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Photoprotection in Plants

Optical Screening-based Mechanisms

Springer

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Dedicated to the memory of Prof. Mark N. Merzlyak (1946–2009)

Preface

The ability of certain plant pigments absorbing in the UV and/or photosynthetically active regions of the spectrum to act as internal light filters has been discussed for quite a time. However, the participation of these compounds in photoprotection of plants has received only occasional attention and is much less studied in comparison with "classic" photoprotective mechanisms such as elimination of reactive oxygen species, thermal dissipation of the excessive excitation energy of chlorophyll, and repair of photooxidative damage.

Until recently, the photoprotective function of different pigments received little attention. However, during the last two decades, the interest of the scientific community in these pigments (generally named "screening" or "sunscreen" pigments) has grown dramatically. According to major citation databases, the number of publications dedicated to various aspects of plant screening pigments increased more than 3 times and there were 5 times more citations of such works. Still, the coverage of the subject is far from uniform: the overwhelming majority of the works in the field were (and so far are) dedicated to UV-screening compounds, their natural occurrence, and physiology, and the number of studies on compounds attenuating visible radiation remains modest in comparison with the number of studies on UV-screening compounds. This situation seemingly stems from an explosion of interest in ozone holes and their consequences for terrestrial and aquatic ecosystems mediated by elevated UV levels. At the same time, potential photoprotective effects exerted by anthocyanins, carotenoids, and flavonols in the visible region were often overlooked.

Recently obtained experimental evidence fostered a rethinking of the physiological significance of a considerable number of well-known compounds, mainly secondary metabolites of plants. This is especially true for secondary carotenoids and many phenolics. Consequently, the photoprotective role of these compounds has been acknowledged in a considerable number of cases. Different mechanisms of photoprotection were discussed and optical screening turned out to be plausible in many situations. The marked achievements in research into screening-based photoprotection in plants became possible owing to recent progress in the development of methods and equipment for the analysis of pigments and changes in plant optical properties induced by accumulation of these pigments. Particularly fruitful was the application of nondestructive optical reflectance-based approaches for quantification of screening pigments in situ.

To date, screening pigments have been discovered almost in all plant species investigated; in many cases, their chemical nature as well as their spectral properties have been documented and, most important, their photoprotective function was experimentally confirmed. The increasing number of works dedicated to anthocyanins and secondary carotenoids together with a large body of data on UV-screening compounds suggests that optical screening is an important defense mechanism of plants integral to the system of mechanisms protecting plants against photooxidative damage.

In spite of the breakthrough in the investigation of the diversity and biochemistry of plant screening pigments, a number of problems related to the spectra in planta, subcellular localization, and the physiological significance of screening pigments remain to be solved. There are also significant gaps in our knowledge about the buildup and relative efficiency of different groups of screening pigments. In particular, information on the in planta spectra of pigments which is crucial for characterization of their photoprotective functions is often lacking at present, especially for pigments absorbing in the visible part of the spectrum.

This monograph represents an attempt to develop an integral (but by no means comprehensive) view of plant photoprotective mechanisms based on optical screening of harmful radiation by extrathylakoid pigments. The first two chapters are dedicated to general questions related to optical screening and its place within the system of photoprotective mechanisms of plants, chemical diversity, and the natural occurrence of the key screening pigments. Chapter 3 addresses the induction and the dynamics of plant pigment composition in the case of accumulation of screening compounds. Chapter 4 discusses the general patterns of localization of screening pigments in cell compartments and their distribution in plant tissues. In Chap.5, the profound effects exerted by the buildup of screening pigments on the optical properties of plants are considered, and Chap.6 elucidates the employment of these effects for nondestructive estimation in situ of the screening pigment content and the efficiency of photoprotection provided by such pigments. The book concludes with a chapter dedicated to the relationships between the accumulation of screening pigments and the resistance of microalgae and higher plants to photoinhibition and photodestruction by high fluxes of UV radiation and photosynthetically active radiation.

I hope this book will be of use for lecturers, students, and specialists in the fields of plant physiology, ecological biophysics, and plant ecology.

Lomonosov State University, Moscow, Russia Alexei E. Solovchenko
April 2010

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Contents

Chapter 1
Optical Screening as a Photoprotective Mechanism

Abstract In this introductory chapter, the concept of photoprotection via "passive" screening of solar radiation by different extrathylakoid pigments is briefly outlined. The key differences between optical screening and other photoprotective mechanisms, such as enzymatic and nonenzymatic elimination of reactive oxygen species, thermal dissipation of the excessive chlorophyll excitation energy, and repair of oxidative damage, are discussed. The importance of screening and screen pigments for long-term photoacclimation is underlined together with specific advantages and drawbacks of this photoprotective mechanism.

The existence of plants as photoautotrophic organisms is characterized by uttermost dependence on the absorption and utilization of solar radiation energy in photosynthetic reactions. The photosynthetic pigments localized in the thylakoid membranes of chloroplasts efficiently capture light quanta and transfer their energy to other components of the photosynthetic apparatus, driving the ATP and NADPH syntheses, CO_2 fixation, etc. On the other hand, photosynthesis proceeds with an optimal rate only within a narrow irradiance range (Fig. 1.1), which is often lower than the fluxes of solar radiation reaching plants under natural conditions (Li et al. 2009; Ort 2001). Therefore, the light energy absorbed by the photosynthetic apparatus cannot be utilized completely in the course of photochemical reactions in many situations (Ensminger et al. 2006). The imbalance between the amount of light energy absorbed and the capacity of the plant to utilize it occurs under high fluxes of solar radiation and/or even under moderate irradiance combined with stresses of different nature, such as extreme temperatures (Ensminger et al. 2006), drought (Georgieva et al. 2010; Yordanov et al. 2000), and mineral nutrition deficiencies (Abadía and Abadía 1993). There are also other situations when plants are rendered sensitive to damage by excessive fluxes of solar radiation. Thus, in juvenile and senescing plants, the regulation of the functioning of the photosynthetic apparatus is not so perfect in comparison with that in mature leaves, making it less efficient in utilization of the absorbed light and therefore prone to photodamage by radiation

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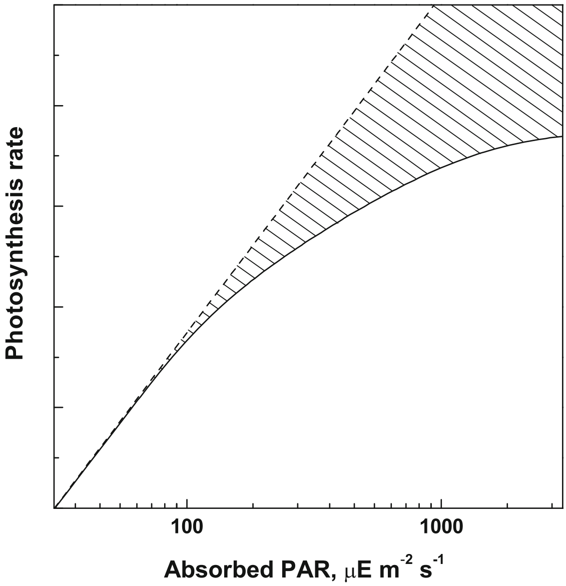

Fig. 1.1 The saturation of photosynthesis at high irradiances leads to the situation where a considerable part of the absorbed photosynthetically active radiation (PAR) cannot be utilized in photochemical reactions (*hatched area*) and imposes the threat of photooxidative damage unless it is removed via a photoprotective mechanism such as thermal dissipation

fluxes which usually do not harm mature plants (Abreu and Munne-Bosch 2007; Hughes et al. 2007; Lu et al. 2003; Munné-Bosch et al. 2001; Woodall and Stewart 1998).

Photodamage to photoautotrophic organisms under unfavorable environmental conditions proceeds primarily via increased generation of reactive oxygen species (ROS) photosensitized in the cells by chlorophylls (Asada 2006; Foyer and Noctor 2000) and a number of endogenous photosensitizers, such as porphyrins, flavins, and pterins (Kreitner et al. 1996; Massey 1994). Apart from the excessive photosynthetically active radiation (PAR), photodamage to plants could be induced by UV radiation, comprising 7–9% of the total energy of solar radiation reaching Earth's surface (Bjorn and Murphy 1985). Short-wavelength UV (UV-C, wavelengths below 280 nm) radiation is absorbed almost completely by the ozone layer of atmosphere. UV-B (280–315 nm) and UV-A (315–400 nm) radiation constitute approximately 5 and 90% of the total solar UV radiation, respectively (Rozema et al. 2002). High-energy UV-B quanta are able to damage plant cells directly, whereas the effects of less energetic UV-A radiation are usually ROS-mediated (Bornman et al. 1997; Rozema et al. 2002).

The essential need for plant survival under variable and often excessive fluxes of solar radiation brought about the development of certain adaptive systems including both regulatory and photoprotective mechanisms (Fig. 1.2) (Asada 2006; Demmig-Adams and Adams 2006; Li et al. 2009). Since the first photoautotrophic organisms on Earth were probably exposed to higher fluxes of harder UV radiation as compared with contemporary species, the enzymatic systems for repair of the UV-induced damage to nucleic acids and important proteins of the

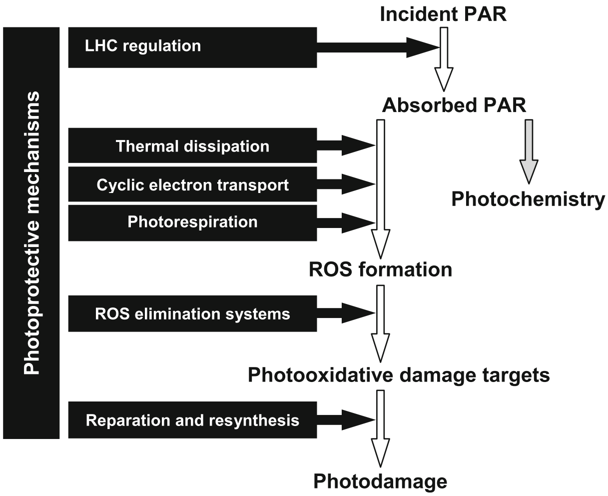

Fig. 1.2 Alternative flows of the energy of absorbed PAR and a multilevel system of "active" photoprotective mechanisms operating in chloroplasts

photosynthetic apparatus are thought to be among the first photoprotective mechanisms that evolved (Bornman et al. 1997; Cockell 1998; Cockell and Knowland 1999). Furthermore, the ROS-detoxifying systems, both enzymatic and nonenzymatic, are ubiquitous in and crucial for the prevention or amelioration of oxidative damage to plants (Asada 2006). Obviously, other mechanisms responsible for the maintenance of efficient photosynthesis in the wide range of radiation wavelengths and fluxes emerged at later stages of evolution (Demmig-Adams and Adams 2006).

It is important to realize that the aforementioned photoprotective mechanisms have certain aspects in common. All of them predominantly cope with the *consequences* of photodamage by UV radiation and PAR, i.e., repair damaged macromolecules, eliminate ROS and products of their reactions *already formed* in the cell (Fig. 1.2). Then, the efficient operation of these mechanisms requires sufficient levels of energy-rich and/or reducing compounds which are necessary for repair of DNA, resynthesis of the membrane lipids and proteins, as well as for regeneration of important low molecular mass antioxidants such as reduced glutathione and ascorbate (Foyer and Noctor 2005).

Over the last two decades, the concept of photoprotective mechanisms based on attenuation or "passive" optical screening of harmful radiation by extrathylakoid pigments has evolved and become widespread (Bilger et al. 2007; Burchard et al. 2000; Cockell and Knowland 1999; Merzlyak et al. 2008b; Morgan-Kiss et al. 2006; Sinha et al. 1998; Solovchenko and Merzlyak 2003, 2008; Steyn et al. 2009). The ability of plants to respond to strong irradiation by the synthesis and accumulation, within different cell compartments and tissue structures, of the compounds selectively absorbing in the UV or the visible part of the spectrum is the foundation of these mechanisms. In higher plants, these compounds are concentrated in superficial structures such as the cuticle and epidermis and/or are distributed within cells and tissues (Lenk and Buschmann 2006; Lenk et al. 2007; Merzlyak et al. 2008a; Solovchenko and Merzlyak 2003). These mechanisms are distinct from the "classic" or "active" photoprotective systems (Fig. 1.2) in a number of ways. Primarily, they prevent photodamage by alleviating its *cause* – the excessive absorption of radiation by the photosynthetic apparatus and other photosensitive cell components (Fig. 1.3).

Plant evolution was accompanied by a continuous expansion of the diversity and an increase of structural complexity of molecules suitable for the photoprotective

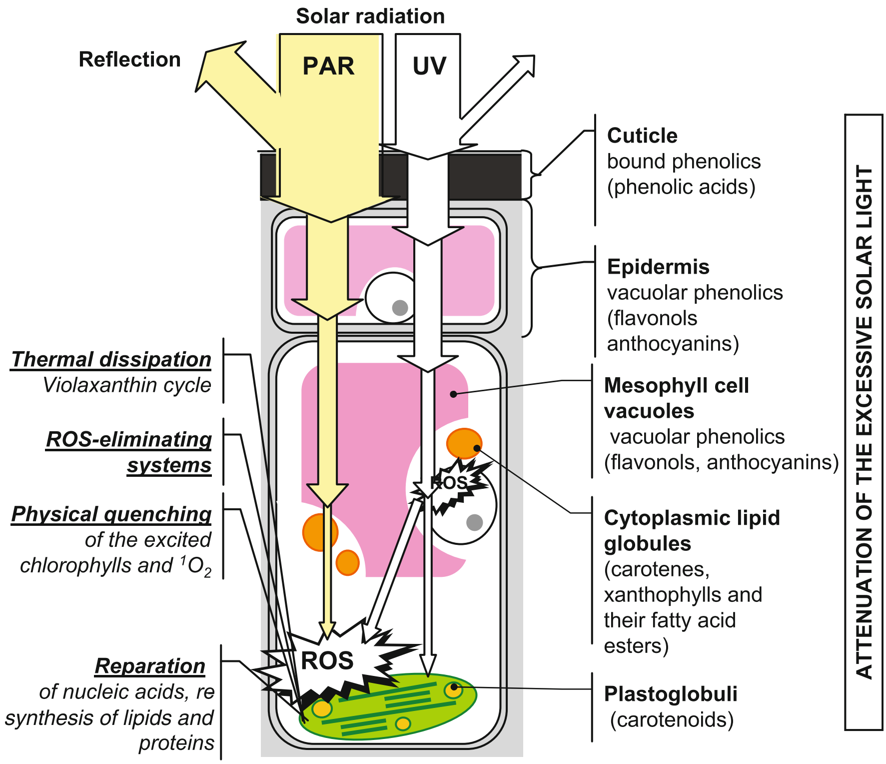

Fig. 1.3 Optical screening – an integral part of a system of photoprotective mechanisms in plants. Under unfavorable environmental conditions and in situations when the regulation of photosynthesis is impaired, high fluxes of solar radiation induce direct or indirect reactive oxygen species (ROS)-mediated damage to plants. Certain mechanisms are responsible for a decrease in ROS levels in the cell and cope with the consequences of photodamage (see also Fig. 1.2). The screening pigments attenuate the incident radiation, thereby removing, to a considerable extent, the cause of photodamage (harmful UV and the excessively absorbed visible quanta). (Reprinted from Solovchenko and Merzlyak (2008) with kind permission from Springer Science + Business Media), Fig. 1

function via radiation screening (which will be covered in detail in the next chapter) (Cockell and Knowland 1999). The vast majority of screening pigments discovered to the date in plants belong to four key groups of compounds differing in chemical structure and the biosynthetic pathways. Among others, they include mycosporine-like amino acids (Sinha et al. 1998) and extrathylakoid (also known as the secondary) carotenoids which do not transfer the absorbed light energy to chlorophylls (Ben-Amotz et al. 1989; Han et al. 2003). Together with carotenoids, the photoprotective function in plants is served by a large number of phenolic compounds (Hoch et al. 2003; Williams and Grayer 2004) and nitrogen-containing heterocyclic betalains (Strack et al. 2003). Different but complementary classes of photoprotective pigments disparate in chemical structure, spectral properties, and localization, e.g., phenolic compounds and carotenoids or phenolics and betalains, are present in many plant species simultaneously (Tanaka et al. 2008). Certain classes of screening pigments such as phenolics are ubiquitous and have been discovered in all plant species studied so far. However, the proposed photoprotective function of a screening compound should be rigorously proved in each case.

Recently obtained evidence suggests that plant screening pigments possess high photostability both in vitro and in planta (Merzlyak and Solovchenko 2002; Merzlyak and Chivkunova 2000). Therefore, a photoprotective screen, once formed, could be maintained with minimal expenditure of energy and valuable metabolites providing a reliable long-term protection against photodamage. It is important, therefore, that the efficiency of “passive” screening of radiation is far less affected by environmental stresses (such as extreme temperatures or drought; Munné-Bosch et al. 2001) which suppress photosynthesis and could impair the ability of the enzymatic systems to provide an adequate level of photoprotection (Asada 2006).

At the same time, the initial buildup of photoprotective compounds demands a considerable amount of photoassimilates and energy to be invested in biosynthesis of screening pigments. The induction of synthesis and accumulation of the pigments in amounts sufficient to accomplish their photoprotective function (as well as decomposition of earlier accumulated screening compounds) is a relatively slow process, which occurs on the timescale of hours and days. Owing to these circumstances, the screening-based mechanisms are warrantable mostly under the prolonged action of a stressor; hence, they are of high importance for long-term adaptation of plants.

To conclude, one can think of radiation screening by extrathylakoid pigments as a photoprotective mechanism relying on principles totally different from those of “classic” photoprotective mechanisms but integral to the whole system of protection of plants against photooxidative stress. Screening-based photoprotection is a first-line defense of plants against potentially harmful solar radiation, which takes a considerable time to deploy as well as to withdraw and is therefore effective for long-term photoacclimation of plants. In the following chapters, the components, the operation, and several approaches for assessment of the efficiency of screening-based photoprotection will be considered.