Review of literature
Plastids in higher plant cells are unique organelles that exist in different forms and perform various functions. All these forms are derived from small, undifferentiated plastids called proplastids, present in meristematic cells. In leaves and other photosynthesising tissues the proplastids develop into chloroplasts. In nonphotosynthetic organs of higher plants, however, the proplastids and chloroplasts are differentiated into other types of plastids such as chromoplasts in flowers and fruits or amyloplasts in roots and tubers (Kirk and Tilney-Basset 1978, Wellburn 1987). Among these, the chloroplasts are well studied and better understood.

1. CHLOROPLASTS
Chloroplasts reside in specialised leaf cells and are associated with one of the most important physiological functions of the plants, the photosynthesis. These organelles are also involved in a variety of secondary processes such as the biosynthesis of starch, lipids, terpenoids, tetrapyrroles, aminoacids and nucleotides. However, all these secondary processes depend on photosynthesis.

1.1 Photosynthesis
Photosynthesis confirms to the biological redox reactions, through which light energy is converted to biologically useable chemical energy. Here donar + acceptor react under the influence of light in the presence of pigments and necessary mechanism. A pigment molecule captures the energy of a photon of light, forming an electronic exited state. In higher plants these excited electrons are used to reduce NADP+. The oxidised pigment is reduced by an electron from water whose H+ accumulates forming a proton concentration gradient, the energy of which is used to produce ATP (Fig 1) (Hall and Rao 1994, Lawlor 1993, Whitmarsh and Govindjee 1999). The chloroplasts are ideally designed to perform this process.
Figure 1: The Z-scheme of photosynthetic electron transport (Witmarsh and Govindjee 1999).
1.2 Structure of chloroplasts

Chloroplasts are 5-10 μm in diameter with an inner and an outer envelope membrane (Kirk and Tinley-Basset 1978, Wellburn 1987). The inner envelope membrane is semi-permeable, controlling the flux of organic and charged molecules in and out of chloroplasts. Inside the chloroplast is a membrane vesicle system known as the thylakoid system. It is embedded in a matrix, the stroma, containing plastidic DNA, ribosomes, plastoglobuli, starch granules and enzymes for CO₂ fixation. Thylakoid membranes are spatially differentiated into appressed granal and non-appressed stromal regions that are inter-connected and show heterogeneity in their composition (Stays 1995).

The thylakoid membrane: Thylakoid membranes are 5-7 nm thick and about half of their mass consist of lipids. It encloses a continuous aqueous phase called lumen. Membrane polypeptide complexes consisting of various non-covalently bound subunits form the scaffolds for the pigments needed for light absorption. Five such supra molecular complexes have been identified, namely, the photosystem II (PSII), the light harvesting complex II (LHCII), cytochrome b₆f complex (Cyt b₆f), the photosystem I (PSI) and ATP synthase (Fig. 2) (Cogdell and Lindsay 2000, Irragang 1999, Staehelin 1986, Staehelin and van der Staay 1996).

PSII: Photosystem II contains proteins and cofactors necessary for the light driven movement of electrons from water to palstoquinone. It has three chlorophyll-protein complexes: the reaction center and two internal chl a light harvesting antenna CP47 and CP43 and a peripheral oxygen evolving complex (OEC) located on the lumenal face of the thylakoid membrane. The reaction center has 4-6 chl a, 2 pheos and 2 quinones bound by a pair of hydrophobic
Figure 2: Schematic picture showing thylakoid structure (Wollman et al 1999).
polypeptides D1 and D2. A number of hypothetical PSII models have been proposed based on the bacterial template (Barber et al 1999). In addition to D1 and D2 the reaction center has three small proteins cyt b-559, Psbl and PsbW. Data shows that CP47 and CP43 have about 20 chl a and 4-5 β-carotenes and a small amount of lutein per polypeptide (Green and Durnford 1996). The OEC of higher plants has three polypeptides of 33, 23 and 16 KDa in 1:1:1 stochiometry. The 33 KDa polypeptide binds to the core complex and stabilizes the Mn-cluster (Coleman and Govindjee 1993). The 23 and 16 Kda enhance the binding of Ca\(^{2+}\) and Cl\(^{-}\). Besides these PSII has also a number of low molecular weight proteins whose exact role is not known.

**PSI:** It catalyzes the photooxidation of plastocyanin and photoreduction of ferridoxin. In PSI the reaction center and internal antenna are combined in a single chlorophyll-protein complex. This has two transmembrane hydrophobic polypeptides, PSI-A and PSI-B, of 82 KDa that bind to a chl a dimer and various electron acceptors and a stromal facing 6 KDa extrinsic protein PSI-C with 2 Fe-S clusters and binds to ferridoxin. These two polypeptides together bind 75-100 additional chla and 12-15 β-carotene molecules, which act as internal antenna lying parallel to the membrane surface (Green and Durnford 1996). There are several other polypeptides either on the luminal or stromal sides of the membrane (Fromme 1996).

**Light harvesting complexes:** LHCII is the most abundant chlorophyll-protein complex and accounts for almost half of the chlorophyll in plastids. LHCII from vascular plants contain three distinct subunits I, II and III in the ratio of 10-20:3:1 depending on the plant growth conditions. The chl \(a/b\) ratio is about 1.4 but vary in different subunits indicating same amount of chla but different chlb. It binds to about 12 chls, 3-4 xanthophylls per polypeptide (Green and Durnford 1996).
Review of literature

minor antenna complexes CP24, CP26 and CP29 exist as monomers binding 8-10 chls with chl a/b ratio between 1.4 to 3 and 2 xanthophylls. These polypeptides are enriched with the xanthophylls, neoxanthin and violaxanthin, compared to LHCII. An additional 22 KDa pigment-binding subunit PsbS has 5 chlorophylls and has been shown to have a role in non-photochemical quenching of fluorescence (Li et al 2000).

LHCI has four distinct polypeptide components in eqimolar ratios forming two dimeric pigment polypeptide complexes LHCI-680 and LHCI-730. Each polypeptide binds to 10 chlorophylls, with a chl a/b ratio of 4, and some catotenoids.

All the polypeptides of antenna proteins except PsbS have a characteristic organization with three a-helical transmembrane domains and stromal exposed N-termini. PsbS has four transmembrane helices (Kuhulbrandt et al 1996).

**Cytochrome b_{6f}:** The cyt b_{6f} complex transfers electrons from reduced plastoquinone to a soluble electron carrier, plastocyanin located in the thylakoid lumen. It is made up of seven subunits of which the contribution of four is known (Trumpower and Gennis 1994). A Rieske iron-sulfur protein, a c-type cytochrome cyt f, cyt b_{6} with two b-type hemes and a subunit IV which together with Rieske protein and cyt b_{6} contributes residues for quinone binding. Recently a chl a molecule has been identified as an additional prosthetic group in cyt b_{6f} complex binding to cyt b_{6} (Wollman et al 1999).

**ATP synthase:** It belongs to the F_{1} family of ATPases and generates ATP from ADP and inorganic phosphate using energy derived from a trans-thylakoid proton
gradient. It has distinct sectors, a membrane-embedded proton channel, CF₀ and a catalytic sector, CF₁ located on the stromal surface of the thylakoid membranes. CF₁ has five subunits α-ε in 3:3:1:1:1 stochiometry and has nucleotide binding sites with ATPase activity. F₀ has four transmembrane subunits I-IV (McCarty et al. 2000).

Electron microscopy techniques such as freeze-fracture and freeze-etching of thylakoids revealed the structural details of its surface and interiors and distribution of functionally active membrane complexes (Staehelin 1986). These techniques along with others have shown that these complexes are not randomly distributed. The ATPase is exclusively found in stroma lamella and the cyt b₆f is distributed evenly in both grana and stroma thylakoids. PSII is predominantly in granal regions and PSI preferably occurs in stromal thylakoids. LHClI is mostly observed in the grana thylakoids though it can migrate laterally between PSII and PSI in response to environmental conditions.

**Thylakoid lipids:** A high protein to lipid ratio characterizes the thylakoid membrane. The unique lipid composition and their physico-chemical characteristics impart thylakoids a highly fluid state that is essential for playing specific and dynamic roles in photosynthesis with the presence of large amounts of glycerolipids. Galactolipids, monogalactosyl diacylglycerol (MGDG) and digalactosyldiacyl glycerol (DGDG), represent up to 80% of glycerolipids, from which MGDG alone constitute about 50% (Webb and Green 1991, Murphy 1986a, 1986b, Siegenthaler and Murata 1998). While the other glycerolipids, sulfoquinovosyl diacylglycerol (SQDG) and phosphatidylglycerol (PG) account for 5-12% (Murata and Siegenthaler 1998). The unique feature of galactolipids is their very high content of polyunsaturated fatty acids mostly 16:3 and 18:3 trienoic acids. SQDG and PG hold mainly 16:0 and 18:3 fatty acids. The plastid
PG has some unique 16:1 trans-3 acid.

Evidences suggest that the bulk lipids, MGDG and DGDG, that predominate the membrane fill the spaces between the various membrane proteins and can be considered as structural lipids (Murata and Siegenthaler 1998). It is explained by the non-bilayer forming MGDG which is shown to form membrane stacking when associated with LHCII (Lee 2000, Simidjiev et al. 2000), leading to gamma formation. This supports the general belief that the lipid-protein interactions play a major role in imposing a bilayer configuration in native membranes (Williams 1998). By contrast, the functional lipids that tend to be less unsaturated than the structural lipids, bound to proteins by specific interactions and ensure an adequate conformation and their orientation in the membrane. These specific associations of lipids with proteins and the lateral heterogeneity of protein components indicate a lateral asymmetry of lipid classes in thylakoids (Siegenthaler 1998).

Similarly, there are experimental evidences suggesting an asymmetric, transmembrane heterogeneity in the lipid class distribution among the two membranes monolayers. They indicate that the outer monolayer is highly enriched in MGDG and PG while the inner one has high levels of DGDG. The lateral and transverse assymmetry in lipid species distribution along with the unusual chemical structures of MGDG and DGDG etc, are proposed to have formed the basis of the unique organization of thylakoid membranes (Siegenthaler 1998).

This organization determines the co-operative interactions among its components and complexes, through their lateral and transverse movements within the membrane. These movements are consequently dependent on the
fluidity of lipid matrix. Membrane fluidity is primarily determined by the length and unsaturation of fatty acid chains of lipids, as well as by the relative composition of its lipids and proteins, and by the temperature. It has been suggested that fatty acid unsaturation is required for maintaining the appropriate membrane fluidity over a wide range of environmental temperatures (Siegenthaler and Tremolieres 1998).

2. ETIOPLASTS
During growth of the higher plants and their seedlings in dark the plastids develop into etioplasts, the typical achlorophyllous plastids, instead of chloroplasts. Although it is not the normal course of plant growth and chloroplast development, the greening of etiolated seedlings and the associated transformation of etioplast to chloroplast provide a convenient experimental system to study the development of thylakoid membrane systems in developing chloroplasts (Bhardwaj and Singhal 1981, Balakrishna et al. 1999).

2.1 Structure of etioplasts
Etioplasts are oval in shape, 1-5 μm long and have a characteristic structure. The membrane structure is much simpler than chloroplasts with a large system of paracrystalline arranged tubules, called prolamellar body (PLB) connected to which are some membranes, the prothylakoids (PT) (Minkov et al 1988, Wellburn 1987). Chlorophyll biosynthesis is stalled at protochlorophyllide (Pchlide) since the enzyme that reduces it to chlorophyllide - protochlorophyllide oxidoreductase (POR) - is a photoenzyme. Carotenoids are synthesized though at much lower levels. They are localized in the plastoglobuli along with the lipids (Goodwin 1977).

PLBs and PTs: The fine structure of the PLB has been extensively studied by
electron microscopy (Selstam 1998). It is formed by a branching tubular membrane. These tubes are formed by a bilayer with a water channel inside. While majority of the Pchlide complexes with POR and reducing agent NADPH along with membranes, resulting in the formation of PLB, a small amount of it is in a free, non-photoreducible form. POR is an extrinsic membrane protein with the lipid protein interaction in the polar region of the membrane (Benli et al. 1991, Birve et al. 1996, Suzuki and Bauer 1995). POR oligomers are probably phosphorylated, and dephosphorylation of POR may regulate its monomer formation and transformation of PLB (Ryberg and Sundquist 1982a, 1982b, 1991, Wiktorsson et al. 1996).

There are two differentially regulated PORs in etioplasts. PORA is present at a high level and is negatively regulated by light; PORB is found at lower, constitutive levels. Recently, it has been reported in barley, PORA and PORB form a stable complex, in a ratio of 5:1, and are called as light harvesting POR-protochlorophyllide complexes (LHPP). In this complex PORA and PORB are specific to Pchlide b and Pchlide a respectively. It was presumed, that Pchlide b funnels light in the initial stages of greening for the reduction of Pchlide a (Willoros 1999, Reinbothe et al. 1999). However, there are several evidences conflicting this model (Armstrong et al. 2000). The PTs lack photosystems and other major thylakoid protein complexes (Lindsten et al. 1988).

Membrane lipids: The lipid composition of the PLBs and PTs is similar to that of thylakoids but the relative amount of MGDG is usually less than the chloroplast. The MGDG to DGDG ratio is 1.6-1.8 and 1.1-1.4 in the PLBs and PTs respectively. There is 30% more lipid per protein in the PLB than in the PT (Selstam and Widell-Wigge 1993). The fatty acid composition of the etioplast membrane lipids shows some variation depending on the age of the plastid.
older the plastid the more the fatty acid composition is similar to that of the chloroplasts. They can synthesize large amounts of highly unsaturated galactolipids and phaspholipids but not the amounts of 16:1(3t) found in mature thylakoids.

3. GREENING
Development of chloroplasts has often been studied during greening of etiolated grass leaves, instead of more natural plastid development in leaves grown under light-dark cycles. This is practical since grasses germinate and develop large leaves in darkness and the number of etioplasts increase with cell age. In the light, the PLB transforms by losing its regular structure and disperses into planar structures. During the dispersal, the membrane is first perforated and latter the holes are closed and thylakoids with grana are formed.

Changes in membrane lipid composition during chloroplast development from etioplasts, in various species under different light conditions have been analyzed (Tevini 1977). During this development the amount of lipid per plastid increases. There are indications that the lipids in the PLBs and PTs are reorganized into the developing thylakoids.

3.1 Thylakoid development during Greening
The development of fully functional thylakoids in plastids from the etioplasts requires the assembly of light-induced and pre-existing components. While some of the thylakoid electron transport chain components such as cytochromes b and f and Rieske Fe-S protein, β-subunit of ATP synthase, ferridoxin-NADP-oxidoreductase and the extrinsic polypeptides of oxygen evolving complex of PSII are synthesised in the dark, the synthesis of chlorophyll and chlorophyll binding proteins such as P700 A and B, D1, D2, CP43 and 47 require the radiant energy.
The transformation and dispersal of the PLB is correlated with a series of reactions that starts in the light. Pchlide is immediately reduced to chlorophyllide and within 10 minutes chla and the apoproteins of P700, CP43, CP47 and D1 are formed (Sundquist and Dahlin 1997, Klein et al 1987, Frank 1993). These intrinsic proteins are supposed to stabilize the lipids to a bilayer.

PSII mutants form Chlamydomonas have suggested that there is a first step in the assembly of PSII where by D1/D2/CP47/Cyt b-559 substructure is formed prior to its association with CP43. These two subcomplexes have been found to migrate to the stacked membrane region independently (de Vitry et al 1989). It has been shown that D1 associates at very early stages with D2 and CP47 in unstacked membrane regions before integrating in larger protein complexes in stacked membrane regions. The D1 protein synthesis and its membrane insertion are redox and membrane fluidity dependent (Wollman et al 1999). At some point of PSII assembly D1 binds chla and β-carotenoids and role of β-carotenoids in the turnover of D1 has been reported (Trebst and Depka 1997). Together with chla, carotenoids accumulation has been shown to play an important role in the stabilization and assembly of newly synthesized photosynthetic complexes including CP47 and CP43 (Wollman et al 1999)).

The PSI subunits PSIA and PSIB are cotranscriptionally inserted into the thylakoid membranes. However, this insertion becomes protease-resistant only upon their association with Chla in greening etioplasts from barley (Kim et al 1991). In developing plastids like the greening of etioplasts PSI is assembled step-by-step. While the PSIA and PSIB can be detected in etioplasts, the peripheral subunits appear subsequently upon exposure to light (Nechushtai and Nelson 1985).
LHCII has been reported to remain in its apoform in the stroma lamella (Yalovsky et al 1992). Thus, it may not require pigment binding for its integration into the thylakoid membranes. However, in vivo and in vitro studies have convincingly demonstrated the critical role of pigment binding for LHCII integration in a protease resistant form. For instance, LHCII which can not maintain a stable conformation when inserted into barley etioplast membranes in vitro, when supplemented with chlorophyll analog Zn-pheophytin a/b turned protease resistant. Thus chl is the only component required for a stable LHCII insertion (Kuttkat et al 1997). Also, several carotenoid and chlorophyll deficient mutants insert but fail to accumulate LHCII in their thylakoid membrane (Wollman et al 1999). It has been suggested that LHCII itself would regulate the final steps of Chl a and Chl b synthesis (Plumley and Schmidt 1995).

It has been shown that the LHCII subunits accumulate first as monomeric pigmented complexes before their oligomerisation in trimers (Dreyfuss and Thornber 1994). Trimer formation of LHCII subunits was also shown to depend on the presence of specific lipid molecules (Wollman et al 1999). Similarly LHCI subunits assemble first as monomeric pigmented complexes before their oligomerization and association with the preexisting PSI. Availability of pigments has been shown to regulate the relative amounts of three types of antenna complexes that differ in their chl a/b ratios. When limiting amounts of chl b are present, the minor antenna accumulates prior to LHCII (Dreyfuss and Thornber 1994).

However, experiments based on in vivo studies using various strains of Chlamydomonas have developed a different view on LHCII assembly. They show that holo-LHCII assembles at the inner chloroplast envelope. LHCII import into
the chloroplast would be arrested when chl delivery is insufficient. Thus membrane integration would be controlled by pigment binding at the inner envelope level. Subsequent translocation to the thylakoid membrane would occur by envelope invagination, budding and vissicle trafficking and fusion in stroma (Hoober et al 1991, Morre et al 1991).

4. CAROTENOIDs

Carotenoids are a class of hydrocarbons and their oxygenated derivatives that consist of eight isoprenoid units forming two tail-to-tail linked C20 units. In many carotenoids the end groups are modified into five or six membered rings at one end or both the ends of the molecule to give monocyclic and dicyclic carotenoids respectively. The hydrocarbon carotenoids are called carotenes and their oxygen derivatives xanthophylls. Both carotenes and xanthophylls have been found in the leaf tissues (Table 1) (Goodwin 1980, Goodwin and Britton 1988, Szabolcs 1990, Young 1993, Frank and Cogdell 1996). However, among xanthophylls only dicyclic xanthophylls are found in photosynthetic tissues. The levels of different carotenoids in leaf tissues are greatly affected by environmental, growth and developmental conditions.

4.1 Carotenoids in photosynthesis

The first and perhaps the essential function of carotenoids in photosynthesis is to prevent the harmful photooxidative reactions in presence of oxygen. This is illustrated by the killing of carotenoidless mutant Rhodobacter sphaeroides R-26 when illuminated in the presence of oxygen (Griffiths et al 1955). This photoprotective function utilises triplet-triplet energy transfer from chlorophyll to carotenoids to prevent the chlorophyll-sensitised formation of singlet oxygen (Edge and Truscott 1999). It was also shown that carotenoids scavenge singlet oxygen directly (Krinsky 1968) in vitro. It has been shown that β-carotene can
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<td>Antheraxanthin</td>
<td>Adinoxanthin</td>
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<tr>
<td>(\alpha)-Carotene</td>
<td>(\beta)-Carotene-5,6-epoxide</td>
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<td>(\beta)-Carotene</td>
<td>(\beta)-Cryptoxanthin</td>
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<td>Neoxanthin</td>
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<td>Semi-(\beta)-carotene</td>
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Table 1: Carotenoids of higher plant photosynthetic tissues (Young 1993)
act as efficient quencher of singlet oxygen generated within isolated PSII reaction centers (Telfer et al 1994, Edge and Truscott 1999). Perhaps the two β-carotenes of the PSII molecules acting as quenchers.

The second function of carotenoids in photosynthesis is to act as accessory light harvesting pigments. They absorb the solar radiation in the 450-570 nm region, where the chls do not absorb efficiently, and transfer the energy to the chlorophylls. Thus carotenoids enhance the wave length range over which light is able to promote photosynthesis. This light harvesting function makes use of singlet - singlet energy transfer between carotenoids and chlorophylls (Frank and Cogdell 1996, Cogdell and Frank 1987, Govindjee 1999, Siefermann-Harms 1987) and generally occurs in the antenna pigment protein complexes like LHCs.

Carotenoids are also involved in photoprotection during photosynthesis through their participation in xanthophyll cycle. This pH dependent thermal dissipation occurs in the LHCII pigments and involves specific deepoxidised xanthophylls (Sarry et al 1994). The increase in the thylakoid ΔpH in excessive light activates the enzyme violaxanthin deepoxidase (VDE), which converts violaxanthin associated with LHCs to zeaxanthin and antheraxanthin. This zeaxanthin may deexcite the excited chl by a direct transfer of energy or along with protons bind to LHCs causing conformational changes leading to concentration quenching by chl or quenching via chl dimer formation (Niyogi 1999)

There are several experimental indications of the direct presence of carotenoids in the photosynthetic membranes particularly those involved in the xanthophyll cycle (Tardy and Havaux 1997; Havaux 1998; Gruszecki 1999). It has been hypothesised that carotenoidss among other terpinoids play
the structural role in biological membranes of prokaryotes (Roohmer et al. 1979). Since chloroplasts share several similarities with prokaryotes it may be expected that carotenoids play a similar role in plastid membranes. The effect of zeaxanthin on the thylakoid membranes fall among phenomena typical of xanthophyll presence in bacterial membranes (Gruszecki 1999). Zeaxanthin, the xanthophyll pigment, is present in photosynthesis apparatus exclusively under conditions of excess illumination or other kind of physiological stress. (Pfundel and Bilger 1994, Eskling et al 1997). Under such conditions due to xanthophyll cycle violaxanthin and zeaxanthin are directly present in the lipid phase of the thylakoid membranes. Their direct presence potentially protects lipid molecules from oxidative damage (Edge et al 1997). As the thickness of the hydrophobic core of the thylakoid membrane closely matches with the length of the zeaxanthin molecule they may act as a filters shielding the pigment protein complexes from excessive illumination (Gruszecki 1999).

As has been observed carotenoids are constitutive components of all pigment protein complexes in plants. Their importance as stabilizing factors suggest that these pigments are also involved in the assembly of PSII and light harvesting complexes. Trebst and Depka (1997) have shown that β-carotene is essential for the assembly of the D1 protein into functional PSII. The chl a/b LHCs in plants also assemble only when carotenoids are present. In some organisms lutein appears to play important role in assembling chl a/b complexes (Bishop et al 1995, Bishop 1996). However, in other organisms the lack of lutein has no obvious effect on the chl a/b antenna (Pogson et al 1996, Niyogi et al 1997). It is presumed that when lutein is missing they are replaced by other carotenoids which effect the stability of LHCs, and different organisms may vary in their ability to tolerate this reduced stability (Paulsen 1999). Carotenoids may
also influence oligomerization and aggregation of LHC II in the thylakoid membrane (Tardy and Havaux 1996, Ruban et al 1997).

4.2 Carotenoid biosynthesis in plants
The biosynthesis of carotenoids found in leaves is located in the plastids and it is assumed to take place in the thylakoid membrane. There are also reports suggesting that it takes place in the plastid envelop as well (Douce and Joyard 1990, Joyard et al.1991).

Formation of carotenoids takes place via the terpenoid pathway using prenyl pyrophosphates as precursors (Fig 3). The enzyme phytoene synthase combines two molecules of geranylgeranyl pyrophosphate to phytoene, the first carotene in the pathway. This carotene is subjected a series of desaturation and two cyclisation steps. The first enzyme involved in the conversion of phytoene is phytoene desaturase, which has shown to be the target for bleaching herbicides. The introduction of oxygen functions and other structural modifications leading to formation of xanthophylls are considered to occur as a final process in carotenoid biosynthesis.

A newly discovered chloroplast alternative oxidase is important for photoprotection during chloroplast development and perhaps also in mature chloroplasts. This oxidase appears to oxidise reduced plastoquinol and the oxidised plastoquinone is thought to be required for phytoene desaturation as an electron acceptor. This function may be critical for efficient carotenoids biosynthesis during the early stages of chloroplast development when PSI dependent oxidation of plastoquinol is less efficient. Also this chlororespiratory path may have a significant role in providing a safety valve by allowing carotenoids biosynthesis to proceed when the photosynthetic electron chain becomes over reduced (Niyogi 2000).
Figure 3: Carotenoid biosynthetic pathway in plants (Carol and Kunz 2001).
Regulation of biosynthesis: Carotenoid biosynthesis is an essential part of the construction of pigment-protein complexes in the thylakoids, and its regulation is closely linked to that of other components such as chlorophylls, proteins and lipids. There are several different carotenoids which have different locations and functions within pigment-protein complexes. There are delicate regulatory mechanisms that determine the specific destination and location of each carotenoid molecule that is made and assembled within the thylakoid pigment-protein complexes.

Etiolated seedlings generally contain substantial amounts of carotenoids, although the distribution of individual pigments differs considerably from that of the green plants. In particular, the etiolated leaves and etioplasts normally contain little or no carotene and a modified collection of xanthophylls. Lutein and violoxanthin are predominant but a substantial amount of antheraxanthin is usually present and there is little or no neoxanthin (Bahl 1977, Barry et al. 1991). The carotenoids are located in prolamellar bodies, prothylakoids, and the envelope membrane of the etioplasts. Among different enzymes of the carotenoids biosynthesis, the phytoene synthase is present in a soluble form in the stroma and the subsequent enzymes in the membrane fraction (Lutke-Brinkaus and Kleinig 1987).

During greening of the etiolated barley seedlings the carotenoid levels seem to be doubled in the rapidly growing bottom zone of the shoot. The levels of β-carotene and neoxanthin increased several-fold, and that of antheraxanthin decreased markedly. The lutein and violaxanthin present in the etiolated seedlings were retained during greening and incorporated into the chloroplasts and thylakoids. β-carotene is synthesized de novo. The neoxanthin appears to be
produced from antheraxanthin or violaxanthin (Britton 1993)

5. OXIDATIVE STRESS AND ANTIOXIDATIVE SYSTEMS IN PLASTIDS

Although oxygen is essential for the aerobic life, it can also cause lethal damage to organisms (Helliwell & Gutteridge 1989). Since thylakoid membrane has highly unsaturated fatty acids and perform oxygenic photosynthesis, it is paradoxical these membranes are highly susceptible to oxidative stress. However, plants have developed advanced defense systems to avoid damage by oxygen free radicals (Foyer et al 1994).

Molecular oxygen in its ground state is triplet, containing two unpaired electrons with parallel spins, and very unreactive. In order to render the ground state oxygen active, the triplet state of oxygen has to be changed to singlet state to circumvent the spin restrictions. In the course of these changes and interactions several active oxygen species such as superoxide anion radical ($O_2^-$), hydrogen peroxide ($H_2O_2$), hydroxylradical ($OH^-$) and singlet oxygen ($^1O_2$) are produced.
**Superoxide radical:** Superoxide radical could be produced in higher plant chloroplasts through the univalent reduction of $O_2$ or univalent reduction of $H_2O_2$ (Elstner 1982, 1987, Asada et al 1998). It can be produced enzymatically by some flavoprotein dehydrogenases or non-enzymatically through the autoxidation of substrates such as ferredoxins, hydroquinones, thiols and reduced hemoproteins (Fridovich 1976). This radical has been implicated as an agent in a number of reactions such as lipid peroxidation, membrane damage, cellular toxicity and single strand breaks in DNA (Fridovich 1976). $O_2^•$ may also play important role in generating more potent oxidants like $^•OH$ by reactions with $H_2O_2$ and in the presence of metals such as iron.

**Hydrogen peroxide:** $H_2O_2$ is produced through dismutation of $O_2^•$ either spontaneously or catalytically with superoxide dismutase (SOD). In addition to dismutation, the reduction of $O_2$ or $O_2^•$ by ascorbate, thiols, ferridoxin and $Mn^{2+}$ ions also produces $H_2O_2$. It is a most stable oxy-intermediate and has a low reactivity. Its toxicity is low at physiological concentrations (Fridovich 1976), however, this toxicity is enhanced in presence of metal catalysts due to hydroxyl radical formation through Fenton reaction.

**Hydroxyl radical:** The major source of $^•OH$ radical in chloroplasts is production from $O_2^•$ and then $H_2O_2$ through the metal catalyzed Haber-weiss or Fenton reaction (Youngman and Elstner 1981, Youngman 1984). They are also produced when Felli or Cull is reduced by reductants such as semiquinone radicals, thiols and ascorbate (Sutton and Winterbourne 1984). It may also be produced in chloroplasts by univalent reduction of $H_2O_2$ (Elstner 1987, Asada and Takahashi 1987). $^•OH$ radicals are very short lived in biological systems, highly reactive and reacts indiscriminately. It interacts with enzymes, small metabolites, nucleic acids...
and membranes right at the site of its production.

**Singlet oxygen:** It is an electronically excited species of \( \text{O}_2 \) where spin reversal to antiparallel spin occurs. \( ^1\text{O}_2 \) is also formed by electron donation of \( \text{O}_2 \) to certain electron acceptors through dismutation. In plastids chlorophylls can act as photosensitizers producing \( ^1\text{O}_2 \). It causes peroxidation of polyunsaturated lipids resulting in weakening and disruption of biomembranes (Takahama and Nishimura 1976).

The active oxygen species (AOS) in biological systems have a very short life time and may generate a number of other reactive species with comparatively longer lifetimes. Oxidized forms of unsaturated fatty acids including lipid peroxiradicals (LOO\(^\cdot\)), hydroperoxides (LOOH) and alkoxy radicals (LO\(^\cdot\)) may be one of these species directly related to the damage. Life times of these lipid derivatives are generally longer than that of free primary AOS and they are regarded as the long life species of active oxygen. They interact with enzyme proteins and other target molecules including DNA to induce cellular damage (Asada and Takahashi 1987).

**Carotenoids as antioxidants:** It is well known that carotenoids can act as antioxidants by quenching sinplet oxygen or photosensitizer triplet status. Quenching by electron exchange energy transfer to produce the carotenoids triplet state (\( ^3\text{Car} \)) is the principal mechanism of carotenoids photoprotection against \( ^1\text{O}_2 \).

\[
^1\text{O}_2 + \text{Car} \rightarrow ^3\text{O}_2 + ^3\text{Car}
\]

\( ^3\text{Car} \) can return to the ground state by dissipating energy as heat or it can be
quenched physically by O$_2$ via intersystem crossing.

Many different carotenoids have been studied, in inorganic solvents and in artificial membranes, to investigate their structural characteristics on the ability to quench O$_2$. The ability to quench increases with chain length of carotenoids and lycopene is most efficient among natural carotenoids. Where as Telfer et al (1994) have shown that β-carotene can act as an efficient quencher of O$_2$ generated within isolated PSII reaction centers. However, the role of carotenoids as free radical quenchers is not well understood. They can interact with free radicals in a number of ways, namely electron transfer, hydrogen atom transfer and addition. The mechanism and rate of scavenging of oxy-radicals by carotenoids is strongly dependent on the nature of the oxidizing species. It has been shown that the carotenoids radicals are converted into the parent carotenoids by ascorbic acid and in some cases α-tocopherol.

**Tocopherols:** α-tocopherols can quench and scavenge $^1$O$_2$, O$_2^-$ and ·OH in the plastid membranes to prevent lipid peroxidation and its chain reactions. These also appear to have some role in controlling membrane fluidity and stability (Fryer 1992).

**Ascorbate:** It prevents oxidative damage through direct quenching of $^1$O$_2$, O$_2^-$ and ·OH, has a role in regeneration of α-tocopherol from α-chromanoxyl radical, and acts as a substrate in VDE and APX reactions. It is very abundant in chloroplasts (Noctor and Foyer 1998, Smirnoff 1996).

**Glutathione:** Glutathione in plastids is capable of detoxifying $^1$O$_2$ and ·OH. It also protects thiol groups in stromal enzymes and is involved in α-tocopherol and ascorbate regeneration through glutathione-ascorbate cycle (Noctor and Foyer
Super oxide dismutase (SOD): The $O_2^{**}$ generated in plastids either due to photo oxidation or due to some other stromal factor mediated reaction is dismutated to $H_2O_2$ by SOD. In almost all plants, chloroplasts contain Cu-Zn-SOD as the major isoform of SOD and several plants also contain Fe-SOD (Asada 1999, Bowler et al 1992). Both these isoforms are soluble proteins, but a major part is attached to stromal thylakoids, where PSI is localised and where $O_2^{**}$ is produced through water to water cycle (Asada et al 1998, Asada 1999, Niyogi 1999). When $O_2^{**}$ fail to be scavenged in the thylakoid system it is dismutated by the stromal enzyme.

Ascorbate peroxidases: $H_2O_2$ produced via the dismutation of $O_2^{**}$ by SOD is reduced to $H_2O$ by ascorbate catalyzed with ascorbate peroxidase (APX). Like SOD, it is also present as thylakoid, bound and soluble stromal isoforms. The bound APX binds to the stromal thylakoids where PSI is localised (Asada et al 1998, Asada 1999, Niyogi 1999).

In addition to SOD and APX plastids also contain phospholipid hydroperoxide scavenging glutathione peroxidase (Eshdat et al 1997) and 2-cys peroxiredoxin (Baier and Dietz 1997). These enzymes may participate in the reduction of lipid hydroperoxide of thylakoid membranes to its alcohol to suppress the chain oxidation of thylakoid phospholipids (Asada 1999).

6. HERBICIDES
Our understanding of photosynthetic process has been greatly enhanced by the use of chemicals as specific inhibitors. Several of these inhibitors are successfully used as herbicides for weed control and are commercially available.
Herbicides that interfere in the primary process of photosynthesis have been classified into five groups: electron transport inhibitors, inhibitory uncouplers, electron acceptors, uncouplers and energy charge inhibitors (Moreland 1980).

Many structurally unrelated herbicides cause bleaching of pigments chlorophylls and carotenoids. These are generally referred to as bleaching herbicides. This chlorosis may result from either inhibition of pigment biosynthesis or from the destruction of existing pigment. Inhibitors such as amitrol, laevulunic acid and diovaleric acid interrupt chlorophyll biosynthesis. Phenylpyridazinones, amitrol etc., are known to inhibit carotenoids biosynthesis.

6.1 Herbicides and Carotenogenesis

Most of the above mentioned bleaching herbicides target the enzyme phytoene desaturase although there are several compounds which inhibit the biosynthesis at other stages like \( \zeta \)-carotene desaturase and \( \rho \)-hydroxi-phenylpyruvate dioxygenase (Boger 1996, Bramley 1993). The effect of bleaching herbicides through inhibition of carotenogenesis has mainly two consequences. First the synthesis of the normal cyclic carotenoids is prevented, resulting in the accumulation of a number of their precursors. Secondly, the absence of these cyclic carotenoids prevents the normal assembly of the photosynthetic apparatus. The plastids that may be formed following the treatment with these inhibitors are generally either inactive or show poor photosynthetic performance. Reports have shown that plants treated with inhibitors of carotenoids biosynthesis are susceptible to photooxidation (Grumbach 1982, Ridley 1982, Ridley and Ridley 1979). Thus after the treatment of herbicides both dark grown and light grown plants are usually bleached.
6.2 Phenylpyridazinones

Phenylpyridazinones are a multifunctional group of compounds. Most of these are rather used as experimental herbicides due to their multifunctional effect on photosynthetic apparatus (Bose and Mannan 1988, Joshi 1993, Karapetyan 1993). Though all these compounds inhibit carotenogenesis directly many of them have been shown to inhibit Hill reaction in isolated chloroplasts. Their mode of action is similar to DCMU in binding to Q\textsubscript{b} binding site of D1 protein in PSII (Tischer and Strotmann 1977, Mannan and Bose 1985). Studies showed that these compounds also inhibit desaturation of linoleic acid and thus increase its content in thylakoid membranes (Murphy \textit{et al.} 1980, Willemot \textit{et al.} 1982, Mannan and Bose 1984, Hanwood \textit{et al.} 1989). Depending on the nature of substitution these herbicides cause bleaching of photosynthetic pigments to varying degree. This bleaching activity is highly dependent on light intensity.

Norflurazon (SAN 9789), 4-Chloro-5-(methylamino)-2-phenyl-3-(2H)-pyridazinone, is known since long as one of the most effective compounds belonging to phenylpyredazinone group, that causes accumulation of phytoene by inhibiting phytoene desaturation (Boger 1996, Sandmann \textit{et al.} 1980, 1981, Vaizberg and Schiff 1976). At higher concentration, however, it was also shown to inhibit desaturation of linolic acid (St John 1976).