PHOTOSYNTHETICA 43 (3): 473-476, 2005

BRIEF COMMUNICATION

# Role of calcium ion in protection against heat and high irradiance stress-induced oxidative damage to photosynthesis of wheat leaves

Hui-Jie ZHAO* and Ji-Fang TAN

*College of Agronomy, Henan Agricultural University, Zhengzhou, Henan, 450002, P.R. China*

## Abstract

Cross stress of heat and high irradiance (HI) resulted in the accumulation of active oxygen species and photo-oxidative damage to photosynthetic apparatus of wheat leaves during grain development. Pre-treatment with calcium ion protected the photosynthetic system from oxidative damage by reducing $O^{-}_{2}$ production, inhibiting lipid peroxidation, and retarding electrolyte leakage from cell. Therefore, high $F_v/F_m$ [maximal photochemical efficiency of photosystem 2 (PS2) while all PS2 reaction centres are open], $F_m/F_0$ (another expression for the maximal photochemical efficiency of PS2), $\Phi_{PS2}$ (actual quantum yield of PS2 under actinic irradiation), $q_P$ (photochemical quenching coefficient), and $P_N$ (net photosynthetic rate) were maintained, and lower $q_{NP}$ (non-photochemical quenching coefficient) of the leaves was kept under heat and HI stress. EGTA (a chelant of calcium ion) and $LaCl_3$ (a blocker of $Ca^{2+}$ channel in cytoplasmic membrane) had the opposite effect. Thus Ca ion may help protect the photosynthetic system of wheat leaves from oxidative damage induced by the cross stress of heat and HI.

*Additional key words*: cross stress of heat and high irradiance; ethyleneglycol-di(2-aminoethylether)-tetraacetic acid; lanthanum (III) chloride; photosynthesis; *Triticum*.

In nature, plants are frequently subject to changes of temperature and irradiance. For instance, the winter wheat grown in northern China often comes up against the cross-stress of heat and high irradiance (HI) during seed-filling. Either heat or HI results in oxidative damage and inhibited photosynthesis (Powles 1984, Havaux *et al*. 1991, Gong *et al*. 1998). Wheat is a temperate cereal with an optimum temperature for photosynthesis of 23–25 °C (Blum 1986). Photosynthesis in wheat leaves begins to decline under temperature between 28 to 35 °C (Al-Khatib *et al*. 1984, Scott *et al*. 1990). In many regions of northern China, air temperature is often higher than the optimum temperature for wheat growth and the sunlight is too high for photosynthesis after anthesis. Severe photo-oxidative damage to photosynthetic apparatus is often caused by the simultaneous occurrence of heat and HI because the excess of photons absorbed by leaves is aggravated under heat stress. Therefore the question how to protect photosynthetic apparatus against damage resulting from heat and HI during seed development has become basic for winter wheat.

During the long term of evolution, higher plants have developed various strategies for preventing damage caused by stresses. For example, there is a defence system in plants to scavenge the active species of oxygen which helps them survive under unfavourable conditions (Bowler and Montagu 1992). A wide range of second messengers have been implicated in response to a variety of stresses. Calcium ion, one of the second messengers, is involved in response to heat stress (Gong *et al*. 1998, Larkindale and Knight 2002). However, Ca is not required for HSP (heat shock protein) production in plants (Gong *et al*. 1997). This suggests that a process other than HSP induction is also required for protection against heat stress and that Ca may be involved in some other signalling pathway, *e.g*. regulating active oxygen metabolism, in the response to heat stress. We experimentally examined the role of Ca ion in protection against heat and

---

*Received* 7 June 2004, *accepted* 7 February 2005.

[*] Corresponding author; e-mail: zhaohj303@163.com

*Abbreviations*: AOS – active oxygen species; Chl – chlorophyll; EGTA – ethyleneglycol-di(2-aminoethylether)-tetraacetic acid; HI – high irradiance; MDA – malondialdehyde; $P_N$ – net photosynthetic rate; PS – photosystem.

*Acknowledgements*: This research was supported by the State Key Basic Research and Development Plan of China (G2000077907) and Natural Science Fund of Henan Province (0111010800).

HI stress-induced oxidative damage to photosynthesis of wheat leaves.

Winter wheat (*Tritivum aestivum* L. cv. Yumai34) plants were grown in the pots (one plant each pot) which were buried in the field of the Research and Teaching Garden of Henan Agricultural University situated in the outskirts of Zhengzhou city, Henan Province, China. Conventional culturing systems were adopted during growth. At the mid grain-filling stage (the 20th d after anthesis), 30 plants with similar growth status were chosen and sprayed with calcium (II) chloride ($CaCl_2$), EGTA [ethyleneglycol-di(2-aminoethylether)-tetraacetic acid], lanthanum (III) chloride ($LaCl_3$) solution, and water, respectively. The next day the plants with pots were brought to laboratory and treated with different temperature and irradiance in a growth box.

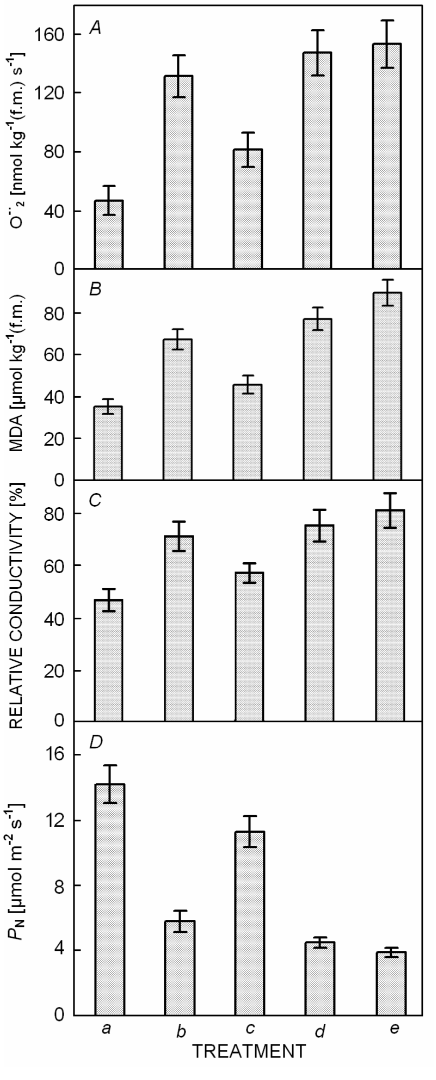

Fig. 1. Influence of calcium, EGTA, and $LaCl_3$ on (*A*) $O^{-\cdot}_2$ production, (*B*) MDA content, (*C*) permeability of membrane, and (*D*) $P_N$ in wheat leaves. *a*: sprayed with water, then treated with normal temperature (25 °C) and moderate irradiance (600 μmol $m^{-2}$ $s^{-1}$), as a control; *b*: sprayed with water, then treated with heat (38 °C) and high irradiance (HI, 1 600 μmol $m^{-2}$ $s^{-1}$) for 3 h; *c*: sprayed with 10 mM $CaCl_2$, then treated with heat and HI for 3 h; *d*: sprayed with 5 mM EGTA, then treated with heat and HI for 3 h; *e*: sprayed with 10 mM $LaCl_3$, then treated with heat and HI for 3 h.

Five treatments were used: (*a*): sprayed with water, then treated with normal temperature (25 °C) and moderate irradiance (600 μmol $m^{-2}$ $s^{-1}$), as a control; (*b*): sprayed with water, then treated with heat (38 °C) and HI (1 600 μmol $m^{-2}$ $s^{-1}$) for 3 h; (*c*): sprayed with 10 mM $CaCl_2$, then treated with heat and HI for 3 h; (*d*): sprayed with 5 mM EGTA, then treated with heat and HI for 3 h; and (*e*): sprayed with 10 mM $LaCl_3$, then treated with heat and HI for 3 h.

After 48 h of treatment, $P_N$ (net photosynthetic rate), chlorophyll (Chl) fluorescence parameters, $O^{-\cdot}_2$ production rate, MDA (malondialdehyde) content, and electrolyte leakage of flag leaf were measured. $P_N$ in the middle part of leaves was determined with a *CIRAS-I* photosynthesis system (*PP-System Company*, UK). Temperature in leaf chamber was regulated to 25 °C and PFD was 800 μmol $m^{-2}$ $s^{-1}$. Chl fluorescence was measured at room temperature (25–26 °C) with the *FM-2* fluorescence monitor (*Hansatech*, UK). Chl fluorescence parameters were calculated according to Schreiber *et al.* (1986) and Genty *et al.* (1989). $O^{-\cdot}_2$ in leaves was determined according to Zhao and Zou (2002): A 0.5 g (fresh mass) leaf sample was homogenized in 2 $cm^3$ of 50 mM phosphate buffer, pH 7.8, at 4 °C. The homogenate was filtered through 4 layers of cheesecloth, and centrifuged at 5 000×*g* for 10 min. 1 $cm^3$ of supernatant, 0.9 $cm^3$ of phosphate buffer (pH 7.8), and 0.1 $cm^3$ hydroxylamine (10 mM) were mixed and incubated at 25 °C for 20 min. Into 0.5 $cm^3$ of incubated solution, 0.5 $cm^3$ of *p*-aminobenzene sulfonic acid (17 mM) and 0.5 $cm^3$ of α-naphthylamine (7 mM) were added. The mixture was incubated at 25 °C for 20 min. After incubation, the liquid became coloured and the same volume of ether was added. Then mixture was stirred and centrifuged at 1 500×*g* for 5 min. The $A_{530}$ absorbance of the pink layer was read. The $O_2$ content was calculated according to a standard curve.

MDA (also known as thiobarbituric acid reactive substance, TBARS) assay was performed following the method of Larkindale and Knight (2002) with some modification. 1 g of leaves was ground in 5 $cm^3$ of 0.6 M trichloroacetic acid and then 5 $cm^3$ of 34 mM thiobarbituric acid was added. The samples were heated at 95 °C for 25 min and then centrifuged at 5 000 rpm for 20 min. The absorbance of supernatant was measured at 532 nm, with a reading at 600 nm subtracted from it to account for nonspecific turbidity. The amount of MDA was calculated using an extinction coefficient of 155 $mM^{-1}$ $cm^{-1}$. Electrolyte leakage was measured by relative conductivity based on the method of Fokar *et al.* (1998).

In PS1, molecular oxygen can be used as the final electron acceptor in the electron transferring chain of photosynthesis, so the superoxide anion, $O^{-\cdot}_2$ is formed by univalent reduction of $O_2$ in Mehler reaction (Asada *et al*. 1974). When photon energy is excessive, this reaction can maintain photosynthetic electron flow and alleviate the damage to photosynthetic system caused by excessive photon energy. But the $O^{-\cdot}_2$ must be scavenged promptly after it is formed. Otherwise, it will be converted to $H_2O_2$ and $O_2$ by SOD (McCord and Fridovich 1969, Monk *et al.* 1989). Moreover, in the presence of metal ions, $O^{-\cdot}_2$ and $H_2O_2$ can form hydroxyl radical ($^{\cdot}OH$), a more active and harmful species of oxygen, by the Haber-Weiss reaction (Bowler and Montagu 1992). These active oxygen species (AOS) can induce oxidative damage to chloroplasts including lipid peroxidation, denaturation of proteins, and mutation of DNA. Our results (Fig. 1*A*) showed that, in comparison with the control, simultaneous occurrence of heat and HI increased $O^{-\cdot}_2$ production significantly. Pre-treatment with Ca ion reduced $O^{-\cdot}_2$ production in wheat leaves remarkably although it was not reduced to the level of the control. In order to prove the effect of calcium, EGTA (a chelant of Ca ion) and $LaCl_3$ (a blocker of $Ca^{2+}$ channel in cytoplasmic membrane) were used. Both EGTA and $LaCl_3$ raised the $O^{-\cdot}_2$ production efficiency in wheat leaves and thus Ca was important for reduction of $O^{-\cdot}_2$ accumulation.

The accumulation of AOS results in lipid peroxidation which causes injury to structure and function of membrane system. MDA, a product of lipid peroxidation, is usually used as the criterion of stress-induced oxidative damage to membrane. Compared with the control, MDA content in wheat leaves was markedly increased under heat and HI stress (Fig. 1*B*). Pre-treatment with Ca ion obviously inhibited the lipid peroxidation induced by heat and HI stress. On the contrary, the use of either EGTA or $LaCl_3$ caused raise of MDA content in wheat leaves.

Cell membrane has a special character of differential permeability by which matter exchange between cell and environment is properly controlled. However, the differential permeability of cell membrane is easily weakened, and even lost under various stresses. In this case, electrolyte leakage increases and relative conductivity in seeped liquid from cell raises. In our experiments, heat and HI stress resulted in significant increase in electrolyte leakage of wheat leaves, suggesting that severe injury to cell membrane was induced (Fig. 1*C*). Pre-treatment with Ca ion noticeably alleviated the electrolyte leakage from cell. EGTA and $LaCl_3$ had an opposite effect. Hence Ca ion could protect cell membrane from injury under heat and HI stress.

Table 1. Influence of calcium, EGTA, and $LaCl_3$ on chlorophyll fluorescence parameters of wheat leaves. For the description of treatments see Fig. 1.

| Treatment | $F_m/F_0$ | $F_v/F_m$ | $\Phi_{PS2}$ | $q_P$ | $q_{NP}$ |
|---|---|---|---|---|---|
| *a* | 3.841 (100.0 %) | 0.788 (100.0 %) | 0.161 (100.0 %) | 0.323 (100.0 %) | 0.511 (100.0 %) |
| *b* | 1.663 (43.3 %) | 0.325 (41.2 %) | 0.071 (44.1 %) | 0.206 (63.7 %) | 0.743 (145.4 %) |
| *c* | 2.751 (71.4 %) | 0.647 (82.1 %) | 0.144 (89.4 %) | 0.279 (86.4 %) | 0.563 (110.2 %) |
| *d* | 1.424 (37.1 %) | 0.309 (39.2 %) | 0.062 (38.5 %) | 0.201 (62.3 %) | 0.807 (157.9 %) |
| *e* | 1.206 (31.4 %) | 0.301 (38.2 %) | 0.059 (36.6 %) | 0.192 (59.4 %) | 0.829 (162.2 %) |

The decreases in $CO_2$ fixation rate, quantum yield, and $F_v/F_m$ reflect the occurrence of photoinhibition, while the decline in $F_m/F_0$ indicates that a partial deactivation of photosystem 2 (PS2) reaction centre has occurred (Powles 1984, Demmig-Adams and Adams 1992, Osmond 1994). $F_m/F_0$, $F_v/F_m$, $\Phi_{PS2}$, $q_P$, and $P_N$ were decreased and $q_{NP}$ was increased markedly under heat and HI stress (Table 1 and Fig. 1*D*). Calcium ion prevented wheat leaves from these changes. Opposite effect was caused by EGTA and $LaCl_3$. Therefore we suggest that Ca ion protects the photosynthetic apparatus against damages induced by the tested cross stress. Nevertheless, some questions remain to be solved. For instance, is the effect of $Ca^{2+}$ on photosynthesis of only secondary importance? Does $Ca^{2+}$ influence the activity of some stroma enzymes? How is Ca taken up by the cell and how is it distributed within the leaf? Does it interact with some proteins of the plasma membrane which are involved in the activation of calcium channels?

## References

Al-Khatib, K., Paulsen, G.M.: Mode of high temperature injury to wheat during grain development. – Plant Physiol. **61**: 363-368, 1984.

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

Blum, A.: The effect of heat stress on wheat leaf and ear photosynthesis. – J. exp. Bot. **37**: 111-118, 1986.

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

Demmig-Adams, B., Adams, W.W., III: Photoprotection and other responses of plants to high light stress. – Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 599-626, 1992.

Fokar, M., Nguyen, H.T., Blum, A.: Heat tolerance in spring wheat. 1. Estimating cellular thermotolerance and its heritability. – Euphytica **104**: 1-8, 1998.

Genty, B., Briantais, J.-M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. biophys. Acta **990**: 87-92, 1989.

Gong, M., Li, Y.J., Chen, S.Z.: Abscisic acid induced thermotolerance in maize seedlings is mediated by calcium level and associated with antioxidant systems. – J. Plant Physiol. **153**: 488-496, 1998.

Gong, M., Li, X.J., Dai, X., Tian, M., Li, Z.G.: Involvement of calcium and calmodulin in acquisition of HS induced thermotolerance in maize seedlings. – J. Plant Physiol. **150**: 615-621, 1997.

Havaux, M., Greppin, H., Strasser, R.J.: Functioning of photosystem 1 and 2 in pea leaves exposed to heat stress in the presence or absence of light. Analysis using *in-vivo* fluorescence, absorbance, oxygen and photoacoustic measurements. – Planta **186**: 88-98, 1991.

Larkindale, J., Knight, M.R.: Protection against heat stress-induced oxidative damage in *Arabidopsis* involves calcium, abscisic acid, ethylene, and salicylic acid. – Plant Physiol. **128**: 682-693, 2002.

McCord, J.M., Fridovich, I.: Superoxide dismutase: An enzymic function for erythrocuprin (hemocuprin). – J. biol. Chem. **224**: 6049-6055, 1969.

Monk, L.S., Fagerstedt, K.V., Crawford, R.M.M.: Oxygen toxicity and superoxide dismutase as an antioxidatant in physiological stress. – Physiol. Plant. **76**: 456-459, 1989.

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

Powles, S.B.: Photoinhibition of photosynthesis induced by visible light. – Annu. Rev. Plant Physiol. **35**: 15-44, 1984.

Schreiber, U., Schliwa, U., Bilger, W.: Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. – Photosynth. Res. **10**: 51-62, 1986.

Scott, A.H., James, A.G., Gray, M.P.: Photosynthetic decline from high temperature stress during maturation of wheat. 1. Interaction with senescence process. – Plant Physiol. **92**: 648-653, 1990.

Zhao, H.J., Zou, Q.: Protective effects of exogenous antioxidants and phenolic compounds on photosynthesis of wheat leaves under high irradiance and oxidative stress. – Photosynthetica **40**: 523-527, 2002.