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Photosynthesis

From Its Evolution to Future Improvements in Photosynthetic Efficiency Using Nanomaterials

Edited by Juan Cristóbal García Cañedo and Gema Lorena López Lizárraga

PHOTOSYNTHESIS - FROM ITS EVOLUTION TO FUTURE IMPROVEMENTS IN PHOTOSYNTHETIC EFFICIENCY USING NANOMATERIALS

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Notice

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Meet the editors

Juan Cristóbal García Cañedo is a biochemical engineer specializing in foods, with Master's and PhD degrees in Biotechnology. He has experience in the culture of microalgae for the production of biocompounds using photobioreactors. He also has 5 years' experience in the food industry in food processing, and in agricultural companies in the areas of food safety, quality assurance, product development, production, and commercialization. Additionally, he has 10 years' experience in research with microalgae in carotenoid production and operation in fed batch mode, and photobioreactor design and scaleup. He has three years' teacher experience as well. Examples of lectures given are microbiology, mass and energy balances, process engineering, mathematics, control and instrumentation, fluid mechanics, bioprocess synthesis and analysis, design topics (ultrafiltration, chromatography, lyophilization), and has lectured in a bioseparation process workshop.
He has presented four international conferences as a speaker and published in four indexed journals and one book chapter on the production of carotenoids and lutein for visual health.

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She has presented three international conferences as a speaker and published in three indexed journals and one book chapter on the production of carotenoids and omega 3 fatty acids for human health.

Contents

Preface

Two lessons I have learned during my research career are the importance of following up unexpected observations and realizing that the most obvious interpretation of such observations can be rational but wrong. When you carry out an experiment there is usually an expectation that the result will fall within a range of predictable outcomes, and it is natural to feel pleased when this turns out to be the case. In my view this response is a mistake. What you should be hoping for is a puzzling result that was not anticipated since with persistence and luck further experiments may uncover something new.

Ellis R. J. (2005).
From chloroplasts to chaperones: how one thing led to another. Govindjee, J. T. Beatty, H. Gest and J.F. Allen (eds.):
Discoveries in Photosynthesis, pp. 745–755.

First, we would like to thank our readers and let them know how happy we are that this book has been published.

The book is a compilation of basic knowledge about photosynthesis. Section 1 deals with the basics of photosynthesis and starts with Chapter 1, which is a brief description of the evolution of photosynthesis, followed by Chapter 2, which is a concise description on the photosynthetic process.

Section 2 discusses the effects of light, nutrients, and cultivation on the photosynthetic process, giving an example in each case. In this sense, Chapter 3 looks at the effects of pulsed irradiation-based LEDs on the growth and photosynthetic light utilization efficiency of lettuce leaves. Because the most important environmental factor for plants is light, Chapter 4 discusses light-capturing pigments, using subtropical plants as an example. Chapter 5 discusses the effect of cultivation in photobioreactors, which are reactors designed for microscopic photosynthetic organisms and are also used for the cultivation of plants under special conditions. Also, salinity has a significant effect under stressful conditions, and Chapter 6 looks at the costs of high salinity tolerance in mangroves, which can tolerate hypersaline environments up to 1600–1800 mmol NaCl kg^{-1}.

Finally, in Section 3 future improvements in the production of photosynthetic organisms are described and discussed. In this sense, Chapter 7 reviews plant nanobionics and its applications for developing plants with improved photosynthetic capacity, explaining how crop productivity can be increased by engineering crop plants for tolerance against various environmental stresses and improving yield attributes, especially photosynthetic efficiency using nanomaterials.

It is important for us to say that during the production of this book the first author suffered serious health problems that led to surgery and several months of rehabilitation. Therefore, this work represents the spirit, effort, and pain of all the authors who have contributed to this excellent work.

We hope you will enjoy reading it as much as we enjoyed writing it.

To my parents Jose Luis García and Delia Cañedo
To my son and daughter
To my uncle Juan Carlos
To BioProducts NN
And for those who always had believe in me and support me

Juan Cristóbal García Cañedo and Gema Lorena López Lizárraga
Bioproductos NN Research and Development of Natural Products
Culiacán, Sinaloa, México

Section 1

Basic Concepts of Photosynthesis

Chapter 1

Introductory Chapter: Evolution of Photosynthesis

Gema Lorena López Lizárraga and
Juan Cristóbal García Cañedo

Additional information is available at the end of the chapter

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1. Evolution of photosynthetic systems

Photoautotrophy is a complex process that some eukaryotic organisms can carry out. Some bacteria, algae, and plants share this capacity to transform light and carbon dioxide into biomass. Photosynthesis requires chlorophyll and other accessory pigments. There are three evolution lines that are recognized according to the types of pigments present in chloroplasts (specialized organelle where photosynthesis is performed) of these organisms (**Figure 1**).

From the above figure,

A. blue lineage is a primary endosymbiosis in which chlorophyll a (Chl *a*) is the only type of chlorophyll present, and chloroplast has cell walls with peptidoglycans is typical of cyanobacteria;

B. in the green lineage, a primary endosymbiosis also occurred, with the difference that in this lineage, Chl *a* is associated to chlorophyll b (Chl *b*). All chlorophyceae algae belong to this group and account for more than 6000 species. From these types of algae, terrestrial plants emerged with the same trait, that is, Chl *a* associated to Chl *b*; and

C. red primary endosymbiosis lineage has only Chl *a* as the only type of chlorophyll present but with different kinds of accessory red carotenoid pigments. Most marine algae belong to this group [1].

It is believed that a second endosymbiosis event occurred and can explain the presence of additional membranes in chloroplasts. The members of the secondary red endosymbiotic event constitute a very diverse group of organisms, and the most important from the pharmaceutical point of view are diatoms (Heterokonta) and the dinoflagellate (Alveolata) [2].

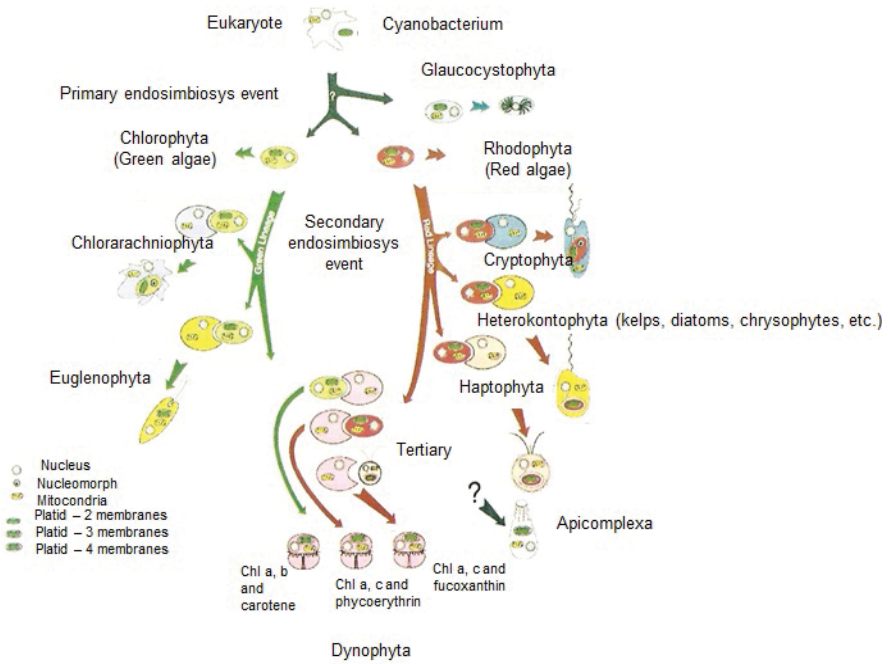

Figure 1. Schematic representation of the primary and secondary endosymbiosis events that gave origin to mayor taxonomic division of organisms that perform oxygenic photosynthesis. All terrestrial plants have their origin from a green primary endosymbiosis event. Most of the aquatic photoautotroph organisms are secondary endosymbionts with red plastids lineage [1].

What are the differences between algae and land plants? Algae are thallophyte, that means they lack of roots, leafs, stomas, and complex reproductive organs [3]. Algae do not form embryos, all reproductive structures are potentially fertile, and protective sterile cells are absent. Development of parenquimatic structures is only present in some groups and finally they exhibit sexual and nonsexual reproduction. By contrast, plants have an elevated level of differentiation like, for example, roots, leafs, vascular network xylem/phloem [4, 5], and other specific characteristics.

2. Protein structure for light harvesting and reaction centers evolved in different pathways

Proteins are encoded in the genetic code of every organism. But new genes must evolve from preexisting genes. This means that through mutations, new biological functions arise that make a protein with one enzymatic or structural function to gain a new function. Some authors have concluded that the proteins of photosynthetic systems may have had originally a photoprotective function. However, given the evolution process, different proteins have been recruited along with different pigment molecules ordered in arrays that enable efficient transfer of excitation energy into the reaction centers where charge separation takes place to fulfill the function of the photosynthetic process [6].

In this sense, reaction centers of photosystems I and II (PSI and PSII) of photosynthetic prokaryotes have similar elements and homologous structures, and therefore it is believed that photosynthetic reaction centers have evolved only once. On the contrary, light harvesting complexes are very different between taxa, share no sequence, and have very little structural similarity (**Figure 2**). This indicates that light harvesting complexes of higher plants, cyanobacteria, purple bacteria, and green sulfur bacteria evolved independently from each other [6, 7]. This means that genes of the antenna may have duplicated and

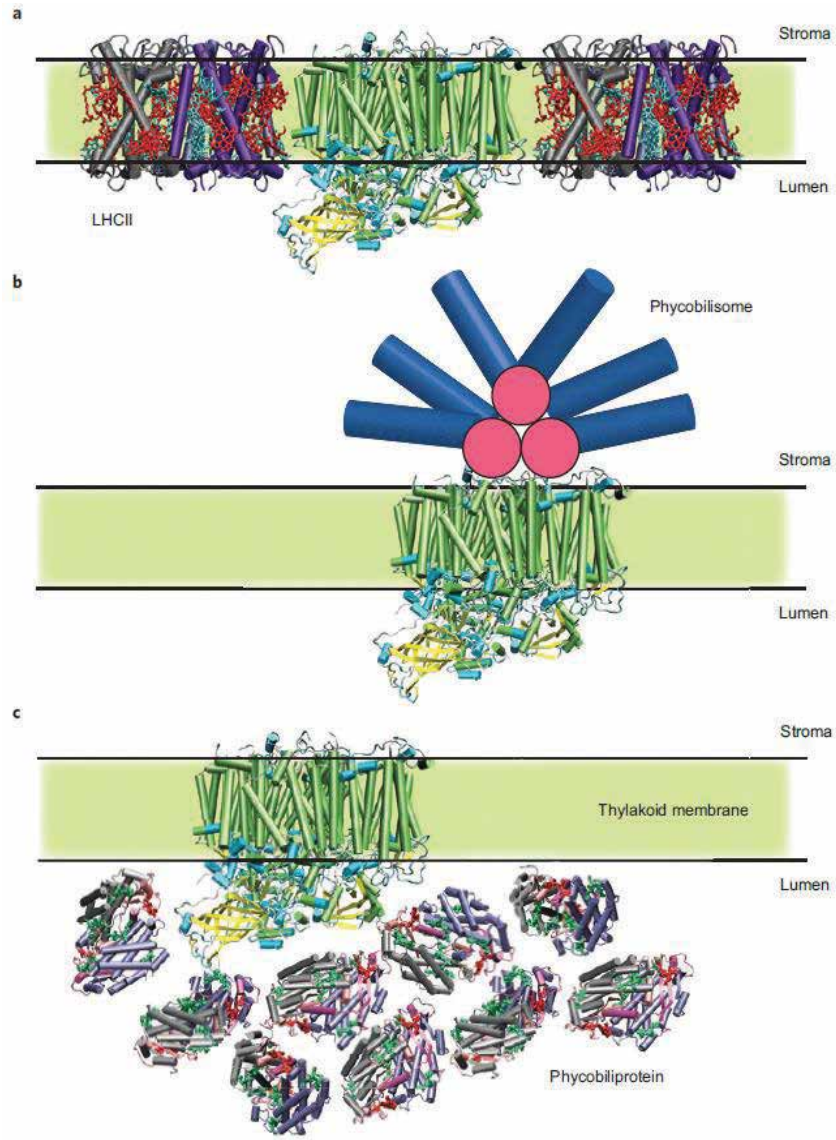

Figure 2. Representation of the reaction center and antenna structures of different taxa of photosynthetic organisms. Reaction centers have similar structures. While the organization of light harvesting antenna complexes relative to the photosynthetic membrane have very different structure. Examples shown are representative of: a, Higher plants and green algae. b, Cyanobacteria and red algae. c, Cryptophytes (Image from Scholes et al., 2011).

diverged, resulting in the multitude of antenna proteins that is present in photosynthetic organisms today. Although the evolution has been from photoprotection to light harvesting, in some branches of the evolutionary tree, there may have been a back conversion into a protective function.

Evolution of light harvesting complex proteins is considered an example of how new protein functions are created by recruitment, and if there is a selective advantage conferred with the new function, it will be fixed in the gene pool. The events creating the recent antenna structure may have took place around 300 million years ago, after which the genes have been fixed in the different organisms with slight variations with adaptive significance that we may find today [6].

Acknowledgements

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Chapter 2

Photosynthesis and Carbon Metabolism

Nimir Eltyb Ahmed Nimir and Zhou Guisheng

Additional information is available at the end of the chapter

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Abstract

Photosynthesis takes place in chloroplasts of green plants and algae and results in the conversion of radiant energy into chemical energy. Water and carbon dioxide are the raw materials; plants can produce sugars by using chlorophyll and light energy. During the first reaction of photosynthesis, ATP and NADPH are produced from light energy. Oxygen and hydrogen are released from water broken during the light reaction. In the dark reaction, CO_2 is converted into glucose by consuming energy that comes from first step of photosynthesis.

Keywords: ATP, NADPH, CO_2, light reactions, dark reactions

1. Light energy

The organism's life on the world depends on photosynthesis process, so it is considered as the essential process. Photosynthesis is defined as the reduction of carbon dioxide into carbohydrate by the green plants utilizing light energy. Diverse types of biological molecules are created directly or indirectly from the photosynthesis products. During respiration process, the organic materials release energy to be used for metabolic processes. The only natural process that can provide oxygen for living organisms is the photosynthesis process [1].

In higher plants, leaves are the organs in which photosynthesis process occurs. Biologists concentrate on organ structure and their biochemical and physiological function. A good example of organ function is plants leaves. Some of the plant leaves alter their shape for distinct purposes, but still their main function is food synthesis. Leaves trend to maximize their area and change their angles to insure to receive enough light. Leaf observed as photosynthetic engine implements photosynthesis efficiently in stress condition [2].

Photosynthesis occurs not only in eukaryotic organisms such as green plants and green algae but also in prokaryotic organisms such as cyanobacteria and certain groups of bacteria. In higher plants and green algae, photosynthesis reactions occur in the chloroplast, which acts as a thermodynamic machine. The chloroplast tricks the radiant energy of sunlight and saves some of it in a stable chemical energy. The reactions that accomplish these energy transformations are identified as the light-dependent reactions or the first stage of photosynthesis. Energy generated by the light-dependent reactions is subsequently used to reduce inorganic CO_2 to organic carbon in the form of sugars. Both the carbon and the energy conserved in these sugars are then used to build the order and structure that distinguishes living organisms from their inorganic surroundings (Duysens et al., 1961).

1.1. Leaf structure

In higher plant, leaf structure especially the upper site, is more suitable to receive much sunlight. However, the lower site reflects and scatters light. Cell arrangement in side leaf is also more important beside leaf morphology. Leaves in dicotyledonous plants have two epidermises: upper and lower covered by cuticle. Mesophyll tissues, which contain the photosynthetic tissues, are found between the two epidermal layers. Generally, there are 1–3 palisade mesophyll cells in upper photosynthetic tissue. Palisade cells are special cells in shape. In lower site, there are spongy mesophyll cells, which have isodiametric shape. Leaf in monocotyledonous is similar to leaf in dicotyledonous, but there are no special mesophyll cells.

Generally, there is more number of chloroplasts in palisade cells, which have high concentration of chlorophyll than spongy mesophyll cells. The presence of more chloroplast does not mean having higher photosynthesis rate, which occurs in the upper part of the leaf. Dicotyledonous leaves have more chloroplasts in their palisade; however, a little cell volume can have more chloroplasts. Much light will pass through the first cell layer without being absorbed because the pigments get combined to the chloroplast.

Light absorption efficiency may be affected by factors that change the light direction. The surface of leaf cell may reflect off the light. If a pigment absorbs light energy, one of three things will occur: first, energy is dissipated as heat. Second, the energy may be emitted immediately as a longer wavelength, a phenomenon known as fluorescence. Third, energy may trigger a chemical reaction, as in photosynthesis. Grana and mitochondria structure in chloroplasts have dimensions parallel to active wavelength in photosynthesis. Light is scattered by grana and mitochondria. Deflection, reflection and scattering increase the effect of light on leaf. Longer light wave increases the number of photons absorbed by leaves [3].

The light absorbed by palisade cell is not much as projected as it is thought and has lower light reduction efficiency. This may be due to the palisade that acts as a light guide. Some of the incident light goes through the intercellular spaces between the palisade cells in much the same way as the light is transmitted by an optical fiber. It is likely that photosynthesis in the highest layer of palisade is light saturated. Any excess of light would be wasteful and lead to photoinhibition. Thus, the increased diffusion of light to the lower cell layers resulting in both scattering light and the light-guide impact would no doubt be advantageous by contributing

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to a more efficient allocation of photosynthetic energy throughout the leaf. Different plants have different leaves. Due to environmental situations, leaves may make some changes for adaptation. Light interception capacity has been compromised in favor of a reduced surface-to-volume ratio, a modification that helps to combat dehydration when exposed to dry air. Leaves become more thicker to store more water in desert land area. In extreme cases, such as the cacti, the leaves have been reduced to spikes and the stem has taken over the double functions of water storage and photosynthesis. Within the leaf mesophyll cells of plants, the chloroplast is the organelle that transforms light energy into ATP and NADPH to convert CO_2 to sugars. ATP is synthesized by chemiosmosis, whereas NADPH is the product of coupled electron transfer reactions in the chloroplast thylakoid membranes. The enzymatic reaction involved in the conversion of CO_2 to sugars takes place in the chloroplast stroma [4].

1.2. Light reactions stage

There are two photosystems in light reactions, photosystems I and II, in the thylakoid membranes. Each photosystem has a complex of numerous chlorophyll and carotenoid molecules (known as light-harvesting antennae), which is associated with membrane proteins. Innumerable units of these photosystems are arranged on the thylakoid membranes in the chloroplast. When light attacks a pigment molecule in each photosystem, the energy is channeled into a reaction center, which consists of chlorophyll, a molecule bound to a membrane protein. In photosystem I, the reaction center is called P700, in the red region of the spectrum, which indicates the wavelength of maximum absorption of light; the center of reaction for photosystem II is P680, again indicating the peak absorbance. Some enzymes and coenzymes, which are associated with photosystem, act as electron carries in thylakoid membranes. Energy is formed in P700 when one photon of light attacks one molecule of pigment in photosystem I. An electron is excited and ejected when P700 absorbs energy leaving P700 in an oxidized state. The emitted electron will be picked by a primary electron acceptor, and then electron will be moved to ferredoxin and finally to NADP+ reducing it to NADPH +H, which are the first products of the light reactions. In photosystem II, another photon of light will be absorbed by a chlorophyll molecule that will transfer its energy to the reaction center P680 (Beardsley and Tim [5]). When P680 absorbs this energy, an excited electron is ejected and passed on to another primary electron acceptor, leaving P680 in an oxidized state. Electron from water will compensate the electron, which is lost by P680, and this reaction is still not clear and is catalyzed by an enzyme on the thylakoid membrane that requires manganese atoms, and water molecules in this reaction will split into H and O_2; the source of both electrons and protons is hydrogen. The electron from primary electron acceptor will push through the other electron carriers that include plastoquinone (Pq), cytochrome complex, and plastocyanin (Pc). The electron is finally passed in photosystem I to oxidize P700. ATP is created during the transfer of electrons, from the thylakoid lumen into the stroma by an ATP synthase in the membrane. The mechanism of ATP production through protons passage remains unclear. The whole process is drives by sunlight energy as showing in Photosystem II to Photosystem I. Water is continually replenished from the electrons that are lost from P680. In noncyclic photophosphorylation, the reduction of NADP is the final step from one-way electron transfers. Cyclic way of electron transfer occurs just in photosystem I. The electrons, instead of

being passed to NADP from ferredoxin, may be passed to the cytochrome complex and then back to P700. Through this process, ATP may be generated, which is known as cyclic photophosphorylation, and electrons' flow begins and ends with P700. The only constant supply of oxygen gas in atmosphere is photosynthesis through diffuses out of the leaves into the atmosphere. The current 20% oxygen content in the atmosphere is the result of 3.5 billion years of photosynthesis. Cyanobacteria are the first photosynthetic organisms that began to accumulate oxygen, in ancient time, when there was no oxygen. Now most of the living organisms depend on oxygen for cellular respiration, and therefore, it is the energy that maintains life. The flow of electrons and moves from water to NADPH is powered by sunlight energy. The result of the light reactions is ATP and NADPH that are needed to drive the biochemical reactions or dark reaction in the Calvin cycle [6].

2. The Calvin cycle (dark reactions)

The atmosphere considers carbon dioxide as the main source, which produces sugar through photosynthesis process. Carbon dioxide occupies approximately 0.035% of earth's atmosphere, which enters the leaf by diffusing through the stomata. Carbon fixation reactions, which reduces CO_2 to the sugar, are sometimes known as Calvin cycle or C3 pathway. These reactions do not use light directly but utilize the ATP and NADPH produced through the light reactions sometimes referred as light-independent reactions or dark reactions. The dark reactions take place in stroma of chloroplast where many reactions were catalyzed by enzymes. This pathway was worked out by Melvin Calvin, in association with Andrew Benson and James Bassham, during the late 1940s and early 1950s. The pathway is named in honor of Calvin, who received a Nobel Prize for his work in 1961. The following discussion will be limited to the main events of the Calvin cycle. The end product of this pathway is the synthesis of a six-carbon sugar, which requires the input of carbon dioxide. To produce single molecule of a six-carbon sugar, it needs to join six molecules of CO_2 by six turns of the cycle. The first process is the cooperation of CO_2 with ribulose-1, 5-bisphosphate (RUBP), a five-carbon sugar with two phosphate groups. The enzyme ribulose bisphosphate carboxylase (RUBISCO) catalyzes carboxylation reaction. The most abundant protein on the Earth is RUBISCO (12.5–25% of total leaf protein). Carboxylation produces an unstable six-carbon transitional that immediately splits into two molecules of a three-carbon compound, with one phosphate group known as phosphoglyceric acid (PGA), or phosphoglycerate. In total, 12 molecules of PGA is yielded from six turns of the cycle, and these molecules of phosphoglyceraldehyde (PGAL) come from the conversion of about 12 PGA molecules. 12 NADPH and 12 ATP are required for this step, which supply the energy for this reaction. Six molecules of RUBP were regenerated from 10 of the 12 glyceraldehyde phosphate molecules in a complex series of interconversions that require six more ATP and allow the cycle to continue. Two molecules of glyceraldehyde phosphate are the net gain from six turns of the Calvin cycle; these are converted into one molecule of fructose-1, 6-bisphosphate, which is converted to glucose. The glucose produced is converted into starch, sucrose, or a variety of other products, thus completing the conversion of solar energy into chemical energy. All steps of photosynthesis process can be concluded in simple

equation, which reflects raw materials and end products of the process. Many plants utilize various carbon fixation cycles that consist of a prefixation of CO_2 before the Calvin cycle. Prefixation occurs in two pathways, C4 and the CAM pathways. Many tropical and subtropical plants, such as maize, sorghum and sugarcane, have the C4 pathway. This pathway takes place in mesophyll cells to fix carbon dioxide into organic acids with a four-carbon compound, so-called C4 pathway; then, this compound is broken down to release carbon dioxide in cells surrounding the vascular bundle. More efficient transfer of CO_2 in C4 pathway and greater photosynthetic rates under conditions of high light intensity, low CO_2 concentrations and high temperature. Crassulacean acid metabolism (CAM) pathway has the same step that functions in a number of plants of desert environments, cacti and succulents. This pathway was initially described among members of the plant family Crassulaceae. CAM plants are unusual in that their stomata are closed during the daytime but open at night. Thus, they fix CO_2 during the nighttime hours, incorporating it into four-carbon organic acids. During the daylight hours, these compounds are broken down to release CO_2 to continue on into the Calvin cycle. This different pathway allows carbon fixation to occur at night when transpiration rates are very low, an obvious advantage in hot, dry desert environments [7].

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Section 2

Effects of Light, Nutrients and Cultivation

Chapter 3

Growth and Photosynthesis under Pulsed Lighting

Michio Kanechi

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75519

Abstract

The effects of pulsed irradiation based-LEDs on the growth and photosynthetic light utilization efficiency of lettuce leaves were studied. Plants were grown under different pulse-cycled irradiations of 0.5–500 Hz, and 1–20 kHz frequencies, at PPFD of 200 $\mu molm^{-2}\ s^{-1}$ with 50% duty ratio (illuminated duration/cycle). The photosynthetic rate (Pn) was maintained relatively constant over the range of measurements at pulsed light at 80 PPFD. At 200 PPFD, Pn gradually decreased by lowering frequency below 2.5 Hz of pulsed light. Pn under pulsed light was slightly higher than that under continuous light. Chlorophyll fluorescence (Fv/Fm, Fv′/Fm′, qP) showed no significant difference between under pulsed light and continuous light except at the lowest frequency (0.2 Hz). The similar quantum yield (ØPSII) and electron transport rate (ETR) of PSII were obtained in a wide range of frequency of pulsed light, which might be an effective illumination strategy for cultivating leaf lettuce by using LEDs. Flashing irradiation did not significantly change chlorophyll content. Results suggested the effectiveness of pulsed light at 50% duty ratio on the growth of leafy vegetables that were richly cultivated in a closed type plant factory with the possibility of saving electricity by using intermittent illumination system with LEDs.

Keywords: chlorophyll fluorescence, CO_2 uptake rate, frequency, lightfleck, quantum yield

1. Introduction

Plants or leaves in their natural state are frequently subjected to large and rapid fluctuations in irradiance. Photosynthetic performance under fluctuating irradiation (pulsed light or irregular sunflecks in forest floor, **Figure 1**) is different from steady-state photosynthesis under continuous or nonfluctuating irradiation at constant light intensity [1]. For example, poplar leaves receive 15% of their light in flecks lasting between 0 and 200 ms and a further 35% in flecks

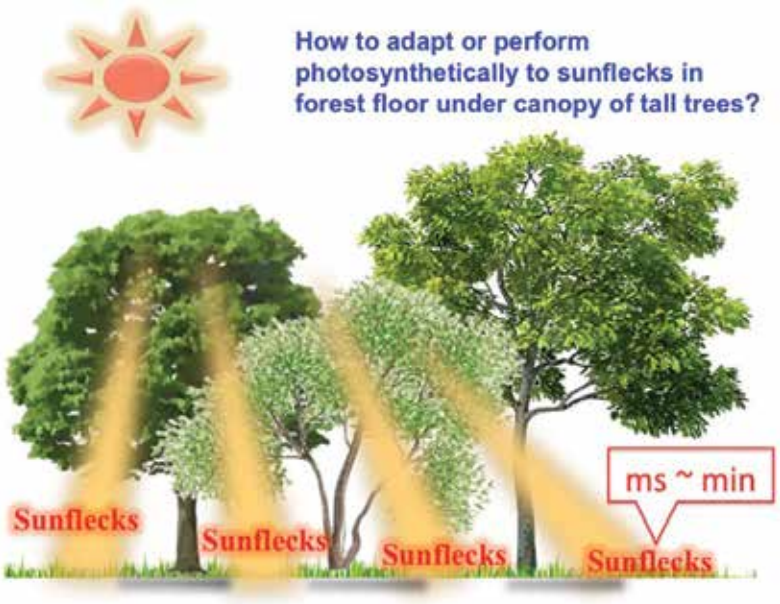

Figure 1. Pulsed light or irregular sunflecks in forest floor.

between 200 and 400 ms [2]. Photosynthesis consists of light reaction and dark reaction as a continuous reaction in this order. The former, which is a chain redox reaction of photosystem II (PSII) and I (PSI), works as light energy harvesting and producing utilizable chemical energy products in the later CO_2 assimilate reaction cycle, which reacts only enzymatically and light independently if adequate amount of these chemical energy products are supplied. The complex web of reactions in photosynthesis have different response times, so that fluxes through some reactions can be much faster than others resulting in fluctuating pool sizes. Furthermore, each reaction process seems to occur very rapidly in nanosecond to millisecond rates in the light reaction [3] as compared to seconds to minute rates in the dark reaction [4]. In recent years, photosynthetic responses to intermittent irradiation have been investigated again by using a developed illumination system with light-emitting diodes (LEDs). Measurement techniques also have a range in response times so that different reactions can be monitored with different temporal resolution.

Most of plant factory systems for producing leafy vegetables have adopted tubular cool-white fluorescent lamps as their light source. Recently, advances in LEDs technology have contributed to grow plants as a new type of light source. For further developing and improving plant factory system, LEDs illumination systems provide a potential alternative to the tubular fluorescent lamps due to their lower energy consumption, wavelength specificity and supposed durability.

Plant growth and development are strongly affected by light intensity (PPFD), quality (wavelength), and duration (photoperiod). The photosynthetic system including chlorophyll content, stomata size and leaf area of lettuce leaves was optimized by adjusting the light spectrum (455, 660, 735 nm) and flux density with high-power LEDs [5]. Light quality was

found critical not only to growth but also to biosynthesis of secondary metabolites in lettuce plants and especially the supplemental irradiation of green LEDs with the combination of red and blue LEDs can improve the growth [6]. Radiation mixture of blue, red, or far-red light with LEDs improved vegetable growth and enhanced the number of floral buds of ornamentals under controlled environmental conditions [7].

LEDs illumination system can blink or flash with a very short period, in which they can be turned fully on and fully off extremely rapidly (μs interval), emitting pulsed light with high intensity. Pulse light by adjusting the frequency and duty ratio (light on period per frequency) of LEDs resulted in optimal growth of potato plantlets *in vitro* with electricity savings and an effective illumination system adjusting light intensity, quality, frequency and duty ratio was developed [8–10]. In the pulsed light technique for growing tomato plants, low frequencies (0.1, 1, 10 Hz) had higher quantum efficiency in PSII than higher frequencies (50, 100 kHz) and continuous light, but the electron transport rate decreased when the frequency of pulse increased [11]. On the other hand, pulsed light of lower duty ratios, combined with lower frequencies, makes the CO_2 uptake rate of cos lettuce lower than that attained in continuous light, inferring that pulsed illumination with such a condition is less advantageous than continuous light for photosynthesis [12].

The objectives of this study were to determine the effects of pulsed light with various frequencies of LEDs illumination system on the growth of leaf lettuce under controlled environmental conditions (PPFD, 200 μmol m^{-2} s^{-1}), and to investigate the leaf photosynthetic responses to pulsed light in comparison with continuous light (PPFD, 0–500 μmol m^{-2} s^{-1}). The study was aimed to provide valuable information regarding the possibility of electricity savings in running plant factory system.

2. Measurements of CO_2 assimilation rates and chlorophyll fluorescence of intact leaves

Leaf lettuce (*Lactuca sativa L. crispa* 'Bio Saradana', Nakahara Seed Product Co., Ltd., Fukuoka, Japan) seeds were sown on watered sponge blocks (10 × 10 × 20 mm). After germination, 20 seedlings that had grown uniformly with three leaves were each transplanted into a polyvinyl pot (90 mm diameter, 80 mm depth) filled with vermiculite and grown for 30 days by bottom irrigation with commercial liquid fertilizer (OAT Agrio Co., Ltd., Tokyo, Japan, EC 1.3 mS cm^{-1}, pH 6.0). The air temperature and relative humidity throughout the cultivation were maintained at 22°C and 60%, respectively. The photosynthetic photon flux density (PPFD) was 200 μmol m^{-2} s^{-1} supplied by LEDs lamps (Legu LED, HRD Co., Ltd. Tootori, Japan) providing a peak wavelength of red (660 nm) and blue (455 nm) with a 16-h day length (**Figure 2**). Fourteen irradiation treatments were examined to determine the effect of wide-range frequencies of pulsed lighting (20, 10, 4, 2, 1.3, 1 kHz, and 500, 50, 5, 2.5, 1.3, 1, 0.5 Hz at 50% duty ratio) and continuous lighting. After measurements of leaf photosynthetic parameters at the end of culture period, all plants were sampled and fresh weights of leaves and roots and total leaf area were recorded.

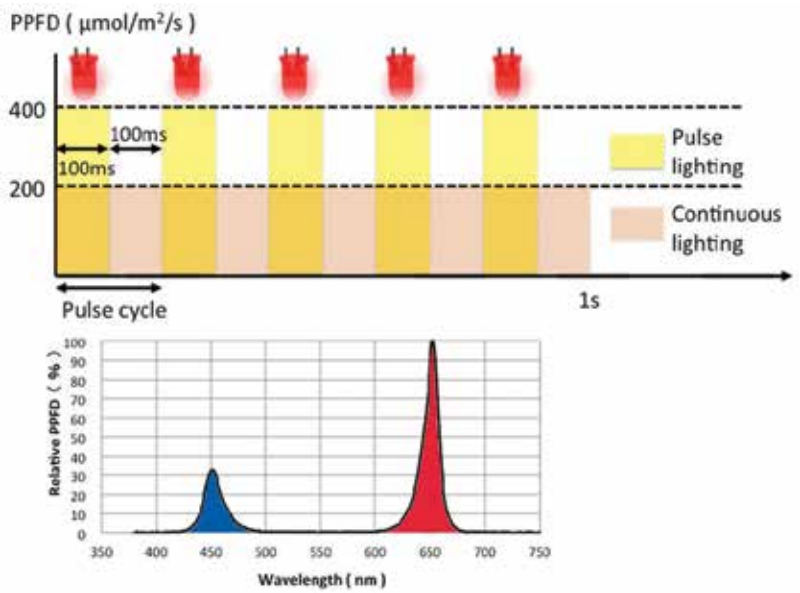

Figure 2. Pulsed lighting by using LEDs under growth conditions. Flashing pulse cycles were 20, 10, 4, 2, 1.3, 1 kHz, and 500, 50, 5, 2.5, 1.3, 1, 0.5 Hz, respectively, with 50% duty ratio (light on period: light off period = 1:1) and 200 μmol m^{-2} s^{-1} effective PPFD at the top canopy of plants. As an example, 5 Hz pulsed or continuous lighting scheme during 1 s is shown in **Figure 2a** by using a LEDs lighting system which irradiates two narrow peak wavelengths of red (660 nm) and blue (455 nm) with 4:1 of light intensity (**Figure 2b**).

The CO_2 uptake rates of a fully expanded mature single attached leaf were measured under varied PPFD and pulsed light conditions supplied by a same type of red-blue LEDs light source used for growing plants at constant leaf temperature, 22°C; leaf vapor pressure deficit, 1.0 kPa; and ambient CO_2 concentration, 400 μmol mol^{-1} using an LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA). The apparent quantum yield (Ø) was estimated as a parameter of the best-fitted non-rectangular hyperbola for the photosynthetic responses to PPFD. Chlorophyll fluorescence has a more rapid response than CO_2 uptakes, which enable photochemical and non-photochemical quenching to be measured during each flash. This can reveal the extent to which the transthylakoid ΔpH gradient builds up with irradiance in continuous and flashing light. For each light treatment, parameters of chlorophyll fluorescence from a fully expanded mature single attached leaf were measured by using a FluorPen FP 100 (Photon Systems Instruments, Drasov, Czech Republic): maximum quantum yield (Fv/Fm) in a dark-adapted leaf, effective quantum yield (Fv′/Fm′) in an actinic light-adapted leaf with each pulse cycle.

3. Plant growth under pulsed light

The growth of lettuce leaves was affected by pulsed illumination as light source for hydroponic cultivation under controlled environmental conditions (**Figures 3, 4**). Shoot fresh weight

and total leaf area increased significantly by up to 20% at high frequencies (20–1.3 kHz) compared to low frequencies (500–0.5 Hz) and compared to continuous illumination. Meanwhile, pulse illumination had little effect on root growth because of its underground development without direct exposure to pulsed light. Leaf thickness was thinner under pulsed light, but the dependence on the frequency was not consistent. The growth estimated by total leaf area per plant of *Arabidopsis thaliana* was previously reported to increase by pulsed light with a frequency at 2.5 kHz and 45% duty cycle compared to continuous light under an average intensity of 20 μmol m^{-2} s^{-1} supplied by LEDs [13]. It was explained that the growth increased

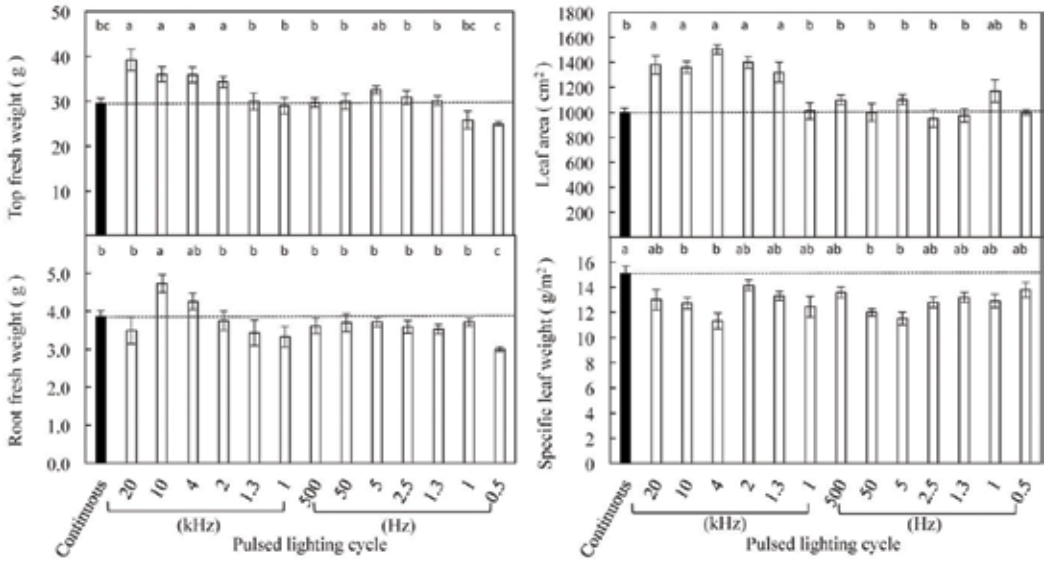

Figure 3. Growth characteristics (top and root fresh weights, total leaf area and specific leaf weight) of plants grown under continuous lighting (black column) and different pulsed lighting (empty columns) conditions at 200 μmol m^{-2} s^{-1} for 20 days. Different lowercase letters represent significant differences ($P \leq 0.05$) among lighting treatments and bars on column represent ± SE (n = 10).

Figure 4. Leaf lettuce hydroponically grown under different pulsed lighting for 20 days.

at higher maximum light intensity of pulsed light than that of continuous light. The growth of lettuce increased by 23% at a pulse frequency of 2.5 kHz (50% duty ratio) under an average intensity of 50 μmol m^{-2} s^{-1} supplied by LEDs [14].

4. Photosynthetic capacity measured at pulsed light

The photosynthetic rates (Pn) of mature leaves grown under continuous light were measured at pulsed light with various frequencies and at continuous light with two intensities (80 and 200 μmol m^{-2} s^{-1}). There were no significant differences between pulsed light with high frequencies (20 kHz–50 Hz) and continuous light, but Pn measured at low frequencies (2.5–0.1 Hz) gradually decreased to 75% of Pn measured at continuous light. Pn showed no difference between pulse and continuous measuring light when it was measured at low light intensity (80 μmol m^{-2} s^{-1}) (**Figure 5**). Pn measured at 80 μmol m^{-2} s^{-1} showed no difference between leaves grown under pulsed light and leaves grown under continuous light, but leaves grown under pulsed light showed no decrease in Pn at 200 μmol m^{-2} s^{-1}. On the other hand, leaves grown under continuous light significantly decreased compared to pulse-irradiated leaves at lower frequencies than 2 Hz. Declined rates of Pn might be due to low scattering light in thicker leaves grown under continuous light compared than leaves grown under pulsed light as shown in **Figure 3**. For Pn-PPFD response curve (**Figure 6**) measured with leaves unfolded under continuous light, light-saturated Pn decreased by lowering pulse frequency of measuring light, resulting in low light saturation. The apparent quantum yield (Ø)

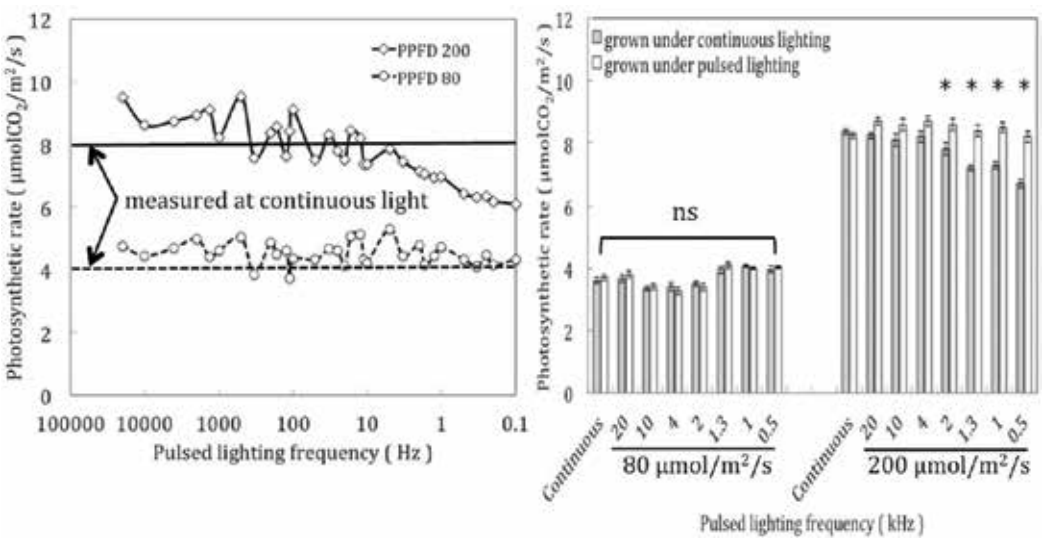

Figure 5. Photosynthetic rates of leaves grown under continuous light (left, broken curve) and under continuous or pulsed light (right, bar graph). PPFD during growing period was 200 μmol m^{-2} s^{-1} with or without pulsed lighting. Measurement conditions of photosynthesis were 80 and 200 μmol m^{-2} s^{-1} PPFD with continuous and with different frequencies of pulse lighting under 400 μmol mol^{-1} CO_2. * and ns show the significant and non-significant differences between two growth conditions.

seemed to be higher at high frequency of pulsed measuring light. The quantum yield is well understood, and the maximum rate can be related to the electron transport capacity of the leaf. It may still influence the Pn-PPFD response via the transthylakoid ΔpH gradient, which slows down electron transport by restricting plastoquinone reoxidation.

The maximum PSII quantum yield (Fv/Fm) showed no difference in a wide range of pulse frequency (10 kHz–0.5 Hz) except for a significant decrease at 0.2 Hz (**Figure 7**), indicating that the function of PSII component might be affected by exposing leaves to slow flash light during their development. The effective PSII quantum yield induced in pulse light (Fv′/Fm′) also decreased at only 0.2 Hz, indicating that there is no relationship between Fv′/Fm′ and high frequency of pulsed light over 0.5 Hz. Similar tendencies were shown in qP and in calculated parameters (ØPSII, ETR). Decreases in these parameters indicate lowered efficiency of light energy utilization by the plant grown under pulsed light at low frequency (0.2 Hz). On the other hand, NPQ, which relates the distribution of light energy into non-photochemical processes by heat dissipation, increased at low frequencies below 1 Hz compared to NPQ measured at high frequencies above 5 Hz and at continuous light (**Figure 8**).

In leaves of tomato plants grown under pulsed light based-LEDs at a wide range of frequencies (100 kHz–0.1 Hz) with 50% duty cycle, the frequencies had both positive and negative

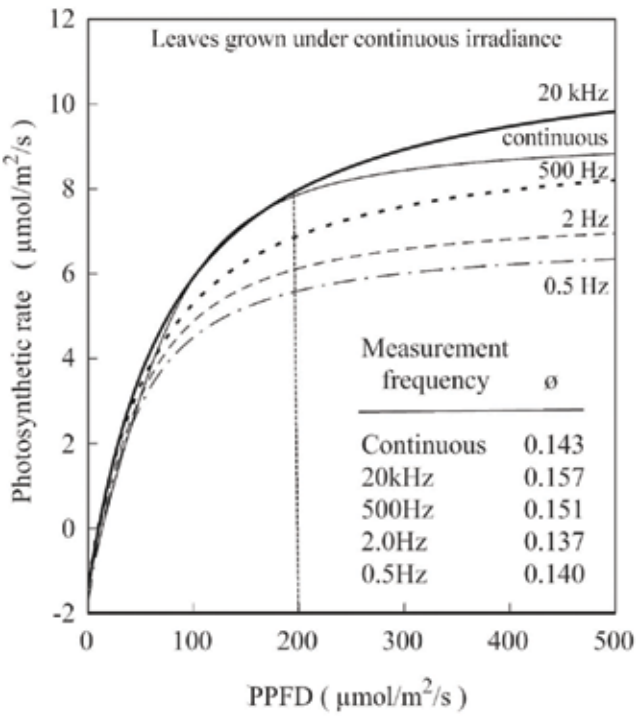

Figure 6. Relationships between photosynthetic rates and PPFD during measurements under different pulsed lighting cycles or continuous lighting of leaves grown under non-pulsed lighting conditions. Ø indicates the apparent quantum yield (the initial slope of response curve). The dotted vertical line shows the growth PPFD (200 μmol m^{-2} s^{-1}).

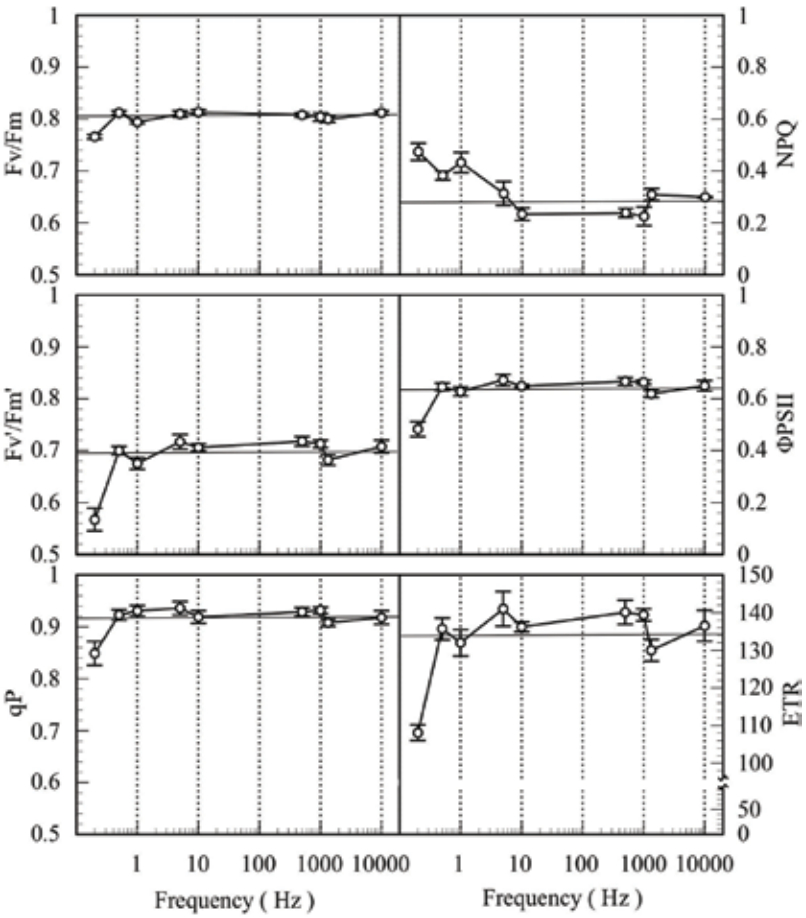

Figure 7. Chlorophyll fluorescent parameters of leaves grown under non-pulsed continuous lighting (horizontal line) and under different pulsed lighting cycles (○). Fv/Fm and Fv′/Fm′ were measured with dark-adapted or exposing leaves to 500 μmol m^{-2} s^{-1} PPFD (saturated irradiance with or without pulse cycles) to estimate photochemical (qP) and non-photochemical (NPQ) quenching.

effects on the chlorophyll fluorescence parameters. Relative to continuous light, the pulse frequencies at 0.1, 1, 100 Hz and 1 kHz were reported to be optimal for growth, productivity and energy consumption [15]. However, earlier report suggested that tomato plants could use intermittent light (in kHz frequencies) as effectively as they use continuous light [16]. Flashing light has been useful as an experimental technique to extract additional information from photosynthesis measurements. The complex web of reactions in photosynthesis have different response times, so that fluxes through some reactions can be much faster than others resulting in fluctuating pool sizes. Furthermore, each reaction process seems to occur very rapidly in nanosecond to millisecond rates in the light reaction compared to seconds to minute rates in the dark reaction (**Figure 9**). When the light is delivered in pulse short enough to only allow a single turnover of PSII, the absorbed quanta are used with maximal efficiency until they

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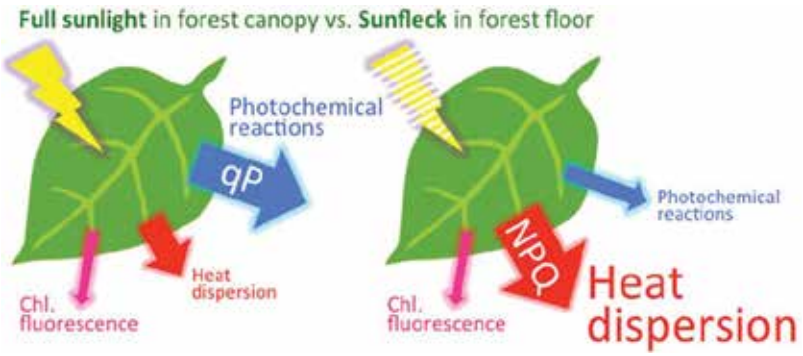

Figure 8. Photochemical reactions of photosynthesis of leaf lettuce are affected by exposing leaves to slow flash pulsed strong lighting (<1 Hz). An instantaneous response of PS by enhancing heat dissipation from leaves exposed to natural sunfleck in forest floor where plants are protected against photoinhibition.

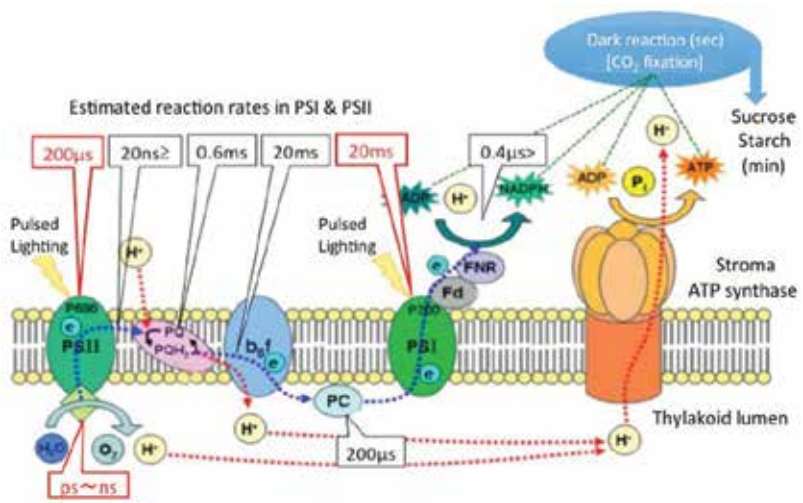

Figure 9. Photochemical reactions of photosynthesis are connected by an electron transport chain (ETC) between Photosystem I (PSI) and II (PSII), and ATP synthase embedded in thylakoid membrane in chloroplast. ETC composes of PSII reaction center (P680), plastquinone (PQ), cytochrome b_6f complex (b_6f), plastocyanin (PC), PSI reaction center (P700), ferredoxin (Fd), ferredoxin-NADP$^+$ reductase (FNR). Each estimated electron transfer rate is an example from Refs. [3, 4].

exceed the surface density of PSII [16]. Providing brighter flashes does not result in any additional photochemistry because the reaction centers do not reopen during the lifetime of the quanta in the pigment array. Consequently, it is possible to quantify the number of functional PSII reaction centers per unit leaf area in intact leaves [17]. With longer pulses of light, photochemical efficiency is reduced because a proportion of reaction centers are closed. However, photochemical efficiency expressed per open reaction center is still maximally efficient. Once a transthylakoid ΔpH gradient develops, photochemical efficiency of open centers begins to decline [18].

The photosynthetic responses to sudden changes in light conditions (lightflecks) were studied and the CO_2 uptake rate found to be maintained at a certain level for several seconds after a sudden decrease in light intensity [4, 1]. When photosynthetic rates have been measured with flashing light, considerable rate increases have been observed at high irradiances in the absence [19] or presence [20] of continuous background light. This has been attributed to post-illumination CO_2 fixation consuming the pools of RuBP [21] as well as triose phosphate that requires some extra ATP synthesis from the ΔpH gradient to convert it to RuBP [22]. If the time between flashes is sufficiently long, the ΔpH gradient is unable to build up as it is dissipated by the post-illumination CO_2 fixation's demand for ATP synthesis. This means that electron transport can occur more rapidly during the pulses of light than in continuous light because plastoquinone reoxidation is not restricted by the ΔpH gradient. If a sufficiently large ΔpH gradient exists at high irradiances even with high CO_2 partial pressures in leaf, then one would expect that flashing light could further enhance the photosynthetic rate. Stitt [23] observed a 30% increase in the rate at 1500 μmol m^{-2} s^{-1} with spinach when long flashing cycles (10s) were used in an oxygen electrode with 5% CO_2, the leaf was kept for 10 s at each light condition. Roden and Pearcy [2] found that the efficiency of post-illumination CO_2 fixation only declined once the intervening dark period exceeded about 1 s. Kriedemann et al. [19] also showed that fluctuating light with 200 ms dark intervening periods enhanced photosynthesis. Electron transport during the flash reduces NADPH and builds up the pool of RuBP and triose phosphate. These pools, termed assimilatory power by Laisk et al. [21] enable post-illumination CO_2 fixation to occur and were equivalent to 5 s of photosynthesis in sunflower leaves at 100–200 μmol m^{-2} s^{-1} PPFD. Sharkey et al. [22] showed the rapid buildup and consumption of these pools during and after lightflecks. For *Phaseolus* leaves grown in sunlight, the RuBP pool was 5 μmol m^{-2} and the total post-illumination CO_2 fixation was 12 μmol m^{-2} when triose phosphate was included. The latter requires additional ATP synthesis that comes from the proton pool stored in the thylakoid lumen [22]. Steady-state pool sizes of 100 μmol m^{-2} for RuBP have been measured in *Raphanus* leaves [24], which are certainly adequate to cope with the maximum of 5 μmol m^{-2} observed here per flash. The balance between Rubisco activity and electron transport rate is effectively increased by the ratio of intervening time to flash length up to the limit set by the pool sizes of RuBP and triose phosphate. Therefore, in flashing light, the dependence of electron transport rate on CO_2 should be small. Thus, photosynthetic intermediates (PIs) were quickly produced by photochemical reactions during lightflecks and consumed thereafter in the CO_2 fixation occurred during next dark or dim light period. An actual estimation of PIs content is difficult under pulsed light, especially at high frequencies. A kinetic model to estimate Pn was developed by considering that photosynthetic intermediates were pooled during light periods and then consumed by partial photosynthetic reactions during dark periods [25]. According to this model, they quantitatively estimated the effects of pulsed light frequency and duty ratio on photosynthetic rates of cos lettuce leaves. The estimated Pn was lower, especially under pulsed light at lower frequencies and did not exceed Pn under continuous light. Accordingly, they concluded that, compared with a constant PPFD, fluctuation in PPFD can theoretically be disadvantageous to photosynthesis, even though the time-averaged PPFD are identical. In this study, lettuce leaves grown under pulsed light at low frequencies (2–0.5 Hz) maintained higher Pn compared to

leaves grown under continuous light when they were measured under pulsed light. It is suggested that photosynthetic adaptations to intermittent radiation might have occurred during leaf development, and that the adapted leaves can more efficiently use flashing light.

5. Conclusions

Pulsed light at high frequencies (2–20 kHz, 50% duty ratio, 200 μmol m^{-2} s^{-1}) positively affected the growth of lettuce leaves under controlled environment. The photosynthetic performances showed differences between leaves developed under pulsed light and leaves developed under continuous light, when the CO_2 uptake rates and chlorophyll fluorescence parameters were measured at lower frequencies (<2 Hz). In the pulsed light technique, it is important to determine both optimal frequency and duty ratio for plants to attain the most efficient use of harvested light. The reason why growth was enhanced under pulsed light at high frequencies has not been resolved by analyzing photosynthetic performances in this study. Further research is required for detecting the pool size of PIs in leaves during their exposure to intermittent radiation. We propose that the pulsed lighting technique by using LEDs could become a useful for the production of leafy vegetables controlled plant factory systems in the near future.

Abbreviations

photosynthetic photon flux density (PPFD)

photosystem I & II (PSI & PSII)

maximum quantum yield of PSII in dark-adapted state (Fv/Fm)

effective quantum yield of PSII induced in light (Fv′/Fm′)

non-photochemical quenching by heat dissipation (NPQ)

photochemical quenching as an estimate of open PSII reaction centers (qP)

quantum efficiency of PSII (ØPSII)

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Chapter 4

Photosynthetic Pigments of Subtropical Plants

Oksana Belous, Kristine Klemeshova and
Valentina Malyarovskaya

Additional information is available at the end of the chapter

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Abstract

This chapter concerns the assessment of environmental and varietal affects on photosynthetic pigment groups. It is revealed that the dynamics of pigment accumulation in subtropical plants within the conditions of Russia's damp subtropics is a complex process and depends on species plants. With this in mind, hazelnut maximum green pigments were achieved in May, and then in August, there was a decline associated with the onset of short periods of drought and increased temperatures. In Actinidia and tea plants, the highest content of green pigments is achieved in August, which is associated with the biology of these crops. However, regardless of species, with the onset of the dry period (July–August), plants increase their accumulation of carotenoids. The high content of carotenoids that accounts for stress during a period of water availability is evidence of their participation in the formation mechanism of subtropical plants' resistance to adverse conditions.

Keywords: subtropical crops, dynamics, pigments, abiotic factors, plasticity

1. Introduction

The most important environmental factor for plants is light, which is the main source of energy for photosynthesis and a regulator of all aspects of vital activity of the plant organism [1–3]. To allow light to influence the plant body and in particular its use in the process of photosynthesis, it needs to be absorbent to photoreceptor-treated pigments. Generally, higher plants have three groups of pigments: carotenoids, chlorophylls, and phycobilins. The main pigments involved in the absorption of light quanta during photosynthesis are the chlorophylls, the pigments that contain the Mg–porphyrin complex. Currently, there are about 10 types of chlorophyll, differing in chemical structure and absorption spectra (higher plants

from 350 to 700 nm and bacteria from 350 to 900 nm). All higher plants contain chlorophyll *a* and *b* [4, 5].

In addition, there are two more bacteriochlorophylls contained in the cells of photosynthetic bacteria: chlorophyll found in diatom algae and chlorophyll *d* in red algae. Yellowing or chlorosis of the leaves is a result of their inability to increase or maintain the content of chlorophyll. Numerous studies have found that this phenomenon depends on a number of internal and external factors (genetic characteristics, temperature, water regime, mineral nutrition, etc.) [6–9].

Along with the green pigments in the chloroplasts, chromatophores contain pigments belonging to the group of carotenoids. Carotenoids represent the yellow and orange pigments of aliphatic structures, derivatives of isoprene. Carotenoids are contained in all higher plants and many microorganisms. These are the most common pigments with various functions, the main ones being: (1) participation in the absorption of light as accessory pigments and (2) to protect the chlorophyll molecules from photooxidation, which is irreversible. Perhaps the carotenoids take part in oxygen exchange in photosynthesis [7, 10].

The biggest differences in the content of photosynthetic pigments are due to the location where plants are growing. Quantitative content and qualitative composition of pigments and the change of their ratio in the leaves are all important characteristics of the physiological condition of the plants and their photosynthetic apparatus, including the orientation of adaptive responses when exposed to stressful conditions. However, the current data found in the scientific literature concern the pigment system of plants within different botanical/geographical zones, in particular a few pigment apparatus of the plants in the subtropical zone that are growing in Russia. At the same time, in the early stages of ontogenesis of the plants of the subtropical zone, it was necessary to adapt simultaneously to two stress factors: high insolation and changes in hydrothermal (particularly hydrological) conditions during the course of the year. The question is: how does adaptation relate primarily to the photosynthetic apparatus?

Thus, since the total amount of photosynthetic pigments varies considerably depending on the locus of the plants and dynamics of their accumulation, which are affected by many factors, we investigated the content of photosynthetic pigments in leaves within a number of subtropical crops grown in the conditions of Russia's damp subtropics. This chapter presents the results of studies of the pigment apparatus of leaves within subtropical plants such as tea (*Camellia sinensis* L.), Actinidia sweet or kiwifruit (*Actinidia deliciosa* Chevalier), and hazelnut (*Corylus pontica* C. Koch), which have an important agronomic value in the subtropics of Russia, as well as decorative subtropical shrubs such as hydrangea large leaf (*Hydrangea macrophylla* [Thunb.] Ser.) and weigela (*Weigela* × *wagneri* L. H. Bailey) of interest in the field of landscape design and urban landscaping.

2. Materials and methods

2.1. Objects

This chapter reflects research that was conducted at the Russian Research Institute of Floriculture and Subtropical Crops, Laboratory of Biotechnology, Physiology and Biochemistry of Plants in 1989–2016.

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Within the research the following objects were used:

1. Tea (*C. sinensis* L.) plants of the local population and varieties "Colchida", "Sochi", "Caratum"
2. Kiwi, Actinidia sweet (*A. deliciosa* Chevalier): varieties Hayward, Monty, Allison, Bruno;
3. Hazelnut (*C. pontica* C. Koch): varieties Chercesskiy-2, President, Futkurami, Lombard red;
4. Hydrangea large leaf (*H. macrophylla* [Thunb.] Ser.): varieties Bichon, Madame Maurice Hamard, Mariesii Perfecta, Draps Wonder, as well as new varieties Madame Faustin, Altona, Admiration, General Patton, Jogosaki;
5. Weigela (*W.* × *wagneri* L. H. Bailey): varieties Augusta, Kosteriana Variegata, Eva Rathke, Mont Blanc, Arlequin, Gustave Malet.

2.2. Methods

The contents of photosynthetic pigments were determined once a month in the extract of green leaves by the method of Shlyk [11]. The allocation of chlorophylls and carotenoids used acetone in the solvent. The density of the extract on a spectrophotometer was measured at the wavelengths corresponding to the absorption ima of chlorophyll *a* (662 nm) and *b* (664 nm) in the red region of the spectrum and at the wavelength of maximum absorption of carotenoids (440.5 nm). When calculating the number of pigments, calculations using Ziegler and Egle formulas were used.

- To calculate the concentration of chlorophyll *a*: C*a* = 11.7E662–2.09E664
- To calculate the concentration of chlorophyll *b*: C*b* = 21.19E664–4.56E662
- To calculate carotid concentration: C*car* = 4.695E440.5–0.268

The sample volume for the studied crops—35–60 pieces with each option—and the selection of laboratory tests to complete the assessment should be carried out with the exposure of the bush (i.e., with four sides).

In varietal evaluation work of photosynthetic pigments, leaf selection is carried out with three to four bushes of each variety, taking into account the principle of selection mentioned above.

The program STATGRAPHICS Centurion XV and mathematical software package MS Excel 7.0 were applied during the processing of research materials and evaluation of experimental results. The following parameters using ANOVA statistics were used: coefficients of data variation,construction of the correlation and regression matrix, and estimation of least statistical difference (LSD [$P \leq 0.05$]).

3. Pigment apparatus of subtropical plants

In tandem with considering the assessment methods of environmental and biological potential within plants, methods based on various physiological parameters were used [8, 12–14].

For example, during complex research of numerous subtropical crops (tea, kiwi, hazelnuts, tangerines, hydrangea, weigela, etc.) the stability of pigment apparatus parameters was proposed [14–22].We show dynamics of accumulation within photosynthetic pigments in all the studied cultures and reveal the dependence of this process on the main factors in the region.

Whereas pigment composition within the plants is extremely labile and depends on many factors for each preculture, it was necessary to set the indicator bodies, taking into account the age of the plant, physiological maturity of the diagnosed organ, its location, etc [23].

As an organ indicator to determine the pigment composition of tea plants, it is necessary to use physiologically mature leaves, which are the first to second leaves, located after the so-called "fish" leaf on the escape of growing season this year's. The "fish" leaf is very different from the normally developed leaves and is a good guide to the selection of samples [24]. In the determination of photosynthetic pigments in the leaves of the kiwi culture the existence of tiers in plants and the location of leaves on the inflorescences and fruits should be acknowledged. According to the results of the conducted research we recommend to use the leaves from the middle layer, preferably further away from the fruit [25]. In the study of the pigment complex of hazelnuts, we proposed to use physiologically mature leaves located on the middle tier escape [26]. For the large-leaved plants hydrangea and weigela it is necessary to use the physiologically formed third to fourth leaves from the apical bud or from the top of the escape [21, 27].

Comparing the pigment composition of different cultures used in our studies, we have concluded that the greatest number of green groups were found in the pigments inherent in the leaves of hazelnut (2.40 mg/g) and tea (2.05 mg/g), while the least amount of chlorophyll was observed in the leaves of hydrangea (1.01 mg/g), which is a characteristic feature of these cultures (**Table 1**). At the same time, hazelnut and tea have lesser amounts of carotenoids (0.49–0.52 mg/g) compared to other studied crops.

3.1. Pigment apparatus of tea (*Camellia sinensis* L.)

In a lengthy study of the pigment complex within tea plants it was not only the regularities of the pigment content and pigment complex dynamics that are dependent on leaf age and plant variety but it was also observed that these findings are somewhat different to the literature data regarding the patterns of accumulation in the photosynthetic pigments group [24].

In a comparative study of the pigment apparatus of sprouts and physiologically mature tea leaves, it was found that the nature of accumulation of chlorophylls and carotenoids in

Culture	Sum (*a* + *b*)	Amount of carotene	*a*/*b*	*a* + *b*/carotene
Tea	2.05 ± 0.05	0.52 ± 0.01	2.04 ± 0.02	4.02 ± 0.09
Kiwi	1.61 ± 0.03	0.76 ± 0.02	1.65 ± 0.03	2.09 ± 0.01
Hazelnut	2.40 ± 0.05	0.49 ± 0.02	1.38 ± 0.05	5.57 ± 0.09
Hydrangea	1.01 ± 0.03	0.78 ± 0.01	1.57 ± 0.03	2.09 ± 0.02
Weigela	1.32 ± 0.04	0.83 ± 0.02	2.23 ± 0.08	2.37 ± 0.02

Table 1. The content of photosynthetic pigments in leaves of studied plants (mg/g).

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sprouts was practically unchanged during the growing season, because the photosynthetic apparatus of leaves is very sensitive to any changes in growing conditions (**Figures 1** and **2**).

The content of pigments in tea leaves is 2.0–3.8 times higher than their number in sprouts. Therefore, for diagnostic purposes in the parameters of the pigment apparatus we stopped at physiologically mature tea leaves.

In tea leaves during the growing season, there was a significant accumulation of green photosynthetic pigments (**Figure 3**).

Moreover, most leaf growth is achieved in August, followed by a slight decline of chlorophyll synthesis. Such quantitative changes in pigments are associated with the biology of the tea bush. It is known that as aging leaves, and this is the second half of the growing season, there is a reduction of spending spare substances for the formation of sprouts. During this period in tea plant there is an attenuation of growth after the active growing season, which is in May. In the third week of June, sprout growth resumes, but is less active than in May, which accounts for about 50% of the entire collection of tea. Green pigments strenuously accumulate in the lamina and this results in a substantial increase in their number. From September there

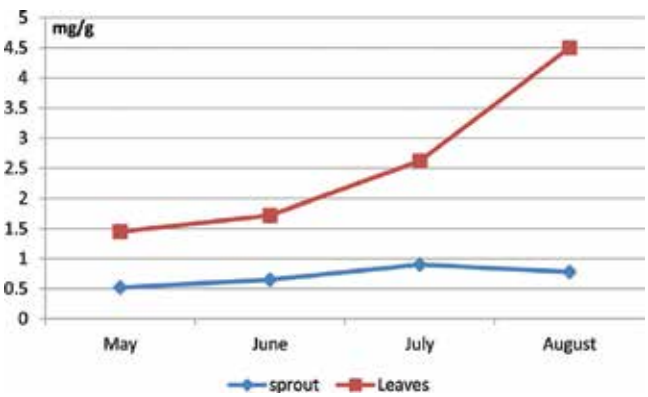

Figure 1. Comparative content of chlorophyll in sprout and tea leaves, average over 3 years.

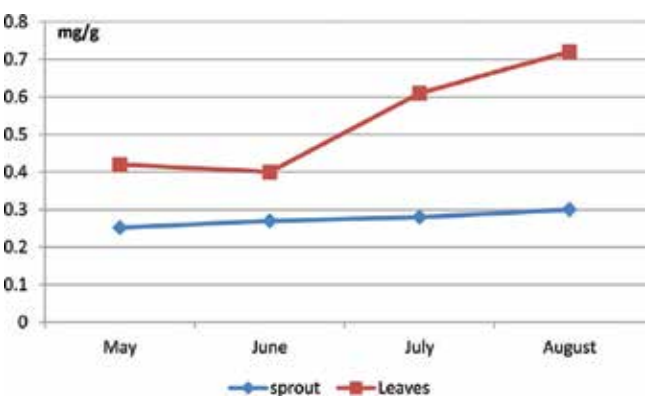

Figure 2. Comparative carotenoid content in sprout and tea leaves, average over 3 years.

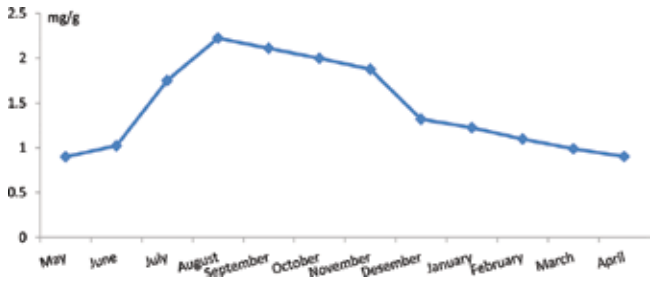

Figure 3. Dynamics of accumulation of chlorophyll (*a* + *b*) in tea leaves, average over 12 years.

is a significant decline in the content of chlorophyll, which continues until the beginning of the new growing season, due to a decrease in the synthesis of green pigments in the winter. As the leaf ages there is a further loss of chlorophyll, as a consequence of activation of the enzyme responsible for degradation. This process continues until April. As is known, tea is an evergreen plant and the lifetime of the leaf is about a year, which contributes to the creation of cosmetic substances and provides full formation of the sprouts in May.

A somewhat different picture is observed in the dynamics of accumulation of carotenoids in the leaves (**Figure 4**). So, the first increase in carotenoids was observed in July–August. This is due to the onset of the dry period, followed by increasing temperature to 30°C and sometimes more, reduced atmospheric humidity of 50–60%, which is more stressful for the tea plant than lack of soil moisture, and increased solar insolation.

A similar increase in the number of carotenoids up to 0.732 mg/g can be observed in winter. It is known that this group of pigments performs a protective role in defense reactions of the plant organism, therefore enhanced accumulation of carotenoids in adverse conditions within the vegetation plant are needed to promote adaptive responses and reduce overall stress.

As studies have revealed, regularities are common to all tea plants [24].In addition, it is found that the characteristics of the culture in a dense planting affect the prevalence of a particular

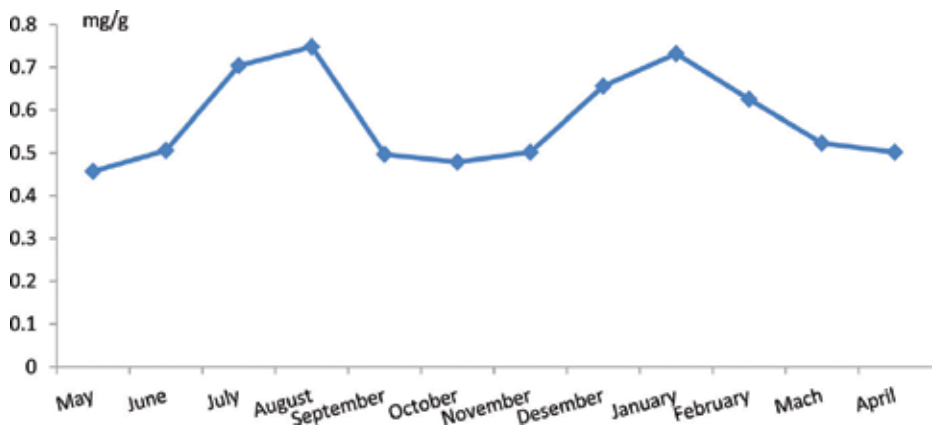

Figure 4. Accumulation dynamics of carotenoids in tea leaves, average over 12 years.

chlorophyll group. For example, the content of chlorophyll *b* indicates the level of adaptation of plants to low light. For a culture in general this is not very important, because it is grown in open spaces and trellis pruning stimulates the growth of leaves on its upper part. However, tightly restricted insular trellis open to the sun may mean that many lateral leaves are in the shade. In this case, the high content of chlorophyll *b* is located in the edges of the plant leaves, preferably for photosynthetic activities [7].

In addition, we identified that the state of the pigment system of tea has an effect on the varietal characteristics of plants and growing conditions (**Table 2**). Significant accumulation of chlorophyll *a* in the leaves is typical for the varieties Caratum and Sochi. The cultivar Colchida contains the least chlorophyll. Chlorophyll *b* accumulated more in varieties Caratum and Sochi, and less in Colchida; and the differences are significant. As is known, not only are the contents of a particular pigment important, but also their ratio because the ratio *a*/*b* can be judged on the predominance of plant I or II to the photosystem. In all the studied tea plants, the ratio *a*/*b* is in the range from 1.75 to 2.50 mg/g, indicating the predominance of photosystem II.

The ratio of total chlorophylls to carotenoids is a more informative sign, because it indicates the degree of adaptation within plants to light and to adverse conditions. A smaller ratio means higher resistance of the varieties. For this indicator, the varieties Keemun and Sochi were allocated, which are quite stable. Within the leaves of given varieties, the ratio of total chlorophylls to carotenoids is 1.8–2.2 times less than in Colchida. Thus, the data of physiological analyses indicates that the cultivar "Colchida" is inferior to the rest varieties.

Regarding the dynamics of accumulation of photosynthetic pigments in cultivars that feature the variety Caratum, a sharp increase in the synthesis of green pigments is observed from June to August, followed by a sharp decline. The synthesis of carotenoids can be quite stable throughout the period of active vegetation, and determines the resistance of the varieties to stress factors.

In leaves of tea varieties in the adverse temperature period (from October to June/July), Keemun accumulates large amounts of carotenoids [24]. With the improvement of climatic conditions (e.g., temperature stabilization, reduction in water scarcity), the carotenoid content

Varieties and clones of tea	**Chlorophyll**			***a*/*b***	**Carotenoids**	***a* + *b*/carotenoids**
	a	***b***	***a* + *b***			
Colchida	1.45 ± 0.48	0.58 ± 0.22	2.03 ± 0.70	2.50 ± 0.07	0.40 ± 0.58	6.25 ± 1.42
Local population	1.72 ± 0.41	0.84 ± 0.22	2.56 ± 0.69	2.05 ± 0.04	0.48 ± 0.11	4.27 ± 1.29
Caratum	1.80 ± 0.53	1.01 ± 0.40	2.81 ± 0.93	1.78 ± 0.11	0.41 ± 0.30	4.35 ± 0.85
Keemun	1.63 ± 0.46	0.72 ± 0.28	2.35 ± 0.74	2.26 ± 0.10	0.64 ± 0.77	3.54 ± 1.98
Sochi	1.78 ± 0.85	1.02 ± 0.34	2.80 ± 0.62	1.75 ± 0.09	0.62 ± 0.68	2.81 ± 0.95
LSD ($P \leq 0.05$)	*0.25*	*0.37*	—	—	*0.18*	—

Table 2. Pigment apparatus characterization of different tea plant varieties (mg/g), average over 6 years.

drops slightly. However, at the onset of the winter period their number increases again. This can explain the stability of varieties not only to water deficit, but also to low temperatures. As for green pigments, over the months their syntheses become much smoother. A native of the North Chinese province of Keemun, this variety has long been established in Krasnodar region and can even be found in more northern regions of Russia (for example, Goith and Adygea) [16]. The only problem is the low quality of the tea produced from the sprouts of this class. However, it is an excellent material for breeding resistant varieties of tea.

In plants of Colchida varieties, which contain fewer photosynthetic pigments, the amount of carotenoids is almost constant during the whole period of vegetation. The synthesis of chlorophyll is similar to the processes occurring in the leaves of Caratum. The only difference is that from June to November the fairly evenly active synthesis gives way to a gentle decline.

An interesting feature was observed in plants of the local population. In general, all varieties of tea maximum green pigments are seen in August, but the leaves of local plants mark another peak of active synthesis—from September to November—and after this there is a uniform decline. The same pattern is observed in the accumulation of carotenoids.

As is known, the power of the pigment system of plants is related to their water exchange. Moreover, the status of chlorophylls and carotenoids in drought periods allows it to use this indicator as a criterion for evaluating the resistance of plants (**Table 3**).

Research of the pigment system in poor water availability periods showed that the content of chlorophylls and carotenoids accurately characterizes the drought resistance of tea plants.

A loss of leaf turgor was accompanied by an increase in the number of photosynthetic pigments [10]. In this case, manifested features are a studied variety, which testifies to their different physiological activity. Note that when there is a water deficit there is increased synthesis of carotenoids in plant varieties Keemun and Sochi, derived on the basis of the variety Keemun (1.5–2.0 times compared with the optimum period). This is closely followed by the variety Caratum. This fact indicates good drought resistance of these plants.

It is important to study the influence of drought on the pigment system to find out not only quantitative characteristics, but also how much variability there is in the content of chlorophylls and carotenoids, since the stability of the synthesis of pigments indicates a physiological state of plants and their ability to resist adverse environmental factors (**Table 3**). Note that greater stability in the synthesis of green photosynthetic pigments is observed in the variety Caratum, which indicates its good adaptive reactions (only 6% compared to the original). At the same time, 48% increases the content of carotenoids, the active synthesis of which is due to stressful conditions. The identified resistance of this variety is confirmed by our data for the study of physiological parameters such as the contents of the forms of water in the plant, increased water scarcity, and enzymatic activity, and as an integral indicator during the growing season.

The variety Colchida and plants of the local population are characterized by the unstable system of green pigments, but low variability of carotenoids.

We have identified that in areas with optimal tea soil conditions, characterized by high fertility, differences in the content of photosynthetic pigments is insignificant. However, more important

Varieties and clones of tea	*a* + *b*			Carotenoids			*a*/b		*a* + *b*/carotenoids	
	Initial	Withering	% to initial	Initial	Withering	% to initial	Initial	Withering	Initial	Withering
Colchida	2.64 ± 0.02	3.35 ± 0.06	27	0.50 ± 0.05	0.55 ± 0.02	10	2.24 ± 0.01	2.14 ± 0.03	5.28 ± 1.02	6.10 ± 0.09
Local population	1.69 ± 0.01	2.10 ± 0.06	24	0.47 ± 0.05	0.55 ± 0.01	17	2.29 ± 0.02	2.08 ± 0.02	3.60 ± 0.02	3.81 ± 0.01
Caratum	1.47 ± 0.05	1.56 ± 0.05	6	0.42 ± 0.02	0.62 ± 0.02	48	1.75 ± 0.01	2.14 ± 0.02	3.50 ± 0.08	2.51 ± 0.01
Keemun	2.22 ± 0.01	2.58 ± 0.04	16	0.74 ± 0.04	0.99 ± 0.01	34	1.91 ± 0.05	1.57 ± 0.05	3.00 ± 0.08	2.61 ± 0.02
Sochi	2.26 ± 0.05	2.69 ± 0.02	19	0.48 ± 0.02	0.66 ± 0.01	38	2.01 ± 0.01	1.75 ± 0.06	4.71 ± 0.09	4.07 ± 0.01
LSD (P ≤ 0.05)	*1.14*	*0.95*	—	*0.10*	*0.12*	—	—	—	—	—

Table 3. Changes in the pigment apparatus of plants due to tea loss of moisture by the leaf (mg/g), average over 6 years.

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are hydrothermal conditions, which in each microsegment are rather peculiar. Plantations are located on slopes, with higher slopes in degrees (from 15 to 20°C, at 5–7°C on the other), the southern exposure of the slope above the solar insolation (50–100 lux) and lower humidity (about 68.7% in 72–81% in other areas). Areas that are warm therefore contain plants that suffer water deficit. This is due to the great liability of the carotenoid content: 30–40% of the original value. Under optimal soil conditions were stimulates the adaptive ability of plants without causing visible oppression of the tea bushes (as evidenced by the high productivity of these plantations).

3.2. Pigment apparatus of the plants of Actinidia sweet (*Actinidia deliciosa* Chevalier)

Within the study of pigment apparatus of the plants of Actinidia sweet (*A. deliciosa*) was installed the dynamic nature of accumulation of chlorophyll ($a + b$) and carotenoid responsive hydrothermal growth conditions [25]. In general, the culture in the leaves during the vegetation period produces a significant accumulation of green photosynthetic pigments, while the highest content of chlorophylls was achieved in August (2.026 mg/g). Enhanced accumulation of carotenoids was also observed in August (0.982 mg/g), which is associated with the onset of the dry period (**Figure 5**).

It is known that carotenoids perform a photoprotective function in defense reactions of the plant organism (they protect the reaction center from the powerful streams of energy at high intensities of light and stabilize the lipid phase thylakoid membranes, protecting them from peroxidation), therefore enhanced accumulation of carotenoids in adverse conditions of a vegetation plant is needed to promote adaptive responses and reduce the overall stress of the plant.

The accumulation of synthetic pigments in Actinidia sweet is no less clear than that of tea appear varietal differences (**Table 4**). As can be seen from the data in the table, the differences between varieties in the accumulation of photosynthetic pigments are essential. The control strain for studies was conducted in the humid subtropical climate for the variety Hayward [28]. As can be seen from **Table 4**, the varieties Monty and Bruno revealed a much smaller number of green pigments, and the same pattern is identified in the content of the leaves of experimental cultivars of carotenoids. The coefficients of variation are large enough to show the dynamic nature of the synthesis of pigments, which depends entirely on hydrothermal factors.

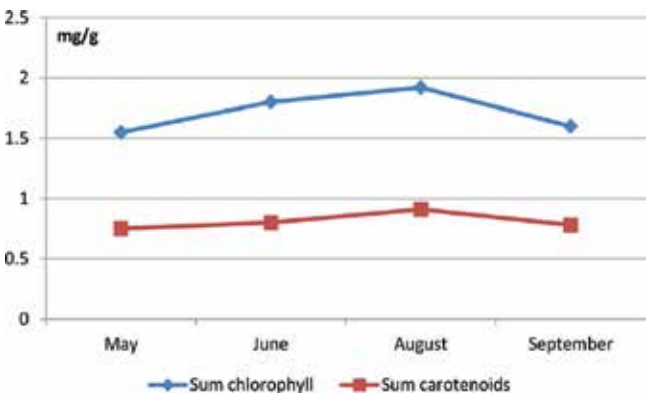

Figure 5. Accumulation dynamics of photosynthetic pigments in leaves of *Actinidia deliciosa*, average over 3 years.

Varieties	Sum chlorophyll		Carotenoids		*a*/*b*	*a* + *b*/carotenoids
	X ± Sx	V, %	X ± Sx	V, %		
Hayward	1.96 ± 0.02	71.4	0.88 ± 0.02	106.9	1.63 ± 0.01	2.02 ± 0.05
Bruno	1.50 ± 0.06	81.6	0.75 ± 0.08	115.7	1.70 ± 0.02	1.98 ± 0.06
Monty	1.25 ± 0.05	89.6	0.63 ± 0.03	126.0	1.51 ± 0.04	2.58 ± 0.07
Allison	1.73 ± 0.02	76.1	0.85 ± 0.09	108.7	1.73 ± 0.09	2.04 ± 0.05
LSD (P ≤ 0.05)	*0.35*		*0.10*		—	—

Table 4. The content of photosynthetic pigments in the leaves of different Actinidia sweet varieties (in mg/g), average over 3 years.

3.3. Pigment apparatus of the plants of hazelnut (*Corylus pontica* C. Koch)

Research of the pigment apparatus of hazelnut leaves showed that the maximum green pigment is in May, followed by a decline associated with the onset of an adverse water availability period in July, accompanied by elevated air temperatures. At the same time, features of the dynamics of carotenoids in the leaves of hazelnut are such that May marks the lowest content of this group of pigments (0.36 mg/g). In this case, the leaves of the hazelnut show certain xeromorphic features associated with a relative resistance of hazelnut to water stress (**Figure 6**).

Furthermore, by August it was observed that there was a significant (twofold) increase in carotenoids in the cells, which is directly associated not so much with the deterioration of the hydrothermal factors, but with aging of the leaf and the destruction of the green pigments of the group [17].

The general pattern, traceable in all cultures, including the hazelnut, is expressed in the presence of varietal differences. However, the overall analysis of the dynamics of chlorophylls made by three-year average data showed that, if varieties such as Lombard red, Cherkesskiy-2, and President as optimization of conditions of humidifying the synthesis of green pigments is restored, grade Futkurami small decline continues further (**Table 5**).

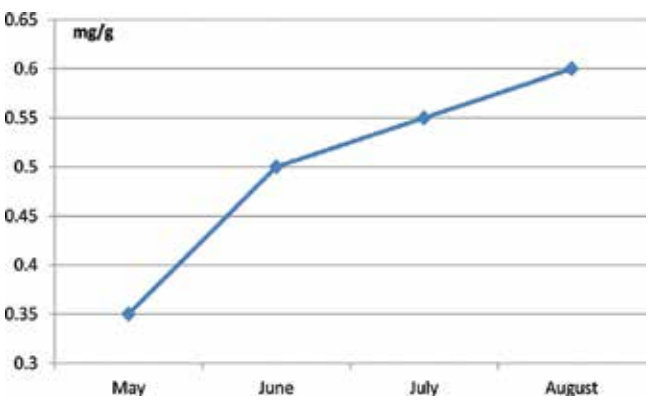

Figure 6. Dynamics of accumulation of carotenoids in the leaves of hazelnut, average over 3 years.

Varieties	Sum of chlorophyll		Carotenoids	
	X ± Sx	V, %	X ± Sx	V, %
Cherkesskiy-2	2.61 ± 0.37	61.9	0.54 ± 0.06	135.7
Lombard red	2.60 ± 0.15	62.0	0.47 ± 0.11	146.0
President	2.55 ± 0.16	62.1	0.48 ± 0.11	144.4
Futkurami	2.59 ± 0.44	62.6	0.51 ± 0.06	140.7

Table 5. The content of photosynthetic pigments in the leaves of different hazelnut varieties (in mg/g), average over 3 years.

In addition, it is revealed that the minimum number of carotenoids over the entire observation period was observed in the varieties of Lombard red and the maximum in the variety Cherkesskiy-2.

3.4. Pigment apparatus of hydrangea large leaf (*Hydrangea macrophylla* [Thunb.] Ser.) and weigela (*Weigela* × *wagneri* L. H. Bailey)

In addition to crops such as kiwifruit, hazelnuts, and tea, we researched the pigment apparatus of ornamental plants hydrangea large leaf and *W.* × *wagneri*. Studies have shown that the dynamics of carotenoids are associated with an adaptive mechanism of protection against stress in hydrangea plants subject to somewhat different patterns than previously considered (**Figure 7**).

As is shown in **Figure 7**, the maximum amount of carotenoids is in May, and by July there is a significant (1.4 times) decrease in the synthesis of this group of pigments. This process is related to the fact that the vegetation of hydrangea starts earlier (February–March) when the air temperature is above 5°C. Consequently, upon reaching the arid period of active vegetative processes the plant is somewhat subsided, leading to suspension of the synthesis of carotenoids; however, further synthesis of carotenoids is enhanced because of its involvement in the activation of defense mechanisms.

In the process of accumulation of photosynthetic pigments there are apparent varietal differences (**Table 6**). Thus, significantly more chlorophyll is contained in the leaves of varieties such

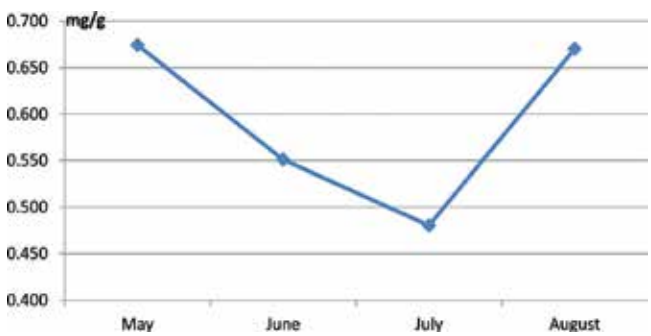

Figure 7. Dynamics of accumulation of carotenoids in hydrangea large leaf, average over 3 years.

Varieties	Chlorophyll			*a/b*	Carotenoids	*a* + *b*/carotenoids
	a	*b*	*a* + *b*			
Altona	0.89 ± 0.04	0.54 ± 0.03	1.43 ± 0.07	1.64 ± 0.03	0.72 ± 0.02	1.93 ± 0.03
Sister Teresa	1.03 ± 0.02	0.67 ± 0.05	1.69 ± 0.03	1.53 ± 0.02	0.90 ± 0.01	1.87 ± 0.02
Bichon	0.87 ± 0.03	0.63 ± 0.02	1.51 ± 0.03	1.38 ± 0.04	0.74 ± 0.03	2.08 ± 0.04
F. rosea	0.80 ± 0.05	0.54 ± 0.08	1.35 ± 0.07	1.48 ± 0.05	0.64 ± 0.05	1.98 ± 0.02
Admiration	1.22 ± 0.07	0.70 ± 0.01	1.92 ± 0.06	1.74 ± 0.04	0.77 ± 0.03	2.49 ± 0.07
Draps Wonder	1.25 ± 0.01	0.75 ± 0.02	1.99 ± 0.01	1.68 ± 0.01	0.90 ± 0.08	2.21 ± 0.02
LSD (P ≤ 0.05)	—	—	*0.55*	—	*0.21*	—

Table 6. Pigment apparatus characterization of different varieties of *Hydrangea macrophylla* (mg/g), average over 3 years.

as Admiration and Draps Wonder, which feature dense dark leaf plates. This fact determines a more active photosynthetic activity in even the smallest shading, since the ratio of chlorophyll *a/b* we can conclude that these varieties are shade tolerant [20]. The highest amount of carotenoids was observed in varieties such as Sister Teresa and Draps Wonder, exhibiting resistance to the action of hydrothermal factors.

The accumulation of carotenoids in these varieties during the period of vegetative decay processes shows active resistance of plants to the accumulation of peroxides in the leaves, which further leads to their more rapid recovery from a stressful situation.

The photosynthetic potential of the new introduced varieties of hydrangea large leaf was assessed by studying within the dynamics of accumulation of photosynthetic pigments in the leaves. According to the data obtained (**Figure 8**), the maximum accumulation of chlorophyll *a* in the cultivars "General Patton" and "Jogosaki" is in May–June (1.11 and 1.07 mg/g wet weight, respectively) and the variety "Altona" recorded two leaps of increase in chlorophyll

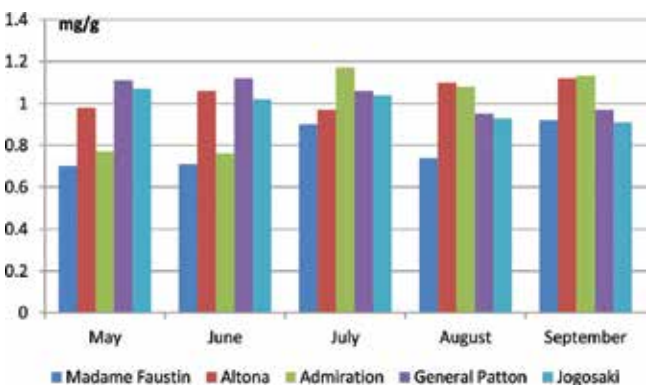

Figure 8. Dynamics of chlorophyll *a* content in leaves of hydrangea large leaf.

a in May–June (0.98 and 1.06 mg/g) and in August–September (1.10 and 1.13 mg/g, respectively). At the same time the variety "Admiration" had low accumulation of this pigment in May–June (0.77 and 0.76 mg/g) and a sharp increase in the summer and autumn months (July–September). During the period of vegetation, all introduced varieties' chlorophyll *a* content was higher than the variety "Madame Faustin" [22]. Note also that the decrease in the content of green pigments in August was accompanied by inhibition of biosynthesis, which is visually manifested in slowing the growth of plants.

Figure 9 shows the dynamics of the content of chlorophyll *b* in the leaves of large-leaved varieties of hydrangea. The results show that the content of the pigment in the optimal hydrothermal conditions for the period (May) averaged 0.48 mg/g. When increasing the stress factors in July–August (by increasing maximum air temperature to 35°C and lowering the humidity of air and soil by 60% and 20%, respectively) a higher content of chlorophyll *b* to an average of 0.74 and 0.78 mg/g, was observed.

While more resistant varieties such as "Altona" and "Admiration" show a marked increase in the content of chlorophyll *b*, a less variable content of the pigment during the period of research was also seen in resistant varieties of "General Patton" and "Jogosaki" (**Figure 9**), in comparison with the control cultivar "Madame Faustin." Our results do not contradict the data of other researchers, who believe that physiological adaptation may manifest itself in an increase in the content of chlorophylls *a* and *b* compared to control. Also, it was experimentally discovered that chlorophyll *b* can perform a protective function; in this case, the higher the content of chlorophyll *b*, the lower the sensitivity to bright light. In addition, during drought, chlorophyll *a* is destroyed to a greater extent than chlorophyll *b*.

An important constituent of the pigment system of plants are the carotenoids. The quantitative content of carotenoids in the leaves of large-leaved varieties of hydrangea showed that this indicator is dynamic. A general trend is the accumulation of yellow pigment in the vegetation period from May to the third week of June in the whole culture (**Figure 10**). Thus, during the optimal period for hydrothermal indicators (May–June), the content of carotenoids on average was at the level of 0.66 mg/g. However, if you increase the action of stress factors on hydrangea plants in the period from the first week of July to the third week of

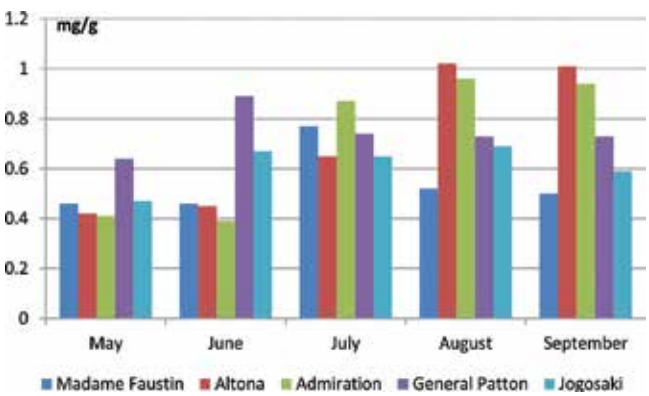

Figure 9. Dynamics of accumulation of chlorophyll *b* in leaves of hydrangea large leaf.

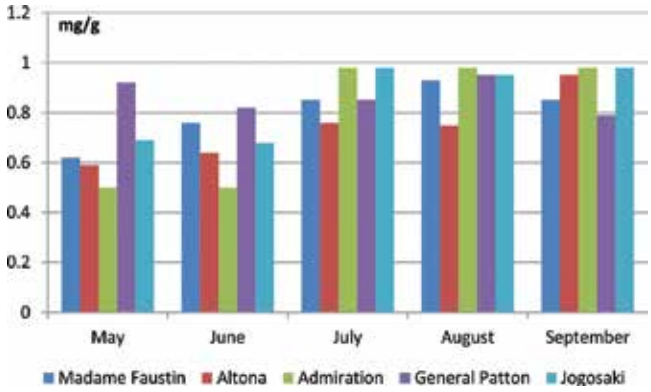

Figure 10. Accumulation dynamics of carotenoids (mg/g wet weight) in leaves of hydrangea large leaf.

September, there was an increase in the accumulation of carotenoids in the average grades to 0.89–0.94 mg/g, respectively. The maximum amount of carotenoids was observed in resistant varieties "Admiration" (1.0 mg/g) and "Jogosaki" (0.98 mg/g) and the minimum amount in the unstable control variety "Madame Faustin" (0.71 mg/g). Increasing the level of carotenoids in relatively resistant varieties in the summer can be explained by adaptive reaction aimed at improving stability of the photosynthetic apparatus and prevention of photodynamic destruction during a drought.

The following informative indicator characterizing the operation of the photosynthetic apparatus is the ratio of chlorophyll *a* to chlorophyll *b* (*a*/*b*). This indicator can characterize the potential photochemical activity of leaves. It is also the photosynthetic activity of chlorophyll *a* and the longer it takes the more intense is the photosynthesis. We found that in the large-leaved varieties of hydrangea the ratio of *a*/*b* ranged from an average over the growing period of 1.49 for "Madame Faustin" (control) to 1.69 for "Altona." Based on the obtained results, we can conclude that for this indicator, the introduced varieties "Altona," "Admiration," and "Jogosaki" have been successfully adapted to the conditions of Russia's damp subtropics [21].

In the accumulation of carotenoids in the leaves of weigela, two periods are clearly seen, completely unrelated to changes in hydrothermal factors (**Figure 11**). In the period June–July, with increasing temperature up to 25–27 OS and a decrease in precipitation, a slight decrease in

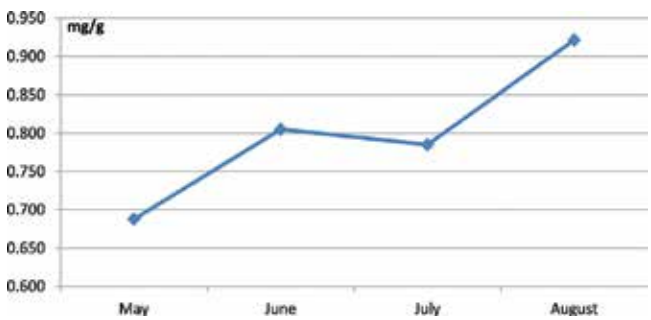

Figure 11. Accumulation dynamics of carotenoids in the leaves of weigela, average over 2 years.

Varieties	Sum of chlorophyll		Carotenoids	
	X ± Sx	V, %	X ± Sx	V, %
Gustav Malet	1.73 ± 0.37	25.4	0.81 ± 0.08	30.7
Arlequin	1.56 ± 0.24	19.9	0.76 ± 0.09	26.0
Eva Rathke	1.78 ± 0.51	20.3	0.82 ± 0.15	24.4
Mont Blanc	1.65 ± 0.29	25.7	0.79 ± 0.07	27.5
LSD (P ≤ 0.05)	*0.45*		*0.31*	

Table 7. Pigment apparatus characterization of different weigela varieties (mg/g), average over 3 years.

carotenoid content is noted. With further tightening of the manifestations of drought, the synthesis of carotenoids increases sharply, reaching a maximum level of 0.921 mg/g wet weight.

As in the previously described cultures, there are varietal differences in the content of photosynthetic pigments, and therefore in the capacity of the pigment system (**Table 7**).

However, as the table shows, the amount of chlorophylls and carotenoids, slightly different weigela varieties, and low values of the coefficients of variation (30%) indicate low variability of the characteristic that can be used in the diagnosis of culture on this indicator [21].

4. The estimation method of ecological flexibility and stability of the varieties on the content of photosynthetic pigment groups

To evaluate the ecological flexibility and stability of studied pigment apparatus variety cultures, we identified the genotype-environmental effects level of significance and the correlation between the studied characteristics that have changed over the years in the target points. In addition, we have assessed the environmental effects on the value of the trait in the two schemes: the variety *x* year and variety *x* points. Of all the currently existing methods of evaluation, we chose a method proposed by Eberhart and Russell [29]. It allows us to estimate the ductility coefficient of linear regression (b_i) using the mean square (variance) deviations from linear regression (S_i^2). This method allows the selection of genotypes by the total of their response to limiting environmental factors. When assessing the flexibility coefficient of the linear regression, the accuracy of this deviation from 1 should be taken into account, i.e., from the average set of grades. If the score is above 1, this indicates a significant increase in the quantitative trait under the influence of improved growing conditions; if b_i is less than 1, varieties show better results in unfavorable conditions. If the values of b_i were not significantly deviated from 1, the variety will exactly follow the change of the environmental conditions. The genotype with negative regression on the environment is flexible; the most valuable genotypes have b_i greater than 1. It should be established that the smaller the dispersion value of the deviation from the regression, the more stable are the characteristics.

4.1. Evaluation of the ecological flexibility and stability of grades of *Actinidia deliciosa* according to the characteristics of the pigment system

In recent years the interest of breeders in the environmental adaptability of varieties has been repeatedly noted. It is recognized that intensive technologies have more stable and

flexible (with high yield potential) cultivars, but they may have a low stability of yield under adverse conditions.

Extensive technology is proposed to use stable, but not as flexible, varieties. Because the goal of the breeder is the creation of varieties, fully realizing their potential in the specific conditions of cultivation, we talk about the need for compliance with selection of the technology level for agricultural production.

In this situation, knowledge of the response to abiotic factors of the main physical characteristics, especially, is closely related to the provision of assimilative capacity, and allows the prediction of expected properties. We defined ecological flexibility characteristics, such as the amount of chlorophylls and carotenoids, depending on varietal facilities of plants of *A. deliciosa* (**Tables 8** and **9**). The analysis of the varieties in the parameters of ecological flexibility of both groups of photosynthetic pigments showed that the varieties, the change of photosynthetic capacity that most fully corresponds to the change in temperature conditions, are types of Monty and the accumulation of carotenoids and Bruno. The linear regression coefficient of the variety Allison suggests a significant increase in the pigments only under the influence of the improvement of thermal conditions of cultivation.

The factor of illumination parameter such as the magnitude of accumulation within chlorophylls ($a + b$) (responsible for synthetic processes) is more adapted to the condition of cultivation in the variety Hayward, while the cultivar Monty shows high ecological flexibility in

Varieties	Air temperature (°C)			Illumination (lux)			Relative humidity (%)		
	r^*	b_i^*	S_i^{2*}	r^*	b_i^*	S_i^{2*}	r^*	b_i^*	S_i^{2*}
Hayward	0.45	0.04	51.2	−0.36	−1.94	9545.6	0.98	−3.20	206.8
Monty	−0.62	−0.26	42.8	0.17	−2.50	7982.6	−0.92	−4.11	173.0
Allison	0.96	0.10	49.5	0.84	−2.20	9231.1	−0.01	−3.62	200.0
Bruno	0.29	0.04	47.0	0.91	−3.48	8776.5	−0.85	−5.75	190.2

Annotation: r^*—correlation coefficient; b_i^*—coefficient of linear regression; S_i^{2*}—variance of the deviations from the linear regression.

Table 8. Evaluation of the ecological flexibility and stability of varieties of *Actinidia deliciosa*, the sum of chlorophylls.

Varieties	Air temperature (°C)			Illumination (Lux)			Relative humidity (%)		
	r^*	b_i^*	S_i^{2*}	r^*	b_i^*	S_i^{2*}	$\mathbf{r}^*$	b_i^*	S_i^{2*}
Hayward	−0.62	0.02	109.08	−0.51	−2.29	109.08	−0.76	−1.84	172.86
Monty	−1.00	−7.45	126.02	0.65	17.85	126.02	−0.79	−7.69	230.69
Allison	−0.74	3.75	108.73	0.71	6.39	108.72	−0.73	−9.76	171.70
Bruno	0.58	−2.83	115.65	0.65	5.99	115.65	−0.94	−1.10	194.28

Annotation: r^*—correlation coefficient; b_i^*—coefficient of linear regression; S_i^{2*}—variance of the deviations from the linear regression.

Table 9. Evaluation of the ecological flexibility and stability of grades *Actinidia deliciosa* on the amount of carotenoids.

relation to accumulation of carotenoids, which is promising for the adaptability of plants of this variety. This is indicated by a high value of coefficient of linear regression, several times higher than 1. Weak responsiveness to changing humidity was observed in the accumulation of pigments in the variety Allison.

Analysis of the obtained values of dispersion indicates a low stability of the capacity of the pigment system in almost all studied varieties, which confirms the variability and flexibility characteristics under the influence of abiotic factors (**Tables 8** and **9**).

In general, given our studies on the power of the pigment system and the data found in **Tables 8** and **9**, it can be concluded that a significant unstable response to changing agroclimatic conditions shows varieties such as Allison, Bruno, and Hayward (control), as indicated (in general, according to both tables), with highest mean square (variance) deviations from the regression line. This allows them to be classified as fragile varieties. In the variety Monty, the dispersion factor in the accumulation of chlorophylls is a minimum for all abiotic factors (S_i^2 = 42.8; 7982.6; 173.0), indicating stability of the trait, while a factor in the accumulation of carotenoids is rather flexible (S_i^2 = 126.02; 126.02; 230.69).

The results of the evaluation of ecological flexibility and stability of grades of *A. deliciosa* on the basic parameters of the power of pigment-based systems analysis model showed complete adherence to the changing conditions of varieties such as Ellison, Hayward (control), and Bruno, which indicates that their instability to the effects of abiotic factors and confirmed the prospectively of this sort, like Monty, are sufficiently adaptive to stress conditions of the vegetation period.

4.2. Evaluation of the ecological flexibility and stability of hazelnut varieties (*Corylus pontica* C. Koch) in the content of carotenoids

Considering the adaptation of the pigment apparatus of plants, including hazelnut, to stress conditions of vegetation, it is possible to show the adaptation of individual plants in ontogenesis and the adaptation of varieties in general. Plants with broad ecological flexibility are better able to adapt to a changing environment. The calculation of ecological flexibility of culture on the content of carotenoids showed a coefficient of linear regression greater than 1, indicating a high flexibility of culture in relation to hydrothermal factors (see **Table 10**).

Varieties	Content of carotenoids, мг/г			
	Temperature (°C)		Precipitation (mm)	
	Coefficient of linear regression (Bi)	The degree of stability (δ^2di)	Coefficient of linear regression (Bi)	The degree of stability (δ^2di)
Cherkesskiy-2	14.60	71.46	24.10	3.92
Lombard red	14.06	73.74	24.51	4.17
President	15.05	91.32	28.80	6.08
Futkurami	12.40	80.68	21.42	4.74

Table 10. The parameters of ecological flexibility experienced with hazelnuts.

Plants of the variety President reveal the highest value of the coefficient in the linear regression in relation to temperature factor and relative to the amount of precipitation. At the same time, less flexible is the grade Cherkesskiy-2; physiological indices have become quite stable over the years, as evidenced by the degree of stability (variance).

Thus, based on indicators of adaptability, it is recommended to grade President as the most sustainable and ecologically flexible. However, for maximum effect, it requires good agronomic conditions as well, and the culture as a whole, judging by the linear regression coefficients significantly greater unit.

5. Conclusion

In this chapter we showed the features of the dynamics of accumulation of photosynthetic pigments in subtropical cultures and revealed the dependence of this process on the main factors of the region. It was revealed that the dynamics of pigment accumulation in subtropical plants within the conditions of Russia's damp subtropics is a complex process, which depends on their species.

Comparing the pigment composition of different cultures used in our studies, we concluded that the greatest number of green pigments were inherent in the leaves of plants of hazelnut (2.40 mg/g) and tea (2.05 mg/g). The least amount of chlorophyll was observed in the leaves of hydrangea (1.01 mg/g), which is a characteristic feature of these cultures. At the same time, hazelnut and tea plants have lesser amounts of carotenoids (0.49–0.52 mg/g) compared to other studied crops.

We also revealed a different pattern of accumulation of chlorophylls and carotenoids of sprouts and physiologically mature tea leaves: the content of photosynthetic pigments in sprouts showed no significant change during the growing season, because the pigment apparatus of leaves is very sensitive to any changes in growing conditions. In addition, the content of pigments in leaves is 2.0–3.8 times higher than their number in sprouts.

In the study of the pigment apparatus of *A. deliciosa*, it was established that the dynamic nature of accumulation of chlorophylls ($a + b$) and carotenoids was responsive to hydrothermal growth conditions.

Studies of the pigment apparatus of the hazelnut leaves showed some regularities associated with relative resistance to water stress, which is manifested in the somewhat different nature of the accumulation of carotenoids.

It is established that the dynamics of carotenoids in hydrangea are associated with an adaptive mechanism of protection against stress, subject to slightly different laws, compared to the previously discussed cultures. This is connected to the period of vegetation of hydrangea that starts earlier than other cultures (February–March).

However, we showed that all subtropical plants noted an increased accumulation of carotenoids. The high content of carotenoids in summer (June–July) is caused by water stress, evidenced by

their participation in the formation mechanism of resistance of subtropical plants to adverse conditions. Active accumulation of chlorophyll in August is a characteristic feature of all subtropical plants in the conditions of Russia's damp subtropics, as confirmed by our research. In August installed the optimal hydrothermal and light conditions: a favorable temperature (26–29°C), optimal humidity (in the range of 78–80%), rainfall increases. This leads to a new period of active photosynthetic activity, which causes the synthesis of groups of green pigments.

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Chapter 5

Pilot Scale of Microalgal Production Using Photobioreactor

Farouk Kamel El-Baz and Hanaa H. Abd El Baky

Additional information is available at the end of the chapter

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Abstract

Microalgal gained much interest as a promising sustainable feedstock for the production of food, feed, bulk chemicals and biofuels. Pilot scale of microalgal is needed to bridge the gap between laboratory scale research and commercial application. Commercial applications of microalgal have been used for a wide array of functions including, pharmaceutical, health sector, nutraceutical, cosmetics and agriculture. Numerous photobioreactors (PBRs) of different volume and shapes have been designed. Cost of PBR has a major influence on production cost for large scale biomass. There are several ways to reduce production cost depends on the type of algal strain, type of PBRs, CO_2 and the production technology of the biomass. Dilution rate is an important factor, which affects the biomass productivity, rate and ultimately what needs to be maximized.

Keywords: photobioreactor, open bonds, biomass production, water sterilization, biofilm, dilution rate

1. Introduction

Microalgal is that the good biotechnological potential for the assembly of huge quantities reasonably compounds like polysaccharides, lipids, proteins, carotenoids and pigments, vitamins, steroids, amongst others. Microalgal species is now used in the manufacture of animal feed, human nutrition, cosmetics, and pharmacy trade parts. Biofuels could also be used to produce biofuels [1–3]. Some authors have faith in microalgal as biodiesel feed stocks [3]. The advantages of microalgal culture compared to superior plants unit listed [4, 5]:

1. Microalgal biological systems square measure thought-about the foremost economical for star lightweight capture and the production of compounds.

2. Several microalgal species manufacture and accumulate compounds of high business value could also be induced, for example, proteins, carbohydrates, lipids, and pigments.
3. The isolation, genetic selection, and strain studies is relatively easy and fewer time intense because microalgal reproduce themselves by a straight forward process and might fulfill their life cycles in precisely several hours or days.
4. Microalgal could also be cultivated with low inorganic nutrients concentration. These make them of express interest as an organic compound provide, encouraging organic compound convenience in regions of low agriculture productivity due to the shortage of water and nutrient-poor soils.
5. Systems for biomass production could also be tailored or scaled up to altogether totally different operation levels, allowing later incorporation of these systems to fully machine-controlled facilities for large scale production.

Microalgal cultivation has received a lot of attention within the past decade [6]. Still, most analysis is completed on the laboratory scale and is geared toward beneath standing the behavior of single cells under controlled conditions. As a central component within the production chain, cultivation ought to even be studied in additional detail beneath business or "large-scale" conditions.

2. Microalgal cultivation systems

2.1. Opens ponds system

Generally, microalgal and true bacteria huge scale mass cultivation is completed in shallow open ponds tanks, of circular or raceway kind, with sun gentle one amongst the vital edges of the employment of open structures is that they are simple to construct, function, and that they have decrease charges than closed systems. Though it's been established that open pool method of life is economically viable, they still have some disadvantages and bounds, they use gentle in a very altogether inefficient method, have evaporation water loses, low dioxide mass transfer value from the system, because of its inefficient mixing mechanisms.

2.1.1. Circular pond

A circular pond is principally used for culturing algal sp. in Asia [7]. The thought of exploitation such a rounded pond with a protracted rotating arm was impressed by the circular reactor in waste matter treatment, so a circular pond is very kind of like the waste matter treatment pond (**Figure 1a**). This type of pool is usually 20–30 cm thorough and 40–50 m in diameter. The long rotating arm is about within the center of the pond that acts sort of a face and performs a paddle wheel perform that is acquainted within the structure to that of a raceway pond. It's obvious that mix of culture media and algal cells square measure additional economical than that in associate ponds, however because the algal is exposed to

Figure 1. Open ponds cultivation system of microalgal cells. (a) Circular open pond and (b) raceway pond.

the surroundings, the contamination is unavoidable. According to the analysis literature, the productivities in circular pond vary between 8.5 g/(m^2 d) and 21 g/(m^2 d) [4].

2.1.2. Raceway pond

During the past 40 years, the raceway pond (**Figure 1b**) has been the foremost common and wide used open system reactor for the large-scale cultivation and business production of microalgal merchandise. The raceway pond was initial raised by Lee [7] and Oswald and Goleuke [8] within the sixties and its look and structure have not been modified a lot of compared with 40 years past till today [8].

2.1.2.1. Challenges in open pond cultivation systems

2.1.2.1.1. Light penetration

Light is the sole supply of radiant strength, bad light penetration into the pond becomes a hassle. Light is regularly considered to be one of the most vital factors that decide algal increase. The critical troubles that are confronted are: the open pond device, mild penetrates most effective the top 3–4 in. (76–100 mm) of the water. Because the algal grow and multiply, the way of life becomes so dense that it blocks mild from reaching deeper into the water. Low intensities might not promote algal boom at the lowest of the pond. Direct sunlight is simply too strong for most algal, which need most effective about 1/10 the quantity of light they acquire from direct sunlight. Excessive intensity may additionally cause picture inhibition and image-oxidation. Lengthy exposure to light may additionally result in minor damage of algal series antenna that may be fast repaired via the cell if placed within the darkish.

Studies have tried that it should be powerful to realize high productivity in open ponds because of the actual fact the temperature and light-weight intensity vary at some purpose of the day and 12 months. Further, even at some purpose of bright summer season days, out of doors algal cultures were shown to be light-limited, to get most productivity, it has been cautioned that it is acceptable to possess artificial resources of sunshine for nights and cloudy days [9]. The sunshine saturation result accounts for the truth that a twenty-fold growth

within the incident energy consequences in simplest a four-fold increase within the amount employed by the algal [10].

2.1.2.1.2. Odor troubles

Developing algal cultivation in open ponds will cause malodour within the ponds. This downside is specially thanks to lack of gas. Alga that square measure suspended below the surface cannot photosynthesize and as a result, they decompose. The decomposition technique consumes dissolved gas, and as a conclusion, gas ranges within the water column decrease, main to the mal-odor to unravel this example deliberate cultivation and harvest home have to be compelled to upset this on this context, right designing refers to harvest home the alga on the right time, before they die and decay.

2.1.2.1.3. Culture infection

Open ponds can get contaminated. Infection of ponds may be a result of: infiltration from other algal strains and alternative organisms, dirt scrap, leaves and alternative mobile materials. as associate instance, though the algal species adult commercially in outside ponds normally grow underneath tremendously selective things *(Nannochloropsis*, *Chlorella* Spp., and *P. tricornutum* adult at excessive nutrient concentrations, *Spirulina* is adult at high hydrogen carbonate concentrations and *D. Salina* is adult at terribly high salinity) contamination by means that of various undesirable algal species isn't uncommon.

Hence, while the capital fees for fixing associate open lake algal farm are low, the chance of contamination of the proper algal oil generating species, with the help of invasive species remains high. The contaminants not solely belong to non-organic sources but to boot from the varied biological and completely different alga species that might come back to be invasive.

Contaminants may have an effect on the hydrogen ion concentration and pH scale of social group medium. Principal contaminants that have an effect on the algal cultivation in open ponds are indexed as follows: protozoans completely different algal species—fauna—insects—different organic resources like bacterium, virus and fungi. The result, the enterprise is to confirm careful tradition protection and things that favor the rise of desired algal species over the contaminating species.

Open ponds, massive contaminants are also removed often by means that of golf stroke appropriately sized show within the water glide. Serious contaminants that sink to the lowest is cornered in pits organized at correct angle to the escort the flow and should then be off from those sediment traps. Those area units controlled through effectively operative the tradition widget as a batch tradition and restarting the social group at regular durations with contemporary, algal matter. That's the first system used for the culture of algal in Japan and H. bird genus in Hawaii. Cautious method of life preservation, presenting things that favor the popular species over the contaminating species conjointly permits lengthy-time amount non-stop method of life. Instance, infection of *Spirulina* cultures with alga is also reduced with the help of holding the hydrogen carbonate concentrations on top of 0.2 m and also the hydrogen ion concentration on top of 10 and running at excessive mobile densities [11]. Raceway cultures of *P. tricornutum* have conjointly been maintained with efficiency in labs for additional than 1 year throughout

trials, by method of holding high concentrations of gas and phosphorus. Contamination of *D. Salina* ponds by method of different algal species of *Dunaliella* (e.g.*D. verdict*, *D. parva*, *D. bioculata*) is controlled significantly by protective high salinities.

Tradition renovation requires continuous watching the utmost primary sort of pursuit is that the everyday microscopic examination to observe any eccentric morphological changes and therefore the presence of contaminating organisms inclusive of various algal and protozoa. Habitual checks of nutrient awareness of the pool should even be completed to stay aloof from sudden nutrient deficiencies. Standard pursuit of modifications in pH and O_2 ranges within the pool over the day can even be a helpful early alarm. Pursuit of O_2 and pH has the gain of obtaining the flexibility to be computerized. Recently, a replacement approach, pulse amplitude modulated fluorometry (pam) has emerge on be had and appears to be terribly touchy to deciding the physiological nation of algal [12].

The contamination with totally different alga can be overcome by employing a gradual population build-up of the required organism. Overlaying ponds with clear membranes or the usage of greenhouses overcomes this bother, permitting the additional effective strains to be fully grown freed from part infection.

2.1.2.1.4. Evaporation associated demanding situations

Cultivation in dry, tropical areas is that the excessive value of evaporation from open pool floor (up to 10 l/m^2/day). This poses a problem each from the purpose of skyrocketing the salt attention within the medium and within the acquisition of sufficient water to form up the water loss. Algal cultivation system locations should be selected in one amongst these manners that they need got a substantial offer of water to form up for evaporation losses.

2.2. Closed system

Photobioreactors (PBR) could also be delineated as an internal, lighted culture vessel designed for controlled biomass production. PBR refers to closed systems that area unit closed to the surroundings having no direct exchange of gases and contaminants with the setting. PBRs, despite their costs, have many major blessings over open systems:

- PBRs minimize contamination and allow axenic microorganism cultivation of monocultures.
- PBRs provide higher management over conditions like pH, temperature, light, greenhouse emission concentration etc.
- PBRs lead to less greenhouse emission loss.
- PBRs stop water evaporation.
- PBRs enable higher cell concentrations.
- PBRs enable the assembly of advanced biopharmaceuticals.

Troubles detected in open systems it has been planned the utilization of closed PBRs. The previous further acceptable for strains which cannot tolerate intense environments or once previous product is noticeably at risk of degradation or infection. Closed systems as well permit

the interference of contamination, permitting the operation in issues for PBR vogue and operation for mass cultivation of microalgal culture modes like photoautotrophic, heterotrophic, or mixotrophic. To boot, closed structures will achieve up to variety of instances larger biomass than open structures, consequently lowering harvest fees [13]. Notwithstanding the excellent advances that area unit done inside the assembly and operation of PBRSs, its generation continues to be in improvement. Around ninety-eight of current biomass production international is obtained in open systems, however the actual undeniable fact that PBRS technology provide an excellent deal of potential in phrases of productivity, manipulate of culture conditions, and pertinences to cultivate numerous traces. Numerous closed BPRS styles that operative in laboratory, pilot plant degrees, and even few had been effectively scaled up to associate in nursing industrial degree. Thought of amongst this made closed BPRS design is that the hollow kind, on this the tubes configuration whereby suggests that of life is hold is taken into consideration one amongst the primary factors touching productivity of chemical process biomass [14]. BPRS built with plastic materials like inflexible clear synthetic resin (p.c), polycarbonate flexible plastic baggage, amongst different substances. They're going to be organized in vertical, horizontal, conical, and willing type, with degasifying devices that allow the elimination of the O_2 created at some stage in chemical process [15]. The tubes could also be organized in photoreactors type comparison of individual open lake or closed PBRSS cultivation appliance each, closed PBRS and open raceway lake cultivation semiconductor diode to 3 primary boom cycles and harvest activities.

Handiest biomass incontestable to be rich in lipids becomes harvested pondering the boom curves for closed PBRS and open raceway lake, a doable forth harvest regarded possible whereas mobile concentrations reached a pair of 106/ml at days 28 and 29, severally, however the biomass throughout this excessive irradiation section become not made in lipids and had, consequently, be harvested some days later. The hybrid cultivation machine light-emitting diode to 6 most significant boom cycles and harvest activities. For that reason, the common increase rate of the hybrid system becomes drastically more than that of every widowed structures. The first cause for this appears to be that biomass increase and lipoid induction levels primarily impartial from each alternative whereas the employment of the hybrid cultivation device. This permits to take care of the life-style in speedy boom at awfully high cellular density. As an alternative, microalgal cultures in character cultivation systems (PBRS or open raceway pond) undergo phases of exponential boom and nutrient starvation to allow lipoid accumulation, discovered through a brief lag section before the subsequent growth phase, resulting in diminished typical growth prices. To work out productivity of all structures, biomass harvests (offered as gram/rectangular meter/day).

2.2.1. Tubular (TPBRs)

Tubular PBRs product of clear tube through that the media is circulated at liquid velocities of usually 0.5 m s^{-1} [16]. To prevent high gas concentrations the clear tubes area unit connected to a degasser or stripper vessel, where gas is removed by air injection. Hollow systems are usually found in varied orientations; horizontal tubes organized throughout one plane and multiple planes of vertically stacked horizontal tubes (fence-like systems). Diameters of the tubes vary with system orientation, diameters larger than 3 cm and smaller than 10 cm area unit usually used [17]. Tubular PBRs area unit costlier to construct than open raceway ponds,

notably vertically minded hollow PBRs. Investment costs for 100 ha horizontal tubular plant were calculable to be 0.51 M€/ha by Norsker et al. [16].

For the selection of a PBR for giant scale production, info on the actual productivity and chemical action efficiency of varied PBR designs is required. Norsker et al. [16] reported outline of chemical action efficiencies obtained with fully totally different reactors, locations and microalgal species; one. You take care of open raceway lake, you take care of horizontal hollow PBRs and you take care of flat panel PBRs [18]. However, for the next comparison of PBR designs information have to be compelled to be gathered at one location with the same microalgal species. Throughout this study, we have a tendency to tend to at identical time compared the performance of pilot-scale out of doors PBRs with *Nannochloropsis* sp. beneath identical climatological conditions at intervals The Netherlands. Four PBRs were place in at the Algal PARC pilot facility; academic degree open raceway pool (OPR), a horizontal annular PBR (HT), a vertical hollow PBR (VT), and a flat panel PBR (FP) [19]. The impact of daily dilution rates and boson flux densities on region productivity and activity efficiency was evaluated for each cultivation system.

2.2.1.1. Helical photobioreactor (HPBR)

HPBR contain of wound clear and versatile tube of very little diameter with separate or connected degassing unit. A pump is utilized to drive the culture through long tube to the degassing unit (**Figure 2**). Travieso et al. [20] technique with fully completely different micro-organism strains. Greenhouse emission gas and matter are circulated from either direction but injection from bottom offers higher natural process efficiency [21]. Tredici and Zittelli [10]

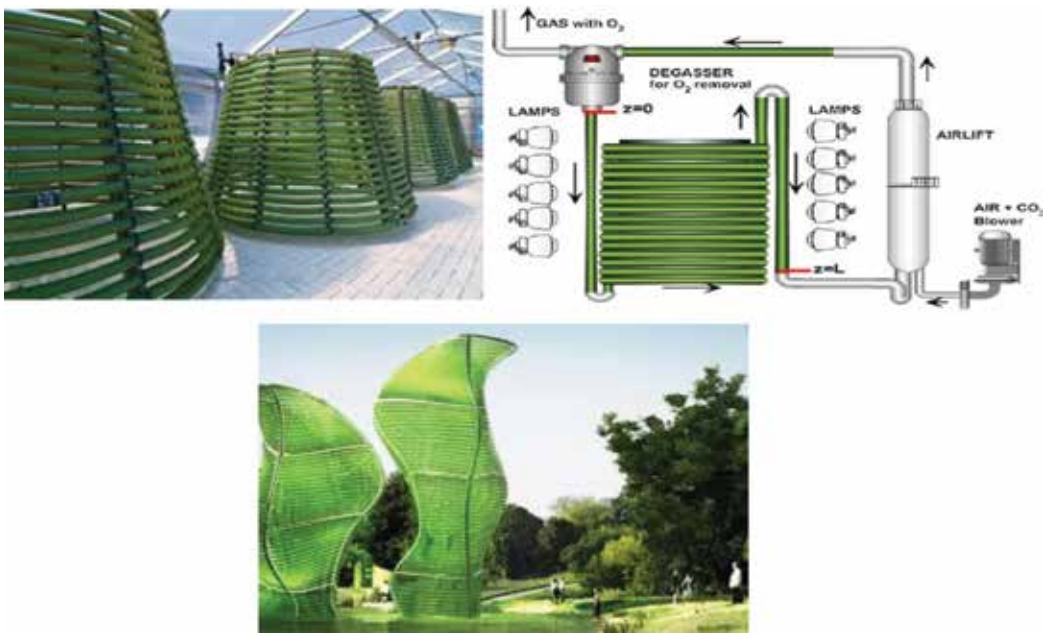

Figure 2. Helical photobioreactor.

designed PVC PBR with 3 cm diameter and vertical structure rigid with 2° with horizontal. It contains a degasser to remove oxygen and gases. Biomass production and photosynthetic potential were found to be 0.9 g L^{-1} d^{-1} and 6.6%, respectively. Light absorption of PVC is important for its higher photosynthetic potential and biomass production. Tredici and Zittelli [10] reported that "the HPBR had surface area to volume ratio of 53 m^{-1} with 23% of total volume was occupied by the gas bubbles" HPBR had advantages:

- Long tubes placed at small rise occupying small ground area,
- Large CO_2 absorption
- Essay scaling-up
- It use a stress limits commercial centrifugal pump [22].

Morita et al. [23] designed a PVC cone shape PBR with angle of 60°, and using air pump for aerated and recirculation of the algal culture, and optimize the temperature by controlling. At angle 60° increase the photosynthetic (6.84%) productivities and biomass productivity.

2.2.1.2. Vertical (VPBRs)

VPBRs are using for outside biomass production, as a result of their giant expanse (**Figure 3**). These PBRs are created from clear VPBRs enable to absorption of the sunshine. The algal media are circulated and aerated with air pump. VPBR**s** are often additional sorted into bubble column and air, based on their mode of algal media flow rate.

2.2.1.3. Horizontal (HPBRs)

HPBRs are the foremost standard PBRs. HPBRs disagree from the vertical tubes in many ways, significantly with tube surface and volume quantitative relation [24]. HPBRs (**Figure 4**) are primarily recognized of tubes organized in multiple potential orientations, like horizontal and helicoidally however all orientations basically add same method. Except for the arrangement

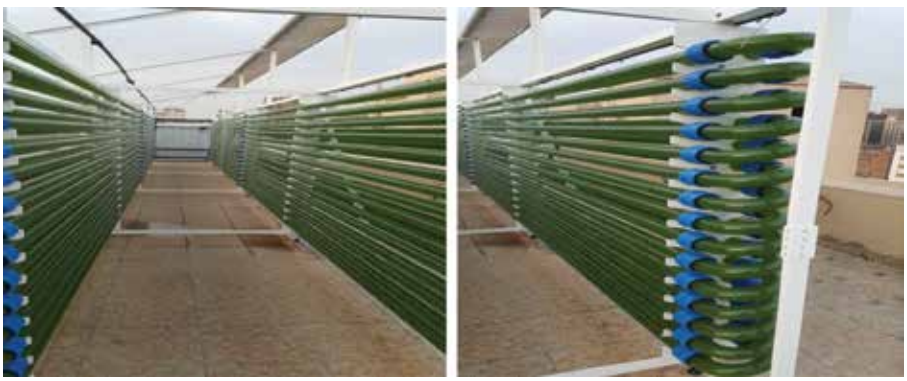

Figure 3. Vertical tubular PBRs.

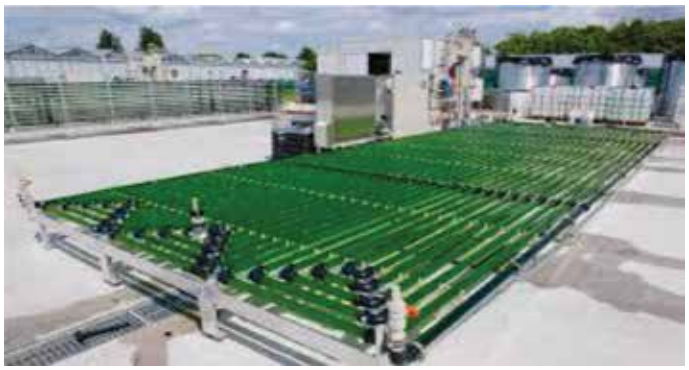

Figure 4. Horizontal tubular PBRs.

of tubes, hollow PBRs disagree within the tube length, flow speed, circulation system, and geometric configuration of the sunshine receiver. Mostly, these tubes have diameters of 10 metric linear unit to maximum 60 metric linear unit, and lengths of up to many 100 m. The utilization of such tubes helps in achieving high surface to volume quantitative relation (above 100/m), that is one amongst them in blessings of this style [25]. Sanchez Miron et al. [24] reported that "the increasing the tube diameter leads to a decrease within the surface/volume ratio, and this issue features a sturdy impact on the culture growth". Moreover, the thus referred to as "lens" or "focusing effect" helps to distribute the sunshine homogenously. In the "focusing effect" the incident light-weight is diluted on the circumference and is, during a radial direction, targeted onto the axis of the tube, so leading to preventing mutual shading and increasing of radiation intensity [25]. One of the main disadvantages of horizontal PBRs includes the buildup of O_2 to repressive levels, oxygen concentrations on top of air usually reduced chemical action in algal strains. HPBRs area unit usually can be the foremost ascendable culture. HPBRs do not seem to be economical possible for giant scale production due to demand of cooling as they need high surface to volume quantitative relation Sanchez Miron et al. [24]. Moreover, picture inhibition due the accumulation of O_2 and high strength leads to reduce production rates.

2.2.2. Flat panel photobioreactors (FPPBR)

FPPBR (**Figure 5**) may be a reasonably common PBR with a rectangular box look that is employed either in algal pure cultivation or alga waste matter cultivation. It can be placed either inside exposed to artificial light weight sources or outside exposed to daylight. Flat panels area unit made by clear or semi-transparent materials (glass, plexiglas, polycarbonate and plastic baggage, etc.). Flat panels have a very-short lightweight path that permits lightweight to easily penetrate the culture liquid. Compounding is principally driven by air bubbles that area unit generated from the air sparser. A pump is typically accustomed supplement air bubbles through the air sparser, current the alga cell suspension. The exhaust gas emission happens at the gas and liquid junction. The reactor is inclined at a precise angle to get the simplest intensity of incident lightweight once the reactor placed outdoors. The main blessings of flat panels include:

a. High surface area to volume ratio

b. Not-too-serious accumulation of dissolved oxygen

c. Convenient to clean

d. The international organization it is versatile and it's suit for scale-up

Meanwhile, the most limitations of flat panel include:

a. It's high-ticket to regulate the temperature

b. Fluid mechanics stress that is generated by aeration

c. Biofoul close to the inner surface

2.2.2.1. Optimizing of PBR growth conditions for a microalgal production

2.2.2.1.1. Major PBR production factors

The following parameters were investigated to optimize the vertical PBR: compounding (intensity of compounding of blending, and mixing pattern), superficial speed, gas holdup, and light-weight availableness. Lightweight availableness within the reactor plays a very important role in increasing the algal density. Lightweight availableness in bubble column is influenced by aeration rates, gas holdup, and liquid speed (mixing and turbulence) [26].

2.2.2.1.2. Light

Light utilization may be an essential issue moving the production of algal biomass. FPPBR area unit usually additional economical in daylight utilization than hollow PBRs as a result

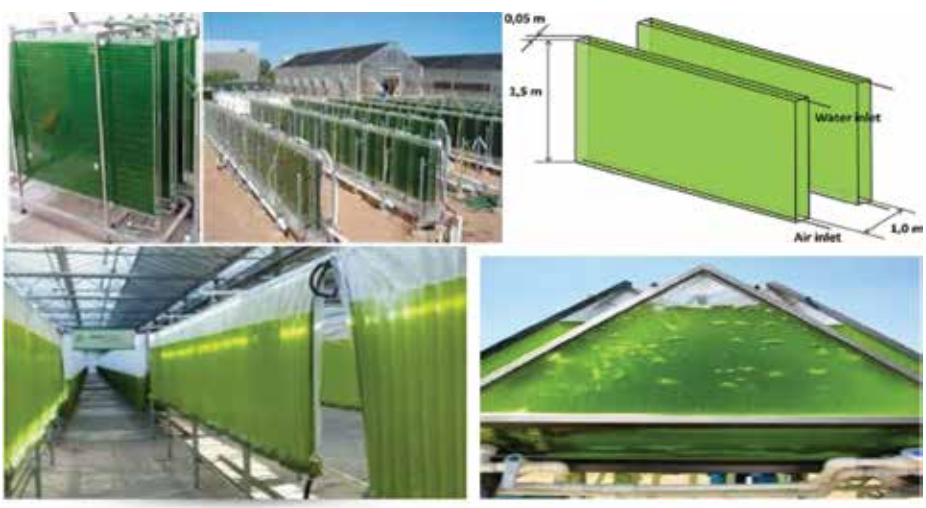

Figure 5. Flat panel photobioreactors.

of they need a wider expanse [10]. Hence, lightweight utilization will be optimized effectively by victimization flat clear panel tubes in numerous configurations and introducing lightweight via fiber optics, and crystal rectifier [27]. Future photo bioreactors have to be improved to realize most chemical action efficiencies near to the theoretical values for achieving higher biomass concentrations with stripped energy and low investment price [18, 28]. The open pond methodology used 1.5 nada alphabetic character with algal productivity 21 ton/ha, whereas hollow PBRs used three nada PE with algal productivity 41 ton/ha and flat panel bioreactor achieved the very best at five nada alphabetic character with algal productivity of 64 ton/ha. This clearly suggests that reducing the light path length (as just in case of hollow and flat panel) is useful for the economical utilization lightweight of sunshine. The orientation of PBRs with relevance the sun is additionally vital, so that the algal receive the utmost quantity of sunshine throughout the day once operated outdoors in step with Sierra et al. [29], for latitudes higher than 35°N the east-faced/west-faced orientation is favorable over north/south orientation. On the contrary, for latitudes underneath 35_N the north/south oriented reactors intercept additional radiation and the distinction is additional pronounced once nearer to the equator.

2.2.2.1.3. Mixing

Mixing keeps algal cells suspended in the nutrient media distribution, and increase gas/liquid/mass transfer to forestall oxygen accumulation, especially in hollow PBRs [18]. The role of blending is suspending algal cells in the sunshine area close to illumination [18, 30]. It will be avoided the harmful effect on the microalgal cells [31]. Mixing effectiveness of makes oxygen transfer in all PBR. There's a general accord that bubble columns and airlift systems supply tight compounding, with low shear stress. However, the sparger and baffles troublesome to clean and repair as a result of baffles connected directly with the reactor wall and hollow fibers gift in sparger create a high risk of biofouling. Circulation is another way to ensure good mixing. Masojidek et al. [32] reported that "the applied a peristaltic pump as circulation equipment to cultivate *Spirulina platensis* and obtained a cell productivity of 0.5 g/L/day, that was thought-about a comparatively high worth by the authors". Ferreira et al. [33] reported that "the utilized three completely different systems for cell circulation, specifically associate in nursing airlift, a motor driven pumping, and a pressurized system, and all over that the normal airlift system might be substituted by the other systems to cultivate *Arthrospira platensis* in hollow PBRs".

2.2.2.1.4. Economics

The PBRs cost is the man influence factor on cost for large scale biomass. The reduction of the PBR value reduced the biomass cost. Acien et al. [34] reported that "the ways to reduce value depends on the sort of algal strain, the sort of PBRs, and therefore the production technology of the biomass". The major value factors square measure irradiation conditions, mixing, photosynthetic potency of the alga, the medium and carbon-dioxide prices. The connectedness consider reducing the cost of PBR is that the consumption of raw materials. The CO_2 is the costliest expendable in production of biomass. Using flue gases from industrial sources

will scale back the cost of greenhouse emission to values as low as zero if flue gases square measure readily on the market [34]. Acien et al. [34] said "that "the bottleneck for the low cost production of microalgal is to develop a lot of productive PBR systems".

3. Conclusion

Production of microalgal at large scale is needs high giant investment and operational prices. The use of open ponds, like raceway ones, may be cheaper to create and operate than other methods, but need giant land area. On the other hand, the restrictions condition for operation of open ponds, such as high condition for temperature limitations, and light intensity, it's value lead to accentuate the efforts in developing outside PBRs. Many research has been done to develop PBRs for protects cultures from contamination, enhance PBRs technologies and enhance mass production. Also, from the economical PBRs view point, the major concerns developing new design of PBRs model have clear and high illumination surface, thus use low energy input and utilizing maximal solar energy. The good PBR models, should be possess high mass transfer rates and high biomass yields within short time of incubation. Other factors like sort of strain, the target product, geographical location, and cost of production are required.

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Conflict of interest

Authors declare no conflict of interest.

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Chapter 6

Mangroves in Contrasting Osmotic Environments: Photosynthetic Costs of High Salinity Tolerance

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Additional information is available at the end of the chapter

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Abstract

Mangrove trees of the salt secreting *Avicennia germinans* and the non-secreting *Rhizophora mangle* were investigated at the northern coast of Venezuela at a low salinity site (127 mmol kg^{-1}) and two hypersaline sites (1600–1800 mmol kg^{-1}). Leaf sap osmolality and mass/area ratio of both species were positively correlated, while size was negatively correlated with soil salinity. Leaf sap osmolality was always higher in *Avicennia* and exceeded soil solution osmolality. Salinity increased the concentration of 1D-1-O-methyl-muco-inositol (OMMI) in *Rhizophora* and glycinebetaine in *Avicennia*. The latter could make up to 21% of total leaf nitrogen (N). Nitrogen concentration was higher in *Avicennia*, but subtracting the N bound in glycinebetaine eliminated interspecific differences. Photosynthetic rates were higher in *Avicennia*, and they decreased with salinity in both species. Leaf conductance (g_l) and light saturated photosynthesis (A_{sat}) were highly correlated, but reduction of g_l at the hypersaline sites was more pronounced than A_{sat} increasing water use efficiency in both species. Lower values of ^{13}C discrimination at the hypersaline sites evidenced higher long-term water use efficiency. Apparent quantum yield and carboxylation efficiency decreased with salinity in both species. *Rhizophora* was more sensitive to high salinity than *Avicennia*, suggesting that glycinebetaine is a better osmoprotectant than OMMI.

Keywords: mangroves, *Rhizophora mangle*, *Avicennia germinans*, soil salinity, compatible solutes, photosynthesis

1. Introduction

Mangrove species in the neotropics are found along large latitudinal ranges including dry and wet coastal environments [1, 2]. Their distribution along steep salinity gradients provide

an opportunity to test *in situ* the impact of soil salinity on osmolyte accumulation in plant tissues, and analyze under similar light, temperature, and air humidity conditions, on their leaf development and photosynthetic performance.

The structural development and complexity of mangrove communities including height, leaf area index, leaf size, stem diameter, branching, litter production, and productivity are inversely related to interstitial soil water salinity. In neotropical mangroves, these properties have been shown to be strongly correlated [3–8].

Photosynthesis decreases significantly with salinity of interstitial water in several mangrove species [7–11]. Some species appear to be more sensitive to soil salinity than others, a characteristic that may be associated with specific metabolic and structural properties such as synthesis of compatible solutes, root permeability, salt excretion, and compartmentation of excess ions [12–17].

We studied the differentiation in leaf morphology, accumulation of osmotically active solutes and the photosynthetic response of two mangrove species, *Rhizophora mangle* L. and *Avicennia germinans* (L.) Stearn, reportedly differing in their salt tolerance [18–20] (**Figure 1**). These species coexist in neotropical mangroves and differ in their mechanisms of salt tolerance. *Rhizophora mangle* is considered as salt excluder, dominant in fringe mangroves throughout the neotropics, whereas *A. germinans* possesses numerous salt secreting glands in their leaves and typically dominates basin mangrove vegetation [20, 21]. Measurements were conducted under field conditions, in contrasting environments regarding fresh water availability and salt concentration of the soil interstitial water. Our objective was to assess quantitatively the impact of high salinity environments on photosynthetic performance and leaf expansion in association with inorganic and organic osmolyte accumulation.

In this chapter, we present results relating leaf sap osmolality and concentration of compatible solutes (cyclitols and glycinebetaine) to leaf morphology and patterns of gas exchange. The compatible solutes are presumably accumulated in the cytoplasm and counteract the osmotic effect of inorganic ions predominantly accumulated in the vacuole [14]. Accumulation of these compounds requires energy and carbohydrates from photosynthesis, and in the case of glycinebetaine, it needs additional amounts of N. The latter probably affects photosynthesis through the reduction of N availability for synthesis of photosynthetic enzymes.

Our hypotheses for this study were: (1) the reduction of photosynthesis resulting from salt accumulation in leaf cell sap is stronger in the species assumed to have lower salt tolerance, *R. mangle*; (2) salinity affects photosynthesis through diminished nutrient uptake, such as N, affecting protein synthesis, and phosphorus (P), possibly affecting N use efficiency; and (3) water use efficiency is higher in the high salinity (drier) environment, as a result of the combined effect of increased cell sap and interstitial soil water osmolalities on leaf conductance, leading to a proportionally larger reduction of transpiration compared to photosynthesis.

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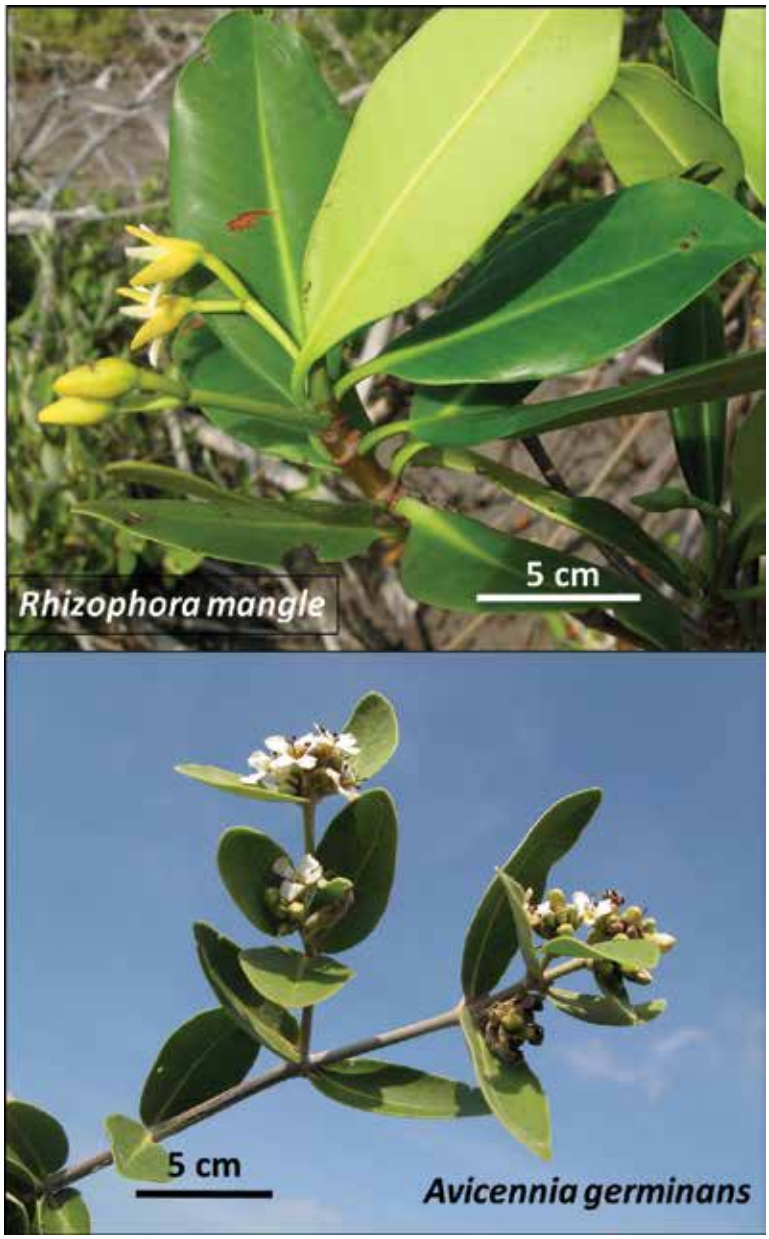

Figure 1. Flowering twig of the species studied. Notice the differences in leaf size.

2. Study sites

Field work was carried out at two locations in the Caribbean coast of northern Venezuela, both in the State of Falcón. The site, further on called Tacuato, is a low stature mangrove

stand (<5 m tall) of the species *Rhizophora mangle* and *Avicennia germinans* growing in a hypersaline lagoon (salinity >1000 mmol kg^{-1} ≈ 35 ppt) located south of the village Tacuato on the Paraguaná peninsula (11°41′40″N, 69°49′52″W). The climate is dry (<400 mm rainfall) with one rainy season from September to December. The lagoon has access to the gulf of Tacuato with an average salinity of 45 ppt (1600 mmol kg^{-1}). The water depth of the lagoon in the sampling area varied between 0 and 20 cm, depending on rainfall events and tides. Diurnal air humidity was about 70–80%, whereas day air temperatures ranged from 27 to 37°C. The tallest trees of both species in the middle part of the lagoon reached a height of 5 m. Trees used for measurements were smaller, but mature, as they were flowering and fruiting. The site was divided into two sub-sites: the fringe-region and an inner site nearly 20 m apart from the fringe, differing in their average osmolality of interstitial soil water (1600 and 1800 mmol kg^{-1}, respectively).

The second study site, further on called Ricoa, is located at the fringes of the estuary of the Ricoa River west to the village of Tocópero (11°30′21″N, 69°12′19″W). Annual precipitation is about twice that of Tacuato (970 mm in average) with peaks in May–July and November–December. The soil water salinity averaged 127 mol kg^{-1} (2–3 ppt), the diurnal air humidity was about 70–80%, like that at Tacuato; day air temperatures were in general lower with highest values around 33°C. Reduced soil salinity was a consequence of higher rainfall and the contribution of the river water run-off. At this site, the trees used for measurements were located at the estuary flood plain, and had approximately the same height as the plants used in Tacuato. Measurements and sample collection of *R. mangle* and *A. germinans* (from now on designated by their genus names) were carried out during seven field trips distributed over 9 months (from October to June), thus including dry and rainy seasons at both sites.

3. Materials and methods

3.1. Interstitial water

Interstitial water was sampled by digging 10–20 cm into the mud with a perforated plastic tube. Water salinity was determined *in situ* with a refractometer (ATAGO) calibrated with distilled water just before the measurement. Osmolality of the sampled water was calculated from salinity (in ppt) as in [22].

3.2. Gas exchange measurements

Gas exchange measurements were carried out with an open IRGA system of the type LCA 3 (ADC3, Analytical Development Co.) combined with a Parkinson leaf chamber of 6.25 cm^2. A photometer and a thermocouple attached to the chamber allowed the measurement of incoming light intensity and leaf temperature. Photosynthetic rates used for correlations with leaf conductance (g_l), and the concentrations of N and chlorophyll, were measured under natural conditions at saturating intensities of photosynthetic active radiation (PAR) ≥ 1000 μmol m^{-2} s^{-1} (A_{sat}). Leaves were oriented at 90° to the incoming radiation during measurements. To obtain a range of quantum fluxes, leaves were shaded in the field by a set of fine wire nets. The wire

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nets covered the photosynthesis chamber until g_l stabilized (2–3 min). A light response curve was a composite of measurements conducted on four leaves. Curves were fitted to the data using Sigmaplot 2.01 (Jandel Corporation 1994) and the following equation [23, 24]:

$$A = \left((\Phi Q + A_{sat}) - \text{sqrt}\left((\Phi Q + A_{sat})^2 - 4\,\theta\,\Phi\,Q\,A_{sat}\right)/2\theta\right) - R_d \quad (1)$$

where Q is the measured quantum flux and A is the rate of photosynthesis. By this procedure, we obtained the maximum photosynthetic capacity at saturating light intensity (A_{sat}) and the apparent quantum yield (ϕ).

To obtain different leaf internal CO_2 concentrations (c_i), the concentration of CO_2 in the air entering the leaf chamber was reduced stepwise below ambient by passing a part of the air flow over soda lime. Photosynthetic rates were found to be higher in the second and third leaves below the branch apex, and these leaves were used for all measurements. Photosynthesis was measured during late morning and early afternoon (10–15 hours).

3.3. Chemical analyses of the samples

After gas exchange measurements, leaves were detached and gently cleaned with a wet tissue to remove salt from their surfaces. About 7–10 leaves were used to obtain one sample. Petioles and midribs were removed. Every leaf was cut into halves of which one was put into a plastic syringe (for leaf sap extraction) and the other was put into a plastic bag (for the determination of chlorophyll, N, and P). The samples prepared in that way were immediately frozen on dry ice. Upon returning to the laboratory, they were stored in a freezer at −5°C.

Fresh mass was determined in the field by a battery powered balance (precision ±0.01 g). Samples were dried at approximately 70°C in a ventilated oven until constant weight. Total chlorophyll (a + b) ($Chlor_{tot}$) concentration of leaf disks was measured by spectrophotometry of acetone extracts [25]. Syringes containing the samples were thawed, and leaf sap was squeezed out with a pressure device [20]. Osmolality of the leaf sap was determined with a dew point osmometer (WESCOR 5500). Total P concentration was measured in acid digested dry leaf material following the procedure of Murphy and Riley [26]. Nitrogen concentration was measured using a standard microKjeldahl procedure [27]. These measurements were contrasted with the parallel analysis of calibrated leaf material (peach leaf or citrus leaf, National Institute of Standards and Technology, USA). Sample preparation for organic compounds analyses and the chromatographic determinations of cyclitols in *Rhizophora mangle* and glycinebetain in *Avicennia germinans* have been described in detail elsewhere [14, 28, 29]. Measurement of carbon isotope ratios ($\delta^{13}C$ values) of leaf material was performed at the Institute of Botany and Microbiology, University of Munich following standard procedures described elsewhere [30].

3.4. Statistical analysis of data

Significant differences between means of species and sites were tested with a one-way analysis of variance (ANOVA) and a multiple range test after Scheffé. Differences were considered

significant when $P \leq 0.05$. Differences between means of measured parameters in the two species at the same site were tested with students t-test at the level of $P = 0.05$. All statistical analyses were done using Statgraphics 5.0.

4. Results

4.1. Leaf morphology

Leaves of *Rhizophora* showed large differences between sites. They were thin and green at Ricoa, but showed a leathery texture and a yellowish color at the two hypersaline sites. At the Tacuato sites, leaves were smaller, had their edges bent downward, and showed an angle well above 45° from the horizontal. In *Avicennia*, differences in leaf morphology were not that obvious, but leaf inclination was also more pronounced in the hypersaline sites. In *Avicennia*, crystals of secreted salt could be observed on the leaf surface at both Tacuato sites.

Leaf length (L) in both species was reduced in the hypersaline sites, while leaf width (W) was reduced only in *Rhizophora* (**Table 1**). As a result, *Avicennia* leaves from hypersaline sites tended to be rounder than those of the low salinity site (smaller L/W ratio).

For both species, average area of a single fully expanded leaf was greater at the low salinity site (**Table 2**). Leaves from the two hypersaline sites differed in size only for *Rhizophora*. Reduction in leaf area from low to high salinity site was more pronounced for *Rhizophora* (37–59%) than for *Avicennia* (26–34%). Leaf dry mass decreased significantly in hypersaline sites only in the case of *Rhizophora*, but fresh mass decreased in both species. The fresh mass/dry mass ratio was higher for *Rhizophora* at all sites. Leaf dry mass/area ratios were significantly lower for both species at the Ricoa site, and the differences between species within sites were only significant in the case of Tacuato-lagoon (**Table 2**).

Site	n	Length (cm)	Width (cm)	L/W
Rhizophora mangle				
Ricoa	21	12.1 ^{a,0}	5.6 ^{a,0}	2.2 ^{a,0}
Tacuato-fringe	78	9.8 ^{b,0}	4.3 ^{b,0}	2.3 ^{a,0}
Tacuato-lagoon	41	8.6 ^{c,0}	3.3 ^{c,0}	2.6 ^{a,0}
Avicennia germinans				
Ricoa	27	7.9 ^{A,1}	2.6 ^{A,1}	3.1 ^{A,1}
Tacuato-fringe	85	5.4 ^{B,1}	2.6 ^{A,1}	2.1 ^{B,1}
Tacuato-lagoon	87	5.1 ^{B,1}	2.5 ^{A,1}	2.1 ^{B,1}

In columns, different superscript letters denote significant differences ($P < 0.05$) between sites and leaves of one species in different sites; different superscript numbers denote significant differences ($P < 0.05$) between species at the same site

Table 1. Average values of leaf dimensions from adult leaves collected at the different sites.

Species and Sites	n	Area	Dry mass	Fresh mass	Dry mass/Area	Fresh/Dry mass
		cm^2	g	g	$g\ m^{-2}$	$g\ g^{-1}$
Rhizophora mangle						
Ricoa	11	50.9 ^{a,0}	0.92 ^{1,0}	2.89 ^{a,0}	180 ^{a,0}	3.16 ^{a,0}
Tacuato-fringe	21	32.1 ^{b,0}	0.77 ^{b,0}	2.11 ^{b,0}	239 ^{b,0}	2.73 ^{b,0}
Tacuato-lagoon	15	21.1 ^{c,0}	0.50 ^{c,0}	1.28 ^{c,0}	237 ^{b,0}	2.58 ^{c,0}
Avicennia germinans						
Ricoa	9	14.8 ^{A,1}	0.28 ^{A,1}	0.75 ^{A,1}	187 ^{A,0}	2.70 ^{A,1}
Tacuato-fringe	20	10.9 ^{B,1}	0.27 ^{A,1}	0.68 ^{AB,1}	250 ^{B,0}	2.41 ^{B,1}
Tacuato-lagoon	14	9.7 ^{B,1}	0.26 ^{A,1}	0.63 ^{B,1}	267 ^{B,1}	2.38 ^{B,1}

Statistical notations as in **Table 1**.

Table 2. Area/mass relationships in adult leaves collected at the different sites.

4.2. Osmotic adaptation to salinity of the soil solution

Salinity of interstitial water differed by more than one order of magnitude between the low and high salinity sites (**Table 3**). Within the high salinity site, the Tacuato-lagoon showed always larger osmolalities than the Tacuato-fringe, because the former was not always in contact with the bay water, so that concentration through evaporation could not be compensated by tides.

Leaf sap osmolality was also higher for both species in the high salinity sites, particularly at Tacuato-lagoon, but absolute values were only 1.5–1.8 times higher than at Ricoa. Average data of **Table 3** show that osmolality was well above 100 mmol kg^{-1} higher in *Avicennia* than in *Rhizophora* for all values of soil salinity. The leaf sap-soil osmolality difference in *Rhizophora* decreased from nearly 900 mmol kg^{-1} in Ricoa to negative values approaching 100 mmol kg^{-1} in Tacuato. In *Avicennia*, the reduction of Δ leaf sap-soil was also very strong, but average values were always positive. The sap-soil differences were larger in *Avicennia*, and the differences between sites were all significant.

Site	n	Osmolality soil solution	*Rhizophora mangle*			*Avicennia germinans*		
			n	Osmolality of leaf sap	Δ sap-soil	n	Osmolality of leaf sap	Δ sap-soil
Ricoa	3	127^{a}	11	1037 ^{a,0}	914 ^{a,0}	9	1226^{A,1}	1103^{A,1}
Tacuato-fringe	6	1666^{b}	21	1631 ^{b,0}	−63 ^{b,0}	20	1859^{B,1}	180^{B,1}
Tacuato-lagoon	5	1862^{c}	15	1893^{c,0}	−92 ^{b,0}	14	2027^{B,1}	54^{C,0}

Units: mmol kg^{-1}
Statistical notations as in **Table 1**

Table 3. Average values of osmolality of soil solution and leaf sap of *R. mangle* and *A. germinans* at the different sites.

4.3. Concentration of compatible solutes

In *Rhizophora*, the main compatible solute is the cyclitol 1D-1-O-methyl-*muco*-inositol (OMMI) [29]. Its concentration in the leaf sap increased with osmolality from nearly 80 mmol L^{-1} in Ricoa to about 160 mmol L^{-1} at Tacuato (**Table 4**). The other cyclitols present in *Rhizophora* (L-quebrachitol, L-*chiro*-inositol, and D-pinitol) were present only as minor components.

The only compatible solute in *Avicennia* is the quaternary ammonium compound glycinebetaine [14]. It reached concentrations of about 120 mmol L^{-1} at Ricoa to 180 mmol L^{-1} at Tacuato. Glycinebetaine contained between 15 and 21% of total leaf N with higher values found at the hypersaline sites (calculated with values from **Tables 4** and **5**). At all sites, concentrations of glycinebetaine in the leaf sap of *Avicennia* were higher than those of cyclitols in *Rhizophora*.

4.4. Total phosphorus, nitrogen, and chlorophyll concentrations

Both total P and N concentrations per unit leaf mass were higher in *Avicennia* than in *Rhizophora* (**Table 5**). In both species, no differences in total P concentration between sites were detected. However, concentrations of P and N per unit leaf area increased with salinity in both species because of the higher leaf mass/area ratios. The N to P molar ratios varied between 23 and 29 in both species, suggesting that P was not a limiting nutrient in these soils.

Concentration of Chl_{tot} per leaf area at a given site was always higher in *Avicennia* than in *Rhizophora*, but there was not a clear pattern relating chlorophyll concentration with salinity (**Table 5**). The ratio $Chlor_{tot}$/N was higher in *Rhizophora*, but the differences disappear if the amount of N invested in glycinebetaine is subtracted, suggesting similar N allocation to photosynthetic structures. In both species, this ratio decreases significantly in hypersaline sites.

Site	n	OMMI	∑cyclitols
		mmol L^{-1}	
Rhizophora mangle			
Ricoa	11	77.2 ^{a}	88.1 ^{a}
Tacuato-fringe	19	125.4 ^{b}	141.3 ^{b}
Tacuato-lagoon	15	159.4 ^{c}	172.6 ^{c}
Avicennia germinans		Glycinebetaine mmol L^{-1}	GB N/Total N %
Ricoa	9	120.1 ^{A}	14.8 ^{A}
Tacuato-fringe	20	165.8 ^{B}	20.1 ^{B}
Tacuato-lagoon	14	178.1 ^{B}	21.1 ^{B}

OMMI = ortho-methyl-muco-inositol (other cyclitols include quebrachitol, chiroinositol and pinitol).
Statistical notations as in **Table 1**

Table 4. Concentration of compatible solutes in *Rhizophora mangle* (cyclitols) and *Avicennia germinans* (glycinebetain) in adult leaves collected at the different sites.

SITE		P		N	N	N/P	$Chlor_{tot}$	Chlor/N	GB
		μmol g^{-1}		μmol g^{-1}	mmol m^{-2}	molar	μmol m^{-2}	mmol mol^{-1}	mmol mol^{-1}
Rhizophora mangle									
RICOA	(5) *	31 ^{a,0}	(11)**	910 ^{ab,0}	161 ^{a,0}	29	328 ^{a,0}	2.04^{a,0}	
TACUATO-fringe	(5)	33 ^{a,0}	(21)	817 ^{a,0}	195 ^{b,0}	25	325 ^{a,0}	1.67^{b,0}	
TACUATO-lagoon	(5)	31 ^{a,0}	(15)	1017 ^{b,0}	240 ^{b,0}	33	383 ^{b,0}	1.61^{b,0}	
Avicennia germinans									
RICOA	(4)	50 ^{A,1}	(9)	1365 ^{A,1}	258 ^{A,1}	27	467 ^{A,1}	1.82^{A,1}	2.1
TACUATO-fringe	(5)	52 ^{A,1}	(20)	1208 ^{B,1}	304 ^{B,1}	23	359 ^{B,1}	1.19^{B,1}	1.5
TACUATO-lagoon	(5)	50 ^{A,1}	(14)	1204 ^{B,1}	324 ^{B,1}	24	411 ^{c,0}	1.29^{B,1}	1.6

*Number of samples for P.

**Number of samples for the rest of the columns.

Statistical notation as in **Table 1**.

Table 5. Average nitrogen, phosphorus, and chlorophyll concentrations of leaf samples taken from the different sites.

4.5. Photosynthetic response to light intensities and internal CO_2 concentration

Light response curves for both species showed a clear reduction in light saturated photosynthesis in hypersaline sites, 36% in *Rhizophora* and 21% in *Avicennia* (**Figure 2**). *Avicennia* had higher rates of A_{sat} than *Rhizophora* at low and high salinity sites. The reductions in photosynthetic light use efficiency (Φ) reached 45% in *Rhizophora* and nearly 37% in *Avicennia*. Light compensation points were not so much affected by salinity, although the number of curves measured (4) does not allow a definitive conclusion.

Photosynthetic response to increasing intercellular CO_2 was measured at CO_2 concentrations near that of ambient air (≈350 ppm) and below (**Figure 3**). Hence, the transition from the linear part of the curve to the plateau of CO_2 saturation was not reached. The initial slope of the curve, representing carboxylation efficiency, was steeper in *Avicennia*, and in this species compensation values were lower than in *Rhizophora*. The carboxylation efficiency was reduced at the hypersaline site by 39% in *Rhizophora* and 26% in *Avicennia*, compared to that at the freshwater site, while compensation values were nearly unchanged.

4.6. Average values of gas exchange parameters

Average light intensities and temperature recorded during the measurement of A_{sat} under natural conditions were similar for both species at each site, but the temperature was higher at the hypersaline sites (**Figure 4**). At any given site, A_{sat} was higher in *Avicennia* than in *Rhizophora*. Differences between sites were significant for both species. At Tacuato-fringe, the values of A_{sat} in both species were only about 70% of those measured at Ricoa. At the Tacuato-lagoon site, A_{sat} was even lower, especially in *Rhizophora*.

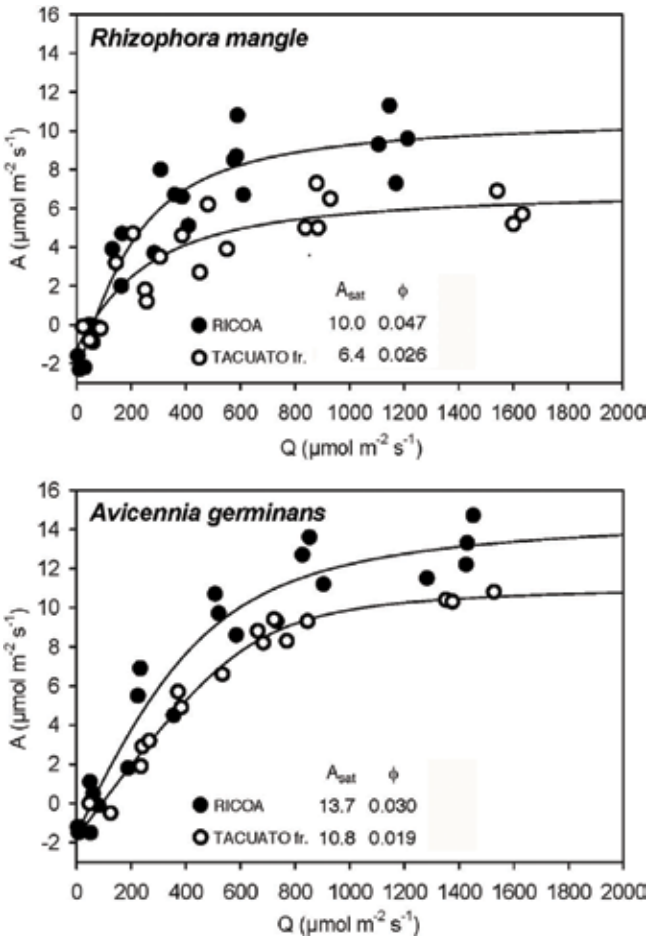

Figure 2. Photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) versus light intensity (µmol m^{-2} s^{-1}) measured in four leaves per species at each site. ϕ: Apparent quantum yield; A_{sat}: light saturated photosynthetic rate.

Leaf conductance to water vapor (g_l) showed a similar pattern to that of A_{sat}; however, relative differences between the low salinity site and the hypersaline sites were more pronounced in the former. Differences between the two hypersaline sites were significant in *Rhizophora* only (**Figure 4**). Intrinsic water use efficiency (A/g_l) was higher in both species at the hypersaline sites, maximum values corresponding to *Rhizophora* at Tacuato.

4.7. Carbon isotope discrimination

As expected, values fo $\delta^{13}C$ followed a pattern opposite to A/gl (**Figure 4**). ^{13}C discrimination (Δ) was calculated from leaf $\delta^{13}C$ values using formulation of Farquhar *et al.* [51] ($\delta^{13}C$ air = −8‰). Discrimination values were lower at the hypersaline sites for both species, whereas they showed no significant differences at the hypersaline site, between Tacuato-fringe and Tacuato-lagoon. Differences between species were only significant at the Ricoa site, with *Rhizophora* having higher Δ values. This pattern in Ricoa was expected as *Rhizophora* showed distinctly higher leaf conductance.

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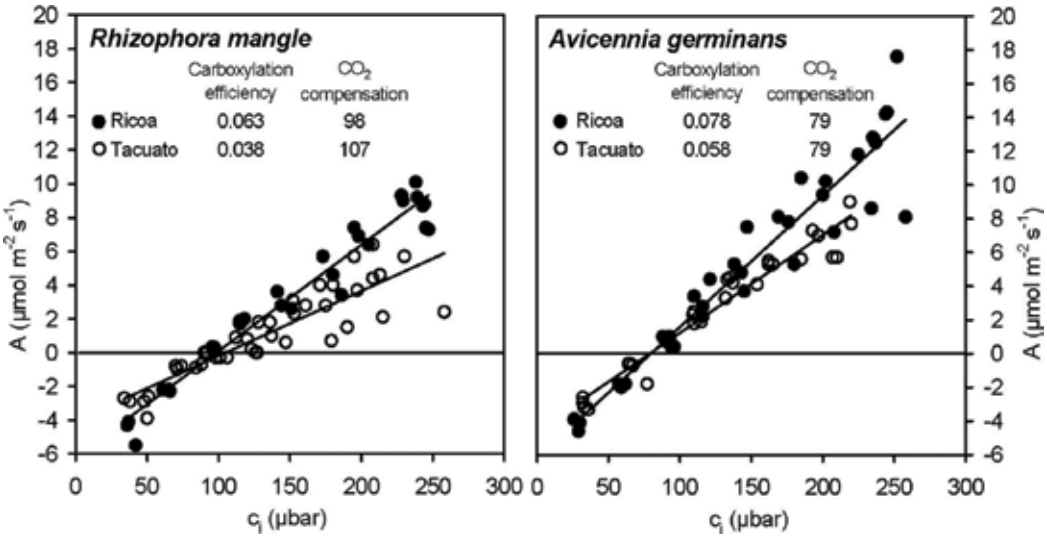

Figure 3. Dependence of photosynthetic rate on intercellular CO_2 concentration measured in four leaves from each species at each site.

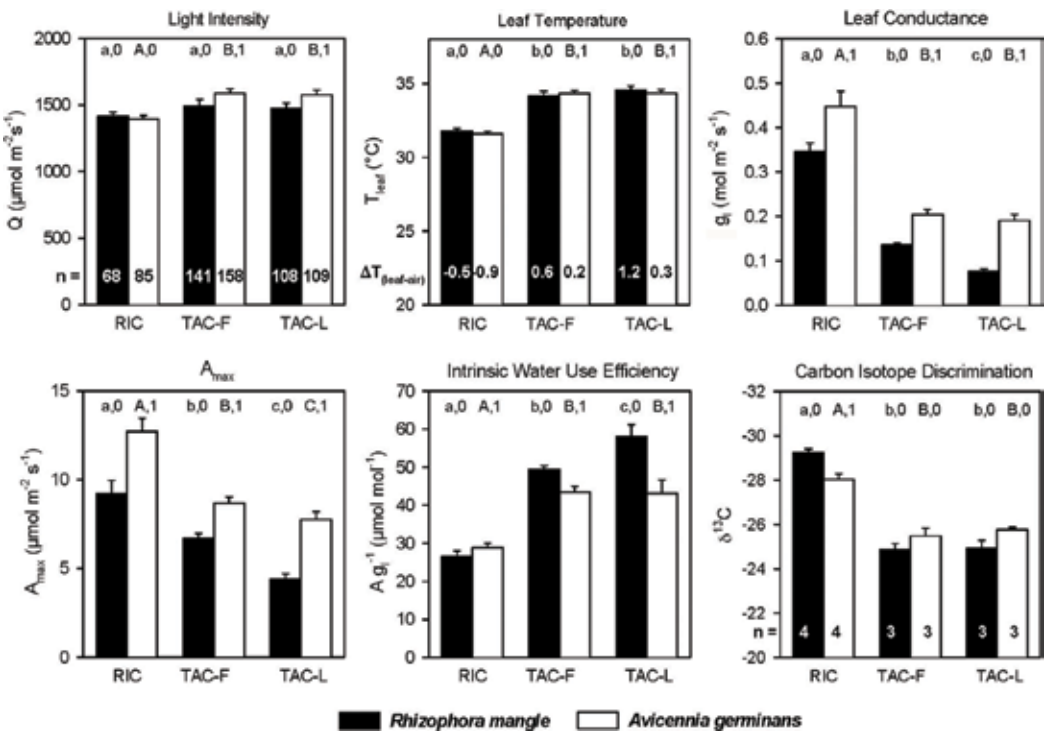

Figure 4. Average light intensity, leaf temperature, and gas exchange characteristics of the investigated species. In the upper left panel, the numbers within the columns indicate the number of leaves measured, while in the right-hand panel, they indicate the difference in temperature between the leaf and the surrounding air. On top of the columns, letters indicate significance of differences between sites for a given species. Numbers indicate differences between species at the same site.

4.8. Efficiency of photosynthetic resource use

Mass-based assimilation rate can be used as a measure of the biomass use efficiency in photosynthesis. As previously shown for A_{sat} per unit area, at any given site, *Avicennia* also showed higher assimilation rates per unit leaf dry mass than *Rhizophora* (**Table 6**). Comparing sites, values were higher at the low salinity site and decreased with salinity.

Water use efficiency was estimated as the ratio of A to transpiration E (calculated using leaf conductance, ambient relative humidity, and temperature). The ratio A/E showed the same pattern of intrinsic water use efficiency for short-term water use, that is, similar for both species at Ricoa, but higher for *Rhizophora* in the hypersaline sites.

Nitrogen use efficiency (A_{sat}/N) was lower in *Avicennia* at all sites, when using total N concentration as basis for calculation. When the amount of N bound in glycinebetaine from total N in *Avicennia* is subtracted, differences between the species disappeared at Ricoa and Tacuato-fringe, but at Tacuato-lagoon, *Rhizophora* still showed a lower A/N index.

4.9. Relationships between leaf gas exchange and specific leaf area, and osmotic properties

We calculated correlations for a complete subset of data including A_{sat} on a dry mass basis, leaf conductance, specific leaf area, osmolality, and N. For these calculations, average values of photosynthesis of the leaves pooled for chemical analyses were used. In all cases, higher correlations were found when using dry mass as a reference basis.

In both species, leaf conductance, specific leaf area, and N concentration were positively, whereas osmolality was negatively, and significantly correlated with photosynthetic rate (**Table 7**). The N-photosynthesis correlation in *Avicennia* increased from 0.67 to 0.79 when the glycinebetaine-N was subtracted from total N.

	n	Asat	A/E	A/N	A/N-GlBet.N	A/Chlor
		μmol CO_2 g^{-1} s^{-1}	mmol mol^{-1}		μmol CO_2 mol^{-1} s^{-1}	
Rhizophora mangle						
Ricoa	11	51.6 ^{a,0}	1.5 ^{a,0}	56.7 ^{a,0}	—	27.7 ^{a,0}
Tacuato-fringe	21	28.3 ^{b,0}	2.5 ^{b,0}	34.6 ^{b,0}	—	20.9 ^{B,0}
Tacuato-lagoon	15	18.6 ^{c,0}	2.8 ^{c,0}	18.4 ^{c,0}	—	11.7 ^{C,0}
Avicennia germinans						
Ricoa	9	68.5^{A,1}	1.7 ^{A,0}	49.7 ^{A,0}	58.8 ^{A,0}	27.2 ^{A,0}
Tacuato-fringe	20	35.0^{B,1}	2.3 ^{B,1}	28.7 ^{B,0}	34.6 ^{B,0}	24.1 ^{A,1}
Tacuato-lagoon	14	29.4^{B,1}	2.2 ^{B,1}	24.3 ^{B,1}	28.1 ^{B,1}	18.8 ^{B,1}

A: Maximum photosynthetic rate; E: Transpiration rate, N: Nitrogen concentration; N-GlBet. N: Nitrogen concentration minus nitrogen bound in glycine betaine; Chlor: Total chlorophyll concentration.
Statistical notations as in **Table 1**.

Table 6. Efficiency of resource use in photosynthesis.

Photosynthesis	*Rhizophora mangle*	*Avicennia germinans*
μmol CO_2 kg^{-1} dry mass. s^{-1} vs	(n = 47)	(n = 43)
Leaf conductance (mol m^{-2} s^{-1})	0.91***	0.90***
Specific leaf area (m^{-2} g^{-1} dry mass)	0.79***	0.88***
Osmolality (mmol kg^{-1})	−0.79***	−0.81***
Nitrogen (μmol g^{-1} dry mass)	0.78** (n = 11)	0.67***
N-glycinebetaine (μmol g^{-1} dry mass)	—	0.79***

Statistical significance:**(P = 0.05);
***(P = 0.01).

Table 7. Correlation coefficients between saturated photosynthesis under field conditions and specific leaf area, and leaf conductance to water vapor and nitrogen concentration.

5. Discussion

5.1. Leaf morphology, leaf size, and leaf water content

A conspicuous visual feature observed in the field was that leaves had a high degree of inclination at the hypersaline sites. Ball *et al.* [31] showed that the high degree of leaf inclination found in mangrove species in nature effectively reduces the intensity of radiant heat loading. Furthermore, in both species, a marked reduction in leaf area in hypersaline sites was observed. This may also improve the energy balance in saline and dry sites, as in smaller leaves convectional cooling is more effective.

Lin and Sternberg [32] found a reduction in leaf size in dwarf scrub mangroves in contrast to tall growing fringe mangroves in Florida. Salinity, as well as nutrient level, may cause reductions in leaf area while neither sulfide nor original growth form had an influence [33].

In the present study, reductions in leaf area and dry mass associated with salinity were, respectively, 59 and 46% in *Rhizophora* compared to the much lower reductions of 34 and 7% in *Avicennia*, suggesting a higher salt sensitivity in the former species. In *Avicennia*, not only leaf size but also leaf shape was affected by high salinity, leaves of high salinity sites being rounder than those of low salinity sites.

Larger leaf dry mass/area ratios at the hypersaline sites have been reported before and attributed to increased succulence [34, 35, 36], although there was also evidence for scleromorphy [34]. We observed in both species a significant decrease in the fresh mass/dry mass ratio that together with the increase in leaf mass/area ratio are rather symptoms of scleromorphy than of succulence. Generally, higher leaf mass/area ratios increase heat capacity and may be of importance in controlling leaf temperature [31].

5.2. Osmotic adaptation

Increases of leaf sap osmolality with soil salinity in both species counteracted the lower soil water potential at the hypersaline sites. In *Rhizophora*, differences between leaf sap and soil

solution osmolalities were small or negative at the hypersaline sites, whereas in *Avicennia* differences were always positive. Scholander *et al.* [21], and Walter and Steiner [37] found that the osmotic potential in mangrove leaves exceeded that of the seawater surrounding them. However, in a field study in Venezuela, Rada *et al.* [38] found that turgor loss occurred at midday in leaves of *Conocarpus erectus* and *Rhizophora mangle* during drought periods. We did not measure water potential in the investigated plants, but both conductance and photosynthetic rates measured did not indicate turgor loss even at the most stressful sites.

At the low salinity site for both species, osmolalities of the leaf sap were found to be about 10 times higher than that of the soil solution, indicating their halophytic (salt accumulating behavior) character [15, 17]. The generally larger values of leaf sap osmolality in *Avicennia* are consistent with the higher salinity tolerance of species of this genus [13, 14, 19, 20, 37].

Concentrations of compatible solutes were clearly correlated to soil salinity and were within the range reported in other field studies [14]. Glycinebetaine concentrations were higher than those of cyclitols at each site, and in both cases their concentration increased with leaf sap osmolality. This is in accordance with their postulated role in keeping osmotic equilibrium between cytoplasm and vacuole. However, the cyclitol/osmolality ratio was 1.25 in *Rhizophora,* while the glycinebetaine/osmolality ratio in *Avicennia* was only 0.7, suggesting a higher osmoprotective efficiency of this compound.

5.3. Phosphorus, nitrogen, and chlorophyll

Studies of nutrient availability in soils under several mangrove stands showed that P availability may be limiting growth, especially under oxidized conditions of well drained soils [39]. Growth limitation by P was confirmed by fertilization studies on dwarf red mangrove in Belize [40], where an increase in growth was brought about only by application of NPK or P. In the present study, leaf P concentration did not differ between sites but was always higher in *Avicennia* compared to *Rhizophora*. Besides, N to P molar ratios were below 35 suggesting that P supply was not limiting mangrove growth in the study sites.

Leaf N concentrations of *Avicennia* were significantly higher than those of *Rhizophora* as has been found earlier [20, 41] and reported for Australian species of these genera by Popp *et al*. [14]. Part of the difference can be explained by the amount of glycinebetaine in *Avicennia,* representing 15–21% of total leaf N. Differences in N concentration between species disappear when this fraction is subtracted from total N.

Differences in N concentrations between sites were mainly related to differences in leaf mass/area ratio in both species. While N concentration decreased with salinity when calculated on a dry mass basis, they increased based on leaf area. *Rhizophora* leaves at Tacuato-lagoon, however, showed a higher concentration of N per leaf area as well as per g dry mass, indicating a strong reduction in nitrogen use efficiency.

Chlorophyll concentrations per leaf area were similar or lower than those reported earlier for the same species in dry and wet habitats [20], or for mangrove species in Australia and India growing on a range soil salinities [42, 43]. The average Chl_{tot}/N ratios decrease markedly in hypersaline sites, pointing to a reduction in N investment in photosynthetic structures due to salt stress. Besides, these ratios were low compared with earlier reports on these species [20], a fact perhaps related to the much lower leaf N/P ratios found in the

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present study. Values of Chl_{tot}/N at a given site did not differ significantly between species, when the amount of N bound in glycinebetaine in *Avicennia* was subtracted from total N. It seems that under similar salinity stress, both species invest a similar N fraction into the construction of photosynthetic structures.

The fractional investment of leaf N into chloroplast protein-pigment complexes can be calculated using the N to chlorophyll ratio in thylakoids estimated by Evans [44] (50 mol thylakoid N/mol chlorophyll). Both species had values ranging from 9% of leaf N in low salinity sites to 5–6% in high salinity sites. *Rhizophora* had always slightly higher values than *Avicennia*. Those values are about half of the average reported for lowland trees in humid tropical forest (107 species, 23.7 ± 0.8% of leaf N) [45]. The large difference underscores the photosynthetic cost of high salinity tolerance.

5.4. Photosynthetic capacity

Avicennia showed consistently higher assimilation rates than *Rhizophora*, in accordance with previous reports on other species of the same genera [11, 46, 47]. Our results showed that both species had lower A_{sat} at the hypersaline sites. However, the depression of A_{sat} related to high salinity was more pronounced in *Rhizophora*.

Light saturated photosynthetic capacity reflects the maximum possible benefits from a given investment in photosynthetic machinery [48]. Zotz and Winter [49] showed a linear relationship between diurnal carbon gain and maximum rate of CO_2 uptake in a range of rainforest canopy species. This would explain the low growth rates and the shrubby stature of the plants at the hypersaline sites. In addition, as constructing and maintaining photosynthetic machinery is energetically expensive, photosynthetic capacity should be tuned to the constraints of the environment [48]. The most prominent factor in mangrove habitats is salinity. In *Aegiceras corniculatum* and *Avicennia marina*, photosynthetic capacity was found to decrease with increasing salinity [50, 51], and A_{sat} was negatively related to salinity in a range of mangrove species under field conditions [11]. Other environmental factors such as low nutrient availability [33] and temporal variation in salinity [52] are also known to depress maximum assimilation rate of mangroves. Extreme low values of A_{sat} in *Rhizophora* leaves at Tacuato-lagoon may be related to a combination of salinity with one or more of the latter mentioned factors.

Values of light saturated A calculated from light response curves confirmed that the photosynthetic capacity was generally higher in *Avicennia* compared to *Rhizophora* and was reduced in both species at the hypersaline sites.

Quantum yield (ϕ) on an incident light basis was depressed at the hypersaline sites in both species. Björkman *et al.* [46] found that quantum yield in mangrove leaves decreased due to the combination of low leaf water potentials with high irradiance; whereas salinity, and the resulting leaf water deficit, had no negative effect on the quantum yield of mangrove leaves protected from direct sunlight.

The C_i-A_{sat} relationships obtained for CO_2 concentrations equal or below ambient were linear, with a slope proportional to the *in vivo* activity of Rubisco (carboxylation efficiency, CE [53]). The lower CE of both species at the hypersaline site indicates that decreases of A_{sat} with salinity were not only due to stomatal limitation, but also due to the result of changes in the biochemical properties of photosynthesis.

CO_2 compensation points were higher in *Rhizophora* than in *Avicennia*, suggesting higher photorespiration rates in the former species. However, species characteristic compensation values were similar at Tacuato and Ricoa. Similar results were obtained by Ball and Farquhar [12] with mangrove species grown at different salinities.

5.5. Water use and N use efficiency in photosynthesis

At the low salinity site, g_l was significantly higher for both species. The generally higher values of g_l of *Avicennia* compared to those in *Rhizophora* are correlated with their assimilation rates. At the hypersaline sites, g_l was reduced to a greater proportion than A_{sat} in both species, but in *Rhizophora*, the reduction of these parameters was more pronounced indicating the lower salinity tolerance of this species.

Intrinsic water use efficiency (A/g_l) evaluates the role of biological components in determining water-carbon exchange relationships [50, 51]. In both species, A/g_l was higher at the hypersaline sites, because of the relatively larger reduction of g_l compared to A_{sat}. Values of A/g_l were similar to those calculated from data from Smith *et al.* [54] for *Avicennia germinans* and *Conocarpus erectus* at a coastal site in northern Venezuela and to those calculated from data from Lin and Sternberg [32] for *Rhizophora mangle* at a site in coastal Florida. In an extensive field study, Clough and Sim [11] found higher water use efficiency in mangroves with increasing salinity and decreasing air humidity. As air humidity in our study did not differ much between sites, changes in A/g_l with salinity were less drastic than in the study mentioned above.

Water use efficiency was higher in *Avicennia* at the low salinity site, but it was higher in *Rhizophora* in the high salinity sites. More conservative water use in the latter species at the hypersaline sites is probably related to its non-salt secreting character. Water loss is minimized as salt exclusion mechanisms at the roots impose a large resistance to water flow [55]. The higher water use efficiency in *Avicennia* at the low salinity site may be related to the association of this species with the more saline soils [15]. However, as in *Avicennia*, leaf-to-soil osmolality difference was at least 50 mmol kg^{-1} at the hypersaline sites, and as accumulation of NaCl can be counteracted by salt secretion, and in this species restriction of water loss from leaves under hypersaline conditions was lower than in *Rhizophora*.

Nitrogen use efficiency in photosynthesis based on total leaf N was higher in *Rhizophora* at all sites. Similar results were reported by Alongi *et al.* [5] in Australian mangrove forests of *R. stylosa* and *A. marina*. However, those differences disappear if the amount of N invested in glycinebetain is subtracted from the total amount of N. In both species, the NUE decreases in hypersaline sites. In an experimental study, Cardona-Olarte *et al.* [16] did not find differences in WUE based on gas exchange of seedling grown in nutrient solutions with salinities between 10 and 40 ppt; however, PNUE decreased from about 85 μmol/mol N at 10 ppt to nearly 60 at 40 ppt.

5.6. Carbon isotope discrimination

The carbon isotope ratio $\delta^{13}C$ is related to a long-term average of c_i and can be taken as an indicator of water use efficiency [56, 57]. Values of $\delta^{13}C$ ranged between −24.3‰ and −29.4‰ (Δ = 16.7 to 22.1‰), with a larger variation found in *Rhizophora*. They were well within the

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range reported for mangroves in the literature [9, 20, 32, 47, 58]. These results confirmed for the long-term, the patterns found for short-term water use efficiency discussed above.

6. Conclusions

The relationships between the set of physiological properties associated with high salinity stress in both mangrove species studied here can be depicted along two sequences of events operating simultaneously (**Figure 5**). Increases in interstitial water salinity affect

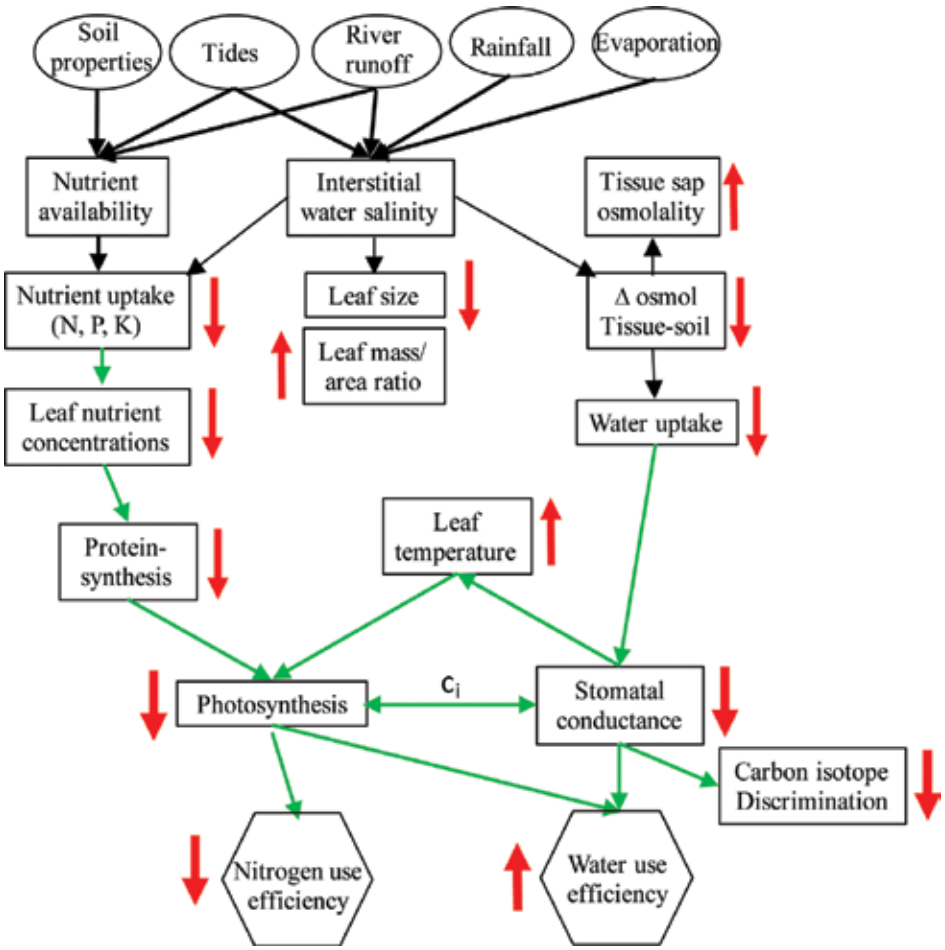

Figure 5. Scheme depicting the assumed sequence of events affecting photosynthesis and resource use efficiency caused by exposure to high salinity conditions. The driving forces for environmental salinity are encapsulated as tides (sea water supply), rainfall (dilution and washing-out effects), atmospheric evaporative demand (air water saturation deficit), and soil properties. Thick red arrows indicate the direction of change resulting from long-term exposure to high salinity. Thin black arrows indicate the plant-environment interface. Green arrows depict the hypothesized dependence of biological processes triggered by increases in cell sap osmolality and leaf nutrient status. The connections between the boxes are not necessarily linear, and processes affected may show differential sensitivity toward interstitial soil water salinity. Generally, mangrove-environment interactions under high salinity conditions lead to higher water use and lower N use efficiencies.

essential nutrients uptake and salt accumulation, determining increases of tissue sap osmolality. Both processes lead to a nutritional limitation of photosynthesis resulting in a strong reduction of nitrogen use efficiency for growth. In addition, the differential soil–plant osmotic potential decreases reducing the amount of water available for transpiration, and inducing an accumulation of compatible solutes that protect cytoplasmic organelles from dehydration and toxic ionic effects. As a result, leaf conductance is reduced to a larger proportion than photosynthesis, thereby increasing leaf temperature and water use efficiency. The connections between the boxes of **Figure 5** are not necessarily linear, and processes affected may show differential sensitivity toward interstitial soil salinity (as shown in the ratio of conductance to photosynthesis). In the processes documented in the present chapter, *Rhizophora* appears to be more sensitive than *Avicennia*, and we speculate that the ultimate cause of this difference may reside in the higher efficiency of glycinebetaine as an osmoprotectant compared to cyclitols.

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Section 3

Future Improvements in the Production of Photosynthetic Organisms

Chapter 7

Plant Nanobionics and Its Applications for Developing Plants with Improved Photosynthetic Capacity

Kusum Khatri and Mangal S. Rathore

Additional information is available at the end of the chapter

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Abstract

In the present scenario, the ever-growing human population, a decreasing availability of land resources and loss of agricultural productivity are the major global concerns, and these possess a challenge for scientific community. To feed the increasing world population, an increase in the crop productivity with available land resources is one of the essential needs. Crop productivity can be increased by engineering the crop plants for tolerance against various environmental stresses and improving the yield attributes, especially photosynthetic efficiency. Nanomaterials have been developed with new functional properties like improved solar energy harvest. With these nanomaterials, nanobionic plants were developed by the facilitated kinetic trapping of nanomaterials within photosynthetic organelle, that is, chloroplast. The trapping of nanomaterials/nanotubes improved chloroplast carbon capture, that is, photosynthesis by improving chloroplast solar energy harnessing and electron transport rate. Besides improving photosynthesis, nanotubes like poly(acrylic acid) nanoceria (PAA-NC) and single-walled nanotube-nanoceria (SWNT-NC) decrease the amount of reactive oxygen species (ROS) inside extracted chloroplast and influence the sensing process in plants, and these are beneficial for a number of physiological processes. The nanobionic approach to engineer plant functions would lead to an era of plant research at the interface of nanotechnology and plant biology. In this chapter, nanobionic approach, transfer of nanomaterial to plants and their offspring and its potential applications to improve photosynthesis will be discussed.

Keywords: nanobionics, photosynthesis, productivity, stress, sustainability

1. Introduction

Nanotechnology is an emerging field of natural science dealing with materials of nano (1–100-nm) scale. NASA defined nanotechnology as 'the creation of functional materials,

devices and systems through a control of matter on the nanometre scale and exploitation of novel phenomena and properties (physical, chemical, biological) at that length scale' [1]. The different applications of nanotechnology include the designing, characterization, production and application of structures, devices and systems. Nanomaterials (NMs) have unique properties like high surface area and improved optical property. For a chemical or a biological reaction, the rate of reaction depends on the surface area of the reactants, and due to the large surface area, nanomaterial-mediated reactions operate at a high rate. Plant biology is one of the oldest branches of science, aiming the study of different aspects of plants. The combination of plant biology and nanotechnology resulted in nanobionics which employs the nanotechnology for the improvement of plant productivity by improving plant growth, development and photosynthetic efficiency [2]. During the synthesis of materials at nanoscale, different properties of these materials get altered, and these altered properties get translated in various applications. Nanobionics is one of the important applications of nanotechnology which involves the improvement of plant or plant productivity using nanomaterials. The nanomaterial can be prepared by direct and synthetic route followed by milling, grinding, homogenization at high pressure and sonication to reduce its size at nanoscale [3, 4]. With unique physicochemical properties, that is, high surface area, high reactivity, tunable pore size, and particle morphology of nanoparticles, the nanomaterials have a large scope of novel application in the field of biotechnology and agricultural industries [5]. The nanomaterials are of different types:

Natural nanomaterial—Materials created independently without the involvement of human being. The natural nanomaterials are sea salt, sea spray, soil dust, volcanic dust, sulphates from biogenic gases, and so on.

Anthropogenic (adventitious) nanomaterial—Material created as a result of human action. The welding fume and particulates (sulphates and nitrates) resulting from the oxidation of gases [6], and soot resulting from the combustion of fossil fuels are the best example of anthropogenic nanomaterial.

Engineered nanomaterial—Nanomaterial designed and manufactured with human interest. The engineered nanomaterials are of organic and inorganic nature.

As the name indicates, the organic nanomaterials consist of carbon atom itself [7] and are polymeric structures with specific nano-characteristics, while inorganic nanomaterials are inorganic by nature. The engineered nanomaterials are of scientific interest because of their huge potential for different applications. The engineered nanomaterials are classified as carbon-based nanomaterials (NMs), metal-based NMs, metal oxides, dendrimers and composites [8]. The nanotubes are linear materials with nanometre size. Carbon nanotubes are long, thin cylinders of carbon molecules having good conductivity of heat, high strength and different electrical properties. The carbon nanotubes (**Figure 1**) are single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs). The double-walled carbon nanotubes are known for higher thermal and chemical stability as compared to single-walled carbon nanotubes [9]. Inorganic nanomaterials are inorganic by nature and consist of metals and metalloid oxides, quantum dots (QD), dendrimers having different kinds of features such as nanofibres, nanowires and nanosheets.

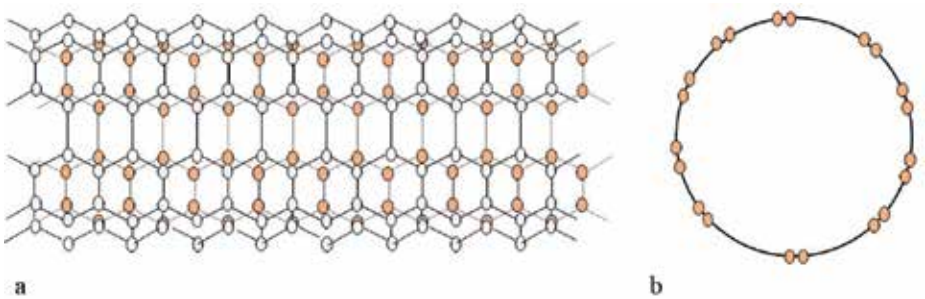

Figure 1. Single-walled carbon nanotube (a) and a cross section of single-walled carbon nanotube (b).

Nanobiotechnology is an emerging field of bioengineering and has enormous potential to modify or augment the plant function by employing the nanomaterial. With nanobiotechnological advancement, plants (1) are capable of imaging objects in their environment, (2) self-powering themselves as light sources, (3) with infrared communication devices and (4) having self-powered groundwater sensors developed [10]. The solar energy harnessing and biochemical sensing can be improved in plants by introducing nanomaterial in them [11], and nanobionic plants were developed for enhanced photosynthesis and biochemical sensing. Nanobionic plants can detect various chemicals present in the environment and have potential use as a plant-enabled sensor for monitoring environmental changes. The nano-encapsulated nutrients commonly referred to as nanofertilizers release the nutrients on demand basis, and thus these are beneficial for crops to regulate plant growth and enhance the target activity [12, 13]. The engineered carbon nanotubes are shown to boost seed germination, growth and development in plants [14, 15]. Comparatively, very few studies have been conducted on nanoparticles which are beneficial to plants. Nanotechnology has a great potential to develop new tools for the incorporation of nanoparticles into plants to augment the existing functions [16].

1.1. Entry of nanoparticles in plant cells

The characteristic feature of plant cell is its cellulosic surrounding, that is, cell wall. The plant cell wall behaves as a barrier for superficial ingression of different external agents including nanoparticles into plant cells. Cell wall possesses pores which provide sieving properties to cell walls, and this range from 5 to 20 nm [17]. Nanoparticles or aggregates of nanoparticles with a diameter less than the pore diameter of the cell wall could pass through pores and can reach the plasma membrane [18]. There is additionally a chance for the enlargement of pores or the induction of new cell wall pores upon interaction with engineered nanoparticles which in turn enhance nanoparticle uptake. Further internalization of nanoparticles or aggregates of nanoparticles occurs through endocytosis by forming a cavity-like structure surrounding the nanoparticles by a plasma membrane. Alternatively, they may cross the membrane via carrier proteins or through ion channels. In the cytoplasm, the nanoparticles may bind with different cytoplasmic organelles and interfere with the metabolic processes [19]. In leaf surface applied nanoparticles, the nanoparticles enter through the stomatal apertures or through the bases of trichomes and thereafter get translocated to tissues [20, 21, 22]. The nanoparticles penetrate

the plant cell wall and enter into the space between plant cell wall and plasma membrane due to small size, capillary action and Van der Waals forces.

1.1.1. Uptake and distribution of nanomaterials in plant cell

Improvement in an agronomic attribute of plant system, that is, photosynthetic efficiency with the help of nanomaterial, needs a successful uptake and transfer of nanomaterials in plant cell. The plant cell wall has pores of an average diameter of 5–20 nm. These pores allow the passage of solutes while constraining the diffusion of massive particles and macromolecules including some enzymes [23]. Plants cell employ several strategies to avail nanomaterial (carbon nanotubes) through cell wall and cell membrane depending on the size of the nanomaterial. The entry of nanoparticle in plant cells depends on size and charge [24, 25]. The single-walled carbon nanotubes are of 1–2 nm and are smaller than cell wall pores (5 nm). These nanotubes could be perceived directly through a spontaneous leakage into the apoplast [26]. Thus, for spontaneous leakage, the single-walled carbon nanotubes must be truncated to a commensurable size [27]. The introduction of wide-diameter carbon nanotubes into walled plant cells could also occur through local hydrolysis of the cellulosic cell wall. The cellulose molecules immobilized on the surface of carbon nanotube generate local lesions in the cell wall which facilitate the uptake of carbon nanotubes [28].

The leakage of carbon nanotubes through the cell wall pores has been reported in cells of *Nicotiana tobacum* and *Catharanthus roseus* [29, 30]. The first experimental evidence for the internalization of single-walled carbon nanotubes (SWNTs) has been shown in *N. tobacum* [31]. Temperature-dependent uptake of single-walled carbon nanotubes in *N. tobacum* suggests the internalization of nanotubes through endocytosis [30]. On the other hand, it has been reported that there is no effect of temperature and light on SWNT transfer to lipid bilayer [11]. Multi-walled carbon nanotubes (MWCNTs) could also penetrate the cell membrane of plant protoplasts [30]. When MWCNTs are in close vicinity of protoplast of *C. roseus*, the nanotube aggregations increase the tonicity of cell medium and facilitate the penetration of MWCNTs. Active transport of nanoparticles has also been reported through the lipid bilayer [32].

The metal oxide nanoparticles may be transported through root to leaf or leaf to root in plants [33], and it was studied in hydroponic [34] and soil [35] culture. The negatively charged nanoceria translocates at a higher rate from root to leaf as compared to positively charged nanoceria [36]. The metal oxide nanoparticles are absorbed by root endodermis through apoplastic and symplastic routes, and these are then transported to stem, leaf, fruit and grains [37–39] through a vascular cylinder [40]. Similarly, the mono-dispersed mesoporous silica nanoparticles penetrate into the roots through symplastic/apoplastic pathways and then to the aerial parts of the plants through vascular system [41]. The uptake of metal oxide nanoparticle has been shown by seeds [42], seedlings [38] and mature tubers [43]. The metal oxide nanoparticles may enter through leaf stomata or cuticle and then to stem and root through phloem sap [44, 45]. The single-walled carbon nanotubes and nanosheets

are transported into cultured plant cells by endocytosis or internalized in plant root cells via non-endocytic pathways [31, 46]. Silver nanoparticle enters in *Arabidopsis* protoplasts through mechanosensitive channels [47].

1.1.2. Uptake of nanomaterial by organelles

Different cellular organelles have been reported to uptake the nanomaterials. Serag et al. [48] reported the vacuolar uptake of SWNTs by labelling the SWNTs with fluorescein isothiocyanate (FITC). Following incubation of plant tissues with FITC-labeled SWNTs, fluorescence signals were detected in the cell vacuoles. Further measurement of diffusion coefficient (Deff) supported vacuolar accumulation. To confirm vacuolar uptake, the Deff was measured using fluorescence recovery in a photobleached area (FRAP). The Deff varied according to the size of a macromolecular complex containing fluorescent label. FRAP helped to study the fractions of molecules capable of recovering in the photobleached area and confirmed the accumulation of SW-F inside the vacuoles [48]. Further, the use of probenecid, an inhibitor of carrier-mediated transport, indicated the accumulation of SW-F in vacuoles.

SWNTs transport passively through chloroplast lipid bilayer through kinetic entrapping or by disrupting lipid bilayers [11, 49]. As SWNTs come in contact with the chloroplast's outer envelope, it wraps around the glycerolipid (forming most of the chloroplast's outer envelope). As nanotubes perforated through the envelopes, they are covered with a layer of lipids that irreversibly binds them to the interior side of the chloroplast. The formation of temporary pores has been noticed in the plasma membrane to internalize the nanoparticles like quantum dots and silica nanosphere [49, 50]. Also, the negatively or positively charged nanoparticles spontaneously penetrate lipid envelopes of the extracted chloroplasts [51].

1.2. Generational transmission nanomaterials

The generational transmission of nanomaterials was studied in rice [52] using a bright field microscopy. Tissue of rice plants at various developmental stages were sampled, washed, sectioned and imaged to track the transmission after 1 week of incubating in 20 mg l^{-1} C_{70} solution. Black aggregates were frequently observed in seeds and roots and less frequently in stems and leaves which indicated that the sequence of nanoparticle uptake was from the plant seeds and roots to stems and leaves. The appearance of black aggregates was mostly found in and near vascular system. It was suggested that the transport of C_{70} occurred simultaneously with the uptake of water and nutrients in the xylem [52]. Further, to investigate generational transmission of nanomaterials, mature seeds from the control plants and C_{70}-treated plants were germinated and second generation was raised. In second generation, black aggregates were also spotted in the leaf tissues, however, with much less frequency [52]. The results were supported by Fourier transform (FT)-Raman and IR spectra from both first- and second-generation rice plants.

2. Employment of nanotechnology for the improvement of photosynthetic activity and plant productivity

Photosynthesis is the most fundamental and vital physiological process in plant kingdom. It converts the light form of energy into chemical form in chloroplasts using chlorophyll and CO_2 and H_2O as raw materials, and stores in the bonds of sugar molecules. This form of energy is later used as the energy currency to regulate various processes. In green plants, chloroplasts are the site of synthesis for chemical energy, that is, carbon-based fuels. With the help of light energy, the captured atmospheric CO_2 is converted into different forms of sugars [53]. Photosynthetic apparatus utilized less than 10% of the sunlight [54], and there are possibilities to improve the solar energy conversion efficiency in photosynthetic organisms. The improvement in photosynthetic efficiency requires broadening the range of solar light absorption [55] particularly in the near-infrared spectra which are able to penetrate deeper into living organisms. With unique properties and higher stability, the nanomaterials can form chloroplast-based photocatalytic complexes having an enhanced and improved functional property under *ex vivo* and *in vivo* conditions [11]. It is clear that neither all the absorbed photons are involved in electron flow under intense light conditions nor chloroplast captures maximum solar energy under non-saturating light [56, 57]. The SWNTs have discrete optical and electronic properties and a broad range of absorption spectra (ultraviolet, visible and near-infrared). The enhancement of light reaction after the insertion of SWNTs in chloroplasts isolated from commercially available baby spinach leaves (*Spinacia oleracea* L.) has been observed [11]. Chloroplast does not have a broad range of absorption spectra and it cannot absorb spectra outside its absorption ranges of spectra. The boosted photosynthetic reactions might be attributed to electronic bandgap of semiconducting the SWNTs which converts the absorbed solar light into photosynthetic excitons [58]. Depending on their inherent light interaction capabilities, nanoparticles (NPs) interfere and alter the photosynthetic efficiency, photochemical fluorescence and quantum yield in plants. Keeping up with the importance of process, the researchers attempted either to mimic the process of photosynthesis artificially or to improve the existing efficiency in planta using nanotechnology-based inventions. The plants have been augmented to harvest more light energy by delivering carbon nanotubes into chloroplast. These carbon nanotubes serve as artificial antennae allowing chloroplast to capture wavelengths of light outside the normal range, that is, ultraviolet, green and near-infrared [11, 16]. Various reports are available on the enhancement of photosynthetic activity in plants through *in vivo* or *ex vivo* approaches. In subsequent text, a few cases will be highlighted to show the relevant progresses made by a nano-technologist for the improvement of agronomic attribute.

Plant photosystems include reaction centres (RCs) and the antenna chlorophylls; they are held in the membrane by weak intermolecular interactions. The antenna chlorophyll absorbs photons and transfers to the RCs and then electrons are transferred to the next electron acceptor. Naturally, photosynthetic machinery absorbs light within certain wavelength intervals. It has been reported that if nanoparticles conjugate with these RCs and antenna chlorophyll, there is an exciton enhancement effect [59]. Nanoparticle conjugate with light-harvesting complex absorbs a wider range of wavelength interval. Nanomaterials conjugated with a

photosynthetic system strongly increase the rate of production of excited electrons due to the plasmon (metal nanoparticle having an oscillating free electron) enhancement effect. This excited electron can be used for photocurrents or chemical reactions. The association of metal nanoparticle with photosynthetic system has been reported to enhance the efficiency of photosystem. The incorporation of metal nanoparticles with light-absorbing chlorophyll molecules enhances the photon field which is referred as plasmon enhancement effect. Thus, the production of exited electrons has been reported to increase due to plasmon resonance and electron-hole separation [60]. In support of this, experimental proofs were generated for the increased rate of the formation of ATP molecules. With hybrid structure, the rate of formation of the excited electron was reported to enhance as compared to photosystem alone [61]. Artificial structures composed of a photosynthetic system and various metal nanoparticles also display strong enhancements of photosynthetic efficiency, and this cause the parallel increases in light absorption by chlorophylls and energy transfer from chlorophylls to nanoparticles [60, 62, 63].

Artificially, the quantum dots (artificial antennae absorbing light efficiently in a wide range of photon energies from solar spectrum) conjugated with a reaction centre complex of *Rhodobactor sphaeroides* purified from natural light-harvesting complexes showed an efficient transfer of excitation energy to reaction centre. The efficient energy transfer from QDs to the bacterial RC clearly offers an opportunity of the utilization of nanocrystals to enhance the photosynthetic biological functioning [59]. A silver nanowire conjugated with light-harvesting complex from the dinoflagellate *Amphidinium carterae* showed strong enhancement in fluorescence intensity of protein-bound chlorophyll molecules [64]. The increase with silver nanowire conjugate was recorded up to an average of 10-fold increase in chlorophyll fluorescence [65], and this indicates a higher rate of generation of excitations in the chlorophylls [66].

Metal nanoparticles have the ability to influence the energy conversion efficiency in photosynthetic systems. The binding to Au and Ag nanoparticles with chlorophyll molecule results in a novel hybrid system, which could produce 10 times more excited electrons due to plasmon resonance and fast electron–hole separation [60]. Electron transfer from excited fluorophore to Au or Ag nanoparticles has been reported [65, 67–69]. The concentration-dependent effects of Au nanoparticles (5–20 nm) on PSII chlorophyll, a fluorescence quenching in soybean leaves, have been observed [70]. Falco et al. [70] observed a shift in fluorescence towards a higher wavelength in Au nanoparticle-treated soybean leaves. An enhanced PSII quantum efficiency was reported in Ag nanoparticle-treated Indian mustard [71].

Giraldo et al. [11] reported 49% increase in electron transfer rate under *ex vivo* conditions (in extracted chloroplast from baby spinach leaves) after treatment with SWNTs. SWNTs also enhanced the light reaction *in vivo* in leaves of *A. thaliana*. Similarly, carbon nanotubes in spinach thylakoid improved photo-electrochemical activity under illumination [72]. Noji et al. [73] reported that nanomesoporous silica compound (SBA) conjugated with photosystem II (PSII) maintained the high and stable oxygen-evolving ability of PSII in *T. vulcanus*. The applied TiO_2 nanoparticles caused the transfer of charges between light-harvesting complex II (LHCII) and TiO_2 NPs because of their photocatalytic properties [74] which induced reduction–oxidation reaction. Ze et al. [75] reported an increased expression of LHCII b and contents of LHCII in the thylakoid membrane of *A. thaliana* after the application of TiO_2 nanoparticles. It was found that TiO_2 NPs promote the light absorption by chloroplast and regulate the distribution of

light energy from PSI to PSII by increasing LHCII content, which in turn accelerate the transformation from light energy to electronic energy, water photolysis and oxygen evolution.

Nadtochenko et al. [62] observed an enhanced electron transfer efficiency in isolated photosynthetic reaction centres using alumina nanoparticles. The bread wheat (*Triticum aestivum* L.) showed an increase in grain number, biomass, stomatal density, xylem-phloem size, epidermal cells and water uptake after seed priming with MWCNT [76]. TiO_2 nanoparticles have been reported to protect chloroplasts from aging during long illumination regimes, promote chlorophyll formation and stimulate Rubisco activity, which in turn results in increased photosynthesis or enhanced photosynthetic carbon assimilation [71, 77, 78]. With exogenous application of TiO_2, Qi et al. [79] observed an improved net photosynthetic rate, water conductance and transpiration rate. Nano-anatase was reported to promote electron transport chain reaction, photoreduction activity of PSII, evolution of O_2 and photophosphorylation of chlorophyll under both visible and ultraviolet light [80]. A higher photosynthetic carbon reaction due to Rubisco carboxylation was observed as a result of nano-anatase-induced marker genes for Rubisco activase mRNA, enhanced protein levels and activities of Rubisco activase [81]. On the contrary, the exogenous application of TiO_2-anatase NPs resulted in a reduced PSII quantum yield, photochemical quenching, electron transfer rate, chlorophyll fluorescence and higher non-photochemical quenching and water loss [82]. Nano-TiO_2 reported to improve water absorption, seed germination, plant growth, nitrogen metabolism and photosynthesis [63, 76, 83, 84]. TiO_2 NPs were reported to alleviate heat stress through regulating stomatal opening [79].

Nano-TiO_2 (rutile) influences the photochemical reaction in spinach chloroplasts [85, 86]. The spinach treated with 0.25% nano-TiO_2 showed improved up-hill reaction and oxygen evolution. The noncyclic photophosphorylation activity was found to be higher than cyclic photophosphorylation in chloroplasts. This increase in photosynthesis with nano-TiO_2 might be associated with the activation of a photochemical reaction in spinach chloroplasts [85, 86]. Similarly, an increase in dry weight, chlorophyll formation, the ribulose bisphosphate carboxylase/oxygenase activity and the photosynthetic rate was reported in aged spinach treated with 2.5% nano-TiO_2 rutile [83]. The nano-anatase TiO_2 improved light absorbance, conversion of light energy to electron energy and ultimately to chemical energy, and this promotes carbon dioxide (CO_2) assimilation. Treatment of nano-anatase TiO_2 improved Rubisco-carboxylase activity 2.67 times in spinach as compared to control, which consecutively activates Rubisco carboxylation and eventually the rate of photosynthesis increase [87]. Pradhan et al. [88] found that Mn-NPs induced an increase in the hill reaction rate in mung bean (*Vigna radiata*).

In the recent time, NMs are used as a vital tool for improving plant growth and productivity under adverse environmental conditions, that is, salt stress. The Si nanoparticles in the soil have been shown to alleviate salt stress, enhance seed germination, improve activities of antioxidative enzymes, photosynthetic rate and leaf water content [89, 90]. Increased leaf, pod dry weight and grain yield were recorded in soya bean using nano-iron oxide [91]. The β-cyclo dextrin-coated iron nanoparticles penetrate the biological membranes of maize and increase the chlorophyll pigments (up to 38%) as compared to control [92]. The spray of citrate-coated

Fe_2O_3 nanoparticle spray on *Glycine max* had positive effects on root elongation and photosynthesis rate. Also, the elongation of root and an increase in seed germination were observed in *Zea mays* L. with silica (SiO_2) nanoparticles treatment [93]. Maize with a treatment of 1500 ppm of ZnO nanoparticulates showed the highest germination and seedling vigor index [94].

3. Future prospects

Nanotechnology has enormous potential to create novel and improved functional properties in photosynthetic organelles and organisms for the enhancement of solar energy harnessing. The upward translocation from root to leaf opens up greater opportunities for their use in various delivery applications. The SWNTs delivered by this spontaneous mechanism have the potential for increasing chloroplast carbon capture by promoting chloroplast solar energy harnessing and electron transport rates. It has been shown that when nanoparticles enter into plant cell, various metabolic changes occur that leads to an increase in biomass, fruit/grain yield, and so on; therefore, further mode and action can be elucidated to evaluate the possibility of their uses. The nanomaterials have the potential to be utilized for the transport of DNA and chemicals into plant cells [95, 96] which offers new opportunity to target specific gene manipulation and expression in the specific cells of the plant. With nanomaterial, the output of a crop can be increased while reducing the input through a better understanding of nanoparticle interaction with plants. The nanobionics approach to engineer plant function will lead to a new area of research at the interface of nanotechnology and plant biology.

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Conflict of interest

The authors declare no conflict of interest.

Abbreviations

Deff	Diffusion coefficient
FITC	Fluorescein isothiocyanate

FRAP	Fluorescence recovery in a photobleached area
MWNT	Multi-walled nanotubes
PAA-NC	Poly(acrylic acid) nanoceria
QD	Quantum dots
SWNTs	Single-walled nanotubes
SBA	Nanomesoporous silica compound
LHCII	Light-harvesting complex II

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This book is a compilation. It starts from the origins of the photosynthetic capacity of organisms with a summary of the evolution of photosynthesis. This is followed by a concise description of the photosynthetic process and a discussion of the role that light, nutrients, and cultivation play in the photosynthetic process using examples in each case. Finally, the book explains future improvements in the field by applying nanotechnology to improve photosynthetic productivity, explaining how crop productivity can be increased by engineering crop plants for tolerance against various environmental stresses and improving yield attributes, especially photosynthetic efficiency using nanomaterials.

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