CHAPTER 1

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INTRODUCTION

1.1. Plants in relation to their environment

The term environment etymologically means surroundings. Thus, environment is a complex of many factors which surround an organism and affect the life of an organism in many ways. These environmental factors can be living (biotic) or non-living (abiotic). These environmental factors exhibit diurnal, seasonal, annual and cyclic variations which the plants are subjected to, and therefore they have to develop strategies to cope with these changes in the environment (Sharma 1997).

1.2. Light as an environmental factor

Light is well known for its effects on the basic physiological processes of plants, such as photosynthesis, transpiration, seed germination, flowering, etc. Sun is the main source of light energy for photosynthesis. The sun radiates energy representing the entire electromagnetic spectrum (ranging from the cosmic rays to radio waves) (Fig. 1.1), but the earth’s atmosphere is transparent only to part of the infra-red and ultraviolet light and all the visible light. The visible portion of the electromagnetic spectrum ranges from the violet at about 380 nm to the far red at 750 nm. Radiation from about 400 to 700 nm is used in higher plant photosynthesis and is called photosynthetically active radiation or PAR and is measured by quantum sensors (Lawlor 1987).

1.3. Photosynthesis

The term photosynthesis literally means building up or assembly by light. In this process green plants in the presence of sunlight synthesise carbohydrates using carbon dioxide and water from the environment with the subsequent release of molecular oxygen, in the process the energy-poor compounds carbon dioxide and water are
Figure 1.1: Electromagnetic spectrum of solar radiation
converted to the energy-rich compound carbohydrates, and oxygen is given out as a byproduct.

1.4. Chloroplast structure and organization

Photosynthetic process occurs in chloroplasts, which are found in mesophyll and palisade cells of the leaves (Fig. 1.2). Chloroplasts are semi-autonomous organelles having a circular genome with a size of 134,525 bp (Hiratsuka et al., 1989). Electron microscope pictures showed that chloroplasts in higher plants are saucer shaped bodies 4 to 10 μm in diameter and 1 μm in thickness (1 μm = 10⁻⁶ m) with an outer double membrane or envelope separating them from the rest of the cytoplasm. The inner envelope membrane of the chloroplast acts as a barrier controlling the flux of organic and charged molecules in and out of the chloroplast. Water passes freely through the envelope membranes, as do other small neutral molecules like carbon dioxide and oxygen (Whitmarsh and Govindjee 1999).

Internally the chloroplast is comprised of a complicated membrane system of lamellae or flattened thylakoids, known as the photosynthetic membrane (or thylakoid membrane), which are arranged in stacks in dense green regions known as grana. The grana are embedded in a colourless matrix called the stroma, and are interconnected by a system of loosely arranged membranes called the stroma lamellae. Four supramolecular protein complexes are embedded in the thylakoids: photosystem II (PS II), cytochrome b₆f (Cyt b₆f), photosystem I (PS I) and ATP synthase (ATPase) (Nelson and Ben-Shem 2004). Figure 1.3 shows the organization of these protein complexes in the thylakoid membrane. The first three complexes participate in electron transport from water to NADP⁺. During photosynthetic electron transport, protons from the stroma are transferred to the luminal space through the thylakoid membrane,
Figure 1.2: Structure of chloroplast
(a) Intact leaf
(b) Leaf cross section
(c) Mesophyll cell
(d) Chloroplast
Figure 1.3: Organization of the protein complexes of the thylakoid membrane. Photosystem II is located predominantly in the stacked regions of the thylakoid membrane; photosystem I and ATP synthase are found in the unstacked regions protruding into the stroma. Cytochrome b₆f complexes are evenly distributed. This lateral separation of the two photosystems requires that electrons and protons produced by photosystem II be transported a considerable distance before they can be acted on by photosystem I and ATP-coupling enzyme.
developing a trans-thylakoid pH gradient essential for ATP synthesis by the ATP synthase.

1.4.1. Lipids and fatty acids in the photosynthetic membranes

The photosynthetic membrane is composed mainly of lipids and proteins (Kirk and Tilney-Basset 1978). The major lipids are polar glycerolipids, such as glycolipids and phospholipids. In plants and eukaryotic algal, glycerolipids and fatty acids are synthesized in chloroplasts (Murata and Siegenthaler 1998). The thylakoid membranes of the chloroplast of higher plants contain three glycolipids, namely, monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG) and sulfoquinovosyl diacylglycerol (SQDG), and one phospholipid, phosphatidylglycerol (PG) (Joyard et al., 1998).

The structural lipids play a role in maintaining the appropriate fluidity of the membrane, which is a consequence of the high degree of unsaturation of these lipids. Furthermore, MGDG can form, under certain conditions, non-bilayer configurations (Williams 1998) and therefore, can influence photosynthetic functions. By contrast, the functional lipids are bound to proteins and tend to be less unsaturated. These molecules are involved in specific interactions with proteins that ensure an adequate maintenance of the confirmation and/or orientation of the proteins in the membrane.

1.4.2. Photosynthetic pigments

The photosynthetic membrane contains one or more organic pigments capable of absorbing visible radiation which will initiate the photochemical reactions of photosynthesis. The major classes of pigments found in plants are the chlorophylls (Chls) and the carotenoids. Chlorophylls are the primary light-capturing pigments which drive the photochemical reactions. Chlorophyll a is the major photosynthetic
pigment in all organisms capable of oxygenic photosynthesis. However, Chl \( a \) has only moderate absorptivity in the green spectral region, causing the green colour of vegetation. It is therefore, supplemented by additional light-harvesting pigments. In green plants Chl \( a \) is accompanied by Chl \( b \), which extends the absorption of light from either side into the ‘green hole’. The presence of Chl \( b \), therefore, narrows somewhat the “green gap” in the absorption spectrum of leaves (Sandmann and Scheer 2000).

The carotenoids are yellow or orange pigments found in all photosynthetic cells. They have triple banded absorption spectra in the region from about 400 to 550 nm. The carotenoids of higher plants include \( \alpha \)-carotene (\( \alpha \)-car), \( \beta \)-carotene (\( \beta \)-car), lutein, neoxanthin (Nx), violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z); their amount varies within the cell depending upon the growth conditions and stress factors (Britton 1990). Carotenoids act as accessory light harvesting pigments (Davidson and Cogdell 1981) and also protect against photooxidative damage (Krinsky 1979). In higher plants Z and A formed by the xanthophyll cycle (Yamamoto et al., 1962), increase non-radiative dissipation of absorbed energy (Demmig et al., 1987a; Sharma and Hall 1991).

1.5. **Organisation and composition of the photosynthetic complex**

Oxygenic organisms have two photosystems namely PS I and PS II. Each photosystem consists of a reaction centre core complex which carries out the primary charge separation, and a light-harvesting complex, which serve to increase the cross-sectional area utilised for light absorption. In higher plants LHC I and LHC II are associated with PS I and PS II respectively. Photon capture by the
photosystem antennae and excitation transfer to the two photosystems, provide the energy for oxidation of water and electron movement to acceptors, which donate electrons to biochemical processes, and for passage of protons into the thylakoid lumen, for synthesis of ATP.

1.5.1. Light-harvesting complexes

Light-harvesting complexes are pigment-proteins whose function is to absorb light energy and to transfer it rapidly and efficiently to the photosynthetic reaction centres (RCs). They bind most of the photosynthetic pigments found in higher plants acting as antennae in trapping and transferring excitation energy.

Light harvesting complex II protein has a trimeric arrangement of monomers in a radially symmetric complex with internal symmetry within each monomer (Fig. 1.4) (Kühlbrandt and Wang 1991). In each monomer there are three membrane-spanning helices, helices A and B showing a high intramolecular similarity, inserted into the membrane with an angle of 30-32° form a cross with each other, while a third helix C lies perpendicular to the membrane plane. Cross-braced to helices A and B, xanthophylls are located in two symmetrical sites called L1 and L2, respectively, connecting the stromal loop with the amphiphilic helix D and the N-terminal domain with the luminal loop. LHC II contains about 8 Chl \( a \) and 6 Chl \( b \) together with 2 xanthophylls (Kühlbrandt and Wang 1991). The 14 Chls have their chlorine rings roughly perpendicular to the membrane plane and are arranged in two layers near the surfaces of the membrane. Helices A and B serve as a scaffold for packing the pigments into a small volume so that the light-harvesting proteins have an unusually large number of pigment molecules, i.e. one Chl per 15 amino acid residues, bound to a single polypeptide (Kühlbrandt et al., 1994).
Figure 1.4:

(a) Organisation of light harvesting complex II, showing the association of specific Chl-binding complexes with PS II.

(b) In PS II an integral core of Chl α-binding proteins, designated CP43 and CP47 are closely associated with the D1/D2 RC complex, several peripheral Chl α/b binding proteins are also present.
Different proteins of LHC II have been identified as closely related pigment-proteins encoded by a family of nuclear genes (Green et al., 1992). All proteins are encoded by nuclear DNA, synthesized in the cytoplasm as precursor proteins with transit sequences which direct the protein into the chloroplast. LHC II preparations yield Lhcb1 and Lhcb2 proteins with various amounts of Lhcb3 and others. Lhcb1 is very homologous to the Lhcb2 protein, and is part of the most peripheral antenna of PS II (Spangfort and Anderson 1989), where it exists with Lhcb1 in mixed trimers (Simpson and Knoetzel 1996). Lhcb3 is the smallest pigment-protein found in LHC II, and in contrast to Lhcb1 and 2 is tightly bound to the reaction centre of PS II. Lhcb4 codes for CP29, the largest of the LHC proteins, and is part of the inner antenna which connects the LHC II to the PS II reaction centre. Lhcb5 and Lhcb6 code for the apoproteins of CP26 and CP24, respectively. The LHC II includes 40-50 Chl a molecules bound to CP43 and CP47 (psbC and psbB); a consensus antenna size value for the Chls organised in the Lhcb proteins is 230-250 Chl (Melis 1991).

The antenna of PS I (LHC I) contains three pigment-protein complexes: a heterodimeric LHCI-730 complex composed of a 21 and 22 kDa polypeptide and two LHCI-680 complexes with single apoproteins of 25 kDa (LHCI-680A) and 23 kDa (LHCI-680B) (Knoetzel et al., 1998; Fig. 1.5). The PS I core is surrounded by a monolayer of 8 LHC polypeptides (Boekema et al., 1990). In PS I, about half of the light-harvesting Chl is associated with the two large polypeptides that contain the RC pigment P700 and form the CPI complex. This consists of two homologous 82 kDa proteins which bind about 90 molecules of Chl a and 12-16 molecules of β-carotene.

Although the primary function of photosynthetic light-harvesting complexes is the absorption of light and the transfer of the excitation energy to the photochemical
Figure 1.5:

(a) Organisation of light harvesting complex I, showing the association of specific Chl-binding complexes with PS I.

(b) In PS I the core complex contain approximately 90 Chl $a$ molecules, additional Chl is present in LHC-I complexes, which contain both Chl $a$ and $b$. 
reaction centres, they are also essential for regulation and distribution of excitation energy within the photosynthetic apparatus, and respond to both short- and long-term fluctuations in light intensity and quality. The LHC II proteins carry out these regulatory functions through different mechanisms: phosphorylation, dissociation into monomers, migration from grana to stroma, binding of xanthophylls, etc. (reviewed by Bassi et al., 1997).

1.5.2. Photosystem II (PS II)

Photosystem II is located in the appressed granal regions of the chloroplast (Anderson 2002), and consists of more than 20 protein subunits (Table 1.1), composed of the reaction centre core complex with a molecule of P680 joined to the oxygen evolving complex and the peripheral light-harvesting antenna assembly (Rhee 2001; Fig. 1.6a). Photosystem II uses light energy to drive two chemical reactions - the oxidation of water and the reduction of plastoquinone. The basic structural unit of PS II is the so-called LHC II-PS II supercomplex (Hankamer et al., 1997). At the heart of PS II, the reaction centre D1/D2 protein heterodimer binds all of the redox factors necessary for stable light-induced charge separation across the thylakoid membrane (Diner and Babcock 1996). These factors are (from the oxidizing side to the reducing side) a tetra manganese cluster with a calcium- and a chloride-ion, two tyrosine residues as referred to YZ and YD, four to six Chl a molecules, two pheophytins, and two plastoquinones. Compared to these, cofactors, such as non-heme iron and bicarbonate anions (Govindjee and Rensen 1993), do not function as a direct electron carrier but are thought to be crucial for this process. Two β-car molecules are present in the RC and are involved in the secondary photochemical reactions (Rhee 2001). The oxygen-evolving core complex comprises other proteins closely associated with the D1/D2 heterodimer. These are the Chl a proteins, CP43 and CP47, α- and β-subunits of
Table 1.1: Photosystem II subunits. The size of each protein is based on its amino acid sequence. The name of the genes and their location, chloroplast (C) or nuclear (N) genome is given. Structural features of proteins are indicated.

<table>
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<th>Gene</th>
<th>Molecular weight (kDa)</th>
<th>Structural features</th>
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<tr>
<td>D1</td>
<td>psbA (C)</td>
<td>32</td>
<td>Reaction center (RC)</td>
</tr>
<tr>
<td>D2</td>
<td>psbD (C)</td>
<td>34</td>
<td>Reaction center</td>
</tr>
<tr>
<td>Cyt b559α</td>
<td>psbE</td>
<td>9</td>
<td>Photoprotection</td>
</tr>
<tr>
<td>Cyt b559β</td>
<td>psbF</td>
<td>4</td>
<td>Photoprotection</td>
</tr>
<tr>
<td>CP47</td>
<td>psbB (C)</td>
<td>47</td>
<td>Chl a-binding RC antenna</td>
</tr>
<tr>
<td>CP43</td>
<td>psbC (C)</td>
<td>43</td>
<td>Chl a-binding RC antenna</td>
</tr>
<tr>
<td>PS II-H</td>
<td>psbH (C)</td>
<td>10</td>
<td>Light-dependent phosphorylation</td>
</tr>
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<td>PS II-I</td>
<td>Psbl (C)</td>
<td>4.8</td>
<td>Reaction centre</td>
</tr>
<tr>
<td>PS II-J</td>
<td>psbJ (C)</td>
<td>4</td>
<td>Stabilization of assembly</td>
</tr>
<tr>
<td>PS II-K</td>
<td>psbK (C)</td>
<td>3.5</td>
<td>Absent in purified oxygen-evolving core</td>
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<tr>
<td>PS II-L</td>
<td>psbl (C)</td>
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<td>Regulation of the P680⁺ reduction</td>
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<td>PS II-M</td>
<td>psbM (C)</td>
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<td>In oxygen evolving core</td>
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<td>PS II-N</td>
<td>psbN</td>
<td>4.7</td>
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<td>PS II-R</td>
<td>Psbr (N)</td>
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<td>OEC1</td>
<td>PsbO (N)</td>
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<td>Regulate oxygen-evolution</td>
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<tr>
<td>OEC2</td>
<td>PsbP (N)</td>
<td>23</td>
<td>Regulate oxygen-evolution</td>
</tr>
<tr>
<td>OEC3</td>
<td>PsbQ (N)</td>
<td>17</td>
<td>Regulate oxygen-evolution</td>
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<td>CP29</td>
<td>Lhcb4 (N)</td>
<td>28</td>
<td>Binds Chl a and b, lutein, Nx, V</td>
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<tr>
<td>CP26</td>
<td>Lhcb5 (N)</td>
<td>27</td>
<td>Binds Chl a and b, lutein, Nx, V</td>
</tr>
<tr>
<td>CP24</td>
<td>Lhcb6 (N)</td>
<td>23</td>
<td>Binds Chl a and b, lutein, Nx, V</td>
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<tr>
<td>CP22</td>
<td>PsbS (N)</td>
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<td>Binds Chl a and b</td>
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cytochrome b559, several small membrane-spanning helical proteins, a cluster of four manganese atoms probably all attached to D1 protein and the 33 kDa, 23 kDa and 17 kDa extrinsic luminal proteins (Barber 2002). In higher plants, and some green algae, the central core is flanked by a family of Chl a/b proteins, monomeric CP29, CP26 and CP24, and the outer antenna trimeric LHC II that regulate light-harvesting (Horton et al., 1996).

1.5.3. Photosystem I (PS I)

Photosystem I is distributed in non-appressed stromal lamellae and peripheral regions of the grana (Anderson 2002) and catalyses the oxidation of plastocyanin, a small soluble copper-protein, and the reduction of ferredoxin, a small iron-sulphur protein. It is made up of the P700 RC and is composed of a heterodimer of proteins that act as ligands for most of the electron carriers (Nelson and Yocum 2006; Fig. 1.6b). The PS I monomer consists of 15 protein subunits (Table 1.2), of these, only PsaA, PsaB, and PsaC bind the cofactors of the electron transfer system. PsaA and PsaB form the core complex around which other subunits are organized. The PsaC, PsaD, PsaH, and PsaE proteins form the stromal peripheral domain that contains the terminal electron donors and the ferredoxin-docking site. PsaN is a lumenal peripheral protein (Chitnis 2001). PsaN and the large luminal domain of PsaF form the plastocyanin docking site of PS I. The remaining proteins of PS I are integral membrane proteins with 1–3 transmembrane helices. The PsaA and PsaB proteins of the PS I core are homologous in their primary sequences and transmembrane topography. They contain 11 transmembrane helices each with their N termini in the stroma. Two domains in these proteins can be defined based on evolutionary and functional considerations. The C-terminal domains of PsaA and PsaB contain five transmembrane helices each and contain residues whose side chains provide coordinating groups for the redox centers.
Table 1.2: Photosystem I subunits. The size of each protein is based on its amino acid sequence. The name of the genes and their location, chloroplast (C) or nuclear (N) genome is given. Structural features of proteins are indicated.

<table>
<thead>
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<th>Components</th>
<th>Gene</th>
<th>Molecular mass (kDa)</th>
<th>Structural features</th>
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<td>PS I-A</td>
<td>psaA (C)</td>
<td>83</td>
<td>Heterodimer binds about 100 Chl a, 12-15 β-carotene</td>
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<tr>
<td>PS I-B</td>
<td>psaB (C)</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>PS I-C</td>
<td>psaC (C)</td>
<td>9</td>
<td>Terminal electron acceptors</td>
</tr>
<tr>
<td>PS I-D</td>
<td>PsaD (N)</td>
<td>17.9</td>
<td>Ferredoxin docking</td>
</tr>
<tr>
<td>PS I-E</td>
<td>PsaE (N)</td>
<td>9.7</td>
<td>Peripheral on stromal side</td>
</tr>
<tr>
<td>PS I-F</td>
<td>PsaF (N)</td>
<td>17.3</td>
<td>Plastocyanin docking</td>
</tr>
<tr>
<td>PS I-G</td>
<td>PsaG (N)</td>
<td>10.8</td>
<td>Found in higher plants only</td>
</tr>
<tr>
<td>PS I-H</td>
<td>PsaH (N)</td>
<td>10.2</td>
<td>Found in higher plants only</td>
</tr>
<tr>
<td>PS I-I</td>
<td>psaI (C)</td>
<td>4.6</td>
<td>One transmembrane α-helix</td>
</tr>
<tr>
<td>PS I-J</td>
<td>psaJ (C)</td>
<td>3.3</td>
<td>One transmembrane α-helix</td>
</tr>
<tr>
<td>PS I-K</td>
<td>PsaK (N)</td>
<td>5.6</td>
<td>Two transmembrane α-helices</td>
</tr>
<tr>
<td>PS I-L</td>
<td>PsaL (N)</td>
<td>15.4</td>
<td>Two transmembrane α-helices</td>
</tr>
<tr>
<td>PS I-M</td>
<td>psaM</td>
<td>3.5</td>
<td>One transmembrane α-helix</td>
</tr>
<tr>
<td>PS I-N</td>
<td>psaN</td>
<td>4.8</td>
<td>Peripheral on the luminal side</td>
</tr>
<tr>
<td>PS I-O</td>
<td>PsaO (N)</td>
<td>9</td>
<td>One transmembrane α-helix</td>
</tr>
<tr>
<td>Ferredoxin</td>
<td>PetG (N)</td>
<td></td>
<td>Fe-S centre</td>
</tr>
<tr>
<td>FNR</td>
<td>PetH (N)</td>
<td>35.4</td>
<td>Ferredoxin-NADP reductase</td>
</tr>
</tbody>
</table>
Figure 1.6:
(a) Structural model of photosystem II reaction center, a schematic representation showing the structure dominated by the two PS II RC proteins D1 and D2.
(b) Structural model of photosystem I reaction center, a schematic representation showing the organisation of the two major portions in this complex the psaA and psaB subunits, designated here as A and B.
Carbon fixing reactions (Stroma)

NADP⁺

hv

cyclic e⁻ transport to cytb₇f

Mem.

Lumen

plastocyanin (PC) from cytb₇f

A₀

A

A₁

P700

Lhca 1-4

E

F

G

H

K

Cytochrome F₅₅₀

Cytochrome b₆₇₃

Cytochrome c₅₅₃

Cytochrome b₆₉₃

Cytochrome f
Interactions among the helices in the C-terminal domains of PsaA and PsaB are primarily responsible for forming the heterodimer. The tilted ten transmembrane helices, along with four parallel helices of the C-terminal domain, make a cage for coordination and protection of the electron transfer chain. The N-terminal domain contains the remaining six transmembrane helices, which resemble the transmembrane topology of the CP43 and CP47 proteins of PS II. These helices, along with the transmembrane segments of other PS I proteins, coordinate the chlorophyll molecules of the internal antenna of PS I (Chitnis 2001).

1.5.5. Electron transport chain

In oxygenic photosynthetic organisms, the two different photosystems, PS II and PS I, operate in series in the non-cyclic electron transport chain to oxidise water, reduce NADP⁺ and generate ATP (Fig. 1.7). Photosystem I can also function independently in the cyclic electron transport pathway to generate ATP. In the light, PS II feeds electrons to PS I by intermediate carriers. The intermediates of the electron transport chain can be divided into several classes: cytochromes (cyt f, b6 and b559), non-haeme iron-sulphur centres (e.g. Ferredoxin and Rieske centre), quinones (eg. plastoquinone), proteins with transition metals as the prosthetic group (plastocyanin, Mn-containing proteins), Chl and pheophytin. The Rieske FeS-protein, functions as the primary oxidant of quinol in cytochrome b₆ complexes (Rieske 1976). It contains a 2Fe-2S-centre with a positive redox potential. The Rieske FeS-proteins of chloroplast are encoded in nuclear genes (Salter et al., 1992). An additional complex in the thylakoid membrane is the ATP synthase, found only in non-appressed region. The ATP synthase may be separated into two parts: coupling factor 1 (CF₁) and CF₀. CF₁ is an oligomeric hydrophilic protein that contains the catalytic sites of the
Figure 1.7:
(a) Schematic representation of the Z-scheme of photosynthesis.
(b) Membrane organisation of the Z-scheme. The various components of the chloroplast electron transport chain and ATP-synthesizing apparatus are illustrated in the thylakoid membrane. Four membrane complexes – PS II, PS I, the cyt b6f complex and the ATP synthase are shown. Electrons are transferred from water to NAD⁺; accompanying this electron transfer, a proton gradient is ultimately utilised for the synthesis of ATP by the ATP synthase.
synthase. It consists of five different polypeptide subunits and a total of nine individual subunits. The subunits are labelled $\alpha$ to $\varepsilon$ in order of decreasing molecular weight. CF$_o$ binds CF$_1$ and functions to conduct protons across the thylakoid membrane. It contains four different polypeptides, labelled in Roman numerals, I-IV (Fromme et al., 1987). Subunits I, II and III are numbered in order of decreasing molecular weight. Subunit IV is the largest CF$_o$ protein. The net reaction is the transfer of electrons from a water molecule to NADP$^+$, producing the reduced form, NADPH. Driven by the H$^+$ gradient built up across the membrane by electron transfer reactions, the ATP synthase converts ADP into ATP.

1.6. Photosynthesis and photoinhibition

All oxygenic photosynthetic organisms exist in an environment in which many factors affecting the rate and capacity for photosynthesis vary on time scales from as short as a few seconds to periods of days or longer (Owens 1994). Principal among these is light intensity. The short-term response of photosynthetic organisms to light intensity is typically described by the photosynthesis-irradiance (P-I) curve (Fig. 1.8a) in which the steady-state rate of photosynthesis is plotted against incident light intensity. At low light intensities (>100 $\mu$mol m$^{-2}$s$^{-1}$) more than 80% of the absorbed quanta would be utilized, in accordance with the maximum measured quantum yields of oxygen evolution (Björkman and Demmig 1987). When the irradiance approaches half of full sunlight (- 1000 $\mu$mol m$^{-2}$s$^{-1}$) as little as 25% of the absorbed quanta are utilized, and at full sunlight, utilization drops to approximately 10%. At the same time, the rate of light absorption by the plants is essentially linear in incident intensity to well beyond the physiological range. The difference between the two curves (shaded area, Fig. 1.8b) represents the absorbed light energy which is in 'excess' of the capacity of photosynthesis to utilize the absorbed energy to drive photosynthesis.
Figure 1.8:
(a) A graph showing the rate of photosynthesis with increasing irradiance.
(b) The predicted response of absorption, utilization and excess of quanta to incident photon flux.
The principal consequences of excess light absorption are photoinhibitory damage to the PS II reaction centre (Krause 1988) and generalized damage to membranes and proteins by singlet oxygen, superoxide and other free radicals (Pell and Steffen 1991). Kok (1956) have defined photoinhibition as the light-dependent reduction in photosynthetic efficiency. Furthermore, photoinhibition of the photosynthetic capacity can become more severe in the presence of an additional stress. Under natural conditions in the field, additional environmental stress factors (other than light) such as low or high temperature, drought, etc., are often present which increase the gap between light absorption and utilization, and further increases the potential for over-excitation of the photosynthetic apparatus whenever environmental conditions suppress photosynthetic carbon metabolism. For instance, heat stress results in the loss of grana stacking due the dissociation of peripheral light-harvesting complexes from the core complex (Gounaris et al., 1984). Reduction of PS II activity by heat stress primarily results from an inactivation of the oxygen evolving complex (Enami et al., 1994), but also perturbations of electron transport within the PS II reaction centers have been documented (Pospišil and Tyystjärvi 1999). Low temperatures sensitize photosynthesis to photoinhibition, so that even low light may induce photoinhibition. This is thought to occur primarily through temperature-imposed thermodynamic constraints on carbon metabolism which prevent excitation energy from being effectively utilized for carbon dioxide assimilation and thus promote photoinhibition (Krause 1994; Huner et al., 1998). Photosynthetic activity is also reduced by water stress. One of the earliest responses to drought is stomatal closure, which limits carbon dioxide diffusion to chloroplasts (Muller and Whitsitt 1996). With short periods of drought, or with relatively mild water stress, stomatal limitations account for most of the decrease in photosynthesis (Cornic and Masacci
1996). However, when drought is prolonged and/or more severe, the breakdown of the photosynthetic apparatus may contribute to the inhibition of carbon dioxide gain in water-stressed plants.

There are two main classes of photoinhibition, largely on the basis of their relaxation times (Osmond 1994). The first is dynamic photoinhibition which occurs most rapidly in sun-grown healthy plants as they are exposed to variations in photon flux that drive their photosynthetic capacity up and down the light response curve without significant damage. Chronic photoinhibition usually predominates in shade grown plants and is more slowly reversible. It occurs following sustained exposure of the photosynthetic apparatus to photon fluxes in excess of those encountered during growth, or to photon fluxes within the growth experienced, but under environmental conditions which themselves impair photosynthetic function. During chronic photoinhibition, the light response curve of photosynthesis is dispersed downwards.

1.6.1. Photoinhibition at PS II

The primary target of high irradiance causing photoinhibition of photosynthesis is PS II (Powles 1984; Krause 1994; Sharma 2002). In this process, two mechanisms are involved affecting either the acceptor side or the donor side of PS II (Fig. 1.9). The two mechanisms are distinguished on the basis of differences in the primary site of electron transport malfunctioning, the subsequent D1 protein degradation, and the oxygen requirement of the process.

Acceptorside-induced photoinhibition of PS II occurs under high irradiance when it exceeds the saturation of photosynthetic electron transport (Barber and Andersson 1992). Excess exposure causes non-physiological over-reduction of the first quinone electron acceptor in PS II. This brings about sequential modifications at
the level of the QA and QB acceptors (Keren et al., 1997). These conditions lead to the recombination of the radical pair, P680* Pheo* (Vass et al., 1992) and the production of the triplet state of P680 (3P680*). This 3P680* is quenched by oxygen and singlet oxygen (1O2*) is thus produced. The 1O2* initiates and also triggers degradation of the reaction centre protein D1, probably by promoting a special conformational change which makes the protein susceptible to proteolytic cleavage (Fig. 1.9). The acceptor-side damage generates a 23.5 kDa N-terminal D1 fragment in vivo (Virgin et al., 1990). Two specific membrane-bound proteases, namely, endoprotease DegP2 and metalloprotease FtsH, degrade the D1 protein in the photodamaged PS II (Haussuhl et al., 2001). Rapid degradation of D1 under strong illumination can destabilize the PS II reaction center and expose D2 to the protease(s) (Ohad et al., 1990). Stability of D2 is necessary to stabilize the PS II complex in the membrane (Erickson et al., 1986). Therefore, whenever D1 and D2 are destabilized, PS II reaction centers may disassemble. The internal antennae, CP43 and CP47, also dissociate from PS II core and appear to move out to the stroma lamellae (Giardi 1993). The specific loss of PS II components is assumed to lead to the destabilization and degradation of LHC II, causing chlorosis (Kilb et al., 1996).

Donor side-induced photoinhibition of PS II occurs when the capacity of water oxidising complex to donate electrons to the RC P680 is inactivated by high irradiance (Minkov et al., 1999) (Fig. 1.9). Under such conditions the water oxidising complex is unable to keep up the rate at which electrons are transferred from P680 towards acceptor side components. This leads to an increase in the lifetime of P680* with a high oxidising potential. The P680* extracts electrons from the surrounding environment (amino acids, primarily histidines) which subsequently leads to
Figure 1.9: Scheme showing the two routes of damage due to acceptor and donor side photoinhibition of PS II.
destruction of D1, Chl and β-car associated with the reaction centre of PS II (Barber 1994).

D1 degradation in vivo, occurs at light fluencies much below the saturation threshold for photosynthesis (Mattoo et al., 1984; Jansen 1993). Photodamage to D1 occurs whenever a plastosemiquinone anion radical is generated in an oxygen-rich environment (Greenberg et al., 1990). The anion may be transiently produced by electron transfer to Q_b (either during cyclic or non-cyclic electron flow) or by direct interaction of UV light with plastoquinone molecules. The anion radical could then interact with oxygen to oxidize D1, possibly through the formation of peroxyl or hydroxyl radicals that can react with amino acid residues or metal ligands in D1. In vitro, a 24 kDa C-terminal D1 fragment can be generated by a cleavage in the loop between helices A and B on the luminal side of the membrane (Shipton and Barber 1992). This fragmentation has been attributed to the donor-side damage.

1.6.2. Photoinhibition at PS I

Photosystem I potential activity is remarkably stable in excessive light as compared to PS II (Aro et al., 1993; Barth et al., 2001). However, many authors have provided evidence that in certain circumstances PS I can be photoinhibited as much as or even faster than PS II (Havaux and Davaud 1994; Barth and Krause 1999; Sonoike 1996). Potential activity of PS I in vivo can be assessed by measuring the P700 absorbance change around 810-830 nm (Klughammer and Schreiber 1994). A preferential photo-inactivation of PS I was observed at chilling temperature in potato leaves (Havaux and Davaud 1994) and in cold-sensitive Cucumis sativus L., when leaves were chilled under low light (Sonoike 1996) or both under low and high light (Barth and Krause 1999). Mechanism of photodamage to PS I involve the destruction of iron-sulphur
centres \((F_A, F_B, F_X)\) (Sonoike et al., 1995), which triggers proteolysis of the PS I-A/B reaction centre proteins and of extrinsic polypeptides of the PS I complex, due to the generation of reactive oxygen species (Sonoike 1996).

1.7. Reactive oxygen species in photosynthesis

In plants the production of reactive oxygen species (ROS), such as superoxide \((O_2^-)\), hydrogen peroxide \((H_2O_2)\), hydroxyl radicals \((\cdot OH)\), and singlet oxygen \((^1O_2^\cdot)\), is an unavoidable consequence when the absorption of light energy exceeds the capacity for photosynthesis (Foyer et al., 1994; Cheeseman 2006). In the light-harvesting antenna of PS II, unused light energy increases the lifetime of the excited state of chlorophyll \((^1\text{Chl}^\cdot)\), which can be converted to the triplet excited state \((^3\text{Chl}^\cdot)\) through the photo physical process of intersystem crossing. \(^3\text{Chl}^\cdot\) itself is not harmful, but it is long-lived and can transfer energy to ground-state oxygen to generate highly reactive singlet oxygen as shown below

\[
\begin{align*}
^1\text{Chl} + hv & \rightarrow ^1\text{Chl}^\cdot \\
^1\text{Chl}^\cdot & \rightarrow ^3\text{Chl}^\cdot \\
^3\text{Chl}^\cdot + ^3\text{O}^\cdot & \rightarrow ^1\text{Chl} + ^1O_2^\cdot
\end{align*}
\]

In addition to the deleterious formation of singlet oxygen, in the Mehler reaction the direct reduction of oxygen by PS I, when the rate of carbon dioxide fixation slows down and the NADP\(^+\) supplement available to accept electrons from the electron transport chain is restricted, results in the formation of superoxide anion radical. Superoxide radical can then undergo a dismutation reaction forming hydrogen peroxide. Superoxide radical is unique in sense that it may act as both an oxidant and reductant. Hydrogen peroxide can readily diffuse across biological membranes and may cause oxidative stress far from site of formation. Both \(O_2^-\) and \(H_2O_2\) are highly reactive, oxidizing cations such as \(Fe^{2+}\), form the hydroxyl radical in the Haber-Weiss
reaction (Halliwell and Gutteridge 1985). Hydroxyl radical is the most reactive of the reactive oxygen species described having capacity to oxidize and react with organic molecules. It is thought that much of the damage that occurs via the production of $O_2^-$ and $H_2O_2$ results from their subsequent conversion to $^\cdot OH$. Singlet oxygen, superoxide, and other reactive forms of oxygen irreversibly oxidize DNA and membrane-bound lipids, proteins and pigments in their immediate vicinity.

1.7.1. Damage to organic molecules by ROS

1.7.1.1. Lipids

The reaction of ROS, especially of hydroxyl radical, with lipids is one of the most prevalent mechanisms of cellular injury and is dependent on the degree of membrane fluidity, which in turn is a function of the saturation state of the lipid bilayer (Halliwell and Gutteridge 1999). The degradation products of lipid peroxidation are aldehydes, such as malonaldehyde, and hydrocarbons, such as ethane and ethylene (Gutteridge and Halliwell 1990). Thylakoid lipids are especially susceptible to oxidative damage because of the abundance of unsaturated fatty acid side chains. Reactive oxygen attack of these lipids initiates peroxyl-radical chain reactions, which eventually can destroy the thylakoid membrane (Knox and Dodge 1985). The mechanism involving lipid peroxidation is that oxygen radicals catalyze oxidative modification of lipids, as shown in Figure 1.10. Presence of double bond adjacent to methylene group makes methylene C-H bonds of PUFA weaker and therefore hydrogen becomes more prone to abstraction. While lipid peroxidation is not initiated by $O_2^-$ and $H_2O_2$, $^\cdot OH$, alkoxy radicals (RO') and peroxy radicals (ROO') result in initiating lipid peroxidation (Halliwell 2006). This lead to self perpetuating process since peroxy radicals are both reaction initiators as well as end products of lipid peroxidation. Lipid peroxy radicals react with other lipids, proteins and nucleic acids,
Figure 1.10: Initiation and propagation reaction of lipid peroxidation.
propagating thereby transfer of electrons and bringing about oxidation of substrates. Cell membranes, which are structurally made up of large amounts of poly unsaturated fatty acids, are highly susceptible to oxidative attack, and consequently, bring about changes in membrane fluidity, permeability and cellular metabolic functions (Halliwell 2006).

1.7.1.2. Proteins

In the PS II RC, the formation of $^1\text{O}_2^*$ under excess light is thought to be the cause of direct damage to structural protein components (D1 and D2), which require de novo protein synthesis to be repaired (Melis 1999). Oxidative attack on proteins results in site specific amino acid modifications, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electrical charge, and increased susceptibility to removal and degradation. The amino acids in a peptide differ in their susceptibility to attack, and the various forms of ROS also differ in their potential reactivity. The primary, secondary, and tertiary structure of a protein determines the susceptibility of each amino acid to attack by ROS (Halliwell and Gutteridge 1999). For many enzymes, the oxidation by $\text{O}_2^-$ of iron-sulphur centres inactivates enzymatic function and other amino acids, such as histidine, lysine, proline, arginine, and serine, and form carbonyl groups when oxidized. A wide range of proteins and their amino acid building blocks are damaged or degraded by ROS, and the accumulation of these proteins in cells has been hypothesized to be part of the aging process (Lesser 2006). Under high irradiance $\text{P}_680^+$ with a high oxidizing potential is capable of extracting electrons from the surrounding amino acids, primarily histidines, which subsequently leads to the destruction of D1 protein of PS II (Barber 1994).
1.7.1.3. DNA

The generation of ROS can induce numerous lesions in DNA that cause deletions, mutations, and other lethal genetic effects. Both the sugar and the base moieties are susceptible to oxidation, causing base degradation, single-strand breakage, and cross-linking to proteins (Imlay 2003). In vitro, $\text{H}_2\text{O}_2$ or $\text{O}_2^-$ cannot by themselves cause strand breaks under normal physiological conditions, and therefore, their toxicity in vivo is most likely the result of Fenton reactions in the presence of a transition metal (Imlay 2003). Both prokaryotic and eukaryotic cells have DNA repair enzymes; for a cell with DNA damage, it is the balance between damage and repair that determines the fate of that cell (Beyer et al., 1991).

1.8. Protective processes

To achieve high efficiency of photosynthesis, plants have developed several adaptive and protective mechanisms to adapt their photosynthetic apparatus to variable light conditions, which can rapidly change both in duration and intensity during the day. These include adjustment of leaf orientation (Öquist and Huner 1991), chloroplast movement as a light-avoidance response (Park et al., 1996), adjustment to a smaller antenna (Park et al., 1997), photorespiration as a safety valve (Osmond and Grace 1995), the Mehler reaction (Park et al., 1996), 

$\text{}$

$\text{de novo}$ synthesis of D1 protein (Greer 1986), scavenging of reactive oxygen species by antioxidants (Asada 1996) and dissipation via zeaxanthin in the xanthophyll cycle (Demmig et al., 1987a; Gilmore 1997). In the following sections, few of these photoprotective strategies are singled out for discussion.
1.8.1. D1 protein repair cycle

To avoid total disassembly and inactivation of PS II, plants have evolved a PS II (or D1) repair cycle to rapidly replace the damaged D1 protein (Barber and Andersson 1992). In the D1 protein repair cycle, non-functional dimeric PS II complexes with damaged D1 protein are phosphorylated, phosphorylated peripheral LHC II Chl a/b proteins are detached (Rintamäki et al., 1999) and then the phosphorylated PS II core dimer is monomerized in the appressed granal domain (Baena-González and Aro 2002). The phosphorylated PS II monomers laterally migrate to non-appressed stroma thylakoids where CP43 is first dephosphorylated and then detached from the damaged PS II cores. The D1 and D2 proteins are initially dephosphorylated, damaged D1 protein is degraded and new D1 protein is simultaneously synthesized on chloroplast ribosomes bound to the stroma lamella membrane, then processed and assembled with its heterodimeric partner, D2 protein. Following photoinactivation the LHC II dissociates from the photoinactivated PS II; cleavage of the D1 protein induces also the dissociation of the oxygen evolving complex proteins that are not degraded and may reassociate with a newly assembled PS II. The PS II complex further dissociates into sub complexes that may diffuse laterally toward the stroma lamellae where either the processed D1 protein may reassemble with PS II subunits; the reassembled complex diffuses within the membrane plane, and upon reaching the grana domain, reassociates with LHC II (Adir et al., 2003). Then CP43 is re-attached and PS II monomers migrate back to appressed granal domains (Baena-González and Aro 2002). Thus, the highly light-regulated cycle between functional PS II dimers and non-functional PS II containing damaged D1 protein located in the appressed granal membranes, and the replacement of damaged D1 protein by D1 protein degradation
and \textit{de novo} synthesis in non-appressed membrane domains, involve marked dynamic structural and compositional heterogeneity of PS II.

Both synthesis and degradation of the D1 protein are light dependent. Under normal conditions, a balance is maintained between the rates of D1 synthesis and degradation, which allows intact and functional PS II. Under photoinhibitory conditions, D1 degradation rates increase. This increment is perceived as either a prime cause for PS II inactivation (Kyle et al., 1984) or a part of the repair cycle (Kettunen et al., 1996).

1.8.2. Antioxidant system

To overcome photooxidative damage mediated by reactive oxygen species, plants have developed an elaborate defense system to scavenge reactive oxygen species and other free radicals that can potentially form in the chloroplast of higher plants. This system of scavengers includes enzymatic and non-enzymatic antioxidants that act in an integrated manner to safely remove these dangerously reactive compounds before they damage cellular constituents. There are mainly three enzyme systems in plant cell to scavenge the free radicals; these are superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase. The non-enzymatic antioxidants are carotenoids, ascorbic acid, reduced glutathione (GSH) and α-tocopherol (vitamin E) which help in the dissipation of excess energy directly where the energy is absorbed, i.e. in the light-collecting chlorophyll/carotenoid-binding complexes.

1.8.2.1. Superoxide dismutase (SOD)

SOD catalyses the dismutation of superoxide to hydrogen peroxide and water.

\[ 2H^+ + 2O_2 \rightarrow H_2O_2 + O_2 \]
Since SOD is present in all aerobic organisms and most sub cellular compartments that generate activated oxygen, it has been assumed that SOD has a central role in the defense against stress (Bowler et al., 1992). There are three distinct types of SOD classified on the basis of the metal cofactor: Copper-zinc, Manganese and iron isozymes. The Manganese-SOD is found in the cytosol, others in the chloroplast of higher plants. Iron-SOD is usually associated with the chloroplast compartment (Bowler et al., 1992).

1.8.2.2. Catalase (CAT)

Catalase is a heme-containing enzyme that carries out the dismutation of hydrogen peroxide into water.

\[ 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]

The enzyme is found in all aerobic eukaryotes and is important in the removal of hydrogen peroxide generated in peroxisomes by oxidase involved in \( \beta \)-oxidation of fatty acids, the glyoxylate cycle (photorespiration) and purine catabolism. The stress conditions which reduce the rate of protein turnover cause the depletion of catalase activity and thus catalase is not very effective enzyme in removing the free radicals under such conditions (Sharma and Singhal 1992).

1.8.2.3. Ascorbate peroxidase (APX)

As catalase is limited in effectiveness to metabolize \( \text{H}_2\text{O}_2 \) by its relatively poor affinity for \( \text{H}_2\text{O}_2 \) and its sub cellular location in the peroxisomes and also susceptible to photo-inactivation and degradation (Streb et al., 1993), ascorbate peroxidase is the main enzyme to scavenge the \( \text{H}_2\text{O}_2 \) in chloroplasts. Simultaneous oxidation and reduction of the ascorbate and glutathione pools when \( \text{H}_2\text{O}_2 \) was added suggested that enzymes of ascorbate-glutathione cycle were involved in coupling electron transport.
to H$_2$O$_2$ destruction. Observation of direct photodestruction of MDHA radical by the thylakoid membranes suggested that this probably is the preferred pathway of ascorbate regeneration. H$_2$O$_2$ generated during the Mehler reaction is considered to be scavenged primarily at the thylakoid level while the stromal ascorbate peroxidase represents a second level of defense against H$_2$O$_2$ escaping thylakoid (Sharma 2002).

1.8.2.4. Ascorbic acid

L-ascorbic acid, or vitamin C, is an essential vitamin in animals and is abundant in plant tissues. All plants and animals, except humans, can synthesize ascorbate *de novo*; animals also can obtain vitamin C through their diet. Ascorbate functions as a reductant source for many ROS, thereby minimizing the damage caused by oxidative stress. Ascorbate scavenges not only H$_2$O$_2$ but also O$_2^-$, HO, and lipid hydroperoxides without enzyme catalysts (Fridovich 1998), and it can indirectly scavenge ROS by recycling α-tocopherol to its reduced form. Ascorbate has been found in plant cell chloroplasts and cytosol, where it also acts as a substrate for ascorbate peroxidase.

1.8.2.5. Glutathione

Glutathione (GSH) is a tripeptide (Glutamine-Cysteine-Glycine) found in animals and plants. It forms a thyl radical that reacts with a second oxidized glutathione, forming a disulphide bond when oxidized (GSSG; Halliwell and Gutteridge 1999). The ratio of GSH/GSSG is often used as an indicator of oxidative stress in cells, and glutathione functions as an antioxidant in many ways by reacting with $^{1}\text{O}_2$, O$_2^-$, and HO$. Glutathione can also act as a chain-breaker of free radical reactions and is an essential substrate for glutathione peroxidase (Halliwell and Gutteridge 1999). The
maintenance of GSH levels, and therefore the reducing environment of cells, is crucial in preventing damage to cells exposed to conditions that promote oxidative stress.

1.8.2.6. Tocopherol

The tocopherols, specifically \( \alpha \)-tocopherol (vitamin E), are lipid soluble antioxidants that scavenge ROS (Halliwell and Gutteridge 1999). This phenolic antioxidant is found in both animals and plants. \( \alpha \)-tocopherol, due to its hydrophobic nature, is located exclusively within the bilayers of cell membranes. \( \alpha \)-tocopherol is generally considered to be the most active form of the tocols. Plants synthesize \( \alpha \)-tocopherol in chloroplasts, with the aromatic ring formed by the shikimic acid pathway - the same pathway that produces ultraviolet-absorbing compounds, the mycosporine-like amino acids, in many marine algae. By contrast, animals must acquire tocopherol through their diet. The antioxidant properties of tocopherol are the result of its ability to quench both singlet oxygen and peroxides (Halliwell and Gutteridge 1999). A marine-derived tocopherol known as \( \alpha \)-tocomonoenol has been isolated from salmon eggs and provides enhanced antioxidant protection because of its ability to diffuse in viscous lipids and prevent lipid peroxidation (Yamamoto et al., 2001).

1.8.2.7. Carotenoids

In photosynthetic organisms, some carotenoids function as accessory pigments in light harvesting, whereas others quench ROS produced as a result of overexcitation of the photosynthetic apparatus by light (Demmig and Adams 1993). Two \( \beta \)-Car molecules are present in the RC of PS II, which absorb light and transfer the excitation energy to Chl \( a \). These \( \beta \)-Car molecules also protect the RC Chl from high light damage (Telfer et al., 1994). \( \beta \)-Car protects the PS II RC against photo-oxidative
damage via quenching of $^1\text{O}_2^*$ or $^3\text{Chl}^*$ but it is unable to quench the triplet excited state of P680 (Yamamoto and Bassi 1996; Telfer 2002).

Chlorophyll triplet quenching

\[
\begin{align*}
^3\text{Chl}^* + \beta-\text{Car} & \rightarrow ^1\text{Chl} + ^3\beta-\text{Car}^* \\
^3\beta-\text{Car} & \rightarrow \beta-\text{Car} + \text{heat}
\end{align*}
\]

Singlet oxygen scavenging

\[
\begin{align*}
^1\text{O}_2^* + \beta-\text{Car} & \rightarrow \text{O}_2 + ^3\beta-\text{Car}^* \\
^3\beta-\text{Car} & \rightarrow \beta-\text{Car} + \text{heat}
\end{align*}
\]

As shown in the above equations, energy is transferred to ground state carotenoids and dissipated by radiationless decay from the resulting triplet carotenoids as heat to the medium (Baroli and Niyogi 2000).

Lutein (Chow 1994; Niyogi et al., 1997), zeaxanthin and violaxanthin (in decreasing order of effectiveness) are also involved in protecting against Chl photobleaching by quenching both $^3\text{Chl}^*$ and $^1\text{O}_2^*$, whereas neoxanthin functions mainly in $^1\text{O}_2^*$ quenching (Croce et al., 1999). Carotenoids thus are an important ‘safety valve’ dissipating the excess energy of the excited pigment and the products of the reaction with oxygen. Carotenoids can also dissipate excess excitation energy through the xanthophyll cycle (Krinsky 1989) that prevents the overexcitation of the photosynthetic apparatus.

1.9. Xanthophyll cycle-dependent energy dissipation

Xanthophylls are oxygen containing carotenoids. The harmless dissipation of excessive absorbed light energy in the light-collecting pigment bed involves the carotenoids of the xanthophyll cycle, consisting of violaxanthin (V), antheraxanthin
(A) and zeaxanthin (Z) (Fig. 1.11), which was discovered by Sapozhnikov in 1957. He described a decrease in the content of V in plants after high light treatment and its subsequent increase in low light or darkness. Yamamoto et al., (1962) showed that decrease in the V concentration in plants in intensive light was connected to the V transformation into a different xanthophyll pigment Z. No function was coupled to the cycle until Demmig et al., (1987a) and Sharma and Hall (1991) found a correlation between Z formation and the decrease in re-emission of light absorbed by chlorophyll, i.e. non-photochemical quenching (qN), whereby excessive excitation energy is siphoned away from chlorophyll and harmlessly dissipated as heat in the light-collecting pigment bed.

The components of the xanthophyll cycle make up from 10 to 40% of the total carotenoids in the leaves, depending on species and growth conditions. Growth in high light results in a larger pool of xanthophyll-cycle pigments (Thayer and Björkman 1990). The cycle is also termed the V cycle to distinguish it from another xanthophyll cycle, which comprises only one de-epoxidation step, the diadinoxanthin cycle of the algae. The pigments of the xanthophyll cycle have been localized to the light-harvesting complexes of both photosystems (Thayer and Björkman 1992; Lee and Thornber 1995). Their location within the antennae presumably allows for effective dissipation of excessive light energy thereby minimizing the formation of singlet oxygen and preventing the over-reduction of the electron transport chain.

During exposure to excess light, violaxanthin (a diepoxide) is enzymatically de-epoxidised to antheraxanthin (a monoepoxide) and further to zeaxanthin which is epoxide free. The conversion of V to Z takes place when higher plants are illuminated with high light intensities, while low light intensities or darkness stimulates the
Figure 1.11: The xanthophyll cycle. Violaxanthin is converted to zeaxanthin through an intermediate antheraxanthin by the enzyme violaxanthin de-epoxidase (VDE). The reverse reaction is catalysed by the enzyme zeaxanthin epoxidase (ZE). Violaxanthin is converted to zeaxanthin in high light and reverts back in the dark.
reverse reaction which leads to the conversion of Z back to V. Two enzymes localized on opposite sides of the thylakoid membrane are engaged in this process: violaxanthin de-epoxidase (VDE) present on the thylakoid lumen side of the membrane, catalyses the de-epoxidation of V to Z and zeaxanthin epoxidase (ZE), carrying out the reverse reaction of epoxidation of Z to V, is localized on the stromal side of the thylakoid membrane (Hager 1966).

Violaxanthin de-epoxidase is a 43 KDa nuclear-DNA encoded protein (Rockholm and Yamamoto 1996) and has a pH optimum of 5.2 (Hager and Holocher 1994). The enzyme requires ascorbate as a co-substrate to reduce the epoxy group, which is then eliminated as water (Neubauer and Yamamoto 1994; Bratt et al., 1995). Violaxanthin de-epoxidase is a water soluble enzyme at neutral or alkaline pH (Hager and Holocher 1994). Under high light conditions, the enzyme is activated by a decrease in the pH of the thylakoid lumen, whereas, it is inactive in the dark, when the pH in the thylakoid lumen is neutral or alkaline (Hager and Holocher 1994). Violaxanthin de-epoxidase can be either unbound or bound to the thylakoid membrane depending on the luminal pH. Connection to the membrane is important for enzymatic activity (Hager and Holocher 1994). At pH lower than 6.0, all VDE molecules are associated with the membrane. If pH increases to 7.0 or more, the VDE exists in an unbound form. ΔpH is caused by proton migration during light-induced electron transport once irradiance exceeds that required to saturate photosynthesis. Therefore once irradiance is excessive and a pH gradient is generated, this in turn triggers the de-epoxidation of V to A and Z, which reduces the efficiency of PS II and protects the RC from photooxidation (Demmig-Adams and Adams 1994). Changes in the de-epoxidation state of the xanthophyll cycle and/or the magnitude of the ΔpH allow plants to alter the level of thermal energy dissipation and closely track changing
light conditions in their natural environment such that only excess absorbed light energy is dissipated and carbon fixation is presumably uncompromised.

Besides the decrease in the lumenal pH, VDE activity also requires the presence of the galactolipid monogalactosyldiacylglycerol (MGDG), which has been proposed to facilitate the solubilization of the substrate V and/or to represent a functional component of the enzyme VDE itself (Yamamoto and Higashi 1978). Zeaxanthin epoxidase requires oxygen and ferredoxin/NADPH as a reductant, and is optimal near pH 7.5 (Siefermann and Yamamoto 1975; Hager 1975). With the build up of protons gradient within the thylakoid lumen, the cycle shifts towards Z and A, and energy dissipation occurs (Gilmore et al., 1996). Both A and Z protect the plants from photoinhibition by dissipating the excess light energy as heat (Gilmore 1997; Goss et al., 1998). This protective function has been demonstrated in plants subjected to various conditions of high light (Demmig-Adams and Adams 1992; Sharma et al., 2002) and light in combination with various other stress factors such as restricted carbon dioxide (Demmig et al., 1987a), chilling (Verhoeven et al., 1996; Havaux and Niyogi 1999), drought or nutrient deficiency (Demmig-Adams and Adams 1992), and high salinity (Björkman et al., 1988). A range of factors influences the degree of conversions of V to Z such as temperature, water stress and light quality, as well as plant species (Thayer and Björkman 1990).

Most of the xanthophylls are bound to the Chl a/b binding antenna proteins of both photosystems, while only a fraction of the pool of xanthophyll cycle pigments V, A and Z may also be present in the lipid phase of the membrane (Havaux 1998; Macko et al., 2002). In higher plants, ten different antenna proteins constitute the family of Chl a/b binding proteins: Lhcb1-6 in PS II and Lhca1-4 in PS I (Jansson
1994). Analysis of X-ray structure of the trimeric LHC II from spinach (Liu et al., 2004), which consists of Lhcb1-3 proteins, identified four different xanthophyll-binding sites in antenna proteins: two lutein are bound to the central L1 and L2 sites associated with the two central transmembrane helices A and B, neoxanthin is bound to the more peripheral helix B and protrudes into the lipid phase, while V is located at the monomer interface (Standfuss et al., 2005). The convertibility of V to Z depends on both the binding site and protein-specific properties: V bound to the V1 site seems to be easily accessible for de-epoxidation in all antenna proteins analysed so far, while V bound to L2 is only slowly or not at all convertible to Z (Jahns et al., 2001). These observations indicate the existence of different pools of antenna-bound V, a convertible fraction involved in photoprotective mechanisms and a non-convertible pool that should serve other functions, either related to light-harvesting and/or the stabilization of antenna proteins.

1.9.1. Molecular mechanism of the xanthophyll cycle

Even though intensive research is being carried out on the molecular mechanism of the xanthophyll cycle, it is still far from being completely understood. To convert V into Z, VDE has to remove two epoxy groups attached to two rings of the V molecule. In accordance with requirement of the MGDG for the xanthophyll cycle, it has been proposed that V is located in the micelles of this galactolipid (Yamamoto et al., 1974; Latowskiet al., 2002). In such structures, believed to consist on average of 28 MGDG molecules, V can oscillate and finally come in contact with substrate binding site of the VDE. Then, one of the two epoxy groups of the ionone rings can be de-epoxidated and V changed into A. For the transformation of A to Z, it has been suggested that A undergoes a flip-flop mechanism. This flip-flop mechanism involves the change in orientation of A molecules within the thylakoid membrane (Gilmore 1997; Fig.
1.12a). Flip-flop of A is necessary for its de-epoxidation because it requires the epoxide group of A to face the VDE enzyme (Yamamoto and Higashi 1978). Researchers (Thayer and Björkman 1992) suggested that in vivo V de-epoxidation occurs within thylakoid pigment-protein complexes, but a great number of experiments demonstrated that the reaction takes place in the lipid part of the membrane. The xanthophyll cycle pigments are not freely located in thylakoid membranes but are bound by proteins of light harvesting complexes, however, these pigments can easily dissociate from their loci (Ruban et al., 2002). Perpendicular location of V in the thylakoid membrane was confirmed by experiments with VDE added from the stromal side of the thylakoids (Akerlund et al., 1995).

For its activity ZE requires NADPH, flavin adenine dinucleotide (FAD), ferridoxin and oxygen (Hager 1975; Bouvier et al., 1996). It has been suggested that electrons from NADPH are transferred through ferridoxin:NADP⁺ oxidoreductase to ferredoxin, then to FAD (Bouvier et al., 1996). Reduced FAD is able to bind oxygen molecule and to form hydroperoxyl moiety. Part of this moiety is transferred to Z, as a hydroxyl radical. Zeaxanthin forms unstable carbocation and then proton from Z is transferred through FAD to oxygen and finally water molecule is formed. After loss of proton Z becomes A in the membrane protein complexes. Similarly to de-epoxidation reaction, A has to perform a flip-flop type movement to be further epoxidated to V. However, until now there is no knowledge how second ionone ring can be exposed to ZE. Two mechanisms are possible: (i) A molecule turns in precincts of protein complex, (ii) it leaves the complex and translocates to lipid domains, containing reversed hexagonal structures where the flip-flop takes place. It is also possible, that both proteins and lipids are engaged in the A flip-flop (Latowski et al., 2004).
1.9.2. **PsbS protein and its role in the xanthophyll cycle**

A light harvesting protein, PsbS, was shown to have a key role in the dissipation of excess energy in higher plants (Li et al., 2000; Külheim et al., 2002). PsbS is a 22-kDa PS II subunit that belongs to the light harvesting complex protein superfamily (Kim et al., 1992). The schematic model for qE in PS II of plants is depicted in Fig. 1.12b. In limiting light, the steady-state thylakoid lumen pH is greater than 6 (Kramer et al., 1999). Violaxanthin is bound mainly to the V1 site in LHC II and the L2 site in other LHC proteins (such as CP29 and CP26) (Caffari et al., 2001; Morosinotto et al., 2002). In excess light, the thylakoid lumen pH drops below 6, driving protonation of carboxylate side chains in VDE and PsbS. Protonation of VDE activates the enzyme and allows for its association with the membrane (Hager and Holocher 1994), where it converts multiple V molecules to Z. Protonation of glutamate residues E122 and E226 in PsbS activates symmetrical binding sites for xanthophylls with a de-epoxidised β-ring end group (i.e. Z). Binding of Z to protonated sites in PsbS results in the qE state in which de-excitation of $^1$Chl* occurs. The binding of Z to PsbS gives rise to a strong red shift in the absorption spectrum, providing an explanation of the $\Delta A_{535}$ which is strongly correlated with qN and which has been shown to at least partially arise from a sub-pool of red-shifted zeaxanthin (Ruban et al., 2002).

There are two main thoughts concerning the nature and mechanism by which a simple alteration in the xanthophylls composition of LHC affects the balance between photon capture and energy dissipation at different irradiances (Young et al., 1997; Horton et al., 1999).
Figure 1.12:

(a) Schematic model showing the flip-flop movement of Antheraxanthin in the thylakoid membrane.

(b) Schematic model showing the role of PsbS in xanthophyll cycle and qE. In excess light, a low thylakoid lumen pH leads to binding of H to the carboxylates of two glutamate residues in PsbS and zeaxanthin synthesis from violaxanthin is induced. Binding of Z to sites in PsbS results in the qE state in which the de-excitation of excited Chl occurs.
1.9.3. Direct quenching

The possibility that differences in the $S_1$ energy levels of V and Z might account for the operation of the xanthophyll cycle was first proposed by Demmig-Adams (1990) and later elucidated by Owens et al., (1992). They suggested that Z has a $S_1$ energy identical to that of $\beta$-Car as these molecules are essentially iso-electronic. The $S_1$ state of Z lies just below that of Chl $a$ and allowing the Z molecule to act as a sink for excitation energy of Chl $a$ (Fig. 1.13a). The energy content of Chl $a$ is lower than the value for the $S_1$ state of V but higher than that of Z. This would suggest that it is energetically possible for the $S_1$ state of Z to quench Chl fluorescence by deactivation of $^1$Chl*. In contrast the higher $S_1$ level of V would only permit it to function as a light-harvesting pigment, transferring its excitation energy to Chl $a$. Thus, at high PFD, when dissipation of excess excitation energy is required, Z is formed within LHC II and its formation serves to deactivate the $^1$Chl* $a$ and dissipate excitation energy harmlessly as heat.

1.9.4. Indirect quenching

The structural differences between V and Z may provide an explanation as to how the xanthophyll cycle pigments could indirectly control energy-dependent quenching (qE). It is suggested that Z induces structural changes of the light-harvesting system of thylakoid membrane (Fig. 1.13b) that favour the radiation-less dissipation process (Ruban et al., 1996). The changes in structure of LHC probably favour their aggregation. This aggregation is possible because of the interactions between the LHC trimers. This mechanism couples the changes in the aggregation of LHC II with the increase in qN. In the light-harvesting Chl $a/b$ binding protein, the Chl molecules are situated at close proximity, but are separated from each other by xanthophyll molecules. These strong anti-quenchers (xanthophylls) prevent close
Figure 1.13:
(a) Comparison of the predicted energy transfer pathways between carotenoids of the xanthophyll cycle and chlorophyll a
(b) The model shows how the structures of xanthophyll cycle pigments (Z) binding to protonated chlorophyll proteins of the PS II inner antennae cause non-photochemical quenching of PS II Chl a fluorescence.
Chl-Chl interaction and quenching (Searle et al., 1991) but do not interfere with optimal energy transfer. The key process of energy dissipation by xanthophyll cycle is protonation-promoted changes in the protein structure leading to a Chl/xanthophyll aggregation and thereby allowing direct quenching of \(^1\)Chl* by Z and energy dissipation (Eskling et al., 1997; Gilmore 1997).

1.9.5. Other functions of Z

Other than energy dissipation Z is proposed to involve in various other functions.

1.9.5.1. As an antioxidant

Antioxidant roles for Z has been reported during long-term high irradiance stress (time-scale of days) (Li et. al., 2002). Using *Arabidopsis npq1* mutants lacking Z, Havaux et al., (2000) showed that the xanthophyll cycle specifically protects thylakoid membranes against lipid peroxidation. The *Arabidopsis npq4* mutants that were defective in qE but possessed a normal xanthophyll cycle activity showed tolerance to lipid peroxidation, demonstrating that photo-protection mediated by the xanthophyll cycle is not solely a result of the involvement of Z in qE (Havaux et al., 2000).

1.9.5.2. Thylakoid membrane fluidity

Modulatory effects of carotenoids on physical properties of model and natural membranes have been known since the seventies. Soon after xanthophyll cycle characterisation, there was a suggestion that this process may regulate physical properties of thylakoid membranes (Siefermann and Yamamoto 1975; Yamamoto 1979). This hypothesis has been confirmed by other studies (Gruszecki and Strzalka 1991; Tardy and Havaux 1997). In natural and model membranes, Z appears to have the strongest influence on such membrane properties as temperature of phase
transition, molecular dynamics, permeability and polarity gradient (Havaux and Gruszecki 1993; Strzalka and Gruszecki 1997).

1.9.5.3. Blue light receptor

Another postulated function of the xanthophyll cycle is blue light reception. It is suggested that Z is responsible for the blue-light-dependent stomata opening (Quinones et al., 1996), chloroplast movement (Tlalka et al., 1999) and phototropism (Quinones and Zeiger 1994). HPLC measurement of Z level as function of chloroplast movement in strong and weak light in *Lemna trisulca* proved a good correlation between these two variables (Tlalka et al., 1999). Because of this finding, Z is supposed to be a photoreceptor in blue-light stimulated chloroplast movement. The degree of increase in Z level (regulated by red light, darkness periods, and use of DTT) correlated well with the blue-light stimulated phototropism of maize coleoptiles (Quinones and Zeiger 1994). The DTT-related inhibition of Z synthesis consequently inhibited blue-light dependent stomata opening in *Vicia faba* epidermis, which is considered to be a proof of the photoreceptor function of Z (Srivastava and Zeiger 1995). However, it has also been shown in experiments correlating the level of protein phosphorylation and blue-light induced phototropism, that there is no connection between Z, or any other carotenoid, and phototropism (Palmer et al., 1996).

1.10. Chlorophyll fluorescence

Light absorption by a Chl molecule transforms it into an energy-rich excited state. An excited Chl molecule is not stable and electrons return rapidly to their ground level releasing absorbed photon energy in a number of ways:
1. Electronic excitation energy can be transferred to another acceptor molecule, this results in photosynthetic electron transport. This part can be measured as photochemical quenching (qP).

2. Excitation energy can be released as heat (thermal dissipation), which can be measured as non-photochemical quenching (qN).

3. It can be released by emitting energy as photon of lower energy content (i.e. higher wavelength), which is known as fluorescence.

4. Another way by which an excited Chl molecule can lose its energy is by transfer from its original excited singlet state (total electronic spin is s=0) into a metastable triplet state (s=1) with a much longer lifetime by a mechanism called intersystem crossing. From the metastable triplet state the chlorophyll molecule can revert to the natural ground state by emitting a photon at a longer wavelength. This weak emission is known as delayed fluorescence or phosphorescence.

The emission maximum of the fluorescence spectrum of a molecule is always at longer wavelength than the wavelength of the corresponding absorption spectrum. The most common physical observable used to assess photosynthetic function and its subsequent down regulation in excess-light conditions is Chl fluorescence, because it is sensitive to a wide range of changes in the overall apparatus (Holt 2004). Photochemistry, heat and fluorescence de-excitation pathways compete for excitation energy. When all PS II RC are capable of a stable charge separation, fluorescence yield is minimal (Fo) and these centers are referred to as open reaction centers. When all reaction centers are in the closed state, i.e., a state in a stable charge separation is not possible, the fluorescence yield is maximal (Fm). The quenching of fluorescence caused by photochemistry in PS II results in variable fluorescence (Fv). The maximum variable fluorescence Fv=(Fm-Fo) is thus a measure for photochemistry in
open PS II centers. The quantum yield of photochemistry in open PS II centres is defined, as the probability of photochemistry after absorption of a photon, and is determined by the efficiency of excitation energy transfer from the antennae to the reaction centre and equals \((Fm-Fo)/Fm\) (Genty et al., 1989).

Processes that decrease the overall Chl fluorescence quantum yield are generally divided into two categories, photochemical quenching \((qP)\), which is exclusively associated with photochemical charge separation in the PS II reaction center, and non-photochemical quenching \((qN)\), which is broadly defined as all fluorescence quenching that is not directly related to charge separation. Factors contributing to \(qN\) are generation of strong pH gradient in thylakoid membrane, phosphorylation and dephosphorylation of light harvesting protein components and associated energy redistribution, concentration of cations \((Mg^{2+})\), photoinhibition, development of xanthophyll cycle, etc.

Different processes that contribute to \(qN\) relax at different rates in darkness following a period of irradiation. The kinetics of relaxation of these different processes can be therefore used to distinguish them. On basis of the kinetics, \(qN\) is divided into three types:

1. Energy-dependent quenching \((qE)\)
2. State-transition quenching \((qT)\)
3. Photoinhibitory quenching \((qI)\)

1.10.1. Energy-dependent quenching \((qE)\) and the role of xanthophyll cycle

The fast component of \(qN\) is called energy-dependent quenching \((qE)\) due to its dependence on the organization of thylakoid membrane (Horton et al., 1996; Müller et al., 2001). \(qE\) is a process of heat dissipation (or non-radiative dissipation) which is
important in the protection of the photosynthetic apparatus against photo-oxidative damage under excess irradiation. For induction of maximal qE, both low thylakoid lumen pH and the presence of xanthophylls are necessary (Krause and Jahns 2003). When the absorption of photon energy exceeds the capacity of the dark reactions to utilise ATP and NADPH produced by a photosynthetic electron transport, synthesis of ATP and NADPH are limited. This subsequently leads to a decrease in pH within the thylakoid lumen and the feedback regulation of light-harvesting by triggering the dissipation of excess absorbed energy as heat. The control by lumen pH allows induction and reversal of the energy dissipation within second to minutes.

qE is associated with the inter-conversion of xanthophyll pigments in the xanthophyll cycle. Low lumen pH activates the enzyme violaxanthin de-epoxidase which converts violaxanthin to zeaxanthin via the intermediate antheraxanthin (Eskling et al., 1997). V de-epoxidation can be blocked by an inhibitor of VDE, dithiothreitol (DTT). Blocking this de-epoxidation reaction results in inhibition of qE (Bilger and Björkman 1990). In addition to the inhibitor studies, the requirement for the de-epoxidized xanthophylls in qE has been proved by using xanthophyll cycle mutants (Niyogi et al., 1998). The involvement of Z and A formation via the xanthophyll cycle in qE was also confirmed in studies using VDE antisense tobacco plants which exhibit suppressed ability to form Z and A (Chang et al., 2000). qE relates better with the amount of Z and A than with the amount of Z alone (Gilmore and Yamamoto 1993). These findings support the idea that both A and Z are involved in qE. In addition to A and Z, also a third xanthophyll, lutein, because of its structural similarity to A, has recently been implicated in qE (Pogson et al., 1998; Niyogi et al., 2001; Lokstein et al. 2002). Direct evidence for the involvement of these pigments in qE has come from studies of mutants of the green alga Chlamydomonas reinhardtii.
(Niyogi et al., 1997) and the laboratory weed *Arabidopsis thaliana* (Niyogi et al., 2001) and from experiments with transgenic tobacco plants with reduced levels of VDE (Chang et al., 2000). The *Arabidopsis nqpl* mutant, which is defective in the gene encoding VDE, has very low levels of qE (Niyogi et al., 1998). The *Arabidopsis lut2* mutant is defective in the lycopene c-cyclase, is unable to synthesize β-carotene and lutein, and exhibits slower induction and a lower extent of qE (Pogson et al., 1998). Essentially all qE is abolished in the *nqpl lut2* double mutant (Niyogi et al., 2001).

In addition, qE requires the presence of a functional version of the PsbS protein (Li et al., 2000; refer to Fig. 1.12). The PsbS protein, presumably acting as a pH sensor (Li et al. 2004), links thermal dissipation to the trans-thylakoid pH gradient as a measure of excess light. Using PsbS-deficient Arabidopsis mutants, PsbS was found to play an important role in plant fitness in a fluctuating light environment, but not in constant high light (Kühlheim et al. 2002). Furthermore, over expression of PsbS in Arabidopsis resulted in greater levels of flexible qN (qE) as well as prevention of sustained depressions in maximal PS II efficiency or photoinhibition (Li et al. 2002).

qE is important for photo-protection by \(^1\text{Chl}\)* quenching, which results in harmless dissipation of excess excitation energy as heat. Up to 80% of the Chl excited states can be dissipated into heat, thereby protecting PS II RCs from over-excitation (Bassi and Caffari 2000). Intersystem crossing is one of possible pathways of \(^1\text{Chl}\)* de-excitaion, by which the \(^3\text{Chl}\)* is formed. \(^3\text{Chl}\)* can interact with ground-state triplet oxygen to produce \(^1\text{O}_2\)*, an extremely damaging reactive oxygen species (Baroli and Niyogi 2000). Thus, qE decreases the lifetime of \(^1\text{Chl}\)* and consequently the probability of \(^1\text{O}_2\)* generation in LHC II. Further, by decreasing the efficiency of
energy transfer to PS II RCs, qE may also prevent the over-reduction of the electron transport chain resulting in the recombination of the primary radical pair, P680⁺Pheo⁻, which can generate the excited triplet states of both the accessory Chl molecule located in the D1 protein and P680, the latter as a minor population (Aro et al., 1993). As in LHC II, interaction between $^3$Chl* and oxygen within PS II RCs results in formation of $^{1}\text{O}_2$*. qE may also prevent the over-acidification of the thylakoid lumen that can inhibit the electron transport at PS II donor side resulting in the generation of long-lived P680⁺ and/or Y$_Z$⁺ (oxidized secondary electron donor of PS II; Niyogi 1999). P680⁺ and Y$_Z$⁺ are themselves capable of oxidizing nearby pigments and proteins, causing the damage to PS II RCs (Aro et al., 1993).

1.10.2. State-transition quenching (qT)

The middle component qT, is associated with the phenomenon of state transitions and is therefore called state-transition quenching (Allen and Forsberg 2001). State-transitions are short-term processes that change the antenna sizes of PS II and PS I. qT induction and reversal caused by these alterations occur over time-scales ranging from minutes to tens of minutes. The basis of state-transitions lies in the reversible phosphorylation of light-harvesting complex of PS II (LHC II) and the movement of phosphorylated LHC II from PS II to PS I. The decrease of the PS II antenna size is accompanied by a reduction of the amount of excitation energy in PS II fluorescence intensity. Unlike qE, an involvement of qT in photo-protection is unclear. It was suggested that LHC II phosphorylation protects the photosynthetic apparatus against photoinhibition. However, some studies show that LHC II phosphorylation is inhibited upon short-term exposure to high irradiance. This effect was observed in higher plants both in vivo (Demmmig et al., 1987b) and in intact chloroplasts (Ebbert and Godde 1994). A contribution to qT to the overall qN is rather negligible under
high irradiance stress in most plants (Müller et al., 2001). qT seems to be important only in low irradiances, when it regulates the distribution of excitation energy between PS I and PS II and thereby optimises the photosynthetic reactions.

1.10.3. Photoinhibitory quenching (qI)

The fast component of qN saturates at high irradiance, whereas the slow component increases as the irradiance is increased (Walters and Horton 1991). The slow component is caused by photoinhibition and is therefore called photoinhibitory quenching denoted as qI (Ruban and Horton 1995). Under high irradiance the protective capacity of qE is saturated, under such conditions qI becomes significant. This quenching shows the relaxation in the range of hours to days and reflects both slowly relaxing photo-protective processes and damage to PS II RCs under stress conditions.

The photo-protective component of qI, like qE, reduces high irradiance induced damage in PS II by dissipating excess absorbed photon energy and it appears to possess some characteristics of qE. Some part of qI may be associated with ΔpH, which is necessary for qE induction. For efficient qE, the inter-conversion of xanthophylls in the xanthophyll cycle together with the formation of ΔpH is necessary. The involvement of the xanthophyll cycle activity in qI was also reported such that relaxation of qI has been correlated with the conversion of Z and A back to V (Jahns and Miehe 1996).

1.11. Thermal energy dissipation within PS I

At high irradiance, when photo-protective thermal dissipation of excess absorbed photon energy within PS II (qE) is engaged, PS I absorbs more photons compared to the electron flux from PS II. This excess photon energy absorbed by PS I can be
dissipated via cyclic electron transport around PS I, which is also suggested to have an important role in photoprotection (Niyogi 1999). In addition to dissipating energy absorbed by PS I, cyclic electron transport may be involved in generating or maintaining the ΔpH that is necessary for down regulation of PS II by thermal dissipation of excess absorbed light energy (Heber and Walker 1992). Biochemical approaches have led to the conclusion that there are at least two pathways of PS I cyclic electron transport, one involving a ferredoxin-plastoquinone oxidoreductase (FQR) and the other involving an NADPH/NADH dehydrogenase (NDH) complex (Bendall and Manasse 1995). The FQR has not yet been identified, although the Psae subunit of PS I is possibly involved. The NDH pathway involves a protein complex bound to the thylakoid membrane that is homologous to the NADH dehydrogenase complex I of mitochondria. Several subunits of this complex are encoded by genes on the chloroplast genome of many plants (Friedrich et al., 1995). Mutants affecting the NDH complex have been generated by disrupting ndh genes in the chloroplast genome of tobacco by homologous recombination (Burrows et al., 1998). These mutants have no obvious phenotype under normal growth conditions. However, measurements of Chl fluorescence and PS I reduction kinetics revealed that cyclic electron transport is partially impaired. Induction of thermal dissipation upon sudden illumination was slightly delayed in mutants subjected to water stress, consistent with the idea that PS I cyclic electron transport is involved in maintaining a ΔpH that is necessary for down-regulation of PS II activity by qE (Burrows et al., 1998; Heber and Walker 1992).

1.12. ABA and the xanthophyll cycle

ABA is a plant hormone involved in many physiological and developmental processes such as transpiration, germination, dormancy and also with the adaptation of plants to
Figure 1.14: The abscisic acid biosynthetic pathway in higher plants, showing relationship with xanthophyll cycle.
environmental stresses (e.g. drought, chilling, salinity and pathogen attack; Cramer and Quarrie 2002). Early on in the similarity in structure between ABA and the end groups of certain carotenoids led to the proposal that ABA may be a breakdown product of carotenoids, with xanthoxin as an intermediate (Hirschberg 2001; Fig. 1.14). This idea was supported by the finding that plants that do not accumulate carotenoids either because of mutation or treatment with inhibitors also lack ABA. Furthermore labelling studies with $^{18}$O$_2$ established that one $^{18}$O$_2$ atom is rapidly incorporated into the carboxyl group of ABA, indicating that there is a large precursor pool (i.e. carotenoids) that already contains the oxygen on the ring of the ABA molecule (Zeevaart et al. 1989). In etiolated leaves and roots, which have low levels of carotenoids, a 1:1 stoichiometry was found between the disappearing of V and Nx and the appearance of ABA and its catabolites (Li and Walton 1990; Parry et al., 1992). Direct evidence for carotenoids being ABA precursors was provided by isolation and characterization of the carotenoid cleavage enzyme, nine-cis-epoxycarotenoid dioxygenase, which converts nine-cis-epoxycarotenoid (9-cis-violaxanthin and/or 9-cis-neoxanthin) to xanthoxin, the committed step in ABA synthesis (Schwartz et al. 1997, Chernys and Zeevaart 2000; Fig. 1.14).

1.13. Acclimation of photosynthesis to the environment

Terrestrial higher plants grow under conditions that vary widely in environmental parameters such as irradiance, temperature, water availability, and soil quality. For instance, with respect to the light environment, plants can be found growing at maximal irradiances that differ by over two orders of magnitude (greater than 2000 μmol photons m$^{-2}$s$^{-1}$ for midday sunlight versus less than 20 μmol photons m$^{-2}$s$^{-1}$ for some deeply shaded environments; Logan et al., 1999). While the basic mechanism of photosynthetic carbon assimilation is invariant, the relationship between its
component processes and the relative emphasis placed upon each is subject to acclamatory alterations and varies widely across differing environments. Photosynthetic carbon assimilation can be separated into the three component processes of light absorption, transduction of absorbed light energy into chemical energy (ATP) and reducing power (NADPH), and the use of ATP and NADPH to fix atmospheric carbon dioxide. Depending upon growth conditions, plants may emphasize light absorption or energy transduction and carbon assimilation. These acclamatory adjustments enhance a plant’s ability to grow and compete in its environment, however, no plant, including rapidly growing crops, is known to utilize all of the excitation energy absorbed during exposure to full sunlight for carbon assimilation (Long et al., 1994). In all but deeply shaded environments, plants regularly have to contend with excitation energy that more than saturates their photosynthetic apparatus and therefore is excessive. To cope up with such excess light plants have developed various adaptive processes (Niyogi 1999).

1.13.1. Acclimation to sun versus shade

Leaves are the site of higher plant photosynthesis and provide the necessary conditions to maintain it. Leaf structure, shape and cell distribution are genetically determined by change, within limits with growth conditions, allowing adjustment to environment. The rate of photosynthesis by the whole leaf and its response to environmental conditions differs greatly between species of plant and is correlated with habitat.

Distinction may be made between plants with high relation of photosynthesis and growth in very intense light, so called sun plants, which are inefficient, with poor photosynthesis and survival in dim light, and shade plants which photosynthesize and
survive only in dim light but are unable to function efficiently in bright light (low maximum rates of photosynthesis and photochemical damage; Lawlor 1987). However many species show flexibility in response to light intensity. They are facultative sun/shade species and may grow in high and low intensity illumination but lack the ability to adapt to the extremes.

It has long been known that plants acclimated to high light environments are capable of higher maximal photosynthetic rates than plants acclimated to low light environments (Björkman 1981). Sun plants which include many crops and plants of tropical regions achieve maximum rates of photosynthesis greater than 30 μmol CO₂ m⁻²s⁻¹ and respiration rates in darkness of 2 μmol CO₂ m⁻²s⁻¹. Not only are maximal rates of photosynthesis higher in high light-acclimated plants, a higher light intensity is required to saturate the photosynthetic apparatus. In order to support their higher metabolic rate, high light-acclimated plants also have a greater respiration rate. Because of this higher respiration rate, high light-acclimated plants have a higher light compensation point (the light intensity where photosynthesis exactly balances leaf respiration), and, as a result have a lower net rate of carbon assimilation in the light-limiting region when compared to low light-acclimated plants. Shade plants may have photosynthesis rates less than 10 μmol CO₂ m⁻²s⁻¹ at light intensity perhaps 1/10th of sun species and maybe damaged by light intensities above half that of sunlight. Shade and sun plants and leaves of the same plant from different illumination differ in chloroplast membrane and light-harvesting and electron transport mechanism.

1.13.1.1. Plant architecture

The productivity of a shade-acclimated plant is often limited by its ability to gather light energy for carbon assimilation. Therefore shade-acclimated plants tend to
allocate more resources to photosynthetic tissues and have a relatively low root to shoot biomass ratio (Givnish 1988). Carbon acquisition in plants growing in exposed environments is likely not limited by light energy absorption. Exposed environments can often be characterized by high temperatures and large leaf-atmosphere vapour pressure deficits, which lead to high transpiration rates in these plants. High growth rates exhibited by sun-acclimated plants require greater amounts of nutrients as well. In order to meet these needs, sun-acclimated plants allocate more resources to root growth and have higher root to shoot biomass ratios than shade-acclimated plants (Poorter and Remkes 1990).

1.13.1.2. Leaf architecture

Sun-acclimated leaves tend to be thicker, due to more highly developed palisade and spongy mesophyll layers (Nobel et al., 1975). They also have a lower internal resistance to carbon dioxide diffusion, allowing for greater carbon dioxide availability necessary to support their high rates of carbon assimilation.

1.13.1.3. Chloroplast movements and morphology

Chloroplasts in the palisade cells of sun-acclimated leaves tend to be located adjacent to the anticlinal cell walls, parallel to the incident light beam, thereby shading each other during exposure to high PFD. They also have a greater fraction of stromal, unstacked thylakoid membrane, providing more membrane surface area for NADP⁺ reduction and ATPase activity needed to support high rates of carbon fixation.

Chloroplasts are usually more numerous in mesophyll cells of shade plants and arranged near the upper leaf surface while leaves of the lower leaf mesophyll have few chloroplasts. However, as the number of cell layers is smaller, shade plants often have less chlorophyll per unit leaf area. Under exposure to low PFD, chloroplast
of palisade cells of shade-acclimated leaves tend to be located adjacent to the periclinal cell walls, perpendicular to the incident light beam, and are therefore in an optimal position to intercept light energy (Chow et al., 1987). Chloroplasts from shade-acclimated leaves have a greater proportion of granal stacked thylakoid membrane (Chow et al., 1987), associated with high concentrations of peripheral light-harvesting complexes of PS II thus increasing the area of light-capturing membrane. Grana are often irregularly orientated which may increase capture of diffuse or variably orientated light.

These chloroplast positions are not static; chloroplast in both shade-and sun-acclimated plants can move in response to changing light conditions in order to maximize light interception when light is limiting and to minimize light interception when it is saturating (Haupt and Scheuerlein 1990).

1.13.1.4. Quantum yields
At low PFD, the photosynthetic apparatus appears remarkably capable of using the vast majority of absorbed photons for photochemistry, independent of the light environment in which the plants were grown or any genetic adaptation to sun and shade environments. It can be concluded that in the absence of stress the maximum quantum yield of sun and shade adapted species, or of plants of a species acclimated to different light environment, do not differ significantly. This conclusion is supported by observation of similar values of the fluorescence ratio Fv/Fm indicating similar maximum quantum yield of PS II photochemistry in both (Björkman and Demmig 1987).

The nearly maximum possible quantum yield indicates that several potential constraints on the efficiency of light use are overcome under the differing light
qualities of sun and shade environments and compositions of the photosynthetic apparatus. These high quantum yields indicate that photons are partitioned equally between PS II and PS I despite the apparent excess of PS II in shade plants and despite differences in light quality. Any other partitioning would reduce the quantum yield. Both light quality and quantity are known to regulate photosynthetic stoichiometry. Comparison of photosynthetic stoichiometries in sun and shade plants from natural environment reverse generally lower PSII/PSI reaction centre ratios but also considerable overlap in values (Chow et al., 1990). Thus potential differences in excitation must be balanced by adjustments in antenna sizes associated with each photosystem. Stress effects such as low nitrogen supply, drought, either too high or low temperature may also reduce quantum yields in high light because of photoinhibitot. These factors may account for much of the differences in quantum yields reported for sun and shade acclimated plants (Pearcy 2000).

1.13.1.5. Light harvesting and electron transport

In light-limited environments a greater emphasis is placed upon light gathering. At the level of the thylakoid membrane this is reflected in a greater concentration of light-harvesting complexes associated with the reaction centres. Shade plants may contain 4 to 5 times more Chl a and b per unit volume of chloroplast and have a higher b/a ratio than sun plants because the LHC increases (Anderson 1986). Both PS I and PS II decrease with shade but the ratio of antenna chlorophyll to reaction centre is slightly larger than in high light grown plants of sun species. The electron transport chain in shade plants is not increased, as there is relatively much less (1/5th) cytochrome f, plastoquinone, ferredoxin and carotenoids per unit of chlorophyll than in sun plants. Shade plants have therefore more light-collecting apparatus, but a smaller complement of electron carriers than sun plants. In dim light the rate of electron
transport is limited by the number of photons falling on the leaf, it would be no advantage for shade plants to produce a large capacity electron transport chain. With a pool of plastoquinone receiving electrons from PS II reaction centres, this rate limiting step is minimized in the shade plants except when the plant is exposed to light of brightness outside the normal range.

Sun plants have less developed thylakoid system, fewer granal stacks and partitions and less LHC so they are less efficient at absorbing light energy at low photon flux than shade plants and so have lower quantum yield. Electron transport in sun plants maybe 15-30 times faster than in shade species (uncoupled rate). Clearly the main differences between the two groups of plants are the capacity of the light-harvesting system and of electron transport. Increases in the concentrations of various electron transport constituents in plants acclimated to higher PFDs partially account for the two-fold increase in photosynthetic rate of these plants (Chow and Anderson 1987). The light absorbing system in shade plants makes them very effective at gathering the light available and passing it to the reaction centre, especially in dim light, but they are limited in bright light by the rate of electron transfer, sun plants in contrast are very efficient at transporting electrons but not at gathering weak light.

1.13.1.6. Rubisco

Plants grown at higher PFDs had Rubisco activities over two-times greater than plants acclimated to lower PFDs (Chow and Andersson 1987).

1.13.1.7. Photosynthetic capacity in sun and shade acclimated leaves

It is now well established that growth in high versus low light results in increased rubisco activity, PS II electron capacity, capacity of cytochrome f and chloroplast coupling factor (CF1) per unit leaf area (Anderson and Osmond 1987). Dynamic
measurements of acclimation following transfer of plants between light environments have revealed a range of responses of light-saturated photosynthetic capacity depending on the species. For some species mature leaves exhibit number or at least only a modest acclimation response (Pearcy and Sims 1994).

The photosynthetic system of strongly illuminated shade plants may be irreversibly damaged by very intense light, whereas sun plants are apparently insensitive. Slow movement of electron through plastoquinone at high rates of electron flow in bright illumination causes backing up of electrons and reaction centre cannot use excitation energy so that the high energy state of Chl accumulate and damage increases. PS II appears more sensitive to photoinhibition than PS I. The structure of shade plants thylakoid makes them more easily damaged. Possibly the structure of the PS or carotenoid complement, which reduces the energy load and provides a safety valve is inadequate in shade plants. Sun plants have relatively smaller light-harvesting system so are inefficient in weak light, but the electron transport chain for electron flow and excess energy dissipating system contribute to their greater efficiency and capacity to assimilate carbon dioxide in intense illumination.

1.13.1.8. Response of energy dissipation

The total pool size of xanthophyll cycle pigments (V+A+Z) changes with the growth light environment as part of the acclamatory response that occurs over a time-scale of days (Björkman and Demmig-Adams 1994). Plants growing in more exposed locations, where they might be expected to have a greater need to dissipate excessive absorbed light energy, tend to have larger pools of xanthophyll cycle pigments (Thayer and Björkman 1990; Logan et al., 1996). In general, plants acclimated to sun-
exposed sites are capable of rapidly converting a greater fraction of their xanthophyll to A and Z in full exposure than are shade-acclimated plants (Brugnoli et al., 1994). These acclamatory adjustments presumably provide sun-acclimated plants with the means to dissipate the excessive absorbed light energy which they experience for hours every day.

1.14. Physiological-ecological response range of PS II to excess light

1.14.1. Short-term response
The first order of defense for a typical PS II unit, of a plant growing in a shade environment when exposed to rapid intermittent doses of excess light, is the xanthophyll cycle-dependent energy dissipation. Typically, otherwise non-stressed, healthy plants show a diurnal time course where the level of xanthophyll cycle-dependent energy dissipation tracks the solar light intensity, peaking at midday when it becomes most excessive. Indeed, it is now clear that xanthophyll cycle-dependent energy dissipation is part of the daily and day-to-day life of almost all terrestrial higher plants and many algae and diatoms (Demmig-Adams et al., 1996).

1.14.2. Long-term responses
As an extension to the highly choreographed short-term (seconds to hours) photoprotective responses of the PS II unit to excess light levels described above, there is also a well defined long-term (hours to seasons) pattern of events involving changes in the overall pigment-protein content and structural composition of the PS II unit. When the duration and or extent of excess light exposure increases, for example, in a fully sun exposed environment, or during suboptimal temperatures, it is possible that the molecular content of the PS II unit is altered such that there is a decrease in the size of the peripheral PS II antenna. This is mostly attributed to a decrease in the
LHC II $b$ and subsequent decrease in its component pigments, namely Chl $a+b$, lutein and neoxanthin. In most cases, long term acclimation of PS II is probably accompanied by no change or a significant increase in the number of xanthophyll cycle pigments, V, A and Z; thus the ratio of $V+A+Z$ to total Chl $a+b$ increases (Demmig-Adams et al., 1996; Gilmore 1997).

1.14.3. Extreme responses - damage and repair of PS II reaction centers

A natural follow up of the above concepts concerning the limitations of the photoprotective avoidance and dissipation mechanisms is to understand what happens when these photoprotective strategies fail under prolonged and/or extreme stresses. It is important to consider that the degree of stress required to inflict failure of PS II function is usually associated with an environmental condition so severe that the primary biochemical functions are almost completely inhibited. The observed degradation of the PS II core ($D_1$, $D_2$, CP43, CP47, etc.) proteins under extremely high-light exposure, at freezing-low temperatures or with limiting electron acceptors ($CO_2$, $O_2$) can be viewed as symptomatic, as opposed to a causal effect, of the inhibited photosynthesis. However, the sustained inhibition of photosynthetic productivity upon return to favorable environmental conditions is a significant and genuine concern and is the primary reason for the intense interest in the photoprotection and photodamage/repair phenomena. It has been demonstrated in many studies that when the rate of reaction center damage exceeds that of repair (reaction center protein synthesis and assembly) accumulation of inactivated PS II units ensues (Chow 1994). Thus, optimizing the balance of PS II damage and damage-repair mechanisms is clearly an important photoprotective strategy in plants exposed to extreme light stress. Further, because inactive or photoinhibited PS II centers have increased rate constants of heat dissipation, it has been proposed that
inactive centers may serve a photoprotective purpose by dissipating excess light that may otherwise cause further damage to intact-functional PS II centers that share excitation energy (Chow 1994).

1.15. Objectives

Most of the work on photoinhibition has been carried out by growing plants under artificial low light conditions and exposing them to artificial constant high light under laboratory conditions. However, very little is known about the extent of photoinhibition and protection under field conditions. In order to obtain a detailed characterization of the extent of damage and protection of the photosynthetic apparatus under field conditions, a study of rice plants grown at three different growth conditions ranging from extremely low to extremely high was carried out. In this study we use natural sunlight as photoinhibitory treatment in order to consider the fluctuations in the light intensities during the course of the treatment.

Based on the measurements of pigment composition and chlorophyll a fluorescence parameters at room temperature we attempted to assess the capacity of acclimation of rice plants to high light and to obtain a more detailed view of the relation between the xanthophyll cycle associated energy dissipation and non-photochemical quenching of chlorophyll a fluorescence in preventing adverse effects of full sunlight on the photochemical system of low light and high light acclimated leaves. More specific objectives were as follows:

♦ To study the rate of photosynthesis, photoinhibition under conditions of excess excitation to determine extent of damage to plants grown under different light intensities
• To measure changes in xanthophyll cycle components and other carotenoids under different growth conditions and treatment conditions and to study the extent of protection

• To study relationship between ABA and xanthophyll cycle with respect to protection against over-energization

• To study changes in lipids of cell membrane and thylakoid membrane, in relation to damage and protection of photosynthesis, under various conditions of over-energization

• To study rate of VDE and ZE under different growth conditions and to relate it to the efficiency of the xanthophyll cycle