PHOTOSYNTHETICA 40 (4): 523-527, 2002

Protective effects of exogenous antioxidants and phenolic compounds on photosynthesis of wheat leaves under high irradiance and oxidative stress

Hui Jie ZHAO* and Qi ZOU**,***

*Department of Agronomy, Henan Agricultural University, Zhengzhou, Henan, 450002, P.R. China**
*Department of Plant Science, Shandong Agricultural University, Tai-An, Shandong, 271018, P.R. China***

Abstract

Infiltration of methyl viologen (MV, source of O^{-}_2) and Na-diethyldithiocarbamate (DDC, inhibitor of SOD) into wheat leaves resulted in the accumulation of active oxygen species and photo-oxidative damage to photosynthetic apparatus under both moderate and high irradiance. Exogenous antioxidants, ascorbate (ASA) and mannitol, scavenged active oxygen efficiently, protected the photosynthetic system from MV and DDC induced oxidative damage, and maintained 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), Φ_{PS2} (actual quantum yield of PS2 under actinic irradiation), q_P (photochemical quenching coefficient), P_N (net photosynthetic rate), and lowered q_{NP} (non-photochemical quenching coefficient) of the leaves kept under high irradiance and oxidative stress. Phenolic compounds used in these experiments, catechol (Cat), resorcinol (Res), and tannic acid (Tan), had similar anti-oxidative activity and protective effect on photosynthetic apparatus as ASA and mannitol. The anti-oxidative activity and the protective effect of phenolic compounds increased with increase in their concentration from 100 to 300 g m^{-3}. The number and the position of hydroxyl group in phenolic molecules seemed to influence their antioxidative activity.

Additional key words: ascorbate; catechol; Na-diethyldithiocarbamate; irradiance; mannitol; photosystem 2; resorcinol; tannic acid; *Triticum*.

Introduction

Radiant energy is the most important environmental factor for plant life. Over a range of photon flux density (PFD), the increase of photon absorption by chlorophyll (Chl) results in an increase in photosynthetic CO_2 fixation. However, when the photon absorbed by the leaves exceeds their utilising capacity, active oxygen species, such as O^{-}_2, H_2O_2, and $^1O_2$, are generated (Asada 1996). They oxidise target molecules and cause photooxidative damage to photosynthetic apparatus (Powles 1984). During the long term of evolution, higher plants have developed various defence systems to scavenge the active species of oxygen; this helps them to survive under unfavourable conditions (Bowler and Montagu 1992). The defence systems are composed of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and several antioxidants such as ascorbate (ASA), GSH, α-tocopherol, carotenoids, *etc.* Therefore, the active oxygen species usually are not formed in dangerous concentrations in chloroplasts under favourable conditions. However, field-grown plant leaves are often exposed to fluctuation of many environmental factors. The most important environmental factor for photosynthesis, the sun irradiation, is very variable, and also other environmental factors, such as water accessibility, temperature, CO_2 concentration, *etc.* are not always favourable to photosynthesis when the sun is bright. High irradiance (HI) stress often takes place in summer, especially during midday. The enzymes involved in CO_2 fixation may be inactivated and the photochemical apparatus may be impaired when the defence systems cannot fully scavenge

Received 25 July 2002, *accepted* 22 October 2002.
***Corresponding author; e-mail: zouqi@sdau.edu.cn
Abbreviations: ASA – ascorbate; Cat – catechol; Chl – chlorophyll; DDC – Na-diethyldithiocarbamate; HI – high irradiance; MI – moderate irradiance; MV – methyl viologen; P_N – net photosynthetic rate; PFD – photon flux density; PS – photosystem; Res – resorcinol; Tan – tannic acid.
Acknowledgements: This research was supported by the State Key Basic Research and Development Plan of China (G1998010100) and Natural Science Fund of Henan Province (0111010800).

the active oxygen (Richter *et al.* 1990, Demmig-Adams and Adams 1992). In these cases, application of exogenous antioxidants will be beneficial for enhancing the ability of plants to scavenge active oxygen and preventing chloroplasts from being damaged (Ye *et al.* 2000, Yordanov *et al.* 2000).

Some natural phenolic compounds in plants, such as flavonoids, caffeic acid, ferulic acid, *etc.* are efficacious endogenous antioxidants (Larson 1988). They can remove active oxygen and retard lipid peroxidation of cell membrane (Salah *et al.* 1995, Zhou *et al.* 2000). Can some exogenous phenolic compounds have antioxidative activity and protect photosynthetic apparatus against photooxidative damage? This is the question we explored. In this paper, the effect of several exogenous phenolic compounds on photosynthesis of wheat leaves under HI and oxidative stress was studied. The aim was to provide an experimental basis for screening of new antioxidants.

Materials and methods

Plants and treatments: Winter wheat (*Tritivum aestivum* L. cv. Yumai66) plants were grown 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 in the field management. At the initial grain-filling stage (the 15th d after anthesis), the flag leaves were cut off and put in different solutions in test tubes (3.5 cm in diameter and 20 cm in length). Methyl viologen (MV, 5 mM) was used as source of O^{-}_{2}, Na-diethyldithiocarbamate (DDC, 20 mM) as an inhibitor of SOD, ascorbate (ASA) and mannitol as exogenous antioxidants, catechol (Cat), resorcinol (Res), and tannic acid (Tan) as exogenous phenolic compounds. Leaves immersed in these solutions were divided into two groups. After 30 min of infiltration, one group was moderately irradiated (MI, 600 μmol m^{-2} s^{-1}) and the other group was treated with high irradiance (HI, 1 500~1 600 μmol m^{-2} s^{-1}) provided for 2 h by a 1 000 W tungsten-halogen lamp and water filter. Temperature was regulated between 28~30 °C.

21 treatments were involved in the experiment:
(*1*) H_2O + MI (control); (*2*) H_2O + HI; (*3*) MV + MI;
(*4*) MV + HI; (*5*) DDC + MI; (*6*) DDC + HI;
(*7*) MV + HI + 100 g m^{-3} ASA;
(*8*) MV + HI + 200 g m^{-3} ASA;
(*9*) MV + HI + 300 g m^{-3} ASA;
(*10*) MV + HI + 100 g m^{-3} mannitol;
(*11*) MV + HI + 200 g m^{-3} mannitol;
(*12*) MV + HI + 300 g m^{-3} mannitol;
(*13*) MV + HI + 100 g m^{-3} Cat;
(*14*) MV + HI + 200 g m^{-3} Cat;
(*15*) MV + HI + 300 g m^{-3} Cat;
(*16*) MV + HI + 100 g m^{-3} Res;
(*17*) MV + HI + 200 g m^{-3} Res;
(*18*) MV + HI + 300 g m^{-3} Res;
(*19*) MV + HI + 100 g m^{-3} Tan;
(*20*) MV + HI + 200 g m^{-3} Tan;
(*21*) MV + HI + 300 g m^{-3} Tan.

Determination of net photosynthetic rate (P_N) and Chl fluorescence: 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 28 °C and PFD was 800 μmol m^{-2} s^{-1}. Chl fluorescence was measured at room temperature (25~26 °C) with *FM-2* fluorescence monitor (*Hansatech*, UK). Chl fluorescence parameters were calculated according to the method of Schreiber *et al.* (1986) and Genty *et al.* (1989).

O^{-}_{2} determination in wheat leaves was done according to the method of Elstner and Heupel (1976) with some modifications. A 0.5 g (fresh mass) leaf sample was homogenised in 2 cm^3 of 50 mM phosphate buffer, pH 7.8, at 4 °C. The homogenate was filtered through 4 layers of cheesecloth, centrifuged for 10 min at 5 000×*g*. 1 cm^3 of supernatant, 0.9 cm^3 of phosphate buffer (pH 7.8), and 0.1 cm^3 hydroxylamine chloride (10 mM) were mixed and incubated at 25 °C for 20 min. Into 0.5 cm^3 of the 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 the mixture was stirred and centrifuged at 1 500×*g* for 5 min. A_{530} of the pink layer was read. The efficiency of O^{-}_{2} production was calculated according to a standard curve.

Results and discussion

Influence of MV and SOD on O^{-}_{2} production and photosynthesis in leaves: In PS1, molecular oxygen can be used as the final electron acceptor in the electron transferring chain of photosynthesis, so the superoxide anion, O^{-}_{2} is formed by univalent reduction of O_2 in Mehler reaction (Asada *et al.* 1974). Under HI, this reaction can maintain photosynthetic electron flow and alleviate the damage to photosynthetic system caused by excess photon energy. But the O^{-}_{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). In the presence of metal ions, O^{-}_{2} and H_2O_2 can form

hydroxyl radical (·OH), a more active and harmful species of oxygen, by the Haber-Weiss reaction (Bowler and Montagu 1992). These active oxygen species can induce oxidative damage to chloroplasts including lipid peroxidation, denaturation of proteins, and mutation of DNA. Our results showed that, compared with the control, HI increased O^-_2 production efficiency of leaves immersed in water. MV and DDC raised the O^-_2 production efficiency further (Fig. 1*A*). The highest production efficiency of O^-_2 was caused by MV under HI, followed by the treatment with DDC + HI.

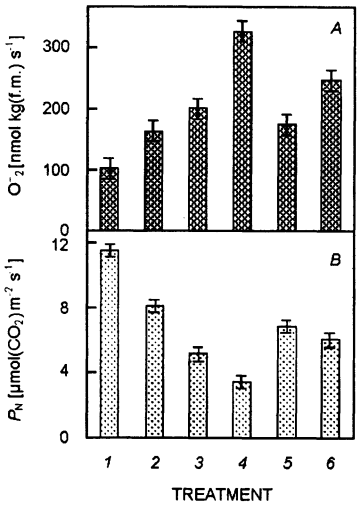

Fig. 1. Influence of MV and DDC on O^-_2 production efficiency (*A*) or net photosynthetic rate, P_N (*B*) in wheat leaves. *1* – H_2O + MI (control), *2* – H_2O + HI, *3* – MV + MI, *4* – MV + HI, *5* – DDC + MI, *6* – DDC + HI.

Table 1 and Fig. 1*B* show that F_m/F_0 (electron transfer through PS2), F_v/F_m (primary photochemical efficiency of PS2), Φ_{PS2} (quantum yield of PS2), q_P (photochemical quenching coefficient), and P_N of leaves in water (control) decreased due to HI, but q_{NP} (non-photochemical quenching coefficient) increased. Hence HI induced photoinhibition and photodamage of photosynthesis in wheat leaves. In comparison with the control, MV and DDC induced noticeable decreases in F_m/F_0, F_v/F_m, Φ_{PS2}, q_P, and an increase in q_{NP} under MI. But severer damages resulted from MV and DDC application under HI.

Influence of exogenous antioxidants and phenolic compounds on O^-_2 production: The photoproduction of radicals and active oxygen in chloroplasts is unavoidable even under the favourable conditions for photosynthesis, so their prompt scavenging is essential to protect the target molecules. In order to increase the ability of plants to scavenge active oxygen, many efforts have been made by researchers. Transgenic plants were tested to raise the activity of the scavenging enzymes (Foyer *et al.* 1994, Allen 1995, Inzé and Montague 1995). In our experiments, both ASA and mannitol scavenged O^-_2 efficiently (Fig. 2). Similarly, the phenolic compounds used in our experiment also decreased the accumulation of O^-_2 (Fig. 2). Over the range of 100~300 g m^{-3}, their ability to scavenge O^-_2 increased with the increase of concentration. Thus applying exogenous antioxidants and phenolic compounds directly to the plants might be a simple and effective way to increase the stress resistance of plants.

Table 1. Influence of MV and DDC on chlorophyll fluorescence parameters of wheat leaves.

Treatment	F_m/F_0	F_v/F_m	Φ_{PS2}	q_P	q_{NP}
H_2O + MI	3.837 (100 %)	0.786 (100 %)	0.156 (100 %)	0.315 (100 %)	0.507 (100 %)
H_2O + HI	2.663 (69.4 %)	0.625 (79.5 %)	0.104 (66.7 %)	0.276 (87.6 %)	0.543 (107.1 %)
MV + MI	1.781 (46.4 %)	0.446 (56.7 %)	0.101 (64.7 %)	0.269 (85.4 %)	0.657 (129.6 %)
MV + HI	1.621 (42.2 %)	0.382 (48.6 %)	0.075 (48.1 %)	0.204 (64.8 %)	0.812 (160.2 %)
DDC + MI	2.216 (57.8 %)	0.505 (67.0 %)	0.099 (63.5 %)	0.244 (77.5 %)	0.639 (126.0 %)
DDC + HI	2.021 (52.7 %)	0.474 (60.3 %)	0.078 (50.0 %)	0.184 (58.4 %)	0.799 (157.6 %)

Table 2. Influence of exogenous antioxidants on chlorophyll fluorescence parameters of wheat leaves.

Treatment	F_m/F_0	F_v/F_m	Φ_{PS2}	q_P	q_{NP}
H_2O + MI (Control)	3.837 (100 %)	0.786 (100 %)	0.156 (100 %)	0.315 (100 %)	0.507 (100 %)
MV + HI	1.621 (42.2 %)	0.382 (48.6 %)	0.075 (48.1 %)	0.204 (64.8 %)	0.712 (140.4 %)
MV + HI +100 g m^{-3} ASA	1.742 (45.4 %)	0.401 (51.0 %)	0.131 (84.0 %)	0.206 (65.4 %)	0.630 (124.3 %)
MV + HI +200 g m^{-3} ASA	2.560 (66.7 %)	0.714 (90.8 %)	0.139 (89.1 %)	0.277 (87.9 %)	0.545 (107.5 %)
MV + HI +300 g m^{-3} ASA	3.415 (89.0 %)	0.742 (94.4 %)	0.145 (92.9 %)	0.301 (95.6 %)	0.524 (103.3 %)
MV + HI +100 g m^{-3} mannitol	1.684 (43.9 %)	0.399 (50.8 %)	0.125 (80.1 %)	0.211 (67.0 %)	0.678 (133.7 %)
MV + HI +200 g m^{-3} mannitol	2.337 (60.9 %)	0.548 (69.7 %)	0.144 (92.3 %)	0.283 (89.8 %)	0.603 (118.9 %)
MV + HI +300 g m^{-3} mannitol	3.261 (85.0 %)	0.703 (89.4 %)	0.148 (94.9 %)	0.302 (95.9 %)	0.516 (101.8 %)

Influence of exogenous antioxidants and phenolic compounds on photosynthesis: 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 PS2 reaction centre has occurred (Powles 1984, Demmig-Adams and Adams 1992, Osmond 1994). F_m/F_0, F_v/F_m, Φ_{PS2}, q_P, and P_N were increased and q_{NP} was decreased by ASA and mannitol (Table 2 and Fig. 3). Therefore we suggest that they protect the photosynthetic apparatus under severe photoinhibition (MV + irradiance). Over the range of 100~300 mg m^{-3}, their protective effect increased with increasing the concentration.

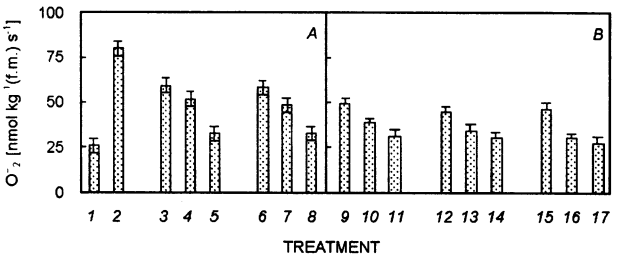

Fig. 2. Influence of exogenous antioxidants or phenolic compounds on O^{-}_2 of wheat leaves. *1* – H_2O + MI (control), *2* – MV + HI, *3* – MV + HI + 100 g m^{-3}ASA, *4* – MV + HI + 200 g m^{-3} ASA, *5* – MV + HI + 300 g m^{-3} ASA, *6* – MV + HI + 100 g m^{-3} mannitol, *7* – MV + HI + 200 g m^{-3} mannitol, *8* – MV + HI + 300 g m^{-3} mannitol,. *9* – MV + HI + 100 g m^{-3} Cat, *10* – MV + HI + 200 g m^{-3} Cat, *11* – MV + HI + 300 g m^{-3} Cat, *12* – MV + HI + 100 g m^{-3} Res, *13* – MV + HI + 200 g m^{-3} Res, *14* – MV + HI + 300 g m^{-3} Res, *15* – MV + HI + 100 g m^{-3} Tan, *16* – MV + HI + 200 g m^{-3} Tan, *17* – MV + HI + 300 g m^{-3} Tan.

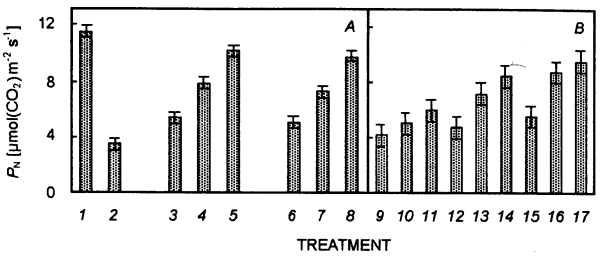

Fig. 3. Influence of exogenous antioxidants on net photosynthetic rate (P_N) of wheat leaves. For description of columns see Fig. 2.

Three kinds of phenolic compounds used in this experiment had a similar protective effect on photosynthesis as ASA and mannitol (Table 3 and Fig. 3). All of them inhibit HI-induced decreases of F_m/F_0, F_v/F_m, Φ_{PS2}, q_P, and P_N and prevent the increase of q_{NP} in wheat plants. The higher the concentration, the better was the effect. Among these three phenolic compounds, Tan exhibited the best anti-oxidative effect, followed by Res and Cat. The number and position of hydroxyl group in the phenolic molecules was probably related to their anti-oxidative activity.

Table 3 Influence of exogenous phenolic compounds on chlorophyll fluorescence parameters of wheat leaves.

Treatment	F_m/F_0	F_v/F_m	Φ_{PS2}	q_P	q_{NP}
H_2O + MI (Control)	3.837 (100 %)	0.786 (100 %)	0.156 (100 %)	0.315 (100 %)	0.507 (100 %)
MV + HI	1.621 (42.2 %)	0.382 (48.6 %)	0.075 (48.1 %)	0.204 (64.8 %)	0.712 (140.4 %)
MV + HI + 100 g m^{-3} Cat	1.491 (38.8 %)	0.401 (51.0 %)	0.062 (39.7 %)	0.203 (64.3 %)	0.643 (126.8 %)
MV + HI + 200 g m^{-3} Cat	1.786 (46.5 %)	0.434 (55.2 %)	0.106 (67.9 %)	0.210 (66.7 %)	0.591 (116.6 %)
MV + HI + 300 g m^{-3} Cat	1.926 (50.2 %)	0.464 (59.0 %)	0.115 (73.7 %)	0.292 (92.7 %)	0.524 (103.4 %)
MV + HI + 100 g m^{-3} Res	1.978 (51.6 %)	0.497 (63.2 %)	0.120 (76.9 %)	0.257 (81.6 %)	0.575 (113.4 %)
MV + HI + 200 g m^{-3} Res	2.036 (53.1 %)	0.509 (64.8 %)	0.137 (87.8 %)	0.283 (89.8 %)	0.547 (107.9 %)
MV + HI + 300 g m^{-3} Res	2.122 (55.3 %)	0.530 (67.4 %)	0.145 (92.9 %)	0.301 (95.6 %)	0.511 (100.8 %)
MV + HI + 100 g m^{-3} Tan	1.818 (47.4 %)	0.491 (62.5 %)	0.130 (83.3 %)	0.249 (79.0 %)	0.558 (110.1 %)
MV + HI + 200 g m^{-3} Tan	2.363 (61.6 %)	0.578 (73.5 %)	0.147 (94.2 %)	0.277 (87.9 %)	0.534 (105.3 %)
MV + HI + 300 g m^{-3} Tan	2.649 (70.1 %)	0.606 (77.1 %)	0.150 (96.1 %)	0.302 (95.9 %)	0.518 (102.6 %)

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