Preface

Rice displays a biphasic growth pattern: half the first phase of vegetative growth precedes the second phase of reproductive growth. The rates at which the phases proceed are strongly influenced by temperature, which largely accounts for different crop durations in temperate and tropical environments. The second phase begins when the rate of vegetative growth reaches a maximum and it reaches a maximum when the rate of growth of the vegetative phase falls to zero. During the first phase, full light interception is reached and the reservoir of nutrients for use in the second phase reaches a maximum. During the second phase of growth, the reservoirs in the vegetative portions of the crop are depleted and the second phase comes to a halt when the crop is mature: the time when most grains are filled and the fewest have been shed. Both empirical and theoretical investigations suggest that the maximum fraction of a crop's total biomass that can be grain is about 50%.

Solar energy captured in photosynthesis over the duration of a crop gives it the capacity to grow. The upper limit to crop biomass is determined by the laws of thermodynamics and mass conservation. At the limit, the total biomass is simply a function of the total quantity of solar energy captured and the efficiency with which that energy is made available for synthetic processes. Total solar energy absorption is largely a function of canopy architecture and crop duration. The efficiency of energy use is largely determined by photorespiration, dark respiration, and losses of biomass that occur owing to senescence. Canopy architecture is usually thought of in terms of leaf erectness and, given that plant breeders have selected for erectness over the past 30 years, little more can be gained in that direction. The opportunities for reducing dark respiration are very limited and senescence is essential in terms of recycling essential nutrients from the vegetative portions of the crop to the reproductive ones. There are many evolutionary examples of plants that have eliminated photorespiration by concentrating CO_2 around the photosynthetic enzyme Rubisco using a four-carbon acid (C_4) cycle. Plants such as rice that do not have a concentrating CO_2 mechanism fix CO_2 into three carbon acids (C_3 plants); their photosynthetic rates in hot environments are about half that of C_4 plants. C_4 plants have double the water-use efficiency of C_3 plants, and use about 40% less nitrogen to achieve 50% higher yields. Evolution

CHARTING NEW PATHWAYS TO C_4 RICE

has made it clear that photorespiration can be eliminated; therefore, it is the obvious candidate for work aimed at significant increases in yields.

The repeated evolution of C_4 photosynthesis indicates that it should be feasible to create C_4 rice plants by engineering C_4 genes into C_3 rice and replicating strong selection pressure for C_4 traits that we think exist in nature. The development of the C_4 system can be seen as an addition to the C_3 system and it is now clear that the C_3 and C_4 syndromes are not as rigidly separated as was first thought. The enzymes that are prominent in the C_4 pathway also exist in C_3 leaves although with very low activity. More surprisingly, there is a well-developed C_4 pathway in certain locations in C_3 plants: in the green tissue around vascular bundles, and probably in rice spikelets. In the opposite direction, maize, a thoroughly C_4 plant, has patches of C_3 tissue wherever a mesophyll cell is not adjacent to a bundle sheath cell, particularly in leaf sheaths and husk leaves. Some of the wild relatives of rice have C_4-like anatomical features and others may have CO_2 compensation points usually associated with C_3-C_4 intermediates. When maize C_4 genes are inserted in rice, they work; the rice genome has been sequenced and sequencing of the maize genome is nearing completion. A large number of genetic resources are available for use in screening programs aimed at detecting genes associated with C_4-ness: 6,000 wild relatives and 500,000 rice mutants. It has been suggested that *Arabidopsis* (C_3) can be used as a test system for transferral of genes from its closest C_4 relative, *Cleome gynandra*. The advantages of this are that all the knowledge of *Arabidopsis* can be used and *Cleome* has a short life cycle.

There are, of course, differences of opinion (contrasting hypotheses) between scientists as to which form of C_4 photosynthesis (single-cell and dual-cell systems) can be achieved most rapidly in rice and the ultimate effectiveness of the different forms in delivering significant increases in yield. This book explores those differences, but begins with a broad perspective of the economic problems surrounding rice and the potential impact on the poor of failing to contain upward pressure on food prices. It continues setting the scene by describing how the rice crop works and the consequences of supercharging photosynthesis.

In the second section of the book, Jane Langdale and her coauthors describe progress in various genetic approaches to understanding chloroplast development and then speculate on solutions to solving the problem of how to convert C_3 systems to C_4 ones.

The chapter of Richard Leegood examines metabolite transport and some of the structural and physiological changes that might be required when adding C_4 systems to C_3 ones.

Susanne von Caemmerer and her coauthors use models of diffusion to explore the effects of leaf anatomy and leakiness of cells on the efficiencies of the two-cell and single-cell forms of C_4 photosynthesis. Finally, they turn their attention to the anatomical and physiological requirements for C_4 rice.

Julian Hibberd advocates dual-track and fast-track approaches to the challenge of producing C_4 rice by inserting genes from *Cleome gynandra* into *Arabidopsis thaliana*.

John Evans and coauthors address the question of how the correct amounts of NADPH (biochemical reducing power) and ATP are provided in C_4 plants by two sorts of chloroplast in two types of cell. They also point out briefly the advantages of the single-cell C_4 system and identify its particular weakness (carbon dioxide leakage from the chloroplast).

The chapter by Rowan and Tammy Sage is a tour de force. It begins by identifying the essential features of a C_4 system and then examines its diversity in *Flaveria* before turning to an examination of the evolutionary factors critical to the emergence of C_4 systems. The chapter ends with a skeleton proposal on how to combine biotechnology and screening to produce C_4 plants from C_3 rice.

The genus *Flaveria* contains not only C_3 and C_4 species, but also several intermediate C_3-C_4 species. Udo Gowik and Peter Westhoff discuss the use of *Flaveria* as a model system for studying the evolution of genes involved in C_4 photosynthesis and the subtle differences between C_3 and C_4 orthologous genes.

D.M. Jiao speculates on ways a C_4 rice could be constructed by various genetic engineering approaches.

The third section of the book is devoted to an examination of single-cell C_4 systems, how they work, and what they might deliver if engineered into rice.

Jim Burnell opens this section by reviewing the early history of attempts to increase the rate of photosynthesis by manipulating the expression of foreign genes and moves on to suggest critical issues that might be examined.

Gerry Edwards and his coauthors briefly review critical features of C_4 plants, paying particular attention to chloroplast position and differentiation in Kranz types and single-cell aquatic types. They provide a more detailed description of single-cell terrestrial C_4 mechanisms and finally suggest some single-cell models for C_4 rice.

John Raven and coauthors describe lessons relevant to C_4 to be learned from diatoms. They provide evidence of high-capacity, low-leakage carbon-concentrating mechanisms in single cells and conclude that single-cell C_4 is a viable aim in engineering C_4 rice.

Continuing with the single-cell C_4 theme, George Bowes and his colleagues describe their work with *Hydrilla verticillata*, an aquatic monocot that operates a facultative, single-cell C_4 system. Their studies suggest that, to design a single-cell C_4 rice, transporter and permeability issues as well as the nuances of enzyme regulation need to be better understood.

Christoph Peterhänsel and his coauthors suggest a novel approach to improving photosynthesis by engineering a bypass of photorespiration in the chloroplast.

The fourth section of the book covers the background of C_4 rice and how it can be delivered.

C_4 physiology is a syndrome of interrelated developmental, anatomical, cellular, and biochemical traits that almost unavoidably must rely on regulatory networks. Tim Nelson and coauthors suggest that laser microdissection of cell types and microarray profiling can provide the comprehensive data for a systems biology approach to understanding differences between rice and C_4 leaf development.

Erik Murchie and Peter Horton draw on experiences of measuring rice photosynthesis in the field and suggest that acclimation to irradiance can result from a signal provided by mature leaves, but the nature of the signal is unknown. They also explore issues surrounding the use of nitrogen for photosynthesis in Rubisco and the conflicting demands for nitrogen in the form of protein in the grain.

D.S. Brar and J.M. Ramos discuss wild species of *Oryza* as an important reservoir of useful genes. Some of these genes have been introduced into indica and japonica rice for resistance to major diseases and insects and for tolerance of various abiotic stresses. It has been suggested that the wild types may contain aspects of C_4-ness and should be screened for anatomical, biochemical, and physiological features associated with the C_4 syndrome.

Parminder Virk and Shaobing Peng explore the consequences of inventing C_4 rice from a plant breeder's perspective. An early step would be to assess the effect of the C_4 syndrome on various agronomic traits such as resistance to pests, emergence of new pests, physical properties of the rice grains, and cooking and eating quality. Second, it would be important to evaluate the amount of expression of the syndrome in different genetic backgrounds and to identify the most promising transgenic event.

Philippe Hervé takes a genetic engineering approach and suggests that improved photosynthesis in rice can probably be achieved by engineering alleles involved in biochemical pathways and plant development. Another suggested strategy may consist of growing transgenic rice plants with C_4 features in different environments and screening for newly acquired C_4 features.

The fifth section leads the way into the formation of a C_4 Rice Consortium.

Richard Bruskiewich and Samart Wanchana deal with the role of bioinformatics in the construction of C_4 rice. They make general observations about sequenced genomes and describe a framework for gene discovery, before brainstorming on possible ways of using genomics information and bioinformatics to introduce C_4 photosynthesis into rice.

This project promises to be a universally important voyage of discovery about the most important of all plant mechanisms: photosynthesis. It will take a consortium of international institutions to make this a reality over the next 10 to 15 years. It is most encouraging that all the authors in this book have agreed to become founding members of a C_4 Rice Consortium. The next task is to build a long-term funding flow that is essential to sustaining research over the one and a half decades we estimate it will take to develop a fully functioning C_4 rice.

The book closes with a critical discussion and evaluation of the new pathways to C_4 rice. In it, all the authors highlighted important points and possibilities for success.