Water stress-sensitized photoinhibition in senescing cotyledons of clusterbean: Changes in thylakoid structures and inactivation of photosystem 2

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Abstract

Seedlings of *Cyamopsis tetragonoloba* were grown on Petri dishes either in water or water *plus* 3 % *PEG-6000* to induce water stress. The senescing cotyledons experiencing the stress exhibited loss in contents of leaf proteins and chlorophyll (Chl) and a decline in oxygen evolution. The effect of PEG treatment was more pronounced at moderate (MI) than low (LI) irradiance. The stress-induced loss in the activity of superoxide dismutase and increase in the thylakoid lipid peroxidation accompanied a change in the physical status of the bilayer membrane as demonstrated by an enhancement of room temperature Chl *a* fluorescence polarization and decrease in energy transfer efficiency in pigment assembly. This resulted in a sustained decrease in photosystem 2 activity blocking channels of energy utilization. The absorbed quanta, thus unutilized, were excess even at MI, leading to photoinhibitory response.

Additional key words: *Cyamopsis tetragonoloba*; fluorescence polarization; lipid peroxidation; malondialdehyde; membrane fluidity; polyethylene glycol; proteins; superoxide dismutase.

Introduction

Chloroplast is the major target of many environmental stress factors (Anderson *et al*. 1995, Mostowska 1997, Biswal 1997a, Biswal and Biswal 1999, Biswal *et al*. 2003). Most of these factors sensitize photoinhibition even under moderate irradiance (Behera *et al*. 2003, Nayak *et al*. 2003), but the cascade of events operating in this response still remains unclear.

Reactive oxygen species (ROS) and their derivatives play multiple roles during stress signalling and photoinactivation of photosynthesis is primarily due to oxidative damage induced by them (Osmond 1994, Minkov *et al*. 1999). The bilayer lipid membrane is considered as the major site of their action (Scandalios 1993). These membranes, in addition to lipids and proteins, also contain several ions and various kinds of receptors. The stress-induced changes in the structure of the membrane may subsequently be transmitted to various types of cellular responses through appropriate biochemical changes. Murata and Loss (1997) critically discussed the role of membranes in perception of temperature stress and transduction of the stress signals to various types of cellular responses. However, a correlative study of water stress-induced alteration in membrane structure, photoinactivation of photosynthesis, and the antioxidative protective system is meagre.

The photo-protective role of carotenoids in general (Young 1991, Biswal 1995) and of β-carotene in particular in protection of the photosystem 2 (PS2) reaction centre against photoinhibitory damage is known (Telfer *et al*. 1994, Biswal and Deo 1995, Nayak *et al*. 2001, 2002). Recently, we have observed a loss in the D1 protein in the cotyledons of clusterbean seedlings experiencing PEG-induced water stress at moderate but not low irradiance. In our study, a stress-induced increase in lipid peroxidation and/or loss of the O_2 evolution leading to a sustained decrease in photon utilization by PS2 were identified as the major factors for sensitization of the chloroplasts for the photoinhibition. The exact mechanism involved in thylakoid lipid peroxidation and impairment of PS2 electron transport activity, however, remains unclear.

We chose senescing system for the study because the

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process is a part of plant development (Buchanan-Wollaston 1997, Thomas 1997, Beers *et al*. 2000) and senescing leaves, like developing and mature ones, also experience stress in nature and are prone to stress (Biswal *et al*. 2003). Second, leaf senescence is characterized by massive loss of pigments and macromolecules and alterations in chloroplast structure and function (Thomas and Stoddart 1980, Nooden *et al*. 1997) that are often correlated with oxidative metabolism (Biswal and Biswal 1988, Dangle *et al*. 2000, Biswal *et al*. 2003). The process is associated with increased membrane permeability, thylakoid lipid peroxidation, and decreased activity of free radical scavenging enzymes (Dhindsa *et al*. 1981, Biswal and Biswal 1990). On the other hand, various scientists consider water stress as a primary stress that subsequently causes oxidative stress because of its ability to generate different kinds of active oxygen species and free radicals (Menconi *et al*. 1995, Sgherri *et al*. 1996). It is, therefore, likely that the stress-induced formation of free radicals may aggravate senescence. Recently, Woo *et al*. (2004) examined several delayed leaf senescence mutants of *Arabidopsis* and observed the tolerance of the senescence-delaying mutants to oxidative stress. We tried to find out how the water stress signal is transduced into a photoinhibitory response through a change in lipid bilayer membrane structure in senescing clusterbean cotyledons.

Materials and methods

Clusterbean (*Cyamopsis tetragonoloba* L. cv. Pusa navbahar) seeds were surface sterilized with 30 % ethanol and kept under running water for 10 h. The imbibed seeds were germinated in dark in wet blotting papers. Healthy germinated seedlings were transplanted in perforated aluminium foils on glass Petri plates containing distilled water or 3 % aqueous polyethylene glycol (*PEG-6000*) solution and were allowed to grow in a temperature controlled room at 25±2 °C as described in Deo and Biswal (2001). Seedlings grown with distilled water under moderate irradiance of 12 W m^{-2} were termed as moderate irradiance (MI) control and those with PEG-induced water stress under the same irradiance as MI+W. Similar sets of the seedlings at 3 W m^{-2} were termed as low irradiance (LI) or low irradiance with water stress (LI+W) seedlings. Age of the seedlings was counted from the date of plating as day zero. Senescing cotyledons of 11–15 d old seedlings were used for the experiment. This phase of cotyledon growth in clusterbean is characterized as senescing phase (Deo and Biswal 2001).

Pigments were extracted with chilled 80 % acetone. Total chlorophyll (Chl) was estimated spectrophotometrically by the method of Arnon (1949) and total leaf proteins were estimated by the Coomassie brilliant blue dye binding method (Bradford 1976).

Chloroplasts were isolated by mechanical grinding of the cotyledons with chilled isolation medium containing 20 mM Tris–HCl buffer (pH 7.8), 5 mM $MgCl_2$, 400 mM sucrose, 10 mM NaCl, and 1 mM EDTA. The homogenate was squeezed through sixteen layers of cheese cloth and the filtrate was centrifuged at 500×*g* for 1 min. The supernatant was then centrifuged at 1 500×*g* for 10 min. The pellet was washed once and suspended with a small volume of the isolation medium.

Extent of lipid peroxidation in the chloroplasts of clusterbean cotyledons was measured in terms of accumulation of malondialdehyde (MDA) following the method of Heath and Packer (1968). Superoxide dismutase (SOD) activity was measured by inhibition of the photoreduction of nitro-blue tetrazolium (NBT) as described earlier (Becana *et al*. 1986). The assay medium in a final volume of 4.4 cm^3 contained 220 nmol Na-EDTA, 8.8 nmol riboflavin, 57 nmol methionin, 0.33 nmol NBT, and 10–100 mm^3 of chloroplast suspension equivalent to 5–50 μg Chl. One unit of the enzyme was defined as the amount inhibiting 50 % of NBT photoreduction.

The O_2 evolution was measured polarographically at 25 °C using $K_3Fe(CN)_6$ as the electron acceptor in a Clark type O_2 electrode (Tripathy and Chakraborty 1991) with slight modifications. The reaction mixture in 2 cm^3 contained 10 mM $MgCl_2$, 50 mM Tris-HCl buffer (pH 7.6), 2 mM $K_3Fe(CN)_6$, and chloroplasts equivalent to 50 μg Chl. The reaction was uncoupled from photophosphorylation by adding 5 mM NH_4Cl.

Room temperature Chl *a* fluorescence emission characteristics of isolated chloroplasts were measured by a spectrofluorimeter *Hitachi 650-40* (Panda *et al*. 1986). For all scannings, a slit width of 5 nm, scan speed of 120 nm per min, and chart speed of 60 nm per min were used. The chloroplast suspension equivalent to 6 μg Chl per cm^3 in 50 mM Tris–HCl buffer containing 175 mM NaCl (pH 7.8) was excited at 450 nm for the measurement of fluorescence emission in the range of 600–750 nm.

Chl *a* fluorescence polarization monitored at F_{685} was measured with the same equipment using polarization accessory. For polarization measurements, a slit width of 10 nm was maintained both at polarizer and analyzer units. The polarization value (P) was calculated from the polarized spectra at the same or perpendicular direction to polarization of incident radiation (Mar and Govindjee 1972). Chloroplasts equivalent to 6 μg Chl per cm^3 of the isolation medium were excited at 620 nm for all such measurements.

Energy transfer efficiency from carotenoids to Chl was measured from the fluorescence excitation spectra of chloroplasts by calculating the ratio of excitation at 475 nm (E_{475}) to excitation at 600 nm (E_{600}) following the method of Gruszecki *et al*. (1991).

Statistical analysis was carried out according to Glantz (1989). Each experiment was repeated four times and Students' *t*-test was carried out to compare the treatment means.

Results

Water stress was imposed by growing the seedlings in 3 % aqueous *PEG-6000* solution under controlled conditions as described in Deo and Biswal (2001). Fig. 1 depicts the changes in contents of Chl and proteins [% of control] in the cotyledons of clusterbean seedlings treated with MI, LI, MI+W, and LI+W. Senescence-induced loss of Chl and protein was more distinct in LI than in MI. Water stress treatment aggravated these losses and the highest decline was observed in the cotyledons experiencing the stress under moderate irradiance (MI+W).

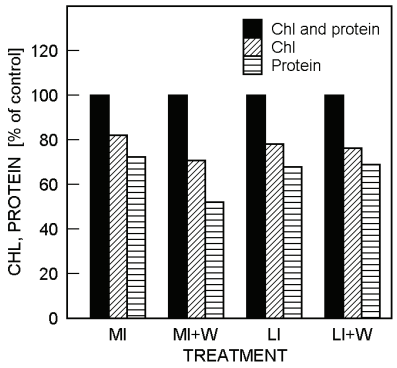

Fig. 1. Changes in contents of total chlorophyll (Chl) and proteins in the senescing cotyledons of clusterbean treated with moderate irradiance (MI), MI with water stress (MI+W), low irradiance (LI), and LI with water stress (LI+W). Measurements were made on the 15th d. The value on the 11th d in each treatment has been taken as 100 % (control). One hundred percent for total Chl corresponds to 17.9±1.2, 12.7±1.1, 15.0±1.0, and 12.2±0.9, and for total protein 104±12, 100±10, 79±8, and 87±6 g kg^{-1}(d.m.), respectively in MI, MI+W, LI, and LI+W. $n = 4$.

To assess senescence and stress-induced changes in the efficiency of the free radical scavenging enzyme, SOD activity was measured in the cotyledons of clusterbean (Fig. 2*A*). The loss in the activity was 17 % in MI and 14 % in LI. Water stress treatment in these two irradiances accelerated the loss to 45 and 36 %, respectively (Fig. 2*A*). Simultaneous with this, thylakoid lipid peroxidation measured as MDA accumulation increased in different cotyledon samples. On the 15th d, these increases were 33, 20, 85, and 56 % in MI, LI, MI+W, and LI+W, respectively (Fig. 3*A*). Thus the oxidative effect of water stress was more prominent in MI than LI.

Table 1 exhibits the changes in room temperature carotenoid to Chl excitation energy transfer that declined during senescence in all the treatments with highest decrease in MI+W. The fluorescence emission intensity (F_{685}) increased in all the cotyledon samples except for MI+W variant, where a quenching of ~18 % of fluorescence was observed. The enhancement of polarization value (P) was similar in MI and LI without the stress treatment (*i.e.* 51 %), but when water stress was administered, the polarization increased to 98 % in MI in contrast to 76 % in LI (Fig. 3*B*).

O_2 evolution declined consistently during the experimental period and remained at 83 and 80 % of the 11-d control value in MI and LI, respectively, at the end (Fig. 2*B*). Water stress accelerated the decline rapidly till 15th d of treatment. However, the decline in O_2 evolution was highest in MI+W with a loss of 57 % and comparatively less in LI+W with a loss of 44 %.

The activity of SOD did not exhibit any significant change during first 24 h of senescence. Stress treatment showed mild effect during this period. The initial stability of SOD may indicate its role on counteracting oxidative stress at 1st phase of senescence and leaves experiencing stress at this phase. An interesting trend was noticed while comparing the data found in MI and LI at the end of the experimental period. Senescence-induced loss in the pigments matched the loss in O_2 evolution and SOD activities which were 14–20 %, and lipid peroxidation increased by 20–35 % in the untreated control cotyledons. These changes were similar in MI and LI without any significant differences and this was in accordance with similar water status in both. Hence, the growth irradiance within short-range level, in absence of any additional stress, does not have much impact on the senescence syndrome of the clusterbean cotyledons. But when water stress was administered, the senescence syndrome was aggravated under both irradiances. However, the stress-induced changes were different in MI and LI.

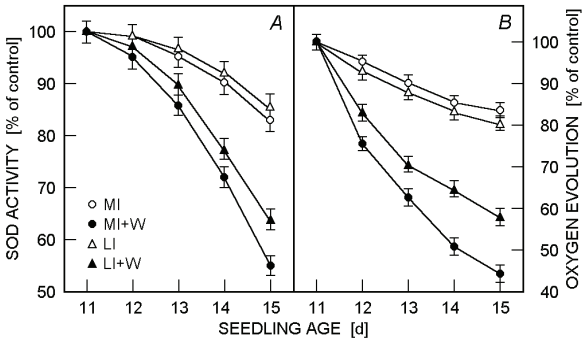

Fig. 2. Senescence-induced loss in (*A*) superoxide dismutase (SOD) activity and (*B*) loss in O_2 evolution in the cotyledons of clusterbean seedlings treated with moderate irradiance (MI), MI with water stress (MI+W), low irradiance (LI), and LI with water stress (LI+W). The value on the 11th d in each treatment has been taken as 100 % (control). One hundred percent for total Chl corresponds to represents (*A*) 2.82±0.09, 2.02±0.06, 2.36±0.05, and 2.06±0.04 enzyme units, and (*B*) 36.9±2.2, 14.7±1.1, 26.1±0.8, and 16.1±0.8 mmol(O_2) kg^{-1}(Chl) s^{-1} in MI, MI+W, LI, and LI+W, respectively. *Vertical bars* represent ± S.D. $n = 4$.

Table 1. Alterations in room temperature chlorophyll (Chl) *a* fluorescence emission and excitation energy transfer from carotenoids to Chl in the senescing cotyledons of clusterbean. The seedlings were treated with moderate irradiance (MI) = control, MI with water stress (MI+W), low irradiance (LI), and LI with water stress (LI+W). Measurements were made on 11th and 15th d of treatment. n = 4, ±SD. Percent changes were calculated with reference to the absolute values on the 11th d in each treatment taken as 100 %.

Parameter	Time [d]	Treatment			
		MI (control)	MI+W	LI	LI+W
Fluorescence emission at F_{685}	11	136±9	180±7	151±6	155±5
	15	163±9	147±8	179±6	190±9
	% change	+20 %	–18 %	+19 %	+23 %
Excitation energy transfer (E_{475}/E_{600})	11	4.26±0.07	3.58±0.05	3.39±0.07	3.13±0.06
	15	3.66±0.05	2.05±0.04	2.94±0.06	2.35 ±0.05
	% change	–14 %	–43 %	–13 %	–25 %

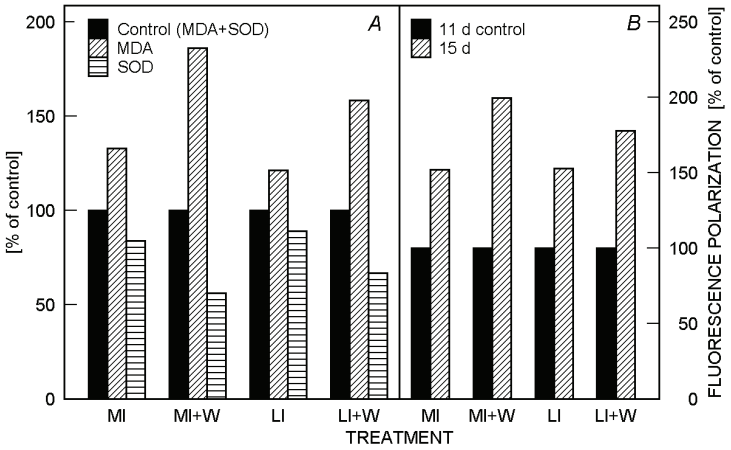

Fig. 3. Alterations in MDA content and SOD activity (*A*) and senescence-induced changes in room temperature chlorophyll *a* fluorescence polarization (*B*) in cotyledons of clusterbean grown in moderate irradiance (MI), MI with water stress (MI+W), low irradiance (LI), and LI with water stress (LI+W). The value on the 11th d in each treatment has been set to 100 % (control). One hundred percent values correspond for (*A*) to 33±2, 54±2.5, 30±2, and 46±3 mmol kg^{-1}(protein), and for (*B*) 0.039±0.002, 0.048±0.002, 0.035±0.002, and 0.042±0.003 [relative] in MI, MI+W, LI, and LI+W, respectively. n = 4.

Photosynthesis was inhibited in MI with a loss of pigments (29 %) as compared to higher loss of O_2 evolution (57 %) and SOD activity (45 %) in the background of a significant enhancement of lipid peroxidation (85 %). These findings suggest a possible sensitization of photoinhibition by water stress at MI.

Discussion

In the present work, senescence in clusterbean cotyledons was associated with a loss of the pigments and proteins (Fig. 1) and a gradual decline in O_2 evolution (Fig. 2*B*) suggesting senescence induced changes in the structure and functional activity of PS2. Biswal *et al*. (2003) critically discussed the possible mechanism of senescence-induced loss in the contents of pigments and macromolecules, and decline in photochemical efficiency of chloroplasts. Water stress aggravated these senescence-induced changes and a down regulation of PS2 activity was observed in the cotyledons experiencing the stress. However, these stress effects are more pronounced in MI than LI.

We found earlier a decrease in the PS2 electron transport activity measured as DCPIP photoreduction and a loss of D1 protein of PS2 in the clusterbean cotyledons grown in MI+W. In the present work, a gradual but significant loss of O_2 evolution in those cotyledons confirmed a possible structural damage and consequently a functional loss of PS2.

An appreciable degree of peroxidation of thylakoid lipids was observed in the chloroplasts isolated from clusterbean cotyledons experiencing water stress in MI (Fig. 3*A*). Lipid peroxidation is initiated by oxidative events arising due to generation of free radicals during senescence (Biswal *et al* 2003) and by various environmental perturbations (Pastori and Trippi 1993, Scandalios 1993). In our work, peroxidation of membrane lipids is a reliable indication of uncontrolled free radical/ROS production in the stressed cotyledons. Although the exact mechanism of the stress-induced formation of free radicals is not known, a loss in thylakoid photochemistry

and/or a decline in the ribulose-1,5-bisphosphate carboxylase/oxygenase activity resulting in a loss in the efficiency of Calvin cycle may favour their formation and create a redox back pressure on the electron transport chain (Biswal 1997b, Biswal *et al*. 2003). We did not examine the dark reaction of photosynthesis in the present work but various workers have observed a decline in activity of the Calvin cycle enzymes and reduced rates of CO_2 fixation during water stress treatment of plants and plant parts (Graan and Boyer 1990, Tuba *et al*. 1996), which may be a factor for overproduction of free radicals resulting in lipid peroxidation in water-stressed plants. In addition, a loss in O_2 evolution during the stress treatment in our experiment (Fig. 2*B*) may also result in the formation of highly oxidizing free radicals at reaction centre of PS2 by the long-lived P_{680}^+ on the donor side (Barber 1995, Biswal 1997a). An enhanced content of free radicals and consequent lipid peroxidation during the stress treatment in MI may also be attributed to the stress-induced suppression of the scavenging potential of the chloroplasts. This notion is supported by a decreased level of β-carotene as reported previously in the clusterbean cotyledons under the same experimental conditions (Deo and Biswal 2001) and a decline in the activity of SOD observed in this study (Fig. 2).

The decline in SOD activity has been reported earlier during senescence of leaves (Dhindsa *et al*. 1981, Bowler *et al*. 1992) but the actual mechanism of this decline still remains unclear. The initial stability of the enzyme both in the senescing cotyledons and cotyledons experiencing the stress (Fig. 2) suggests a role of the enzyme to counter oxidative stress. Subsequent loss in the activity may be attributed to the loss in capacity of the cotyledons to synthesize the enzyme protein. However, it is difficult to establish a precise relationship between oxidative damage and ROS and SOD activities. Woo *et al*. (2004) found that in some senescence-delaying mutants of *Arabidopsis*, the activity of SOD and other antioxidative enzymes did not have any link with the increased tolerance of the mutants to oxidative stress.

An enhanced rate of thylakoid lipid peroxidation in the background of poor scavenging environment may inhibit mobility of the lipid acyl chains and cause rigidification of the bilayer membrane by inducing a gel phase and altering the ultrastructure of the thylakoid membrane (Li *et al*. 1989). The fact that the physical state of the membrane has been altered by water stress treatment is evident from the observed data on room temperature Chl *a* fluorescence polarization (Fig. 3*B*). A greater extent of Chl *a* fluorescence polarization in MI+W in the present experiment (Fig. 3*B*) thus can be correlated with a higher lipid peroxidation (Fig. 3*A*). On the other hand, the lower level of gel phase formation in LI- than MI-grown cotyledons with the stress treatment, as evident from fluorescence polarization data (Fig. 3*B*), may be due to less accumulation of free radicals and consequently a low lipid peroxidation (Fig. 3*A*).

Judging from the data on stress-induced changes in fluorescence polarization (Fig. 3*B*) and decrease in excitation energy transfer in the thylakoid in our study (Table 1), it can be inferred that the association of the Chl and carotenoids with the pigment-protein complexes has been changed as a result of decreased fluidity of the membrane. This indicates a dislocation of the pigments on thylakoid complexes leading to a loss in the efficiency of utilization of photon energy and consequently loss in the photochemical potential of thylakoids (Fig. 2*B*). The excess photons unused, thus, caused further damage to PS2 in the senescing cotyledons of clusterbean experiencing the stress.

In addition to the accumulation of unutilized quanta due to decrease in CO_2 assimilation as recently reported by Lu *et al*. (2003), we propose that senescence and stress induced membrane gel phase formation and consequently a change in the topology of the pigments on thylakoids may cause closure of channels of photochemistry. This may bring about accumulation of excess quanta leading to photoinhibitory response.

References

Anderson, J.M., Chow, W.S., Park, Y.-I.: The grand design of photosynthesis: Acclimation of the photosynthetic apparatus to environmental cues. – Photosynth. Res. **46**: 129-139, 1995.

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. – Plant Physiol. **24**: 1-15, 1949.

Barber, J.: Molecular basis of the vulnerability of photosystem II to damage by light. – Aust. J. Plant Physiol. **22**: 201-208, 1995.

Becana, M., Aparicio-Tejo, P., Irigoyen, J., Sanchez-Diaz, M.: Some enzymes of the hydrogen peroxide metabolism in leaves and root nodules of *Medicago sativa* L. – Plant Physiol. **82**: 1169-1171, 1986.

Beers, E.P., Woffenden, B.J., Zhao, C.: Proteolytic enzymes: possible roles during programmed cell death. – Plant mol. Biol. **44**: 399-415, 2000.

Behera, S.K., Nayak, L., Biswal, B.: Senescing leaves possess potential for stress adaptation: the developing leaves acclimated to high light exhibit increased tolerance to osmotic stress during senescence. – J. Plant Physiol. **160**: 125-131, 2003.

Biswal, B.: Carotenoid catabolism during leaf senescence and its control by light. – J. Photochem. Photobiol. B **30**: 3-13, 1995.

Biswal, B.: Chloroplasts, pigments and molecular responses of photosynthesis under stress. – In: Pessarakli, M. (ed.): Handbook of Photosynthesis. Pp. 877-885. Marcel Dekker, New York – Basel – Hong Kong 1997a.

Biswal, B.: Chloroplast metabolism during leaf greening and degreening. – In: Pessarakli, M. (ed.): Handbook of Photosynthesis. Pp. 72-81. Marcel Dekker, New York – Basel – Hong Kong 1997b.

Biswal, B., Biswal, U.C.: Photosynthesis under stress: stress signals and adaptive responses of chloroplasts. – In: Pessarakli, M. (ed.): Handbook of Plant and Crop Stress. 2nd Ed. Pp. 315-336. Marcel Dekker, New York – Basel 1999.

Biswal, B., Deo, P.M.: ß-carotene protects D1 protein of thylakoid during senescence and water stress. – In: Mathis, P. (ed.): Photosynthesis: from Light to Biosphere. Vol. IV. Pp. 573-576. Kluwer Academic Publ., Dordrecht – Boston – London 1995.

Biswal, U.C., Biswal, B.: Ultrastructural modifications and biochemical changes during senescence of chloroplasts. – Int. Rev. Cytol. **113**: 271-321, 1988.

Biswal, U.C., Biswal, B.: Plant senescence and changes in photosynthesis. – Biol. Educ. **3**: 33-61, 1990.

Biswal, U.C., Biswal, B., Raval, M.K.: Chloroplast Biogenesis: From Proplastid to Gerontoplast. – Kluwer Academic Publ., Dordrecht 2003.

Bowler, C., Montagu, M.V., Inze, D.: Superoxide dismutase and stress tolerance. – Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 83-116, 1992.

Bradford, M.M.: A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. – Anal. Biochem. **72**: 248-254, 1976.

Buchanan-Wollaston, V.: The molecular biology of leaf senescence. – J. exp. Bot. **48**: 181-199, 1997.

Dangle, J.L., Dietrich, R.A., Thomas, H.: Senescence and programmed cell death. – In: Buchanan, B., Gruissem, W., Lones, R. (ed.): Biochemistry and Molecular Biology of Plants. Pp. 1044-1100. American Society of Plant Physiologists, Jones 2000.

Deo, P.M., Biswal, B.: Response of senescing cotyledons of clusterbean to water stress in moderate and low light: Possible photoprotective role of β-carotene. – Physiol. Plant. **112**: 47-54, 2001.

Dhindsa, R.S., Plumb-Dhindsa, P., Thorpe, T.A.: Leaf senescence, correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. – J. exp. Bot. **32**: 93-101, 1981.

Glantz, S.A.: Primer of Biostatistics. 2nd Ed. – McGraw Hill, New York 1989.

Graan, T., Boyer, J.S.: Very high CO_2 partially restores photosynthesis in sunflower at low water potentials. – Planta 181: 378-384, 1990.

Gruszecki, W.I., Veeranjaneyulu, K., Zelent, B., Leblanc, R.M.: Energy transfer process during senescence: fluorescence and photoacoustic studies of intact pea leaves. – Biochim. biophys. Acta **1056**: 173-180, 1991.

Heath, R.L., Packer, L.: Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. – Arch. Biochem. Biophys. **125**: 189-198, 1968.

Li, G., Knowles, P.F., Murphy, D.J., Nishida, I., Marsh, D.: Spin-labelled ESR studies of lipid-protein interactions in thylakoid membranes. – Biochemistry **28**: 7446-7452, 1989.

Lu, Q., Wen, X., Lu, C., Zhang, Q., Kuang, T.: Photoinhibition and photoprotection in senescent leaves of field-grown wheat plants. – Plant Physiol. Biochem. **41**: 749-754, 2003.

Mar, T., Govindjee: Decrease in the degree of polarization of chlorophyll fluorescence upon addition of DCMU to algae. – In: Forti, G., Avron, M., Melandri, A. (ed.): Photosynthesis, Two Centuries After its Discovery by Joseph Priestley. Vol. 1. Pp. 271-281. Dr W. Junk Publ., The Hague 1972.

Menconi, M., Sgherri, C.L.M., Pinzino, C., Navari-Izzo, F.: Activated oxygen production and detoxification in wheat plants subjected to a water deficit programme. – J. exp. Bot. **46**: 1123-1130, 1995.

Minkov, I.N., Jahoubjan, G.T., Denev, I.D., Toneva, V.T.: Photooxidative stress in higher plants. – In: Pessarakli, M. (ed.): Handbook of Plant and Crop Stress. 2nd Ed. Pp. 499-525. Marcel Dekker, New York – Basel 1999.

Mostowska, A.: Environmental factors affecting chloroplasts. – In: Pessarakli, M. (ed.): Handbook of Photosynthesis. Pp. 407-426. Marcel Dekker, New York – Basel – Hong Kong 1997.

Murata, N., Loss, D.A.: Membrane fluidity and temperature perception. – Plant Physiol. **115**: 875-879, 1997.

Nayak, L., Biswal, B., Ramaswamy, N.K., Iyer, R.K., Nair, J.S., Biswal, U.C.: Ultraviolet-A induced changes in photosystem II of thylakoids: effect of senescence and high growth temperature. – J. Photochem. Photobiol. B **70**: 59-65, 2003.

Nayak, L., Raval, M.K., Biswal, B., Biswal, U.C.: Photoprotection of green leaves by zeaxanthin, a two-channel process. – Curr. Sci. **81**: 1165-1166, 2001.

Nayak, L., Raval, M.K., Biswal, B., Biswal, U.C.: Topology and photo protective role of carotenoids in photosystem II of chloroplast: a hypothesis. – Photochem. Photobiol. Sci. **1**: 629-631, 2002.

Nooden, L.D., Guiamét, J.J., John, I.: Senescence mechanisms. – Physiol. Plant. **101**: 746- 753, 1997.

Osmond, C.B.: What is photoinhibition? Some insights from comparisons of shade and sun plants. – In: Baker, N.R., Bowyer, J.R. (ed.): Photoinhibition of Photosynthesis: from Molecular Mechanisms to the Field. Pp. 1-24. Bios Scientific Publ., Oxford 1994.

Panda, S., Raval, M., Biswal, U.C.: Manganese-induced modification of membrane lipid peroxidation during aging of isolated wheat chloroplasts. – Photobiochem. Photobiophys. **13**: 53-61, 1986.

Pastori, G.M., Trippi, V.S.: Antioxidative protection in a drought-resistant maize strain during leaf senescence. – Physiol. Plant. **87**: 227-231, 1993.

Scandalios, J.G.: Oxygen stress and superoxide dismutase. – Plant Physiol. **101**: 7-12, 1993.

Sgherri, C.L.M., Pinzino, C., Navari-Izzo, F.: Sunflower seedlings subjected to increasing stress by water deficit: Changes in $O_2^{\cdot-}$ production related to the composition of thylakoid membranes. – Physiol. Plant. **96**: 446-452, 1996.

Telfer, A., Dhami, S., Bishop, S.M., Phillips, D., Barber, J.: β-carotene quenches singlet oxygen formed by isolated photosystem II reaction centers. – Biochemistry **33**: 14469-14474, 1994.

Thomas, H.: Chlorophyll: a symptom and a regulator of plastid development. – New Phytol. **136**: 163-181, 1997.

Thomas, H., Stoddart, J.L.: Leaf senescence. – Annu. Rev. Plant Physiol. **31**: 83-111, 1980.

Tripathy, B.C., Chakraborty, N.: 5-aminolevulinic acid induced photodynamic damage of the photosynthetic electron transport chain of cucumber (*Cucumis sativus* L.) cotyledons. – Plant Physiol. **96**: 761-767, 1991.

Tuba, Z., Lichtenthaler, H.K., Csintalan, Z., Nagy, Z., Szente, K.: Loss of chlorophylls, cessation of photosynthetic CO_2 assimilation and respiration in the poikilochlorophyllous plant *Xerophyta scabrida* during desiccation. – Physiol. Plant. **96**: 83-388, 1996.

Woo, H.R., Kim, J.H., Nam, H.G., Lim, P.O.: The delayed leaf senescence mutants of *Arabidopsis, ore 1, ore 3,* and *ore 9* are tolerant to oxidative stress. – Plant Cell Physiol. **45**: 923-932, 2004.

Young, A.J.: The photoprotective role of carotenoids in higher plants. – Physiol. Plant. **83**: 702-708, 1991.