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Photoinhibition of photosynthesis and mechanism of protection against photodamage in crop plants**.

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Photosynthesis is the process by which chlorophyll containing plants convert solar energy into photochemical energy. This energy is stored in the form of carbohydrates providing food for humans and all other heterotrophic organisms. In addition it provide us with fuel as well as fibre. The productive potential of this process has, until recent years, seems endless. However, the burgeoning human population with its constantly increasing demand on both present and past products of photosynthetic activity threatens our future survival. An understanding of this process under changing environmental conditions which put plant under environmental stress resulting in an aberrant change in physiological processes brought about by either one or combination of environmental factors such as light (UV and visible), temperature, salt, and drought etc. and understanding of the processes involved in protection of photosynthesis against these stresses may help us in improving the efficiency of photosynthesis and engineer plants to withstand unfavourable environmental conditions without significant loss of productivity. In this lecture we discuss mechanism of photoinhibition and processes by which plants protect itself against photoinhibitory damage is discussed.

The light dependent inhibition of the light dependent reaction of photosynthesis is known as photoinhibition (Sharma and Hall, 1990; Powles, 1984; Osmond, 1994). The photoinhibition occurs when the plants are exposed to high light than that can be utilised (for ATP/NADPH formation) or dissipated in the form of chlorophyll fluorescence) by their normal photochemical processes. The photoinhibition can also take place at low light level if plants are predisposed to any additional stress such as temperature (Ludlow and PBjorkman, 1984; Sharma and Hall 1993) water stress (Bjorkman and Powles, 1984; Sharma and Singhal 1993) salt stress (Sharma and Hall 1991), thereby, further decreasing the efficiency of photosynthesis to utilise the light.

Proposed site for photoinhibition:

The interaction between light and photosynthetic membrane appears complex.It is reported that primary photochemistry of photosynthesis is involved in the photoinhibitory inactivation (Cleland 1988; Krause 1994, Sharma et al. 1994). This may include oxidising side of the reaction centre (Sharma and Hall 1992, Barber and Andersson 1992, Vass et al. 1992), reducing side of the reaction centre (Kyle 1987, Mattoo et al. 1984, Trebst et al. 1988) or the reaction centre itself (Andersson and Barber 1996). The evidence show that the inhibition of photochemistry proceed from an initial damaging event that under stress condition could ramify other components including the oxygen evolving system and D1 protein. There are two possibilities concerning the nature of the primary event that is caused by an aberrant chemical species such as oxygen radicals or there is some change in the geometry of the reaction centre that result in the inhibition. Observation indicate that the initial damage to PS II involves photochemical processes that inactivates its function and trigger the D1 and D2 proteins for enzymatic degradation. The multitude of sites originally proposed were based on experiments carried out either *in vivo* or *in vitro* with isolated thylakoid or relatively large PS II preparation, capable of oxygen evolution. However with the elucidation of the structure of PS II

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reaction centre, these conflicting theories of site of damage have been rationalised (Fig. 1).

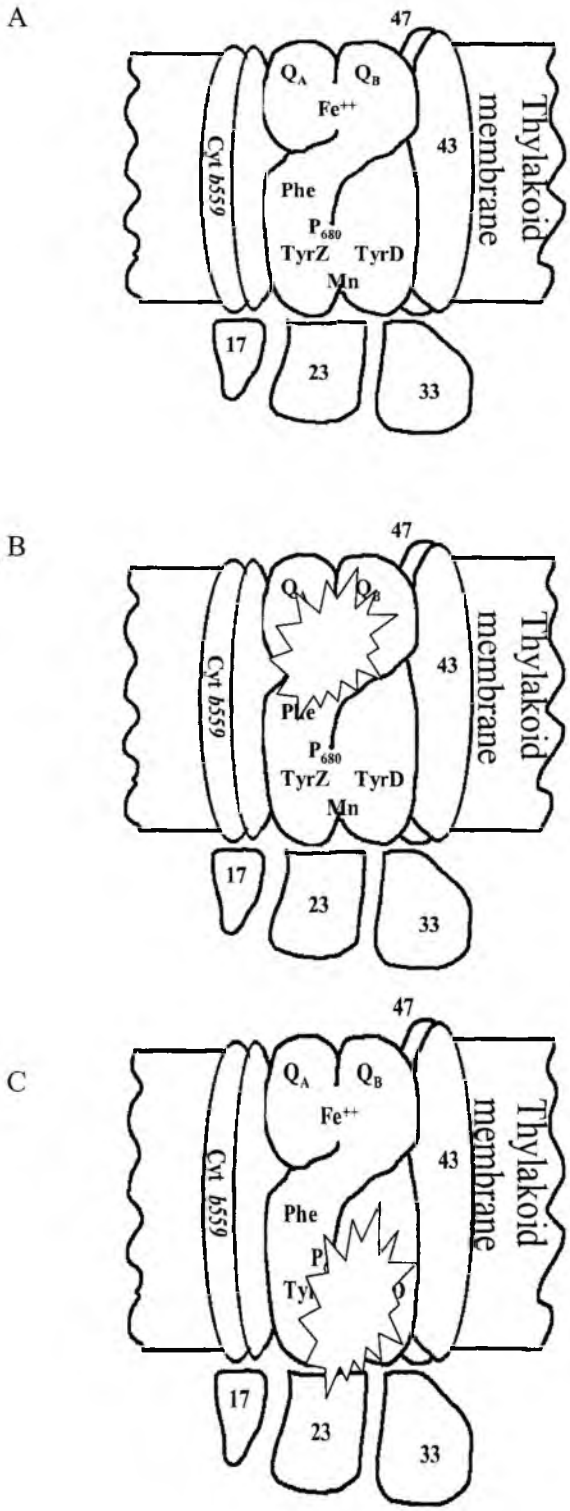

Fig. 1: Schematic diagram of photosystem II (A) Normal, (B) and (C) photoinhibitory damage to reducing and oxidising side of PS II reaction centre respectively, under high light conditions.

Acceptor side- induced photodamage.

Support for the damage to acceptor side of the PS II under photoinhibitory conditions were provided by Kyle et al. (1984) who suggested the turnover of D1 protein under high light and suggested that damaging mechanism may involve a reactive form of plastoquinone bound to QB site. Further it was confirmed by the work of Mattoo et al. (1984), Trebst et al. (1988) that herbicides which bind to D1 protein and displace plastoquinone from QB pocket, prevent rapid turnover of D1 protein. They also suggested that photoinhibition was due to the interaction of molecular oxygen with the plastoquinone anion in the QB site and that the resulting oxygen radicals would in turn lead to protein damage there by inhibiting electron transport (Kyle et al. 1984). No degradation of D1 protein was observed when chloroplasts were photoinhibited under *in vitro* condition in the absence of oxygen (Arntz and Trebst 1986; Hundel et al. 1990; Kunn and Boger 1990; Nedbal et al. 1992; Sharma and Singhal 1992) indicating role of oxygen radical in the photoinactivation. It has been shown that exposure of isolated thylakoids to photoinhibitory illumination is accompanied by singlet oxygen production (Hideg et al. 1994), moreover, generation of singlet oxygen by isolated PS II reaction centre has been shown by detection of luminescence at 1270 nm (Macpherson et al. 1993, Telfer et al. 1994) and by an indirect chemical trapping method (Telfer and Barber 1994). Oxygen radical could be generated from the triplet state of chlorophyll. Chlorophyll molecule absorbs quantum of light and is excite to the singlet state and could also be converted to the triplet form by intersystem crossing which then interacts within the oxygen molecule to form oxygen radical. The radical subsequently attacks P680 and there by inactivate it. Alternatively P680 itself forms a triplet, which subsequently interact with oxygen forming a radical which again attack the closest molecule. A triplet state is formed more often when Q is reduced (Moore and Smith 1984). In other case oxygen directly alters P680 itself by absorbing second quanta by

oxidising P680. Many of these modifications of P680 would affect its electrical properties. In high light excessive excitation might generate ^{3}Chl in the antenna which can transfer its excitation energy to ground state $^3O_2$ forming $^1O_2$ as long as triplet chlorophyll is not quenched by carotenoids.

$$^1Chl^+ \longrightarrow {}^3Chl \quad (1)$$
$$^3Chl + {}^3O_2 \longrightarrow {}^1Chl + {}^1O \quad (2)$$

Superoxide ($O2^{\cdot -}$) and hydroxide ($OH^{\cdot}$) radical is formed by reducing the oxygen under conditions where reductant generated in the photochemical reactions are not utilised by biochemical reactions. Though superoxide ($O2^{\cdot -}$) radical are rather non-reactive but can give rise to the formation of hydroxyl radicals in the Haber-Weis reaction (Youngman 1984).

$$O_2 + Fe^{3+} \longrightarrow Fe^{2+} + O_2^- \quad (3)$$
$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH + OH^{\cdot} \quad (4)$$

Further study of isolated system has also indicated that the target of singlet oxygen attack is the P680 chlorophyll which may be at its conjugated ring system. Alternatively it could ligate with histidine, which is known to react with singlet oxygen (Halliwell and Gutteridge 1989). Another possibility is the formation of bityrosine cross links (Davies 1987; Prasil et al. 1992).

The concept that acceptor side photoinhibition is due to damage by singlet oxygen generated when radical pair recombination occurs is recognised with various observations from a number of laboratories. However, the conclusion is that the situation is not fully clear since the role of primary QA and secondary QB plastoquinone acceptors during photoinactivation remains to be conclusively established particularly with respect to the situation *in vivo*.

Donor side - induced photodamage:

Some of the difficulty experienced in the interpretation of data in terms of the acceptor side/ singlet oxygen mechanism are due to the fact that there seems to be a second oxygen independent route by which PSII can be photoinactivated (Barber and Anderson 1992). Experimental evidence for the donor side inactivation has come mainly from *in vivo* studies. This situation may occur during normal steady state electron flow even at low light intensities and most certainly under conditions which destabilise the water splitting system such as low temperature. Such stabilisation results in an increased life time for the $P680^+$ state, which is a high oxidising potential and has the capacity to extract electrons from its surrounding potential and has the capacity to extract electrons from its surrounding and thus cause irreversible oxidative damage. Three kinetically different phases of inactivation are suggested. The first phase was to be a decrease in the rate of electron transfer between Yz and $P680^+$, second was a loss of Yz^+ formation. The third phase was very slow and was observed as a loss of Yd^+ formation (Blubaugh et al. 1991). They also identified the formation of carotenoids and chlorophyll radicals under conditions of donor side induced photoinhibition.

In brief, it is suggested that acceptor side mechanism involves recombination of radical pair $P680^+$ pheophytin$^-$. The recombination lead to the production of the $P680^+$ triplet which is not quenched by carotenoids but instead lead to the formation of highly toxic singlet $O_2^{\cdot}$

$$^3P680^+ + {}^3O_2 \longrightarrow P680 + {}^1O_2 \quad (5)$$

As a consequence, the D1 protein is modified by triggering of proteolytic degradation. The degradation process involves an initial cleavage in the loop joining transmembrane segments D and E near to the QB binding site (C-terminal side of residue 238). The donor side mechanism, however, is not dependent on the presence of oxygen and result from damage due to long lived oxidation states $P680^+$. Extensive oxidation of this type destabilises the D1 and D2 protein and pattern of degradation products observed is different from that generated by the acceptor side

mechanism. The primary cleavage occurs on on the D1 protein in the loop transmembaren segement A and B. There is no evidence that either side induced degradation of protein is due to indirectge photochemical cleavage. Rather it seems that the detrimental photochemical processes give rise to confirmational changes in D1 and D2 proteins that signal proteolytic reaction.

These two views are not difficult to reconsile as main components of both reducing side and donor side of the PSII reaction centre are housed in D1 protein and in both type of damage, D1 protein get degraded.

Photoinhibition repair cycle:

It has been shown that main target for photoinhibition is PS II with the rapid turn over of the D1 protein. Three major discoveries have modified this concept of turnover of the D1 protein. First the D1 protein was identified as one of the subunits in the reaction centre hetrodimer (Nanba and Satoh 1987). Thus, fast turnover of this subunit, while other PS II subunits are maintained and probably re-utilised in the assembly of novel PS II complexes, is carried out by an organised and specialised proteolytic machinery able to cut out the D1 protein from damaged PS II complexes. Second, as it was thought that photoinhibition of electron transport was a consequence of D1 degradation, several groups set out to time resolve the reaction leading to photoinhibition. A third important discovery was that at least two independent mechanisms exist that lead to photoinhibition and subsequent degradation of D1 protein (Styring et al 1990). One oxygen-dependent set of reactions as explained in acceptor side induced photodamage and another oxygen-independent set of reaction as described in donor-side induced photodamage. With emerging understanding of the reaction that damage the D1 protein, it has become clear that D1 degradation is actually part of the what is known as the photoinhibition repair cycle. Several different steps can be resolved between the light-dependent damage and the actual degradation of the D1 protein. In reaction that might be coupled to degradation of the D1 protein, new copies of D1 are synthesised and later inserted into PS II complexes devoid of D1. Activation of the new PS II centres demands rebinding of chlorophylls, pheophytins, quinones, the acceptor side iron and the Mn ions. How these activation reactions are accomplished after the specific depletion and re-insertion of the D1 protein during the photoinhibition repair cycle is a quite untouched field. Results indicate that there is a lag of about 1 h between synthesis of the D1 protein and the appearance of fully functional PS II centres. During this hour, the redox chemistry is re established. Probably the reactivation half of the photoinhibition repair cycle involves assembly of the PS II subunits in a defined order (Styring and Jegerschold 1994).

Oxygen radicals and damage to photosynthesis:

Oxygen play dual role in photoinhibition, one as a protective role against photoinhibition and another as a enhancement of photoinhibition. The protective role is in the form of photorespiration and Mehler reaction. Studies have shown that levels of oxygen present in the atmosphere which promote photorespiration diminish the extent of photoinhibition (Fig. 2). The protective effect of photorespiration has been attributed to the consumption of photosynthetic energy, NADPH and ATP, (Scheuramann et al. 1991). Oxygen also serves as an alternative electron acceptor of PS I when other acceptor such as NADP are insufficient to reoxidise to reduce the ferrodoxin (Mehler 1951; Fig. 2). This predominant pathway of oxygen reduction proceeds from reduced ferrodoxin and generate superoxide radical. In excess light oxygen reduction contributes to the electron drain from PS I thereby preventing photodamage to a large extent (Heber et al. 1990).

Increasing the level of oxygen during the photoinhibition exacerbate the damage. Atmospheric oxygen in its ground state is distinctive

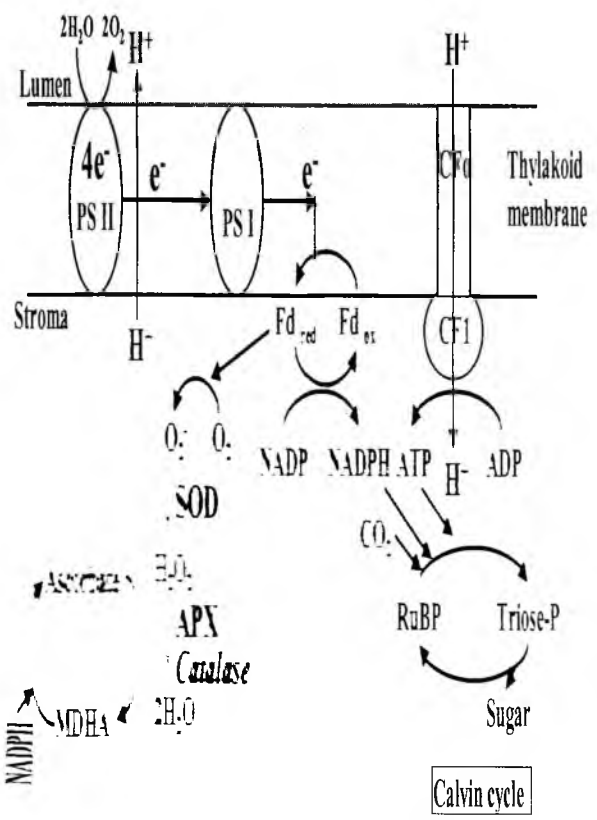

Fig. 2: The Mehler Reaction showing oxidation of oxygen under conditions of over reduction resulting in production of superoxide radiacals and follow up reactions.

among the gaseous elements because it has two unpaired electrons. This makes oxygen very unlikely to participate in reaction with organic molecules unless it is activated. The requirement for activation occurs because the two unpaired electrons in oxygen have parallel spins. According to Pauli's exclusion principle, unless reductant also has two unpaired electrons with parallel spin (triplet state) opposite to that of the oxygen, which is a very rare occurrence. Hence oxygen is usually non-reactive to organic molecules (Youngmen 1984).

Activation of oxygen may occur by two different mechanisms. Absorption of sufficient energy to revcerse the spin on one of the unpaired electrons, or monovalent reduction. If triplet oxygen absorbs sufficient energy to reverse the spin of one of its unpaired electrons, it will form the singlet state. The second mechanism of activation is by stepwise monovalent reduction of oxygen to form superoxide ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) and finally water. The first step in the reduction of oxygen forming superoxide is endothermic but subsequent reduction are exothermic (Krause 1994).

The damage to photosynthesis by these oxygen radical is mainly caused by way of oxidising the organic substances such as lipids and proteins. The oxidation of organic substances, mainly lipids, may proceed by two possible reaction. One by addition of $OH^{\cdot}$ to the organic molecule and second by abstraction of a hydrogen atom from it. In the addition reaction, the hydroxyl radical adds to an organic substrate forming a hydroxylated product that is further oxidised by ferrous ions or oxygen to a stable oxidised product.

$$OH^{\cdot} + R \longrightarrow {}^{\cdot}ROH \quad (6)$$

$$^{\cdot}ROH + Fe3+ \longrightarrow ROH + Fe2+ + H+ \quad (7)$$

$$^{\cdot}ROH + O_2 \longrightarrow ROH + O_2^{-} + H^{+} \quad (8)$$

In the abstraction reaction, the radical oxidises the organic substrate forming water and an organic radical. The organic radical can react with O_2 in the triplet ground state, which can lead to the formation of a peroxyl radical which can readily abstract hydrogen from another organic molecule leading to the formation of a second carbon radical. This is the reason why oxygen free radicals cause damage far in excess of their initial concentration (Fig. 3).

$$^{\cdot}OH + RH \longrightarrow R^{\cdot} + H_2O \quad (9)$$

$$^{\cdot}R + O_2 \longrightarrow ROO^{\cdot} \quad (10)$$

$$ROO^{\cdot} + RH \longrightarrow R^{\cdot} + ROOH^{\cdot} \quad (11)$$

Oxidative attack on protein results in site specific amino acid modification, fragmentation of the peptide chain, aggregation of cross linked reaction products, altered electrical charge and increased susceptibility to proteolysis. The amino acids in a peptide differ in their susceptibility to

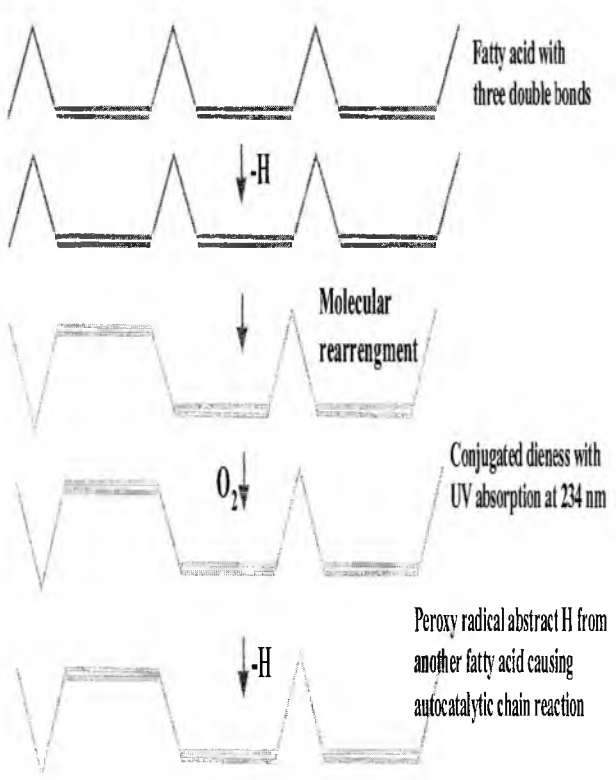

Fig. 3: Initiation and propagation reaction of lipid peroxidation by extraction of hydrogen atom.

attack and the various forms of activated oxygen differ in their potential reactivity. Sulphur containing amino acids and thiol groups specifically, are very susceptible sites. Activated oxygen can abstract H atoms from cystine residue to form thiyl radicals that will crosslink to form disulfide bridges. Alternatively oxygen can add to a methionine residue to form methionine sulphoxide derivatives, Reduction of both of these may be accomplished in microbial systems by thioredoxin and thioredoxin reductase (Farr and Kogoma 1991). The oxidation of iron-sulphur centres by superoxide destroys enzymatic function (Gardner and Fridovich 1991). Many amino acids undergo specific irreversible modifications when protein is oxidised. Histidine, lysine, proline, arginine and serine form carbonyl groups on oxidation. The oxidative degradation of protein is enhanced in the presence of metal cofactors that are capable of redox cycling, such as Fe. In these cases metal binds to a divalent cation binding site on the protein. The metal then react with hydrogen peroxide in a Fenton reaction to form a hydroxyl radical that rapidly oxidises an amino acid residue at or near the cation binding site of amino acid. This site specific alteration of an amino acid usually inactivates the enzyme by destruction of the cation binding site. Oxidative modification of specific amino acid is one mechanism of marking a protein for proteolysis. (Farr and Kogome 1991).

Protection against photodamage:

Plants have developed various strategies to protect itself against excess light. There are morphological adaptation, physiological and biochemical processes which prevent excess light to reach the sensitive site. The morphological changes help in preventing excess light reaching the PS II reaction centre either by transmitting the light or decreasing the surface area available for light exposure. These changes are;

(a) Thick cuticle
(b) Movement of leaves
(c) Curling and shading of leaves
(d) Movement of chloroplasts within the cell
(e) Smaller LHC in sun plants

Once light reaches the light harvesting complex there are other set of protective mechanisms which protect plants against light by either dissipating the excess radiation away from the reaction centre or by scavenging the toxic products formed as a result of over energization. These processes are;

(a) LHC get physically dissociated from reaction centre
(b) Chlorophyll a to chlorophyll b ratio is changed
(c) Xanthophylls
(d) Antioxidants
(e) Cytochrome *b*559

So plants have two means by which they can

prevent photodamage and thereby loss to productivity, i.e. either by preventing the excess light reaching the photosynthetic reaction centre, the site of photodamage or by preventing damage to organic molecules (lipids, proteins etc.) by removing the causal agents, free radicals, generated as a result of photochemical process. The former is largely done by dissipating the light energy within the light harvesting complex by xanthophyll cycle and later is done by various enzymatic and non-enzymatic processes.

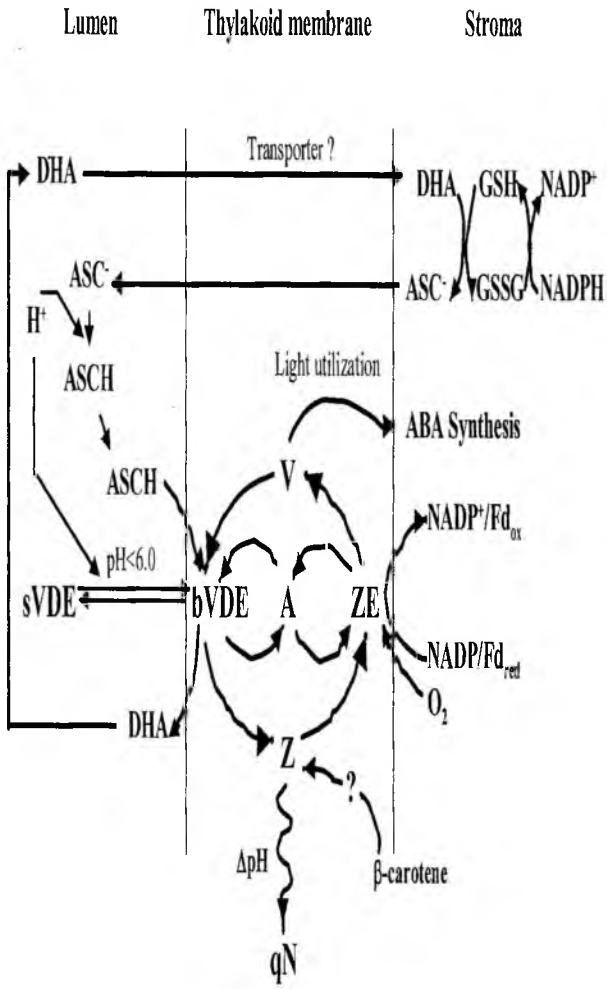

Fig. 4: Model of the regulation of the xanthophyll cycle and its relation to ABA synthesis. V, violaxanthin; Z, Zeaxanthin; A, Antheraxanthin. VDE; Violaxanthin deepoxidase (s) soluble or (b) bound; ZE, Zeaxanthin epoxidase;

Xanthophyll cycle:

Xanthophylls are O_2 containing carotenoids. During photoinhibitory treatment violaxanthin is de-epoxidised to zeaxanthin via antheraxanthin, which is reversed in the dark. Violaxanthin (V) is a functional component of the xanthophyll cycle of thylakoids and is phototransformed via antheraxanthin (A) to its de-epoxidised form zeaxanthin (Z). The conditions for the operation of xanthophyll cycle have been established(Fig. 4). The forward reaction i.e. the photoconversion of V to Z is catalysed by the membrane bound enzyme deepoxidase and requires an acid pH in the thylakoid lumen and only proceeds when the irradiance is sufficiently high to provide the proper acid pH. In the dark or low light conditions which do not maintain ?pH in the thylakoid lumen, the back reaction takes place, i.e. epoxidation of Z to V, which also requires O_2 and NADPH and another membrane bound enzyme epoxidase. In recent years a photoprotective role is ascribed to this cycle (Demmig et al. 1987, Sharma and Hall 1991). The dissipation process within the pigment complex involves xanthophyll cycle (Demmig et al. 1987, 1989). A correlation was found between the calculated activity of radiationless energy dissipation represented by qN and the content of xanthophyll cycle (synthesis of Z). Further evidence to the hypothesis came from the knowledge that Z acts as a competitor for the excitation energy under excess light, since increase in radiationless energy dissipation resulted in a decrease in photochemical efficiency (Bjorkman and Demmig 1987). The capacity for a rapid removal of Z facilitated a rapid increase in the qP upon return to conditions under which light is no longer excessive. When leaves were treated with dithiothreitol (DTT; which was administered through cut petiole) it completely inhibited de-epoxidation of V to Z without affecting photosynthetic O_2 evolution (Fig. 5). It was observed that such leaves has an approximately 30% greater reduction state of PS II compared to that of leaves containing Z (Bilger et al. 1989) indicating an increased amount of energy reaches the PS II reaction cen-

tre when violaxanthin de-epoxidation is inhibited . Work with certain lichens (algae in association with fungi naturally lacking xanthophyll cycle) and compared with green algae which possess the xanthophyll showed that upon exposure to excess light the cyanobacteria associated with lichens which lack zeaxanthin, did not form zeaxanthin and also did not exhibit rapidly developing and relaxing non-photochemical quenching and the reduction state of PS II centre remain high under excess irradiance, whereas zeaxanthin containing green algae showed greater tolerance to larger exposure at high irradiance by forming zeaxanthin rapidly from de-epoxidation of V and also exhibited greater non-quenching and the reduced state of PS II reaction centre was maintained at low level cycle (Demmig-Adams, 1990). Work by Chowdary et al. (1993), Sharma and Hall (1996), Sharma and Sankhalkar (2001) also indicated a relationship between xanthophyll cycle and photoprotection through better dissipation of excess radiation under *in vitro* conditions. However, there seems to be a limitation to the role of xanthophyll cycle as a protection mechanism against photoinhibition (Sharma and Hall 1990, 1993), since Z content after a photoinhibition treatment at 1600 μmol m^{-2} s^{-1} at 5°C was not further increased when plants were photoinhibited at a higher PPFD (2500 μmol^{-2} s^{-1}). It was also reported that changes in xanthophyll cycle were temperature dependent showing better rate of de-epoxidation at 20°C that at 5°C (Sharma and Hall 1990).

Enzymatic Processes:

There are mainly three enzyme system in plant cell to scavenge the free radicals these are superoxide dismutase, catalase and ascorbate peroxidase.

Superoxide dismutase (SOD): The enzyme was identified by a number of names, erythrocuprein, indophenol oxidase and terazolium oxidase untill its catalytic function was discovered by McCord and Fridovitch (1969). SOD is now known to catalyze the dismutation of superoxide to hydrogen peroxide and water.

$$2H^{+} + 2O_2 \longrightarrow H_2O_2 + O_2 \qquad (12)$$

Since SOD is present in all aerobic organism and most sub cellular compartments that generate activated oxygen, it has been assumed that SOD has a central role in the defence against stress (Beyer et al. 1991; Bowler et al. 1992; Scandalias 1993). There are three distinct types of SOD classified on the basis of the metal cofactor: Copper-Zink (Cu/Zn SOD), Manganese (Mn-SOD) and iron (Fe-SOD) isozymes (Bannister et al. 1987). The Mn-SOD is found in the cytosol, others in the chloroplast of higher plants. Fe-SOD is usually associated with the chloroplast compartment (Bowler et al. 1992). The prokaryotic Mn-SOD and Fe-SOD and the eukaryotic Cu/Zn-SOD enzymes are dimers, whereas the Mn-SOD of mitochondria are tetramers (Scandalias 1993). All forms of SOD are nuclear encoded and are targeted to their respective sub cellular compartments by an amino terminal targeting sequence. Each of SOD isozymes are independently regulated according to the degree of oxidative stress experienced in the respective sub cellular compartments, but how this is communicated at the molecular level is unknown. This role may be served by unique lipid peroxidation products from each organelle that diffuse from the site of oxidative damage to the nucleus where they would enhance transcription of specific SOD genes.

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

$$2H_2O_2 \longrightarrow 2H_2O + O_2 \qquad (13)$$

The enzyme is found in all aerobic eukaryotes and is important in the removal of hydrogen peroxide generated in peroxisomes by oxidase involved in β–oxidation of fatty acids, the glyoxylate cycle (photorespiration) and purine catabolism. All forms of catalase are tetramers in excess of 220 kDa. Multiple forms of catalase

has been described. Catalase is very sensitive to light and has a rapid turn over similar to that of D1 protein of PS II. The stress conditions which reduce the rate of protein turnover cause the depletion of catalase activity and thus catalase is not very effective enzyme in removing the free radicals under such conditions (Sharma et al. 1998).

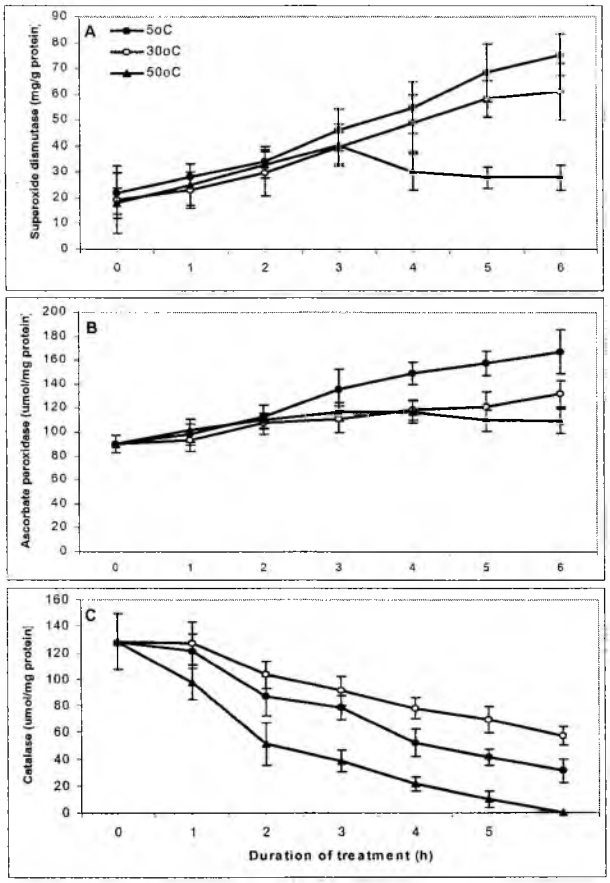

Fig. 5: Effect of photoinhibition on intact leaves at 3600 μmol m^{-2} s^{-1} PAR at 30°C in leaves pretreated with water, 5 mM ascorbate, a promoter ox xanthophyll cycle and 4 mM DTT, an inhibitor of xanthophyl cycle. The data represent average of five experiments.

Ascorbate Peroxidase: As catalase is limited in effectiveness to metabolize H_2O_2 by its relatively poor affinity for H_2O_2 and its sub cellular location in the peroxisomes and also susceptible to photo inactivation and degradation (Streb et al. 1993; Sankhalkar 2001). Ascorbate peroxidase is the main enzyme to scavenge the H_2O_2 in chloroplasts. Simultaneous oxidation and reduction of the ascorbate and glutathione pools when H_2O_2 was added suggested that enzymes of ascorbate-glutathione cycle were involved in coupling electron transport to H_2O_2 destruction. Observation of direct photoreduction of the MDHA radical by the thylakoid membranes suggested that this is probably the preferred pathway of ascorbate regeneration (Foyer and Libendias 1993). H_2O_2 generated during the Mehler reaction is considered to be scavenged primarily at the thylakoid level while the stromal ascorbate peroxidase represents a second level of defence against H_2O_2 escaping the thylakoid.

Non-enzymatic system:

These are mainly carotenoids which quench the free radicals generated during the photochemical reactions in the chloroplasts. The carotenoids are yellow or orange pigments found in all photosynthesizing cells. Carotenoids contain a conjugated double bond system of the polyene type. They are usually either hydrocarbons (carotene) or oxygenated hydrocarbons (carotenols or xanthophyll) of 40 carbon chains built up from addition of isoprene subunits. They have triple-bonded absorption spectra in the region from about 400-530 nm. The energy absorbed by the carotenoids may be transferred to chlorophyll lamellae in the close proximity to the chlorophyll. In addition to functioning as accessory light harvesting pigments (Davidson and Cogdell 1981), carotenoids play an important role in protection of photosynthetic apparatus against oxidative damage (Krause 1994). Carotenoids are substance with special and remarkable properties that form the basis of their varied functions and actions in all kinds of living organism. Carotenoids have the ability to act as chain breaking anti-oxidant and thus protect cells and organisms against photodamage. The ability of carotenoids to quench singlet molecular oxygen is suggested by various workers (Bohm et al. 1995, Hill et al. 1995), however, β-carotene at high O_2 partial pressure may act as a pro-oxidant (Burton and

Ingold 1984). The reaction of carotenoids with a free radical lead to electron transfer

$$R^{\bullet} + Car(H) \longrightarrow RH + Car \quad or \quad (14)$$
$$R^{\bullet} + Car \longrightarrow R^{-} + Car^{\bullet +} \quad (15)$$

These are two proven photoprotective reactions of carotenoids, one is to dissipate the excessive enrgy by transferring energy from the triplet to ground state and the other is to scavenge the O_2 radicals generated under photoinhibitory conditions.

$$\text{Chlorophyll} \longrightarrow {}^3Chl^* \quad (16)$$
$${}^3Chl^* + {}^1Car \longrightarrow {}^1Chl + {}^3Car^* \quad (17)$$
$${}^3Car^* \longrightarrow {}^1Car + Heat \quad (18)$$

The quenching of ${}^3Chl^*$ by carotenoids to prevent the generation of signlet O_2 through triple sensitization is a protection mechanism. The another process is to remove singlet O_2 generated during photoinhibition by mechanism of epoxidation as seen in the equation below.

$$\text{Carotenoids} \xrightarrow[\text{Dark}]{h\nu/O_2} \text{Epoxy-carotenoids} \quad (19)$$

Beside carotenoids certain other molecules such as ascorbate and glutathione can also prevent oxidative damage to the photosynthetic apparatus by utilising the reductant energy in various cellular reactions. Phenolic compounds such as flavonoids etc. may also play a role in preventing oxidative damage to photosynthetic system under stress conditions.

ABA as a regulatory molecule in energy dissipation?

Recently there has been a renewed interest in the possibility of xanthophylls may act as precursor for ABA in higher plants. Various corn mutants have been described which lack the ability to synthesize carotenoids and which accumulate little or no ABA. In addition, inhibitors of carotenoids biosynthesis such as norflurazon and fluridone also inhibit the accumulation of ABA under some conditions (Moore and smith 1984). A recent report suggest very convincingly a precursor role for xanthophylls (Schwartz et al. 1997). Earlier elucidation of the structure of the ABA and its biosynthetic derivation from carotenoids has been proposed in higher plants (Taylor and Smith 1967), however, the enzymatic cleavage of carotenoids has been difficult to demonstrate *in vitro*, for this reason the cleavage reaction remain controversial. Direct evidence for a cleavage enzyme in ABA biosynthesis is lacking. Labelling experiments with ${}^{18}O_2$ (Zeevaart and Creelman 1988) suggest that ABA is synthesised from large precursor pool that contains two of the four oxygen found in the molecule (Creelman and Zeevaart 1984). Oxygen derived from the hydroxygen and epoxide of neoxanthin was suggested to account for the observed ${}^{18}O_2$ labelling pattern (Marin et al. 1996,

Table 1: Non-Photochemical quenching and xanthophyll cycle (violaxanthin and zeaxanthin) content in sorghum seedlings grown without and with ABA and photoinhibited at 2200 and 3600 μmol m^{-2} s^{-1} PAR at 30°C for 6 hour. The xanthophyll contents are given as μg pigments FW^{-1} using β-carotene as external standard.

	Without ABA			With ABA		
	Control (0 h)	2200 PAR	3600 PAR	Control (0 h)	2200 PAR	3600 PAR
qN	0.366	0.443	0.509	0.564	0.648	0.654
Xanthophylls						
Violaxanthin	19.33	17.56	15.68	24.28	21.1	20.8
Zeaxanthin	0.0	1.9	4.6	0.0	4.9	6.32

Schwartz et al. 1997, McCarty 1995, Parry et al. 1992). A propose biosynthetic pathway of ABA is shown in Fig. 6.

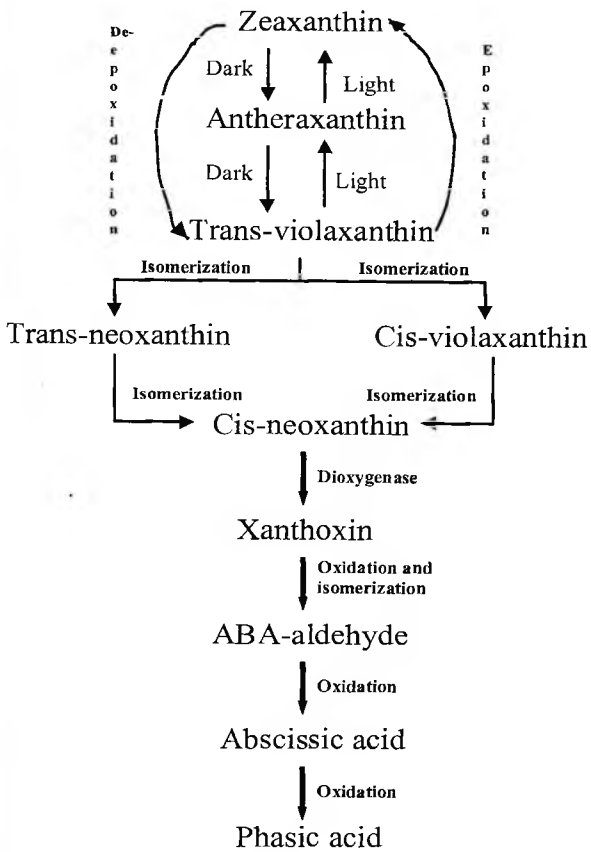

Fig. 6: The abscissic acid biosynthetic pathway in higher plants. Common precursor violaxanthin is synthesizinf zeaxanthin on one hand and ABA at another end.

Since the role of xanthophyll cycle in the protection of photosynthetic apparatus from photoinhibition is well documented and since the strong evidence for the pathway ABA biosynthesis from epoxy-carotenoids has been reported, it is of interest to investigate the relationship between endogenous xanthophylls and ABA levels under photoinhibitory conditions. Work in our laboratory using exogenously applied ABA on the light-dependent zeaxanthin formation indicate a possible role for ABA in stimulating the xanthophyll cycle under photoinhibitory conditions. and facilitating the better energy dissipation (Sharma and Sankhalkar 2001; Table 1).

Cytochrome *b*559 and phtoprotection:

Cytochrome *b*559 is a heme potein that is composed of two polypeptides, the ?- and ?- subunits, and is integral component of all PS II reaction centres. It's proximity to PS II reaction centre is demonstrated (Butler et al. 1973) and it is part of purified reaction centre preparations (Nanba and Satoh 1987). Some studies reveal two cytochrome *b*559 hemes/PS II (Boerner et al. 1992) while other reveals only one cytochrome *b*559 hemes/PS II (Buser et al. 1992a). The three-dimensional structure of cytochrome *b*559 is unknown. Spectroscopic analysis of the isolated cytochrome *b*559 protein, the gene-derived amino acid sequences of the α- and β- subunits and immunological experiments, have led to suggest a heme cross linked hetrodimer structure (α- and β; Vallon et al. 1989). Understanding the functional role of cytochrome *b*559 has proven difficult, largely because its light induced turnover under physiological conditions is slow compared to other redox carriers involved in linear electron transfer. Genetic modification of cytochrome *b*559 indicate that it plays a crucial structural role in the assembly/or stability of PS II (Cannani and Havaux 1990). Work by Nedbal et al. (1992) shows that a redox component can exert strong control over the sensitivity of PS II to photoinhibition. Changing the redox state of a one-electron component from the reduced to the oxidised state decrease the rate of photoinhibitionn in continuous light by more than an order of magnitude.

One model that provides a molecular mechanism to account for photoinhibition focuses on the reducing anion radicals formed in the sequential reactions. In this model, the trigger for photoinhibition is the creation of stable $pheo^-$, which can occur when QA is reduced and PS II is in the state of $P680^+$ $Pheo^-$ QA^-. Since reduced pheophytin is a strong reductant it can reduced QA^- to QA^{2-} which trigger subsequent

steps that result in removal of D1 protein (acceptor side induced photodamage). The photoprotective scheme that provide molecular role for cytochrome *b*559 for redox control of photoinhibition is based on an alternative electron transfer pathway which drains electrons from $pheo^-$, thereby preventing the formation of QA^2. The pathway depends on a redox component with an operating midpoint potential around 20mV (low potential form) being in the oxidised state. Rich and Bendall (1980) reported that the low potential form of cytochrome *b*559 is a one electron acceptor with a mid-point potential of +20 mV that is pH independent between 6.0 and 8.0, which slowly mimics the redox behaviour we found for the component controlling photoinhibition (Fig. 7).

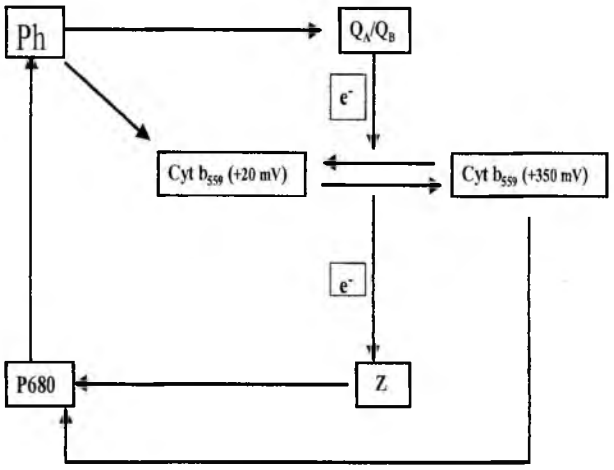

Fig. 7: Involvement of cytochrome b559 in electron transfer within the PS II reaction centre.

Another model of photoinhibition suggest oxidising side-induced photodamage (donor side of the PS II), with in this scheme Buser et al. (1992) have proposed that high potential cytochrome *b*559 (+300 mV; Paliwal and Singhal 1989) provides a protective pathway in PS II by reducing the damaging cation radicals $P680^+$). Photooxidation of high potential cytochrome *b*559 in PS II can be observed when the main electron donation pathway from water to p680 in impaired by low temperatures. Whether any of these two reactions protect PS II against photodamage remains to be proven. It has also been proposed that PS II cyclic electron transport involving cytochrome *b*559 as a mechanism to protect the reaction centre from damage due to high light intensities (Whitmarsh and Chylla 1990, Tyystjarvi and Aro 1990). In some cases, cyclic electron transport is proposed to dissipate excess energy that would otherwise damage the reaction centre or neighbouring component, however, there is no direct evident that cytochrome *b*559 can provide such protection (Fig. 7).

Photoinhibition and consequences for crop productivity:

Photosynthesis is fundamental to plant productivity. There are many factors that modify the magnitude of production attained in the field. However, four main factors determine the net biomass gain or net productivity (Pn): the quantity of incident light (Q), the proportion of that light intercepted by green plant organs (β), the efficiency of photosynthetic conversion of the intercepted light into biomass (ε) and respiratory losses of biomass (R). The relationship between plant productivity and these factors can be presented as

$$Pn = Q.\ \beta.\varepsilon - R. \qquad (20)$$

Photoinhibition which affect photosynthesis by affecting β and ε factors may be one of the reason why the achieved net primary productivity of phototrophic oxygen evolved under optimal conditions in nature falls short of the maximum predicted from the maximum photon yield of photosynthesis and the incident PFD (Raven 1994). Thus an idealised tropical habitat might provide a mean PFD of 1500 $\mu mol\ m^{-2}\ s^{-1}$ for the 12 hour of the each day of the year, permitting (with a photon yield of 0.09 mol C fixed per mole photon incident: Long et al. 1993) a gross photosynthetic fixation of 5.83 mol C $m^{-2}\ d^{-1}$ with 24 hour respiration consuming 25% of this (Raven 1976) and carbon equal to 45% of the dry weight, the net primary productivity is 117 g

dry weight m^{-2} d^{-1} or 426 tonne dry eight ha^{-1} y^{-1}. The highest observed productivity are around 99 tonne ha^{-1} y^{-1} for C4 grass *Echinochoea*

polystachya (Piedade et al. 1991). While there are many possible contributions to this shortfall (e.g. higher respiratory costs, incomplete photon interception) it is possible that photoinhibition could play a part since photoinhibition occur in a constant, high PFD in which the plants are developed, therefore, photoinhibition could also restrict productivity under less than optimal conditions of temperature or water or nitrogen supply.

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