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PHOTOSYNTHETIC CAPACITY, ANTIOXIDANT ACTIVITY AND MOLECULAR RESPONSES IN *PARTHENOCISSUS QUINQUEFOLIA* (L.) PLANCH UNDER HIGH TEMPERATURE STRESS AND SUBSEQUENT RECOVERY

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Abstract

Photosynthetic capacity and photosystem II (PSII) activity decreased with increasing temperature, whereas antioxidant enzyme activity showed the opposite trend. High temperature stress induced a significant increase in $\Phi_{f,D}$, and D_1 protein turnover rate. Photosynthetic capacity, PSII activity, and antioxidant enzyme levels in plants treated at 35 and 40°C were restored to control levels upon stress relief, whereas those in plants grown at 45°C were only partially restored. Therefore, the temperature limit for heat tolerance in *Parthenocissus quinquefolia* is between 40 and 45°C. Further, it was observed that antioxidant enzymes were crucial for high-temperature stress resistance in *P. quinquefolia*, with DEGP1 protein playing a major role in the rapid turnover of D_1 protein for PSII repair.

Parthenocissus quinquefolia (Vitaceae) is considered as an important climbing woody species for three-dimensional greening due to its advantage of high coverage and rapid growth (Emerine *et al.* 2013). However, global warming poses an increasingly serious threat to this kind of plant species growing on walls (Islam 2015).

Photosystem II (PSII) activity is commonly used as an indicator of plant health status in warm environments (Chen *et al.* 2017). Non-regulated thermal energy loss ($\Phi_{f,D}$) reflects photodamage happened to PSII (Osório *et al.* 2013). Antioxidant enzyme, such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD), can eliminate superoxide free radicals (O^{2-}) and hydrogen peroxide (H_2O_2). Previous studies have confirmed that the decrease of PSII activity at high temperature is caused by the inhibition of PSII repair which is closely related to D1 protein turnover (Li *et al.* 2017). However, most of this research focused on economically important crops, whereas a few studies have been conducted on lianas. In this study, responses of photosynthetic capacity, the antioxidant system, and related proteins in *P. quinquefolia* to elucidate the effects of heat on this species were investigated.

Experiments were conducted at North China University of Science and Technology, China (39°37′ N; 118°37′ E). The uniform two-year-old *P. quinquefolia* plants with 10 - 15 fully expanded leaves were divided into four groups (five replicates per group), and each group was subjected to either 25 (control) 35, 40 or 45°C for 1 d (from 09:00 to 16:00 hrs). After the stress period, each group was returned to control treatment conditions for 2 d. All parameters were collected at the end of the stress period, and after the 2 d recovery period. Net photosynthetic rate (P_n), the maximum quantum yield of PSII photochemistry (F_v/F_m), actual quantum yield of PSII

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Table 1. Effects of high temperature on gas exchange and chlorophyll fluorescence parameters and malondialdehyde (MDA) content and antioxidative enzyme activity in leaves in *Parthenocissus quinquefolia.*

Temp. (°C)	P_n /mol(CO_2)/ m^2/sec[1]		Fv/Fm		Φ_{PSII}		$\Phi_{f,D}$		MDA content (μg/g[1)]		SOD (U/g[1)]		POD (U/g[1)]		CAT (μmol H_2O_2/g[1]/min[1)]	
	HS	RC	HS	RC	HS	RC	HS	RC	HS	RC	HS	RC	HS	RC	HS	RC
25	9.7 ± 0.2a	9.7 ± 0.2a	0.814± 0.00219a	0.815± 0.00176a	0.706± 0.00265a	0.692± 0.00666b	0.103± 0.00821b	0.109± 0.00793b	2.2± 0.04d	2.2± 0.05c	550± 7d	551± 4c	1184± 18d	1167± 22b	0.57± 0.026d	0.55± 0.02b
35	9.2 ± 0.5a	9.6 ± 0.2a	0.809± 0.00306a	0.816± 0.00088a	0.632± 0.00493b	0.706± 0.00321a	0.125± 0.00809b	0.102± 0.00843b	2.6± 0.02c	2.2± 0.04c	582± 2c	561± 1b	1580± 15c	1190± 19b	0.76± 0.012c	0.58± 0.01b
40	8.6 ± 0.2a	9.5 ± 0.3a	0.798± 0.00318b	0.814± 0.00353a	0.531± 0.00606c	0.677± 0.00328bc	0.135± 0.00260b	0.122± 0.00756b	2.9± 0.03b	2.4± 0.02b	620± 4a	569± 2b	1695± 14b	1226± 22b	0.99± 0.012b	0.75± 0.01a
45	6.4 ± 0.2b	7.4 ± 0.1b	0.780± 0.00410c	0.802± 0.00296b	0.362± 0.00227d	0.550± 0.00561c	0.346± 0.00812a	0.182± 0.00487a	3.4± 0.03a	2.8± 0.05a	602± 3b	583± 2a	1913± 5a	1320± 10a	1.16± 0.023a	0.80± 0.02a

Each value represents the mean ± S.E. of five replicates. Different letters on error bars indicate significant differences ($p < 0.05$).

photochemistry (Φ_{PSII}), and non-regulated thermal energy loss ($\Phi_{f,D}$) were measured and calculated according to Lazár (2015). Malondialdehyde (MDA) content, and POD, SOD, and CAT activities were measured as described by Chen *et al.* (2017). The identification of proteins was based on multiple reaction monitoring (MRM) (Wang *et al.* 2016). All data were expressed as means ± standard errors of five replicates. One-way ANOVA was performed using the SPSS version 19.0 software (IBM, Chicago, IL, USA) and Duncan's multiple comparison ($p < 0.05$) test was used to determine significant differences among means.

Table 2. Effect of high temperature on relative protein abundance of D1, D2 and DegPs and FtsHs protease families in leaves of *Parthenocissus quinquefolia.*

Protein accession	Fold-change		Plant species	Protein description
	HS	RC		
O22609	1.4592	1.1701	*A. thaliana*	Protease Do-like 1, chloroplastic GN=DEGP1 PE=1 SV=2
Q0ZJ40	1.0339	1.067	*V. vinifera*	Photosystem II protein D1 GN=psbA PE=3 SV=1
Q39102	1.1805	0.8091	*A. thaliana*	ATP-dependent zinc metalloprotease FTSH 1, chloroplastic GN=FTSH1 PE=1 SV=2
Q39444	1.1805	0.8091	*C. annuum*	ATP-dependent zinc metalloprotease FTSH, chloroplastic (Fragment) GN=FTSH PE=2 SV=1
Q655S1	1.0952	0.6005	*O. sativa. japonica*	ATP-dependent zinc metalloprotease FTSH 2, chloroplastic GN=FTSH2 PE=3 SV=1
Q8W585	1.0925	0.569	*A. thaliana*	ATP-dependent zinc metalloprotease FTSH 8, chloroplastic GN=FTSH8 PE=1 SV=1
Q9FH02	1.1805	0.8091	*A. thaliana*	ATP-dependent zinc metalloprotease FTSH 5, chloroplastic GN=FTSH5 PE=1 SV=1

HS and RC represent high temperature stress and recovery, respectively.

With increasing temperature, leaf P_n and the Φ_{PSII} decreased (Table 1). This indicated that the photosynthetic capacity and photosynthetic efficiency of PSII reaction centers were negatively affected by high temperature stress (Feng *et al.* 2014, Haque *et al.* 2014). The decrease in F_v/F_m was slight at 35 and 40°C but significant at 45°C (4.18%). This indicated that significant inhibition occurred at PSII reaction centers at 45°C. Moreover, the $\Phi_{f,D}$ showed an increasing trend with increasing temperature. This indicated that photodamage are still inevitable when ambient temperature exceeds the suitable temperature range for plant growth (Osório *et al.* 2013). During recovery, chlorophyll fluorescence parameters were restored to pre-stress levels at temperatures lower than 45°C, which indicated that the PSII activity of *P. quinquefolia* tolerated 40°C.

High temperature induced membrane-lipid peroxidation damage in *P. quinquefolia* and increased antioxidant enzyme activity, which helped plants to overcome oxidative stress. Interestingly, SOD content showed an initial increase followed by a decrease with increasing temperature (Table 1), thus suggesting that the synthesis of H_2O_2 was inhibited and that the 45°C temperature treatment exceeded the capacity of the antioxidant system of *P. quinquefolia* (Sgobba *et al.* 2015).

During the stress period, a significant increase was observed in DegP1, FtsH, FtsH1, and FtsH5 levels (Table 2). This indicated that the turnover rate of D1 protein was enhanced and that DegP1 played a major role in D1 turnover for the repair of PSII (Li *et al.* 2017). FtsH, FtsH1,

FtsH2, FtsH5 and FtsH8 levels decreased significantly upon stress relief compared to those under stress, indicating that the turnover rate of D1 protein decreased. It is inferred that D1 protein turnover did not play a major role at this stage.

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