Review Article

Molecular Mechanisms of Photoinhibition in Plants: A Review

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Abstract | Through the process of electron transport along a series of redox processes, sunlight energy is transformed into chemical energy that is then stored during photosynthesis. Photoinhibition is a significant and extremely complex phenomenon, which is basically light-induced damage to the photosynthetic machinery that principally affects the Photosystem II complex and leads to less photosynthetic productivity. A light-independent photosynthetic activity restricts the consumption of electrons produced in the early photoreactions, which appears to be the principal cause of photoinhibition by visible light. There is a chance that excessive photosynthetic electron transport could lead to an overabundance of reactive oxygen species (ROS). Reactive oxygen species such as H_2O_2 and O_2, that develops in photosystem II as a result of exposure to intense light, start to damage electron transfer system components and protein structure. Plants have adapted several protective mechanisms like production of antioxidants, enzymes and carotenoids to face reactive oxygen species and avoid photoinhibition. This article provides overview of molecular mechanisms involved in photoinhibition and its protective elements.

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Introduction

Photoinhibition is the term used to describe the decrease in photosynthetic activity brought on by light. The thylakoid membrane-encased PSII complex catalyses, generating one O_2 molecule and four protons for every two H_2O molecules oxidized. There are around 20 unique protein subunits, approximately 36 molecules of chlorophyll, and plastoquinone that is bounded to protein, and light-driven electron transfer make up PSII. The reaction center of PSII comprises of D1 and D2 proteins. These proteins contain redox cofactors that are used in electron transport chain. The charge separation that occurs between the pheophytin (Phe) electron acceptor molecule and the excited reaction centre chlorophyll assembly (P680•) is the fundamental factor that drives the light-driven PSII electron shift. The fundamental separated charge

radical state ($P680^{+\bullet}Phe^{\bullet}$) is created by this light-induced event and is subsequently stabilized on both the donor and acceptor sides of PSII. In photosystem II (PSII), charge separation happens in picoseconds, whereas the gradual process is catalyzed by Rubisco in 101 s. Photosynthesis is a complicated mechanism with stages catalyzed in lifetimes spanning picosecond (1012 s) to second time spans. Plastoquinol (PQH2) undergoing oxidation by cytochrome b6f (Cytb6f) between these two points and takes place over a period of milliseconds (103 s) (Tjisu *et al.*, 1998). Photoinhibition decreases photosynthetic efficiency by reduction in scavenging mechanism in response to excess temperature and light (Tjisu *et al.*, 1998).

Recent studies have provided new insights into the molecular mechanisms of photoinhibition in plants (Levin *et al.*, 2021). The role of the thylakoid electron transport chain (ETC) in regulating the redox state of the chloroplast and preventing oxidative stress has been understood (González *et al.*, 2021). The photoprotective pigment zeaxanthin has been found to play a crucial role in dissipating excess light energy and preventing oxidative damage to chlorophyll and other photosynthetic pigments (Bassi and Dall'Osto, 2021; Simkin *et al.*, 2022). Key regulatory pathways involved in the dynamic adjustment of the antenna size of chlorophyll molecules and in the regulation of energy transfer between chlorophyll and other pigments have been identified (Kolodny *et al.*, 2021). The role of chlorophyllases in regulating the levels of chlorophyll and other pigments involved in light harvesting and in the response to oxidative stress has been elucidated (Jahan *et al.*, 2022). New approaches, such as fluorescence imaging, mass spectrometry, and transcriptomics, have allowed for a more complete understanding of the complex molecular processes involved in photoinhibition. These insights have important implications for improving photosynthetic efficiency in crops and mitigating the effects of light stress in plants grown under challenging conditions (Priyadarshan and Priyadarshan, 2019).

The objectives of this review are to provide a comprehensive and current understanding of the topic. The paper will summarize previous findings and conclusions, evaluate the strengths and weaknesses of existing research, synthesize the current knowledge of the molecular mechanisms involved, discuss the implications for improving photosynthetic efficiency and mitigating light stress, identify areas for future research, and provide a useful resource for researchers and practitioners in the field of plant biology and agriculture.

Photoinhibition caused by UV light

Ultra violet light mainly damages the nucleic acid molecules, enzymes and PSII complex of the photosynthetic organisms as presented in Figure 1 (Shomali *et al.*, 2023). Potential UV-induced damage targets in PSII include tyrosine electron donors, catalytic Mn_4Ca clusters for water oxidation, and quinone electron acceptors (Soitamo *et al.*, 2017). High ROS formation occurs mainly under UV-B light spectrum. The most harmful UV range among the three is UV-C, which has a shorter wavelength (200–280 nm). However, solar radiation with a wavelength light with wavelengths below 280 nm is prevented from penetrating the atmosphere and reaching the surface of the earth. UV-C radiation has no physiological significance. When compared to UV-A light, UV-B radiation has a roughly 50% higher harmful efficiency (Zhang *et al.*, 2016).

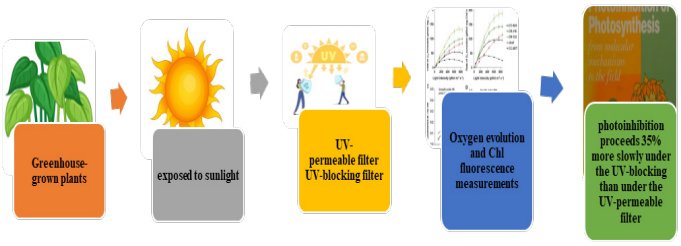

Figure 1: *Photoinhibition caused by UV light* (Hakala-Yatkin *et al.*, 2010).

Visible spectrum of light a causal factor of photodamage

Contrary to the generally held mainstream view, in the context of visible light, the situation is far more complicated, where the Mn_4Ca cluster plays a primary role in sensitizing UV-induced photodamage of PSII (400–700 nm). The intricate interaction of photodamage, photoprotection, and healing of injured PSII centers, its equilibrium ensures that the net loss of PSII activity, further complicates the situation (Zavafer *et al.*, 2017).

Receptor side events in photoinhibition

The catalytic Mn_4Ca apparatus involved in water oxidation is destructed by photodamage, which do not require the presence of excess excitation and solely depends solely on the visible light (Zavafer *et al.*, 2017). Anaerobiosis decreases the activity on the PSII acceptor site, allowing for the study of light-induced alterations at the acceptor side. The

double reduced form of Q_A can also take place under substantially reducing conditions. This is accompanied by the dissociation of Q_AH_2 from its binding location. Under the availability of oxygen, the synthesis of stable $Q_A^{\bullet}$ is a crucial stage in the photo inhibitory process, although under physiological circumstances, twofold decrease of Q_A has presumably low harvest. The inhibition of electron transfers forwardly, a temporary phenomenon in the case of stabilized $Q_A^{\bullet}$ and a permanent phenomenon in the case of Q_AH_2 creation and release, is the most significant effect of the alterations that arise at the Q_A and Q_B position. Through restricted forward electron transit enables the production of the triplet state of excited P680. When ^{3}P680 interacts with molecular oxygen, extremely combative singlet form of oxygen is created, which harms the protein environment and actually inhibits PSII electron transport (Krause, 2019). This inhibition can only be restored by the synthesis of the D1 protein dependent PSII repair (Li *et al.*, 2021).

Triple chl formation in photoinhibition

Carotenoids, which also serve as $^1O_2$ quenchers and protect the Chls in light harvesting systems from ^{3}Chl creation, have been shown to produce $^1O_2$ in isolated LCHII complexes. Additionally, it has been demonstrated that weakly linked Chl pigments play a role in photoinhibition PSII (Orekhova *et al.*, 2021). Additionally, damage of photosystem of PSII with temporarily compromised donor side has been shown to result in the elimination of one Chl in PSII other than P680. In comparison to ^{3}Chl generation, which is not directly regulated by electron transfer reactions, ^{3}P680 formation is affected by transmission of electron events via excitation back pressure. As a result, ^{3}P680 participates in the photoinhibitory process in a dynamic, electron transport-regulated manner. The state of $P680^{+\bullet}Pheo^{\bullet}$ is created after the excitation reaches the PSII reaction centre. Even if $1[P680^{+\bullet}Pheo^{\bullet}]$ is rapidly stabilized by acceptor and donor side electron transfer mechanisms, may take place during the entire life of the primary radical pair, which can change $1[P680^{+\bullet}Pheo^{\bullet}]$ to its triplet state (Lingvay *et al.*, 2020).

Donor side events in photoinhibition

When the rate of electron extraction by $P680^{+\bullet}$ exceeds the rate of donation of an electron from the Mn4Ca cluster, which is involved for oxidation of water, tempted PSII inactivation can occur due to illumination circumstances. As a result, there is an increase in long-lasting oxidizing radicals on the PSII donor side quickly inactivate electron transport, resulting in protein damage. The oxidizing complex of water and PSII can be also visible in its sequestered reaction centre particles that do not comprise the Mn_4Ca cluster when Mn is artificially inhibited by washing with Tris, NH_2OH treatment, or by removing Cl. PSII light sensitivity is caused by single amino acid alterations in the C-terminal region of the D1 protein that partially or totally block the activity of oxygen generation (Nawrocki *et al.*, 2021). Previous research has indicated that PSII electron shift is inhibited by non-interacting processes when subjected to both detectable and short-wavelength UV-B radiation at the same time, both of which have been proven to negatively affect the Mn4Ca cluster. Dissimilar target sites are impacted by the UV-B and visible spectral regions differently in terms of the functionality of PSII reaction centres and oxygen generating activity (Nawrocki *et al.*, 2021).

Mechanisms ensuring protection against photoinhibition

Protective role of carotenoids: An essential component of chloroplast protection is provided by two different forms of Carotenoids are found in thylakoids: (i) a free fraction of Cars that serves as an antioxidant to scavenge reactive oxygen species (ROS) created by LHCs and RC complexes. This free fraction can make up to 15% of the overall Car pool. and (ii) cars that are attached to the apparatus of photosynthesis, where they are in close proximity to chlorophyll molecules. The xanthophyll content of terrestrial plants is very well maintained in terms of both the general composition and location in chloroplast structures, despite the enormous variation produced by evolution. Cars are largely attached to photosynthetic multiplexes and have a consistent distribution among their many parts: The RCs bind -carotene, whereas the LHCs are responsible for the binding of the xanthophylls lutein, violaxanthin, and neoxanthin, as well as zeaxanthin, which accumulates as a result of exposure to high levels of light. A distinctive function for each molecular species is suggested by the maintenance of Car structure and site of binding throughout a diverse collection of plant genus and species (Izuhara *et al.*, 2020).

ROS scavenging

The ($^3O_2$) and ^{3}Car ground triplet states are created as a result of the energy transfer from ROS to Car. Although the energy of triplet excitation is effectively

transformed into heat and into the ground state (Zavafer *et al.*,, 2017; Krause, 2019). Neo, which makes up around 15% of all cars, is specifically designed to quench $^1O_2$; in contrast, Viola's purpose in photoprotection is to scavenge $^1O_2$. Zea, whose scavenging activity is increased and also possesses a ^{3}Chl* quenching activity, is created when Viola is de-epoxidized in EL (de Souza *et al.*, 2017).

Prenylquinols function as $^1O_2$ scavengers. Prenylquinols, including tocopherol and plastoquinols, mediate chemical scavenging by electron transport, as opposed to Cars, which additionally makes scavenging easier by facilitating the transfer of excitation energy (quenching). Within the thylakoid lipid matrix, tocopherols are found in a free state, whereas pigment-protein complexes are mostly responsible for binding to cars. Tocopherol and ascorbate work together in plants; mutants with lower ascorbate content exhibit a corresponding rise in tocopherol (Pinnola and Bassi, 2018). Young leaves that have been exposed to intense light in the Arabidopsis thaliana npq1 mutant have been shown to develop larger levels of -tocopherol. This demonstrates that high quantities of tocopherol can compensate for lower levels of 1O2 detoxification by Zea. Tocopherol and Cars have complementary protective roles in vivo. Instead, compared to WT, the tocopherol-deficient *A. thaliana vte1* mutant accumulates more Zea in EL.

Ascorbate (vitamin C), can build up to exceptionally high amount (20-300 mM) during EL adaptation. Violaxanthin de-epoxidase (VDE) requires ascorbate to function properly as a cofactor, a direct quencher of $^1O_2$, O_2.-, and OH, and a scavenger of OH through ascorbate peroxidase (Stahl-Rommel *et al.*, 2022). It also serves as an electron donor to PSII (APX). It also functions to regenerate -tocopherol from -tocopheryl radicals. The phenotype of ascorbate-deficient mutants of *A. thaliana*, also known as vtc, which are extremely sensitive to several different oxidative stresses like ozone, UV light, and others, is one source of in *vivo* supportive data.

Enzymatic antioxidants

The enzymes glutathione peroxidase, peroxiredoxin, APX, SOD, and CAT (catalase) are examples of enzymatic antioxidants. Every compartment of the subcellular structure contains these enzymes. Typically, an organelle has many enzymes working to neutralize distinct ROS. $O_2^{\cdot-}$, the primary oxidant produced by PSI, is quickly converted to H_2O_2 by SOD. APX is a major enzyme in the ascorbate-glutathione cycle which operates the H_2O_2detoxification system in chloroplasts. Ascorbate used by APX as a particular electron donor to convert H_2O_2 to H_2O.In this situation the water-water cycle is crucial to prevent photodamage in PSI. $O_2^{\cdot-}$ is the major result of photoreduction of $O_2^{\cdot-}$ in PSI in EL, that can then be transformed into H_2O_2 by SOD, which is an enzymatic process (Guidi *et al.*, 2017; Guo *et al.*, 2018).

Conclusions and Recommendations

The process of photoinhibition in plants, which results in damage to photosystem II (PSII) under high light conditions, has been the subject of extensive research over the past three decades. This research has uncovered several key mechanisms that contribute to photoinhibition, including a lack of electron sinks, photoinactivation of PSII and other cell components, inactivation of the Mn_4Ca cluster, and the overall regulation of photoinhibitory damage by the PSII repair cycle. While these processes can occur simultaneously, the spectral dispersion of light can influence their effectiveness. The photodamage caused by Mn in the visible (blue) range has been well-studied, but there are also compelling arguments against the notion that photoinhibition is only caused by the inactivation of the Mn_4Ca cluster. Other photodamage mechanisms, such as those mediated by singlet oxygen and reactive oxygen species, also exist and may contribute to photoinhibition in different ways. Despite a lack of direct comparisons of these pathways, it is possible that both the Mn-based mechanism and other mechanisms may occur simultaneously and contribute to photodamage. Overall, the study of the molecular mechanisms of photoinhibition in plants has advanced our understanding of this complex process and highlights the need for continued research in this area.

Novelty Statement

This review article provides an thorough and updated insight towards understanding the molecular mechanism of photoinhibition in plants. It will help the scientific community and students to thoroughly develop their concepts towards photoinhibition and conduct their experiments.

Author's Contribution

Muhammad Asim Bhutta: Provided technical guidence during research work
Amna Bibi and Zarmeena Amjad: Helped in data analysis
Nadia Hussain Ahmad: Proofread the manuscript.
Sadia Kanwal: Helped in data analysis.
Hafeez UR Rehman, Muhammad Nouman Khalid, Syeda Fiza Nayab and Umar Farooq: Helped in manuscript write-up

Conflict of interest
The authors have declared no conflict of interest.

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