PHOTOSYNTHETICA 39 (2): 183-189, 2001

Photosynthetic electron transport, photophosphorylation, and antioxidants in two ecotypes of reed (*Phragmites communis* Trin.) from different habitats

X.Y. ZHU, G.C. CHEN, and C.L. ZHANG*

Department of Biology, Lanzhou University, Lanzhou 730000, Gansu, P. R. China

Abstract

We compared chloroplast photochemical properties and activities of some chloroplast-localised enzymes in two ecotypes of *Phragmites communis*, swamp reed (SR, C_3-like) and dune reed (DR, C_4-like) plants growing in the desert region of north-west China. Electron transport rates of whole electron transport chain and photosystem (PS) 2 were remarkably lower in DR chloroplasts. However, the electron transport rate for PS1 in DR chloroplasts was more than 90 % of the activity similar in the SR chloroplasts. Activities of Mg^{2+}-ATPase and cyclic and non-cyclic photophosphorylations were higher in DR chloroplasts than in the SR ones. The activities of chloroplast superoxide dismutase (SOD) and ascorbate peroxidase (APX), both localised at or near the PS1 complex and serving to scavenge active oxygen around PS1, and the content of ascorbic acid, a special substrate of APX in chloroplast, were all higher in DR chloroplasts. Hence reed, a hydrophytic plant, when subjected to intense selection pressure in dune habitat, elevates its cyclic electron flow around PS1. In consequence, it provides extra ATP required by C_4 photosynthesis. Combined high activities of active oxygen scavenging components in DR chloroplasts might improve protection of photosynthetic apparatus, especially PS1, from the damage of reactive oxygen species. This offers new explanation of photosynthetic performance of plant adaptation to long-term natural drought habitat, which is different from those, subjected to the short-term stress treatment or even to the artificial field drought.

Additional key words: antioxidant; ascorbate peroxidase; chloroplast; cyclic electron flow; Mg^{2+}-ATPase; natural drought; photosystem 1 and 2 activities; stomatal resistance; superoxide dismutase; transpiration rate.

Introduction

Modifying the developmental properties in consequence to environmental transition is the main factor for plant adaptation to special habitat. The responses to changed environment usually include quantitative changes in growth, differentiation of cells and organs, and even the changes in metabolic pathway within the tissues. Water deficit, as a common and main stress factor, can induce the transformation of carbon metabolic pathway in higher plants. For example, some species, such as cactus and *Mesembrianthemum*, can change its pathways to Crassulacean acid metabolism (CAM) or "latent CAM" modes (Winter 1974, Cushman and Bohnert 1999). In other species, such as maize and purslane, night-time accumulation of malic acid decreased to a similar degree as leaf water potential that decreased to –0.4 MPa regardless of leaf age; this shows a tendency of transformation of C_4 into C_3 pathway (Shang-Guan 1998). Obviously, as plants cope with changing environment, their ability to alter carbon metabolism offers great flexibility and adaptability for survival.

C_4 metabolic pathway is regarded a superior carbon assimilation mode for plant survival under conditions that cripple C_3 pathway such as high temperature, high irradiance, and water deficit (Hatch 1992, Furbank and Taylor 1995, Ku *et al.* 1996). The amphibious leafless sedge *Eleocharis vivipara* developed Kranz anatomy and conducted C_4 photosynthesis under terrestrial conditions, but developed C_3-like traits and operated C_3 photosynthesis when submerged in water (Ueno 1996). Exposed to higher temperature and drought treatment for two weeks, two C_3 plants, *Haberlea rhodopensis* and *Romondic servica,* showed significantly elevated C_4 photosynthesis (Shang-Guan 1998).

In contrast to the situation for C_3 plants, it is essential to enhance the ATP pool to satisfy the need of C_4 photosynthesis. Under water stress, reductions in primary

---

Received 2 October 2000, *accepted* 20 March 2001.

*Author for correspondence; e-mail: zhangcl@lzu.edu.cn

Abbreviations: Aa, ascorbic acid; APX, ascorbate peroxidase; Chl, chlorophyll; DCPIP, 2,6-dichlorphenol indophenol; DTT, dithiothreitol; MV, methyl viologen; PETR, photosynthetic electron transport rate; PPFD, photosynthetic photon flux density; PS, photosystem; SOD, superoxide dismutase.

Acknowledgement: The research was supported by the China National Key Basic Research Special Funds No. G 1999011700.

events of photosynthesis result from the depression of PS2 function (Govindjee *et al.* 1981, Matorin *et al.* 1982, Havaux *et al.* 1986). Exposed to drought treatment, both wheat and maize seedlings exhibited lowered PS2 activities in our laboratory (He *et al.* 1995, Hao *et al.* 1996). Natural drought is always accompanied by other limiting factors, such as high temperature and high irradiance. The combination of these factors favours photoinhibition of PS2 (Björkman and Powles 1984, Valladares and Pearcy 1997). This will undoubtedly affect electron flow from PS2 to PS1, and thus influence ATP synthesis coupled with it. Moreover, decreased PS2 activity and lowered carbon assimilation capacity induced by drought will substantially increase electron flow to O_2 and result in an imbalance between production and scavenging of activated O_2. In consequence, it will induce the oxidative damage mediated by reactive oxygen species in chloroplasts (Smirnoff 1993, Asada 1996). However, few experiments have given satisfying insights into the regulation relationships among photosynthetic electron transport, ATP supply, and antioxidant defences in plant adaptation to long-term drought and with changed photosynthesis mode (from C_3 to C_4).

P. communis Trin. is a hydrophytic species whose typical habitats are the fresh and brackish water area of swamp, riversides, and lake sides. However, reed plants can adapt to adverse terrestrial habitats and evolve various ecotypes exhibiting genetic difference (Haslam 1970, 1975, Matoh *et al.* 1988). In addition to swamp reed, there are other three terrestrial reed ecotypes growing in the desert regions of north-west China. Studies on these four reed ecotypes conducted in our laboratory have proved some stable variations of morphological and physiological characteristics in response to drought and salinity in different terrestrial ecotypes. To comprehensively understand the effects of drought on photosynthesis in plants, we studied the ecophysiology of two wild reed ecotypes from contrasting habitats, *i.e.*, common swamp reed and arid-resistant dune reed.

Materials and methods

Plants and sampling site: Two ecotypes of *P. communis* Trin., referred to as SR (swamp reed, growing in 1-m-deep swamp) and DR (dune reed, growing on 5 to 10-m-high sand dune) according to the traits of their respective habitats, were selected for experiments at the Desert Research Institute of Chinese Academy of Sciences, Linze Research Area (39°31'-58'N, 100°4'-36'E; 1 300 m elevation). This region is a typical desert landscape as previously described (Wang *et al.* 1998). During July 1 to 4, the second leaves of the two reed ecotypes were simultaneously collected at midday and frozen in liquid N_2 except those for preparing intact chloroplasts.

Isolation of chloroplasts: Intact chloroplasts were prepared quickly as described by Reeves and Hall (1980). 2 mM ascorbic acid was added before isolation. The pellets were suspended in the 0.33 M sorbitol, 2 mM EDTA, 1 mM $MgCl_2$, 50 mM Hepes-NaOH (pH 7.6). Intactness of chloroplasts (approximately 60 %) was determined from measurements of ferricyanide-dependent oxygen evolution before and after osmotic shock of the chloroplasts (Lilley *et al.* 1975). Aliquots of chloroplasts were frozen in liquid N_2 until assayed for photochemical activities, photophosphorylation, and chloroplast-localised antioxidant enzymes.

Assays of electron transport rate and photophosphorylation: An aliquot of chloroplasts was thawed and centrifuged at 66.7 rps for 2 min and re-suspended in the medium as above. The concentration of Chl was determined spectrophotometrically in 80 % acetone as described by Arnon (1949). The rates of electron transport for whole-chain, PS1, and PS2 were determined by monitoring O_2 uptake or evolution with Clark-type O_2 electrode connected to a recorder (Tripathy and Mohanty 1980). The assays with chloroplasts corresponding to 20 µg of Chl in a final volume of 1.2 cm^3 were carried out at 24.5 °C using a temperature-controlled cuvette and irradiance of 900 µmol m^{-2} s^{-1} PPFD. Non-cyclic photophosphorylation was measured in medium according to Mayoral *et al.* (1981) with the exception that ^{32}P was omitted. 0.2 cm^3 of chloroplasts corresponding to 30 µg of Chl were added to 0.8 cm^3 reaction mixture containing 30 mM Tricine-NaOH (pH 8.0), 43 mM NaCl, 4 mM $MgCl_2$, 1.6 mM ADP, 25 µM ferricyanide, and 4 mM sodium phosphate. Cyclic photophosphorylation was assayed in the same way as the non-cyclic one except that 10 µM PMS and 20 µM DCMU were substituted for ferricyanide. All samples were irradiated at 25 °C for 90 s with 1 000 µmol m^{-2} s^{-1} PPFD that had been filtered through 10 cm of H_2O. After the light was turned off, 0.1 cm^3 of 20 % trichloroacetic acid was added, and the test tubes were centrifuged for 10 min at 66.7 rps. Aliquots (0.2 cm^3) of supernatant were used for determinations of ATP formation as described by Lundin *et al.* (1976), measuring luminescence of luciferin in the presence of firefly luciferase.

Activity of chloroplast Mg^{2+}-ATPase and its Arrhenius plots: Aliquots of chloroplasts (1 cm^3) corresponding to 100 µg Chl were added to 1 cm^3 of activation medium containing 0.25 M Tris-HCl (pH 8.0), 0.5 M NaCl, 0.05 M $MgCl_2$, 50 mM DTT, and 0.5 mM PMS. Samples were irradiated at room temperature for 5 min with 900 µmol m^{-2} s^{-1}. Enzyme activity was determined according to McCarty and Racker (1968). The reaction tubes were incubated in a temperature range from 0 to 40 °C (an interval of 4 °C) for 20 min, the reactions were stopped by addition of trichloracetic acid to a final concentration of 2 %. The content of inorganic phosphorus was determined by the method of Taussky and Shorr (1953). The Arrhenius plots of chloroplastic Mg^{2+}-ATPase were drawn using the logarithm of the enzyme reaction rate as the ordinate and reciprocal of absolute temperature as abscissa.

Measurements of ascorbic acid (Aa) and ATP contents in leaves: Ascorbic acid contents in leaves were measured according to the method of Shigeoka *et al.* (1979). For measurement of ATP content in leaves, 1.5 g of leaf tissues pulverised in liquid nitrogen were homogenised in 10 cm^3 of steam water. The tubes containing homogenate were loosely fitted with corks and heated in vigorously boiling water bath for 15 min, then cooled and centrifuged at 66.7 rps for 5 min. ATP contents were determined by the method of bioluminescence as above.

Activities of antioxidant enzymes in leaves and chloroplasts: The SOD activity was measured spectrophotometrically as described by Dhindsa *et al.* (1981). Leaf tissue pulverised in liquid nitrogen was homogenised in extraction medium containing 50 mM KPO_4 (pH 7), 0.1 mM EDTA, and 5 % polyvinylpyrrolidone. After centrifugation (1500×g, 20 min), aliquots of the supernatant were taken away to determine enzyme activity and protein concentration (Lowry *et al.* 1951). Activity of APX was assayed from the decrease in absorbance at 290 nm as reported by Nakano and Asada (1981), with 0.5 mM ascorbate as a donor. For chloroplast-localised enzyme analysis, isolated intact chloroplasts were thawed, centrifuged, and re-suspended in the appropriate enzyme extraction solutions. Aliquots were used to determine Chl content.

Determination of stomatal resistance and transpiration rate: On midday (from 12:00 to 14:00) of July 1, 1998, the gas exchange parameters of the second leaves of mature shoot of two reed ecotypes were examined individually with *Li-1600* Steady State Porometer (*Li-Cor*, USA).

Results

Stomatal resistance and transpiration rate of SR and DR leaves: Even though both SR and DR plants shared similar meteorological conditions, they markedly differed in growth and development. SR had relatively tall and big shoots with wide leaves, while DR produced short and small shoots with narrow leaves. Therefore, the population density of DR was much thinner than that of SR. Besides the soil water content, different habitat and growth characteristics of plants also lead to differences in relative humidity and photon-flux density in canopy of the community. This indicates that DR was subjected not only to water stress, but also to high temperature and high irradiance (Table 1).

Table 1. Environmental conditions, stomatal resistance, and transpiration rate in the second leaves of two ecotypes of *P. communis* measured around noon, on July 1, 1998. Each value is the mean±SE of 11-13 leaves. SR: swamp reed; DR: dune reed. $^{**}p < 0.01$, $^{*}p < 0.05$. --- not determined or not calculated.

Ecotype	Soil water content [%]	Environment temperature [°C]	Canopy relative humidity [%]	Photon flux density [µmol m^{-2} s^{-1}]	Leaf temperature [°C]	Stomatal resistance [s cm $^{-1}$]	Transpiration rate [µmol m^{-2} s^{-1}]
SR	---	24.4±0.41	59.86±4.13	800.0±35.53	23.66±1.20	0.86±0.03	16.69±2.94
DR	13.7±0.33	27.7±0.78*	16.72±1.50**	872.4±40.06*	26.72±0.61**	1.51±0.09**	14.76±2.43*

Electron transport rate and photophosphorylation of chloroplasts: Photosynthetic electron transport rates (PETR) of whole-chain and PS2 were remarkably lower, being 55.7 and 72.5 %, respectively, in chloroplasts isolated from DR plants than from the SR ones. However, PETR for PS1 maintained 91.8 % activity of SR's, indicating that PS1 was more stable than PS2 under natural drought (Fig. 1). Non-cyclic and namely cyclic photophosphorylation activities of DR were higher than these activities of SR (Fig. 1). We observed considerably less PS2 and whole-chain activities in DR than SR, while PS1 was still relatively very active in chloroplasts of DR. The cyclic electron flow around PS1 may thus greatly contribute to photophosphorylation in DR chloroplasts.

Arrhenius plots of chloroplastic Mg^{2+}-ATPase: In the range from 0 to 40 °C, the activities of chloroplast Mg^{2+}-ATPase in DR were significantly higher than those in SR, being [mmol(P_i) kg^{-1}(Chl) s^{-1}] 38.18 in DR and 13.65 in SR at 24°C, respectively. Arrhenius plots, presented as the logarithm of Mg^{2+}-ATPase reaction rate *versus* the reciprocal of absolute temperature, showed that DR and SR possessed different break points (about 15 °C in DR and 19 °C in SR). This was generally regarded as a lipid-phase transition temperature or conformational change temperature of membrane-bound enzyme (Fig. 2). Since the membrane lipid-phase transition temperature is reciprocally related to the membrane fluidity, this result also suggests that the DR's thylakoid membrane has a higher membrane fluidity.

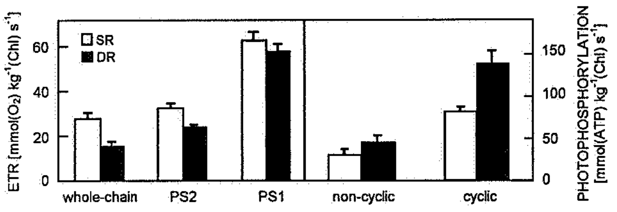

Fig. 1. The PS1, PS2, and whole-chain electron transport rates and the cyclic and non-cyclic photophosphorylations in chloroplasts isolated from two ecotypes (SR, swamp reed; DR, dune reed). Means±SE of three independent chloroplast preparations from leaves collected on July 1 to 4, 1998.

The content of ATP in leaves of SR and DR: ATP content on the basis of unit dry mass in DR leaves was 6.2-fold lower than in SR leaves [31.5±1.22 *vs.* 196.8±8.24 mmol(ATP) kg^{-1}(d.m.)]. Combining this with the above results of chloroplast Mg^{2+}-ATPase activities and photophosphorylation, we suggest that the turnover of ATP in DR leaves is much higher than in SR leaves.

The activities of SOD and APX and the content of Aa in SR and DR: Expressed on a unit protein basis, SOD activity in DR leaves was slightly higher than that in SR leaves, while per unit Chl it was 3.3-fold higher in DR than in SR (Table 2). Being consistent with a considerable increase of the content of ascorbic acid in its leaves, DR also showed a higher activity of APX, another main antioxidant enzyme, which specifically demands ascorbate as a reductant and reduces H_2O_2 to H_2O in chloroplasts (Table 2). This result corresponds to that of Gupta *et al.* (1993), who observed an enhancement of active oxygen-scavenging system in chloroplasts of tobacco; this increase in oxidative stress protection could result not only from enhanced SOD content but from the combined increases in SOD and APX activities. The content of ascorbate acid, a special substrate of APX in chloroplasts, was 2.5-fold higher in DR leaves than in SR.

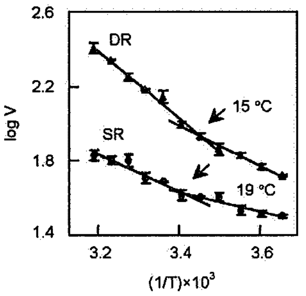

Fig. 2. The Arrhenius plots of the thylakoid-bound Mg^{2+}-ATPase in chloroplasts isolated from the leaves of swamp reed (SR) and dune reed (DR) *P. communis*. Each point represents the mean of three individual measurements. Bars±SE. The temperatures of break points on the plots, which are calculated by equations of two tendency lines, correspond to about 15 °C for DR and 19 °C for SR. V: enzyme reaction velocity; T: absolute temperature.

Table 2. Specific activities of SOD and APX in extracts of whole leaves and isolated chloroplasts and ascorbic acid (Aa) content in two ecotypes of *P. communis* (SR, swamp reed; DR, dune reed). Means±SE; *n* = 5 for SOD, and *n* = 3 for APX and Aa.

Ecotype	Active oxygen-scavenging enzymes Leaf SOD [Unit mg^{-1}(protein)]	Chloroplast SOD [Unit mg^{-1}(Chl)]	Chloroplast APX [mmol(ascorbate oxidized) kg^{-1}(Chl) s^{-1}]	Ascorbic acid [g kg^{-1}(d.m.)]
SR	32.55±3.10	50.91±6.80	15.0±0.81	0.050±0.002
DR	47.96±2.30	167.80±10.70	28.5±1.00	0.124±0.005
DR/SR	1.5	3.3	1.9	2.5

Discussion

The ability of plants to acclimate to long-term drought in their environment is crucial for determining their resistance to stress, and is a significant factor in their habitat preference (Anderson and Osmond 1987, Murchie and Horton 1997). Evolution of C_4 plants from C_3 ones had to find several precursor enzymes to modify, amplify, and relocate in specific cell compartments to catalyse various special reactions of C_4 photosynthesis. At the same time, substantial modifications of leaf anatomy had to occur co-ordinated with the development of this specialised biochemistry (Hatch 1992). Recently, comparative studies on the photosynthetic characteristics of four reed ecotypes from the north-west China were conducted in our laboratory. On the basis of $\delta^{13}C$ values, activities of photosynthetic enzymes, leaf anatomy, and ultrastructure, we conclude that this reed species evolved the C_3-like ecotype in swamp environments, and the C_4-like C_3-C_4 intermediate in dune desert environments (Zheng *et al.* 2000).

The relative rates of ATP consumption and production are very important in photosynthetic regulation (Furbank and Foyer 1986, Furbank and Horton 1987). In contrast to C_3 plants, C_4 plants require extra ATP for C_4 photosynthesis pathway. Decreased activities of PS2 and whole-chain PETR will inevitably result in the decline in photophosphorylation that couples with the linear electron transport from PS2 to PS1. The decrease in photophosphorylation under water stress results from the changes in activity and conformation of thylakoid-bound ATPase (Mayoral *et al.* 1981, Sharkey *et al.* 1982). We found that cyclic phosphorylation around PS1 in DR plants was markedly higher than that in the SR ones (Fig. 1), and chloroplastic Mg^{2+}-ATPase in the leaves of DR showed higher activity than that in SR leaves (Fig. 2). This result was different from those reporting that photophosphorylation is more seriously affected by water stress than the electron transport.

The existence of cyclic photophosphorylation in higher plants and its potential for ATP production are well established (Furbank and Horton 1987, Hebert *et al.* 1990, Heber and Walker 1992, Katona *et al.* 1992). This cyclic electron flow appears to operate preferentially when the electron supply from PS2 is limited, for example, by down-regulation of PS2 (Hebert *et al.* 1990, Asada 1999) or when the linear electron transport is restricted by lower acceptor availability in leaves whose stomata are closed (Katona *et al.* 1992). When carbon dioxide limits photosynthesis as a result of water stress in combination with high irradiance, coupled cyclic electron transport helps to establish a proton gradient large enough to overcome decreased electron flow from PS2 to PS1 (Heber and Walker 1992). Furbank *et al.* (1990) also suggest that in C_4 plants an important additional role of cyclic electron flow provides the extra ATP needed for photosynthetic pathway. Studies on Chl fluorescence in *Synechocystis* PCC 6803 show that higher fluidity of thylakoid membrane can not only ensure conformational adjustments of membrane-bound components but also promote the transport of the cyclic electron flow around PS1 (Mi *et al.* 1997). Although the energy storage capacity of cyclic photophosphorylation alone might be small in comparison with the capacity of energy storage by linear and cyclic electron flow combined, it might be significant under such conditions as DR's, which has evolved to C_4 pathway with decreased PS2 activity under long-term drought. At this point, we agree with the view of Hebert *et al.* (1990), *i.e.*, even if the amount of ATP produced by cyclic electron flow through PS1 is small, it is very important for repair or compensation of stress-damaged or down-regulated PS2 units.

In chloroplasts, dioxygen is univalently photoreduced to the superoxide anion radical in PS1 (Asada *et al.* 1974, Asada 1999). This indicates that PS1 is the most threatened site in chloroplasts under the oxidative stress originated from drought. So it is very important to scavenge active oxygen in chloroplasts at the site of their generation quickly and effectively, and thus to protect PS1 complex, the stroma enzymes, and scavenging system itself from the damage by the reactive oxygen species (Asada 1999).

Experiment with immuno-gold labelling showed that in spinach chloroplasts the local concentration of SOD on the stroma-facing thylakoid membranes (PS1) was about 1 mM (Ogawa *et al.* 1995). Thylakoid-bound APX was also located in the vicinity of PS1 complex at a ratio of 1 : 2 (Miyake *et al.* 1993), and the content of Aa in chloroplasts accounted for 40 % of the total protoplasm (Foyer *et al.* 1983). Thus elevated enzyme and non-enzyme systems for scavenging active oxygen in DR chloroplasts are capable of scavenging $O_2^{\cdot -}$ and H_2O_2 at the site of its generation prior to their interaction with the target molecules and PS1 complex, thus ensuring that PS1 functions normally even under long-term drought. Simultaneously, elevated concentration of anti-oxidative systems in DR chloroplasts can also protect thylakoid membrane lipids from oxidative damage, and in turn, ensure the structural completeness of membrane and compartmentation of protons, and bring all enzymes on the thylakoid membranes to the best conformational state that ensures high activities.

In summary, when reed, a hydrophilic plant, was translocated from water to dune habitat, its photosynthetic apparatus, mainly PS2, was partly damaged or down regulated. However, in order to adapt to long-term natural drought, which induced stomata closing, DR plants gradually evolved its photosynthetic pathway from C_3 to C_4 so as to make full use of limited CO_2 within the tissues. Together with cyclic electron transport and cyclic photophosphorylation around PS1, this facilitated the extra ATP synthesis needed for C_4 photosynthesis and energy supply for normal growth and development of plants. Among these processes, increased level of anti-oxidative system in DR chloroplasts was important for ensuring normal PS1 function. All these components of physiological response are better fitted together and give optimum performance for DR plants in drought habitat.

References

Anderson, J.M., Osmond, C.B.: Shade-sun responses: compromises between acclimation and photoinhibition. – In: Kyle, D.J., Osmond, C.B., Arntzen, C.J. (ed.): Photoinhibition. Pp. 1-38. Elsevier, Amserdam – New York – Oxford 1987.

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

Asada, K.: Radical production and scavenging in the chloroplasts. – In: Baker, N.R. (ed.): Photosynthesis and the

Environment. Pp. 123-150. Kluwer Academic Publ., Dordrecht – Boston – London 1996.

Asada, K.: The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. - Annu. Rev. Plant Physiol. Plant mol. Biol. **50**: 601-639, 1999.

Asada, K., Kiso, K., Yoshikawa, K.: Univalent reduction of molecular oxygen by spinach chloroplasts on illumination. – J. biol. Chem. **249**: 2175-2181, 1974.

Björkman, O., Powles, S.B.: Inhibition of photosynthetic reactions under water stress: interaction with light level. - Planta **161**: 490-504, 1984.

Cushman, J.C., Bohnert, H.J.: Crassulacean acid metabolism: molecular genetics. – Annu. Rev. Plant Physiol. Plant mol. Biol. **50**: 305-332, 1999.

Dhindsa, R.S., Plumb-Dhindsa, P.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.

Foyer, C., Rowell, J., Walker, D.: Measurement of the ascorbate content of spinach leaf protoplasts and chloroplasts during illumination. – Planta **157**: 239-244, 1983.

Furbank, R.T., Foyer, C.H.: Oscillations in levels of metabolites from the photosynthetic carbon reduction cycle in spinach leaf disks generated by the transition from air to 5% CO_2. – Arch. Biochem. Biophys. **246**: 240-244, 1986.

Furbank, R.T., Horton, P.: Regulation of photosynthesis in isolated barley protoplasts: the contribution of cyclic photophosphorylation. – Biochim. biophys. Acta **894**: 332-338, 1987.

Furbank, R.T., Jenkins, C.L.D., Hatch, M.D.: C_4 photosynthesis: quantum requirement, C_4 acid overcycling and Q-cycle involvement. – Aust. J. Plant Physiol. **17**: 1-17, 1990.

Furbank, R.T., Taylor, W.: Regulation of photosynthesis in C-3 and C-4 plants: A molecular approach. – Plant Cell **7**: 797-807, 1995.

Govindjee, Downton, W.J.S., Fork, D.C., Armond, P.A.: Chlorophyll *a* fluorescence transient as an indicator of water potential of leaves. – Plant Sci. Lett. **20**: 191-194, 1981.

Gupta, A.S., Webb, R.P., Holaday, A.S., Allen, R.D.: Overexpression of superoxide dismutase protects plants from oxidative stress. Induction of ascorbate peroxidase in superoxide dismutase-overexpressing plants. – Plant Physiol. **103**: 1067-1073, 1993.

Hao, L., Wang, H., Wen, J., Liang, H.: Effects of water stress on light-harvesting complex II (LHCII) and expression of a gene encoding LHCII in *Zea mays*. – J. Plant Physiol. **149**: 30-34, 1996.

Haslam, S.M.: Variation of population type in *Phragmites communis* Trin. – Ann. Bot. **34**: 147-158, 1970.

Haslam, S.M.: The performance of *Phragmites communis* Trin. in relation to temperature. – Ann. Bot. **39**: 881-888, 1975.

Hatch, M.D.: C_4 photosynthesis: an unlikely process full of surprises. – Plant Cell Physiol. **33**: 333-342, 1992.

Havaux, M., Canaani, O., Malkin, S.: Photosynthetic responses of leaves to water stress, expressed by photoacoustics and related methods. I. Probing the photoacoustic method as an indicator for water stress *in vivo*. – Plant Physiol. **82**: 827-833, 1986.

He, J.X., Wang, J., Liang, H.G.: Effects of water stress on photochemical function and protein metabolism of photosystem II in wheat leaves. – Physiol. Plant. **93**: 771-777, 1995.

Heber, U., Walker, D.: Concerning a dual function of coupled cyclic electron transport in leaves. – Plant Physiol. **100**: 1621-1626, 1992.

Hebert, S.K., Fork, D.C., Malkin, S.: Photoacoustic measurements in vivo of energy storage by cyclic electron flow in algae and higher plants. – Plant Physiol. **94**: 926-934, 1990.

Katona, K., Neimanis, S., Schönknecht, G., Heber, U.: Photosystem I-dependent cyclic electron transport is important in controlling Photosystem II activity in leaves under conditions of water stress. – Photosynth. Res. **34**: 449-464, 1992.

Ku, M.S.B., Kano-Murakami, Y., Matsuoka, M.: Evolution and expression of C_4 photosynthesis genes. – Plant Physiol. **111**: 949-957, 1996.

Lilley, R.McC., Fitzgerald, M.P., Rienits, K.G., Walker, D.A.: Criteria of intactness and the photosynthetic activity of spinach chloroplast preparations. – New Phytol. **75**: 1-10, 1975.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the Folin-phenol reagent. – J. biol. Chem. **193**: 265-275, 1951.

Lundin, A., Rickordsson, A., Thore, A.: Continuous monitoring of ATP-converting reactions by purified firefly luciferase. – Anal. Biochem. **75**: 611-620, 1976.

Matoh, T., Matsushita, N., Takahashi, E.: Salt tolerance of the reed plants *Phragmites communis*. – Physiol. Plant. **72**: 8-14, 1988.

Matorin, D.N., Ortoidze, T.V., Nixolaev, G.M., Vendiktov, P.S., Rubin, A.B.: Effects of dehydration on electron transport activity in chloroplasts. – Photosynthetica **16**: 226-233, 1982.

Mayoral, M.L., Atsmon, D., Shimshi, D., Gromet-Elhanan, Z.: Effect of water stress on enzyme activities in wheat and related wild species: carboxylase activity, electron transport and photophosphorylation in isolated chloroplasts. – Aust. J. Plant Physiol. **8**: 385-393, 1981.

McCarty, R.E., Racker, E.: Partial resolution of the enzymes catalyzing photophosphorylation. III. Activation of adenosine triphosphatase and ^{32}P-labeled orthophosphate-adenosine triphosphate exchange in chloroplasts. – J. biol. Chem. **243**: 129-137, 1968.

Mi, H.L., Ye, J.Y., Wang, Y.J., Li, D.Y., Shen, Y.K.: Study on the mechanism of temperature affecting post-illumination transient increase in chlorophyll fluorescence in *Synechocysis* PCC 6803. – Chin. Sci. Bull. **42**: 1106-1109, 1997. [In Chin.]

Miyake, C., Cao, W.-H., Asada, K.: Purification and molecular properties of the thylakoid-bound ascorbate peroxidase in spinach chloroplasts. – Plant Cell Physiol. **34**: 881-889, 1993.

Murchie, E.H., Horton, P.: Acclimation of photosynthesis to irradiance and spectral quality in British plant species: chlorophyll content, photosynthetic capacity and habitat preference. – Plant Cell Environ. **20**: 438-448, 1997.

Nakano, Y., Asada, K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. – Plant Cell Physiol. **22**: 867-880, 1981.

Ogawa, K., Kanematsu, S., Takabe, K., Asada, K.: Attachment of CuZn-superoxide dismutase to thylakoid membranes at the site of superoxide generation (PSI) in spinach chloroplasts: Detection by immuno-gold labeling after rapid freezing and substitution method. – Plant Cell Physiol. **36**: 565-573, 1995.

Reeves, S.G., Hall, D.O.: Photosynthesis and nitrogen fixation. Higher plant chloroplasts and grana: general preparative procedures (excluding high carbon dioxide fixation ability chloroplasts). – In: Colowick, S.P., Kaplan, N.O. (ed.):

Methods in Enzymology. Vol. 69. Pp. 85-104. Academic Press, New York – London – Toronto – Sydney – San Francisco 1980.

Shang-Guan, Z.P.: [Effects of drought on the photosynthesis of crops.] – In: Shan, L., Cheng, P.Y.: Ecophysiological Basis of Dryland Agriculture. Pp. 68-77. Science Publishing House, Beijing 1998. [In Chin.]

Sharkey, T.D., Badger, M.R.: Effects of water stress on photosynthetic electron transport, photophosphorylation, and metabolite levels of *Xanthium strumarium* mesophyll cells. – Planta **156**: 199-206, 1982.

Shigeoka, S., Yokota, A., Nakano, Y., Kituoka, S.: The effect of illumination on the L-ascorbic acid content in *Euglena gracilis* Z. – Agr. biol. Chem. **43**: 2053-2058, 1979.

Smirnoff, N.: The role of active oxygen in the response of plants to water deficit and desiccation. – New Phytol. **125**: 27-58, 1993.

Taussky, H.H., Shorr, E.: A microcolorimetric method for the determination of inorganic phosphorus. – J. biol. Chem. **202**: 675-685, 1953.

Tripathy, B.C., Mohanty, P.: Zinc-inhibited electron transport of photosynthesis in isolated barley chloroplasts. – Plant Physiol. **66**: 1174-1178, 1980.

Ueno, O.: Structural characterization of photosynthetic cells in an amphibious sedge, *Eleocharis vivipara*, in relation to C_3 and C_4 metabolism. – Planta **199**: 382-393, 1996.

Valladares, F., Pearcy, R.W.: Interactions between water stress, sun-shade acclimation, heat tolerance and photoinhibition in the sclerophyll *Heteromeles arbutifolia*. – Plant Cell Environ. **20**: 25-36, 1997.

Wang, H.L., Hao, L.M., Wen, J.Q., Zhang, C.L., Liang, H.G.: Differential expression of photosynthesis-related genes of reed ecotypes in response to drought and saline habitats. - Photosynthetica **35**: 61-69, 1998.

Winter, K.: Evidence for the significance of Crassulacean Acid Metabolism as an adaptive mechanism to water stress. – Plant Sci. Lett. **3**: 279-281, 1974.

Zheng, W.J., Zheng, X.P., Zhang, C.L.: A survey of photosynthetic carbon metabolism in 4 ecotypes of *Phragmites australis* in northwest China: leaf anatomy, ultrastructure, and activities of ribulose 1,5-bisphosphate carboxylase, phosphoenolpyruvate carboxylase and glycolate oxidase. – Physiol. Plant. **110**: 201-208, 2000.