REVIEW OF LITERATURE
Photosynthetic light reaction

In chloroplast of higher plants, photosystem I (PS I) and photosystem II (PS II) in the thylakoid membrane are the sites where the primary photochemical reactions occur. These reactions include capture of photons by chlorophyll molecules, excitation energy transfer among light-harvesting chlorophyll molecules, trapping of the excitation energy by reaction center chlorophyll molecules, charge separation at the reaction center and transfer of electrons through several electron transport components. The main function of these successive process is to oxidize water molecules to derive electrons and to transfer the electrons to NADP^+. ATP molecules and reduced NADP^+ produced in the course of the electron transport reaction are used for assimilation of CO_2.

The electron transport components in oxygenic photosynthesis are organized in membrane bound supramolecular complexes (McCarty and Carmeli 1982, Andréasson and Vännberg 1988). The Z scheme proposed earlier by Hill and Bendall (1960) aligned the electron transport components according to their redox potential. Over the past 30 years our knowledge of molecular mechanisms related to Z-scheme has expanded enormously.

Primary electron acceptor of PS II

The primary reaction that is taking place in the PS II is the charge separation between the reaction center (P680) and the acceptor molecule pheophytin. Existence of intermediate primary
electron acceptor between P680 and the first stable acceptor \( Q_A \) was suggested by many workers (Klimov et al. 1980a, Eckert and Renger 1980). Latter it was found to be pheophytin (Pheo). It is a specialized chlorophyll molecule lacking \( Mg^{2+} \). The presence of Pheo was confirmed by optical absorption spectroscopy (Fajer et al. 1980), EPR (Klimov et al. 1986), ENDOR studies (Fajer et al. 1980). The Pheo is located on the same side as P680 (Trissl and Kunze 1985). Two Pheo molecules per reaction center have been detected in higher plants and cyanobacteria by chemical methods (Murata et al. 1986).

**The first stable electron acceptor of PS II (\( Q_A \))**

The first stable electron acceptor of PS II, \( Q_A \) (plastoquinone), oxidize Pheo\(^-\) by accepting an electron from it in 250-350 ps (Nuijis et al. 1986, Eckert et al. 1988). \( Q_A \) is a single electron acceptor and reduced to semiquinone anion (\( Q_A^- \)) form (Vermaas and Govindjee 1981, Mathis and Rutherford 1987, Andréasson and Vännård 1988). EPR signal studies indicate that \( Q_A \) is located close to a \( Fe^{2+} \) in reaction center of PS II (Okamura et al. 1982, Vermaas and Rutherford 1984). \( Fe^{2+} \) is suggested to have a role in stabilization of semiquinone form of \( Q_A \) and \( Q_B \) (Rutherford and Zimmerman 1984). Absorbance changes at 320 nm was used to characterize \( Q_A \) (Weiss and Renger 1984, Renger et al. 1986).
Two electron gate quinone acceptor \( Q_B \)

The reduced electron acceptor \( Q_A^- \) is oxidized by a second quinone, \( Q_B \). Both the reduced \( Q_A \) and \( Q_B \) exhibit typical semiquinone optical UV spectra (Schatz and Van Gorkom 1985). Electron transfer to \( Q_B \) takes place in the ms regime. The \( Q_B \) can be reduced by two electrons to form a quinol. The existence of such a two electron gate between \( Q_A \) and PQ pool was postulated by Velthuys and Amesz (1974). The redox properties of \( Q_B \) are different from that of PQ. The oxidation of semiquinone \( Q_B^- \) to \( Q_BH_2 \) is accompanied by a true protonation. The kinetics of electron transfer from \( Q_A^- \) to \( Q_B \) was faster than that of \( Q_A^- \) to \( Q_B^- \). The electron transfer through \( Q_B \) is found to be regulated by HCO\(_3^-\) (Eaton-Rye et al. 1986). The mechanism of action of DCMU like inhibitors is thought to be through binding of the herbicide in or close to the \( Q_B \) site on the protein in competition with \( Q_B \) binding (Mullet and Arntzen 1981, Lavergne 1982, Purcell et al. 1991).

\( Q_{400} \)

The electron acceptor \( Q_{400} \), designated after its mid-redox potential of 400 mV at pH 7.0. It has been identified as an iron of Fe-Q by EPR spectroscopy and redox studies (Petrouleas and Diner 1986). \( Q_{400} \) exists in the form of \( Fe^{3+} \) (Brettel et al. 1985). Ferricyanide and various quinone acceptors oxidize the iron via the \( Q_B \) site (Zimmermann and Rutherford 1986, Diner and Petrouleas 1987).
The donor side of PS II

The primary electron donor of PS II is a specialized Chl a molecule known as P680. It was detected as a flash induced absorption change attributable to Chl a oxidation. In vitro studies have suggested that P680$^+$ could be a ligated monomeric Chl a (Davies et al. 1979). The triplet state of P680 formed by the recombination of P680$^+$-Pheo$^-$ was detected as EPR signals when PS II preparations were illuminated at liquid helium temperature (Rutherford et al. 1981). The Em is +1.1V due to its high potential nature.

The reduction of P680$^+$ at room temperature takes place in the time scale of 10 to 100 ns. The optical properties of the oxidized Z resemble those of plastosemiquinone (Weiss and Renger 1984) and also possibly influenced by an additional electron carrier D on the donor side. The midpoint potential of Z/Z$^+$ couple and D/D$^+$ couple is shown to be +1.12 V and +500-760 mV, respectively (Tsu et al. 1987).

Plastoquinone

Plastoquinone (PQ) is suggested to be a possible mobile electron carrier between the Q$_B$ and Cyt b$_6$-f complex (Anderson 1981, Whitmarsh 1986). It has a quinone ring with two methyl groups and an isoprenic side chain attached to it. It is present in relatively higher amount than the other electron transport
intermediates. During electron transport, PQ accepts two electrons from $Q_BH_2$ and is protonated to form PQH$_2$ (Haehnel 1984, Rich and Moss 1987). Apart from electron transport, PQ is engaged in other important functions in the chloroplast, viz., translocation of protons into the lumen, which generates proton gradient across the membrane (Velthuys 1987) and phosphorylation of LHCP through the activation of a kinase (Larsson et al. 1987a, Bassi et al. 1988) and helps in the regulation of energy distribution between the two photosystems (Fork and Satoh 1986).

Cytochromes

Cytochromes are chromoproteins arranged in between the two photosystems. Cyt b559 consists of two protein subunits of 9 and 4 KDa (Cramer et al. 1986). It is coupled to PS II (Ke et al. 1972) and has an intimate association with the D1/D2 proteins (Barber et al. 1987a, Hansson and Wydrzynski 1990). Cyt b559 may be involved in the electron flow around P680 by accepting electrons from the $Q_B$ (Tsujimoto and Arnon 1985, Falkowski et al. 1986). Loss of oxygen evolving activity of PS II is connected with the conversion of Cyt b559 into a low potential form (Ghanotakis et al. 1986). However, no correlation between the high potential form of Cyt b559 and $O_2$ evolving activity has been observed (Bergstrom and Franzen 1987). Cyt b$_6$ and Cyt f function as the intermediates between PS II and PS I (Hauska et al. 1983).
Plastocyanin

Plastocyanin (PC) is a Cu-containing protein and is suggested to be loosely bound to the lumen side of the thylakoid membrane. However, latter studies with inside out vesicles confirmed that PC is bound to the inner thylakoid surface (Haehnel et al. 1981), but consistent with its function in the lumen (Haehnel 1984). PC is a mobile electron carrier between Cyt f and P700 as evidenced by oxidation-reduction kinetics of P700 (Whitmarsh 1986). The reduction of P700 by PC was decreased with the increase in the viscosity of lumenal fluid (Olsen and Pedersen 1983). Raljczak et al. (1988) presented evidences for the mechanism of interaction of PC with PS I. It was shown to be due to electrostatic nature of aminoacids on the protein. The negative charged Tyr 83 region was suspected to be involved in the PC interaction with PS I reaction center (Farver et al. 1982). Hippler et al (1989) have shown that PC was crosslinked very near to P700 and in the proper orientation for electron transfer.

P700

P700 is the PS I reaction center in the Z scheme. It is bound to the large polypeptide of the reaction center I. It was for many years thought to consist of Chl a molecule (Malkin 1982). From optical and electron paramagnetic spectroscopic
studies, it has been suggested that P700 probably consists of two molecules of Chl a. But Hiyama et al. (1987) suggested that it consists of a Cl0 epimer of Chl a. Dornemann and Senger (1986) suggested that the chemical nature of P700 as a chlorinated chlorophyll, 13-hydroxy-20-chloro-Chl a. By using hole burning experiments, Gille et al. (1987) have shown that P700 is a dimer with a charge transfer excited state having a large electric dipole movement. The chemical nature of P700, either as monomer or dimer, is still in debate (Ikegami and Itoh 1988). Despite differing ratios of Chl a to P700 and different protein composition in different PS I preparations, the stoichiometry of Chl a to P700 was found to be 1:1 (Kobayashi et al. 1988).

**Electron acceptors A_o and A_1**

The first two known electron acceptors of PS I are A_o and A_1. These are associated with the two larger polypeptides (Ia and Ib subunits) of PS I reaction center (Rutherford and Heathcote 1985, Schoeder and Lockau 1986). A_o^- and A_1^- had EPR g values typical of a chlorophyll anion and a semiquinone, respectively (Thurnauer and Gast 1985). Photoaccumulation experiments showed that A_o has a strong absorption at 670 nm indicating a chlorophyll monomer (Mansfield and Evans 1985, Lagoutte and Mathis 1989). The optical spectroscopic studies suggested that A_1 as a quinone component especially vitamin K1 (phyloquinone) (Zeigler et al. 1987, Biggins and Mathis 1988).
The Fe-S centers $F_X$, $F_A$ and $F_B$

There are three membrane bound Fe-S centers in PS I acceptor side, namely $F_X$, $F_A$ and $F_B$ (Mathis and Rutherford 1987). They are one electron carriers (Malkin and Blarden 1978). By using EPR signal the component $F_X$ has been identified as an unusual Fe-S center (Evans 1982). The $F_X$ component is either 2Fe-2S or 4Fe-4S Fd or Fe-Q (McDermott et al. 1988). $F_A$ and $F_B$ are identified as clusters of 4Fe-4S type (Andréasson and Vännård 1988, Scheller et al. 1989). These centers are located on a single 9 KDa polypeptide (Oh-Oka et al. 1988). $F_A$ and $F_B$ interact closely with one another and it is not known whether they transfer electrons in series from X ($X \rightarrow A \rightarrow B$) or in parallel (Haehnel 1984).

Terminal acceptors of PS I

The final electron acceptors from PS I are the extrinsic proteins Fd and Fd-NADP reductase. Fd is an electron carrying protein which contains an 2Fe-2S center and reduced by electrons from $F_A$ or $F_B$. Recently it has been identified that a 20 or 22 KDa protein subunit is interacting with Fd (Zilber and Malkin 1988, Andersen et al. 1990). The Fd-NADP reductase is a flavoprotein containing FAD (Carrillo and Vallerjos 1987). This protein complex is an extrinsic protein found to be attached to the thylakoid membrane (Karplus et al. 1984). The FAD and NADP in the enzyme is bound in a non-covalent manner (Dykes and Davis 1982).
Structural and functional organization of thylakoid membrane proteins

The higher plant thylakoid membrane proteins are organized into five membrane spanning complexes, three of which are pigment-protein complexes and the remaining two are not associated with pigments. The complexes are Chl a binding PS II complex, Chl a binding PS I complex, the Chl a/b binding light harvesting complexes, the Cyt b_{6}-f complex and the ATP synthase complex. These integral protein complexes together with a number of closely associated extrinsic proteins like water oxidizing enzyme system are responsible for light harvesting, electron transport, proton gradient formation, ATP synthesis and NADP^{+} reduction (Barber et al. 1987b, Chapman et al. 1989, Hansson and Wydrzynski 1990).

PS I complex

The PS I complex comprises the PS I reaction center protein complex and PS I antenna complex (LHCP I complex). Apart from these complexes other protein moieties such as PC, Fd, Fd-NADP reductase do form part of the PS I complex.

The reaction center I protein complex

PS I reaction center isolated from higher plants contains polypeptides designated as Ia, Ib, II, III, IV, V, VI and VII in order of decreasing molecular weight ranging from 82 KDa to about
8 KDa (Mullet et al. 1980, Lagoutte and Mathis 1989). The function of PS I reaction center is to oxidize PC and to reduce Fd. The subunit I of PS I reaction center consists of two homologous proteins (Ia and Ib) and function as the primary photoschemical reaction center. These subunits are encoded by the chloroplast genes psa A and psa B. They both have approximate molecular masses of 82 KDa and present in equimolar amounts (Fish and Bogorad 1986, Bruce and Malkin 1988, Cushman et al. 1988). The reaction center P700 and the electron acceptor A_0 and A_1 are all located on subunits of I_a and I_b (Thornber et al. 1976, Lagoutte and Mathis 1989).

The early electron transfer components F_A and F_B are located on a single 9 KDa polypeptide, probably the subunit III of RC I (Høj et al. 1987, Oh-Oka et al. 1988, 1989). Recently, a 9 KDa protein containing two Fe-S centers was isolated in a functionally intact form (Oh-Oka et al. 1991). This polypeptide is also encoded by chloroplast genome (psa C). The remaining subunits of PS I reaction core polypeptides are encoded by nuclear genes (Okkels et al. 1989). The subunit IV is a peripheral membrane protein located at the stromal side of thylakoid membrane. It is known to interact with Fd (Andersen et al. 1990). The subunits V and VI have been suggested to have close association with PC (Munch et al. 1988, Hippler 1989). The functions of the rest of the subunits are much more illusive and unknown.
Light harvesting chlorophyll-protein complex I (LHCP-I)

The LHCP I is a Chl a/b protein complex with a Chl a/b ratio of 3.5-3.7. Preliminary characterization of LHCP I was made by Mullet et al. (1980). This complex consists of five subunits, ranges from 18 to 27.5 KDa (Wollman and Bennoun 1982). LHCP I was proposed to be the site of 736 nm fluorescence of higher plants at 77 K. Among the protein subunits, 27 KDa was suggested to be the peripheral antenna protein which in association with the other four subunits of LHCP I and mediate energy transfer between LHCP I and the reaction center of PS I. All the Chl b in PS I preparation was shown to be associated with LHC I (Anderson et al. 1983).

PS II complex

More than 22 polypeptides have been suggested to be associated with PS II (Majojidek et al. 1987). One possible scheme to describe the organization of PS II involves the subdivision of PS II into three functional parts.

1. Reaction center or core complex which contains the primary reactants, the donor and acceptor side.
2. The O₂ evolution system
3. The antenna pigment-protein complex of PS II.

Reaction center complex of PS II

PS II complex is made up of intrinsic reaction center
polypeptides and extrinsic polypeptides. The primary reactants and the reducing side components of PS II have been assigned to be so called D1 and D2 intrinsic proteins. D1 and D2 polypeptides are of 32 and 34 KDa size, respectively. This assignment was based on homology arguments for the gene sequences between the D1 and D2 proteins and L and M subunits of the reaction center complex of purple bacteria (Hearst 1987). Isolated D1/D2/Cyt 559 complex (Nanba and Satoh 1987, Seibert et al. 1988) contained minimal amount of Chl and Pheo and which can undergo photochemistry (Chapman et al. 1988, Wasielewski et al. 1989). Stoichiometric analysis indicates that D1/D2/b559 complex contains Chl a, Pheo, β-carotene and monogalactolipids at a molecular ratio of 6:2:2:1, but no PQ or Mn (Kobayashi et al. 1990, Murata et al. 1990).

The first electron donor to P680 is known as Z, which gives rise to EPR signal II very fast is most likely a tyrosine residue located on D1 protein (Debus et al. 1988a). The chemical identity of dark stable free radical giving rise to EPR signal II slow (D⁺) has also been assigned to a tyrosine residue located on a D2 protein (Vermass et al. 1988, Debus et al. 1988b). Thus the primary reactant (Z and Pheo⁻), the quinone electron acceptors (QA and QB), the associated electron donor to (Fe) and the tyrosine electron donor to P680 are all shown to be located on D1/D2 heterodimer within the reaction core. Studies of the electric field generated by the electron transfer from P680 to
the primary acceptor Pheo and from Pheo\textsuperscript{−} to the first stable electron acceptor, Q\textsubscript{A}, indicate that the distance between P680 and Pheo and Pheo and Q\textsubscript{A} are approximately equal (Trissl et al. 1987). In addition to D1 and D2 subunits, a cap like polypeptide with apparent molecular mass of 9-10 KDa was suggested to be present above the acceptor side of D1/D2 (Farchaus and Dilley 1986).

PS II reaction core contains at least one heme Cyt b559 (Cramer et al. 1986). It has been suggested that Cyt b559 may have a photoprotective role (Thompson and Brudvig 1988). PS II contains Mn which is required for O\textsubscript{2} evolution. The functionally active Mn binds to the D1/D2 heterodimer (Colemann and Govindjee 1987, Dismukes 1988) and is situated towards the luminal side of the membrane, between the reaction core and the extrinsic 33 KDa protein of the water oxidation complex (Yamamoto 1988a, Seibert et al. 1988, Miyao and Murata 1989).

**The oxygen evolution system of PS II**

Oxygen evolution activity is closely associated with PS II. Oxidizing equivalents produced by the photo-induced charge separation at the reaction center Chl a of PS II are accumulated at Mn atoms and water molecules coordinated with them to evolve O\textsubscript{2}. In the earlier study with a sensitive O\textsubscript{2} electrode and pulse flashes, four oxidizing equivalents accumulated in PS II were shown to be required for producing one molecule of O\textsubscript{2} from water.
(Joliot et al. 1969). The so called S-cycle model of O\textsubscript{2} evolution also proposed where water oxidation is supposed to occur only after formation of S4 state, i.e., after four positive charge equivalents have been accumulated (Kok et al. 1970, Styring and Rutherford 1987). Highly active O\textsubscript{2} evolving PS II membrane fragments were prepared (Berthold et al. 1981, Kuwabara and Murata 1982a) and they were proved to be a quite promising material for analyzing the O\textsubscript{2} evolution system. The O\textsubscript{2} evolving PS II complex is believed to consist of six intrinsic proteins (47,43 KDa, D1, D2 proteins and two subunits of Cyt b559) and three extrinsic proteins (33, 23 and 18 KDa) (Inoue et al. 1983, Murata and Miyao 1985). Following Tris (Yamamoto et al. 1981) or NaCl (Akerlund et al. 1982, Kuwabara and Murata 1983) or CaCl\textsubscript{2} (Ono and Inoue 1983) treatment, specific release of the three extrinsic proteins from the membrane was shown. As release of the three extrinsic proteins from the PS II particles was accompanied by release of Mn atoms of PS II and complete inhibition of O\textsubscript{2} evolution, it was suggested that the three proteins are involved in the O\textsubscript{2} evolution system. More direct evidence of Mn functioning in O\textsubscript{2} evolution system was obtained from the measurement of the EPR signal of Mn at liquid helium temperature (Dismukes and Siderer 1981).

Among the three proteins 33 KDa protein is responsible for stabilizing the catalytic property of Mn atoms in PS II.
The number of Mn atoms per reaction center of PS II is four (Cheniae 1980, Yocum et al. 1981, Yamamoto 1989). To elucidate the roles of these three extrinsic proteins of PS II in O₂ evolution, many reconstitution experiments were carried out in the protein-deleted PS II membranes (Murata and Miyao 1985, Miyao and Murata 1989). The millisecond kinetics of O₂ evolution after third flash was retarded by the removal of 33 KDa protein (Miyao et al. 1987).

The roles of the 23 and 18 KDa proteins seem to be auxillary. There are reports suggesting a close relationship between the two extrinsic proteins and Ca²⁺ and Cl⁻ binding at the catalytic site (Ghanatakis et al. 1984, Imaoka et al. 1986). The depletion of Ca²⁺ and concomitant inhibition of the S2 → S3 transition was observed (Tamura et al. 1989). The inactive complexes are converted to active by dark incubation with Ca²⁺.

Experiments with cross-linking and affinity chromatographical studies suggest that the 33 KDa protein binds to the reaction center-carrying polypeptides directly (Gounaris et al. 1988). Reconstitution experiments with the 23 and 18 KDa proteins indicated that the 23 KDa protein has two binding sites, one to the 33 KDa protein and the other to PS II intrinsic proteins (Miyao and Murata 1983). The 18 KDa protein is bound to the 23 KDa protein (Miyao and Murata 1989).
The antenna pigment-protein complex of PS II

The light harvesting pigment-protein complex of PS II is divided into two major parts the proximal antenna and the distal antenna. The proximal antenna was characterized as a long-wavelength absorption form that couple energy transfer from the bulk of the light-harvesting pigments in the distal antenna to the reaction core (Pearlstein 1987, Hansson and Wydrzynski 1990).

The proximal antenna consists of two pigment-protein complexes, namely CP47 and CP43 (Green 1988). These two proteins have apparent molecular masses of 45-51 and 40-45 KDa, respectively. Only the CP43 can be phosphorylated (Ikeuchi et al. 1987). Each protein binds 20-25 Chl a and about 5 carotene, but no Chl b or Pheo (Breton and Katoh 1987). The fluorescence at 77 K peaks at 685 and 695 nm for CP43 and CP47, respectively (Nakatani et al. 1984). The CP47 and CP43 proteins are always present in O₂ evolving preparations from higher plants. These two complexes are shown to be responsible for the assembly of the PS II reaction core (Vermaas et al. 1988).

The distal antenna consists of two parts, LHCP II and ACP II. The LHC II contains at least six separable oligomeric components. The apparent molecular masses of these proteins are 25 to 27 KDa (Peter and Thornber 1988). It has both Chl a and Chl b and contains xanthophyll as the major carotenoid. The LHC II is involved in the stacking of the granal membrane (Staehlin
and PS I (Braintais et al. 1986). LHC II can be phosphorylated by membrane-bound kinases, then forms a mobile fraction which dissociates from PS II and migrates from the appressed to the non-appressed region of the membrane. The migrated LHC II increases the light harvesting capability of PS I (Larsson et al. 1987b, Bassi et al. 1988, Williams and Doming 1990). There are a number of other chlorophyll proteins associated with PS II distal antenna that are not part of LHC II, known as accessory chlorophyll-proteins. Among these components are CP 29, CP26 and CP24 are well known (Dunahay and Staehlin 1986, Bassi et al. 1987, Green 1988). These components function as linkers for the LHC II to the proximal antennae.

**Cyt b₆-f complex**

This complex functions as an intermediate between PS II and PS I. This complex has been well characterized in spinach thylakoids (Hauska et al. 1983). It is also required for cyclic electron flow supported by PS I. It is capable of operating the Q-cycle electrons/H⁺ exchange process.

Cyt b₆-f complex consist of at least five polypeptides (34, 33, 23, 20 and 17.5 KDa), one Cyt f, two Cyt b₆ and Rieske Fe-S protein. The 20 KDa component is nuclear encoded and harbours the Rieske Fe-S center. The two Cyt b563 hemes are bound to the 23 KDa, chloroplast encoded polypeptide. Cyt f is contained in a
34 KDa polypeptide which again is encoded by the chloroplast genome (Vallejos et al. 1984). The reoxidation of PQH\(_2\) gives rise to the release of 2\(H^+\) and 2\(e^-\). The first electron is passed to the Rieske center. The Rieske center then reduces Cyt f which is reoxidized by PC. The second electron could either be donated to the reoxidized Rieske center or passed to Cyt b\(_6\) (Rich 1984).

**ATP-synthase complex**

The ATP synthase complex in chloroplast consists of two portions namely, CF\(_o\) and CF\(_1\). The CF\(_o\) is an intrinsic component of the membrane having hydrophobic polypeptides, whereas CF\(_1\) is an extrinsic peripheral membrane complex (Nelson 1980). The function of CF\(_o\) is to conduct protons across the membrane that will enable the CF\(_1\) (catalytic site) to divert the stored energy into proton motive force to form ATP from ADP and Pi. In chloroplasts, at least 8-9 polypeptides catalyze the reaction of ATP. It was shown that the phosphorylation activity of this enzyme depends upon the presence of all subunits (Richter et al. 1984, Nalin et al. 1985). CF\(_1\) complex is composed of five different polypeptides. The molecular mass of these subunits ranges from 14 to 55 KDa (Nelson 1987). CF\(_o\) is composed of four different polypeptides designated as subunit I, Ia, II and III (Pick and Racker 1979), with molecular mass of 27, 20, 14 and 8 KDa, respectively. The function of subunit III is proton conduction, whereas subunit II is involved in binding of CF\(_1\) with subunit II.
and III to form a functional proton channel (Nelson 1981). Subunits I, Ia and III are chloroplast genomic products, whereas subunit II may be nuclear encoded.

**Dl-protein**

The thylakoid membranes of chloroplast contain more than 40 distinct size classes of intrinsic polypeptides (Leto et al. 1982). Among these polypeptide exhibiting an apparent molecular mass of 32 KDa on PAGE is a PS II reaction center component, known as Dl-protein. As this polypeptide is rapidly labelled in isolated chloroplasts of several plant species (Ellis et al. 1979, Mattoo et al. 1984), it appears to be rapidly turned over in vivo (Ellis 1977) and is biosynthetically regulated at the level of transcription (Leto and Arntzen 1981). This polypeptide is encoded by chloroplast genome (Psb A) and the aminoacid sequence of the PsbA gene is well established (Ajlani et al. 1989, Etienne et al. 1990). The amino acid changes in herbicide resistant plants led to the suggestion that D1 protein has five transmembrane segments (Trebst and Depka 1985). The confirmation of D1 protein is modulated depending on whether the Q_B site is occupied or not (Trebst et al. 1988).

The rate of turn over of the 32 KDa protein is strongly influenced by light intensity (Mattoo et al. 1984). The mechanism of its light dependent metabolism is largely unknown. Greenberg et al. (1989) have quantified the rate of 32 KDa
protein degradation over a broad spectral range (UV, visible and far-red). The quantum yield for degradation is highest in the UV-B (280-320 nm) region in *Spirodela oligorhiza*. D1 protein is the primary target of photoinhibition. Photoinhibitory damage of PS II complex appears initially to involve impairment of quinone reduction followed by degradation of the D1 protein (Kyle et al. 1984, 1987; Bradbury and Baker 1986, Richter et al 1990). In order to repair PS II complexes, it has been proposed that the damaged complexes migrate from an appressed to non-appressed thylakoid domain, where the protein can be degraded by a membrane-bound protease. Newly synthesised D1 polypeptides are inserted into the damaged PS II, which then migrate back to appressed region of thylakoid membrane (Adir et al 1990, Guenther and Melis 1990).

**D2-protein**

This polypeptide with the molecular mass of 32-34 kDa is difficult to resolve on PAGE due to its diffuse and poorly stained nature (Satoh et al. 1983). It also co-migrate with 32 KDa herbicide binding protein and 33 KDa extrinsic polypeptide (Haehnel 1984). This polypeptide is known to harbour QA, Pheo, Fe and P680 (Trebst 1986). D2 polypeptide has five membrane spanning helices (Deisenhofer et al. 1985, Trebst and Draber 1986). The subunit nature of D1 for this protein also being suggested because of its affinity with certain herbicides (Metz
et al. 1986). All the carotenes which are associated with PS II reaction center complex are present in D1/D2 proteins and they transfer their absorbed light energy to chlorophylls in the D2 polypeptide (Brody 1988).

Chlorophyll fluorescence

It has been demonstrated in numerous studies that a close relationship exists between processes of Chl a fluorescence induction and functioning of photosynthetic apparatus. Chl a fluorescence measurements have been extensively used as a non-destructive, sensitive and intrinsic probe to study the photosynthetic reactions (Govindjee and Papageorgiou 1971, Schreiber 1983, Krause and Weiss 1984, Cao and Govindjee 1990). Chlorophyll molecules, upon absorption of light, reach an excited state and return to the ground state from their first excited state in many ways, such as photochemical reaction, fluorescence emission, energy transfer to the neighbouring molecules and thermal deactivation. The emission of fluorescence is around 685 nm are in actively photosynthesizing organisms, it oscillates with time. This pattern is known as 'Kautsky effect' (Govindjee and Papageorgiou 1971, Papageorgiou 1975). This oscillation in fluorescence emission observed by Kautsky can be divided into two phases, a fast phase which completes in a few seconds and a slow phase which lasts for few minutes. At room temperature fluorescence emission is mainly from PS II and LHC II and the
contribution of PS I being insignificant (Karapetyan and Bukhov 1986).

In dark adapted chloroplasts or leaves upon illumination, the fluorescence level rises very fast to a low fluorescence level called $F_0$ followed by a sigmoidal increase to a temporary maximum called 'P'. The 'O' to 'P' rise is known as variable fluorescence (Krause and Weiss 1984, 1991). The $F_0$ level which is also known as the constant fluorescence is not associated with functioning of electron transport. It represents the emission by excited antenna molecules (Mathis and Paillotin 1981). Only the variable part of fluorescence emitted by PS II chlorophylls is associated with photochemical activity (Duysens and Sweers 1963). The changes in variable fluorescence are determined by redox conversions of $Q_A$. The rapid increase of fluorescence after $F_0$ (O-I) reflects photochemical reduction of $Q_A$. The reoxidation of $Q_A^-$ by intersystem electron acceptors causes its decline (I-D) (Satoh et al. 1977). Again the D-P raise indicates a steady state, in which electrons are fed to the intermediate pool by PS II and abstracted by PS I at equal rates (Papageorgiou 1975). When the photosynthetic electron transport chain between $Q_A$ and $Q_B$ is blocked by a herbicide such as DCMU, the Chl a fluorescence rises very fast to a high (Fm) plateau (Duysens and Sweers 1963, Melis and Homann 1978, Sinclair and Spence 1988). The fast fluorescence transient (O-P) in algae and higher plant is succeeded by a slower change during which the fluorescence yield
rises to a maximum, \( M \) and then declines slowly to a low terminal level, \( T \) (PSMT). At this end, the rate of \( O_2 \) evolution attain a steady state (Mohanty 1972).

**Effect of herbicides on photosynthetic apparatus**

Herbicides are phytotoxic to susceptible plants by interfering with metabolic processes. The action of many commercial herbicides are on bioenergetic reactions or on the biosynthesis and structure of lipids and pigments (Sandmann and Böger 1983). These herbicides are useful tools for the study of chloroplast structure and function. DCMU, atrazine and DBMIB are classical inhibitors of photosynthetic electron transport and they are used to establish the arrangement of redox components in the 'Z' scheme.

Many herbicides decrease the chlorophyll and carotenoid contents of chloroplasts. This occurs either by inhibiting pigment biosynthesis or by pigment destruction (Sandmann et al. 1984a, Milivojevic and Markovic 1989). Herbicides, such as norflurazon, fluridan and difunon inhibit \( \alpha \) and \( \beta \) -carotene synthesis with concurrent accumulation of phytoene and phytofluene (Khan et al. 1979, Sandmann and Böger 1982). Substituted pyridazinone type chemicals, like norflurazon (SAN 9789), potentially block the synthesis of carotenoids and prevent the formation of LHCP and CPs of PS II (Lekhotski et al. 1982, Lebedev et al. 1988). Herbicides like oxadiazon and MK-616
(Sandmann et al. 1984b) and amitrole (Feierabend 1984) exert phytotoxic activities by interfering directly with chlorophyll biosynthesis. Linuron and Monolinuron were reported to induce strong deficiency in β-carotene and cause damage to the reaction center of PS I and PS II (Milivojevic and Markovic 1989). It has been assumed that DCMU type herbicides could have direct interaction with reaction center Chl of PS II (Izawa 1977). The decrease in Chl a by irreversible photooxidation was observed by Szigeti and Medgyesi 1981. It has been shown that a sublethal concentration of atrazine greatly increased the polar lipid content of CP complexes in Lemna (Grenier et al. 1987). The number of grana per chloroplast and the length of grana lamellae increased when plants were grown under high light intensity with low concentration of atrazine (Beaumont et al. 1980, Mattoo et al. 1984).

Many herbicides like bipyridylium salts and p-nitro or chloro-substituted diphenyl ethers initiate cellular and chloroplast membrane damage in crop plants (Orr and Hess 1982). These herbicides causes chlorosis in green plants by triggering oxidative photodestruction of chloroplast membranes and associated pigments. During this reaction active oxygen species are formed. They are converted to \( \text{H}_2\text{O}_2 \) by superoxide dismutase and further to very active hydroxy radicals. Such phytotoxic effects mediated by diphenyl ethers are inhibited by DCMU (Lambert et al. 1984). The herbicides atrazine and DCMU also
partially protect the D1 protein against active oxygen species mediated degradation (Kuhn and Boger 1990). Herbicides like nitrogen and p-nitro diphenyl ethers inhibit photophosphorylation by binding to the coupling factor (Huchzermeyer and Loehr 1983). It has been shown that oxyfluorfen inhibits the energy transfer from light harvesting Chl-protein complex to PS II (Sharma et al. 1990).

Herbicide interaction with PS II

During the last decade remarkable progress has been achieved in understanding the mechanism of herbicide interaction with PS II of cyanobacteria and higher plants. DCMU and atrazine are well known herbicides, also known as PS II herbicides, at a particular concentration fully inhibit O₂ evolution (Yamashita and Butler 1969, Pfister and Arntzen 1979, Mullet and Arntzen 1981). These herbicides along with other triazines, urea and phenolic compounds, bind to the D1 polypeptide at the Qg site (Mullet and Arntzen 1981, Mattoo et al. 1981, Purcell et al. 1991). A competitive herbicide binding at the Q_B site is currently assumed to be responsible for the blockage of electron transfer between Q_A and Q_B, although, allosteric effects on the particular polypeptide cannot be totally neglected (Trebst 1987, Kleier et al. 1987). Kleier et al. (1987) have proposed a detailed structural schemes for the binding niche of Q_B and PS II herbicides. The models are mainly based on three different lines of experimental evidences:
1. The striking sequence homologies and hydropathy pattern similarities between L and M subunits of the reaction centers of purple bacteria and the D1 and D2 polypeptides of higher plants respectively (Rochaix et al. 1984, Youvan et al. 1984).

ii) the x-ray structure of *Rhodopseudomonas viridis* reaction centers (Michel et al. 1986), and

iii) the point mutations of D1 in herbicide resistant mutants (Erickson et al. 1984, Golden and Haselkorn 1985, Etienne et al. 1990).

The most frequent mutation site in the D1 protein is at serine 264. In higher plants the serine 264 is replaced by a glycine (Hirschberg and McIntosh 1983), whilst it is replaced by an alanine in the DCMU mutant of *C. reinhardtii* (Erickson et al. 1984). The position of amino acid change modify the affinity constant of herbicide for its binding site. The affinity of herbicide binding can be modified by the interaction of D1 with other proteins or its lipoidic environment. The redox state of the non-heme iron located between Q_A and Q_B is another parameter which can affect herbicide binding (Renger et al. 1983). The oxidation of Fe^{2+} reduced atrazine binding in PS II membrane fragments (Renger et al., 1988). Using radioactively labelled herbicides, it has been shown that herbicide binding was used as one of the methods to estimate the PS II reaction centers in thylakoid membrane, assuming that there is a fixed number of herbicide binding sites per PS II reaction center (Tischer and Strotman 1977, Oettmeier et al. 1982, Chow et al. 1990).
Mattoo et al. (1984) have demonstrated that incubation of isolated chloroplasts with DCMU resulted in the protection of a labelled 32 kDa polypeptide from the action of trypsin. Degradation of this protein was linked to photosynthetic electron transport and this process being effectively blocked by PS II inhibitors such as DCMU and atrazine (Gaba et al. 1987). The white and far-red light induced degradation of D1 protein is also inhibited by DCMU. The herbicide is thought to act by displacing $Q_B$ from its binding environment (Vermaas et al. 1983, Mattoo et al. 1984). It is assumed that the continuous formation of potentially damaging semiquinone anions in white and far-red light are the candidates that activate the 32 kDa protein degradation (Crofts and Wraight 1983). Herbicide mediated UV-resistance in cyanobacteria has been shown by David et al. (1988).

Rashid and Carpentier (1990) have shown that selective removal of 23 and 18 kDa extrinsic polypeptides from PS II membranes renders high susceptibility to atrazine mediated PS II inhibition. Depletion of these extrinsic polypeptides results in reduced $Q_B$ affinity to D1 polypeptide and enhanced atrazine penetration into the vicinity of the PS II complex. While the binding of herbicides to 32 kDa shows a high binding affinity, additional low affinity binding to other proteins is indicated (Tischer and Strotmann 1977). Two atrazine binding sites, with high and low affinities, have been observed on the D1 and D2 polypeptides, respectively (Chow et al. 1990).
Temperature effect on photosynthetic apparatus

Low temperature effect

Photosynthesis may be irreversibly inhibited by exposure to chilling temperature (Smillie et al. 1978, Long et al. 1983). The photosynthetic characteristics of plants which develop at low temperatures may be non-adaptive and these plants are known as 'chilling sensitive' plants. Smillie et al. (1978) observed inhibition of normal chloroplast development of barley at low temperature. As the growth temperature is lowered, the fluidity of membrane will drop and diffusional processes will be hindered. Low growth temperature of 7°C has caused significant variations in the lipid to protein ratio of thylakoid membranes (Chapman et al. 1982). Lynch and Thomson (1982) showed an increase in fatty acid unsaturation in Dunaliella chloroplast phospholipids during low temperature acclimatization. The level of DGDG in the chloroplast membrane showed significant increase during low temperature growth. These alterations of the membrane lipids offer temperature tolerance to the chloroplasts (Lyutova et al. 1988) and to the crop plants (Pike and Berry 1980).

Severe impairment of the synthesis of some chloroplast proteins was observed at chilling temperature in rice and rape (Meza-Basso et al. 1986, Hahn and Walbot 1989). Accumulation of 31 kDa polypeptide in the thylakoid has been observed in cold stressed maize leaves. This polypeptide was considered to be an unprocessed precursor of the 29 kDa apoprotein of the CP29
Hayden et al. 1988). Thylakoids isolated from plants grown at 14 and 17°C exhibited a decreased accumulation of many polypeptides when compared to those grown at 25°C. Such reduced polypeptide synthesis was accompanied by a loss of PS I and PS II activity (Nie and Baker 1991).

**High temperature effect**

High temperature treatment of leaves has caused a progressive inactivation of chloroplast activities (Berry and Björkman 1980, Sayed et al. 1989a). Adequate evidences are available to indicate that inhibition of whole leaf photosynthesis by heat stress is caused by disruption of the functional integrity of the photosynthetic apparatus (Berry and Björkman 1980). High temperature treatment causes the following to the photosynthetic machinery of higher plants:

a) a reduction in the quantum yield of whole leaf photosynthesis (Schreiber and Berry 1977),

b) a sudden rise in the Fo level (constant fluorescence) of Chl a fluorescence (Ruppel 1982, Melø and Faamundshytten 1990), and


The PS II mediated electron transport is much more sensitive to high temperature treatment than that of PS I (Santarius 1975, Berry and Björkman 1980, Sayed et al. 1989b). Incubation of leaves or isolated chloroplasts at high temperature (35-55°C)
results in marked inhibition of $O_2$ evolution, $CO_2$ fixation and photophosphorylation (Reynolds et al. 1990). Several other studies indicate that the water-splitting complex of PS II is especially sensitive to high temperature (Santarius 1975, Gounaris et al. 1983, Krishnan and Mohanty 1984). Cheniae and Martin (1970) provided supporting experimental evidences for the loss of PS II mediated electron transport and concomittant loss of Mn. It has been widely reported that the PS I mediated electron transport is stimulated at temperatures above 30°C (Santarius 1975, Thomas et al. 1986, Sabat and Mohanty 1989) and inhibited at temperatures above 55°C (Sayed et al. 1989b). Such increase in PS I activity is associated with uncoupling of chloroplast by heat and also heat induced alteration of thylakoid membrane structure imposing changes in the electron donor site (Sabat et al. 1986, Thomas et al. 1986, Sabat and Mohanty 1989).

Chlorophyll fluorescence studies were used to rank species for their temperature tolerance (Seeman et al. 1984, Melø and Faemundshytten 1990). The observed increases in Fo level in the chloroplasts as a result of continuous high temperature treatment presumably occur due to dissociation and destruction of pigment-protein complexes associated with PS II. A heat induced decrease in the efficiency of excitation energy transfer from Chl $b$ to Chl a and changes in the distribution of energy transfer between PS II and PS I were observed (Armond et al. 1979, Raison et al. 1980). Above 30°C the PS II and a portion of its bound LHC II
antennae migrate to the PS I rich non-appressed region. A
detachment of LHC II from PS II has also been suggested by
fluorescence studies on intact leaves or isolated thylakoid
membranes (Sane et al. 1984, Weis 1985). It was therefore
suggested that temperature dependent changes in the organization
of the light-harvesting apparatus may play a physiological role
by preventing overexcitation of PS II under high light with
elevated leaf temperature (Sundby and Andersson 1985). Visible
light acting as an efficient protector of the photosystems
against its inactivation by heat (Havaux et al. 1991).

High temperature acclimatization of the chloroplasts induces
modification of thylakoid membrane mobility which in turn
increase surface potentials by conformational changes in membrane
protein or alteration in phospholipid composition (Goltsev et al.
1987). Thylakoid membranes show low degree of fatty acid
unsaturation and become rigid under high temperature (Raison et
al. 1982, Goltsev et al. 1987). In vitro degradation of the 32
kDa (D1 protein) is temperature dependent. At 30°C both the
synthesis and degradation of 32 kDa protein was higher than at
10°C under photo inhibitory fluence level (Gong and Nilson 1989).

**Stratospheric ozone depletion and UV-B radiation**

It is widely agreed that a portion of earth's protective
stratospheric ozone layer is being depleted, largely by man-made
Although the list of chemical pollutants responsible for the loss of stratospheric ozone is long, one major group, the chloro-fluorocarbons (CFC), stands first. They percolate into the stratosphere where, on reacting with UV radiation produce free chlorine which destroys ozone. Each CFC molecule acts in a catalytic fashion to destroy about 100,000 ozone molecules. The major effect of this ozone loss will be an increase in the amount of UV radiation reaching the biosphere. This increase will be completely contained within the UV-B (280-320 nm) region. The short wavelength portion of UV radiation, known as UV-C (<280 nm), is completely absorbed by ozone. Increased solar UV-B radiation has been reported to cause detrimental effect on different crop plants.

Morphological and anatomical changes in higher plants

The biological effects of UV-B radiation on lower and higher plants are well reviewed by Caldwell (1971) and Klein (1978). The effects of UV-B radiation on terrestrial plants have been extensively reviewed by Tevini and Teramura (1989). In general UV-B radiation reduces plant height, fresh and dry weights and influence shortening of internode, curled leaf margins, thickened lamina, secretion of wax and abscission of the leaf.

One of the most common observation on seedlings growth after UV-B radiation is stunting or dwarfing resulting in decreased in internode length. Exposure to high levels of UV-B alters photo-
synthesis and normal growth and development in many economically useful crop plants (Van et al. 1977, Brandle et al. 1977). A marked reduction of hypocotyl length was observed in many plants grown under greenhouse or in field conditions with supplemental UV-B (Tevini et al. 1983a, Teramura and Murali 1986, Teramura and Sullivan 1987, Barnes et al. 1988, Ros 1990). Leaf area was found to be reduced significantly in 60% of crop species tested for UV-B sensitivity (Biggs and Kossuth 1978). In most of the higher plants apical meristem is well protected from direct exposure to solar UV-B by the young leaves, therefore, it is likely that UV radiation acts primarily on leaves and the primary symptoms also observed in leaves. Symptoms like bronzing, scorching, glazing and chlorosis have been observed in pea (Vu et al. 1978), soybean (Biggs et al. 1981, Sullivan and Teramura 1990), barley (Tevini et al. 1981) and cotton (Krizek 1975). The mechanisms involved in the reduction and inhibition of cotyledon and hypocotyl growth are UV-B mediated inhibition of cell division and effects on cell elongation factors like alteration of water potential and destruction of phytohormones, especially auxins (Tevini and Iwanzik 1986, Kulandaivelu et al. 1989).

UV-B mediated sensitivity of crop plants depends on the growth conditions of particular crop (Sullivan and Teramura 1990). In general, plants grown in growth chambers and greenhouse were more susceptible to UV-B than plants grown in the field conditions. The susceptibility to UV-B radiation under
controlled environment was due to the fact that the plants fail to develope UV-B protective mechanisms as found in field grown crops (Mirecki and Teramura 1984). The orientation of leaf also influence the UV-B irradiance reaching the leaf surface (Caldwell 1981). The epidermis of leaf has been assumed to protect mesophyll photosynthesis from UV radiation by decreasing the penetration of UV (Caldwell et al. 1983). The mechanism involves induction of synthesis of UV-B absorbing compounds and storage of these compounds in the vacuoles of epidermal cells (Flint et al. 1985, Björn et al. 1986, Cen and Bornmann 1990). Leaf scattering and reflectance in the UV-B region vary from species to species depending on the leaf texture. Species such as Dudleya brittoni and Picea pungens have dense pubescent leaves with UV-B reflectance in the order of up to 70% (Clark and Lister 1975, Mulroy 1979).

The amount of visible light also plays a role with regard to the extent of UV-B radiation damages to plants. Teramura (1980) found that after 6 or 7 weeks exposure to a range of moderate UV-B irradiance, reduced growth of wheat and soybean was observed in plants grown in shade condition with low PAR. Under high light conditions, bean plants appeared as most resistant to the enhanced UV-B radiation (Cen and Bornman 1990). Specific leaf weight (SLW) is also one of the adaptation features of crop species to UV-B radiation (Teramura 1983). In a screening experiments for UV-B sensitivity, crops like bean, pea, soybean,
cowpea, cucumber, watermelon, kohlrabi, rhubarb brussel sprouts and rutabaga were found to be more sensitive in which the growth and leaf expansion were reduced by 50-70%. Species like rice, wheat, barley, millet, oats, cotton and sunflower were shown to be UV-B resistant (Biggs et al. 1981, Tevini et al. 1981).

Brandle et al. (1977) have found that dry weight in pea was significantly reduced after only 2 days of UV-B radiation. They have also found an increase in dry weight of leaf as a result of increase in SLW in soybean, bean and cucumber. An altered dry weight accumulation and biomass reallocation was reported in green house and growth chamber studies (Biggs and Kossuth 1978, Vu et al. 1978). In field experiments, Caldwell et al. (1975) found that above ground dry weight was reduced in barley and pepper, while below ground reduction was observed in soybean and corn. Approximately one-third of among sixteen rice cultivars tested showed significant decrease in biomass with UV-B radiation (Teramura et al. 1991).

Biochemical changes

UV-B radiation is readily absorbed by nucleic acids and protein chromophores (Caldwell 1971). The important biochemical parameters which are influenced by UV-B radiation are photosynthetic and non-photosynthetic pigments, cuticular lipid composition, endogenous level of phytohormones, cellular and chloroplast membrane lipids and proteins and soluble proteins, especially enzyme composition of chloroplast stroma.
Pigment composition

Decrease in photosynthetic activity may also be due to photodestruction of pigments. The photosynthetic pigments, Chl a, Chl b and carotenoids, are adversely affected by UV-B radiation, with carotenoids generally being less affected than chlorophylls (Teramura 1983). Selective destruction of Chl b and carotenoids was observed in some plant species (Basiouny et al. 1978, Teramura and Caldwell 1981). This may be due to selective destruction of Chl b biosynthesis from Chl a or degradation of precursors by UV-B (Tevini et al. 1981). It has been reported that Chl a/b ratio decreased with increasing UV-B irradiance in soybean but increased in pea (Vu et al. 1984). Basiouny et al. (1978) found parallel reduction in net photosynthesis and total chlorophyll. When soybean plants were exposed to moderate levels of UV-B and 750 umol.m$^{-2}$ of PAR, an increase in chlorophyll concentration was observed (Teramura and Caldwell 1981). Similar increase in chlorophyll concentration was also reported by Mirecki and Teramura (1984). Tevini et al. (1989) have shown an increase in chlorophyll content on unit leaf area basis under solar UV-B simulating to 12% ozone depletion.

UV-B radiation in many plant species induce the synthesis of non-photosynthetic pigments namely phenyl propanoids (Tevini et al. 1983b, Wellmann 1983). Under high UV-B irradiance during growth Tevini et al. (1981, 1983) have observed high concentrations of flavonoids, which developed a resistance to UV-B
penetration to the inner mesophyll cells in barley and radish. Similar increase in flavonoid and anthocyanin content due to UV radiation was also observed by others (Bennett 1981, Mohr and Drumm-Herrel 1983, Beggs et al. 1986, Braun and Tevini 1991). These selective UV-B filters are located mainly in vacuoles. However, chloroplast location of these pigments have also been reported (Weissenbock et al. 1976, Wollenweber 1982).

Biochemical composition

Increased UV-B radiation has been shown to influence biomass and carbohydrate status in a number of field grown plant species (Teramura 1983, Tevini and Teramura 1989). The leaf soluble protein and carbohydrate content were found to be affected in many of the UV-B irradiated crops. Esser (1980) showed an increase in the carbohydrate pool in bean but decrease in spinach and radish. Crops like barley, corn and radish (Tevini et al. 1981), potato, spinach and tomato (Vu et al. 1982a) showed increased amounts of protein after UV-B treatment. Vu et al. (1982b) found that UV-B induced decrease in leaf soluble protein was parallel to that of RuBPcase activity. Chloroplast membrane lipids were greatly reduced in barley, bean, corn and radish seedlings exposed to UV-B radiation (Esser 1980). Tevini et al. (1981) reported a reduction in total protein and glycerolipids in a number of crop plants and suggested a correlation with UV-B induced process of senescence. UV induced ageing and damage to subcellular membranes were observed in many crop plants.
(Roshchupkin et al. 1975). The quality of yield in Essex was affected by supplemental UV-B radiation. While seed protein content was much affected in almost all the species tested lipid content was reduced only in some species (Teramura et al. 1990b).

Epicuticular waxes play an important role in minimizing cuticular transpiration and water diffusion. Enhanced UV-B radiation caused 25% increase in total wax in cucumber, bean and barley (Steinmuller and Tevini 1982, 1986). The mode of UV-B action was suggested to be on the biosynthetic pathway of waxes (Tevini and Steinmuller 1987).

Since the visible symptom of a plant species for UV-B radiation is reduction in plant height and shortening of internodes, it seems to indicate that UV-B radiation acts by inhibiting the auxin level (Kulandaivelu et al. 1989). Witztum et al. (1978) have shown that UV radiation significantly lowers IAA content in fronds of Spirodela oligorhiza. Similar possible inactivation of auxin in plant tissue by UV radiation was also suggested by many workers (Fridburg and Eriksson 1975, Rajagopal et al. 1987, Karez and Stolarek 1988). Recently, under artificial UV-B irradiation, UV-dependent destruction of IAA and formation of growth-inhibiting IAA photoproducts were observed in sunflower (Ros 1990).

**Physiological effects**

The photosynthetic light reaction and net CO₂ assimilation
rates have been shown to be detrimentally affected by UV-B radiation. Net photosynthesis is affected by high UV-B under low PAR (Mirecki and Teramura 1984). The trend is similar for direct measurement of RuBPcase and PEPcase, where most reductions occurred with high UV-B and low PAR levels (Vu et al. 1982b, Vu et al. 1984). In general exposure to enhanced UV-B radiation alters net photosynthesis and normal growth of many economically important crop plants (Van et al. 1977, Teramura et al. 1980, Sullivan and Teramura 1990).

The reduction in photosynthetic rate was found to be due to increase in mesophyll diffusion resistance (Teramura et al. 1980). The effect of UV-B on guard cell activity, as measured by stomatal diffusion resistance, has been variable (Van et al. 1977, Teramura et al. 1983). Comparing the effect of UV-B radiation on C_3 and C_4 plants, Basiouny et al. (1978) found that among the four C_3 plants Hill reaction was significantly reduced in oats, collards and soybean, whereas no reduction had occurred in peanuts (C_3), sorghum (C_4) and maize (C_4). Such lower sensitivity displayed by C_4 plants may be due to sclerification of tissue, vertical orientation of leaves with protective basal sheaths. Kulandaivelu et al. (1991) reported that the relative resistant in C_4 chloroplasts could be also due to the organizational differences of the thylakoid membrane.

High UV-B levels were reported to have strong effect on the amount of carboxylating enzyme present, rather than on the
inactivation of the enzyme itself (Vu et al. 1984). When simultaneous high PAR and lower level of UV-B were used, RuBPcase activity has been reported to be unaffected (Mirecki and Teramura 1984). The activity of PEPcase has significantly reduced in maize exposed to high UV-B doses while low UV-B radiation level enhanced the activity of this enzyme (Vu et al. 1982b). UV-B radiation also reduce the organic acids like glycerate, succinate, fumarate and soluble sugars like glucose, sucrose and fructose (Takeuchi et al. 1989). Sisson and Caldwell (1976) reported that dark respiration was increased in Rumex patientia. On the contrary, Teramura et al. (1980) found that UV-B radiation did not affect the dark respiration.

Target sites of UV-B radiation in photosynthetic apparatus

There are many reports on the possible target site of UV-B in photosynthetic apparatus. In addition, UV-B induced damage to membrane integrity is likely to be the contributing factors in photosynthetic damage (Skokut et al. 1977, Allen et al. 1978, Noorudeen and Kulandaivelu 1982, Bornman et al. 1986). Electron micrographs of chloroplasts from UV-B irradiated leaves revealed the disintegrity of thylakoid membranes at various regions (Vu et al. 1982a), dilation of thylakoids (Brandle et al. 1977), and rupture of chloroplast double membrane (Bornman et al. 1983, 1986). These changes will conceivably cause changes in membrane permeability. The damage at the level of membrane integrity was
attributed to the formation of endogenous free radicals which remove electrons from polysaturated lipids resulting in lipid peroxidation.

There is a general consensus that UV-B radiation mainly influences PS II. Most studies related to UV-B radiation effects on photosynthetic light reactions have found that PS I activity is not affected by UV-B (Renger et al. 1982, Iwanzik et al. 1983, Kulandaivelu and Noorudeen 1983, Kulandaivelu et al. 1991). There have been some reports of marginal inhibition of PS I, only in cases where high intensity of UV-B radiation was administered (Van et al. 1977, Brandle et al. 1977). However, UV-B induced inhibition of PS I mediated cyclic photophosphorylation was reported (Van et al. 1977, Iwanzik et al. 1983).

A number of studies have been performed to identify the primary target of UV-B radiation in PS II (Brandle et al. 1977, Noorudeen and Kulandaivelu 1982, Iwanzik et al. 1983, Renger et al. 1989). Some studies showed that UV-B radiation predominantly attacks the charge separation reaction sequence

\[ \text{Z-P680} - \text{Pheo} - Q_A \longrightarrow Z^+\text{-P680} - \text{Pheo} - Q^- \]

(Noorudeen and Kulandaivelu 1982, Renger et al. 1986, Tevini et al. 1988, Renger and Eckert 1991), so that these centers are blocked and transformed into dissipative sinks for excitation energy (Iwanzik et al. 1983) and no longer capable of supporting normal electron flow.
The measurement of chlorophyll fluorescence induction (Kautsky effect) has been used as a tool for determining the sites of UV-B damage. A reduction in the variable fluorescence yield (Fv) was attributed to the inhibition of energy transfer within PS II reaction center (Noorudeen and Kulandaivelu 1982). The impairment is due to the altered redox state of PQ pool in the sequence PQH₂ \( \rightarrow \) PQ + H₂. However, the PQ/PQH₂ ratio shows not much change after UV-B treatment (Tevini and Iwanzik 1983). It was shown that Fo values tend to remain unchanged (Katoh and Kimimura 1974, Renger et al. 1986) except at high levels of UV-B radiation (Tevini and Iwanzik 1983). An increased Fo (constant fluorescence) level was observed in UV-B treated *Helianthus* cotyledons and it could be due to reduced exciton transfer in the antenna pigment molecules (Tevini et al. 1989). UV-B induced fluorescence quenching, the photochemical quenching (qQ) and non-photochemical quenching (qE) have also been studied (Braintais et al. 1980, Horton 1983). Tevini et al. (1988) reported that with UV-B radiation the photochemical quenching due to oxidation of Qₐ, was increased initially and then decreased during steady state fluorescence, however, the non-photochemical quenching was shown to be enhanced during UV-B exposure up to 10 days.

Since PQ absorbs in the UV range, the Qₐ and Qₐ were also screened for UV targets. Renger et al. (1986) found that the amplitude of the flash-induced absorption change at 320 nm decreases with UV-B exposure. It was therefore suggested that
either the reaction center was blocked or UV-B radiation had caused a structural modification of the $Q_A$ and $Q_B$ binding apoproteins. D1 and D2 were shown to be UV-B sensitive (Greenberg et al. 1989).

Studies also indicate that UV-B radiation acts at the $O_2$ evolving system on the oxidizing side of PS II (Renger et al. 1989, Nedunchezhian and Kulandaivelu 1991, Renger and Eckert 1991). The amplitude of the flash-induced absorption changes at 830 nm an indication of the number of reaction centers carrying out stable charge separation from P680 to $Q_A$, was shown to be only slightly decreased by UV-B. The Mn content of the interface of reaction center and 33 kDa extrinsic protein decreases only partly at UV-B irradiation times where the $O_2$ evolution capacity is almost completely lost (Renger et al. 1989, Renger and Eckert 1991). Renger et al. (1989) also studied the possibility of an altered D1/D2 configuration causing functional disruption of the Mn association with the regulatory 33 kDa extrinsic protein. UV-B radiation also reduce the number of herbicide binding sites in D1 protein. These observations provide further evidences for a modification of D1/D2 complex by UV-B radiation. Nedunchezhian and Kulandaivelu (1991) have shown that loss of PS II mediated electron transport activity and $O_2$ evolving capacity was found to be primarily due to the depletion of 23 and 17 kDa extrinsic polypeptides during in vitro UV-B treatment. Chloroplasts isolated from DCMU grown wheat seedlings showed greater
resistance to UV-B radiation indicating same site of action at PS II reaction center (Kulandaivelu and Annamalainathan 1991).

Since the LHC plays an important role with regard to light absorption, thylakoid stacking and distribution of energy, any change or damage to these complexes will have strong effects on the photosynthetic system. UV-B radiation may alter LHC and modify the functional connection between LHC and PS II reaction core (Renger et al. 1986). The effect of UV-B on LHC could also be due to overall changes in membrane organization (Tevini et al. 1989b).

UV-B defence mechanism

In light of the differences in UV-B sensitivity found among crops and native plants, it is assumed that plants have the capacity to develop various protective and repair mechanisms against UV-radiation (Beggs et al. 1986). One of the most important and sensitive targets of UV radiation is DNA. Action spectra for UV damage in most organisms suggest strongly that DNA is the primary target (Caldwell 1971, Setlow 1974). Photo-oxidation and DNA pyrimidine dimers are the general damage induced by UV-B radiation in microorganisms, algae and higher plants. To overcome UV damages, organisms develop important repair mechanisms. Plants have numerous biochemical mechanisms, which include enzyme mediated repair mechanisms to protect the cells from the harmful effects of photooxidation (Elstner 1982). Photo-
reactivation is another repair mechanism commonly exist in higher plants. UV induced production of DNA pyrimidine dimers can be repaired by DNA photolyase, which is light-regulated in higher plants (Langer and Wellman 1990). Coumesterol (isoflavonoids) formation in plant cell was taken as an indicator for UV induced DNA product. The formation of coumesterol was shown to be reversed by white light given after UV-B irradiation (Beggs et al. 1985).

As mentioned earlier, pigments like flavonoids and anthocyanin are synthesized in upper leaf epidermal cells of higher plants after UV-B treatment (Wellmann 1971, Beggs et al. 1986). They are selective UV filters located mainly in vacuoles and chloroplasts. These pigments do not absorb visible radiation (Mclure 1976). Recently, Tevini et al. (1991a,b) found that photosynthetic function was low in few crop species where the UV-B induced accumulation of screening pigments was very low.

Many organisms known to induce the synthesis of a set of proteins, known as stress proteins, upon exposure to temperature increase or a number of other environmental stresses like, water, heavy metal, abscisic acid, pathogen, etc. (Neumann et al. 1989, Vierling 1991). Such stress proteins, are found to be synthesized under UV-B radiation at normal temperature in Anacystis nidulans (Shibata et al. 1991) and in Vigna (Nedunchezhian et al. 1992). It has been assumed that these UV-inducible proteins have a protective role against the damaging effects of UV-B radiation.
UV-B radiation and other stresses

Crop plants in field commonly experience multiple stresses simultaneously during their growth period from germination to yield. In some cases the response to one stress may compensate for the response to another stress. In this compensatory phenomenon, a plant encountering two different stresses would be less affected than a single stress. In some other case, it is likely that multiple stresses have synergistic effects where plants would be more adversely affected than plants subjected to only a single stress.

Water stress affects plant growth through several physiological processes viz., cell expansion, stomatal conductance, photosynthesis, dark respiration, etc. (Hanson and Hitz 1982, Kramer 1983). UV-B radiation potentially increase the susceptibility to water loss in cucumber, but not in radish (Teramura et al. 1983, Tevini et al. 1983a). The observed UV-B resistance in radish may be related to high flavonoid biosynthesis under these two stresses (Tevini et al. 1983a). Green house studies have shown that under water stress soybeans are less susceptible to UV-B radiation (Teramura et al. 1984). Murali and Teramura (1986a) have shown that the anatomical and biochemical changes induced by water stress were the reason for the insensitivity of growth and net photosynthesis to UV-B radiation. Combination of drought and UV-B radiation did not result in additive effects on total plant growth or seed yield in soybean (Sullivan and
Field studies have shown that sensitivity to UV-B radiation can also vary markedly from one growing season to the next. The seasonal variation closely parallels the variability in precipitation during particular growing season (Murali and Teramura 1986b).

Bogenrieder and Doute (1982) compared the effects of UV-B radiation on the growth and photosynthetic response of lettuce and alpine sorrel grown under four different mineral concentrations. The sensitivity of net photosynthesis to UV-B radiation increased with increasing mineral concentration in lettuce, but not in alpine sorrel. Murali and Teramura (1985) also showed less susceptibility to UV-B radiation under mineral deficiency in soybean. UV-B sensitivity is influenced by existing microclimatic conditions including ambient levels of visible radiation, where UV-B sensitivity was higher in low PAR grown plants (Warner and Caldwell 1983, Mirecki and Teramura 1984). Sensitivity to UV-B radiation may also vary depending upon the phenological stages of development.

UV-B radiation and CdCl₂ applied simultaneously decreased the dry weight, height of seedlings, pigment content and CO₂ assimilation to a greater extent than found under UV-B treatment alone (Bornmann and Dubé 1991, Dubé and Bornman 1991). Biomass and yield were increased significantly in soybean, rice and wheat when grown in elevated CO₂. However, with concurrent increases in UV-B and CO₂, these CO₂ induced increases remained in soybean
but eliminated in rice and wheat (Teramura et al. 1990a). Therefore, the combined effects of CO$_2$ and UV-B are species specific. Recently, Tevini et al. (1991c) have performed experiments in growth chambers with two temperature regimes of 28 and 32°C and raised plant species under UV-B enhanced radiation. The results indicate that high temperature regimes could ameliorate the UV-B effects in two (maize and rye) among three species tested.