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Research article

Damage of photosynthetic apparatus in the senescing basal leaf of *Arabidopsis thaliana*: A plausible mechanism of inactivation of reaction center II

Pranab Kishor Mohapatra [a,1], Padmanava Joshi [b], Nemmara Krishnan Ramaswamy [c], Mukesh Kumar Raval [d], Udaya Chand Biswal [a], Basanti Biswal [a,*]

[a] School of Life Sciences, Sambalpur University, Jyoti Vihar, 768019, Odisha, India
[b] Department of Physics, Anchal College, Padampur, P.O. Rajborasambar, 768036 Bargarh, Odisha, India
[c] Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Center, Trombay, 400485 Mumbai, India
[d] Department of Chemistry, Gangadhar Meher College (Autonomous), Sambalpur 768004, Odisha, India

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ABSTRACT

Significant decline in oxygen evolution and DCPIP photoreduction and a marginal restoration of the later with DPC as an electron donor suggest the inactivation of reaction center of photosystem II. The declines in the height of thermoluminescence bands support the view and the damage of reaction center II could be central to the senescence process in *Arabidopsis* leaves. The enhancement in the number of reduced quinones, signifying a loss in redox homeostasis in the electron transport chain between photosystem II and I leads to the creation of an energy imbalance. The view is supported by the decline in actual quantum yield of photosystem II in the light adapted state and maximum quantum yield of primary photochemistry in the dark adapted state of chlorophyll fluorescence. An increase in chlorophyll *a* fluorescence polarization and decline in carotenoid to chlorophyll energy transfer efficiency suggest the perturbation in thylakoid structure. A plausible mechanism illustrating the senescence mediated inactivation of oxygen evolving complex has been proposed.

1. Introduction

It is well established that alterations in the structure and function of chloroplasts occur during leaf senescence [1–7]. The alterations include the degradation of photosynthetic pigments and proteins, inactivation of both photosystems (PS) I and II, and down regulation of enzyme activities associated with Calvin-Benson cycle [5,6,8–11]. Reports demonstrating the damage of PS II [7,12–14], with oxygen evolving complex (OEC) as one of the initial events during leaf senescence in many plant systems are exquisitely available [6,7,15].

On the other hand, perusal of relevant literature reveals that the study on alterations in the structure and function of chloroplasts during senescence of leaf in *Arabidopsis thaliana*, a plant extensively used as a model system for senescence related genomic study, is meager. In a recent work from our laboratory, senescence-mediated decline in O_2-evlution, photochemical potential of PS II, stomatal conductance, CO_2 fixation and up regulation of certain enzymes have been reported in this plant system [16]. However, no attempt has yet been made to understand the mode of inactivation of PS II during senescence of the leaf. In fact, in course of this work we observed a marginal restoration of DCPIP photoreduction with DPC as an electron donor letting us to believe that the damage of reaction center (RC) II may not be inconsequential in the inactivation of OEC. Thus our objectives have been to examine the inactivation of RC II during senescence of *Arabidopsis* leaf and if the inactivation has a role in the decline in O_2-evolution and the disorganization of thylakoid membrane.

2. Results

2.1. Changes in Chl and protein contents

Fig. 1 describes the changes in the content of total Chl and total leaf protein in course of growth and development of basal leaf of *Arabidopsis* seedlings. Both these parameters increased, although

Abbreviations: Chl, chlorophyll; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP, 2,6-dichlorophenolindophenol; DPC, 1,5-diphenylcarbazide; F_v/F_m, maximum quantum yield of primary photochemistry in the dark adapted state; F_0, initial fluorescence; TL, thermoluminescence; qP, photochemical quenching; MDA, malondialdehyde; OEC, oxygen evolving complex; PSII, photosystem II; ROS, reactive oxygen species; RC II, reaction center II; Φ_{PSII}, quantum yield of photosystem II in the light adapted state.

* Corresponding author. Tel.: +91 663 2432060; fax: +91 663 2430158.
E-mail address: basanti_b@hotmail.com (B. Biswal).
[1] Present address: Department of Chemistry, CV Raman College of Engineering, Bidya Nagar, Mahura, Janla, Bhubaneswar 752054, Odisha, India.

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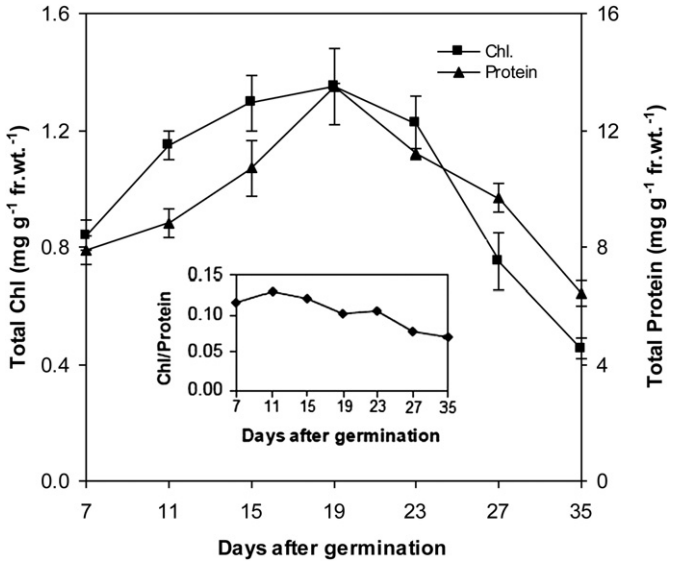

Fig. 1. Alteration in the content of total Chl and total leaf protein in course of development of the basal leaf of *Arabidopsis thaliana* seedlings. The alteration in the ratio, total Chl/total protein is inserted. Each point is a mean of three independent estimates ($n = 3$). Bar indicates ± SD.

with different kinetics, up to d 15 (developing) reached a peak value on d 19 (mature) and then declined indicating senescence phase of the leaves. On d 35, the content in total Chl and total leaf protein were 34% and 47% of the corresponding peak values as on d 19. Therefore, the measurements of all photosynthetic parameters were carried out on d 19 for mature leaf and on d 35 for senescing sample. The pattern of change in the ratio of total Chl to total protein (total Chl/total protein) is depicted as an in set in Fig. 1. The ratio was more than 0.1 during developmental phase of the leaf growth, equal to 0.1 from d 19 to 23 and less than 0.1 there after.

2.2. Alteration in O_2 evolution and PS II-mediated DCPIP photoreduction

Fig. 2 exhibits a comparison of the rate of O_2-evolution and the rate of transport of two electrons in PS II as measured in terms of DCPIP photoreduction with H_2O or DPC as electron donor between mature and senescing samples. While the photosynthetic oxygen evolution (Fig. 2A) was measured directly from the leaf discs, the DCPIP photoreduction (Fig. 2B) was measured from chloroplasts isolated from the basal leaf. In senescing sample, the rate of O_2-evolution was 11% ($p < 0.005$) of the mature one while the rate of DCPIP photoreduction with H_2O and DPC as electron donors in senescing sample was 21.5% ($p < 0.001$) and 26.6%($p < 0.001$) respectively of the corresponding mature sample.

2.3. Alterations in Car-to-Chl energy transfer efficiency, fluorescence polarization and MDA accumulation

Table 1 describes the alterations in Car-to-Chl energy transfer efficiency, fluorescence polarization and MDA accumulation in chloroplasts isolated from the basal leaf of *A. thaliana* seedling during senescence. The Car-to-Chl excitation energy transfer efficiency in both PS I and PS II measured in terms of [E_{475}/E_{600} (F_{685})] and [E_{475}/E_{600} (F_{735})], respectively declined by 41% ($p < 0.005$). On the other hand, the fluorescence polarization P_{735} and P_{685} increased by 99 ($p < 0.005$) and 112 ($p < 0.005$) %, respectively in senescing leaves, while the accumulation of MDA increased by 117% ($p < 0.005$) on d 35.

2.4. Changes in Chl *a* fluorescence transients and fluorescence parameters

The kinetics of double normalized Chl *a* fluorescence transients indicating the alteration in the pattern of the O–K–J–I–P curve during senescence are shown in Fig. 3. Different phases of the kinetics in mature sample are distinctly observable in Fig. 3 while the differentiation of these transients were diminished in senescent leaf. The K-band, which occurred at 0.3 m, in double-normalized fluorescence induction curve of senescent sample became dominant over the mature one. Subsequently the induction curve in J–I–P steps of senescent leaves remained low with a large separation from the mature one.

Table 2 describes the results of fluorescence transient parameters (i) total flux absorbed by PS II antenna pigments per reaction center (ABS/RC), (ii) the number of active PS II reaction centers per excited cross section (RC/CS) and (iii) electron transfer per excited cross section (ET/CS). The parameters ABS/RC increased by 53.5% ($p < 0.005$) while RC/CS and ET/CS decreased by 39 ($p < 0.005$) and

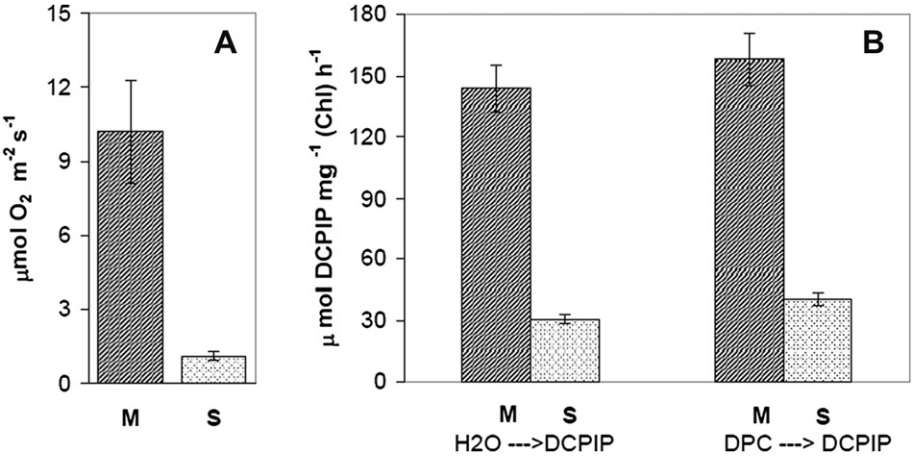

Fig. 2. (A) Senescence induced decline in the rate of oxygen evolution measured from mature (M) and senescent (S) leaf discs of *Arabidopsis* (B) represents PS II mediated electron transport activity measured in terms of DCPIP photoreduction with H_2O or DPC as the electron donor in chloroplasts isolated from d 19 old (M) and d 35 old (S) basal leaves of *Arabidopsis thaliana*. Vertical bars represent ± SD ($n = 4$).