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Overproduction of photosynthetic electrons is associated with chilling injury in green leaves

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Abstract

Employing the non-invasive techniques of infra-red gas analysis and pulse amplitude modulated chlorophyll fluorometry, we determined the partitioning of photosynthetic electrons between photosynthetic carbon reduction and other reductive processes resulting in the formation of active oxygen species (AOS) in intact green leaves. This we studied in plant species that are adapted to two different agro-climatic conditions, namely the warm plains (76°36'E, 9°32'N) and the cool mountains (1 600 m a.s.l.) in the south Indian state of Kerala. Ground frost and low temperature were more harmful to those species adapted to the warm plains than the ones adapted to the cool mountains. Exposure to low temperature decreased leaf photosynthetic carbon assimilation rates and quantum yield of photochemical activity in species naturally adapted to the warm plains. High irradiances further aggravated the harmful effects of low temperature stress possibly by overproducing AOS. This resulted in severe peroxidative damage as inferred by the accumulation of malondialdehyde (MDA) in the leaves.

Additional key words: active oxygen species; chlorophyll fluorescence; cool mountains and warm plains; frost; low temperature stress; malondialdehyde; net photosynthetic rate; photosystem 2; stomatal conductance; quantum yield.

Introduction

Low temperatures can seriously inhibit several metabolic processes and thus can be stressful to plants (Holaday *et al.* 1992). This stress is particularly severe in plants with a tropical or subtropical evolutionary background (Long *et al.* 1983, Jacob *et al.* 1999). Green leaves are extremely vulnerable to low-temperature stress and this is aggravated by high solar irradiances (Powles *et al.* 1983).

Leaf net photosynthetic rate (P_N) is dependent on ambient temperature, because Calvin cycle enzymes are temperature sensitive (Holaday *et al.* 1992). Chilling temperatures inhibit P_N more than the photochemical activities and this causes an imbalance in the utilisation of the absorbed energy for photosynthetic carbon reduction and other processes (Powles 1984). The problem of imbalance in energy utilisation is further aggravated when the absorbed solar energy is in excess of what is required to maintain the leaf P_N which is the case when a plant is exposed to an environmental stress (Powles 1984, Huner *et al.* 1993). The excess energy in the photosynthetic apparatus causes formation of various active oxygen species (AOS) which are extremely harmful to cells if they are not safely scavenged (Asada 1996). The cumulative damaging effects of AOS and various free radicals (FR) are collectively called oxidative stress (Asada 1996). Green leaves experiencing an abiotic stress such as chilling are powerful sources of AOS and FR formation. If they are not checked, oxidative stress can result in premature ageing and senescence of these leaves (Aro *et al.* 1993, Fryer *et al.* 1998).

In the present study we investigated the harmful ef-

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Abbreviations: AOS – active oxygen species; Chl – chlorophyll; ETR – electron transport rate across PS2; F_v/F_m – ratio of variable to maximum fluorescence obtained after 20 min dark adaptation of the leaves; g_s – stomatal conductance; MDA – malondialdehyde; P_N – net CO_2 assimilation rate; PAM – pulse amplitude modulated fluorometer; PPFD – photosynthetic photon flux density; PS2 – photosystem 2; Φ_{PS2} – apparent PS2 quantum yield; T – leaf temperature.
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fects of concomitant occurrence of low temperature and high irradiance on the photosynthetic apparatus of different plant species adapted to distinct agro-climatic conditions.

Materials and methods

From September 1999 to January 2000, we grew 150 young plants of *Hevea brasiliensis* in large polybag containers (0.75 m^3) filled with garden soil (equal mixture of red laterite soil, river sand, and farm yard manure) following all standard agronomic practices (Rubber Grower's Companion 1995) on the farms of Kerala Livestock Development Board (Indo-Swiss Project) located in Mattupetty (76°36'E, 9°32'N, altitude 1 600 m a.s.l.), a hill station in the Western Ghats in the south Indian state of Kerala. The winter temperature here can be quite cool with occasional ground frost during the months of December and January. A parallel set of plants was kept as control at Rubber Research Institute of India, Kottayam (76°36'E, 9°32'N, altitude 73 m a.s.l.). Kottayam is on the plains about 200 km west of Mattupetty and does not experience any low temperature stress. At the start of the experiment all the plants were pruned to an equal height from the bud union to bring uniformity in growth in all the plants for both the environments. We also examined rose (*Rosa hybrida*), *Setaria sphacelata*, Napier grass (*Pennisetum purpureum*), glory of the garden (*Bougainvillea spectabilis)*, plum (*Prunus domestica*), clover (*Trifolium alexandrinum*), and bottle brush (*Calistemon lanceolatus*) that were growing naturally and therefore adapted to either the low temperature mountain conditions of Mattupetty or the warm climate of the plains of Kottayam.

The average minimum temperatures for December 1999 and January 2000 in Mattupetty were 9.5 and 10.6 °C, respectively, and the maximum temperature was 19.3 °C in both the months. The corresponding minimum temperatures in the plains of Kottayam were 22.4 and 22.3 °C, while the maximum temperatures were 33.3 and 34.0 °C. Temperatures below 18.0 °C can be stressful and therefore affect the optimal growth of *Hevea* plants (Zongdao and Xueqin 1983). Thus, *Hevea* plants grown in Mattupetty experienced low temperature stress while their counterparts in Kottayam were free from it.

A comprehensive study was made on green leaves of the above species during December 1999-January 2000. Two days before these measurements there was a mild early morning surface frost with a temperature of –2 °C for about three hours in the early morning in Mattupetty while Kottayam continued to remain warm. P_N and stomatal conductance (g_s) were measured using a portable photosynthesis system (*Li-6200*, *Li-Cor*, USA). Chlorophyll (Chl) fluorescence was measured with a pulse amplitude modulated fluorometer (*PAM-2000*, *Walz*, Germany). Maximum potential photochemical efficiency defined as the ratio of variable to maximum fluorescence emitted by chlorophyll (F_v/F_m) was estimated after dark adaptation of leaves for 20 min. Apparent PS2 quantum yield (Φ_{PS2}), *in vivo* photosynthetic electron transport across PS2 (ETR), and the rate of photosynthetic electron flow through PS2 to processes not linked to photosynthetic C metabolism were calculated (Genty *et al.* 1989, Jacob and Lawlor 1993, Jacob and Karaba 2000). The gas exchange and Chl fluorescence measurements were conducted both under low and high photosynthetic photon flux densities (PPFD). The low PPFD was in the range of 100-200 µmol m^{-2} s^{-1} and the high PPFD was in the range of 700-1 200 µmol m^{-2} s^{-1}. After these measurements, the contents of Chl and malondialdehyde (MDA) in leaves were estimated according to the methods of Arnon (1949) and Heath and Packer (1968), respectively. Standard errors were calculated and independent *t*-test was used to find the statistical significance of the means.

Results and discussion

Low temperature stress inhibited P_N more in *Hevea* than in the other species naturally acclimated to the cool conditions of the mountain (Table 1). The low temperature-induced inhibition in P_N was further aggravated at high PPFD in *Hevea* grown at Mattupetty. All the native species found in Mattupety, except Napier grass, had high P_N in spite of the occurrence of frost two days before the measurements were made suggesting their intrinsic tolerance to chilling stress. In Napier grass, the distal portion of the leaf blade exposed to the ambient air and thus exposed to the frost showed much smaller P_N than the basal portion not exposed to frost. This suggests that Napier grass is intrinsically susceptible to chilling stress. But by keeping the tender shoots and growing tips well protected and not exposed to the frost, this species can escape from frost damages and survive although the exposed blades may die.

Like leaf photosynthesis, the maximum potential quantum yield of PS2 (dark adapted F_v/F_m) also showed a decrease in response to low temperature stress in *Hevea* and the exposed blades of Napier grass which were susceptible to low temperature stress (Table 2). But in the other species which were apparently chilling tolerant, dark F_v/F_m (Table 2) remained close to the theoretical maximum value of around 0.83 (Björkman and Demmig 1987). The effective quantum yield of PS2 was also the lowest in the susceptible species at any given PPFD (Fig. 1).

The inhibitory effect of low temperature stress on leaf photosynthesis is well known (Huner *et al.* 1993). Such effects are more pronounced in species evolved in the tropics which are not adapted to chilling conditions (Baker *et al.* 1994). In the present study, the two tropical species *Hevea* and Napier were more vulnerable to the low temperature stress as evidenced from the inhibitions in P_N, dark-adapted F_v/F_m, and Φ_{PS2}. Strong irradiance can aggravate these inhibitory effects of environment on green leaves (Long *et al.* 1994). Strong irradiance inhibits photosynthesis in green leaves experiencing an abiotic stress such as chilling (Fryer *et al.* 1998). The inhibitory effects of low temperature and strong irradiance are observed both in the photosynthetic carbon metabolism as well as in the photochemical activities (Fryer *et al.* 1998). PS2 is particularly sensitive to abiotic stresses (Baker 1996). In the present study, the maximum potential quantum yield of PS2 in the dark-adapted state and effective quantum yield at a given PPFD were markedly decreased in the low temperature sensitive species when they were exposed to low temperature stress.

In spite of the down regulation in the photochemical activity, there was relatively more photosynthetic electrons formed in the leaf for every mole of CO_2 assimilated in the low temperature susceptible plants (Fig. 2*A*). This indicates that in such plants there were relatively more excited electrons than required to sustain their photosynthesis rates. These excess electrons were apparently

Table 1. Combined effects of low temperature and high irradiance on gas exchange in some plant species in Mattupetty (low temperature stress) and Kottayam (control). PPFD = photosynthetic photon flux density [μmol m^{-2} s^{-1}], T = leaf temperature [°C], stomatal conductance, g_s [mol m^{-2} s^{-1}], and net photosynthetic rate, P_N [μmol m^{-2} s^{-1}]. Mattupetty experienced a mild ground frost two days before the measurements. In Napier grass, the basal ends of the leaf blades were not exposed to frost and hence protected. The distal ends were exposed to frost. nex = the basal portions of the leaves not exposed to frost, exp = the distal end of the leaves exposed to frost. Values under parentheses are standard errors. *n* = 10-20.

Species	Mattupetty PPFD	T	g_s	P_N	Kottayam PPFD	T	g_s	P_N
Hevea	199	22.3	0. 12	1.02	214	31.2	0.50	5.70
	(25.2)	(0.3)	(0.01)	(0.18)	(12.6)	(0.3)	(0.03)	(0.26)
	1206	30.2	0.10	0.60	1100	34.6	0.50	11.08
	(30.9)	(0.4)	(0.01)	(0.24)	(15.9)	(0.2)	(0.01)	(0.23)
Rose	916	25.9	0.50	15.91	1140	34.7	1.69	19.33
	(54)	(1.7)	(0.11)	(2.21)	(35.6)	(0.1)	(0.10)	(0.72)
Setaria	640	26.8	0.58	21.33				
	(38)	(0.2)	(0.02)	(0.86)				
Napier (nex)	627	27.2	0.39	15.98				
	(6)	(0.1)	(0.02)	(0.93)				
Napier (exp)	772	27.3	0.27	03.45				
	(25)	(0.2)	(0.03)	(2.02)				
Clover	755	26.4	2.09	26.13				
	(25)	(0.2)	(0.20)	(0.87)				

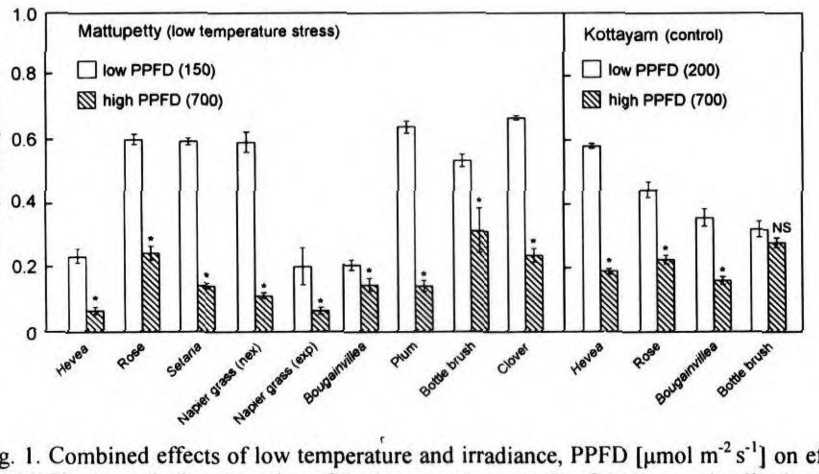

Fig. 1. Combined effects of low temperature and irradiance, PPFD [μmol m^{-2} s^{-1}] on effective PS2 quantum yield. ±S.E. bars shown. *n* = 10-20. nex = the basal portion of the leaves not exposed to frost, exp = the distal end of the leaves exposed to frost. $^*p<0.01$, NS = not significant.

used for AOS production under low temperature stress when carbon assimilation was impaired. For example, the low temperature exposed leaves of *Hevea* grown at Mattupetty had as many as 2.32 electrons going for AOS formation for every electron used for carbon reduction, but this diversion for AOS formation was only 0.55 electrons per electron used for C reduction in Kottayam when measured at a sub-saturating PPFD (Table 3). At high PPFD this diversion of photosynthetic electrons for AOS formation showed a marked increase in the low temperature exposed *Hevea* leaves grown at Mattupetty but not in Kottayam. These results indicate that strong irradiance aggravated the effects of low temperature stress by diverting more electrons for AOS production that resulted in oxidative stress.

Table 2. Effects of low temperature on the maximum potential quantum yield of PS2 photochemistry (dark adapted F_v/F_m) in a few plant species at Mattupetty (low temperature stress) and Kottayam (control). Values under parentheses represent standard errors. n = 10-20. nex = the basal portion of the leaves not exposed to frost, exp = the distal end portion of the leaves exposed to frost.

Species	F_v/F_m Mattupetty	Kottayam
Hevea	0.50 (0.03)	0.80 (0.09)
Rose	0.82 (0.001)	0.81 (0.05)
Setaria	0.80 (0.03)	
Napier (nex)	0.75 (0.01)	
Napier (exp)	0.26 (0.11)	
Bougainvillea	0.81 (0.02)	0.80 (0.01)
Plum	0.81 (0.004)	
Bottle brush	0.82 (0.004)	0.82 (0.04)

Production of AOS is an inevitable consequence of aerobic life but is not necessarily a sign of damage or ill health (Slain 1988, Asada 1996). However, the effective scavenging of AOS depends on the antioxidant capacity of the organism and this determines the metabolic health of a cell (Slain 1988, Scandalios 1990, Asada 1996). One would expect that such protective capacity was more expressed in the stress tolerant than stress susceptible species (Scandalios 1990). Even under congenial conditions, there could be production of excited electrons which is in excess of what is required to sustain the rate of carbon assimilation, as we have observed in the control *Hevea* plants grown at Kottayam (Table 3). These excess electrons might not cause any adverse effects as the leaves were not experiencing any low temperature stress and their AOS scavenging capacity was adequate to take care of these excess electrons. When this capacity breaks down, as we suspect in *Hevea* and Napier grass exposed to low temperature stress in Mattupetty, there is enhanced AOS production and hence oxidative stress is unavoidable. The high MDA/Chl ratio (Fig. 2*B*) suggests that the large production of AOS may have resulted in oxidative stress leading to peroxidative damage in these two low temperature susceptible species grown in Mattupetty. Thus, our results indicate that production of excited photosynthetic electrons in excess of what is needed to sustain photosynthetic carbon metabolism is associated with low temperature stress in green leaves.

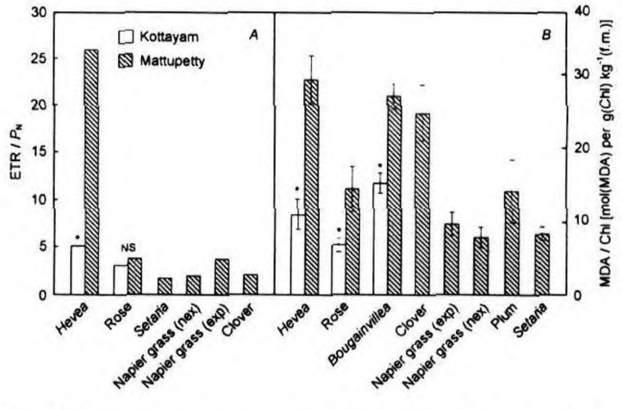

Fig. 2. Effect of low temperature stress on (*A*) the ratio of total electron transport rate (ETR) to net CO_2 assimilation rate (P_N) and on (*B*) the ratio of malondialdehyde (MDA) to chlorophyll (Chl) contents in the different plant species grown in Mattupetty (low temperature stress) and Kottayam (control). nex = the basal portions of the leaves not exposed to frost, exp = the distal end of the leaves exposed to frost. $^*p<0.01$, NS = not significant.

Table 3. High irradiance concomitant with low temperature stress led to the formation of AOS (active oxygen species) through over-producing photosynthetic electrons in the green leaves of *Hevea*. PPFD = photosynthetic photon flux density [μmol m^{-2} s^{-1}], P_N = net CO_2 assimilation rate [μmol m^{-2} s^{-1}], ETR = electron transport rate across PS2 [μmol m^{-2} s^{-1}], and $\frac{\text{ETR} - 4P_N}{4P_N}$ = number of electrons for AOS formation per electron used for CO_2 reduction. Values under parentheses represent standard errors. n = 10-20.

PPFD	Kottayam (control) P_N	ETR	$\frac{\text{ETR} - 4P_N}{4P_N}$	Mattupetty (low temperature stress) P_N	ETR	$\frac{\text{ETR} - 4P_N}{4P_N}$
Low PPFD 200	5.7 (0.26)	35.3 (1.62)	0.55	1.0 (0.18)	13.6 (0.80)	2.32
High PPFD 900	11.1 (0.23)	56.6 (2.39)	0.28	0.60 (0.24)	15.6 (3.69)	5.48

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Mauch, J.E., Birch, J.W.: **Guide to the Successful Thesis and Dissertation.** 4th Ed. – Marcel Dekker, New York – Basel 1998. ISBN 0-8247-0169-0. 21 + 335 pp., USD 59.75.

The reviewed book is the 58th volume of the series of monographs and textbooks "Books in Library and Information Science". This volume should help both the university students and faculty teachers. It has become popular as seen from existing four editions (this one was revised and expanded). For us who live in Europe where the tradition of universities started mostly in the 13th or 14th century, already the introductory historical chapter brings interesting information: In the U.S. the Doctor of Philosophy degree was first offered as late as in 1861 at Yale University, and even the courses of study leading to the master's degree started only in 1858 at the University of Michigan. Also some of the present professional U.S. degrees are certainly very rare in Europe, such as Master of Nursing or Master of Urban and Regional Planning.

The main text is divided into ten chapters, that deal with individual steps of preparing proposal of either thesis (T) for obtaining master's degree or dissertation (D) for reaching the doctor's degree, conducting the study, writing the manuscript, and defending it. Each chapter is opened by a box showing on which pages answers to three to five specific questions can be found. The content of this manual deals more with T/D in philosophy, history, and other social sciences than with T/D in natural sciences. In natural sciences, in Europe and certainly also in the U.S., possible T/D topics are mostly limited by the research scope of the respective institute or research team and by the available apparatuses and instruments. Hence the selection of T/D topic starts usually with recommendations of the advisor, not with ideas of the student. The possibility of honours programs for outstanding undergraduate students is more or less a speciality of the U.S.; in Europe such bachelor's thesis is mostly a necessity for all students who wish to reach this degree and continue in undergraduate studies.

First two chapters deal with getting started and selecting a proper research advisor. Distinctions between research in academic and professional disciplines are summarised in Table 1-1, distinctions between qualitative and quantitative research are on pp. 18-19. Developing the proposal by an interaction of student and advisor and preparing it by the student is the topic of next two chapters. The reader can find here, *e.g.* things dissertation advisors hate to hear (pp. 38-39), problems of sexual harassment (p. 42), ethical responsibilities (pp. 48-50) and responsibilities of higher-education institutions (pp. 54-57), criteria of selection of the research advisor (pp. 58-60), recommendations for disabled and handicapped (pp. 65-67) or foreign (pp. 76-77) students, checklists of T/D topic sources (p. 68) or topic feasibility and appropriateness (pp. 74-75), recommendations for database searches (pp. 81-84), how to prepare table of contents for the proposal (p. 97). Further recommendations are how to prepare literature review, make research design and select methodology, collect data and treat them. On pp. 116-122 nineteen examples of research types are given.

Chapter 5 deals with the T/D committee that controls the progress of preparing the T/D and continuously gives advice and consults the candidate. The form for evaluation of T/D (pp. 133-135) and the progress report memorandum (p. 137) are certainly interesting tools to maintain communication of student, advisor, and committee. The criteria for selecting committee members and their roles (*e.g.* to encourage clear writing) are also given here. Chapter 6 is on the approval of the overview and chapter 7 on conducting the study. One can find here how can word processing programs help in work, how to use private information, what are the obligations to human (and animal) subjects, *etc.*

Very important is the topic of chapter 8 entitled "Writing the manuscript", but the space reserved to this question is very limited (only 25 pp.). Interesting is the recommended table of contents for T/D (pp. 219-220): implications for practice and scholarly understanding, and recommendations for further research or modifications in accepted theories are often omitted by students. Constructing a "dummy" of the finished document (pp. 220-221) is a sound idea. Also the notice on copyrighted material is very important (pp. 221-222) as well as the checklist for T/D (pp. 228-229). Chapter 9 deals with T/D defence, including oral examination, and chapter 10 is on publication of the finished dissertation, responsibilities to professional improvement, *etc.* A list of 18 points as guidelines for articles or papers (pp. 268-270) is certainly useful.

There are three appendices: a very good list of research related telecommunication and computer terminology, suggested proposal and project guidelines, and course outline. The bibliography contains 279 items. An author index and a subject index are included.

According to my opinion it was not a very good idea to prepare book that should simultaneously serve students, advisors, and committee members. I think that half of the book size would be enough for students, certainly with more emphasis to the writing of T/D manuscript and to the problems of students of natural sciences. Nevertheless, everybody can find some interesting information in this book.

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