Photoprotective function of photorespiration in several grapevine cultivars under drought stress

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Abstract

Four grapevine cultivars, *i.e*. Cabernet Sauvignon (a member of the Western Europe cultivar group), Rizamat (a member of the East cultivar group), Red Double Taste (a hybridized cultivar from *Vitis vinifera* L. and *V. labrusca* L.), and 1103Paulsen (a hybridized rootstock), were treated by three severity orders of drought stress for 25 d. Then net photosynthetic rate (P_N), maximal photochemical efficiency (F_v/F_m), actual photochemical efficiency (Φ_{PS2}) of photosystem 2, total electron transport rate (J_T), and electron transport flows used in carboxylation (J_C) and in oxygenation (J_O) reactions catalysed by ribulose-1,5-bisphosphate carboxylase/oxygenase were determined. P_N was determined again after re-watering for 2 d by gas exchange measurement. Along with the increase in severity of drought stress, P_N, F_v/F_m, Φ_{PS2}, J_T, and J_C in all four cultivars decreased. The range of decrease differed among cultivars. J_O expressed various trends from cultivar to cultivar. In Rizamat that received slight and moderate drought stress, P_N evidently decreased, but J_O markedly increased, thus maintaining high values of J_T and Φ_{PS2}. Prior to the moderate drought stress, the F_v/F_m was high in Rizamat, indicating that the photodamage had not happened ahead of the moderate drought stress given. Under the severe drought stress, the photorespiration rate in Rizamat decreased by 70 %, and J_T, Φ_{PS2}, and F_v/F_m also dropped to very low values, *i.e*. the photodamage of photosynthetic apparatus has taken place. This suggested that the photorespiration has consumed the excessive assimilatory power and the photo-protective function of photorespiration is very important for Rizamat. When Cabernet Sauvignon grew under drought stress, its J_O decreased in a small range, thus maintaining higher values of J_C, J_T, Φ_{PS2}, and F_v/F_m; hence no serious photodamage occurred. Despite of the fact that P_N of cv. Red Double Taste decreased markedly under the slight drought stress, J_O still increased under the severe drought stress. This suggests that photorespiration is important in photoprotection under drought stress. J_O in cv. 1103Paulsen markedly decreased under slight stress. Accordingly, P_N, F_v/F_m, Φ_{PS2}, J_T, and J_C decreased to extremely low values. Thus photorespiration effectively protects the photosynthetic apparatus from photo-damage under drought, assists in maintaining a relatively high Φ_{PS2}, and helps P_N to be rapidly recovered after re-watering.

Additional key words: chlorophyll fluorescence; gas exchange; net photosynthetic rate; photoinhibition; *Vitis vinifera* L.

Introduction

Drought stress always induces a decline in saturation irradiance of a plant. The high irradiance and high temperature which appear simultaneously with the drought may lead to increase of excessive photon energy and decrease of photochemical efficiency, and finally to damage of photosynthetic apparatus when the stress is severe. Multiple photo-protective mechanisms have been formed in plants during the long-term evolution. Xanthophyll cycle-dependent thermal dissipation under drought is an important mechanism protecting photosynthetic apparatus from high irradiance damage (Demmig *et al*. 1988). Osmond and Björkman (1972) proposed that photorespiration might be an important photoprotective mechanism.

Photorespiration was initially found in tobacco (Decker 1955), and its main process, named glyoxylate cycle (*i.e*. biosynthesis and oxidation process of glyoxylate), was made clear after subsequent researches. The first step of the glyoxylate cycle is the synthesis of phosphoglyoxylate from ribulose-1,5-bisphosphate (RuBP) and O_2 catalyzed by ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO). Since it consumes plenty of anabolites just assimilated by plants,

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photorespiration used to be considered as a pure waste process. Many experiments were conducted to improve maize yields *via* inhibiting photorespiration rate with chemicals or selecting cultivars with low photorespiration rate by breeding (Zelitch 1966, 1989, 1992). However, some important physiological functions of photorespiration were found afterwards. In addition to the photoprotective function, as an alternative pathway for consumption of excessive assimilatory power, photorespiration is also important in ammonia metabolism (Orea *et al.* 2002), and acts as a main source of glycine, the precursor of glutathione (Noctor *et al*. 1999).

There has existed a controversy about the photoprotective function of photorespiration for many years (Osmond and Björkman 1972). Trials with mutants made by Kozaki and Takeka (1996) proved that photorespiration was essential in protecting the photosynthetic apparatus under high irradiance. With help of immunoabsorbent techniques, Wingler *et al*. (1999) tested the increase of photorespiratory activity under drought. By using $^{18}O_2$, Haupt-Herting and Fock (2000) found that the transfer of excessive photosynthetic electrons to O_2 was an important energy dissipation pathway in tomato plants during drought stress. However, many authors showed that the photorespiration had no significant effects on protection of photosynthetic apparatus from damage. For example, Brestic *et al*. (1995) found no obvious effects in F_v/F_m after inhibiting photorespiration by low O_2. Nogués and Alegre (2002) also proposed that the decline of oxidative reaction of RuBP was accompanied with the decline of carboxylation reaction. Obviously, the key difference between the two opposing viewpoints is whether photorespiration decreases markedly under drought stress. The method for estimating photorespiration by lowering O_2 concentration (2 % O_2) neglects the recycling of O_2 and CO_2 by integrated photosynthesis-photorespiration model, and the poor ventilation between intercellular and outside air induced by the decrease of stomatal conductance (g_s) enlarges this recycling. Tekeba and Kozaki (1998) estimated that this recycling might occupy up to 75 % of J_T under drought stress. Therefore, the error of photorespiration rate measured by this method would be enlarged. Combined measurements of gas exchange and chlorophyll (Chl) fluorescence could exactly estimate the photorespiration activity, and evaluate the photo-protective function of photorespiration according to the change of photorespiratory activity.

To clarify the changes of photorespiration in grapevines under different severity of drought stress and the role of photorespiration in photo-protection, we conducted an experiment with different grapevine cultivars. J_O and photorespiration rate were calculated using combined measurements of gas exchange and Chl fluorescence.

Materials and methods

Plants: Experiments were conducted from May to June 2003 in the small orchard of Shandong Agricultural University with 2-year-old potted grapevine younglings of four cultivars, *i.e*. Cabernet Sauvignon (a member of Western Europe cultivar group), Rizamat (a member of East cultivar group), Red Double Taste (a hybridized cultivar from *V. vinifera* L. and *V. labrusca* L.), and 1103Paulsen (a hybridized rootstock). The grapevines cultured in ceramic pots of 20 cm diameter and 15 cm depth were moved to the experiment plot for drought treatments at different levels when the 10th leaves of new growth expanded. For experiment, two of the fifth leaves below the tips of the new growths in each pot were randomly chosen. The experimental plot was mulched with plastic to avoid the roots spreading into ground and thereby absorb moisture from it. A rain shed was built over the potted younglings and covered with plastic cloth in the evening and during precipitation. Four treatments were set up with two pots each, including slight drought stress (T1, 65–70 % of relative water content, RWC, in soil), moderate drought stress (T2, 40–45% RWC in soil), severe drought stress (T3, 30–35 % RWC in soil), and well watered (CK, 80–85 % RWC in soil). On the surface of soil for each pot four holes at about 2 cm of diameter and *ca*. 10 cm in depth were made. Water lost during the day was supplied through the holes with a funnel each evening.

Gas exchange and Chl fluorescence combined measurement: A gas exchange system *CIRAS-1* (*PP Systems*, UK) and a modulated fluorescence monitor system *FMS-2* (*Hansatech*, UK) were used for combined gas exchange and Chl fluorescence measurement after drought stress for 25 d. The cuvette conditions were controlled by *CIRAS-1* at air temperature of 25±2 °C, relative humidity (RH) of 75±5 %, CO_2 concentration of 350±5 cm^3 m^{-3}, and irradiance of 800 μmol(photon) m^{-2} s^{-1}. Net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), and dark respiration rate (R_D) were recorded by *CIRAS-1*. Steady-state fluorescence (F_s), maximal fluorescence (F_m'), and actual photochemical efficiency ($\Delta F/F_m'$ or Φ_{PS2}) were simultaneously recorded by *FMS-2*. Initial fluorescence (F_0), maximal fluorescence (F_m), variable fluorescence (F_v), and maximal photochemical efficiency (F_v/F_m) were also recorded by *FMS-2* after putting leaves in dark for 20 min. P_N was recorded again by *CIRAS-1* after re-watering for 2 d. Three leaves per treatment per cultivar were measured, and the average ± standard deviation was regarded as final data.

Data analysis: Total electron transport rate (J_T), electron transport used for carboxylation (J_C), and electron transport used for oxygenation (J_O) were calculated according to Valentini *et al.* (1995):

$J_T = \Delta F/F_m' \times PPFD \times 0.84 \times 0.5$
$J_C = 1/3[J_T + 8(P_N + R_D)]$
$J_O = 2/3[J_T - 4(P_N + R_D)]$
where PPFD is photosynthetic photon flux density.

Results

***C*i and *P*N in different cultivars treated with different drought stress**: Stomatal conductance (g_s) of every cultivar decreased (values not shown) along with severity of drought stress, and so was P_N. Among the four cultivars, P_N in cvs. 1103Paulsen and Red Double Taste decreased markedly, which suggested that a great excess of photons existed in them. The increase of C_i in these two cultivars implied that non-stomatal limitation occurred even at slight drought stress. The cvs. Rizamat and Cabernet Sauvignon maintained higher P_N under the slight and moderate drought stress, and C_i increased only after severe drought.

Maximal photochemical efficiency and actual photochemical efficiency of photosystem 2 (PS2) in different cultivars treated with drought stress: F_v/F_m and Φ_{PS2} of the all four cultivars decreased with increase in drought stress. F_v/F_m of Cabernet Sauvignon, Rizamat, and Red Double Taste maintained high at the moderate drought stress. For Cabernet Sauvignon the value was still high at the severe drought stress, while for Rizamat and Red Double Taste it decreased. F_v/F_m of cv. 1103Paulsen decreased markedly at the slight drought stress.

Φ_{PS2} in Cabernet Sauvignon and Red Double Taste decreased slowly as the severity of drought stress went on, and reached the lowest value at the severe drought stress (Fig. 1). While Φ_{PS2} in the other two cultivars had a marked decreasing trend in different severity drought stress treatments, the decline in 1103Paulsen appeared even at the slight drought stress, and that in Rizamat at the severe drought stress (Fig. 1).

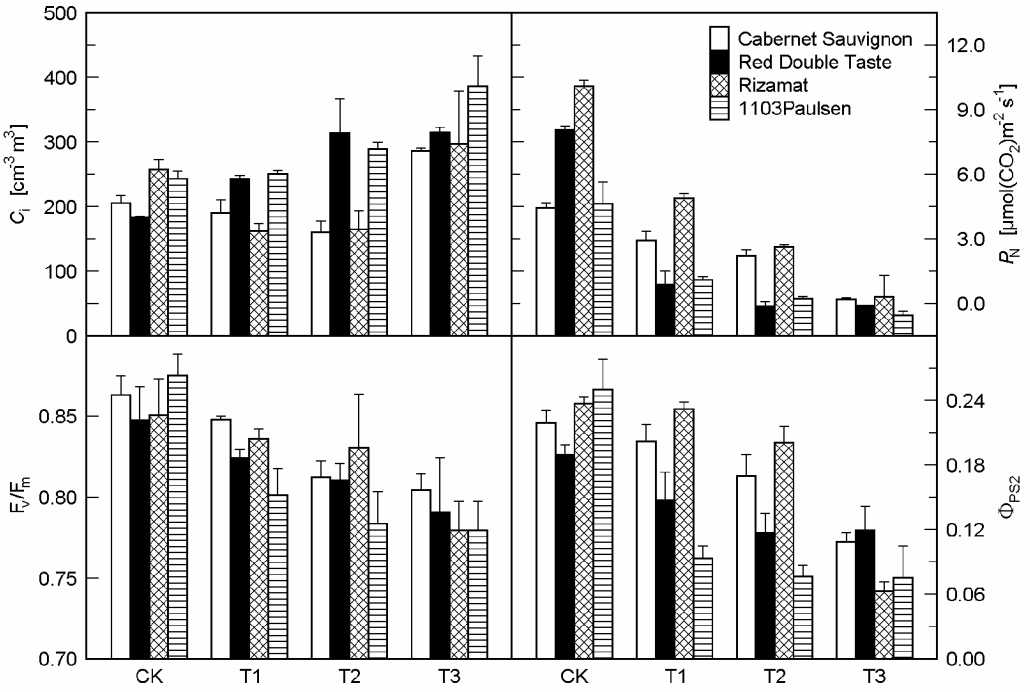

Fig. 1. Substomatal CO_2 concentration (C_i), net photosynthetic rate (P_N), and maximal (F_v/F_m) and actual photochemical (Φ_{PS2}) efficiencies in different grapevine cultivars enduring different drought stress treatments: CK (RWC 80–85 %), T1 (RWC 65–70 %), T2 (RWC 40–45 %), and T3 (RWC 30–35 %).

J_T, J_C, and J_O in different cultivars under different drought stress: Total electron transport rate of each cultivar decreased with increase in drought stress severity, but differed distinctly among cultivars. Under the slight drought stress, J_T, J_C, and J_O in 1103Paulsen decreased markedly, while J_O in Red Double Taste and Rizamat increased, which suggested that drought stress induced increase in photorespiration and thus strengthened the photoprotective function. J_T, J_C, and J_O in cvs. 1103Paulsen and Rizamat decreased under severe drought stress to a low level, proving that the photoprotective function offered by photorespiration was weakened seriously. In Cabernet Sauvignon and Red Double Taste these characteristics maintained a high level, implying that the photorespiration still offered some photoprotection (Fig. 2).

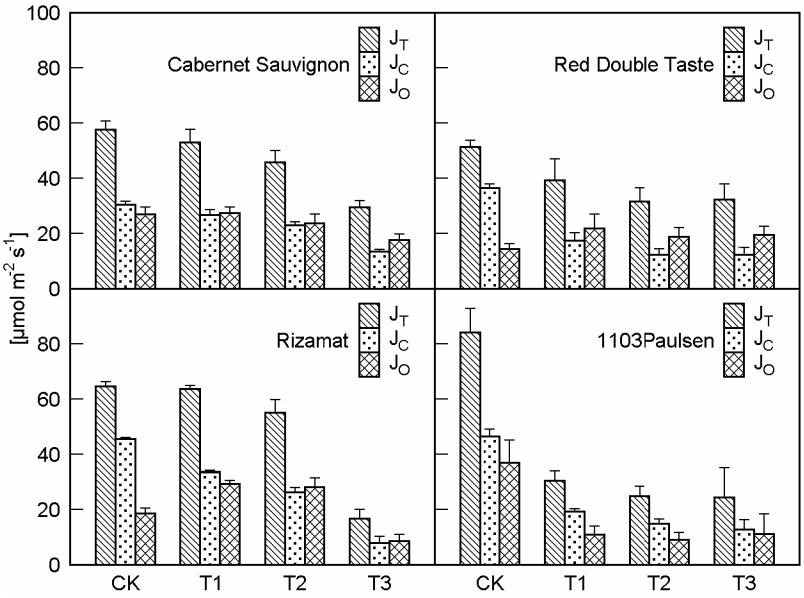

Fig. 2. Total electron transport rate (J_T), electron transport flow used in carboxylation (J_C), and electron transport flow used in oxygenation (J_O) of different grapevine cultivars enduring different drought stress treatments: CK (RWC 80–85 %), T1 (RWC 65–70 %), T2 (RWC 40–45 %), and T3 (RWC 30–35 %).

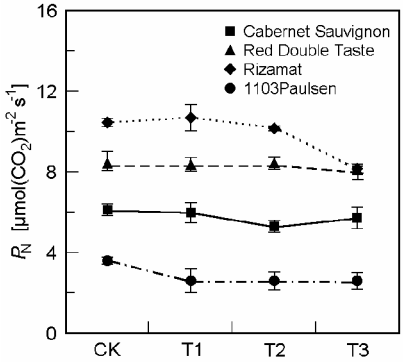

Fig. 3. Recovery of net photosynthetic rate (P_N) after re-watering for two days in different grapevine cultivars enduring different drought stress treatments: CK (RWC 80–85 %), T1 (RWC 65–70 %), T2 (RWC 40–45 %), and T3 (RWC 30–35 %).

Recovery of photosynthetic capacity in different grapevine cultivars after re-watering: Potted grapevine younglings were re-watered after drought stress treatment for one month and measurements were made after 2 d of re-watering. Photosynthetic capacity of all cultivars recovered in varying degrees after this re-watering, but cv. Rizamat enduring severe drought stress and 1103Paulsen treated with any drought stress showed a relatively low recovery of photosynthetic capacity, probably because of a relatively serious photodamage in these two cultivars. This implied that photorespiration provided photoprotection by consuming excessive assimilatory power, which not only assisted in maintaining a high Φ_{PS2} during drought stress, but also benefited the recovery of photosynthetic capacity after re-watering (Fig. 3).

Discussion

Criteria to define photoinhibition: Photoinhibition was defined as the decrease of photochemical efficiency and photodamage of photosynthetic apparatus induced by excessive photons (Long *et al.* 1994). The decrease of F_v/F_m implied the photodamage of photosynthetic apparatus, and was used as the criterion to define photoinhibition in early researches conducted with detached chloroplast. Jia (2001) suggested that Φ_{PS2} is a more reasonable criterion to define photoinhibition than F_v/F_m because it reflects the decrease of photochemical efficiency induced by non-photochemical dissipation of excessive photons. In our experiment, F_v/F_m decreased slightly and Φ_{PS2} decreased pronouncedly in each treatment of every cultivar. Considering the great part of photosynthetic capacity recovered after re-watering, we believe that F_v/F_m really reflects the photodamage of photosynthetic apparatus and Φ_{PS2} reflects the decrease in short-term-recoverable photochemical efficiency. These two indexes should be used together to express the photoinhibitory degree and type undergone by the leaves.

Accuracy of photorespiration estimated by combined measurement of parameters of gas exchange and Chl fluorescence: The photorespiratory estimation method used in this experiment would be more accurate than the low oxygen method, especially under the drought stress with decreased g_s and poor ventilation between intercellular and outside air, because it avoided the error brought

by recycling of integrated photosynthesis-photorespiration. The shortage of this method is the neglect of the electron transport consumption by Mehler reaction. J_O calculated by this method should include the part of electron transport consumed by photorespiration and Mehler reaction. Photorespiration calculated by this method must be more exact owing to the electron transport consumption by Mehler reaction being far lower than that by photorespiration (Biehler and Fock 1996).

Photoprotective function of photorespiration and differences among cultivars: Photorespiration could prevent photosynthetic apparatus from photodamage by consuming excessive assimilatory power, a prerequisite of which should be the increase of photorespiratory activity, or at least no obvious decrease. The increase of photorespiratory activity could be induced by high irradiance (Gerbaud and Andrè 1980) and CO_2 deficit (Tourneux and Peltier 1995). Flexas *et al*. (1999) found that O_2 uptake by grapevine leaves increased at low night temperatures and/or water stress, proposing a possible explanation of increasing photorespiration and Mehler reaction. The reason for increase of photorespiratory activity at drought stress should be the decrease of intercellular CO_2 concentration (Farquhar and Sharkey 1982, Cornic *et al.* 1992) and increase of RuBPCO catalyzed RuBP oxygenase reaction (Hartman and Harpel 1994) induced by decrease in g_s. Along with the aggravated drought stress, the decrease of electron transportability induced a deficit in NADPH and ATP supplies (Haupt-Herting and Fock 2000). Severe drought stress even inactivated the whole enzyme system of the plant (Portis *et al.* 1986), resulting in decrease of photorespiratory activity, so as to cause a weakening or disappearing of the photoprotective function.

Adopting combined measurement of gas exchange and Chl fluorescence to calculate J_O and photorespiration rate in this experiment, we found that J_O of Rizamat and Red Double Taste increased (*i.e*. the photorespiration was activated by the stimulation coming from the drought stress) under the slight and moderate drought stress, and that of Cabernet Sauvignon decreased slightly (*i.e*. no obvious decrease of photorespiration rate happened). Correspondingly, these three cultivars sustained relatively high F_v/F_m and Φ_{PS2} levels, and P_N recovered quickly after re-watering. But under the severe drought stress, the photorespiration rate of Rizamat decreased markedly and so did F_v/F_m and Φ_{PS2}, and the photosynthetic capacity recovered to a relatively low degree after re-watering. The same cases appeared in 1103Paulsen even at the slight drought stress, proving that photorespiration really played an important role in photoprotection, helped to sustain relatively high F_v/F_m and Φ_{PS2}, and became the reason of quick recovery of photosynthetic capacity after re-watering.

Obvious differences in photoprotection of photorespiration existed among cultivars. Photorespiration did not react effectively in all cultivars. In 1103Paulsen, photorespiration rate decreased markedly at the slight drought stress, which could not provide effective photoprotection, and so became an important reason for its leaves to be unendurable to drought stress and high irradiance. For Cabernet Sauvignon, although photorespiration rate decreased slightly along with the drought stress increase, its photoprotective role was still assignable. Photorespiration in Rizamat and Red Double Taste increased under the slight drought stress, then decreased slightly as the severity of drought stress went on, showing a very important role in photoprotection. The J_O, J_T, J_C, F_v/F_m, and Φ_{PS2} in Rizamat decreased significantly at the severe drought stress, resulting in loss of photoprotective function, and being an important reason for occurring of photodamage.

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