1 • Terrestrial photosynthesis in a changing environment

J. FLEXAS, F. LORETO AND H. MEDRANO

1.1. HISTORICAL PERSPECTIVE

The study of plant physiological responses to the environment (i.e., 'ecophysiology') has attracted researchers since early times, starting from Hales' proposal that plants took their nourishment from the surrounding air (Hales, 1727), through Charles Darwin's observations on leaf and chloroplast movements in response to external conditions and experimental manipulations (Darwin, 1881a,b; 1882), to Darwin's son Francis' early works on the relationship between transpiration and stomatal aperture (Darwin and Pertz, 1911; Darwin, 1916). From its very early re-foundations in modern times, plant ecophysiology has focused on photosynthesis – and transpiration, its undissociable process in terrestrial environments – as the most central physiological characteristic of plants changing in response to the environment. In parallel, considerable progress in the structural and biochemical basis of photosynthesis enabled improvement in the understanding of photosynthetic processes (Calvin and Benson, 1948), the integration of photosynthesis with transpiration and respiration and the view of photosynthesis as the basis for quantitative models of plant growth and crop production. Monsi and Saeki (1953) developed a theoretical frame to describe the distribution of light within plant communities, as Gaastra (1959) worked on photosynthesis in terms of gas exchange along a series of resistances in and out of leaves. Studies on leaf energy balance were initiated (Raschke, 1956; Gates, 1962) and later developed (Montieth, 1973), when portable infrared (IR) gas analysers for measuring photosynthesis became available (Bosian, 1960), allowing field campaigns of measuring photosynthesis in natural environments around the globe (Tranquilini, 1957; Lange *et al.*, 1969; Björkman *et al.*, 1972; Billings, 1973). In the late sixties and early seventies, the C_4 pathway was discovered (Hatch and Slack, 1966) as well as the oxygenase activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Ogren and Bowes, 1971), revealing a greater diversity of photosynthetic pathways than thought. Meanwhile, photosynthesis-based plant growth models were developed (Brouwer and de Wit, 1969; Penning de Vries *et al.* 1974). In the late seventies, an optimisation-theory model to explain stomatal behaviour linking photosynthesis to transpiration was developed by Cowan and Farquhar (1977), and the most widely used leaf photosynthesis model was presented (Farquhar *et al.*, 1980a). After those decades, the importance of photosynthesis and related processes (such as transpiration, respiration and growth) was such that, according to several authors, plant ecophysiology in the eighties was fully focused on photosynthesis-related subjects, such as energy and mass exchange (Mooney *et al.*, 1987; DeLucia *et al.*, 2001; see Fig. 1.1).

Over the last thirty years, the boost of 'molecular biology' studies, in particular addressing the plant's genome and functional genetics development, offered new tools to explore photosynthesis ecophysiology and evolution. The studies on plant ecophysiology in general, and photosynthetic response to the environment in particular, moved from an organismal focus to scales below and above the organism (DeLucia *et al.*, 2001; see Fig. 1.1). The new scenarios of climatic change challenged our capacity to predict plants, communities and global atmospheric changes in the near future. Studies on the ecophysiology of photosynthesis are now frequently backed by phylogenetics, molecular genetics and biochemical analysis, and serve as the basis to describe population dynamics and ecosystem processes (Fig. 1.1). Recent new directions in photosynthesis research include expanding current bi-dimensional gas-exchange models to tri-dimensional views (Verboven *et al.*, 2008; Kaiser, 2009; Tholen and Zhu, 2011), using systems biology approaches for the analysis of potential metabolic responses to the changing

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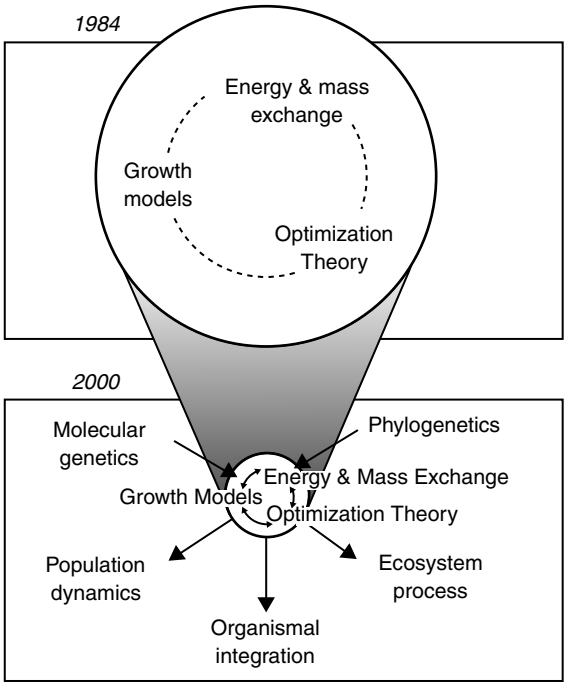

Fig. 1.1. Evolution of the focus of studies on plant ecophysiology from the 1980s to the current century, highlighting the importance of photosynthesis-related aspects, such as energy and mass exchange and growth (after DeLucia *et al.*, 2001).

environment (Luo *et al.*, 2009), and designing and developing both genetically modified plants with improved photosynthesis (Zhu *et al.*, 2010) and synthetic photosynthesis (Bar-Even *et al.*, 2010; Larom *et al.*, 2010). Despite many recent advances essential basic questions remain unresolved, such as which is the natural variation in plant photosynthesis (Flood *et al.*, 2011) or why is the photosynthetic process less energy efficient than human-built photovoltaic devices (Blankenship *et al.*, 2011). For these reasons, knowledge of photosynthesis is of general interest to researchers in plant biology, environmental sciences, agronomy or forestry, among others. Owing to this expansion, the response of photosynthesis to the environment is a very active area of research, producing more than a thousand publications per year. Over the last few years, up to four of the highest impact plant journals published Special Issues on this subject (Annals of Botany Vol. 103, Issue 4; Journal of Experimental Botany Vol. 60, Issue 8; Plant and Cell Physiology Vol. 50, Issue 4; Plant Physiology Vol. 155, Issue 1).

1.2. IMPORTANCE OF TERRESTRIAL PHOTOSYNTHESIS

The reasons why photosynthesis in terrestrial plants deserves so much attention are multiple. Perhaps the most obvious is the fact that photosynthesis has been the cornerstone of evolution of current life on earth, and is nowadays the process supporting most of the Earth's primary production. The world's human population has raised exponentially, overpassing 7 billion people, whereas changing patterns of land use have resulted in a reduced crop area. During the so-called 'Green Revolution', starting in the sixties, global crop productivity increased mostly because of better knowledge of plant nutrition, plant defence against pathogens and genetic improvement of plant structure and harvest index (i.e., the proportion of total plant biomass that is harvestable), rather than by improvements of photosynthesis and total plant biomass (Zhu *et al.*, 2010). However, an increased use of chemical fertilisers and pesticides are now known to have negative effects on the environment and ecological resources (Tilman *et al.*, 2002). The world population has now doubled since the 'Green Revolution' started, largely increasing the global demands for food. Most predictions suggest that such demand will double again by 2030 (Edgerton, 2009; Murchie *et al.*, 2009). Moreover, the demand for plant materials besides food has expanded for many years owing to the increasing importance of, for instance, plant-based biofuels (Edgerton, 2009). On the other side, the strategies implemented during the 'Green Revolution', which had led to steady increases in global cereal production from 1960 to 1990, appear now almost saturated, leading to only minor additional increases in global production (Tilman *et al.*, 2002). Overall, to meet the global demands for plant resources in the immediate future, only two options appear to be available (Edgerton, 2009): (1) expanding the area under production; and (2) improving productivity on existing farmland. Although these two options are not mutually exclusive, the second is particularly challenging as genetic improvement of plant structure and harvest index seems to have already achieved an optimum. Hence, improving productivity would require improving leaf photosynthesis and photosynthetic efficiency in the use of carbon, water and nutrients (Long *et al.*, 2006a; Peterhansel *et al.*, 2008; Murchie *et al.*, 2009; Zhu *et al.*, 2010).

Besides feeding humans, photosynthesis is of broad importance for many other reasons. For instance, the evolution of photosynthesis-related plant traits in response to the

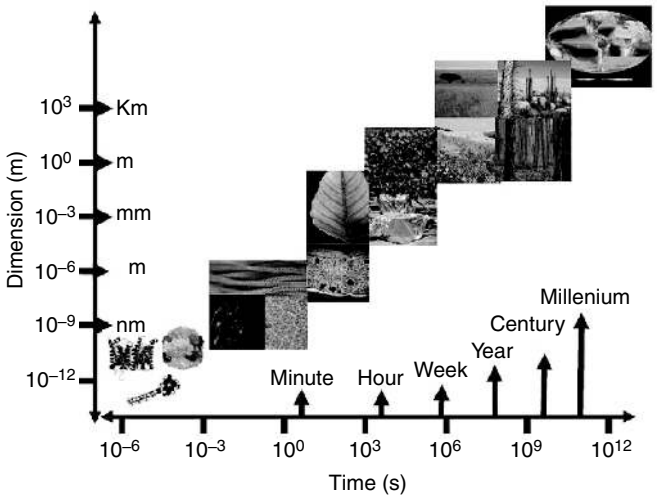

Fig. 1.2. Different time and dimension scales for photosynthetic events (modified from Osmond *et al.*, 2004).

changing conditions during the Earth's history is recognised to have started the oxygenic environment that allowed life as we know it now, and have contributed to shape the current physiognomy of vegetation and its diversity (Beerling *et al.*, 2001; Brodribb *et al.*, 2007; Franks and Farquhar, 2007; Sack *et al.*, 2008; Hohmann-Marriott and Blankenship, 2011). Photosynthesis is therefore an essential component of plant survival and species fitness (Athanasiou *et al.*, 2010; Donovan *et al.*, 2011; McDowell, 2011). Alternatively, terrestrial photosynthesis constitutes about half of the total Earth carbon sinks excluding the atmosphere (Canadell *et al.*, 2007; Beer *et al.*, 2010). Moreover, whereas the ocean sink strength is very controversial, estimations of the terrestrial sink, contributed to by photosynthesis, is rather solid (IPCC, 2007). Therefore, terrestrial photosynthesis is the main natural tool to counteract climate change and to contribute, together with transpiration (Hetherington and Woodward, 2003) and ecosystem carbon dynamics (Heimann and Reichstein, 2008), to plant-driven climate feedbacks (Bonan, 2008; Cao *et al.*, 2010; Rotenberg and Yakir, 2010). This is the reason why current arguments by the COP 15 at the Copenhagen meeting (http://en.cop15.dk/) have addressed issues other than forestation, which is still regarded as the main option to reduce CO_2 increase in the atmosphere. Moreover, because of the tight relationship between photosynthesis and transpiration, which results from the central role of stomata in both processes, photosynthesis is also pivotal for determining WUE (i.e., the amount of biomass obtained per unit water used). Owing to increasing scarcity of fresh water on a global scale (IPCC, 2007), improving water use in crop production through improving photosynthetic efficiency is a requisite for a sustainable agriculture (Rockström *et al.*, 2007; Morison *et al.*, 2008). But on the other hand, and against all these needs, there is ample evidence that human activities are acting in detriment of photosynthetic carbon fixation, delaying for example the carbon cycle of forests (Magnani *et al.*, 2007), or competing for the use of fertile soils by agro-forestry. Clearly, the development of a social consciousness will be required, in addition to research, to achieve the goal of a sustainable but sufficiently productive agriculture coupled with plant-biodiversity preservation.

1.3. RECENT ADVANCES IN STUDIES ON TERRESTRIAL PHOTOSYNTHESIS AND ITS RESPONSES TO THE ENVIRONMENT

In recent decades, important advances have been made in the knowledge of photosynthetic responses to the environment. Studies in this area are currently performed using multidisciplinary approaches, and range from the smallest time-and-space scales to the largest (Fig. 1.2).

At the smallest scales, photosynthetic processes occurring in fractions of seconds can now be properly tracked, even under field conditions, using available spectroscopic techniques (see Chapter 10). The recent and rapid development of molecular analytical tools has also allowed genomic and proteomic analysis that strengthen the links between

genotype and phenotype when measuring the photosynthetic capacity and the resistance of the photosynthetic apparatus to stress conditions (e.g., Bogeat-Triboulot *et al.*, 2007; see Chapter 9). At the molecular level too, important advances are being made in understanding the existing variability in the structure and kinetics of photosynthetic enzymes (Galmés *et al.*, 2005) and in photoprotective mechanisms (Bode *et al.*, 2009). Perhaps the most significant advances in recent years have been made thanks to the rapid development of genetically engineered plants, which considerably increases the precision with which single photosynthetic components and their effects on the functionality of photosynthesis can be studied (Kozaki and Takeba, 1996; Horváth *et al.*, 2000; Kebeish *et al.*, 2007; Merlot *et al.*, 2007; Rivero *et al.*, 2007, 2009; etc.).

At the organelle and leaf levels, advances in the last few decades include improved knowledge of stomatal diversity and its functional significance (Franks and Farquhar, 2007; Brodribb *et al.*, 2009; Brodribb and McAdam, 2011); understanding the inter-relations between leaf structure, hydraulic architecture and photosynthetic function (Nikopoulos *et al.*, 2002; Brodribb *et al.*, 2007; Sack *et al.*, 2008); highlighting the crucial importance of mesophyll conductance to CO_2 as a significant and variable limiting factor for photosynthesis (Loreto *et al.*, 1992; Flexas *et al.*, 2008); highlighting the importance of the interactions between photosynthesis and respiration (Krömer *et al.*, 1993; Gallé *et al.*, 2010; Nunes-Nesi *et al.*, 2011); improving basic knowledge about the genetics and physiology of different photosynthetic pathways (Tanz *et al.*, 2009); and further developing leaf photosynthesis models (Yin *et al.*, 2009). Of particular interest, because of the generality of its implications, has been the description of a 'worldwide leaf economics spectrum', consisting of retrieval of tight relationships among leaf traits (including photosynthesis) that are largely independent of the world's regions and climates (Fig. 1.3). These relationships represent trade-offs for which any existing plant can present at a definite time high growth capacity and high resistance to stressful environments. The existence of such a spectrum sets the boundaries of leaf functioning, and helps predict plant distribution and vegetation boundaries under changing land use and climate. It also shows how limited the possibilities are of improving a given leaf trait such as photosynthesis without co-improving or impairing other leaf traits. For example it proves very difficult to improve photosynthesis without increasing stomatal conductance and hence water losses, or to improve photosynthesis of herbivore-resistant

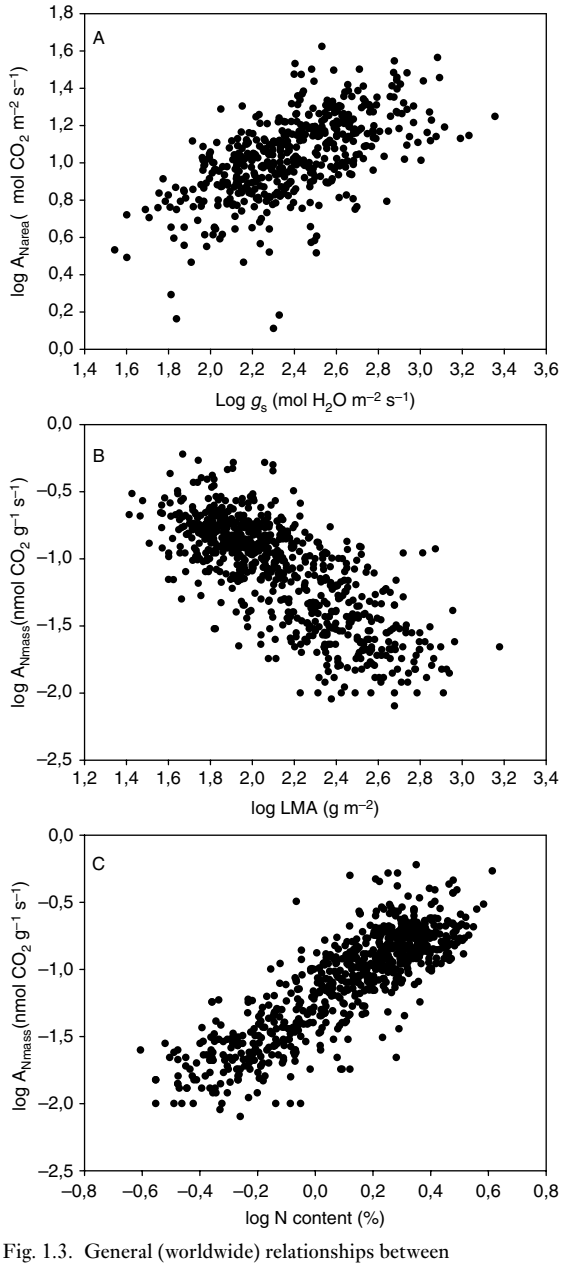

Fig. 1.3. General (worldwide) relationships between photosynthesis and other leaf traits across a large number of species belonging to different functional groups and inhabiting different biomes. **(A)** Log-scale relationship between net photosynthesis on an area basis and stomatal conductance. **(B)** Log-scale relationship between net photosynthesis on a mass basis and leaf mass per area. **(C)** Log-scale relationship between net photosynthesis on a mass basis and leaf nitrogen content. LMA, Leaf dry mass per unit leaf area. Data from Wright *et al.* (2004).

leaves that are characterised by a high leaf mass per area. Nevertheless, there is some scattering in these relationships, suggesting that some variability can be explored in the ratios between any pair of these leaf traits, further improving the photosynthetic efficiency in limiting conditions (see Chapters 16–22).

At the organism and population level, photosynthesis has been recognised as a crucial determinant of genotype fitness in segregating populations. In this sense, photosynthetic differences between male and female plants (Nicotra *et al.*, 2003; Letts *et al.*, 2008) or the impacts of polyploidy (Vyas *et al.*, 2007; Li *et al.*, 2009) on photosynthesis are just a few examples of novel described features of photosynthetic responses. Alternatively, numerous studies have increased our understanding of photosynthesis in organs other than leaves (Chapter 7), and highlighted photosynthetic adaptations of particular plants, increasing the known range of diversity in photosynthetic strategies to cope with environmental constraints (Chapter 7). It is also worth mentioning that important advances have been made relating to systemic signalling systems, including reactive oxygen species (ROS) (Miller *et al.*, 2010) and electrical impulses (Grams *et al.*, 2007) that connect the photosynthetic responses of distant plant parts. Many molecules that have previously been seen as indicators of photosynthetic damage, such as ROS, are indeed now emerging as important signalling molecules activating hypersensitive responses and other reactions of plants to stressful agents (Miller *et al.*, 2010).

Finally, in recent years photosynthesis studies have extended to the largest scales, the canopy and ecosystem levels. At these scales, the introduction of flux-level gas-exchange measurements has been crucial (e.g., Misson *et al.*, 2007; Baldocchi, 2008; see Chapter 14), as well as the development of suitable remote sensing techniques (e.g., Grace *et al.*, 2007; Damm *et al.*, 2010; see Chapter 15). Currently, efforts are being made to map CO_2 fluxes, photosynthetic light and water-use efficiencies at regional and global scales, aided by remote sensing tools, especially to allow assessment of photosynthesis from the space (Gamon *et al.*, 2004; Fuentes *et al.*, 2006; Drolet *et al.*, 2008).

1.4. SCOPE AND STRUCTURE OF THE PRESENT BOOK

The contents of the present book have been designed to try to exhaustively cover the entire span of photosynthesis studies, from the molecular to the ecosystem level, including the basics of terrestrial photosynthesis, its responses to the environment and the most recent advances in the field. The reader may feel lost in the wealth of information of this book, but we have adopted a modular strategy to drive the reader to the information that is specifically needed and at a well-defined level of investigation. Thus, the book has been comprehensively structured in parts comprising 34 chapters, their contents being in increasing order of biological complexity. Hence, the present introductory chapter provides a brief summary and overview of the structure and contents of the book. Part 1 'Photosynthesis: the process' (Chapters 2 to 8) offers a succinct review of the photosynthetic basic of, with a particular focus on models of leaf photosynthesis and photosynthetic limitation analysis, both important aspects for ecophysiology. Part 2 'Measuring photosynthesis' (Chapters 9 to 15) addresses how to measure photosynthesis and related aspects in ecophysiological studies, providing a detailed presentation of the most useful techniques, their principles and procedures. Part 3 'Photosynthesis response to single environmental factors' (Chapters 16 to 22) reviews current state-of-the-art techniques while taking the simplest approach in ecophysiology of photosynthesis. The following Parts, 4 'Photosynthetic in time' (Chapters 23 to 25) and 5 'Photosynthesis in space' (Chapters 26 to 32), show more complex and interacting aspects of photosynthesis related to photosynthesis variations in time, from ontogeny to evolutionary aspects (Part 4), and photosynthetic variations in space, covering photosynthetic in crops, as well as in the most important biomes of the world (Part 5). Finally, increasing the complexity to the global scale, Part 6 'Photosynthesis in a global context' (Chapters 33 and 34) covers worldwide aspects of photosynthesis, such as its implications for WUE and its responses in a climate-change context.

The book is designed to offer a basic, yet informative and updated, view of the primary process driving life on earth to students of all university courses, and to scholars of all biological fields. By transferring current knowledge of photosynthesis to new generations we hope to seed the perception of the importance of this process for the environment, and for the future of agriculture and forestry, the paramount activities sustaining human life on Earth and the mitigation of global change. As for photosynthesis, time and space are crucial parameters that will indicate whether our intents will be met!

Part I
Photosynthesis: the process

2 • Biochemistry and photochemistry of terrestrial photosynthesis: a synopsis

T.D. SHARKEY, J.-M. DUCRUET AND M.A.J. PARRY

2.1. INTRODUCTION

Photosynthesis is typically understood as the light-dependent production of sugar from carbon dioxide (CO_2). The endosymbiotic chloroplast is the cellular location for most of this metabolism in plants, but some additional metabolism occurs in the cytosol to make the sugars that will be transported around the plant, mainly sucrose and also sugar alcohols, such as sorbitol and manitol. There are many processes that can properly be called photosynthesis, but a core set of processes underlie most of the considerations in this book. This chapter will provide an overview of those processes, and many topics covered in this chapter are the subject of more in-depth chapters later on. This chapter begins by describing the initial capture and temporary storage of light energy as highly reactive molecules (nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP)) on carbon. By reducing (i.e., by adding electrons to) carbon from its most oxidised state (CO_2) to the status of sugars $(CH_2O)_n$, the energy initially stored as NADPH and ATP can be stored on the carbon. Additional energy can be stored on each carbon atom by reducing it fully, as happens in the synthesis of oils ($R\text{-}CH_2\text{-}R$), but this is generally not considered when describing photosynthesis. Finally, issues surrounding uptake of the CO_2 will be addressed.

2.2. PHOTOCHEMISTRY SYNOPSIS

Photochemistry, the capture of light energy and its conversion to chemical energy suitable for reducing CO_2 to sugar, is the source of nearly all energy available to living things. Energy captured by absorbing molecules is stored as the high-energy intermediates NADPH (reducing power) and ATP (sometimes called the energy currency of the cell).

2.2.1. The pigment antennae and photochemical centres I and II

In the first step of photosynthetic electron transport (PET), light is absorbed by chlorophylls (chl.) and auxiliary pigments organised in pigment-protein complexes that constitute the antennae of two photosystems (photosystem I and II (PSI and PSII)) (Figure 2.1). Most chl.s (>99%) are antennae chl.s and do not participate directly in photochemistry. These antenna chl.s are found attached to 'light-harvesting' complexes (LHC), and to core antenna subunits. In cyanobacteria the antennas are found outside of the photosynthetic membrane as phycobilisomes, whereas in algae and plants the antennas are integral membrane protein complexes. Higher-plant LHCIIs (light-harvesting complexes of PSII) are trimers, with each trimer having three membrane-spanning α-helices. Each monomer has a lutein molecule that can absorb light, but its main role is likely protection of the chl. molecules from photodestruction. LHCIs (light-harvesting complexes of PSI) are associated with PSI; LHCII is normally are associated with PSII, but can move within the thylakoid membrane from PSII to PSI. Chl. *b* absorbs shorter red wavelengths and longer blue wavelengths than chlorophyll *a* (Figure 2.1) and is located in the LHC, mainly LHCII, and in the minor chl.-protein complexes (CP) of PSII. LHCI in the peripheral antenna of PSI contains CP complexes with a red absorption shifted towards longer wavelengths. Normally, light excitation is balanced between PSII and PSI, but spectral differences between the two photosystems depending on the spectrum of incoming light or excessive rates of cyclic electron flow around PSI (CEFI) may result in an imbalance. Phosphorylation of some LHCII, which causes it to migrate from PSII to PSI, results in a transfer of light energy from PSII to PSI. This is called a state 1 to state 2

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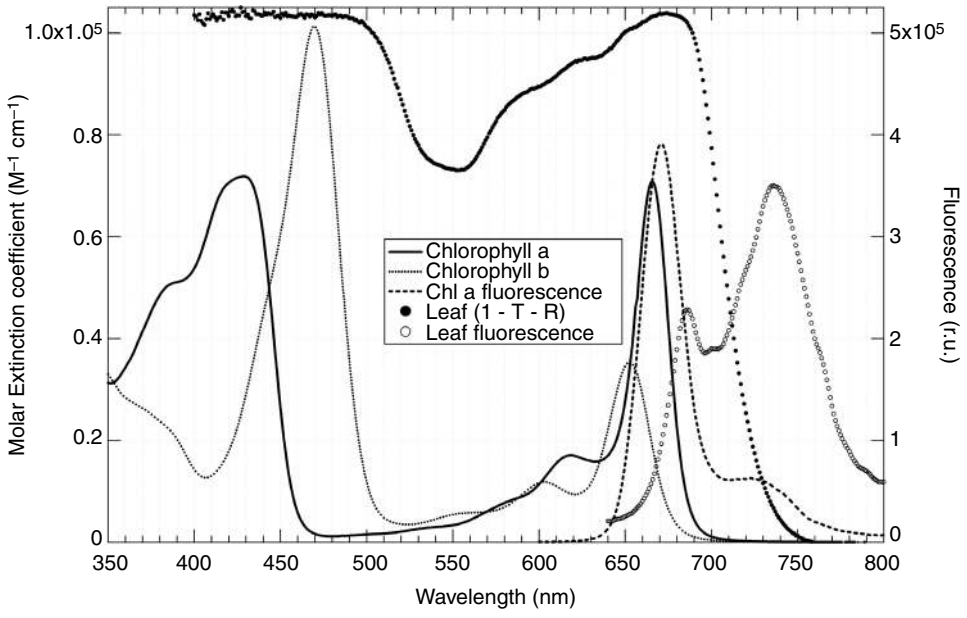

Fig. 2.1. Absorption and fluorescence emission spectra of pure chlorophylls in methanol (lines) and of a pea leaf (circle). Light absorbed by leaf is the fraction of incoming light normalised to one that is not transmitted (T) nor reflected (R). In leaf fluorescence, the 685-nm peak is more strongly decreased by chlorophyll re-absorption than the 740-nm peak.

transition (Allen and Forsberg, 2001; Jensen *et al.*, 2007) and requires a specific subunit of PSI (Lunde *et al.*, 2000).

Light quanta are absorbed, and then migrate randomly in antennae by resonance transfer between chl.s, first to antenna chl.s in the core photosystem complexes, and then to the photochemical traps, called centres, containing a special chl. *a* dimer (except in some bacteria). At the reaction centre (RC), an electron is transferred from the excited singlet Chl* to a primary acceptor, transiently producing a chlorophyll$^+$ cation, which is re-reduced by a primary electron donor. This separation of +/– charge pairs stabilised on electron carriers constitutes the primary step of photosynthesis. The Chl* ↔ chl.$^+$ + e$^-$ charge separation step corresponds to an absorption change of centre chl. dimers, at 680 nm for PSII (P680) and 700 nm for PSI (P700). The P700$^+$/P700 ratio can be measured *in vivo* in the region of 810 to 830 nm in order to monitor PSI activity, and this underlies an optical method for assessing PSI function (Chapter 10). Alternatively, P680$^+$ appears only transiently in undamaged PSII, and is rapidly re-reduced in the submicrosecond range by the primary donor TyrZ connected to the oxygen evolving complex (OEC). PSII is, however, endowed with the unique property of emitting both a variable fluorescence and a luminescence, which reflect its functioning. The variations of the fluorescence yield results from those of the light-induced high fluorescence P680Q_A^- state of PSII centres (Krause and Weis, 1991; Govindjee, 1995; Baker, 2008). A weak delayed fluorescence, generally called luminescence, originates from two Chl* that is created in darkness by the +/– charge pairs separated by a previous illumination and stabilised on PSII electron carriers. Fluorescence reflects the fate of the light quanta in the antenna, whereas luminescence is a probe of stabilised charge pairs produced by a previous illumination.

PSII acts as a photo-water-oxidase-plastoquinone-reductase. It extracts four electrons from two water molecules and raises them successively to a more reducing (negative) redox potential, allowing them to reduce plastoquinone (PQ) to plastohydroquinone (PQH_2), i.e., two electrons per PQ. This requires two buffer systems to store the + and – charges after a photochemical event (Figure 2.2).

Positive charges are stored on the OEC. A tyrosine (called Z) transfers an electron to the oxidised P680$^+$, and then accepts an electron from the OEC. The OEC stores positive charges on a Mn_4O_4Ca cluster endowed with four oxidation steps, the S states S_0 to $S_{4(+)}$ (Rutherford and Boussac, 2004). The transient last step performs water photolysis:

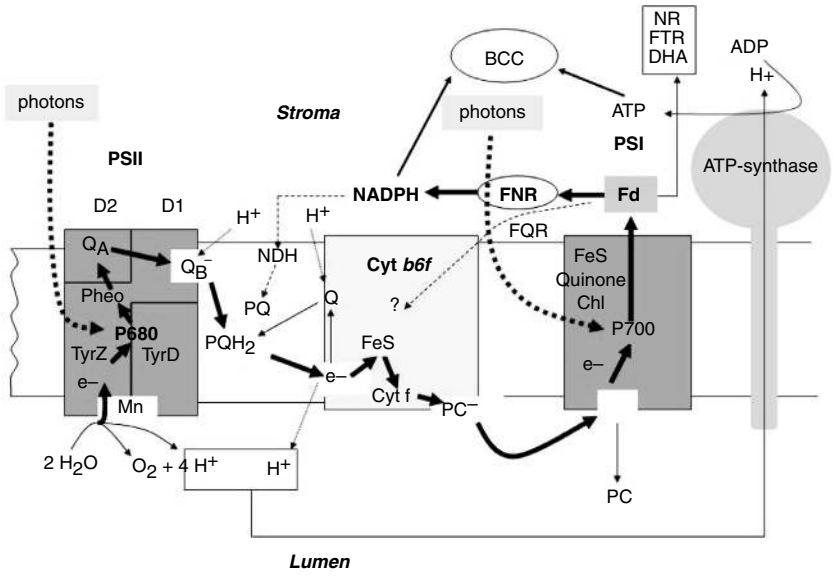

Fig. 2.2. The photosynthetic electron-transfer chain. Electrons extracted to water are raised to a more reducing potential by the two photosystems, PSII and PSI, functioning in series, to finally reduce ferredoxin, then NADPH. This electron transfer is coupled to a proton pumping from stroma to lumen. The proton flow through the ATP synthase drives ATP synthesis. Inducible cyclic pathways are FQR (ferredoxin-plastoquinone-reductase) and NDH (NADPH dehydrogenase). Chl, chlorophyll; Cyt, cytochrome; DHA, mono-dehydro-ascorbate; FNR, ferredoxin; NADP Reductase; FTR, ferredoxin thioredoxin reductase; NR, nitrite reductase; Pheo, pheophytin; $PQ_$, plastoquinone; PQH_2, plastohydroquinone; Q, Q cycle (that involves PQ/PQH_2 and amplifies proton pumping by the cyt b_6f); Tyr, tyrosine.

$$Z^+ + S_3 \rightarrow Z + S_4 + 2H_20 \rightarrow S_0 + O_2 + 4H^+ \quad \text{[Eqn. 2.1]}$$

The OEC is exposed to the lumen, in which protons are released fractionally during the S_0 to S_4 oxidation steps.

The electron charge is stabilised on the secondary acceptor Q_B formed by a PQ loosely bound to a protein pocket, which is also the binding site for diuron- or atrazine-like herbicides, until a second arrives on the primary acceptor Q_A:

$$Q_A^- + Q_B^- \rightarrow Q_A + Q_B^{2-} + 2H+ \rightarrow Q_A + Q_B\text{–}empty + PQH_2 \quad \text{[Eqn. 2.2]}$$

The quinonic acceptors are facing the stroma, from which the protons are taken up (Figure 2.2).

In addition to the variable chl. fluorescence and luminescence that reflects its functioning (Krause and Weis, 1991; Govindjee, 1995; Baker, 2008), PSII has the other noteworthy property of being highly sensitive to various climatic or chemical stresses (Aro *et al.*, 1993). The reasons for the latter are many: (1) an elaborate but fragile OEC complex; (2) a fairly long (several nanoseconds) excitonic lifetime in the antenna, increased when the light conversion is slowed by downstream inhibition of electron flow, which produces singlet oxygen; (3) a high local concentration of O_2 in grana where PSII is located; (4) a loose Q_B pocket interacting with the highly unsaturated, therefore fluid and oxidisable, lipids of the thylakoid membrane. This can explain the basic architecture of the core PSII, formed by two 32-kDa polypeptides, reaction-centre proteins D1 and D2, encoded by the chloroplastic genes *PsbA* and *PsbD* respectively. D1 is the high-turnover protein in the whole plant cell, the only one that is rapidly marked by ^{35}S-methionine in a leaf submitted to a strong illumination (Mattoo *et al.*, 1981); it acts as a fuse that bears the centre chlorophyll pair, the OEC and the stable Q_B acceptor, and that it is frequently disassembled and replaced when a high-energy pressure produces damaging reactive species. Photoinhibition occurs when the repairing process cannot cope with damage to D1 caused by high light intensities; it is favoured by stress factors such as cold (see Chapter 18) that both slows down energy draining towards the Calvin cycle and increases the membrane rigidity, which impairs the replacement of D1 (Kanervo *et al.*, 1997).

The core of PSI consists of a large pigment-protein complex of 15 integral proteins, including a central heterodimer of sub-units encoded by genes *PSaA* and *PsaB*

(Jensen *et al.*, 2007). Owing to spectral properties of PSI antenna, FR light (>700 nm) can be used to excite PSI preferentially to PSII. An electron is transferred from the excited dimer chl. centre P700* to a primary acceptor, another chl. molecule (A_0), then to a phylloquinone (A_1) and to iron-sulfur centers (F_X, F_A, F_B). The final electron acceptor of PSI is ferredoxin (Fd), soluble in the stroma, which reduces $NADP^+$ (nicotinamide adenine dinucleotide phosphate) to NADPH via the enzyme ferredoxin-NADP-reductase (FNR; Fig. 2.2). The PSI centre P700 is located close to the lumen, and a soluble plastocyanin molecule has to dock at the interface to re-reduce the $P700^+$ resulting from a charge separation (Bottin and Mathis, 1985). PSI contrasts with PSII by a slower re-reduction of its oxidised dimer $P700^+$ by the mobile plastocyanin and by a faster reoxidation of its primary chl. acceptor A_0 by the secondary phylloquinone acceptor A_1, which leads to an increase of the light-induced $P700^+A_0$ state; PSI has no variable fluorescence. The absorption change owing to $P700 \leftrightarrow P700^+$, generally measured on a secondary band around 820 nm, provides a tool to monitor PSI activity (Haveman and Mathis, 1976; Harbinson and Woodward, 1987; Schreiber *et al.*, 1989). Ferredoxin is also an electron donor to other metabolic pathways: nitrite reductase, the ascorbate cycle by reduction of the monodehydroascorbate (MDHA) radical and the thioredoxin redox regulatory system. PSI is less sensitive to heat than PSII and generally considered more tolerant to stresses, although a photoinhibition of PSI also occurs in some species in cold conditions (Havaux and Davaud, 1994; Terashima *et al.*, 1998; Scheller and Haldrup, 2005).

The transmembrane cyt b_6f complex mediates the electron transport from PSII to PSI, receiving electrons from reduced PQ and transferring them to plastocyanin, with separate pathways around PSI for the two electrons and the involvement of a Q cycle (Section 2.1.3). Cyt b_6f appears to also be the entry point of electrons from reduced Fd to the intersystem chain, constituting the specific step of the cyclic electron-transfer pathway around PSI (CEFI). The CEFI was discovered by Arnon (1959) in isolated thylakoids but its role *in vivo* was questioned until optical methods (Chapter 10) allowed it to be monitored in leaves (Bukhov and Carpentier, 2004). It corresponds to the putative ferredoxin-plastoquinone-reductase or FQR (Bendall and Manasse, 1995), an electron-transfer activity to which no protein support could be ascribed up to now, although related mutations have been recently characterised (Shikanai, 2007). A second pathway, the NAD(P)H-dehydrogenase (NDH) that reduces the PQ pool, is supported by a well-characterised membraneous protein complex; it is also active in the dark and constitutes a first step of the chlororespiratory pathway, of which a second step is the oxidation of PQH_2 via a plastid terminal oxidase (PTOX) (Peltier and Cournac, 2002). CEFI performs proton pumping without generation of reducing power, which has two consequences: (1) an increase of the lumen acidity that enhances the protective non-photochemical quenching (NPQ); and (2) an increase of ATP synthesis when needed, for example, for protein synthesis and repair. Indeed, CEFI is triggered in various stress situations (Rumeau *et al.*, 2007).

2.2.2. Provision of reducing power

The photons captured by antennas and transferred to *RC*s provide the energy that allows electrons to flow from water to $NADP^+$ in a process called linear electron flow (LEF). In LEF, the rates of PSII and PSI activity must be matched and there are a number of regulatory mechanisms to achieve this balance. LEF provides reducing power that is used primarily for carbon reduction, but also for other important reduction reactions such as nitrite reduction. Up to 20% of reducing power can be used in nitrogen metabolism (Bloom *et al.*, 2002). The reducing power stored on carbon as CO_2 (fully oxidised) is changed to the redox level of a sugar (partially reduced carbon), and represents the bulk of the energy stored as a result of photosynthesis. Fully reduced carbon (e.g., lipids) is formed in anabolic reactions not strictly associated with photosynthesis.

2.2.3. Provision of adenosine triphosphate

The LEF from water to the reducing side of PSI generates reduced Fd, then NADPH, the source of electrons for the Calvin cycle. However, the Calvin cycle also requires ATP to assimilate CO_2. The photosynthetic electron transfer proceeds along vectorially organised transporters, so that several steps of electron transfer are coupled to proton pumping from the stroma into the lumen, creating a pH gradient (ΔpH) and a transmembrane electric field $\Delta\Psi$. Together, these constitute a chemiosmotic potential, or proton motive force (*pmf*), that drives synthesis of ATP as described by Peter Mitchell. In chloroplasts this is called photophosphorylation to distinguish it from the similar process in mitochondria, termed oxidative phosphorylation. The relationship between the ΔpH and $\Delta\Psi$ is described by the Nernst equation: