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Photosynthesis and activity of photosystem II in response to drought stress in Amur Grape (*Vitis amurensis* Rupr.)

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Abstract

The Amur Grape (*Vitis amurensis* Rupr.) cultivars 'ShuangFeng' and 'ZuoShanyi' were grown in shelter greenhouse under natural sunlight and subjected to drought. Sap flow rate, net photosynthetic rate (P_N), and chlorophyll (Chl) fluorescence were measured on Amur Grape leaves subjected to different drought treatments. Significant decreases in P_N were associated with increasing intercellular CO_2 concentration (C_i), suggesting that the reduction in P_N was caused by nonstomatal limitation. Analysis of OJIP transients according to the JIP-test protocol revealed that specific (per PSII reaction center) energy fluxes for light absorption, excitation energy trapping and electron transport have significantly changed. The appearance of a pronounced K-step and J-step in polyphasic rise of fluorescence transient suggested the oxygen-evolving complex and electron transport were inhibited. Drought stress has relatively little effect on the parameter maximal quantum yield of PSII photochemistry (F_v/F_m), but the performance index (PI_{ABS}) is more sensitive in different drought treatment. There are cultivar differences in the response of PSII activity to drought, the photosynthetic apparatus of 'ZuoShanyi' cultivar is more resistant to drought than that of 'ShuangFeng', and JIP-test could be a useful indicator for evaluation and selection to drought tolerance.

Additional key words: Amur Grape; drought stress; JIP-test; net photosynthetic rate; photosystem II.

Introduction

Water is the most limiting resource in the arid and semiarid area, drought is one of the most limiting environmental factors for crop growth, so drought stress severely affects crop growth and reduces average yields (IPCC 2001, Chaves *et al.* 2003). With global warming, arid and semiarid area will be further expanded. The Amur Grape (*Vitis amurensis* Rupr.) as an economic crop is native to the Far East (China, Russia, Japan, Korea, and Siberia). It is very resistant to cold, but not tolerant to drought and sometimes need abundant watering.

Drought stress often results in stomatal closure, which is the initial cause for decrease of P_N (Cornic and Briantais 1991, Chaves 1991, Yordanov *et al.* 2000), but nonstomatal limitation may play a dominant role in decreased P_N as stress develops. Nonstomatal limitation has been attributed to the reduced carboxylation efficiency or to the inhibited functional activity of PSII. Yu *et al.* (2009) thought that the decrease in P_N might be associated with the increase in leaf to air vapour pressure deficit and the depression of effective quantum yield of PSII photochemistry (Φ_{PSII}) on grapevine. However, Vos and Oyarzun (1987) showed that the decrease in P_N is related to stomatal limitation on potato.

Photosynthetic capacity and quantum yield show little or no change in low drought stress (Yordanov *et al.* 2003), but moderate and severe drought stress often inhibits activity of enzymes related to Calvin cycle and

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Abbreviations: ABS – absorption energy flux; C_i – intercellular CO_2 concentration; Chl – chlorophyll; CK – control; CS – optical cross-section; DI – dissipation energy flux; E – transpiration rate; ET – energy flux for electron; F_0 – minimal fluorescence of dark-adapted state; F_m – maximal fluorescence of dark-adapted state; g_s – stomatal conductance; LD – low drought; MD – moderate drought; OEC – oxygen evolving complex; P_N – net photosynthetic rate; PI_{ABS} – performance index; M_0 – net rate of PSII closure; PSII – photosystem II; RC – reaction center; RWC – relative water content; SD – severe drought; SWC – soil water content; TR – energy flux for trapping; Φ_{PSII} – effective quantum yield of PSII photochemistry; φp_o – maximum quantum yield of primary photochemistry ; ψ_0 – efficiency that a trapped electron can move further ahead of Q_A^-.

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photochemistry (Maroco *et al.* 2002, Lawlor and Cornic 2002, Dias and Brüggemann 2007). Although plant have efficient mechanisms to protect photosynthetic apparatus from injury, for example, the over-excited energy can be consumed by Mehler reaction and photorespiration (Thomas and Stoddart 1980, Demmig-Adams and Adams 1992, Foyer and Noctor 2000, Foyer and Noctor 2003), severe drought stress still will result in damage of photosynthetic apparatus (Valladares and Pearcy 2002). Flexas *et al.* (2000) studied the change of F_m in drought stress on grape. Christen *et al.* (2007) thought that the JIP-test analysis may offer more information about photosynthetic processes and supplied a sensitive means to detect drought stress on mature grapevine.

Chl *a* fluorescence measurements represent a sensitive and reliable method to monitor and quantify the changes induced in the photosynthetic apparatus by drought stress (Van Heerden *et al.* 2007, Naumann *et al.* 2007, Oukarroum *et al.* 2007, Oukarroum *et al.* 2009). Although effects of drought stress on other plants have been extensively studied, growing ability of Amur grape on drought-prone areas has barely been studied. For this reason, two Amur Grape cultivars were compared: 'ZuoShanyi' and 'ShuangFeng' are main Amur grape cultivars in northeast area of China, they have shown a high productivity. Many farmers in the area use the two cultivars. In recent years, Amur grape have been planted gradually in arid regions, for example Mongolia, which decrease the probability for downy mildew infection. But in these regions Amur grape is often subjected to severe drought stress as well as other environmental constraints, such as high temperature and excessive light, which cause severe decrease of yield and fruit quality. Nondestructive methods to evaluate drought of Amur grape are still missing. Therefore, the purpose of this study was to analyze photosynthetic efficiency and Chl *a* fluorescence transients of Amur grape affected by drought stress, and to assess fluorescence monitoring as a possible tool to evaluate drought tolerance of Amur grape. In this frame, it can be used to adapt agricultural and forestry practices to cope with fluctuations of local environmental conditions as a result of predicted global climate changes.

Materials and methods

Plants: A rain shelter greenhouse experiment was conducted from May 2010 till August 2010, annual cutting seedlings of Amur Grape (*V. amurensis* Rupr.) cultivars 'ShuangFeng' and 'ZuoShanyi' were grown in plastic pots (290 mm in diameter and 180 mm high) under natural daylight conditions at the Institute of Special Wild Economic Animal and Plant Science. Jilin city, Jilin province was selected as experimental site. It is situated between 44º04'N and 126º05'E, annual precipitation is 679 mm, annual highest and lowest temperatures are 35°C and –30°C, respectively. When the plants were in direct sunlight they received a PPFD of 1,400–1,600 µmol m^{-2} s^{-1}. Average night- and day-time temperatures in shelter greenhouse were 16°C and 30°C, and relative air humidity was 85% and 60%, respectively. Plant materials of two varieties were uniform and of the same size about 200 mm high. Prior to the start of the experiment, the cutting seedlings were taken out of sandy bed, the roots washed and individually transplanted in pots filled with matrix which was composed of deer manure, vermiculite, sand, and garden soil (1:1:1:4, v/v/v). All seedlings were irrigated daily. After 7 weeks of growth, the drought treatments were initiated. The drought stress treatments were gradually imposed. Three drought-stress levels were considered and compared to a control (CK), in which the soil water potential was kept constant because seedlings were well watered daily. Drought-stressed plants showed low water deficit after 6 d without watering (low stress, LD), moderate stress after 12 d without watering (moderate stress, MD) and severe stress after 24 d without watering (severe stress, SD). Control and drought-stressed seedlings were arranged in a completely randomized design with five replications. Drought stress (four levels, including the control treatment) and varieties (two) were considered as treatments. In total, 40 seedlings were used. The measured plant had 8 leaves.

Absolute water content in soil: For the pots under different drought level, SWC was calculated gravimetrically. Immediately after measurement of plant sap flow rate, CO_2 assimilation and Chl *a* fluorescence transient terminated, soil wet mass (SWM) was determined. Then all the pot soil was oven-dried to constant mass at 105 ± 2°C for over 24 h for dry mass determinations of pot soil (SDM). Soil water content (SWC) was calculated from the equation: SWC [%] = (SWM – SDM)/SDM × 100.

Plant sap flow rate: The sap flow rate of Amur Grape has been observed by advanced Photosynthesis Monitor (*PTM-48M, Daletown Company Ltd.,* Nicosia, Cyprus), sap flow collectors were *SF-5P* Sensor (suitable for 4- to 10-mm stem diameter). The sensors were installed about 0.5–1.0 m above ground, depending on stem diameter, as recommended by the manufacturer. The sensors were covered carefully with two or three layers of aluminium foil in order to protect the sensor from external heat effects.

CO_2 assimilation: Net photosynthetic rate (P_N) was measured with a portable photosynthesis system (*CIRAS-2; PP-Systems,* Hitchin, UK). At the same time, intercellular CO_2 concentration (C_i), transpiration rate (E), and stomatal conductance (g_s) were automatically recorded. Leaves were illuminated by the *CIRAS-2* light source with a PPFD of 1,200 µmol m^{-2} s^{-1}, at 360 µmol $mol^{-1}(CO_2)$

concentration, a leaf temperature of 30°C, 70% relative humidity, and flow rate of 196 ml min^{-1}.

Chl *a* fluorescence transient measurement and JIP-test: Chl *a* fluorescence transient (OJIP) was measured with a Plant Efficiency Analyzer (*Pocket-PEA, Hansatech Instruments,* King's Lynn, Norfolk, UK). Fluorescence transients were recorded during a 1-s pulse of red radiation (3,500 μmol m^{-2} s^{-1}). The fluorescence signals were recorded within a time scan from 10 μs to 1 s with a data acquisition rate of 100 readings ms^{-1} for the first 2 ms, and 1 reading ms^{-1} after 2 ms. All the measurements were done on full dark-adapted (20 min) leaves.

Fluorescence transients were analyzed according to the JIP-test, which is a Chl *a* fluorescence analysis program (*BIOLYZER HP3*, *Bioenergetics Laboratory*, University of Geneva, Switzerland) (Strasser and Strasser 1995, Strasser 1997, Strasser *et al.* 2004). The following data from the original fluorescence measurements were used: the minimal fluorescence of dark-adapted state or the fluorescence intensity at 50 μs (considered as F_o); the maximal fluorescence of dark-adapted state (F_m); the fluorescence intensities at 300 μs (K-step). The JIP-test represents translation of the original recorded data to biophysical parameters that quantify the stepwise energy flow through PSII (Van Heerden *et al.* 2007). The following parameters refer to time zero (onset of fluorescence induction): (*1*) The specific energy fluxes (per RC) for absorption (ABS/RC), trapping (TR_o/RC), electron transport (ET_o/RC) and dissipation (DI_o/RC); (*2*) the maximal quantum yield of PSII photochemistry (F_v/F_m). The formulae used to calculate these biophysical parameters are detailed in Appendix.

The performance index (PI_{ABS}), is also calculated (Strasser *et al.* 2000). The PI_{ABS} considers the three main steps that regulate photosynthetic activity by a PSII reaction centre (RC) complex, namely absorption of light energy (ABS), trapping of excitation energy (TR) and conversion of excitation energy to electron transport (ET) (Van Heerden *et al.* 2007).

Statistical analysis was conducted with the software of *SAS* (*JMP 6.0, SAS Institute*, Cary, NC, USA). In data sets with parametric distribution, significant differences between means of the treatments means were determined using Student's *t*-test at a significant level set to 0.05. Sap flow rates of stem are the averages of three replicates from 3 plants per experiment (drought stress and control); gas-exchange data are the average of individual measurements of 3 plants per experiment; Chl *a* fluorescence parameters are the average of individual measurements of 6 plants per experiment.

Results

Changes of soil water content in different treatments: SWC of different treatment decreased significantly from 25.71% in well watered treatment to 17.44%, 12.18%, and 9.44%, in LD, MD, and SD stress treatment, respectively (Table 1).

Table 1. Changes of soil relative water content (RWC) in different drought treatments of Amur Grape. Each value is the mean ± SD (*n* = 5). *Different letters* indicate significant difference ($P<0.05$). CK – control, LD – low drought, MD – moderate drought, SD – severe drought.

Treatment	RWC [%]
CK	25.71 ± 0.68[a]
LD	17.44 ± 0.95[b]
MD	12.18 ± 1.20[c]
SD	9.44 ± 1.29[d]

Effects of drought stress on sap flow rate: Drought stress decreased sap flow rate of Amur Grape (Fig. 1*A*). Sap flow rate decreased from 1.69 ml h^{-1} in well watered Amur Grape to 1.49 ml h^{-1} and 0.93 ml h^{-1} in MD and SD stress in 'ZuoShanyi' seedlings respectively, but had a relative increase in LD stress. For 'ShuangFeng' seedlings, change of sap flow rate is similar to 'ZuoShanyi', but sap flow rate of 'ShuangFeng' is lower than 'ZuoShanyi' in different treatment.

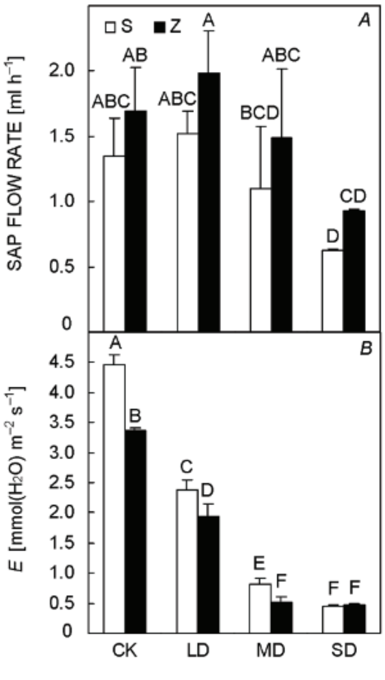

Fig. 1. Effects of drought stress on sap flow rate in the Amur grape stem (*A*) and transpiration rate (*B*) in 'ZuoShanyi' and 'ShuangFeng', at the end of low, moderate and severe drought stress, values are means ± SD (*n* = 3). *Different letters* indicate significant difference ($P<0.05$). *E* – transpiration rate, CK – control, LD – low drought, MD – moderate drought, SD – severe drought, S – ShuangFeng, Z – ZuoShanyi.

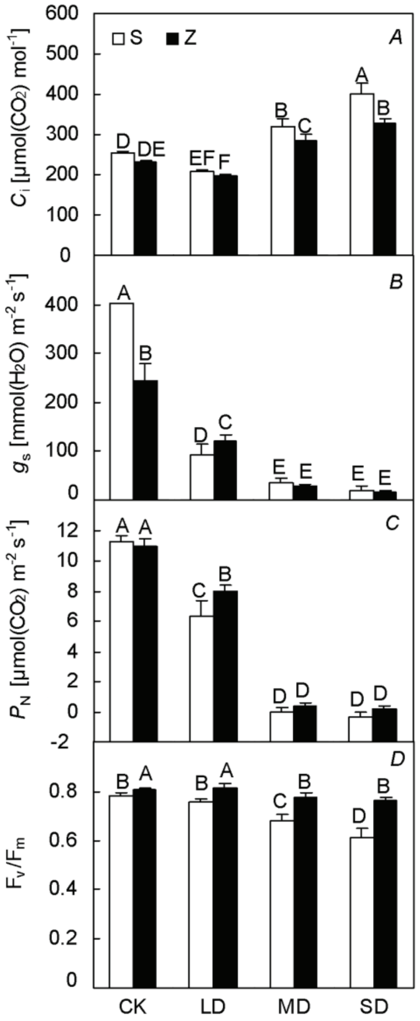

Fig. 2. Effects of drought stress on intercellular CO_2 concentration (C_i) (*A*), stomatal conductance (g_s) (*B*), net photosynthetic rate (P_N) (*C*). Values are means ± SD ($n = 3$), and maximal quantum yield of PSII photochemistry (F_v/F_m) (*D*) of the Amur Grape, values are means ± SD ($n = 6$). *Different letters* indicate significant difference ($P<0.05$). CK – control, LD – low drought, MD – moderate drought, SD – severe drought, S – ShuangFeng, Z – ZuoShanyi.

Effects of drought stress on P_N, g_s, E, and C_i: C_i increased by 23.78% and 40.78% in MD- and SD-stressed 'ZuoShanyi' seedlings respectively, but decreased 15.7% in LD, compared to the 'ZuoShanyi'. For 'ShuangFeng' seedlings C_i increased by 25.86% and 57.78%, respectively, in MD and SD stress treatment (Fig. 2*A*) compared to well watered plants, but decreased 17.94% in LD.

g_s and E also decreased significantly as stress developed (Figs. 1*B*, 2*B*), but E of 'ShuangFeng' is higher than 'ZuoShanyi' in different treatment.

P_N decreased from 10.97 μmol m^{-2} s^{-1} in well watered 'ZuoShanyi' to 7.97 μmol m^{-2} s^{-1}, 0.37 μmol m^{-2} s^{-1} and 0.17 μmol m^{-2} s^{-1} in LD, MD, and SD stress treatments, but P_N of 'ShuangFeng' cultivar decreased from 11.23 μmol m^{-2} s^{-1} in well watered seedlings to 6.27 μmol m^{-2} s^{-1}, –0.07 μmol m^{-2} s^{-1} and –0.33 μmol m^{-2} s^{-1}, in LD, MD, and SD stress treatment respectively (Fig. 2*C*).

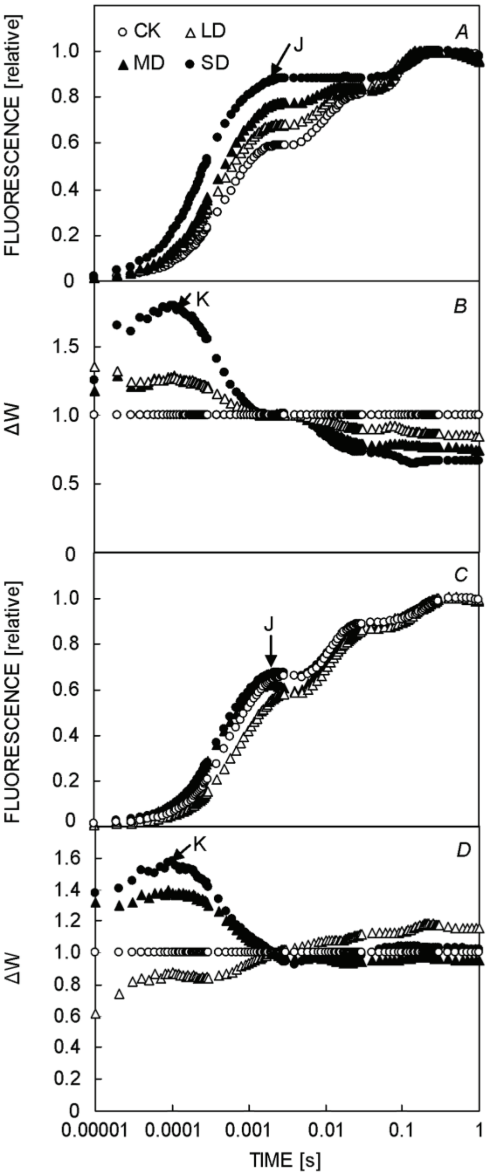

Fig. 3. Effects of drought stress on the polyphasic chlorophyll fluorescence transients plotted on a logarithmic time scale. The relative variable fluorescence at any time is defined as: $V_t = (F_t - F_o)/(F_m - F_o)$. 'ShuangFeng' (*A*), 'ZuoShanyi'(*C*). Changes in the amplitude of the K-step, which is expressed as the ratio $\Delta W = W(\text{treatment})/W(\text{control})$, $W = (F_t - F_o)/(F_j - F_o)$. 'ShuangFeng' (*B*), 'ZuoShanyi'(*D*). K-step – at about 300 μs, J-step – at 2 ms, CK – control, LD – low drought, MD – moderate drought, SD – severe drought.

Changes of Chl fluorescence transients: The polyphasic rise of fluorescence transients (OJIP) was measured with a plant efficiency analyzer. Drought tolerance of two Amur Grapes in response to days of stress was evaluated through analyzing several leaf fluorescence parameters

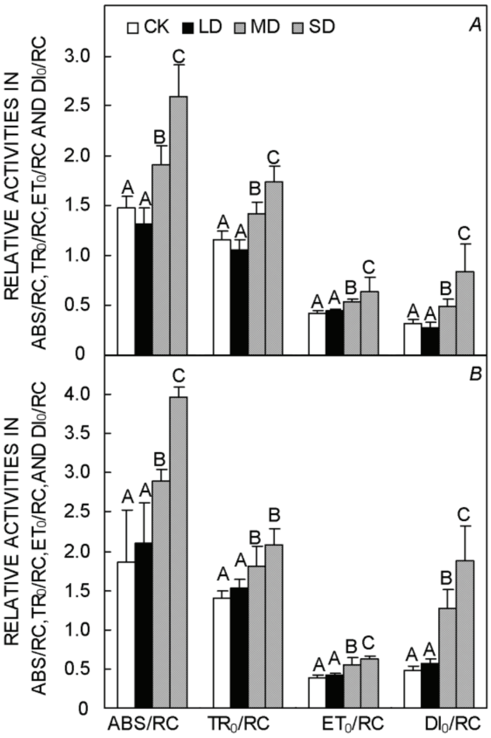

Fig. 4. Relationships of the relative activities in absorption flux per reaction center (ABS/RC), trapped energy flux per RC (TR_0/RC), electron transport flux per RC (ET_0/RC) and dissipated energy flux per RC (DI_0/RC) of Amur grape leaf in different drought treatments. Each value is the mean ± SD ($n = 6$). 'ZuoShanyi'(*A*), 'ShuangFeng' (*B*). *Different letters* indicate significant difference ($P<0.05$). CK – control, LD – low drought, MD – moderate drought, SD – severe drought.

determined after 20-min full dark adaption.

In order to compare changes in fluorescence rise kinetic more clearly, original fluorescence curves were normalized between F_0 and F_m (Fig. 3*A*,*C*). Variable fluorescence (V_t) curves of two Amur Grape cultivars are different, the curve of 'ShuangFeng' showed a significant difference between control and treatment, however, the curve of 'ZuoShanyi' has no significant difference compared to 'ShuangFeng'. For SD stress treatments, a very clear K-step appeared in 'ShuangFeng'. In order to compare the changes in the amplitude of the K-step (at about 300 μs) during drought stress, the fluorescence curves were normalized between F_o and F_j (at 2 ms), *i.e.* $W = (F_t - F_o)/(F_j - F_o)$. The amplitude of the K-step (W_k) was expressed as the ratio W (treatment)/W (control) = ΔW. A higher W_k was observed in SD stress than in LD and MD stress, otherwise, a ΔW_K of 'ShuangFeng' is higher than that of 'ZuoShanyi' in SD stress (Figs. 3*B*,*D*).

In order to determine the sensitivity and dynamic ranges of the fluorescence parameters in different drought stresses and different cultivars, we compared the different fluorescence parameters in two cultivars under different drought-stress conditions.

The OJIP fluorescence transients were analyzed according to the JIP-test protocol. Fig. 2*D* shows the changes of maximal quantum yield of PSII photochemistry (F_v/F_m). Results demonstrate that F_v/F_m showed a significant difference only under moderate and severe drought condition in 'ShuangFeng' but a slight change in 'ZuoShanyi'.

The effects of drought stress on specific (per excited cross-section of leaf) energy fluxes for light absorption, excitation energy trapping and electron transport are presented in the form of column diagram (Fig. 4*A*,*B*). ABS/RC, TR_0/RC, ET_0/RC and DI_0/RC had increased significantly, for acclimation responses to drought stress in 'ShuangFeng', but increased significantly only under MD and SD condition in 'ZuoShanyi'.

Table 2. Changes of F_0, F_m, F_j, dv/dt, and PI_{ABS} in different drought treatments of Amur Grape. Each value is the mean ± SD ($n = 6$). *Different letters* indicate significant difference ($P<0.05$). CK – control, LD – low drought, MD – moderate drought, SD – severe drought, F_0 – minimal fluorescence of dark-adapted state; F_m – maximal fluorescence of dark-adapted state, F_j – fluorescence intensity at the J-step (at 2 ms), dv/dt – the initial slope of the relative variable fluorescence, PI_{ABS} – performance index.

Variety	Treatment	F_0	F_m	F_j	dv/dt	PI_{ABS}
ShuangFeng	CK	369 ± 6^c	$1,668 \pm 112^a$	$1,122 \pm 21^c$	0.774 ± 0.078^d	13.3 ± 3.06^b
	LD	402 ± 20^b	$1,656 \pm 100^a$	$1,134 \pm 19^{bc}$	0.992 ± 0.091^c	7.57 ± 1.05^{dc}
	MD	416 ± 32^b	$1,306 \pm 67^c$	$1,150 \pm 37^{abc}$	1.308 ± 0.19^b	2.16 ± 1.07^e
	SD	475 ± 33^a	$1,221 \pm 68^c$	$1,177 \pm 32^a$	1.688 ± 0.32^a	0.92 ± 0.76^e
ZuoShanyi	CK	334 ± 7^e	$1,729 \pm 7^a$	$1,154 \pm 37^{abc}$	0.761 ± 0.11^d	15.8 ± 2.9^b
	LD	323 ± 19^e	$1,746 \pm 13^a$	$1,164 \pm 69^{ab}$	0.808 ± 0.11^d	21.7 ± 5.1^a
	MD	341 ± 8^{de}	$1,520 \pm 80^b$	$1,169 \pm 23^{ab}$	0.941 ± 0.09^{dc}	8.3 ± 1.3^c
	SD	361 ± 11^{cd}	$1,535 \pm 33^b$	$1,183 \pm 25^a$	1.293 ± 0.09^b	5.2 ± 2.2^d

Table 2 shows the changes in F_0, F_m, F_j, dv/dt and PI_{ABS} in 'ShuangFeng' and 'ZuoShanyi' in different treatments. F_0 and dv/dt increased significantly comparable to CK in 'ShuangFeng', but increased significantly only in MD and SD stress in 'ZuoShanyi'. F_m, F_j and PI_{ABS} had respectively decreased in both varieties. But F_m and F_j decreased significantly only in MD and SD stress, PI_{ABS} decreased significantly in LD stress in 'ShuangFeng', increased significantly in LD stress in 'ZuoShanyi'.

Discussion

Drought affects many physiological and biochemical processes within plants. There are many studies to develop some new screening methods in order to evaluate severity of drought (Boussadia *et al.* 2008, Van Heerden *et al.* 2007). In the present study, our results show that the photosynthetic apparatus of 'ZuoShanyi' becomes more resistant to drought stress than 'ShuangFeng' when grown in arid and semiarid areas. This can be seen by the significantly enhanced resistance of PSII, as reflected in the changes of the various fluorescence parameters related to PSII function.

Drought stress effects on sap flow: Sap flow rate of 'ShuangFeng' is lower than 'ZuoShanyi' in different treatments, but E of 'ShuangFeng' is higher than 'ZuoShanyi' in different treatments. In addition, air vapour pressure deficit of 'ShuangFeng' is higher than 'ZuoShanyi' in different treatment (data not shown). These results indicated 'ShuangFeng' losed more water than 'ZuoShanyi', the more water inside 'ZuoShanyi' may keep leaf and whole plant survive. Further, 'ZuoShanyi' may be more drought-tolerant.

Drought stress effects on leaf photosynthesis: A significant relationship was determined between P_N, g_s, and C_i. P_N and g_s decreased significantly as drought stress developed, but C_i shows a decrease in LD stress and increase in MD and SD stresses, suggesting that C_i might not be the limiting factor for photosynthesis in the stressed plant (Dias and Brüggemann 2010). Many studies confirmed that C_i remained constant when P_N decreased apparently (Correia *et al.* 1990, Sinha *et al.* 1997). The decrease in P_N might be due to photodamage, while photodamage is often associated with environmental stress, such as drought or temperature (Ohashi *et al.* 2006, Gamon and Pearcy 1990). P_N in the leaves is depressed due to stomatal limitation in LD stress, but in MD and SD stresses nonstomatal limitation will be a main factor which depressed the P_N (Galmés *et al.* 2007), although it has been reported by Flexas *et al.* (2000) that decreases in P_N were mainly due to stomatal closure and not to decreased photochemical efficiency of PSII in grapevines. The nonstomatal limitation factor may be inhibition in activity of various enzymes of the Calvin cycle and PSII photochemistry (Chaves *et al.* 2003, Maroco *et al.* 2002) .

The importance of fluorescence parameters for water-stress assessment: In order to gain more insight into the enhanced resistance of PSII to drought stress, the structure and function of PSII were evaluated according to the JIP test by measuring the fast Chl fluorescence transients, which can be used as a tool to detect and estimate the PSII behavior. The parameters of phenomenological energy fluxes can be visualized by means of an energy pipeline model of the photosynthetic apparatus in the form of the leaf model (Strasser 1987, Strasser *et al.* 1996, Krüger *et al.* 1997). However, more importantly, we observed that the decrease in the number of active RCs was much greater in severe drought stress than that in control. The well watered plants have no inactivation of the RC.

Many studies demonstrated that drought stress resulted in injuries to oxygen-evolving complex (Guissé *et al.* 1995). Oukarroum *et al.* (2007) considered that changes of the L and K-bands of the fluorescence transients OJIP could predict the vitality of leaves and the tolerance of the varieties to drought stress. The effect of the different treatments on 'ZuoShanyi' and 'ShuangFeng' was evident by comparing the shape of the corresponding fluorescence transient curves. A similarity between the two Amur Grape cultivars was the appearance of a clearly defined K-peak at around 300 µs (ΔW-peak), which suggests inhibition or damage of the oxygen-evolving complex (OEC) (Stasser 1997, Wen *et al.* 2005). Previous studies have found that there is appearance of a K-peak when leaves are exposed to elevated temperature (Jiang *et al.* 2006, Li *et al.* 2009). Appearance of K-step under drought stress may be due to lower stomatal conductance and transpiration rate, which increase leaf temperature (Van Heerden *et al.* 2007). One difference, however ΔW value of 'ShuangFeng' is higher than 'ZuoShanyi', suggesting oxygen-evolving complex of 'ZuoShanyi' have greater resistance than 'ShuangFeng'. Thereby, the appearance of K-step can be used as a convenient stress indicator and specifically attributed to a damage on the oxygen-evolving complex on a practical basis (Strasser 1997, Wen *et al.* 2005).

As noted before, drought stress has relatively slight effect on the parameter F_v/F_m, which decreases only under SD stress condition (Lu and Zhang 1999, Zlatev and Yordanov 2004, Oukarroum *et al.* 2007). Many studies confirmed parameters derived from the JIP-test to be very sensitive to indicate the physiological status of the plant under drought stress (Wen *et al.* 2005). PI_{ABS} may be a good parameter for monitoring and evaluating drought stress (Van Heerden *et al.* 2007). Our results showed that PI_{ABS} had a highly significant change comparable to CK in 'ShuangFeng', but decreased significantly only in MD and SD stress in 'ZuoShanyi'. Similar changes were observed in barley, where PI_{ABS} of Aït Baha had an increase in drought stress, suggesting the cultivar is more tolerant to drought stress (Oukarroum *et al.* 2009). Our results further indicated that the photosynthetic apparatus of 'ZuoShanyi' becomes more resistant to drought stress than 'ShuangFeng'.

In addition, ABS/RC, TR_0/RC, ET_0/RC, DI_0/RC, F_0, F_m, and dv/dt started to change significantly only in MD or SD stress in both cultivars. It must be stressed that TR_0/RC expresses the maximal rate of the closure of RCs as a fractional expression over the total number of RCs that can be closed. This is because it is possible under stress conditions that some RCs are inactivated in the sense of being transformed to quenching sinks without reducing QA to QA^- reducing centers. In such a case TR_0/RC refers only to the active (QA to QA^- reducing) centers. The same is valid also for two other specific fluxes ABS/RC and ET_0/RC, since their derivation is based on the expression for TR_0/RC. Thus, an inactivation of PSII RCs will result in an increase in TR_0/RC and thereby ABS/RC and ET_0/RC. However, it is possible that an increase in TR_0/RC may be due to a decrease in the re-oxidation of QA especially. Increase in the trapping flux of the still-active RCs (TR_0/RC) is due to decrease of total number of active RCs a simultaneous under drought stress. Significant increase of F_0 in low stress in 'ShuangFeng' indicated RCs of PSII may be due to an accmulation of Q_B^- nonreducing centers under water stress (Lu *et al.* 1998) and could be an indicator of the relative abundance of Q_B^- nonreducing centers (Flexas *et al.* 2000). Increase of F_j had caused appearance of J-step, which indicated the inhibition of electron transport chain after Q_A in PSII. dv/dt reflects the slope at the origin of the fluorescence rise, further, increase of dv/dt indicated that the reoxidation of Q_A^- to Q_A is inhibited under drought stress.

It should be noted that the limitations of the system might exist. In the future, the materials will be mature plants and be planted in field, which is very interesting. But the results of this study clearly revealed changes of photosynthetic efficiency and Chl *a* fluorescence transients.

In conclusion, nonstomatal limitation is main factor in decreased P_N in MD and SD stress. The K and J steps at fluorescence transient were increased by drought stress, which indicated the inhibition of oxygen-evolving complex (OEC) and electron transport chain after QA in PSII. PI_{ABS} may be a more sensitive parameter for monitoring and evaluating drought stress. 'ZuoShanyi' may be more resistant to drought stress, while 'ShuangFeng' can be sensitive to drought stress.

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Appendix

Summary of JIP-test formulae using data extracted from the OJIP transients (Van Heerden *et al.* 2007):
Specific fluxes or specific activities

$ABS/RC = M_0(1/V_J)(1/\varphi p_o)$
$TR_0/RC = M_0(1/V_J)$
$ET_0/RC = M_0(1/V_J)\ \psi_0$
$DI_0/RC = (ABS/RC) - (TR_0/RC)$

The initial slope of the relative variable fluorescence $(dv/dt)_0$ can be expressed as the initial increment per ms using the time interval of 250 µs where: $(dv/dt)_0 \approx 4\ (F_{300\,\mu s} - F_0)/(F_m - F_0)$.
In the JIP-test the Equation $[4(F_{300\,\mu s} - F_0)/(F_m - F_0)]$ is represented by the parameter M_0, and is defined as the net rate of PSII closure.

Performance indexes

$PI_{ABS} = (RC/ABS)[\ \varphi p_o/(1 - \varphi p_o)][\ \psi_0/(1 - \psi_0)]$