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# Article 16. Original Review of Environmental Physiology: Photosynthesis and Systematic Metabolomics

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## Abstract

This article has comprehensively summarized the photosynthesis system in plant, illustrated by representative case studies. The improvement in both study theory and research method of physiological responses to various environmental stress have been substantially discussed in each part. Especially, metabolomics theory and method are improved to systematically indicate the biochemistry pathways in physiological responses to various environmental stress, with a bio-informatics model established by using C++ Language.

**Key words:** Photosynthesis, Environmental Physiology, Environmental Stress, Metabolic Pathways, Systematic Metabolomics.

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## 1. Introduction

This article firstly critiques the past life evolution theory in our earth planet, pointing out that the 21% content of oxygen in atmosphere must rely on the terrestrial plant's photosynthesis to maintain. Photosynthesis chemistry system is the most advanced organ process in the biological kingdom, as trees' population must be the first species settled in this planet with the longest evolutionary history [7]. Consequently it is to review the biochemistry process with emphasis on the enzyme catalyzing in photosynthesis chemistry; Plant physiology response to various environment stress is regulated via complicated pathways, and this issue is paid more attention due to climate change impact, which is also systematically reviewed; Finally, the metabolomics theory and method are re-discussed and modified, aiming to improve the indicator of biochemistry pathways in metabolic process, with the establishment of bio-informatics model.

Photosynthesis is the physiological process for green plants (including algae) to absorb light energy and synthesize rich organic compounds from carbon dioxide and water, subsequently releasing oxygen, which are of great significance for achieving energy conversion in earth ecosystem and for maintaining carbon-oxygen balance in

the atmosphere. The reaction conditions of photosynthesis include cytochrome enzyme molecules and carbon dioxide (or hydrogen sulfide) under visible light radiation. Photosynthesis is mainly divided into two physiological stages, including both light reaction chain and dark reaction chain [1].

The characteristics of the photo-reaction stage is that the electrons' energy released by the oxidation of water molecules driven by light are converted to $NADP^+$ through the electron transfer system that is similar to the mitochondrial respiratory, and this electron transfer chain causes $NADP^+$ to be reduced to NADPH; Another product of electron transfer is that protons' energy in the matrix are pumped into the cystic cavity, forming transmembrane proton gradient that drives phosphorylation, which converts ADP to ATP [2].

The chemistry reaction equation of light reaction stage is: $H_2O+ADP+Pi+NADP^+ \rightarrow O_2+ATP+NADPH+H^+$ [2]

In this chemistry reaction equation, NADPH is a coenzyme species called as reduced coenzyme II (reduced nicotinamide adenine dinucleotide phosphate), in which N refers to niacinamide, A refers to adenine, D is dinucleotide and P is a phosphate group [11]; Correspondingly, NADP+ (Niacinamide adenine dinucleotide phosphate) is the oxidized form of reduced coenzyme II (NADPH), with the symbol of '+' indicating the loss of an electron's energy and the addition of a positive charge [12]; ATP is adenotriphosphate, synthesized from both adenosine diphosphate (ADP) and phosphate (Pi) [9].

The dark reaction stage is also named as carbon reaction stage, utilizing the products of light reactions to generate and assimilate carbon, which reduces the gaseous carbon dioxide to sugars ($CH_2O$). Due to the fact that this carbon reaction stage basically does not rely directly on light radiation, but only relies on the provision of light reaction stage, it is called the dark reaction stage [2].

The chemistry reaction equation of carbon reaction stage includes: $CO_2+ATP+NADPH+H^+ \rightarrow (CH_2O) + ADP+ Pi + NADP^+$, so the overall synthesized reaction equation of photosynthesis is: $CO_2+H_2O \rightarrow (CH_2O) + O_2$ [2] (Figure 1).

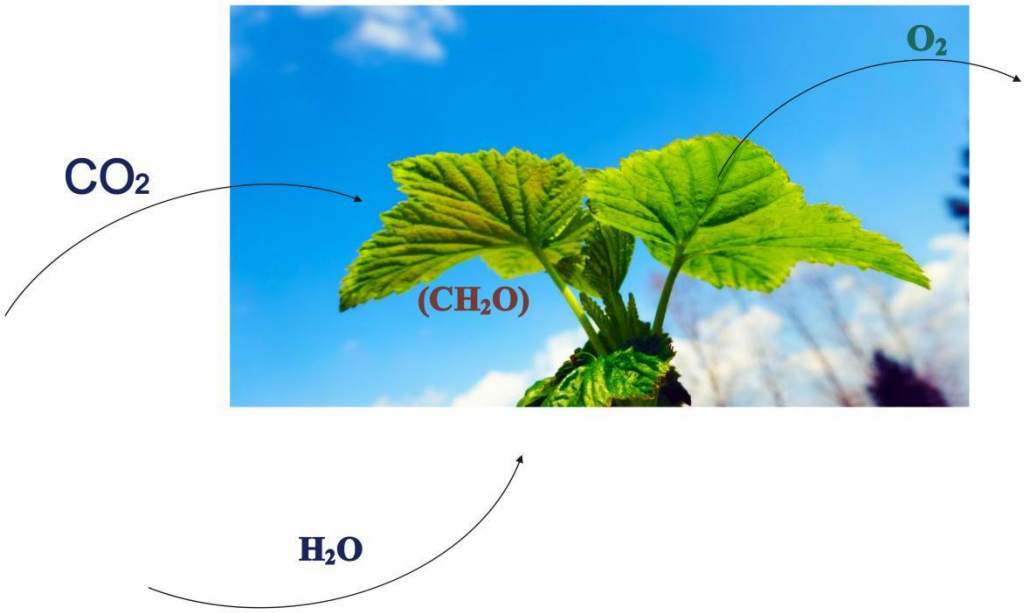


**Figure 1. The overall chemistry reaction chain of photosynthesis in ecosystem.**

## 2. Electron transfer chain

This section describes the electron energy flow in reaction chain process. My another quantum chemistry article has pointed out that the formation of ionic bond is caused by the electron energy transfer between the outermost electrons of adjacent different atoms rather than the electron itself transferring across different atoms [4], so this article will interpret the electron energy flow in photosynthesis reaction chain according to my new definition of ionic bond.

The main carriers in the chain of photosynthetic electron transfer include plastoquinone (PQ), Cytochrome b6 (Cyt. b6), Plasmocyanin (PC), Ferredoxin (Fd) and Fd NADP reductase (FNR). In photosynthesis, the transfer process of electrons' energy commences from $H_2O$ to coenzyme II (NADP+), which is driven by light excitation. After absorbing light energy, photosynthetic pigments concentrate the energy at the reaction center, formed by the molecules of chlorophyll a that causes electric charge separation and photochemical reactions. In this process, water is oxidized to release oxygen, while electrons are transferred to coenzyme II (NADP+) on the other hand, which is reduced to NADPH through a series of intermediate electrons' energy carriers (also known as donors) [6]. In this section, each elementary part of photosynthesis systems is introduced below.

### 2.1.Primary reaction

When the complex molecule of chlorophyll a (P) is excited by light radiation, it turns to be the excited state (P*), releasing electron energy to the primary electron energy acceptor (A). Chlorophyll a (P) is consequently oxidized into a positively charged ($P^+$) oxidized state, while the receptor is reduced to the negatively charged reduced state ($A^-$), but oxidized chlorophyll ($P^+$) can recover its reduced state by obtaining electrons'

energy from secondary electron donors (D) after losing electrons. In this continuous redox process, the primary electron acceptor releases high-energy from electrons into the electron transfer chain until the final electron acceptor $NADP^+$ is synthesized. Similarly, the oxidized electron donor (D+) also needs to take electrons' energy from the previous donor, in all the way to the primary electron donor: water ($H_2O$) [3].

### 2.2.Light collecting composite

Light collecting composite is composed of approximately 200 chlorophyll molecules and some peptide chains. Most photo-pigment molecules capture light energy and transfer it to pigment molecules of the reaction center through induced resonance, which are therefore called antenna pigments. All the chlorophyll b and most of chlorophyll a in chloroplasts are classified into antenna pigments. In addition to chlorophyll, carotenoids and lutein molecules also play a role in capturing light energy, known as auxiliary pigments [3].

### 2.3.Photo-Systems

In green plants, photosynthetic electrons' energy transfer is completed by the coordination of two photo-response systems: One is the special molecule of chlorophyll a that absorbs far-red light, with a maximum absorption peak at 700 nanometers, called P700. The photo reaction system composed of P700 and other auxiliary complexes is called Photosystem I (PS I); The other photo-response system is the molecule of chlorophyll a that absorbs red light, with an absorption peak at 680 nanometers, called P680. The photo reaction system composed of P680 and other auxiliary complexes is called Photosystem II (PS II). A complex composed of cytochrome b6-f and ferritin connects these two photo-systems [6].

### 2.4.Photo System I (PS I)

PS I can be excited by light at the wavelength of 700nm. Containing multiple peptide chains, PS I is located in the stroma thylakoid membrane of the contact area between the grana and matrix, composed of the collector complex I and the center of action. Except for a few special chlorophyll as central pigments, all other chlorophylls are classified into antenna pigments combining approximately 100 molecules of chlorophyll. The three electronic carriers include A0 (one chla molecule), A1 (vitamin K1), and three different 4Fe-4S [3].

### 2.5.Photo System II (PS II)

The absorption peak of PS II is at a wavelength of 680nm. At least 12 polypeptide chains are involved, which are located on the thylakoid membrane in the non-contact area between the grana and matrix, including a light harvesting complex (LHC II), a reaction center and an oxygen releasing complex containing manganese atoms. D1 and D2 refer to two core peptide chains that bind to the central pigment consisting of P680, pheophytin and plastoquinone [3].

### 2.6.Cytochrome b6/f complex

Cytochrome b6/f complex formation is still unclear, which may exist in dimeric form with each monomer containing four different sub-units, including cytochrome b6(b563), cytochrome f, ferritin and sub-unit IV that is speculated to be the binding proteins of plastoquinones [3].

## 3. Electron transfer chain classification

This type of electron transferring from water, driven by two light systems and a series of electron transfers, is ultimately converted to the electron transferring pathway of NAD+, known as non-cyclic electron transfer. If the electrons excited by PS Ⅰ are transferred to Fd that is not used for NAD+ reduction but returned to PQ, then a closed loop electron transfer is formed, where the electron carrier cytochrome b6 (Cyt. b6) participates, becoming cyclic electron transfer. When electrons' energy transfers from high potential to low potential between PS I and PS II, they are coupled with phosphorylation, converting a portion of electrical energy into biochemical energy in ATP, which are used to promote the reduction of $CO_2$ under the catalysis effects of NADPH in the photosynthetic carbon cycle, thus completing the energy conversion from light energy into electrical energy and eventually into biochemical energy [6]. The details in both non-cyclic and cyclic electron transfer chain is described below:

### 3.1.Non-cyclic electron transfer chain

The process of non cyclic electron transfer chain is generally described as follows:
Electrons' energy departs from photo system II: Photosystem II → Primary acceptor → Plasmid quinone (Pq) → Cytochrome complex → Plasmid blue pigment (copper containing protein, Pc) → Photosystem I → Primary acceptor → Ferriredox protein (Fd) → NADP reductase, so the overall reaction process is that the non cyclic electrons' energy transfer chain starts from the photosystem II and will cleave water, releasing oxygen and producing ATP, NADPH [3] (Figure 2).

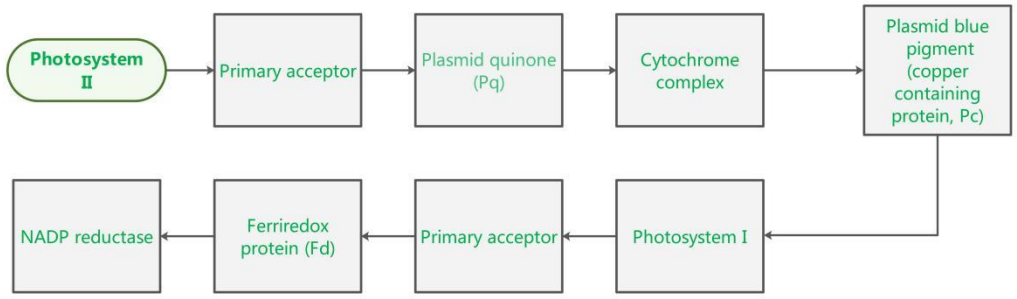


**Figure 2. The process of non cyclic electron transfer chain in photosynthesis.**

### 3.2.Cyclic electron transfer chain

The process of cyclic electron transfer chain is interpreted as follows:
Electrons' energy depart from photo system I: Photosystem I → Primary Receiver → Ferroredoxin (Fd) → Cytochrome Complex → Plasmid Blue (Copper containing Protein) (Pc) → Photosystem I. In comparison to non-cyclic chain, the cyclic

electrons' energy transfer chain does not produce oxygen because the electrons' energy source is not the cracked water, but finally ATP will be produced by cyclic electron transfer chain [3] (Figure 3).

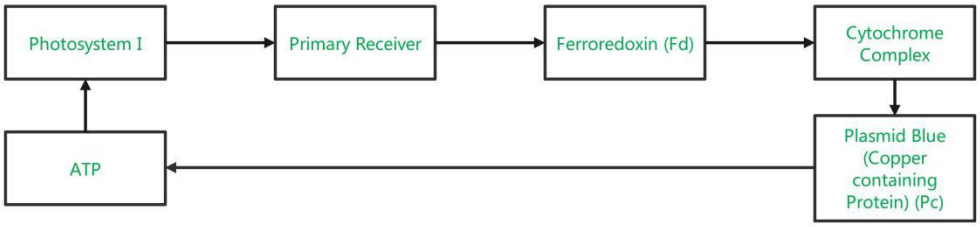


**Figure 3. The process of cyclic electron transfer chain in photosynthesis.**

**4.Z-shaped photo-system process**

So far the Z-shaped scheme is widely accepted by scientist community for explaining the pathway of photosynthetic electron transfer, which have believed that the photosynthetic electron transfer chain is composed of PS Ⅱ and PS Ⅰ, as well as a series of electron carriers connecting the two photo-systems, with each carrier on the electron transfer chain arranged in a Z-shaped series according to its oxidation-reduction potential [6].

The direct electrons' energy donor of PS II is assumed to be Z, which is connected with the decomposition of water and the release of molecular oxygen, and manganese is required to participate in this reaction. The primary electrons' energy acceptor is pheophytin (pheo), and the secondary electrons' energy acceptor is quinone (QA, QB). PS-II generates a strong oxidation potential, grabbing electrons' energy from water and oxidizing water, and finally generates molecular oxygen. The primary electrons' energy donor of PS Ⅰ is Plasmocyanin (PC) associated with Cytochrome f protein (PetA) of thylakoid Cyt b6/f-complex (algal), which is located on the inner side of the chloroplast thylakoid membrane together with iron sulfur protein (Fe SR) [6].

The primary electrons' energy acceptor (A0, A1) is the monomer of chlorophyll a, while the secondary electrons' energy acceptor X may also be the binding state of ferritin (Fe-SA, Fe-SB). Ferredoxin (Fd) is located on the outer side of the thylakoid membrane, which binds loosely to the membrane and is consequently easy to separate for movement. Chlorophyll a (P) generates a strong reducing potential, leading Fd to be reduced and then transferring electrons' energy to Fd NADP reductase (FNR) and NAD+. An important electron carrier connecting two photo systems is plastoquinone (PQ), which can move back and forth across the thylakoid membrane. When PQ is oxidized, it receives both electrons' energy from quinone (Q) and protons' energy from outside the thylakoid membrane near the outer side of the membrane; under the reduced state, PQ moves to the inner side of the membrane, transferring electrons' energy to Cyt. f, and discharges protons' energy into the cavity of the thylakoid. PQ is

reduced and oxidized back and forth in this way, transferring electrons' energy while it also transfers protons' energy from outside the thylakoid membrane into the cavity, causing the variation in proton concentration between inside and outside the cavity, so that photosynthetic phosphorylation is promoted to synthesize adenosine triphosphate (ATP) [6] (Figure 4). The details in photosynthetic phosphorylation is described below:

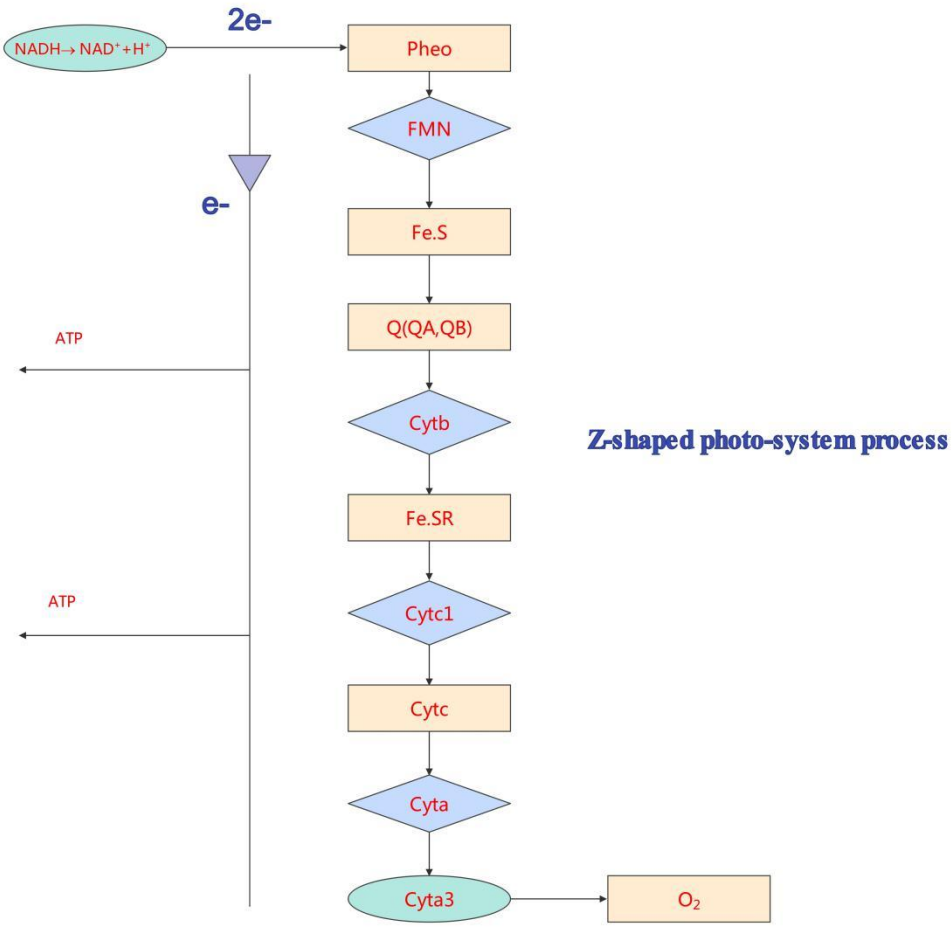


**Figure 4. Z-shaped photo-system process**

**5.Photosynthetic phosphorylation**

Photo-phosphorylation is the reaction for the thylakoid membrane of plant chloroplasts or the chromophore of photosynthetic bacteria to catalyze the formation of adenotriphosphate (ATP) from both adenosine diphosphate (ADP) and phosphate (Pi) under light. In the photo-reaction of photosynthesis, in addition to transferring a portion of light energy into NADPH for temporary storage, another portion of light energy is used to synthesize ATP, coupling photosynthesis with the phosphorylation of ADP, which is called photosynthetic phosphorylation. The main difference between

oxidative phosphorylation and mitochondrial oxidative phosphorylation is that oxidative phosphorylation is driven by the oxidation of high-energy compound molecules, while photosynthetic phosphorylation is driven by photons [9].

Correspondingly to photosynthetic electron transfer classification, photosynthetic phosphorylation is divided into three types: firstly, the reaction coupled between non cyclic photo-phosphorylation and non cyclic electron transfer chain to produce ATP; secondly, the reaction coupled between cyclic photo-phosphorylation and cyclic electron transfer chain to produce ATP; third, the reaction coupled between pseudo cyclic photo-phosphorylation and pseudo cyclic electron transfer chain to produce ATP [9].

The relationship between photo-phosphorylation and electron transfer can be represented by the indicator ATP/e2- or P/O. Ratio indicator of ATP/e2- represents the amount of ATP molecules formed by each pair of electrons passing through the photosynthetic electron transfer chain, while ratio indicator P/O represents the amount of ATP molecules that can be formed by every release of one oxygen atom in a photo reaction. The larger ratio will indicate the tighter coupling between phosphorylation and electron transfer [9].

### 5.1.Non cyclic photo-phosphorylation

The relationship between non cyclic photosynthetic phosphorylation and absorption quantum amount can be expressed by the following equation: $2NADP+3ADP+3Pi+2H_2O \rightarrow 2NADPH+2H+3ATP+O_2$. In the non cyclic photo phosphorylation reaction the system does not only generates ATP, but also produces NADPH and releases oxygen. Non cyclic photosynthetic phosphorylation is uniquely found in oxygen-releasing organisms containing grana layers, which plays a major role in photosynthetic phosphorylation [9]. The electrons' energy conversion chain coupled with non cyclic photo-phosphorylation is described in detail below:

After receiving radiation energy, P680 converts from the ground state to the excited state (P680*), and then transfers electrons' energy to the primary electrons' energy acceptor, demagnesium chlorophyll, in whose process P680* concurrently carries a positive charge and obtains electrons from the primary electron donor Z, located at a tyrosine side chain on the D1 protein at the reaction center, for reduction. The electrons' energy transfer chain Z+ in photosynthesis obtains energy from the oxygen-releasing complex, while the oxidized oxygen-releasing complexes obtain electrons' energy from water, which causes water to undergo photolysis: $2H_2O \rightarrow O_2+2\ (2H)+4e$ [3].

In another direction, magnesium chlorophyll transfers electrons' energy to QA binding with D2, which in turn quickly transfers electrons' energy to QB on D1. The reduced plastoquinone dissociates from the photosystem II complex, while another oxidized plastoquinone occupies the reduced plastoquinone position to form a new QB. In this

energy-carrying chain process, plasmid quinones transfer electrons' energy to the cytochrome b6/f complex, concurrently transferring protons' energy from the matrix to the thylakoid cavity. The electrons' energy are then transferred to Cu in the copper-containing protein plastocyanin (PC) located on one side of the thylakoid cavity, which are subsequently transferred to photosystem II [3].

Differed from P680, the high-level energy electrons released by P700 being excited under light radiation are transmitted sequentially along the direction of A0 → A1 → 4Fe-4S, whose reaction is undergone from one side of the thylakoid cavity to the ferriredoxin (FD) on the side of the thylakoid matrix. Finally, under the effects of ferriredoxin NADP reductase, electrons' energy are transferred to NADP, forming NADPH, while the P700 that has lost electrons will obtain electrons' energy from the PC and reduce them [3].

In the process of photosynthetic phosphorylation, a pair of electrons' energy is transferred from P680 to NADP via P700, synthesizing 4 additional H atoms in the cystic cavity, among which two H atoms source from $H_2O$ photolysis and another two H atoms are transferred from PQ located in the matrix, but there is one H atom used for reduction outside the matrix NADP, so there is a high level of H (pH ≈ 5) in the cystic cavity, forming a proton kinetic potential (matrix pH ≈ 8) between cystic cavity and matrix. H atoms infiltrate the matrix through ATP synthase, promoting the binding of ADP and Pi to form ATP. ATP synthase, also known as CF1-F0 coupling factor, possesses the structure that is similar to mitochondrial ATP synthase. CF1 is also composed of 5 sub-units of α3β3γδε, while the structure of CF0 is embedded in the membrane and consists of four sub-units, serving as the channel for protons to pass through the thylakoid membrane [3].

### 5.2.Cyclic photo-phosphorylation

The process of Z-shaped electrons' energy transfer described above is the non cyclic photosynthetic phosphorylation, but when plants lack of NADP, electrons' energy flows within Photosystem I, only synthesizing ATP without producing NADPH, which turns to be the cyclic photosynthetic phosphorylation [3]. The chemistry reaction equation is described as:

$ADP+Pi \rightarrow ATP+H_2O$

Cyclic photosynthetic phosphorylation is the only form of light energy conversion for non-photosynthetic-oxygen-releasing organisms, mainly occurring within the matrix layer [9].

### 5.3.Pseudo cyclic photo-phosphorylation

Pseudo cyclic photo-phosphorylation releases and absorbs oxygen, and the reduced electron acceptor is ultimately oxidized by oxygen with the chemistry reaction below [9]:

$ADP+Pi+H_2O \rightarrow ATP+O_2\text{-}+4H+NADP+$

If the supply is low (for example the oxidation of NADPH is hindered), it is conducive to the pseudo cyclic electron transfer [9].

**5.4.Summary of three types of photo-phosphorylation**

Among three types of photo-phosphorylation, both non cyclic and pseudo cyclic photophosphorylation are regulated and inhibited by DCMU (dichlorophenyl dimethylurea) with the product name of Diuron (a kind of herbicide), whereas cyclic photosynthetic phosphorylation is not inhibited by DCMU. All three types of photosynthetic phosphorylation are coupled with electron transfer chain. Consequently, if electron transfer inhibitors are added to the chloroplast system, photosynthetic phosphorylation will be eliminated; Inversely, when phosphorylation is coupled with electron transfer chain, whose efficiency is accelerated (such as adding phosphorylated substrates into the system), it will promote electron energy transfer efficiency and oxygen releasing [9]. The photophosphorylation process can refer to the figures of both non cyclic and cyclic electron transfer chain for better understanding (Figure 2 and Figure 3).

**6.Chemiosmotic theory**

There are various theories aiming to explain the mechanism of oxidative phosphorylation, with representative theories such as intermediate product theory, conformational theory, chemiosmotic theory, etc, among which the widely accepted one is the chemiosmotic theory [10].

The chemiosmotic theory was proposed by Mitchell (1961) in the United Kingdom after extensive experiments, which hypothesized that energy conversion and coupling mechanisms had expressed as the following characteristics: firstly, membranes composed of phospholipids and protein peptides showed selectivity against ions and protons; secondly, electron energy transporters with oxidation-reduction potential were unevenly embedded within the membrane; thirdly, proton energy transfer systems was coupled with electron transfer on the membrane; fourthly, proton energy transfer is coupled with ATPase on the membrane [10].

As it is to explain the mechanism of photosynthetic phosphorylation, chemiosmotic theory emphasizes that when oxidation occurs, the respiratory chain acts as a proton pump, and protons' energy are pumped out of the outer side of the mitochondrial inner membrane (membrane gap), causing a trans-membrane electro-chemical potential difference between the inner and outer sides of the membrane, which is utilized by ATP synthase on the membrane to synthesize ATP from ADP and Pi. This means that electron energy transfer in the photosynthetic electron transfer chain need to be accompanied by proton energy motion force (pmf) on both sides of the membrane, so that the ATP synthesis can be driven. The energy released by every 4 protons enters

the mitochondrial matrix through the membrane gap along the electro-chemical gradient, which can synthesize an ATP molecule. After passing through the electron transfer chain, a molecule of NADH+H+ can accumulate 10 protons, thus generating a total of 2.5 ATP molecules, while a $FADH_2$ molecule only accumulates 6 protons, resulting in only 1.5 ATP molecules after passing through the electron transfer chain [10].

There are several types of experiments to yield evidence supporting chemiosmotic theory below:

### 6.1.Stage Photosynthetic Phosphorylation Experiment

Photosynthetic phosphorylation can be relatively divided into light and dark stages, in whose process phosphorylated substrates are not added to chloroplast suspension under light radiation, but when light is cut off, adding substrates can form ATP in the experiment. At first, it was believed that Z* was a chemical substance, thus proposing the theory of photosynthetic phosphorylation intermediates. By gaining the knowledge that the high-energy state of Z* is the electro-chemical potential of H+ both inside and outside the membrane, the two-stage photosynthetic phosphorylation essentially provides evidence that electron energy transfer on the thylakoid membrane under light, generating transmembrane H+ electro-chemical potential, whereas subsequently in the dark, the H+ electro-chemical potential is utilized to synthesize ATP from the added ADP and Pi. In 1962, Shen Yungang etc from China used this experiment to detect the presence of high-energy states (Z*) in photosynthetic phosphorylation. In 1963, Jagendorf et al. also observed the existence of high-energy states of photosynthetic phosphorylation [9].

### 6.2.Acid alkali phosphorylation experiment

Jagdorff et al. (1963) neutralized the thylakoids of chloroplasts in the weakly acidic solution at pH 4, causing the pH of the thylakoid membrane cavity to decrease into 4, in which subsequently another buffer solution containing ADP and Pi at pH 8 was added. It was found that this instantaneous pH change resulted in the H+ gradient between the inside and outside of the thylakoid membrane, which could generate ATP between ADP and Pi without illumination or electron transfer. The pH difference inside and outside the thylakoid drove ATP synthesis to be formed in vivo by photosynthetic electron transfer and H+ transport. This acid-base phosphorylation experiment provided the most important evidence supporting the chemiosmotic theory hypothesis [9].

### 6.3.Experimental study on the absorption of protons' energy by thylakoids under light

Under illumination, the pH increase of chloroplast suspension that initially does not contain pH buffer solution can be measured by pH meter, which is due to the transport of protons' energy from the suspension to the thylakoid membrane cavity caused by photosynthetic electron transfer, resulting in the high concentration of H+ inside the

membrane while a lower concentration of H+ is maintained outside the membrane. After electron transfer generates the proton gradient, protons' energy tends to undergo reverse transmembrane transfer. When protons' energy undergoes reverse transfer, the energy stored in the proton gradient is utilized to synthesize ATP [9].

## 7.**ATPase Enzyme**

### 7.1.Conformational structure of ATPase

Proton reverse transfer and ATP synthesis is catalyzed by ATPase (adenosine triphosphate), which is also known as coupling factor or CF1-CF0 complex on the inner membrane of chloroplasts. The enzyme that catalyzes the synthesis of ATP in chloroplasts is very similar to the ATPase in mitochondria, which is located on the outer side of the thylakoid membrane like a doorknob in chloroplasts, existing in non-stacked capsule like membranes. The ATPase structure of chloroplasts is also very similar to that of mitochondria and bacterial membranes, composed of two protein complexes: one is the hydrophilic 'CF1' protruding from the membrane surface and the other is the hydrophobic 'CF0' embedded in the membrane. ATPase is composed of nine sub-units with a molecular weight of approximately 550 000, which catalyzes the formation of phosphonic anhydride bonds, synthesizing into ATP from ADP and Pi. In addition, ATPase catalysis reaction is also reversible, catalyzing hydrolysis of ATP coupled with H+ transport into the thylakoid membrane. For the two parts of ATPase, CF0 is inserted into the membrane acting as a proton channel, while CF1 is composed of α3, β3, γ, δ, and ε sub-units. Among these sub-units, the α and β sub-units have the function of binding to ADP, while the γ sub-unit controls proton flow; The δ sub-unit binds to CF0, while the ε sub-unit functions as inhibiting catalysis in the dark, to limit ATP hydrolysis and avoid wasted energy. CF1 is located on one side of the matrix, which facilitates newly-synthesized ATP to release into the matrix. CF0 is composed of at least three sub-units, and oligomycin can inhibit the activity of ATPase, thereby blocking photosynthetic phosphorylation [9].

The molecular weight of CF1 is about 400 000, containing five sub-units of α (60 000), β (56 000), γ (39 000), δ (19 000) and ε (14 000). The alpha (α) sub-unit may function as the binding sites to nucleotides, which undergoes conformational changes during catalysis; it is found that the β sub-unit is the catalytic site for the synthesis and hydrolysis of ATP molecules; γ sub-unit plays the role in controlling proton flux; The binding of δ sub-units may be related to CF0, while it is speculated that the ε sub-unit may be capable of inhibiting the activity undertaken by the CF1-CF0 complex under the dark, preventing ATP hydrolysis. Both δ and ε sub-units also have the blocking effect on proton (H+) leakage through CF0 that contains four sub-units, including I, II, III, and IV. III is a polymer that may contain 12 peptides with a total molecular weight of 100 000, which is speculated to be the main channel for proton transfer in CF0, and the functions of sub-units I, II, and IV may be related to the establishment of proton transfer channels or binding to CF1[9].

When the thylakoid membrane loses CF1, it loses its phosphorylation function, but

the phosphorylation function can be restored, if CF1 is added again, whose process is driven by the proton channel: the thylakoid membrane that reduces CF1 will leak protons (H+), but once CF1 is added back to the membrane or an inhibitor of CF0 is added, proton leakage potential stops. This concentration variation on proton potential indicates that CF0 is the 'channel' for protons, supplying them to CF1 to synthesize ATP. Boyer (1993) proposed that it was the rotational conformation changes of the sub-units on CF1 caused by the concentration gradient of proton (H+) that catalyzes ATP synthesis, becoming the way of CF1 to utilize the energy released by H+ transmembrane for ATP synthesis [9].

### 7.2. The binding and transformation mechanism of ATP synthesis

The rotation of the γ sub-unit of ATPase causes conformational changes of the β sub-unit in the order of tightness (T), relaxation (L), and opening (O), regulating the catalytic synthesis of the three processes: ADP and Pi binding, ATP formation, and ATP release [9].

### 7.3.Inhibitors of Photosynthetic Phosphorylation

As discussed in chemiosmotic theory above, the photosynthetic phosphorylation of chloroplasts requires: firstly, electron's energy transfers on the thylakoid membrane; secondly, there is the proton gradient both inside and outside the thylakoid membrane; thirdly, reaction is under the catalysis of active ATPase, so any reagent that disrupts one of these three conditions will cause the cessation of photosynthetic phosphorylation, which become the inhibitors of photosynthetic phosphorylation [9]. There are several types of inhibitors summarized below:

#### 7.3.1.Electron transfer inhibitor

Electron transfer inhibitor is a kind of reagents that are capable of inhibiting photosynthetic electron's energy transfer, such as hydroxylamine ($NH_2OH$) that is capable of cutting off electron's energy flow from water to PS II; DCMU that can inhibit electron's energy transfer from quinone (Q) to PQ on PS II; KCN and Hg play the role in inhibiting the oxidation of PC; some herbicides (such as simazine, atrazine, bromacil, isocil, etc.) also regulate the photo-phosphorylation electron transfer as inhibitors that block electron's energy transfer so that they can kill plants [9].

#### 7.3.2.Uncoupling agent

Uncoupling agent is another type of inhibitor reagent that is capable of splitting the coupling between phosphorylation reaction and electron transfer, with common types of uncoupling reagents including DNP (dinitrophenol), CCCP (carbonyl cyanide-3-chlorophenyl hydrazone), short peptide D, Nigerian bacteriocins, NH4+, etc. These uncoupling reagents can increase the permeability of the thylakoid membrane to protons or increase the ability of coupling factors to leak protons. As the result it is to eliminate the transmembrane H+ electro-chemical potential, so that phosphorylation is no longer carried out, although electron's energy transfer can still occur, which can be even faster due to the reduction of the inhibition effects of high

internal H+ concentration on electron transfer [9].

### 7.3.3.Energy transfer inhibitors

Energy transfer inhibitors are the reagents that directly impose effects on ATPase to inhibit phosphorylation, such as dicyclohexylcarbodiimide (DCCD); p-chloromercuriobenzene (PCMB) acting on CF1 and oligomycin acting on CF0, all of which block photosynthetic phosphorylation by inhibiting ATPase activity [9].

### 7.3.4.The inhibitory sites of chloroplast electron transfer chain inhibitors

The formation of super-oxidized and other reactive oxygen chemistry species by electron formation inhibitors are usually considered as the active functional group sites to inhibit electron energy transfer [9].

## 8.Carbon assimilation

### 8.1.Calvin cycle

#### 8.1.1.Carboxylation stage

$CO_2$ must enter the carboxylation stage to be synthesized into carboxylic acids, which is reduced by the acceptor, Ribulose 1,5-diphosphate (RuBP). Under the catalytic effects of ribulose 1,5-diphosphate carboxylase/oxygenase (Rubisco), RuBP forms an intermediate product with $CO_2$, then reacting with 1 molecule of $H_2O$ to form 2 molecules of glyceryl-3-phosphate (PGA), which is the $CO_2$ carboxylation stage [3].

#### 8.1.2.Restoration stage

Glyceraldehyde-3-phosphate is phosphorylated by ATP, catalyzed by glyceraldehyde-3-phosphate kinase to synthesize into glyceraldehyde-1,3-diphosphate (DPGA), which is then reduced by NADPH+H under the catalytic effects of glyceraldehyde-3-phosphate dehydrogenase, eventually forming into glyceraldehyde-3-phosphate (PGAld) [3].

#### 8.1.3.Update phase

The update stage is the process in which PGAld undergoes a series of transformations and finally forms RuBP, which is the regeneration stage of RuBP starting Calvin cycle again [3].

### 8.2. C4 pathway

#### 8.2.1.Carboxylation

The $CO_2$ receptor of the C4 pathway is PEP located in the cytoplasm of mesophyll cells. Under the catalysis of enol acetone phosphocarboxyl kinase (PEPC), $HCO_3$- ($CO_2$ dissolved in water) is immobilized to produce oxaloacetic acid (OAA), which is a dicarboxylic acid molecule containing four carbon atoms, becoming the symbol of the C4 dicarboxylic acid pathway [3].

#### 8.2.2. Transformation

The oxaloacetic acid species in the chloroplasts of mesophyll cells are reduced to

malic acid by NADP malate dehydrogenase, but there are also some other oxaloacetic acid species and glutamic acids in the cytoplasm to synthesize into aspartic acid and ketoglutarate under the effects of aspartate transaminase. After the formation of C4 acids such as malic acid and aspartic acid, they are transferred to the vascular sheath cells [3].

### 8.2.3. Decarboxylation and Reduction

Tetracarbodicarboxylic acid undergoes decarboxylation in the vascular bundle sheath to form pyruvate or alanine, while the released $CO_2$ is reduced to sugars through the Calvin cycle[3].

### 8.2.4. Regeneration

The C3 acid (pyruvate or alanine) that is the products of decarboxylation of C4 acid is then transported back to the mesophyll cells, which is catalyzed by pyruvate phosphokinase (PPDK) and ATP to generate $CO_2$ receptor PEP in the chloroplasts, consequently re-starting a reaction cycle of C4 pathway[3].

## 8.3. Sedum acid metabolism pathway (CAM)

Plants in the Sedum family, such as Sedum and Arabidopsis, are regulated by unique $CO_2$ fixation mechanisms in their leaves. At night, stomata open and absorb $CO_2$, and under the effects of PEP carboxylase plants combine with PEP to form OAA, which is further reduced to malic acid and accumulated in vacuoles. During the daytime when the stomata close, malic acid in the vacuoles is transported to the cytoplasmic sol, and it oxidizes decarboxylation catalyzed by NADP malate enzyme, releasing $CO_2$ and participating in the Calvin cycle to form starch. In addition, triphosphate forms PEP through glycolysis and further circulates, so the organic acid content of plants is very high at night, while the sugar content decreases; During the day, organic acids decrease while sugar content increases on the contrary. This metabolic type of diurnal variation in the synthesis of organic acids was first discovered in the young root of Sedum family plants, hence called Sedum acid metabolism [3].

## 9. Types of photo-pigments

Chloroplasts are the organ in which photosynthesis happens, and chloroplast thylakoids contain two types of photo-pigments, including chlorophyll and orange yellow carotenoids (carotenoids and lutein). Typically, the ratio of chlorophyll to carotenoids is about 3:1, while the ratio of Chlorophyll a to Chlorophyll b is also about 3:1 in plant leaves. In addition to chlorophyll a and b, there are also chlorophyll c, chlorophyll d and phycocyanin in many types of algae such as phycoerythrin and phycocyanin. In photosynthetic bacteria, there are bacterial chlorophyll existing to undertake photosynthesis. Chlorophyll a, chlorophyll b and bacterial chlorophyll are all composed of a magnesium chelated porphyrin ring and a long-chain alcohol, only with a small difference between them. Carotenoids are tetraterpenes composed of isopentene units, while phycobilinoids are a class of pigment proteins whose chromophores are the chains composed of pyrrole rings without containing metals.

Photo-pigments also have many conjugated double bonds, embedded all chlorophyll and almost all carotenoids in the thylakoid membrane, whereas proteins are bound through non-covalent bonds. Several photo-pigment molecules can be bound to a single peptide chain, and the distance and orientation between each pigment molecule are fixed, facilitating conducive energy transfer. Carotenoids and lutein can affect chlorophyll a and b, playing the certain protective role. The absorption spectra of several types of photo-pigments are different: chlorophyll a and b absorbs red, orange, blue, purple light, but carotenoids absorb blue purple light, with the lowest absorption rate in green light. Especially, the absorption spectra of both phycoerythrin and phycocyanin differ greatly from chlorophyll, indicating the ecological adaptiveness for algae living in the ocean under the different light conditions from the terrestrial plants [3].

The absorption peak process of both Chlorophyll a and b: there are two sets of photosynthesis systems on the chloroplast membrane, including both photosynthesis system I and photosynthesis system II. Photosynthesis system I is more primitive than photosynthesis system II, but electrons' energy transferring begins in photosynthesis system II. Under light radiation conditions, photons with wavelengths between 680nm and 700nm are absorbed respectively, which mainly contains blue-purple light waves, accompanied by a small amount of red light. They are utilized as energy to continuously transfer electrons' energy obtained from the water molecule photolysis process, which is capable of converting by only a few special states of chlorophyll a, finally transferred to coenzyme NADP II [3].

The hydrogen ions obtained from water photolysis move outward from the thylakoid to the substrate through the protein complex on the thylakoid membrane due to the concentration variation, resulting in a decrease in potential energy between them, which is used to synthesize ATP for dark reactions. At this time the hydrogen ions with reduced potential energy are carried away by the hydrogen carrier $NADP^+$. A molecule of NADP can carry two hydrogen ions and chemistry reaction equation is NADP+2e+H=NADPH, while the reducing coenzyme NADPH acts as a reducing agent in this dark reactions [3].

## 10.Photosynthesis organisms

### 10.1.C3 plants

$CO_2$ solidification reaction of C3 plants can quickly transform carbon dioxide into organic compounds, 3-phosphoglycerate (PGA), an intermediate in glycolysis. Carbon dioxide firstly enters the leaves of C3 plants (such as rice and wheat) through stomata and after this carbon dioxide directly enters the mesophyll for the Calvin cycle, whereas the vascular bundle sheath cells of C3 plants are very small, containing very little chloroplasts, so the Calvin cycle does not occur here [3].

### 10.2.C4 Plants

C4 plants are mainly those living in arid tropical regions. In the 1960s, Australian

scientists, Hatch and Slake, discovered that tropical green plants such as corn and sugarcane, did not only undertake the Calvin cycle like other green plants, but also firstly fixed carbon dioxide through a special pathway, which is called as the Hatch-Slack pathway or the tetracarboxylate pathway. Under the arid tropical environment, plants have to shorten their opening time of stomata for absorbing carbon dioxide, to avoid rapid loss of water through transpiration, so the intake of carbon dioxide is inevitably low due to the limitation of adverse environment. The evolution need is that plants must utilize this small amount of carbon dioxide for photosynthesis to synthesize the substances needed for their own growth. Differed from C3 plants, around the vascular bundles of C4 plant leaves, there are vascular bundle sheaths surrounding them, whose cells contain chloroplasts but there are no grana or developmental abnormalities inside, in which the main focus is on the Calvin loop [3].

In its mesophyll cells, there is a unique enzyme species namely phosphoenolpyruvate carboxylase, which causes carbon dioxide to be firstly assimilated by a three carbon compound - phosphoenolpyruvate, then synthesizing into a molecule of four-carbon compound oxaloacetic acid. This is the reason to name this dark reaction as C4. After being converted into malate, oxaloacetic acid enters the vascular sheath and is decomposed into carbon dioxide and a molecule of pyruvate. Carbon dioxide subsequently enters the Calvin cycle, followed by the C3 process, while pyruvate will be re-synthesized into phosphoenolpyruvate, which consumes ATP. That means that C4 plants can open their stomata at night or at low temperatures to absorb $CO_2$ and synthesize C4 compounds firstly and then Calvin cycle uses the $CO_2$ provided by C4 compounds to synthesize organic compounds during the day when there is sunlight [3].

This arrangement of Calvin cycle in C4 plants makes the carbon dioxide fixation efficiency much higher than C3 plants, becoming the environmental adaptiveness for plant growth in arid environment. As comparison and contrast, the starch obtained from photosynthesis in C3 plants is stored in mesophyll cells where the Calvin cycle takes place and the vascular bundle sheath cells do not contain chloroplasts, whereas the starch of C4 plants will be stored within the vascular bundle sheath cells in which the Calvin cycle of C4 plants occurs [3].

### 10.3.Crassulaceae acid metabolism (CAM)

As discussed above, C4 plants are spatially staggered between carbon dioxide fixation and the Calvin cycle, while the CAM cycle is temporally staggered between carbon dioxide fixation and the Calvin cycle. The CAM plants that undergo this pathway are those with swollen fleshy leaves, such as pineapples. These plants open their stomata at night to absorb carbon dioxide that is also fixed through the Hatch-Slake pathway. In the morning, the pores of CAM plants choose to close to avoid rapid water loss, while the Calvin cycle begins in mesophyll cells. CAM plants also keep high efficiency in fixing carbon dioxide due to the evolutionary adaptation [3].

### 10.4.Algae and bacteria

Eukaryotic algae, such as red algae, green algae, brown algae, etc., have chloroplasts like higher advanced plants, engaging in oxygen-producing photosynthesis in earth ecosystem. Light is absorbed by chlorophyll, and many algae have different pigments in their chloroplasts, leading them to different colors [3].

In comparison to plants, bacteria that carry out photosynthesis do not have chloroplasts, which is directly carried out by the bacterial cells themselves. Blue algae (also known as cyanobacteria) belonging to prokaryotes also contain chlorophyll and undergo oxygen-producing photosynthesis like chloroplasts. Other photosynthetic bacteria have a variety of pigments called bacterial chlorophyll or bacterial green, but do not oxidize water to produce oxygen in photosynthesis, which utilize other substances such as hydrogen sulfide, sulfur, or hydrogen as electron donors instead of water. Non-oxygen-producing photosynthetic bacteria that have been discovered so far include purple sulfur bacteria, purple non-sulfur bacteria, green sulfur bacteria, green non-sulfur bacteria and sun bacteria [3].

## 11.Photosynthetic rate

The photosynthetic rate usually is defined as the amount of $CO_2$ or $O_2$ absorption per unit time or per unit leaf area, which can also be expressed as the accumulation of dry matter per unit leaf area or per unit time. The factors influencing the photosynthesis rate are divided into both internal and external factors [3].

### 11.1.Internal factors

There are mainly two internal factors influencing photosynthesis: firstly, leaf development and structure significantly affects photosynthesis rate. The newly-grown tender leaves at leaf age have a low photosynthetic rate, but subsequently the relative photosynthetic rate between different parts of leaves varies at different growth stages, and generally the photosynthetic rate follows a ‘low-high-low’ pattern with increasing leaf age; In addition to leave age, the structure of leaves, including leaf thickness, the ratio of palisade tissue to sponge tissue, and the number of chloroplasts and thylakoids, all results in the effect on the photosynthetic rate, which is controlled by both the genetic factors and environmental conditions [3].

Secondly, the output rate of photosynthetic products (such as sucrose) from leaves can affect the photosynthetic rate of the leaves. The reasons for this effect would include feedback inhibition, which refers to the inhibitory effect of the endpoint photosynthesis product caused by the regulation of enzyme activity in the biosynthetic pathway [5], and influence of starch granules [3].

### 11.2.External factors

There are several external environmental factors affecting photosynthesis rate.

### 11.2.1.Light radiation

The influence of light intensity on photosynthesis is expressed as the light-intensity-photosynthetic-rate curve: Under dark conditions, leaves do not undergo photosynthesis, only releasing respiration effects. With the increase of light intensity, the photosynthetic rate will also correspondingly increase, but only when the specific threshold of light intensity is reached, the photosynthetic rate of the leaves is equal to the respiration rate, which means that the amount of carbon dioxide absorbed is equal to the amount of carbon dioxide released at this critical point. Then if the light intensity exceeds a certain level, the increase in photosynthetic rate slows down. When the critical level of light intensity is reached, the photosynthetic rate no longer increases, reaching the light saturation point [3] (Figure 5).

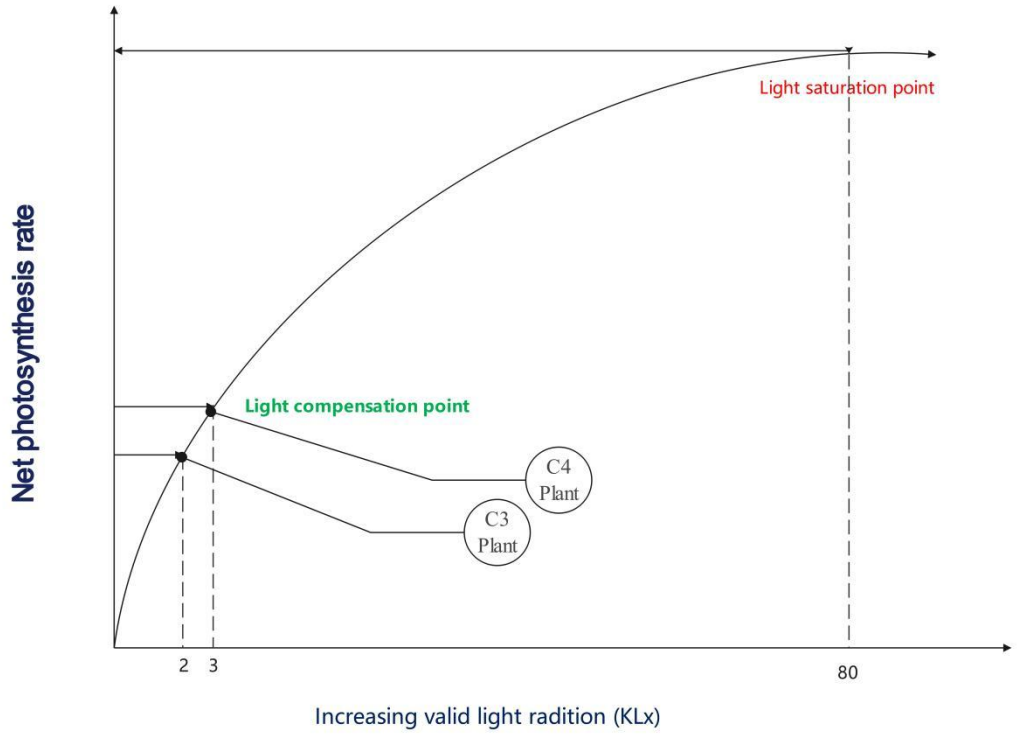


**Figure 5. Light intensity photosynthetic rate curve.**

### 11.2.2.Photo-inhibition of photosynthesis

While insufficient light can become the limiting factor for photosynthesis, the excessive light energy can also have adverse effects on photosynthesis. If the amount of light received by photosynthetic organs exceeds the amount they are capable of utilizing, the excessive light energy can cause a decrease in photosynthetic rate. The impact of light quality on photosynthesis is also known: in solar radiation, it is usually considered that only visible light can be utilized by plant photosynthesis, because the spectrum of photosynthesis is generally consistent with the absorption spectrum of chloroplast pigments [3].

### 11.2.3.Carbon dioxide

The supply of carbon dioxide effect on photosynthesis can be expressed as carbon-dioxide-photosynthetic-rate curve, because carbon dioxide is one of the raw and original materials for photosynthesis, resulting in significant impact on the rate of photosynthesis [3]. There are two examples of carbon-dioxide-photosynthetic-rate curve under normal condition and salty stress condition for comparison below:

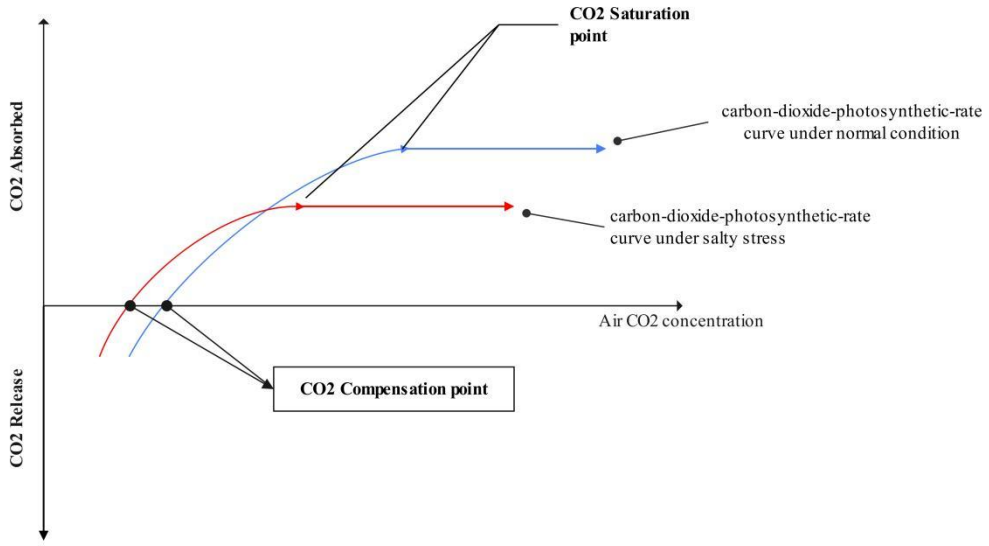


**Figure 6. The carbon-dioxide-photosynthetic-rate curve under normal (control) condition and under salty stress for *Sophora japonica L.* The figure is drawn according to the data of Table 1 [37].**

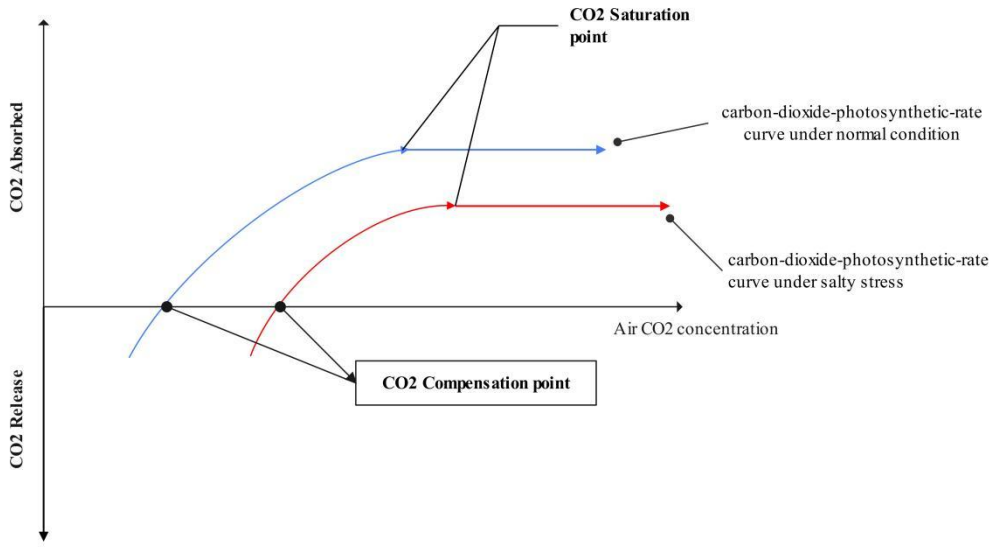


**Figure 7. The carbon-dioxide-photosynthetic-rate curve under normal (control) condition and under salty stress for *Juglans regia L.* The figure is drawn according to the data of Table 1 [37].**

Carbon dioxide mainly enters the leaves through stomata, and externally strengthening ventilation or increasing the application of carbon dioxide on leaves can significantly improve the photosynthetic rate of crops, especially for C3 plants [3].

### 11.2.4.Temperature

The dark reaction in the photosynthetic process is a kind of biochemical reaction catalyzed by enzymes that will be sensitively influenced by temperature [3].

### 11.2.5.Moisture

The main reasons for water deficit reducing photosynthesis include decreased stomatal conductivity, slow output of photosynthetic products, damage to photosynthetic pathways and damage on the expansion of photosynthetic area [3].

### 11.2.6.Mineral nutrition

Mineral nutrients have a wide range of functions in photosynthesis, which are caused by the components of chloroplast structure, important components of electron transporters, the important role of phosphate groups, activation or regulatory factors [3].

### 11.2.7.Daily variation of photosynthetic rate

The environmental factors are continuously changing throughout the day, and the variation in environmental conditions can cause significant effects on the photosynthetic rate. Among these environmental conditions, the diurnal variation in light intensity results in the greatest impact on the diurnal variation of photosynthetic rate [3].

## 12.Photosynthesis and ecosystem role in earth

### 12.1.Energy conversion

Plants convert solar energy into both biochemical and biophysical energy while assimilating inorganic carbides, which is stored in the formed organic compounds. The amount of solar energy assimilated by photosynthesis every year is about 10 times than the energy required for human consumption. The bio-energy stored in organic matter is not only used by plants themselves, but also is utilized by all the heterotrophic organisms especially for human nutrition and activities [3].

Energy conversion between light radiation and biochemical energy with the total reaction formula for photosynthesis is [9]:

$6CO_2+6H_2O \rightarrow C_6H_{12}O_6+6O_2$ ;

$\Delta G0' = 2881$ kilo-joules per mole;

As can be seen from the reaction formula, synthesizing a molecule of oxygen requires

4 electrons and 8 photons, so 6 oxygen molecules require a total of 6 × 8 = 48 photo-quanta. Each mole of light quantum contains $6.02 \times 10^{23}$ light quantum, but the energy of light quantum varies at different wavelengths, and short wavelength light waves carry higher energy. If it is calculated on the basis of the light waves at 700nm wavelength, 48 light quanta carry the mean energy of 48 × 170 = 8265 kilo-joules per mole. Under standard conditions, 1 mole of glucose requires 2881 kilo-joules of free energy (Δ G0'), so the energy utilization efficiency of photosynthesis is 288l/8265 × 100% = 35% [9].

### 12.2.An important pathway for inorganic substances to become organic matter

Plants in earth planet can absorb approximately $7\times10^{11}$ t carbon dioxide, synthesized into organic matter of $5\times 10^{11}$ t every year. The food, oil, fiber, wood, sugar, fruits, etc that human need all source from photosynthesis. Without photosynthesis, there would be no human survival and development [3].

### 12.3.Photosynthesis and atmosphere

Photosynthesis is the source of oxygen production in atmosphere, and maintaining 21% of oxygen content in atmosphere mainly depends on plant photosynthesis (the amount of oxygen released during photosynthesis is about $5.35\times10^{11}$ t/a). Consequently, photosynthesis does not only provide conditions for aerobic respiration, but also forms the ozone layer on the outer layer of the atmosphere due to the gradual accumulation of oxygen, which can absorb strong ultraviolet radiation harmful to living organisms from sunlight. Although photosynthesis of plants can absorb and utilize a large amount of $CO_2$ pollutant from the atmosphere, the $CO_2$ concentration in the atmosphere is still increasing mainly due to the excessive emission of $CO_2$ in the process of urbanization and industrialization [3].

## 13.Discussion of plant species evolution and earth ecosystem

### 13.1.Biotic energy and abiotic energy

The past biology knowledge describes the energy conversion in photosynthesis as chemical energy or physical energy without distinguishing abiotic energy from biotic energy, so my article has modified the terms of both chemical energy and physical energy into biochemical energy and bio-physical energy respectively, and the intrinsic differences between abiotic energy and biotic energy have been discussed and compared in my another article [8]. The abiotic energy of light radiation is converted into biotic energy by the photosynthesis of plants, which is considered as the primary/original biotic energy firstly utilized by plants themselves. Then herbivores consume plant biomass, converting plant bio-energy into herbivorous bio-energy, and finally herbivorous biotic energy is eaten by carnivorous animals, which consequently forms the food chain and bio-energy consuming cycle in ecosystem. Based on the biotic energy consuming cycle in biological kingdoms, it is easily deduced that there is the intrinsic difference and boundary between biotic energy and abiotic energy. Biotic energy is utilizable by creatures themselves, but only plant photosynthesis system is capable of utilizing abiotic energy (light energy), changing into biotic

energy utilized by creatures.

Correspondingly, there is also intrinsic difference between biotic chemistry reaction and abiotic chemistry reaction, which has been substantially discussed by my another article [8]. One of the key difference is that biotic chemistry reaction actively and selectively binds different molecules at the intersection of active chemistry bonds between them, determined by creature's intelligence, whereas abiotic chemistry reaction makes different molecules collide each other randomly, so my arguing supports the 'Chemiosmotic theory' discussed in section 6 of this article, which hypothesizes that "membranes composed of phospholipids and protein peptides showed selectivity against ions and protons".

My article has also improved the interpretation of both electron and proton transfer chain according to the new definition of ionic bond in my quantum chemistry article [4], which states that "the real nature of electricity is the fluids or pulse of electromagnetic waves, rather than electron movement across atoms in conductor materials (otherwise, there will be a large number of positively charged protons bursting out at high speed, so that all electric conductors are the same as radioactive elements, which is obviously out of date)." Obviously, life can hardly bear the rays' radiation, so my article has changed the electron transfer into electron energy transfer by electromagnetic waves. In nature, the free forms of electron that can be transferred between atoms is usually produced by high intensity of voltage such as lightning, coupled with radioactive substances, which is definitely not suitable for the life activity.

### 13.2. Evolution of life in our planet

Further more, my article disagrees with that plant species are evolved from the ocean creatures in earth planet. Firstly, my another article has defined that different species can be differentiated by the various range of bio-signals' wave frequency among different species [7], so my article does not believe that a species with the specific range of bio-signal wave frequency can be evolved into other different frequency of bio-signals, which is the bounder to hardly cross over. Secondly, according to the role played by plant species in earth ecosystem, "the survival of other higher multicellular organisms in both terrestrial ecosystem and marine ecosystem on the earth must rely on the large and sufficient amount of oxygen production by trees after the establishment of large-scale tree population [7]." Although some microbes can also generate oxygen in earth, the amount is too little to keep the constant 21% of oxygen content in atmosphere, and the amount of oxygen in atmosphere produced by microbes may be easily exhausted by frequent fires events in earth planet at the earth development stage (for example, frequent submarine magma eruptions during geological plate movements). Consequently, my article will insist that trees population must be the first species settled in this planet with the longest evolutionary history, so photosynthesis organs possess the most complicated organic process among earth organisms.

In addition to the visible light waves, the UV-B light radiation would also be the utilizable light waves for photosynthesis in the plant species that are evolved from plateau origin under intensive UV-B radiation, which can be deduced by the experiment results that enhanced UV-B treatment increase the biomass growth of Caucasian clover in my another article [13][14].

## 14.Environmental interactions with photosynthesis

This section will emphasize the case studies aiming to reveal the environmental interactions (especially for the environmental stress) with plant photosynthesis.

### 14.1.Cold stress

The impact of cold stress (0~20℃) on photosynthesis can be divided into both direct and indirect effects. Firstly, low temperature directly affects the structure and activity of photosynthetic systems, mainly including: the impacts on the composition, permeability and fluidity of chloroplast thylakoid membranes, the impacts on submicroscopic structure of chloroplasts, the impacts on the chlorophyll content and the changes in the ratio of chlorophyll a to chlorophyll b, the impacts on the light photochemical reactions and the dark reactions in photosynthesis that mainly affects enzyme activity in the Calvin cycle process; Secondly, low temperature affects other physiological processes in plant body, indirectly affecting photosynthesis, for example causing water stress, increasing in $CO_2$ diffusion resistance caused by pores, eliminating the transportation of photosynthetic products, accumulating starch and sucrose in the leaves [19].

#### 14.1.1.Case studies on cold stress physiology

Hu and Yu (2021) assessed the effects of low temperature on photosynthesis system of tomato plants, and found that the Plastochron Index (PI) value of plant leaf growth remained basically unchanged under the cold stress treatment at 10 ℃ and 5 ℃, indicating that growth of plant leaf completely stopped by treatment at low temperature. After 8 days of recovery, the PI value of the 10 ℃ treatment increased rapidly, with an increase of 15.3%, which was comparable to the increase in the control group (without cold stress treatment) within 8 days before (15.9%), but after 8 days of plant recovery at 5 ℃, the increase in PI value was only 8.4% in comparison. The results indicated that tomato plants under 10 ℃ low temperature stress were capable of recovering their growth rapidly after stress relief, while plants under 5 ℃ low temperature stress still could not fully recover to its normal growth after 8 days of stress relief [15].

During this period, the chlorophyll content in the upper leaves of the control plant remained unchanged, while the chlorophyll content in the lower leaves rapidly decreased with the whole plant growth, but both 10 ℃ and 5 ℃ treatments resulted in the decrease in chlorophyll content. After 8 days of recovery, the chlorophyll content in the upper leaves was fully restored in both 10 ℃ and 5 ℃ treatments. Compared

with the upper leaves, the restoration of chlorophyll content in lower leaves was not effective, which could be revealed by the evidence that the chlorophyll content in the lower leaves of plants slightly increased in the plants treated at 10 ℃, while the chlorophyll content in the lower leaves of plants treated at 5 ℃ continued to decrease [15].

The upper leaf of the control plant was the growing leaf, with its net photosynthetic rate increasing over time, but the net photosynthetic rate of lower leaves gradually decreased over time, going towards aging. Both 10 ℃ and 5 ℃ treatments resulted in the decrease in net photosynthetic rate. After 8 days of treatment at 10 ℃, the upper leaves decreased by 18.4% while the lower leaves decreased by 34.4%; After 8 days of treatment at 5 ℃, the decrease was 48.6% and 66.1% for upper and lower leaves, respectively. Low temperature treatment led to the greater impact on the photosynthesis of lower leaves compared with upper leaves [15].

During the subsequent period of recovery after cold temperature treatment ceased, the net photosynthetic rate, stomatal conductance and intercellular $CO_2$ concentration of the upper leaves treated at 10 ℃ quickly returned to the control level, but the upper leaves treated at 5 ℃ recovered slowly and remained below the control level until the 8th day after recovery. In comparison to upper leaves, the net photosynthetic rate of the lower leaves of plants treated at 10 ℃ gradually increased during the recovery process, reaching a higher level than before treatment on the 8th day, whereas the net photosynthetic rate of the lower leaves of plants treated at 5 ℃ recovered slightly during the recovery process, but it no longer could recover to the pre-treatment level, which was only equivalent to the lower leaf of control plants with aging characters at the same time [15].

In order to further explore the mechanism how low temperature reduced photosynthesis, the effect of low temperature stress on chlorophyll fluorescence in upper leaves was further observed. According to the results, the photochemical efficiency (Fv/Fm) of PSII did not decrease under 10 ℃ treatment, and the quantum efficiency of photosynthetic electron transfer (Φ PSII) was also slightly reduced at the 8th day under 10 ℃ treatment, but it quickly recovered to the control level after stress was relieved; Under the treatment at 5 ℃, both Fv/Fm and Φ PSII significantly decreased ($P=0.05$), which recovered to the control level on the 4th day of recovery. These results showed that 10 ℃ low temperature stress did not cause damage to the photosynthetic apparatus, while 5 ℃ treatment has led to partial inactivation or damage of the photosynthetic apparatus [15].

Gu et al., (2024) study simulated cold stress on two *Stenotaphorum secundatum* strains of lateral blunt leaved grass, including S13 (cold resistant variety) and S55 (sensitive variety) which expressed as significant difference in cold resistance in the past. Their photosynthetic performance was measured under the cold temperature (8 ℃) for 0, 3, 7 and 14 days respectively, and two varieties' differences in

photosynthesis were analyzed. The results showed that with the extension of low temperature stress duration, the net photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr) of the two variety materials all significantly decreased ($P<0.05$), but the inter-cellular $CO_2$ concentration (Ci) of S13 showed significant ($P<0.05$) decrease trend firstly (0 ~7 days) and then significantly ($P<0.05$) increased (14 days), while the Ci of S55 variety remained stable in the early stage (0~7 days) and significantly increased in the later stage (14 days) ($P<0.05$). These findings indicated that the early stage of low temperature stress was caused by stomatal limiting factors, because Ci would indicate the efficiency in the $CO_2$ uptake by leave stomata, which resulted in a decrease in Pn of lateral blunt leaved grass, but in comparison the impact of later stage (14 days) was caused by non-stomatal factors. The chlorophyll content (Chl), carotenoid content (Car), and chlorophyll fluorescence parameters (Fv/Fm) all showed the gradually decreasing trend, indicating that the lateral blunt leaved grass was inhibited by light. The physiology responses between the two varieties were basically consistent, but there was significant difference in their response to low temperature stress ($P<0.05$): after 3 days of low-temperature treatment, the Chl, Car, and Fv/Fm of S13 were significantly higher ($P<0.05$) than those of S55; After 7 days of low-temperature treatment, the Pn, Gs, Tr, Chl, Car, and Fv/Fm of S13 were significantly higher ($P<0.05$) than those of S55; After 14 days of low-temperature treatment, the Pn, Chl, and Car of S13 were still significantly higher ($P<0.05$) than those of S55, but the differences in other indicators were not significant ($P>0.05$) [20].

Under low temperature stress, the level of plant damage was considered to be positively correlated with the decrease in chlorophyll, which was consistent with this experiment results, and low temperature treatment gradually reduced the Chl and Car of the lateral blunt leaved grass with the extension of cold stress treatment time, indicating that low temperature could hinder the synthesis of chlorophyll and carotenoids in the lateral blunt leaved grass, thereby directly negatively affecting photosynthesis, but the chlorophyll content of different varieties showed significant differences ($P<0.05$), which revealed that the decrease in Chl content in variety S55 was more severe than the cold resistance variety (S13)[20][22].

The maximum photochemical efficiency (Fv/Fm) that was measured by chlorophyll fluorescence instrument could be used as an indicator to assess the degree of abiotic stress, as the main causal factors for the decrease in Pn reflected by Fv/Fm were non stomatal factors. The reason for the decrease in Fv/Fm might be that low temperature stress reduced the ability of leaves to assimilate $CO_2$, thereby reducing the demand for ATP and NADPH in chloroplasts of lateral blunt leaved grass. Then PS Ⅱ received feedback from redox reactions, consequently leading to excess light energy and the decrease in Fv/Fm [20][21].

### 14.1.2.Discussion

The physiology mechanism caused by low temperature has been substantially

discussed by the article [15]. However, my article further complements the cold temperature effects on the plant physiology: by comparing with the results in the net photosynthetic rate of the lower leaves between the control plants and the recovery plants after treatment at 10 ℃, it is further deduced that the cold temperature at the moderate level of 10 ℃ would slow down the aging process of plants, refreshing and even enhancing the net photosynthesis rate during recovery period compared with the control plants with normal aging process. This findings may help to improve the plant cultivation practices for the selection of tomato maturing period, depended on the market needs.

The study by Br ü ggemann et al. showed that the long-term growth arrest and decrease in photosynthetic capacity of tomatoes under low light conditions and low temperatures of 6-10 ℃ were not due to the degradation in the enzyme activity of both Rubisco-1,5-diphosphoribulose RuBP carboxylase and oxygenase Ribulose-1,5-bisphosphophase larboxlass/oxygen nase, nor were because of the acceleration of leaf senescence. The reason was because of the photosynthesis of mature leaves which was difficult to recover to the normal levels during the recovery period after low temperature treatment ceased [16][17][18]. However, this recovery rate was aging-dependent and temperature-dependent: according to the findings of study [15], the damage to the lower leaves under 10 ℃ low temperature and damage to the upper leaves under both 10 ℃ and 5 ℃ low temperature was reversible, which could be fully or partially restored during recovery periods, but the damage to the lower leaves under 5 ℃ low temperature was irreversible, and this irreversible recovery was attributed to the acceleration of the aging of the lower leaves by this study [15], which became inconsistent with Br ü ggemann et al.'s explanation [16][17][18]. In my article, I agreed with Br ü ggemann et al.'s explanation by proposing that cold stress would slow down the aging process rather than accelerating aging process as discussed in above paragraph. However, the findings in article [15] indicated that younger leaves were recovered efficiently after both moderate (10 ℃) and severe (5 ℃) cold stress were relieved, while older leaves could be hardly recovered from severe cold stress.

**Table 1. Summary of plant physiology indicators/treatment under various environmental stress (Cold stress).**

| Indicators/Treatment of Plant Physiology | Plant Species | | Environmental stress types |
|---|---|---|---|
| | Tomato [15] | Lateral blunt leaved grass [20] | |
| Plastochron Index (PI) | Select | | Cold stress |
| Chlorophyll content (Chl) | Select | Select | Cold stress |
| Net photosynthetic rate (Pn) | Select | Select | Cold stress |
| PS I and PS II | | | |

| | | | |
|---|---|---|---|
| Stomatal conductance (Gs) | Select | Select | Cold stress |
| Inter-cellular $CO_2$ concentration (Ci) | Select | Select | Cold stress |
| Photochemical efficiency (Fv/Fm) | Select | Select | Cold stress |
| Upper and lower leaves | Separately measuring | | Cold stress |
| Environmental stress simulation | 8 days at 10 ℃ and 5 ℃ | 0, 3, 7 and 14 days at 8 ℃ | Cold stress |
| Recovery treatment | 8 days after cold stress | | Cold stress |
| Transpiration rate (Tr) | | Select | Cold stress |
| Carotenoid content (Car) | | Select | Cold stress |
| The initial fluorescence (Fo) | | | |
| Non photochemical quenching (NPQ) | | | |
| Electron transfer rate (ETR) | | | |
| Antioxidants activity | | | |
| AsA-GSH cycle | | | |
| Lutein cycle | | | |

### 14.2.Plant physiology under hot temperature

On the contrary, plant photosynthesis physiology responses to hot temperature has been paid more attention due to the climate change impact.

#### 14.2.1.Case studies on plant photo-physiology to hot stress

In order to understand the impacts of high temperature stress on the photosynthesis and antioxidant system of *Carya cathayensis Sarg.* seedlings, provided scientific reference for the cultivation of *Carya cathayensis* during the high temperature season, Ye et al., (2024) study treated *Carya cathayensis* seedlings at different temperatures (25 ℃, 30 ℃, 35 ℃, 40 ℃) as hot stress simulation, and the parameters of both gas exchange and chlorophyll fluorescence, as well as the changes in antioxidant enzymes and antioxidants, were measured in *Carya cathayensis* leaves. The results showed that with the increase of temperature, the net photosynthetic rate (Pn) and stomatal conductance (Gs) of walnut leaves ascended firstly and then descended, but the inter-cellular $CO_2$ concentration (Ci) displayed the continuous upward trend; The initial fluorescence (Fo) and non photochemical quenching (NPQ) of walnut significantly increased under high temperature stress, while the maximum photochemical efficiency (Fv/Fm) and electron transfer rate (ETR) significantly

decreased above 35 ℃; Under the influence of high temperature stress, the contents of hydrogen peroxide ($H_2O_2$) and malondialdehyde (MDA) in walnut leaves significantly increased. However, when the plants were treated at 25 ℃ to 35 ℃, the activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbic acid peroxidase (APX), glutathione peroxidase (GPX), and glutathione reductase (GR), as well as the contents of reduced glutathione (GSH) and oxidized glutathione (GSSG), increased with the increasing temperature, but these antioxidant enzymes and antioxidants' activity significantly decreased at 40 ℃. These results indicated that high temperature stress caused damage and led to decrease in electron transfer rate in the PSII response of walnuts, resulting in the reduction in photosynthetic rate. At the same time, it quickly activated the protective responses of heat dissipation and antioxidant systems under moderate hot stress (below 35 ℃), but this hot stress response was not effective under higher temperature stress at 40 ℃, which could reflect that the antioxidant system was damaged, exceeding the tolerance threshold of walnuts at this temperature [23].

The increase in Fo is mainly attributed to the variation in the structure of the capsule membrane, adjusting excessive light energy that causes damage to photosynthetic systems. The indicator of Fv/Fm represents the potential maximum photosynthetic capacity of plants, functioned by the inactivation of PSII reaction centers, which is usually related to plant photo-inhibition, while NPQ represents the heat dissipation capacity of plants, and the higher the value, the stronger the ability to dissipate excess light energy. Both indicators reflect plant photo-protection mechanism under excessive light radiation or heat (the two adverse conditions are usually associated with each other in the field) [23][24][25].

High temperature stress can stimulate the formation of reactive oxygen species (ROS) in plant cells, causing oxidative damage to membranes, lipids, proteins, and nucleic acids, but a small amount of ROS helps plant growth and development. When plants are subjected to high temperature stress, they accumulate a large amount of ROS, which in turn causes damage to the plants [23][26].

This study showed that under 30 ℃ conditions, although the MDA and $H_2O_2$ contents (ROS species) significantly increased, Pn increased at this time, indicating that mild level ROS induced an increase in the photosynthetic capacity of walnut at 30 ℃. Under moderate hot stress, the damage caused by reactive oxygen species was reduced or eliminated by increasing the activity of antioxidant enzymes in order to avoid the toxic effect of ROS accumulation on plants. Due to the effects of plant antioxidant mechanism, the membrane lipid peroxidation of walnut leaves retained at a low level, and the cell structure and function were relatively complete, allowing for normal metabolism. However, when hot stress lifted, excessive ROS could not be completely eliminated, and plants suffered irreversible damage [23][27].

The ascorbic acid - glutathione cycle (AsA-GSH cycle) can remove $H_2O_2$, thereby

preventing the formation of highly active ·OH. APX uses AsA as a specific electron's energy donor to reduce $H_2O_2$ into water. Enzyme species GR plays the crucial role in the clearance of reactive oxygen species in oxidative stress response, and in addition it is also involved in the ascorbic acid - glutathione cycle pathway, which maintains the ratio of GSH to GSSG content in plants by catalyzing NADPH that reduces GSSG into GSH. GPX is one of the main enzymes that catalyze the oxidation of reduced glutathione (GSH) in the redox cycle of glutathione. GPX does not only catalyze the generation from GSH into GSSG, but also promotes the decomposition of $H_2O_2$ to protect biofilms from ROS damage, reducing toxic peroxides to non-toxic hydroxyl compounds, so that normal cell function is maintained [23][28].

Usually, the ratio of GSH to GSSG in plants is stable, mainly because plants' metabolism regulates its glutathione balance as the adaptiveness to external environment. When the ratio value increases, it indicates that the antioxidant capacity in the plant body is enhanced, which is capable of improving the efficiency of AsA-GSH cycle and avoiding plant damage caused by external stress. Consequently, the ratio of GSH/GSSG in plant cells can be utilized to indicate the efficiency of the ascorbic acid glutathione (AsA-GSH) cycle as well as the degree of environmental stress [23][29].

The GSH/GSSG of walnut increased under both mild and moderate stress (35 ℃), but its ratio began to significantly decrease at severe hot stress (40 ℃). The above results indicated that under moderate temperature stress, the GSH/GSSG ratio of walnut increased and the ability of AsA-GSH cycle to clear ROS also increased correspondingly, but this physiological protection mechanism was not effective under severe hot stress [23].

Using autumn eggplant (*Kandelia obovata*) with cold tolerance and heat-resistant olive (*Bruguiera gymnorrhiza*) from two geographical populations: Guangxi, China (lower latitude) and Okinawa, Japan (higher latitude) respectively, the photosynthetic activity parameters, including Photosystem I (PS I) and Photosystem II (PS II), lutein cycle components, and chlorophyll a and b contents, were measured, based on which the differences in their photosynthetic physiological responses after high-temperature treatment were analyzed. The results showed that high temperature treatment (45℃ /35℃) significantly reduced the maximum photochemical potential (Fv/Fm) of plant PSII, with a reduction amplitude order: lower latitude population < higher latitude population; olive seedlings < autumn eggplant seedlings. The redox state (Pm) of P700 decreased, with descending amplitude order: lower latitude populations < higher latitude populations, but the non photochemical quenching (NPQ) of leaves increased, manifested as lower latitude population > higher latitude population, as well as olive seedlings > autumn eggplant seedlings. High temperature treatment caused the increase in the capacity of lutein storage (V+A+Z) and the state of de-epoxidation (A+Z)/(V+A+Z) occurred, with lower latitude populations being greater than higher latitude populations. Indicator of (A+Z)/(V+A+Z) was negatively correlated with

Fv/Fm, but positively correlated with NPQ. On the second day of recovery at room temperature, the Fv/Fm of high latitude autumn eggplant seedlings remained at 0.69, indicating irreversible damage to their PSII. However, the Fv/Fm of other seedlings returned to normal levels, and all seedlings maintained the high level of NPQ and lutein de-epoxidation ratio, still playing the photo-protective role on the second day of recovery. Compared with Savannah and Mediterranean habitats, mangroves possess the largest lutein pool. The research results found that low latitude populations showed the high adaptability to high temperature stress, partly due to the strong photo-protective effect of the lutein cycle, but there was trade-off between cold and heat tolerance among species and their origins [30].

Hot stress that exceeds the suitable temperature for photosynthesis can cause deactivation of enzymes such as Rubi sco. Especially when the leaf temperature exceeds the temperature threshold of 42 ℃, it significantly increases the fluidity of the thylakoid membrane and leads to protein denaturation, resulting in direct damage to the photosynthetic apparatus. Under hot stress, both light and dark reactions in plant photosynthesis are negatively affected, so the plant photosynthetic system is subject to excessive light energy stress correspondingly, leading to excessive production of reactive oxygen molecules and damage to the biofilm. Under stress conditions, increasing non photochemical quenching in metabolic process, mainly through the lutein cycle, can dissipate excessive light energy and avoid damage to photosynthetic mechanisms. The lutein cycle converts epoxidized violaxanthin (V) into both epoxidized zeaxanthin (A) and zeaxanthin (Z), accompanied by non radiative energy dissipation, which consequently plays a crucial role in regulating the efficiency of solar energy conversion into photosynthetic products and keeping antioxidant processes effective [30].

**Table 2. Summary of plant physiology indicators/treatment under various environmental stress (Hot stress).**

| Indicators/Treatment of Plant Physiology | Plant Species | | Environmental stress types |
|---|---|---|---|
| | *Carya cathayensis Sarg.* [23] | *Kandelia obovata; Bruguiera gymnorrhiza* [30] | |
| Plastochron Index (PI) | | | Hot stress |
| Chlorophyll content (Chl) | | Select | Hot stress |
| Net photosynthetic rate (Pn) | Select | | Hot stress |
| PS I and PS II | | Select | Hot stress |
| Stomatal conductance (Gs) | Select | | Hot stress |
| Inter-cellular $CO_2$ concentration (Ci) | Select | | Hot stress |

| | | | |
|---|---|---|---|
| Photochemical efficiency (Fv/Fm) | Select | Select | Hot stress |
| Upper and lower leaves | | | Hot stress |
| Environmental stress simulation | 25,30,35,40℃ | 35,45℃ | Hot stress |
| Recovery treatment | | 2 days of recovery | Hot stress |
| Transpiration rate (Tr) | | | Hot stress |
| Carotenoid content (Car) | | | Hot stress |
| The initial fluorescence (Fo) | Select | | Hot stress |
| Non photochemical quenching (NPQ) | Select | Select | Hot stress |
| Electron transfer rate (ETR) | Select | | Hot stress |
| Antioxidants activity | Select | | Hot stress |
| AsA-GSH cycle | Select | | Hot stress |
| Lutein cycle | | Select | Hot stress |

**14.2.2.Discussion**

The experiment parameters used in hot stress simulation have been summarized in Table 2. However, the hot stress in the field is usually associated with excessive light radiation due to the weather conditions (for example, the atmospheric temperature is usually increased by the enhancing light radiation intensity), and the hot stress simulation of these cases' experiment has been isolated, from the 'real' weather condition of excessive light radiation in the field, which would be definitely the deficiency of environmental physiology experiment design, so that the conclusion of these cases may not be consistent with the physiology responses in the field conditions, especially when the climate change lets this planet face much more light radiation than before.

From the discussion of enzyme analysis in both AsA-GSH cycle and Lutein cycle, it indicates that the enzyme regulating pathways to hot stress would go through the same pathways as the metabolic regulating pathways to excessive light radiation. This findings would further support the argument proposed by my another article [31], which argues that "the memory of cells, in terms of identifying the bio-signals of a specific environmental factor (can be biotic or abiotic) triggering the gene expression for environmental adaptiveness or immunology, can be trained and strengthened by the biophysical simulation of other environmental factors, because multiple gene traits of environmental adaptiveness or immunology should be located in the same linkage group of genome." Consequently, the seedlings cultivated under hot stress simulation

would increase the stress tolerance to excessive light radiation when the seedlings are transferred to grow in the field land under climate change.

As discussed in AsA-GSH cycle, the enzyme function of GR and GRX plays the role in two inverse reaction chains respectively (GR: from GSSG into GSH; GRX: from GSH into GSSG). Consequently, my article further complements that the decreased ratio of GSH/GSSG under severe hot stress condition in the case study is caused by the quicker deactivation of GR than GRX, which means that the enzyme GR is more sensitive to the severe hot stress than GRX in AsA-GSH cycle. This complementary conclusion can be supported by the case study results.

The trade-off has been reported between cold and hot tolerance in above case study, which means that a variety cannot possess the gene traits of both cold and hot tolerance. This findings is consistent with my conclusion in another article that argues: "the selection of a specific gene trait would lead to the lose of other gene traits as compensation nature due to the linkage effects of the whole genome pool"[32].

### 14.3.Plant photo-physiology to increased atmospheric $CO_2$

Climate change leads to increased atmospheric $CO_2$ concentration in the earth, with the notable impacts on plant photosynthesis system, which has been comprehensively studied in the past research.

#### 14.3.1.Case studies

Based on the platform of Chinese Rice FACE (Free air $CO_2$ enrichment), Xu et al.,(2023) study investigated the effects of elevated atmospheric $CO_2$ concentration (ambient atmosphere+200 μ mol/mol) on rice photosynthesis, antioxidant enzyme activity, and copper and zinc absorption by two fertilization methods, including chemical fertilizers and 50% organic nitrogen substitution fertilizers. Compared with the atmospheric $CO_2$ concentration at normal level, the treatment of increased atmospheric $CO_2$ concentration only (without 50% organic substitute fertilizer treatment) significantly increased the net photosynthetic rate of rice leaves at heading stage (17.9%), but significantly reduced stomatal conductance (41.7%). Under the treatment of 50% organic fertilizer that replaced chemical fertilizer treatment, it was found that the increase in atmospheric $CO_2$ concentration had no significant effect on both chlorophyll content and net photosynthetic rate of rice leaves at heading stage, but significantly reduced the stomatal conductance (59.3%) and transpiration rate (42.0%) of rice leaves, by comparing the normal atmospheric $CO_2$ concentration with the increase treatment in $CO_2$ concentration [33].

Under chemistry fertilizer treatment, the increase of atmospheric $CO_2$ concentration significantly reduced the activities of both SOD and POD antioxidants in rice leaves at heading stage, so that the content of MDA (the products of peroxidation) was significantly increased. However, under the 50% organic fertilizer substitution treatment, it was indicated that the increase in atmospheric $CO_2$ concentration

significantly increased POD activity so it significantly reduced MDA content, by comparing the normal atmospheric $CO_2$ concentration with the increase in $CO_2$ concentration [33].

Under both normal atmospheric $CO_2$ concentration and elevated atmospheric $CO_2$ concentration levels, the application of 50% organic fertilizer and other nitrogen substitutes significantly increased the available Cu and Zn content in the soil, compared with the application of chemical fertilizers. The increase of atmospheric $CO_2$ concentration only (without 50% organic substitute fertilizer treatment) significantly increased the Zn content in rice grains, which was consistent with the significant increase in available Zn content in soil due to the increase in atmospheric $CO_2$ concentration only. However, the increase in atmospheric $CO_2$ concentration only (without 50% organic substitute fertilizer treatment) had no significant effect on the available Cu content in soil. Under the treatment of 50% organic fertilizer replacing chemical fertilizer, it was revealed that the increase in atmospheric $CO_2$ concentration significantly reduced the Cu content in rice stems and grains, as well as the Zn content in grains, by comparing the normal atmospheric $CO_2$ concentration with the increase of $CO_2$ concentration [33].

It was concluded that the application of 50% organic fertilizer and other nitrogen substitutes that replaced chemical fertilizers could help to improve the adaptability of rice to increased $CO_2$ concentration [33].

The response of photosynthetic rate of red palm leaf (*Anthurium andrae anum Arizona*), including plant growth and photosynthetic enzyme activity, to high $CO_2$ concentration was studied by using the facility of open top plastic film greenhouse. The results showed that after 30 days of treatment, the plant height, single leaf area and fresh weight of treatment group T1 (700 ± 100 μ mol $CO_2$ mol-1) increased by 12.8%, 2.39%, and 29.2% respectively compared to the control group that was placed under the atmospheric $CO_2$ concentration of 360 ± 30 μ mol $CO_2$ mol-1, while the plant height, single leaf area and fresh weight of treatment group T2 (1000 ± 100 μ mol $CO_2$ mol-1) increased by 8.7%, 1.81%, and 27.2% respectively compared to the control group. The net photosynthetic rates of Group T1 and Group T2 measured under their increased $CO_2$ treatment conditions increased by 27.0% and 33.8% respectively compared to the control, and the net photosynthetic rates of both Group T1 and Group T2 measured under the control conditions (the increased $CO_2$ treatment condition ceased temporally during this measurement) were also higher than the control Group. Both stomatal conductance and transpiration rate of the treatment Group T1 and Group T2 decreased, but it promoted the accumulation of soluble sugars and starch in the leaves, while the chlorophyll content did not show significant changes. High concentrations of $CO_2$ could promote an increase in Rubisco activity, whereas the activity of glycolate oxidase significantly decreased [34].

**Table 3. Summary of plant physiology indicators/treatment under various environmental stress (elevated $CO_2$ concentration).**

| Indicators/Treatment of Plant Physiology | Plant Species | | Environmental stress types |
|---|---|---|---|
| | Rice (Wuyunjing 23)[33] | Red palm leaf[34] | |
| Plastochron Index (PI) | | | Elevated $CO_2$ |
| Chlorophyll content (Chl) | Select | Select | Elevated $CO_2$ |
| Net photosynthetic rate (Pn) | Select | Select | Elevated $CO_2$ |
| PS I and PS II | | | Elevated $CO_2$ |
| Stomatal conductance (Gs) | Select | Select | Elevated $CO_2$ |
| Inter-cellular $CO_2$ concentration (Ci) | | | Elevated $CO_2$ |
| Photochemical efficiency (Fv/Fm) | | | Elevated $CO_2$ |
| Upper and lower leaves | | | Elevated $CO_2$ |
| Environmental stress simulation | (ambient atmosphere +200 μ mol CO2/mol | 700 ± 100 μ mol $CO_2$ mol-1; 1000 ± 100 μ mol $CO_2$ mol-1 | Elevated $CO_2$ |
| Recovery treatment | | | Elevated $CO_2$ |
| Transpiration rate (Tr) | Select | Select | Elevated $CO_2$ |
| Carotenoid content (Car) | | | Elevated $CO_2$ |
| The initial fluorescence (Fo) | | | Elevated $CO_2$ |
| Non photochemical quenching (NPQ) | | | Elevated $CO_2$ |
| Electron transfer rate (ETR) | | | Elevated $CO_2$ |
| Antioxidants activity | Select | | Elevated $CO_2$ |
| AsA-GSH cycle | | | Elevated $CO_2$ |
| Lutein cycle | | | Elevated $CO_2$ |
| Fertilizer treatment | chemical fertilizer and 50% organic nitrogen substitution fertilizer | | Elevated $CO_2$ |
| Heavy metal content | Cu and Zn in soil and plants | | Elevated $CO_2$ |

| | | | |
|---|---|---|---|
| Rubisco enzyme | | Select | Elevated $CO_2$ |
| Glycolate oxidase | | Select | Elevated $CO_2$ |
| Soluble sugars and starch in the leaves | | Select | Elevated $CO_2$ |

**14.3.2.Discussion**

According to the rice case study results in Fig1, elevated $CO_2$ concentration increases the photosynthesis rate under the chemical fertilizer application, but reduces the photosynthesis rate under the substitution fertilizer application of 50% organic nitrogen; based on the results in Fig2, under the 50% organic fertilizer substitution treatment, the increase in atmospheric $CO_2$ concentration significantly increases POD activity so it significantly reduces MDA content, whereas chemical fertilizer treatment decreases POD activity, which increases MDA content, by the increasing $CO_2$ concentration. By comparing and contrasting the above results, my article further concludes that the application of 50% organic nitrogen substitution fertilizers effectively activates the metabolic pathways of antioxidant in plant defense physiology, whereas the application of chemical fertilizers cannot activate this antioxidant pathway, but the activation of antioxidant metabolic pathways becomes the main reason to reduce the photosynthesis rate under elevated $CO_2$ concentration. As summarized in above content of my article, various environmental stress conditions are capable of activating antioxidant pathways in plant metabolic system, including hot stress, increased atmospheric $CO_2$ concentration etc, and the activation of plant metabolic defense pathways under various environmental stress conditions will usually decrease the plant photosynthesis rate and plant growth.

Under the treatment of 50% organic fertilizer replacing chemical fertilizer in the rice case study, the increase in atmospheric $CO_2$ concentration significantly reduces the Cu content in rice stems and grains, as well as the Zn content in grains, which has been attributed to the variation in the biomass rather than the uptake of Cu and Zn by plants in Xu et al.,(2023) study. However, my article disagrees with this argument: according to the results in Fig3, under the chemical fertilizer treatment, it is shown that the increase in atmospheric $CO_2$ concentration increases the available Zn in soil by comparing CF. A with CF. F results, and the Zn concentration in grains are correspondingly increased, which means that the increased available Zn in soil leads to enhanced uptake of Zn by plant grains; In comparison, under the 50% organic fertilizer substitution treatment, the increase in atmospheric $CO_2$ concentration increases both available Cu and Zn in soil by comparing COF. A with COF. F results, but both Zn and Cu concentrations in grains are NOT correspondingly increased (both concentrations decrease apparently), which would reveal that the plant uptake capacity of both Zn and Cu in soil tends to decrease by the 50% organic fertilizer substitution treatment, and this may be attributed to the activation of the metabolic pathways of antioxidant in plant defense physiology as well.

To compare and contrast both case studies above, it can be concluded that the

photosynthesis rate of plants tends to be increased by the enhanced atmospheric $CO_2$ concentrations, but both stomatal conductance and transpiration rate usually decrease under the enhanced atmospheric $CO_2$ concentrations, and the final yield of crops would tend to benefit from the moderate stress of $CO_2$ concentrations, in terms of plant growth, nutrition accumulation and soil mineral element uptake.

It is further concluded that under increased atmospheric $CO_2$ concentrations, the metabolic antioxidant pathways may be activated when the stress conditions reach the threshold level, but the activity of carbon assimilation enzyme Rubisco tends to be enhanced in the carbon cycle so that the photosynthesis rate is increased correspondingly [33][34]. However, my article here further complements the plant metabolic defense mechanism under enhanced atmospheric $CO_2$ concentrations: from the results that increased $CO_2$ concentrations reduces both stomatal conductance and transpiration rate, it is deduced that the plant response to the stress condition of enhanced atmospheric $CO_2$ concentrations would go through the same metabolic pathways as its response to water deficit and hot stress at severe level, because plant physiology response to water deficit and hot stress at severe level also usually leads to the stomatal closure and limits the respiration function in leaves. Consequently, the reduction of respiration function (indicated by the decreased stomatal conductance and transpiration rate) becomes the reason to explain that the enzyme activity of glycolate oxidase which plays the key role in respiration function is significantly decreased, under the increased atmospheric $CO_2$ concentrations in the red palm leaf case study.

### 14.4. Plant photo-physiology to water deficit

In addition to hot stress, excessive light radiation and elevated atmospheric $CO_2$ concentrations discussed above, water deficit is also becoming the thorny issue faced by agriculture under the impacts of global climate change.

#### 14.4.1. Case studies

Two maize varieties including Zhengdan 958 (strong drought resistance) and Shaandan 902 (weak drought resistance) were selected as test materials to conduct a pot water control experiment, which simulated three drought stress levels (mild drought, moderate drought, severe drought) and normal irrigation. The effects of drought stress on the photosynthetic rate, chlorophyll fluorescence and related physiological indicators of maize leaves during the seedling stage were analyzed. The results showed that under drought stress, the net photosynthetic rate (Pn) and stomatal conductance (Gs) of the leaves of both varieties significantly decreased, while the inter-cellular $CO_2$ concentration (Ci) firstly decreased and then increased, but the stomatal limitation value (Ls) increased and then decreased. This indicated that the decrease in leaf Pn under moderate drought stress level was attributed to stomatal factors, but the decrease in Pn under severe drought stress level was mainly caused by non stomatal factors; With the intensification of drought stress level, the actual quantum yield ( $\Phi$ PS II), electron transfer rate (ETR) and photochemical quenching

(qP) of photosystem II (PS II) in the leaves of two varieties had been continuously decreasing, while non photochemical quenching (NPQ) increased firstly and then decreased, indicating that heat dissipation triggered important photo-protective mechanism in plants under moderate drought, but subsequently leaf's photosynthetic electron energy transfer was hindered and PSII was damaged under severe drought; Under drought stress, the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in the leaves of two varieties firstly increased and then decreased, while the content of malondialdehyde (MDA) continued to increase, revealing that the early stage of drought stress resulted in the induction effect on the increase of protective system enzyme activity. Under severe stress, the activity of scavenging enzymes against reactive oxygen species decreased, leading to cell membrane damage. These results advised that under mild and moderate drought stress, the two maize varieties stabilized the function of photosynthetic system by reducing the synergistic effects of light capture, heat dissipation and enzyme activity regulation, becoming the stomatal limiting factor for the decrease in Pn, while the damage to photosystem II and antioxidant enzyme system under severe drought stress was the non stomatal limiting factor to explain the decrease in Pn. The physiological parameters of Zhengdan 958 variety were less affected by drought than those of Shaandan 902, showing the drought resistance due to its high photosynthetic efficiency and strong protective enzyme activity under drought stress [35].

In the semi-arid loess hilly area, a pot water control experiment was conducted on 3-year-old seabuckthorn seedlings to measure the photosynthetic rate, chlorophyll fluorescence, antioxidant enzyme activity and other photosynthetic physiological indicators under 8 types of soil moisture gradients. The changes and mechanisms of seabuckthorn leaf photosynthesis during the gradual increase of soil drought stress levels, as well as its quantitative relationship with soil moisture, were explored. The results showed that within the range of soil's relative water content (RWC) from 38.9% to 70.5%, the net photosynthetic rate (Pn), stomatal conductance (Gs), and inter-cellular $CO_2$ concentration (Ci) of seabuckthorn all significantly decreased with the aggravation of drought stress, while the stomatal limitation value (Ls) significantly increased, indicating that the decrease in Pn was mainly attributed to stomatal limitation; When RWC was less than 38.9%, drought stress continued to intensify, and both Pn and Ls decreased with Ci significantly increased, indicating that the main reason for Pn decline had shifted to non stomatal factors; It was found that moderate soil water stress could improve the water use efficiency (WUE) of seabuckthorn leaves, and the RWC range for maintaining high levels of Pn and WUE in seabuckthorn was 58.6%-82.9% and 48.3%-70.5%, respectively; With the aggravation of soil drought stress levels, the maximum fluorescence (Fm), maximum photochemical efficiency of PSII (Fv/Fm), actual photochemical efficiency of PSII (ΦPS II), and photochemical quenching (qP) of seabuckthorn all showed the gradually decreasing trend, but the initial fluorescence (Fo) significantly increased, while non photochemical quenching (NPQ) showed the trend of firstly increasing and then decreasing; When RWC was within the range from 38.9% to 70.5%, heat

dissipation was an important photo-protective mechanism for seabuckthorn; When RWC was less than 38.9%, PSII was damaged and electron transfer was obstructed; With the aggravation of soil drought stress levels, the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in seabuckthorn leaves all showed the trend of firstly increasing and then decreasing, while the content of malondialdehyde (MDA) showed the gradually increasing trend; When the soil drought level was between 48.3% and 70.5% RWC, it induced the activity of antioxidant enzyme system in seabuckthorn leaves, but when soil drought reached severe stress (RWC<38.9%), the antioxidant enzyme system of seabuckthorn leaves was damaged, so antioxidant enzyme activity decreased and cell membranes were damaged. When the soil drought level was between 48.3% and 70.5% RWC, seabuckthorn leaves could stabilize the normal function of photosynthetic apparatus through the synergistic effect of heat dissipation and enzyme activity regulation [36].

**Table 4. Summary of plant physiology indicators/treatment under various environmental stress (Water deficit).**

| Indicators/Treatment of Plant Physiology | Plant Species | | Environmental stress types |
|---|---|---|---|
| | Two maize varieties[35] | Seabuckthorn [36] | |
| Plastochron Index (PI) | | | Water deficit |
| Chlorophyll content (Chl) | | | Water deficit |
| Net photosynthetic rate (Pn) | Select | Select | Water deficit |
| PS I and PS II | ϕPS II | ϕPS II | Water deficit |
| Stomatal conductance (Gs) | Select | Select | Water deficit |
| Stomatal limitation value (Ls) | Select | Select | |
| Inter-cellular $CO_2$ concentration (Ci) | Select | Select | Water deficit |
| Photochemical efficiency (Fv/Fm) | Select | Select | Water deficit |
| Upper and lower leaves | | | Water deficit |
| Environmental stress simulation | Mild, moderate, severe drought stress levels | 8 types of soil moisture gradients | Water deficit |
| Recovery treatment | | | Water deficit |
| Transpiration rate (Tr) | | | Water deficit |
| Carotenoid content (Car) | | | Water deficit |
| The initial fluorescence (Fo) | | Select | Water deficit |

| | | | |
|---|---|---|---|
| Non photochemical quenching (NPQ) | Select | Select | Water deficit |
| Electron transfer rate (ETR) | Select | Select | Water deficit |
| Antioxidants activity | Select | Select | Water deficit |
| AsA-GSH cycle | | | Water deficit |
| Lutein cycle | | | Water deficit |
| Fertilizer treatment | | | Water deficit |
| Heavy metal content | | | Water deficit |
| Rubisco enzyme | | | Water deficit |
| Glycolate oxidase | | | Water deficit |
| Soluble sugars and starch in the leaves | | | Water deficit |
| Water use efficiency (WUE) | | Select | Water deficit |

### 14.4.2. Discussion

In this section, to compare and contrast above case studies, it can be concluded that hot stress, excessive light radiation, elevated atmospheric $CO_2$ concentrations and water deficit stress all trigger the antioxidant metabolic pathways indicated by the activity of antioxidant enzymes such as SOD, POD or CAT. At severe level of all the above environmental stress conditions, the reactive oxygen species indicator (MDA) reaches the highest concentration, reflecting destructive effects on cell membranes as non stomatal factor limiting photosynthesis. However, the physiology responses of stomatal factors vary among these environmental stress conditions: under mild and moderate hot stress, stomatal conductance (Gs) tends to increase, which may reflect the activation of heat dispersion function, whereas stomatal conductance (Gs) continuously decreases with the increasing stress level of water deficit, excessive light radiation and elevated atmospheric $CO_2$ concentrations, which means that heat dispersion function would not be induced at mild and moderate levels of these environmental stress, unlike hot stress. Consequently, my article's conclusion disagrees with the discussion of the above two case studies which argue that heat dispersion function is activated at the mild and moderate stress levels of water deficit as one of stomatal limiting factors. In my article, the decreasing stomatal conductance (Gs) is considered as the indicator of limiting respiration function to avoid transpiration lose of water at both mild and moderate stress levels of water deficit, which is different from the heat dispersion function as its physiology response under hot stress.

## 14.5. Plant photo-physiology to salty stress

### 14.5.1. Case studies

The effects of salty stress on the photosynthesis of semi annual vermiculite potted seedlings in two tree species populations *Sophora japonica L.* and *Juglans regia L.*

under salt stress conditions (NaCl solution treatment concentrations of 50, 100, 200 m mol·$L^{-1}$) were studied. The results showed that the net photosynthetic rate of *Juglans regia L.* significantly decreased after the treatment of salt stress, and the decrease rate was becoming greater with the increasing salt concentration and prolonged stress time. After salt stress, the net photosynthetic rate of *Sophora japonica* increased, with more increase under short-term stress and smaller increase with prolonged stress. The content of chlorophyll a and b in *Sophora japonica* and *Juglans regia L.* did not change significantly under 200 mmol·$L^{-1}$ NaCl stress for 24 days, but the ratio of chlorophyll a/b value in *Juglans regia L.* decreased. The carotenoid content of *Sophora japonica* increased firstly and then returned to be the same as the control after salt stress, while that of *Juglans regia L.* decreased significantly. The main difference in $CO_2$ response curves between the two tree species was found to be that the photo-respiration rate of *Sophora japonica* decreased by 43.7% compared with the control, while that of *Juglans regia L.* increased by 71.60% compared with the control; The $CO_2$ compensation point of *Sophora japonica* decreased by 13.58% in comparison to the control, while that of *Juglans regia L.* increased by 194.15% compared with the control. The difference in light response curves between the two tree species was that the dark respiration rate of *Sophora japonica* increased by more than three times in comparison to the control, while that of *Juglans regia L.* decreased to 65.28% of the control, indicating that *Juglans regia L.* responded as significant photo-inhibition to salty stress [37].

Two plant species of *Querc us aliena var . peki ngensis* and *Q.aliena var . ac uteserr* were selected to conduct the experiment with different concentrations of NaCl, and the effects of salt stress conditions on their 2-year-old seedlings' growth, photosynthesis, and chlorophyll fluorescence characteristics was studied correspondingly. The results showed that with the increase of salt concentration, the total leaf area, fresh and dry weight of plants, net photosynthetic rate of leaves (Pn), stomatal conductance (Gs), stomatal limitation value (Ls), chlorophyll content (Chl), and quantum efficiency of photosynthetic electron transfer in photosystem II (Φ PSII) all significantly decreased, while the initial fluorescence (F0) and photochemical efficiency (Fv/Fm) of photosystem II showed no significant changes; The growth of *Querc us aliena var . peki ngensis* was less affected by salt stress than that of *Q.aliena var . ac uteserr*, in terms of relatively less reduction in net photosynthetic rate and less stress on its photosynthetic structure [38].

**Table 5. Summary of plant physiology indicators/treatment under various environmental stress (Salty stress).**

| Indicators/Treatment of Plant Physiology | Plant Species | | Environmental stress types |
|---|---|---|---|
| | *Sophora japonica L.* and *Juglans regia L.*[37] | *Querc us aliena var . peki ngensis* and *Q.aliena var . ac uteserr* [38] | |
| Plastochron Index | | | Salty stress |

| | | | |
|---|---|---|---|
| (PI) | | | |
| Chlorophyll content (Chl) | Select | Select | Salty stress |
| Net photosynthetic rate (Pn) | Select | Select | Salty stress |
| PS I and PS II | | ΦPS II | Salty stress |
| Stomatal conductance (Gs) | | Select | Salty stress |
| Stomatal limitation value (Ls) | | Select | Salty stress |
| Inter-cellular $CO_2$ concentration (Ci) | | | Salty stress |
| $CO_2$ response curves | Select | | Salty stress |
| light response curves | Select | | Salty stress |
| Photochemical efficiency (Fv/Fm) | | Select | Salty stress |
| Upper and lower leaves | | | Salty stress |
| Environmental stress simulation | NaCl solution treatment concentrations of 50, 100, 200 mmol · L -1 | 0, 0.1%, 0.2%, 0.3%, 0.4% of NaCl | Salty stress |
| Recovery treatment | | | Salty stress |
| Transpiration rate (Tr) | | | Salty stress |
| Carotenoid content (Car) | Select | | Salty stress |
| The initial fluorescence (Fo) | | Select | Salty stress |
| Non photochemical quenching (NPQ) | | | Salty stress |
| Electron transfer rate (ETR) | | Select | Salty stress |
| Antioxidants activity | | | Salty stress |
| AsA-GSH cycle | | | Salty stress |
| Lutein cycle | | | Salty stress |
| Fertilizer treatment | | | Salty stress |
| Heavy metal content | | | Salty stress |
| Rubisco enzyme | | | Salty stress |
| Glycolate oxidase | | | Salty stress |
| Soluble sugars and starch in the leaves | | | Salty stress |

| | | | |
|---|---|---|---|
| Water use efficiency (WUE) | | | Salty stress |

### 14.5.2. Discussion

Tree species of *Sophora japonica L.* yields increased concentrations on carotenoid content under the salt stress conditions, which has been explained as the protective substances in plant defense physiology under salt stress by the case study [37]. However, my article further complements this physiology response to salty stress in more details: as described in the section 2.2. of my article, the carotenoid is also one of light collecting composites as the auxiliary pigments to capture light energy, so the enhanced synthesis of carotenoid in tree species *Sophora japonica L,* which is induced by salty stress, becomes the main metabolic pathways to increase the net photosynthesis rate due to the increased light energy capturing capacity under salty stress, whereas species *Juglans regia L.* can not induce the synthesis pathway of carotenoid by the salty stress (the carotenoid content decreases under the salt stress conditions in species *Juglans regia L.*). However, there is inter-dependent compensation mechanisms among these organic functions inside plant, which has been discussed in my other articles [32][39], so as the compensation to enhanced light energy capturing capacity, tree species *Sophora japonica L.* decreases the photo-respiration rate correspondingly that is shown in $CO_2$ response curves, whereas species *Juglans regia L.* increases the photo-respiration rate as the compensation to the decreased light energy capturing capacity accordingly.

## 14.6. Plant photo-physiology to radiation stress (including UV-B)

Climate change and increased incidence of Solar Flare cause the plants of our planet to grow under various stress conditions of light radiation, commonly including UV-B radiation stress, strong light intensity stress, low light intensity stress.

### 14.6.1. Case study

My another article had discussed the plant physiology responses under both water deficit and UV-B stress by conducting an environmental stress simulation experiment in New Zealand: “Caucasian clover (*Trifolium ambiguum M. Bieb.*) and two populations of white clover (*Trifolium repens L.*) were grown for 9 weeks with supplemental application of UV-B radiation at a rate of approximately 13 kJ $m^{-2}$ $day^{-1}$. Parameters of total aerial biomass, net photosynthesis, conductance, transpiration, water use efficiency, relative chlorophyll content, water solute potential ΨW, canopy temperature were tested in this research. Drought stress was also simulated during the last four weeks. Compared with the control, the total aerial Dry Matter (DM) production across clovers decreased by 81% under drought condition. However, Caucasian and Tienshan clover showed higher drought tolerance in terms of osmotic adjustment. Under well-water condition, the total aerial biomass yield of Tienshan clover was not significantly affected by UV- B, while Kopu II was sensitive to UV-B. By the intra-specific comparison within white clover species, Tienshan clover, which showed less UV-B sensitivity and higher tolerance to drought, was less productive and

had a original habitat with multiple forms of stress. Further more, drought stress reduced UV-B sensitivity in both clover species. On the other hand, UV-B treatment also improved water-deficit tolerance across clovers by 43% under drought. In comparison, for Caucasian clover, UV-B increased the total aerial biomass yield by 84% under drought conditions. This indicated that UV- B might lead to a higher improvement of drought tolerance in Caucasian clover than in white clover. However, results also indicated that the pathways of physiological adjustments would differ between UV-B radiation and drought stress conditions [14]."

Especially, my article had explained the metabolic pathways under both water deficit and UV-B stress as: "In addition, Hofmann et al., (2003) reported that UV-B increased proline levels, which played a role in decreasing the water solute potential for osmotic adjustment under water-deficit condition, in white clover under well-water treatment [41]. However, in this experiment, UV-B did not significantly decrease the water solute potential under well-water conditions (Figure 3 and 4), which indicated that the pathways of adjusting physiological response would differ between UV-B radiation and water stress. Further more, the synthesis of solute species proline, triggered by the bio-signal of UV-B, would be more sensitive, explaining the mutual enhancement of UV-B and drought tolerance (however, this was considered as the minor mechanism as compared to the discussion below), whereas other compatible solute species involving in osmotic adjustment would be less sensitive to UV-B (UV-B even tended to eliminate the synthesis of some solute species revealed by the slight increase of water solute potential in Caucasian clover in Figure 3 and by the increased water solute potential in Tianshan clover by 27% (not listed) during well-water conditions). Nevertheless, the significantly increased water solute potential by UV-B radiation during drought conditions, which had been previously explained by the UV-B-induced changes of leaf morphology and different growth reduction rate among plant organs [41][42], would be attributed to the UV-B-induced elimination of synthesis of some solute species in cell as well, but this lead to positive effects on DM production, because it is hypothesized that the accumulation of compatible solutes during water stress condition (without UV-B stress) would be usually excessive for maintaining the cell turgor [14]."

**Table 6. Summary of plant physiology indicators/treatment under various environmental stress (UV-B radiation stress).**

| Indicators/Treatment of Plant Physiology | Plant Species | Environmental stress types |
|---|---|---|
| | Caucasian clover (*Trifolium ambiguum M. Bieb.*) and two populations of white clover (*Trifolium repens L.*) [14] | |
| Plastochron Index (PI) | | UV-B and water deficit |
| Total aerial biomass | Select | UV-B and water deficit |
| Chlorophyll content | Select | UV-B and |

| | | |
|---|---|---|
| (Chl) | | water deficit |
| Net photosynthetic rate (Pn) | Select | UV-B and water deficit |
| PS I and PS II | | UV-B and water deficit |
| Stomatal conductance (Gs) | Select | UV-B and water deficit |
| Stomatal limitation value (Ls) | | UV-B and water deficit |
| Inter-cellular $CO_2$ concentration (Ci) | | UV-B and water deficit |
| $CO_2$ response curves | | UV-B and water deficit |
| light response curves | | UV-B and water deficit |
| Photochemical efficiency (Fv/Fm) | | UV-B and water deficit |
| Upper and lower leaves | | UV-B and water deficit |
| Canopy temperature | | UV-B and water deficit |
| Environmental stress simulation | Well-water (WW): Plants were watered at a daily rate of 5% below field capacity of the soil; Drought (DR): Plants were watered when 2% above permanent point wilting occurred; UV+: Plants were exposed to UV-B radiation at a rate of approximate 13 kJ m-2 day-1 from 12 Philips UV lamps; UV-: The UV- room contained a dummy rig with un-energised lamps. | UV-B and water deficit |
| Recovery treatment | | UV-B and water deficit |
| Transpiration rate (Tr) | Select | UV-B and water deficit |
| Carotenoid content (Car) | | UV-B and water deficit |
| The initial fluorescence (Fo) | | UV-B and water deficit |
| Non photochemical quenching (NPQ) | | UV-B and water deficit |
| Electron transfer rate (ETR) | | UV-B and water deficit |
| Antioxidants activity | | UV-B and water deficit |

| | | |
|---|---|---|
| AsA-GSH cycle | | UV-B and water deficit |
| Lutein cycle | | UV-B and water deficit |
| Fertilizer treatment | | UV-B and water deficit |
| Heavy metal content | | UV-B and water deficit |
| Rubisco enzyme | | UV-B and water deficit |
| Glycolate oxidase | | UV-B and water deficit |
| Soluble sugars and starch in the leaves | | UV-B and water deficit |
| Water solute species (Proline) | Select | UV-B and water deficit |
| Water use efficiency (WUE) | Select | UV-B and water deficit |
| Water solute potential ΨW | Select | UV-B and water deficit |

Under the background of frequently global warming and extreme weather, the southern double-season rice area is prone to the stress of high temperature and strong light weather conditions during grain-filling period, but turns to be subject to low temperature and weak light weather conditions during late rice season, which restricts the yield and quality of high-quality indica rice in the southern double-season rice area. This study employed multiple rice cultivars as research materials, including Xiangzaoxian 45 (high-quality), Zhongjiazao 17 (non-high-quality), and late indica varieties Taiyou 871 (high-quality) and Tianyou Huazhan (high-quality). Through pot experiments conducted in an artificial climate chamber, there were three treatments that simulated the stress weather conditions during the early rice grain-filling stage: control sample (CK), high temperature with normal light (HT), and high temperature with strong light (HT+SL). For the late rice grain-filling stage, there were three types of environmental conditions set up: control (CK), low temperature with normal light (LT), and low temperature with weak light (LT+LL). The experiment analyzed the effects of environmental stress on rice leaf photosynthetic characteristics, grain filling dynamics, and key enzymes in carbon and nitrogen metabolism. For double-cropping early rice, environmental stress of both HT and HT+SL treatments significantly reduced net photosynthetic rate, transpiration rate, stomatal conductance, and maximum photosynthetic efficiency (PSI) as compared to CK sample. Compared with HT only, the combined stress treatment of HT+SL markedly increased transpiration rate while decreasing PSI. Grain filling dynamics showed that HT and HT+SL treatments significantly accelerated initial grain filling rates, but led to no significant impact on grain weight at both upper and lower nodes, possibly because of the

significantly reduced time to reach maximum filling rate. In summary, HT and HT+SL combined stress treatments significantly inhibited photosynthesis and grain filling in early rice during the filling process [59].

According to the enzyme activity analysis, under HT and HT+SL stress, enzyme activities of SSS (Soluble Starch Synthase), GBSS (Granule-Bound Starch Synthase) and SBE (Starch Branching Enzyme) showed a significant increase in the early filling stage but decreased markedly in the late stage. Compared to HT alone, the HT+SL combined stress treatment significantly enhanced SSS and GBSS activities in the early filling stage, while DBE (Starch Debranching Enzyme) activity showed no significant change. Under HT and HT+SL stress, GOT (Glutamic-Oxaloacetic Transaminase) activity increased significantly only in the early filling stage, but enzyme of GPT (Glutamic-Pyruvic Transaminase) activity remained consistently elevated throughout the filling period, and no significant difference was observed in GPT activity between HT alone and HT+SL combined stress treatments [59].

For double-cropping late rice, LT only and LT+LL combined stress treatments significantly reduced the net photosynthetic rate, transpiration rate, stomatal conductance, and maximum photosynthetic efficiency (MPE) of the late rice, as compared to the control (CK). Compared to the single LT stress treatment, the LT+LL composite stress treatment significantly lowered the net photosynthetic rate. Both LT and LT+LL treatments showed the tend of reducing grain weight in both upper and lower parts, with the LT+LL composite stress resulting in more severe impact due to significant decrease in the initial grain-filling rate. Under LT and LT+LL combined stress treatments, SSS, GBSS and SBE activities were significantly reduced during the early grain-filling stage, whereas GBSS activity increased markedly in the late grain-filling stage, but SBE activity decreased significantly throughout the grain-filling period. Additionally, there was no significant difference in GBSS activity between LT and LT+LL treatments, whereas SBE activity was significantly reduced under the LT+LL combined treatment compared to the single LT treatment. Both LT and LT+LL treatments led to significant decrease in GOT activity during the full grain-filling stage, while GPT activity showed an initial increase followed by a decline and there was no significant difference in GPT activity between LT and LT+LL treatments. In summary, LT and LT+LL stress treatments resulted in significant inhibitory effects on late rice photosynthesis, grain-filling and enzyme activities related to carbon and nitrogen metabolism during the grain-filling process [59].

**Table 7. Summary of plant physiology indicators/treatment under various environmental stress (radiation and temperature stress).**

| Indicators/Treatment of Plant Physiology | Plant Species | Environmental stress types |
|---|---|---|
| | Xiangzaoxian 45 (high-quality), Zhongjiazao 17 (non-high-quality), and late indica varieties Taiyou 871 (high-quality) and | |

| | | |
|---|---|---|
| | Tianyou Huazhan (high-quality)[59] | |
| Plastochron Index (PI) | | High temperature and strong radiation; low temperature and low radiation |
| Total aerial biomass | Rice grain-filling (yields only) | |
| Chlorophyll content (Chl) | | |
| Net photosynthetic rate (Pn) | Select | |
| PS I and PS II | | |
| Stomatal conductance (Gs) | Select | |
| Stomatal limitation value (Ls) | | |
| Inter-cellular $CO_2$ concentration (Ci) | Select | |
| $CO_2$ response curves | | |
| light response curves | | |
| Photochemical efficiency (Fv/Fm) | Select | |
| Upper and lower leaves | | |
| Canopy temperature | | |
| Environmental stress simulation | For the early rice grain-filling stage, high temperature with normal light (HT), and high temperature with strong light (HT+SL); For the late rice grain-filling stage, low temperature with normal light (LT), and low temperature with weak light (LT+LL). | |
| Recovery treatment | | |
| Transpiration rate (Tr) | Select | |
| Carotenoid content (Car) | | |
| The initial fluorescence (Fo) | | |
| Non photochemical quenching (NPQ) | | |
| Electron transfer rate (ETR) | | |
| Antioxidants activity | | |
| AsA-GSH cycle | | |

| | | |
|---|---|---|
| Lutein cycle | | |
| Fertilizer treatment | | |
| Heavy metal content | | |
| Rubisco enzyme | | |
| Glycolate oxidase | | |
| Soluble Starch Synthase | Select | |
| Granule-Bound Starch Synthase | Select | |
| Starch Branching Enzyme | Select | |
| Starch Debranching Enzyme | Select | |
| Glutamic-Oxaloacetic Transaminase | Select | |
| Glutamic-Pyruvic Transaminase | Select | |
| Soluble sugars and starch in the leaves | | |
| Water solute species (Proline) | | |
| Water use efficiency (WUE) | | |
| Water solute potential ΨW | | |

### 14.6.2. Discussion

The findings of this clover species case study, which reports that both UV-B stress and water deficit stress mutually enhance stress tolerance for each other, further supports the argument of my another article: "the memory of cells, in terms of identifying the bio-signals of a specific environmental factor (can be biotic or abiotic) triggering the gene expression for environmental adaptiveness or immunology, can be trained and strengthened by the biophysical simulation of other environmental factors, because multiple gene traits of environmental adaptiveness or immunology should be located in the same linkage group of genome [31]." More specifically, this mutual enhancement of stress tolerance is caused by the strengthened synthesis pathway of water solutes in cells such as proline. From the results that water solute potential in cells tends to be increased by UV-B stress treatment, rather than decreasing due to the induced synthesis pathway of proline, it is further deduced that when the product of proline synthesis pathway reaches the threshold level induced by double environmental stress conditions, the inhibitor enzyme of water solutes synthesis pathways will be activated to reduce excessive water solutes in cells as inverse reaction cycle, so that multiple cell functions can be maintained at the balanced status.

In summary of enzyme studies involving in the plant resistance under radiation stress, strong light stress usually causes excessive reactive oxygen species (ROS), so antioxidant enzyme species are chosen as common indicators to analyze the plant resistance pathways, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbic acid peroxidase (APX), glutathione peroxidase (GPX), glutathione reductase (GR), reduced glutathione (GSH) and oxidized glutathione (GSSG) [23], as well as the lutein cycle study [30]. In addition, the plant resistance against excessive radiation stress can be also improved by Si application [62]. In the case study, the various enzyme activities have been discussed with focus on the synthesis and accumulation of starch and protein, including SSS (Soluble Starch Synthase), GBSS (Granule-Bound Starch Synthase), SBE (Starch Branching Enzyme), DBE (Starch Debranching Enzyme), GOT (Glutamic-Oxaloacetic Transaminase) and GPT (Glutamic-Pyruvic Transaminase)[59]. The cellular function of glutamyl transpeptidase (GPT) is to transfer the amino group between α-amino acids and α-keto acids, and the relevant studies suggest that GPT may provide ketone acids for amino group assimilation. The enhanced activity of GPT facilitates the amino group assimilation in the glutamine synthetase/glutamate synthetase (GS/GOGAT) cycle and reduces the toxic effects of amino radicals within rice plants [60][61].

There are totally six enzyme species that are related with starch synthesis tested in case study [59], but unlike the antioxidant enzyme species, not all the starch-related enzyme species are the specific enzyme regulating the plant resistance/tolerance against stress conditions. Consequently it is to firstly select the resistance-regulated enzyme species by my article from the above six enzyme species: Among six enzyme species tested in case study, only GBSS activity increases under three types of stress conditions: hot temperature, strong light, low temperature, and GPT activity increases under both hot stress and cold stress. Consequently it is deduced that both GBSS and GPT are the specific enzyme species regulating the resistance/tolerance pathway induced by multiple stress conditions, whereas the other enzyme species are not. Based on the my hypothesis that both GPT and GBSS would play the specific role in the plant resistance induced by various stress conditions, it is to re-analyze the case study's results with emphasis on the biochemistry pathways regulating plant resistance below:

According to the results that compared to HT alone, the HT+SL combined stress treatment significantly enhances GBSS activities in the early filling stage, it is deduced that the plant resistant response under HT only or under SL only would go through the same biochemistry pathways, regulated by enzyme species GBSS, so combined stress treatment (HT+SL) would enhance the enzyme expression and correspondingly the plant stress resistance would be mutually increased, which is induced by doubly combined stress treatment in comparison to single stress condition (HT only or SL only); In comparison to the above doubly mutual enhancement of plant resistance, there is the pathway induced by single stress only: based on the results that enzyme of GPT activity is consistently elevated throughout the filling

period, and no significant difference is observed in GPT activity between HT alone and HT+SL combined stress treatments, it is revealed that enzyme expression of GPT would also play the role in plant resistance under HT stress, but this enzyme species may not be induced by strong light stress condition, so the biochemistry pathways regulated by GPT would be different between HT and SL stress conditions.

According to the results that GBSS activity increases markedly in the late grain-filling stage under LT and LT+LL stress treatments and there is no significant difference in GBSS activity between LT and LT+LL treatments, it is deduced that GBSS up-expression may play the role in stress tolerance to low temperature via the pathway of starch synthesis in the late grain-filling stage, but this enzyme species' up-regulation is not induced by low radiation stress; Compared between HT+SL and LT+LL treatments, GBSS up-regulation is activated by HT or SL stress conditions in the early grain-filling stage, whereas GBSS up-regulation is induced by LT stress condition only in the late grain-filling stage; In combination with the results that GPT activity shows an initial increase followed by a decline under both LT and LT+LL treatments and there is no significant difference in GPT activity between LT and LT+LL treatments, it is further concluded that under low temperature stress, GPT up-regulation expression enhances plant stress resistance in the early grain-filling stage and subsequently GBSS up-regulation plays the role in plant stress tolerance in the late grain-filling stage, both of which regulate the synthesis of starch accumulation in grain-filling, but low light stress condition does not trigger the up-regulation of both GPT and GBSS enzyme.

In summary, the case study [59] has only discussed each enzyme activity of six starch-synthesis-related enzyme species, without specifying which enzyme species regulate the plant resistance under various stress conditions, so my article has tried to fill in this knowledge gap as complement.

### 14.7. Plant photo-physiology to biotic stress in ecosystem

In addition to the abiotic factors of environmental stress that have been selected to discuss above, the biotic stress faced by plants in ecosystem mainly includes pathogen invasion, grazing by animals, deforesting by human activity etc. My other articles have substantially discussed the host-pathogen interactions [43][44], the biodiversity conservation, as well as the synthesis of toxicity against animals' grazing correspondingly [45], so this article will not discuss this theme in details again. The difference in plant physiology responses between abiotic and biotic stress in environment is pointed out by my another article: "Compared with the bio-signal of environmental gradient simulation, the bio-signal of biotic factor identified by cells is specific, whereas the bio-signals of abiotic factors show the patterns of 'environmental gradient' (the gene expression patterns vary gradually in response to the gradual change of environmental factors along the environmental gradient) [40]." The specificity of host-pathogen interaction is similar to the artificial medicine whose chemistry composition and dose are also specific against specific disease infection,

while the enzyme activities reported in the case studies of this article have further supported my above argument with regards to the gene expression patterns along environmental gradient.

### 14.8. Comprehensive discussion of photo-physiology under various environmental stress

From Table 1 to Table 7, net photosynthesis rate is the primary indicator to measure the efficiency of plant photosynthesis apparatus, which have been used in nearly all the case studies. Net photosynthesis rate decreases in most case studies under various environmental stress conditions, except that Pn tends to increase under moderate hot stress or under increased $CO_2$ concentration, revealing positive effects on Pn to some extent.

Stomatal conductance (Gs) have been measured in nearly all the case studies as a common indicator to reveal the stomatal factor affecting photosynthesis. It is noted that Gs decreases under most types of environmental stress conditions, except that it tends to increase under moderate hot stress, revealing the thermal dispersion function adjusted by plant stoma.

Chlorophyll content (Chl) is the primary antenna pigments capturing light radiation energy, which becomes another common indicator to assess the plant photosynthesis capacity. It is reported that Chl decreases significantly under cold stress, hot stress, salty stress, but this indicator is less sensitive to the increased $CO_2$, water deficit and UV-B radiation, which is shown according to the results of above case studies.

Auxiliary pigments of both carotenoids and lutein molecules have been also selected in above case studies, which are positively correlated with stress tolerance of plants, including cold stress tolerance, hot stress tolerance and salty stress resistance according to the above case studies.

Photochemical efficiency (Fv/Fm) is the non stomatal factor affecting the photosynthesis efficiency and it decreases significantly under severe cold stress, severe hot stress, water deficit in the above case studies.

Antioxidant enzyme species have been measured under hot stress, increased $CO_2$ concentrations and water deficit by the above case studies to reveal the metabolic pathways of plant physiology responses to environmental stress, and the activity of these enzyme species usually ascends with the increasing stress levels but decreases under severe stress conditions due to the irreversible damage impacts.

It is worthwhile noting that not all the environmental stress conditions will negatively affect the photosynthesis rate or plant yield growth: mild level hot stress or increased $CO_2$ concentration at moderate level tends to increase photosynthesis rate, and double stress conditions of both water deficit and UV-B do not more severely decrease the

plant growth, but increase the stress tolerance to each other in above case studies.

From above case studies, it is concluded that the specific enzyme species regulating the plant resistance/tolerance pathways, induced by multiple stress conditions, have antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbic acid peroxidase (APX), glutathione peroxidase (GPX), glutathione reductase (GR), reduced glutathione (GSH), oxidized glutathione (GSSG), AsA-GSH cycle, and three photo-physiology enzyme species (Rubisco enzyme, lutein cycle, and Glycolate oxidase), as well as two starch-synthesis-related enzyme species (GBSS and GPT).

**15. Environmental physiology and development on systematic metabolomics**

Cell starts its metabolic process from the genetic resource that is mainly carried by the DNA molecules. Then this genetic information undergoes transcriptions to mRNA, tRNA, rRNA, etc, which are considered as the primary metabolic molecules in cell; Subsequently various metabolic pathways are activated by cell with the synthesis of secondary metabolites (such as glutamic acid like Proline in the above case study) under the changing environmental conditions; finally these secondary metabolites are synthesized into tertiary metabolites (such as polysaccharide compounds), and in plants' study the final metabolites can be approximately estimated by the dry matter of biomass production. In the whole metabolic process, enzyme species play the major role in regulating these metabolic pathways in cell, which consequently become the optimal bio-molecules to indicate the specific metabolic process in cell with study emphasis on its molecular structure and activity of enzymology [39].

Previous botanists created and adopted metabolomics theory to systematically study cellular metabolism from the phenotype to genotype in a bottom-up way (Lecture notes instructed by Hofmann, R.W, 2007), but enzyme species was not the study emphasis of previous metabolomics studies. After this, a 3 - dimension (I × E × N) matrix was designed by myself [39], aiming to quantify the zymograms across different isozyme families among different bio-samples. After several steps of matrix transformation, this 3 - dimension (I × E × N) matrix is decomposed into three square matrix: I×I dimension; n×n dimension; e×e dimension, which provide the new tools of statistic analysis on the zymograms across different isozyme families among different bio-samples.

For the different enzyme species containing the same functional group within an isozyme family, the activation of the specific enzyme species usually corresponds to specific environmental conditions along the changing environmental gradient, which has been illustrated by my another article [44]. In the AsA-GSH cycle of above case study, the enzyme function of GR and GRX plays the role in two inverse reaction chains respectively (GR: from GSSG into GSH; GRX: from GSH into GSSG), so it is deduced that enzyme species GR and GRX belong to the same isozyme family, which will be triggered separately to maintain the balance between GSSG and GSH.

Consequently, it is concluded that the different enzyme species within an isozyme family together regulate the cyclic reaction chain in cell's metabolism. To further study the gene expression on these enzyme regulators, systematic analysis across different isozyme families are compulsory, because multiple cell functions must be activated concurrently in responses to the external environmental stress conditions. My newly designed 3 - dimension (I × E × N) matrix definitely meets the needs of systematic metabolomics study. A bio-informatics model has been developed by using C++ language to facilitate this matrix calculation process in the appendix of this article.

In the Table 1 of my another article [39], the enzyme function in plant resistance to environmental stress is summarized. After the case studies of my current article, this table can be further complemented:

**Table 8. Summary of the enzyme function in plant resistance to various environmental stress** (the complement to the Table 1 of article [39])**.**

| **Isozyme Families** | **Function in Plant Resistance to Environmental Stress** |
|---|---|
| Peroxidase (POD) | Disease Infection [47]; Elevated CO2 stress [33]; Water deficit stress [35][36] |
| Superoxide dismutase (SOD) | Radiation stress [23]; Elevated CO2 stress [33]; Water deficit stress [35][36] |
| Ascorbic acid peroxidase (APX) | Radiation stress [23] |
| Glutathione peroxidase (GPX) | Radiation stress [23] |
| Catalase (CAT) | Drought Stress; Temperature Stress (both cold and hot); Salinity Stress; Disease Infection; Ozone; Radiation Stress [48]; Water deficit stress [35][36] |
| Malate dehydrogenase (MDH) | Acid soil; Aluminum toxicity[49]; Elevated CO2 stress [33]; Water deficit stress [35][36] |
| Alcohol dehydrogenase (ADH) | Waterlogging Stress; Salinity Stress; Cold Stress; Drought Stress; Anaerobic Stress [50] |
| NAD-dependent isocitrate dehydrogenase （ICDH） | Drought Stress; Salinity Stress; Heavy Metal Stress; Anti-Oxidation [51] |
| Lactate dehyderogenase (LDH) | Heavy Metal Stress [52] |

| | |
|---|---|
| Glucose-6-phosphate dehydrogenase (G6PDH) | Anti-Oxidation; Drought Stress; Salinity Stress; Cold Stress [53] |
| Glutamate dehydrogenase (GDH) | Drought Stress; Salinity Stress; Cold Stress; Disease Infection [54] |
| Malic Enzyme (ME) | Drought Stress; Salinity Stress; Cold Stress; UV-B Radiation Stress; Physical Injury [55] |
| Beta-Amylase (BAM） | Drought Stress; Temperature Stress (both cold and hot); Salinity Stress [56] |
| AsA-GSH cycle (GR and GRX enzyme) | Hot stress [23] |
| Reduced glutathione (GSH) | Radiation stress [23] |
| Oxidized glutathione (GSSG) | Radiation stress [23] |
| Lutein cycle (VDE and ZE enzyme) | Hot stress [30][57] |
| Rubisco enzyme | Elevated $CO_2$ [34] |
| Glycolate oxidase | Elevated $CO_2$ [34] |
| GBSS (Granule-Bound Starch Synthase) | hot temperature, strong light, low temperature [59] |
| GPT (Glutamic-Pyruvic Transaminase) | hot temperature, low temperature [59] |

Additionally, the experiment methods for the analysis of 110 families of isozyme in total has been fully summarized by Nie et al., (2023) [46] with 'Public License' for re-use.

## 15.Conclusion

Plant photosynthesis reaction converts light energy into bio-energy that is considered as the primary/original bio-energy being utilizable by the advanced creatures in our planet. Because the definition of electron transfer chain in photosynthesis system is just a kind of weaker electric current' s definition, according to the new definition of electric current in my another article [58], the electron transfer chain process is re-defined as: the abiotic energy from light waves excites the free electrons inside the molecules of antenna pigments firstly, and then the excited state of free electrons

emits electromagnetic waves into other biological molecules, which leads the free electrons of other bio-molecules to be the excited state as conduction effect, achieving the energy conversion along the electron transfer chain in photosynthesis and finally becoming the biotic energy. This primary/original biotic energy is firstly utilized by plants themselves, and then herbivores eat plant biomass and convert plant biotic energy into herbivorous bio-energy, which will be finally consumed by carnivorous animals, forming the food chain and bio-energy consuming cycle in ecosystem.

According to my quantum chemistry article, "the exited process of free electrons is defined by equation: $\Delta E = E2\text{-}E1 = h\nu = hc/\lambda$, where $\Delta E$ is the energy absorption by electrons, E2 is the electron orbital energy of exited state, E1 is the electron energy at lower energy orbital, h is the Planck constant, v is the light wave frequency, c is the light speed and $\lambda$ is the light wavelength [4]." Although this equation is deduced to calculate the light absorption experiment, it is also applicable on the calculation of excited energy conversion between the molecules of antenna pigments and other bio-molecules (such as Plasmid quinone (Pq) in the non cyclic electron transfer chain or Ferroredoxin (Fd) in the cyclic electron transfer chain), which has been discussed above. In this calculation, v is not the light wave frequency, but is the frequency of electromagnetic waves emitted by the free electrons of excited state inside the molecules of antenna pigments, which will be absorbed by the Pq or Fd molecules. Consequently, this newly discussed energy conversion under excited state among bio-molecules along electron transfer chain would be the future research gaps for quantum chemistry or biophysics studies, rather than studying light wave absorption only. To study this electromagnetic wave emitted by the free electrons of excited state inside the molecules of antenna pigments, quantum resonance technology is adopted to analyze the frequency and intensity [63]. It is hypothesized that the electromagnetic waves emitted by the free electrons of excited state inside the molecules of antenna pigments is controlled by the intelligence of plant cells, revealing important life function in plant photosynthesis system, which may more accurately indicate the photosynthesis efficiency. This hypothesis is further discussed in Figure 8 below:

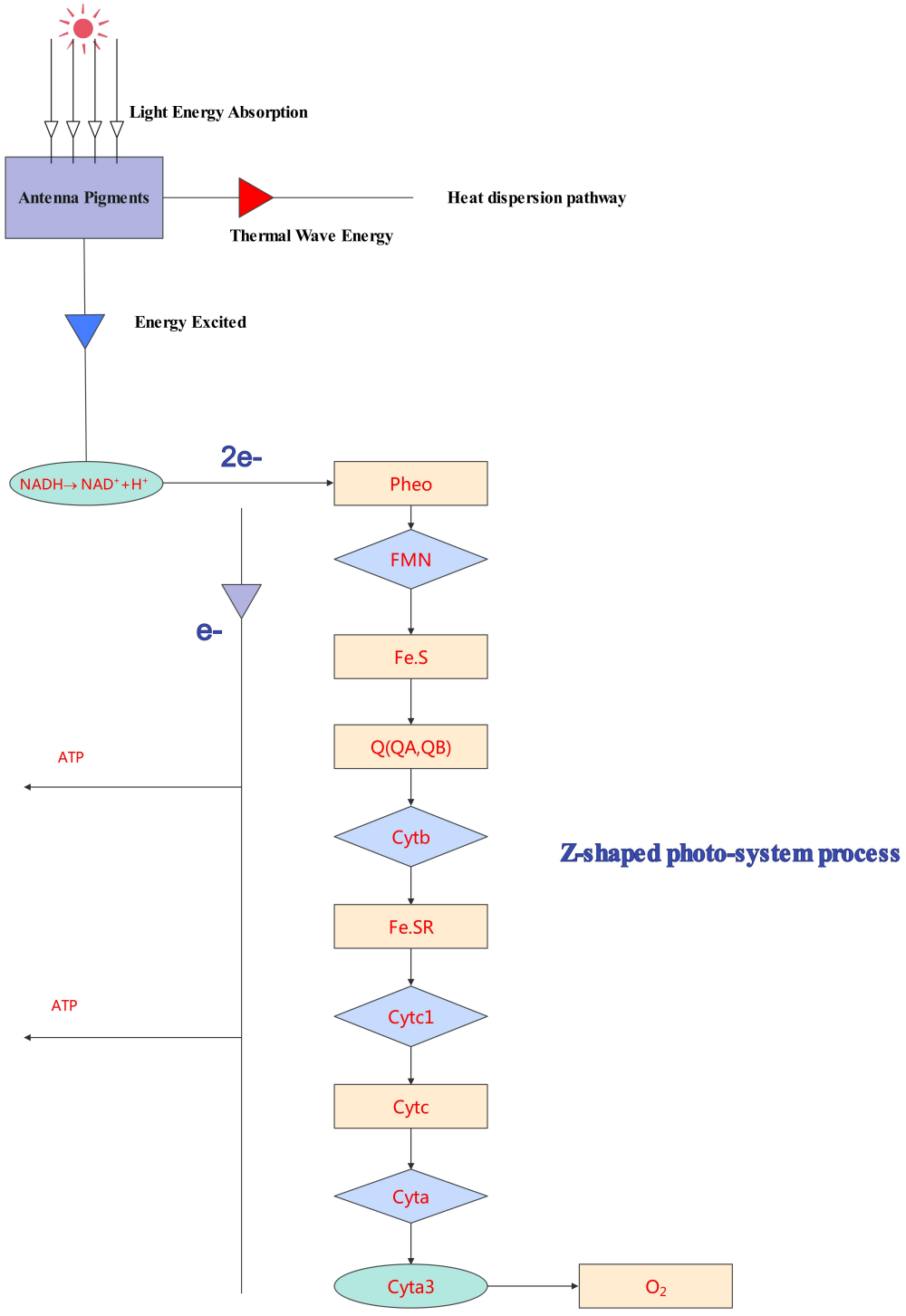


**Figure 8. Conceptional model of energy flow in photosynthesis system.**

As shown in Figure 8, light wave energy is firstly absorbed by antenna pigments, and then the absorbed energy is divided into two parts: one is the energy excited by the free electrons that pass on the energy conversion along the reaction chain of Z-shaped photo-system, and the other is thermal wave energy, generating heat inside plant cells, which subsequently enters heat dispersion pathway. Plant cell's life function must

adjust the ratio between these two parts of energy. Consequently, it is hypothesized that the measurement of the energy excited by the free electrons of antenna pigments would more accurately indicate photosynthesis efficiency, compared with light energy absorption measurement.

Plants regulates their metabolic pathways in response to various environmental stress conditions, including both abiotic and biotic factors, so that the photosynthesis system can function steadily, which ensures the survival pre-condition of other advanced creatures in our planet --- the 21% of oxygen in atmosphere. Compared with microbial kingdom, plant species (especially tree species) are required to operate their oxygen-producing photosynthesis system more steadily over life periods, even when they face environmental stress, but the oxygen-producing photosynthesis in microbial kingdom may cease temporally (turn to be dormant state) under environmental stress conditions, which can not stably produce oxygen supply for other advanced multicellular creatures' survival needs in earth ecosystem.

Consequently, the long-term changing environmental conditions enforce plant defense physiology to be the most complicated organic process with the longest evolutionary history over the development of earth planet, which have been substantially discussed in this article by selection of representative case studies under various environmental stress.

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**Appendix. Matrix algorithm and bioinformatics**

This article adopts both 2-dimensional arrays and 3-dimensional arrays to calculate the zymograms designed in my article [39], which is developed by C++ language. To facilitate the data processing, an example of 3×4×5 matrix is assumed as the input of this bioinformatics modeling.

A 3-dimensional matrix of |Xnei| is defined as: variable n is from 1 to N, and N represents the total amount of bio-sample types. Each bio-sample type is collected from different environmental conditions, and each bio-sample type yields a copy of zymogram in electrophoresis experiment. In this example, N is assumed to be 3, and matrix |X1ei|, |X2ei| and |X3ei| represents a copy of zymogram yielded by the bio-sample 1, bio-sample 2 and bio-sample 3, respectively; variable e is from 1 to E, and E refers to the total amount of isozyme families tested in this electrophoresis experiment. In this example, the electrophoresis pipe is placed horizontally, so the variable at each row represents each isozyme family and in total 4 rows represent 4 different isozyme families in each copy of zymogram; variable i is from 1 to I, and I is the total amount of enzyme species at each locus. In this example, the electrophoresis pipe is placed horizontally, so the variable at each column represents each enzyme species at the same locus and in total 5 enzyme species are found, which means that there are totally 5 columns in each copy of zymogram; If a electrophoresis band clearly appears in the electrophoresis pipe, the value is given to be 1; otherwise it is zero.

$$|X1ei| = \begin{matrix} 0 & 1 & 0 & 0 & 1 \\ 1 & 0 & 1 & 0 & 1 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 1 & 0 \end{matrix}$$

$$|X2ei| = \begin{matrix} 0 & 0 & 1 & 1 & 1 \\ 0 & 1 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 \end{matrix}$$

$$|X3ei| = \begin{matrix} 1 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 & 1 \\ 0 & 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 \end{matrix}$$

Then the above three matrices are input into the program below, and a 5×5 matrix, a 3×3 matrix and a 4×4 matrix are finally derived for the principal components analysis (PCA) to analyze variable i, variable n, variable e, respectively.

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```
#include <bits/stdc++.h>
using namespace std;
int a[101][101][101], a1[101][101], a2[101][101], a3[101][101], b1[101][101], b2[101][101], b3[101][101], c1[101][101], c2[101][101], c3[101][101], d1[101][101], d2[101][101], d3[101][101], E1[101][101], E2[101][101], E3[101][101], F1[101][101], G1[101][101], H1[101][101], I1[101][101], I2[101][101], I3[101][101];
int main ()
{
    int n,e,i,t;

    cout << "\n=== Input the values (0 or 1) for matrix X[n][e][i] (leaving a space between values) ===" << endl;
    for(n=1;n<=3;n++)
        for(e=1;e<=4;e++)
            for(i=1;i<=5;i++)
        cin>>a[n][e][i];    //input the value of matrix Xnei£¬ a[n][e][i] is zero or one ; 3*4*5 Matrix

        cout<<endl;

    for(n=1;n<=3;n++)
          for(i=1;i<=5;i++)
              b1[n][i]=a[n][1][i];    //select the value of matrix Xni, when e is 1 ; 3*5 Matrix

    for(n=1;n<=5;n++)
          for(i=1;i<=3;i++)
               b2[n][i]=b1[i][n];    // the tronspose of matrix Xni ; 5*3 Matrix

    for(n=1;n<=5;n++)
        for(i=1;i<=5;i++)
            for(t=1;t<=3;t++)
                b3[n][i]+=b2[n][t]*b1[t][i];    // the value of the transpose of matrix Xni (5*3) multiplied by matrix Xni (3*5); final value are 5*5 Matrix

    for(n=1;n<=3;n++)
          for(i=1;i<=5;i++)
              c1[n][i]=a[n][2][i];    //select the value of matrix Xni, when e is 2 ; 3*5 Matrix

    for(n=1;n<=5;n++)
```

```
        for(i=1;i<=3;i++)
              c2[n][i]=c1[i][n];    //the tronspose of matrix Xni ;    5*3 Matrix

    for(n=1;n<=5;n++)
        for(i=1;i<=5;i++)
            for(t=1;t<=3;t++)
                c3[n][i]+=c2[n][t]*c1[t][i]; // the value of the transpose of matrix Xni (5*3) multiplied by matrix Xni (3*5); final value are 5*5 Matrix

    for(n=1;n<=3;n++)
        for(i=1;i<=5;i++)
            d1[n][i]=a[n][3][i]; //select the value of matrix Xni, when e is 3 ; 3*5 Matrix

    for(n=1;n<=5;n++)
        for(i=1;i<=3;i++)
            d2[n][i]=d1[i][n]; //the tronspose of matrix Xni ; 5*3 Matrix

    for(n=1;n<=5;n++)
        for(i=1;i<=5;i++)
            for(t=1;t<=3;t++)
                d3[n][i]+=d2[n][t]*d1[t][i]; // the value of the transpose of matrix Xni (5*3) multiplied by matrix Xni (3*5); final value are 5*5 Matrix

    for(n=1;n<=3;n++)
        for(i=1;i<=5;i++)
            E1[n][i]=a[n][4][i];    //select the value of matrix Xni, when e is 4 ; 3*5 Matrix

    for(n=1;n<=5;n++)
        for(i=1;i<=3;i++)
            E2[n][i]=E1[i][n];    //the tronspose of matrix Xni ; 5*3 Matrix

    for(n=1;n<=5;n++)
        for(i=1;i<=5;i++)
            for(t=1;t<=3;t++)
                E3[n][i]+=E2[n][t]*E1[t][i];      // the value of the transpose of matrix Xni (5*3) multiplied by matrix Xni (3*5); final value are 5*5 Matrix

    cout << "\n=== The matrix (5*5) for variable i ===" << endl;
    for(n=1;n<=5;n++)
    {
        for(i=1;i<=5;i++)
        cout<<b3[n][i]+c3[n][i]+d3[n][i]+E3[n][i]<<" ";    //output the value of the
```

```
sum of matrix for PCA ; 5*5 Matrix;
            cout<<endl;
      }

             cout<<endl;

      for(n=1;n<=3;n++)
           for(i=1;i<=5;i++)
             a1[n][i]=b1[n][i]+c1[n][i]+d1[n][i]+E1[n][i]; //to calculate the value of
Matrix sum Xni ; 3*5 Matrix

      for(n=1;n<=5;n++)
           for(i=1;i<=3;i++)
                  a2[n][i]=a1[i][n];     // the transpose of Matrix sum Xni ; 5*3 Matrix

      for(n=1;n<=3;n++)
           for(i=1;i<=3;i++)
               for(t=1;t<=5;t++)
                    a3[n][i]+=a1[n][t]*a2[t][i];                //the value of Matrix sum Xni
(3*5) multiplied by its transpose (5*3); final values are 3*3 Matrix

      cout << "\n=== The matrix (3*3) for variable n ===" << endl;
      for(n=1;n<=3;n++)
      {
           for(i=1;i<=3;i++)
           cout<<a3[n][i]<<" ";    //output the value for PCA ; 3*3 Matrix;
           cout<<endl;
      }

           cout<<endl;


             return 0;
           }
```

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```
#include <bits/stdc++.h>
using namespace std;
int a1[101][101], a2[101][101], a3[101][101], b1[101][101], b2[101][101], b3[101][101];
int main ()
{
    int e,i,t;

    cout << "\n=== Input the values (0 or 1) for matrix X[1][e][i] (leaving a space between values) ===" << endl;
        for(e=1;e<=4;e++)
            for(i=1;i<=5;i++)
              cin>>a1[e][i];    //input the value of matrix Xei£¬ a1[e][i] is zero or one ; 4*5 Matrix

    cout << "\n=== Input the values (0 or 1) for matrix X[2][e][i] (leaving a space between values) ===" << endl;
        for(e=1;e<=4;e++)
            for(i=1;i<=5;i++)
              cin>>a2[e][i]; //input the value of matrix Xei£¬ a2[e][i] is zero or one ; 4*5 Matrix

    cout << "\n=== Input the values (0 or 1) for matrix X[3][e][i] (leaving a space between values) ===" << endl;
            for(e=1;e<=4;e++)
              for(i=1;i<=5;i++)
               cin>>a3[e][i]; //input the value of matrix Xei£¬ a3[e][i] is zero or one ; 4*5 Matrix

            cout<<endl;

    for(e=1;e<=4;e++)
        for(i=1;i<=5;i++)
       b1[e][i]=a1[e][i]+a2[e][i]+a3[e][i]; // the sum of Matrix Xei; 4*5 Matrix

    for(e=1;e<=5;e++)
        for(i=1;i<=4;i++)
        b2[e][i]=b1[i][e];    // the transpose of Matrix sum Xei; 5*4 Matrix

    for(e=1;e<=4;e++)
        for(i=1;i<=4;i++)
            for(t=1;t<=5;t++)
```

```
        b3[e][i]+=b1[e][t]*b2[t][i]; //the value of the Matrix sum Xei (4*5 Matrix), multiplied by its transpose (5*4 Matrix); final values are 4*4 Matrix

    cout << "\n=== The matrix (4*4) for variable e ===" << endl;
    for(e=1;e<=4;e++)
    {
        for(i=1;i<=4;i++)
        cout<<b3[e][i]<<" ";    //output the value for PCA ; 4*4 Matrix
        cout<<endl;
    }

       return 0;

    }
```