans J.R. and von Caemmerer S. 1996. Carbon dioxide diffusion inside leaves. Plant Physiology, 110, 339–346.

Flexas J., Ribas-Carbo M., Hanson D.T., Bota J., Otto B., Cifre J., McDowell N., Medrano H. and Kaldenhoff R. 2006. Tobacco aquaporin NtAQP1 is involved in mesophyll conductance to CO_2 in vivo. Plant Journal 48, 427–439.

Furbank R.T., Chitty J.A., Jenkins C.L.D., Taylor W.C., Trevanion S.J., von Caemmerer S. and Ashton A.R. 1997. Genetic manipulation of key photosynthetic enzymes in the C_4 plant *Flaveria bidentis*. Australian Journal of Plant Physiology 24, 477–485.

Furbank R.T., von Caemmerer S., Sheehy J. and Edwards G.E. 2009. C_4 rice: a challenge for plant phenomics. Functional Plant Biology 36, 845–856.

Leegood R.C. 2002. C_4 photosynthesis: principles of CO_2 concentration and prospects for its introduction into C_3 plants. Journal of Experimental Botany 53, 581–590.

Monson R.K., Edwards G.E. and Ku M.S.B. 1984. C_3–C_4 intermediate photosynthesis in plants. BioScience 34, 563–574.

Monson R.K. and Moore B.D. 1989. On the significance of C_3–C_4 intermediate photosynthesis to the evolution of C_4 photosynthesis. Plant, Cell and Environment 12, 689–699.

Price G.D., Badger M.R., Woodger F.J. and Long B.M. 2008. Advances in understanding the cyanobacterial CO_2-concentrating-mechanism (CCM): functional components, C_i transporters, diversity, genetic regulation and prospects for engineering into plants. Journal of Experimental Botany 59, 1441–1461.

Quick P., Sheehy J. and Ferrer A. 2013. Potential for enhanced photosynthetic efficiency to increase yield potential in C_3 crops by the addition of C_4 photosynthetic pathways. In 'Applying photosynthesis research to improvement of food crops', ed. by J.E. Gready, S.A. Dwyer and J.R Evans. ACIAR Proceedings No. 140, 36–42. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Rawsthorne S. and Bauwe H. 1998. C_3–C_4 intermediate photosynthesis. Pp. 150–162 in 'Photosynthesis. A comprehensive treatise', ed. by A.S. Raghavendra. Cambridge University Press: Cambridge.

Sage R.F. 2004. The evolution of C_4 photosynthesis. New Phytologist 161, 341–370.

Sheehy J., Mitchell A.K. and Hardy B. 2007. Reconfiguring the rice plant's photosynthetic pathway. IRRI: Los Baños, Philippines.

Spalding M.H. 2008. Microalgal carbon-dioxide-concentrating mechanisms: *Chlamydomonas* inorganic carbon transporters. Journal of Experimental Botany 59, 1463–1473.

Taniguchi Y., Ohkawa H., Masumoto C., Fukuda T., Tamai T., Lee K., Sudoh S., Tsuchida H., Sasaki H., Fukayama H. and Miyao M. 2008. Overproduction of C_4 photosynthetic enzymes in transgenic rice plants: an approach to introduce the C_4-like photosynthetic pathway into rice. Journal of Experimental Botany, 59, 1799–1809.

Uehlein N., Otto B., Hanson D.T., Fischer M., McDowell N. and Kaldenhoff R. 2008. Function of *Nicotiana tabacum* aquaporins as chloroplast gas pores challenges the concept of membrane CO_2 permeability. Plant Cell 20, 648–657.

von Caemmerer S. 1989. A model of photosynthetic CO_2 assimilation and carbon-isotope discrimination in leaves of certain C_3–C_4 intermediates. Planta 178, 463–474.

von Caemmerer S. 2000. Biochemical models of leaf photosynthesis (vol. 2). CSIRO Publishing: Melbourne.

von Caemmerer S. 2003. C_4 photosynthesis in a single C_3 cell is theoretically inefficient but may ameliorate internal CO_2 diffusion limitations of C_3 leaves. Plant Cell and Environment 26, 1191–1197.

von Caemmerer S., Evans J.R., Cousins A.B., Badger M.R. and Furbank R.T. 2007. C_4 photosynthesis and CO_2 diffusion. In 'Reconfiguring the rice plant's photosynthetic pathway', ed. by J.E. Sheehy, P.L. Mitchell and B. Hardy. IRRI: Los Baños, Philippines.

von Caemmerer S. and Furbank R.T. 1999. Modeling of C_4 photosynthesis. Pp. 169–207 in 'C_4 plant biology', ed. by R.F. Sage and R. Monson. Academic Press: San Diego, USA.

von Caemmerer S. and Furbank R.T. 2003. The C_4 pathway: an efficient CO_2 pump. Photosynthesis Research 77, 191–207.

Woodger F.J., Bryant D.A. and Price G.D. 2007. Transcriptional regulation of the CO_2-concentrating mechanism in a euryhaline, coastal marine cyanobacterium, *Synechococcus* sp strain PCC 7002: Role of NdhR/CcmR. Journal of Bacteriology 189, 3335–3347.

Zhu X.G., Long S.P. and Ort D.R. 2008. What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? Current Opinion in Biotechnology 19, 153–159.

Additional references

Price G.D., Badger M.R. and von Caemmerer S. 2011. The prospect of using cyanobacterial bicarbonate transporters to improve leaf photosynthesis in C_3 crop plants. Plant Physiology 155, 20–26.

Price G.D. 2011. Inorganic carbon transporters of the cyanobacterial CO_2 concentrating mechanism. Photosynthesis Research 109, 47–57.

Price G.D., Pengelly J.J.L., Forster B., Du J., Whitney S.M, von Caemmerer S. et al. 2012. The cyanobacterial CCM as a source of genes for improving photosynthetic CO_2 fixation in crop species. Journal of Experimental Botany 64, 753–768.

Enhancing Rubisco activity at higher temperatures by re-engineering Rubisco activase

Michael E. Salvucci[1]

Summary

This paper discusses the possibility of increasing plant performance under moderate heat stress by improving the thermal stability of Rubisco activase.

- The research is driven by the observation that photosynthesis is acutely sensitive to inhibition by moderate heat stress and that this inhibition can cause a significant reduction in grain yield. Data from several studies show that recent increases in global surface temperatures have already had a negative impact on crop yield and more severe reductions are predicted under even the most conservative climate warming scenario.
- Inhibition of net photosynthesis by moderate heat stress correlates with a decrease in the activation state of Rubisco. At elevated temperatures, processes that inactivate Rubisco (i.e. catalytic misfire and decarbamylation) accelerate while the rate of reactivation of Rubisco by the chaperone, Rubisco activase, decreases.
- Inhibition of Rubisco activase activity occurs at elevated temperatures and is a consequence of: (1) the inherent thermal instability of the activase protein; (2) heat-induced changes in the chloroplast environment that reduce the activity and/or thermal stability of Rubisco activase; or (3) a combination of the two.
- Natural and engineered variations in Rubisco activase thermotolerance provide evidence that improvements in the thermal stability of Rubisco activase can lead to better photosynthetic performance under moderate heat stress.
- Gaps still exist in our understanding of the role of Rubisco activase in the inhibition of photosynthesis by moderate heat stress, including questions about the mechanism for deactivation of Rubisco (catalytic misfire or decarbamylation) and the mechanistic basis for thermal inactivation of Rubisco activase.
- Strategies for improving photosynthetic performance under moderate heat stress in crop plants include: (1) increasing the thermotolerance of activase; (2) modifying Rubisco to reduce catalytic misfire; and (3) stabilising activase during episodes of heat stress.

Introduction

This section addresses how heat stress reduces productivity and affects photosynthesis, against the backdrop of global climate change.

Plant productivity and grain yield

Heat stress, even of moderate intensity and/or duration, reduces plant productivity with a significant reduction in harvestable yield (Lobell and Asner 2003; Lobell and Field 2007). While vegetative growth is reduced by elevated temperatures, episodes of heat stress occur more commonly during the mid to late stages of the plant life cycle, during flowering, seed-set and grain-fill. Thus, heat stress is usually encountered and therefore has its greatest effect during the

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reproductive stages of plant development when the harvestable yield is generally produced. Photosynthesis supplies carbohydrate for the developing reproductive structures of the plant, as well as for maintenance of the vegetative portions. Consequently, inhibition of photosynthesis during reproductive development has a direct and negative consequence on yield (Wang et al. 2006). The effect is most obvious during grain-fill when recently fixed carbon is transported directly from source leaves to the developing sinks. However, even pollen production and viability, which are both acutely sensitive to inhibition by high temperature (Satake 1995; Kakani et al. 2005), have a strong reliance on photosynthesis since the carbohydrate content of pollen grains affects their viability during heat stress (Firon et al. 2006).

Heat stress and photosynthesis

The impact of abiotic stress on plant processes depends on the intensity and duration of the stress (Berry and Björkman 1980; Larcher 1995). Photosynthesis has a broad temperature optimum, above which rates decrease with increasing temperature (Berry and Björkman 1980). For a plant species, the temperature optimum for photosynthesis generally corresponds to the prevailing high temperature during the growing season, whereas temperatures just a few degrees higher are supra-optimal. In contrast, the temperature optimum for dark respiration generally exceeds the highest temperatures encountered during the day or night (Bernacchi et al. 2001).

From practical and mechanistic points of view, it is important to distinguish between moderate and severe heat stress. Moderate heat stress is experienced 1–10°C above the thermal optimum and is characterised by a readily reversible effect on photosynthesis (Berry and Björkman 1980; Weis 1981). In contrast, severe heat stress occurs at higher temperatures and the damage to photosynthesis persists even after the stress subsides. In the natural environment, moderate heat stress is encountered much more frequently than severe heat stress. In fact, because of plants' ability to cool by transpiring H_2O, severe heat stress is probably rare for crop plants except in tropical or subtropical environments or during prolonged drought. Mechanistically, moderate heat stress involves just a few targets (i.e. weak links) that, if modified, would improve photosynthetic performance under heat stress. In contrast, severe heat stress affects myriad biochemical processes, some of which are permanently damaged and require replacement by de novo protein or lipid synthesis.

Global climate change

Increases in global temperatures over the past few decades have reduced grain yield (Lobell and Asner 2003; Lobell and Field 2007). That the impact has been negative indicates that the 'fertilising' effect of elevated CO_2 has not yet offset the inhibitory effects of elevated temperatures and suggests that a downward trend in yield will continue as temperatures continue to increase. It is well known that stomatal closure occurs with elevated CO_2 concentration, reducing transpiration, the driving force of leaf cooling. The collateral effect of elevated CO_2 on leaf temperature indicates that maintaining world food production requires modifications to crop plants that optimise their performance in a warmer and more CO_2-enriched world. For example, a recent analysis of maize, soybean and cotton production in the USA predicted that the yields of these crops, if grown in their current locations, will decrease by 30–60% before the end of the century under the slowest warming scenario (Schlenker and Roberts 2009).

Inhibition of photosynthesis by moderate heat stress

Response of net photosynthesis to elevated leaf temperature

It is widely accepted that Rubisco limits the rate of net photosynthesis under conditions of high light and ambient and sub-ambient CO_2 concentrations (Farquhar et al. 1980; Quick et al. 1991). Figure 1 compares the measured response of net photosynthesis to leaf temperature to the response calculated from the kinetic properties of Rubisco (Salvucci and Crafts-Brandner 2004a). The calculated rate increases with temperature because of higher rates of catalytic turnover by Rubisco, which offset the increased rates of photorespiration (Table 1). The difference between the calculated and measured rates represents the extent of inhibition of photosynthesis by high temperature from inhibition of one or more of the partial reactions.

Partial reactions of photosynthesis

The rates of biological processes increase with temperature to an optimum and then decrease as the

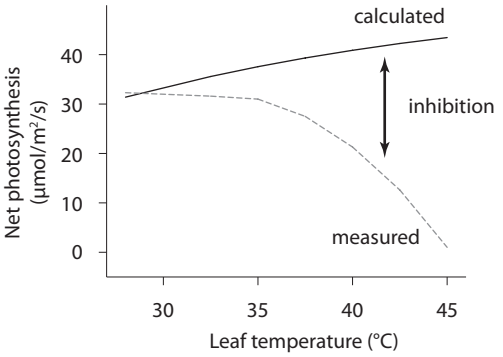


Figure 1. Response of net photosynthesis to elevated leaf temperature in air. The response of cotton leaf photosynthesis to temperature was measured directly and was calculated assuming a limitation by Rubisco. The difference between the calculated and measured rates represents the extent of inhibition of photosynthesis.

enzymatic proteins that catalyse the various steps in the process become inactivated because of loss of structural integrity (protein denaturation) (Somero 1995). Rates of photosynthesis are determined by: (1) 'light reactions' of photosynthesis, which include light harvesting, electron transport, synthesis of ATP and NADPH for the Calvin cycle; (2) CO_2 fixation catalysed by Rubisco; and (3) Calvin cycle reactions that regenerate the sugar–phosphate substrate, ribulose-1,5-bisphosphate (RuBP) (Figure 2). It is likely that the three processes exhibit different temperature optimums because of differences in the temperature responses of their individual partial reactions.

Three lines of evidence indicate that Rubisco is the early target of moderate heat stress. Under moderate heat stress: (1) Rubisco deactivates, correlating with the inhibition of net photosynthesis; (2) net photosynthesis can be increased by increasing the CO_2 concentration even under non-photorespiratory conditions (Wise et al. 2004; Yamori et al. 2005); and (3) the ratio of RuBP to 3-phosphoglycerate (PGA) increases (Weis 1981; Kobza and Edwards 1987). These results indicate that the capacity for RuBP regeneration is in excess under moderate heat stress, suggesting limitation by Rubisco.

Rubisco activation—early target of heat stress

Although there is some controversy about the precise cause (Sage et al. 2008), clear evidence exists for inactivation of Rubisco under very moderate heat stress (reviewed in Salvucci and Crafts-Brandner 2004a). Inhibition of Rubisco activase activity coupled with faster rates of inhibitor formation and possibly decarbamylation lead to inactivation of Rubisco by the mechanism shown in Figure 3 (see also Salvucci and Crafts-Brandner 2004a,b). Although the rate of catalysis by Rubisco increases with temperature because of the high thermal stability of Rubisco (Crafts-Brandner and Salvucci 2000; Salvucci and Crafts–Brandner 2004c), a loss of available sites for catalysis (i.e. inactivation of Rubisco) leads to a net reduction in CO_2 fixation when RuBP resupply is non-limiting (e.g. under high irradiance). Inhibition of Rubisco activase activity has been attributed to an inherently low temperature optimum for the enzyme (Salvucci and Crafts-Brandner 2004c), which is related to an acute thermal instability of the protein

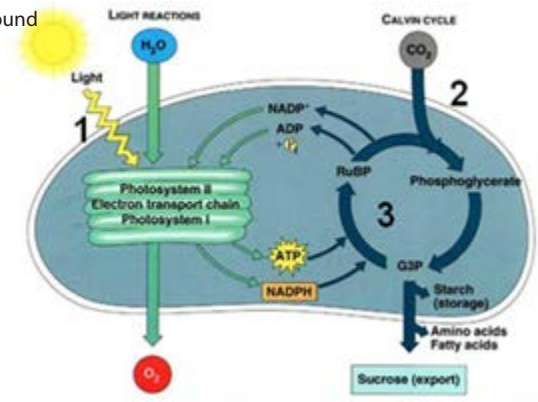
Table 1. Effect of elevated temperature on factors influencing Rubisco activity and their net effect on photosynthesis under physiological conditions

Factor	Response to high temperature	Effect on photosynthesis
CO_2 solubility	Decreases	Decrease
CO_2/O_2 specificity	Decreases	Decrease
k_{cat}	Large increase up through 50°C	Increase
K_C	Increases	Decrease
Inhibitor production (catalytic misfire)	Faster rates	Decrease
Inhibitor binding	Less tight[a]	Increase
Carbamylation	Decreases[a]	Decrease
RuBP regeneration	Faster rates	Increase
Rubisco activase activity	Severely inhibited	Decrease

[a] depends on Mg^{2+} concentration
k_{cat} = Rubisco catalytic rate; K_C = Michaelis–Menten constant for carboxylation by Rubisco

1. Light reactions

- primarily membrane-bound or associated reactions
- provide high energy equivalents for RuBP regeneration
- inhibited by *severe* heat stress



2. Rubisco

- soluble enzyme
- catalyses CO_2 or O_2 fixation
- activation state inhibited by *moderate* heat stress

3. Calvin cycle reactions

- catalysed by soluble enzymes
- regenerates RuBP and produces carbohydrate for export
- sensitivity to heat stress *unknown*

Figure 2. Chloroplast showing the reactions of photosynthesis and their characteristics, function and response to elevated temperature

(Salvucci et al. 2001). Others have suggested that inhibition of Rubisco activase activity is a secondary consequence of heat-induced changes in the chloroplast environment (i.e. stromal oxidation, metabolite levels and energy charge), which influence the activity and/or thermal stability of Rubisco activase (Schrader et al. 2007; Sage et al. 2008).

Tolerance to moderate heat stress

Evidence that reducing the extent of Rubisco inactivation through improvements in Rubisco activase improves tolerance to moderate heat stress is as follows:

- Natural variations:
  - Species from contrasting thermal environments (desert to Antarctic) have activases with different thermal stabilities, but the temperature response of their Rubiscos was remarkably similar (Salvucci and Crafts-Brandner 2004c).
  - Rubisco activase and net photosynthesis in a temperate poplar species was more stable under moderate heat stress than activase from an alpine poplar species (M.I. Hozain, M.E. Salvucci, M. Fokar and A.S. Holaday, unpublished data).
  - Maple genotypes from Florida and Minnesota differ in the response of maximum rates of carboxylation by Rubisco (V_{Cmax}) to temperature and the more thermal stable Florida genotypes had a greater concentration of the long activase isoform (Weston et al. 2007).
- Transgenic plants not targeted for activase ('unintended improvement in activase'):
  - Transgenic tobacco plants that produce glycine betaine in the chloroplast have improved heat tolerance through protection of Rubisco activase against denaturation (Yang et al. 2005).
  - Transgenic rice plants that overexpress sedoheptulose biphosphatase in the chloroplast have improved heat tolerance through protection of Rubisco activase against denaturation by an unknown mechanism (Feng et al. 2007).
- Transgenic plants with improved activase:
  - Transgenic *Arabidopsis* were made more heat tolerant by introducing a more heat stable activase produced by DNA shuffling. Compared with wild-type, the number of siliques per plant under moderate heat stress was two to eight times higher in the transgenic plants (Kurek et al. 2007).
  - Transgenic *Arabidopsis* were rendered more thermally stable by introducing a more heat stable chimeric activase made from tobacco activase with the sensor II region from *Arabidopsis*. Compared with wild-type, the total seed mass under moderate heat stress was four times that in the transgenic plants (Kumar et al. 2009).

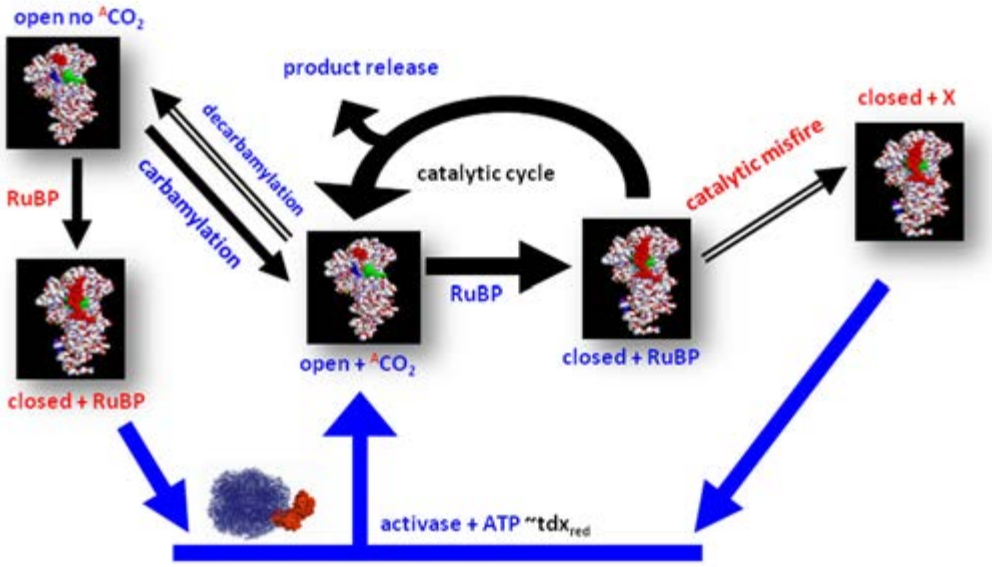


Figure 3. Important features of the mechanism of Rubisco activase. Rubisco activase (lower blue lines) alters the conformation of dead-end forms of Rubisco (closed conformation containing bound xylulose 1,5-bisphosphate (XuBP) or RuBP), restoring activity to inactive sites by facilitating release of bound inhibitors (i.e. 'opening' the sites). Under moderate heat stress, activase activity is severely impaired, while the rates of catalytic misfire and decarbamylation increase (double-line arrows). The molecular images show Rubisco large subunits in either the open conformation (active-site is solvent accessible) or closed conformation (active-site is shielded from solvent by loop 6 in green and the C-terminus in red).

Gaps in knowledge

This identifies gaps in knowledge about Rubisco and Rubisco activase as targets of moderate heat stress.

Rubisco

Rubisco-related questions fall into two groups:

1. Importance of catalytic misfire (fall-over) to thermal inactivation:
   – To what extent does catalytic misfire occur in vivo under moderate heat stress? What are the products that inactivate Rubisco under heat stress? Are they enediol- or oxygenase intermediate-derived?
   – How do physiological levels of Mg^{2+}, CO_2 and metabolites, and pH, for example, affect inactivation of Rubisco by catalytic misfire?
   – Do natural inhibitors like 2-carboxy-d-arabinitol 1-phosphate (CA1P) have any role in inhibiting Rubisco under moderate heat stress?
2. Importance of decarbamylation to thermal inactivation:
   – How do physiological levels of Mg^{2+}, CO_2 and metabolites, and pH, for example, affect the carbamylation state?
   – To what extent is inhibitory RuBP binding to the catalytic site affected by temperature?

Activase

Activase-related questions fall into three groups:

1. Structure and mechanism of activase:
   – What is the three-dimensional structure and mechanism of activase?
2. Mechanistic basis for thermal instability of activase:
   – Why is activase so unstable? Does flexibility compromise structural integrity?
   – What factors in the chloroplast (e.g. chaperones, Mg^{2+}, adenosine-5'-triphosphate–adenosine-5'-diphosphate (ATP/ADP) ratio, Rubisco, metabolites and a reducing environment) affect

the activity and thermal stability of activase during heat stress?
   – Do changes in activase amounts, structure or isoforms occur during thermal acclimation?
   – To what extent do chaperonins like cpn60 (Salvucci 2008) stabilise activase during episodes of heat stress?
   – Do species differ in the extent of activase vs. non-activase limitations above the thermal optimum?
3. Function of the two isoforms and their influence on thermal stability:
   – Does moderate heat stress inhibit Rubisco activase through perturbation of its redox regulation?
   – Does overexpression of the longer isoform improve photosynthesis under non-stress conditions as reported (Wu et al. 2007)? What happens under moderate heat stress?

Other questions

Further questions not related directly to either activase or Rubisco are:

- What is the effect of temperature on carbohydrate transport, particularly phloem loading and unloading?
- To what extent is pollen sterility related to decreased carbohydrate supply from lower photosynthetic activity and increased respiration in the leaves?

Strategies for improving crop productivity

Strategies for improving crop productivity under moderate heat stress by modifying Rubisco and/or activase are:

- Improve the thermal stability of Rubisco activase (already documented for *Arabidopsis*). This strategy might require replacing endogenous activase genes in a crop plant or synthesising improved activase behind a heat shock promoter.
- Reduce inhibition of Rubisco by catalytic misfire by making the higher plant enzyme more *Synechoccus*-like in inhibitor formation or more *Rhodospirillum rubrum*-like for inhibitor binding (Pearce 2006), but with higher plant-type substrate specificity.
- Prevent decarbamylation by elevating CO_2 at the site of Rubisco (i.e. by a C_4 or inorganic carbon concentrating mechanism). A collateral effect would be faster turnover, particularly at higher temperatures, especially if combined with less misfire or greater reactivation capacity by activase.
- Stabilise activase through episodes of heat shock by improving interactions with chaperones and hasten activase recovery by supplementing the amount through de novo synthesis.
- Modify the composition of the thylakoid membranes to prevent ion leakage. This strategy was attempted in *Arabidopsis* without effect (Kim and Portis 2005), but leakage seems to occur (Zhang et al. 2009) and is thought to negatively impact Rubisco activation.

Acknowledgments

I thank Drs A.R. Portis, Jr., R. Sage, T. Sharkey, W. Yamori, S. von Caemmerer, A. Leakey, R. Spreitzer, A. Kumar, R. Wachter, S.A. Dwyer, J.R. Evans and A.S. Holaday for their suggestions and opinions.

References

Bernacchi C.J., Singsaas E.L., Pimentel C., Portis A.R. Jr and Long S.P. 2001. Improved temperature response functions for models of Rubisco-limited photosynthesis. Plant, Cell and Environment 24, 253–259.

Berry J. and Björkman O. 1980. Photosynthetic response and adaptation to temperature in higher plants. Annual Review of Plant Physiology 31, 491–543.

Crafts-Brandner S.J. and Salvucci M.E. 2000. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. Proceedings of the National Academy of Sciences USA 97, 13430–13435.

Farquhar G.D., von Caemmerer S. and Berry J.A. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149, 78–90.

Feng L., Wang K., Li Y., Tan Y., Kong J., Li H., Li Y. and Zhu Y. 2007. Overexpression of SBPase enhances photosynthesis against high temperature stress in transgenic rice plants. Plant Cell Reports 26, 1635–1646.

Firon N., Shaked R., Peet M.M., Pharr D.M., Zamski E., Rosenfeld K., Althan L. and Pressman E. 2006. Pollen grains of heat tolerant tomato cultivars retain higher carbohydrate concentration under heat stress conditions. Scientia Horticulturae 109, 212–217.

Kakani V.G., Reddy K.R., Koti S., Wallace T.P., Prasad P.V.V., Reddy V.R. and Zhao D. 2005. Differences in in vitro pollen germination and pollen tube growth of cotton cultivars in response to high temperature. Annals of Botany 96, 59–67.

Kim K. and Portis A.R. Jr 2005. Temperature dependence of photosynthesis in *Arabidopsis* plants with modifications

in Rubisco activase and membrane fluidity. Plant Cell Physiology 46, 522–530.
Kobza J. and Edwards G.E. 1987. Influences of leaf temperature on photosynthetic carbon metabolism in wheat. Plant Physiology 83, 69–74.
Kumar A., Li C. and Portis A.R. Jr 2009. *Arabidopsis thaliana* expressing a thermostable chimeric Rubisco activase exhibits enhanced growth and higher rates of photosynthesis at moderately high temperatures. Photosynthesis Research 100, 143–153.
Kurek I., Chang T.K., Bertain S.M., Madrigal A., Liu L., Lassner M. and Zhu G. 2007. Enhanced thermostability of *Arabidopsis* Rubisco activase improves photosynthesis and growth rates under moderate heat stress. Plant Cell 19, 3230–3241.
Larcher W. 1995. Stress due to extreme temperature. Pp. 340–378 in 'Physiological plant ecology: ecophysiology and stress physiology of functional groups', 3rd edition, ed. by W. Larcher. Springer-Verlag: Berlin.
Lobell D.B. and Asner G.P. 2003. Climate and management contributions to recent trends in U.S. agricultural yields. Science 229, 1032.
Lobell D.B. and Field C.B. 2007. Global scale climate-crop yield relationships and the impacts of recent warming. Environmental Research Letters 2, 014002. Available at URL: iopscience.iop.org/1748-9326/2/1/014002/.
Pearce F.G. 2006. Catalytic by-product formation and ligand binding by ribulose bisphosphate carboxylases from different phylogenies. Biochemical Journal 399, 525–534.
Quick W.P., Schurr U., Scheibe R., Schulze E-D., Rodermel S.R., Bogorad L. and Stitt M. 1991. Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with "antisense" *rbcS* - I. Impact on photosynthesis in ambient growth conditions. Planta 183, 542–554.
Sage R.F., Way D.A. and Kubien D.S. 2008. Rubisco, Rubisco activase, and global climate change. Journal of Experimental Botany 59, 1581–1595.
Salvucci M.E. 2008. Association of Rubisco activase with chaporonin 60-β: a possible mechanism for protecting photosynthesis during heat stress. Journal of Experimental Botany 59, 1923–1933.
Salvucci M.E. and Crafts-Brandner S.J. 2004a. Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. Physiologia Plantarum 120, 179–186.
Salvucci M.E. and Crafts-Brandner S.J. 2004b. Mechanism for deactivation of Rubisco under moderate heat stress. Physiologia Plantarum 122, 513–519.
Salvucci M.E. and Crafts-Brandner S.J. 2004c. Relationship between the heat tolerance of photosynthesis and the thermal stability of Rubisco activase in plants from contrasting thermal environments. Plant Physiology 134, 1460–1470.
Salvucci M.E., Osteryoung K.W., Crafts-Brandner S.J. and Vierling E. 2001. Exceptional sensitivity of Rubisco activase to thermal denaturation in vivo and in vitro. Plant Physiology 127, 1053–1064.
Satake T. 1995. High temperature injury. Pp. 805–812 in 'Science of the rice plant, Vol 2. Physiology', ed. by T. Matsuo, K. Kumazawa, R. Ishii and H. Hirata. Food and Agricultural Policy Research Center: Tokyo.
Schlenker W. and Roberts M.J. 2009. Nonlinear temperature effects indicate severe damages to U.S. crop yields under climate change. Proceedings of the National Academy of Science USA 106, 15594–15598.
Schrader S.M., Kleinbeck K.R. and Sharkey T.D. 2007. Rapid heating of intact leaves reveals initial effects of stromal oxidation on photosynthesis. Plant, Cell and Environment 30, 671–678.
Somero G.N. 1995. Proteins and temperature. Annual Review of Physiology 57, 43–68.
Wang J., Gan Y.T., Clarke F. and McDonald C.L. 2006. Response of chickpea yield to high temperature stress during reproductive development. Crop Science 46, 2171–2178.
Weis E. 1981. Reversible heat-inactivation of the Calvin cycle: a possible mechanism of the temperature regulation of photosynthesis. Planta 151, 33–39.
Weston D.J., Bauerle W.L., Swire-Clark G.A., Moore B. and Baird W.V. 2007. Characterization of rubisco activase from thermally contrasting genotypes of *Acer rubrum* (Aceraceae). American Journal of Botany 94, 926–934.
Wise R.R., Olson A.J., Schrader S.M. and Sharkey T.D. 2004. Electron transport is the functional limitation of photosynthesis in field-grown Pima cotton plants at high temperature. Plant, Cell and Environment 27, 717–724.
Wu H.R., Li L.B., Jing Y.X. and Kuang T.Y. 2007. Over- and anti-sense expressions of the large isoform of ribulose-1,5-bisphosphate carboxylase/oxygenase activase gene in *Oryza sativa* affect the photosynthetic capacity. Photosynthetica 45, 194–201.
Yamori W., Noguchi K. and Terashima I. 2005. Temperature acclimation of photosynthesis in spinach leaves: analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions. Plant, Cell and Environment 28, 536–547.
Yang X., Liang Z. and Lu C. 2005. Genetic engineering of the biosynthesis of glycinebetaine enhances photosynthesis against high temperature stress in transgenic tobacco plants. Plant Physiology 138, 2299–2309.
Zhang R., Cruz J.A. and Kramer D.M., Magallanes-Lundback M.E., Dellapenna D., Sharkey T.D. 2009. Moderate heat stress reduces the pH component of the transthylakoid proton motive force in light-adapted, intact tobacco leaves. Plant, Cell and Environment 32, 1538–1547.

Additional references

Carmo–Silva A.E., Gore M.A., Andrade–Sanchez P., French A.N., Hunsaker D.J. and Salvucci M.E. 2012. Decreased CO_2 availability and inactivation of Rubisco limit

photosynthesis in cotton plants under heat and drought stress in the field. Environmental and Experimental Botany 83, 1–11.

Carmo–Silva A.E. and Salvucci M.E. 2012. The temperature response of CO_2 assimilation, photochemical activities and Rubisco activation in *Camelina sativa*, a potential bioenergy crop with limited capacity for acclimation to heat stress. Planta 236, 1433–1445.

Parry M.A.J., Reynolds M., Salvucci M.E., Raines C., Andralojc P.J. and Zhu X.G. et al. 2011. Raising yield potential of wheat: increasing photosynthetic capacity and efficiency. Journal of Experimental Botany 62, 453–467.

Scafaro A.P., Yamori W., Carmo–Silva A.E., Salvucci M.E., von Caemmerer S. and Atwell B.J. 2012. Rubisco activity is associated with photosynthetic thermo-tolerance in the wild rice *Oryza meridionalis*. Physiologia Plantarum 146, 99–109.

Yamori W., Masumoto C., Fukayama H. and Makino A. 2012. Rubisco activase is a key regulator of non steady-state photosynthesis at any leaf temperature and, to a lesser extent, of steady-state photosynthesis at high temperature. The Plant Journal 71, 871–880.

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