Pak. J. Bot., 52(6): 1965-1971, 2020. DOI: http://dx.doi.org/10.30848/PJB2020-6(40)

SALIX VIMINALIS MALES MAINTAIN HIGHER PHOTOSYNTHETIC CAPACITY THAN FEMALES UNDER HIGH TEMPERATURE STRESS

FEI-FEI ZHAI[1,2], YUN-XING ZHANG[1], HAI-DONG LI[1], JIN-MEI MAO[2,3], ZHEN-JIAN LI[2], JUN-XIANG LIU[2], YONG-QIANG QIAN[2], LEI HAN[2], ZHEN-YUAN SUN[2*]

[1]*School of Architectural and Artistic Design, Henan Polytechnic University, Jiaozuo 454000, P.R. China*
[2]*Research Institute of Forestry, Chinese Academy of Forestry; Key Laboratory of Tree Breeding and Cultivation, State Forestry Administration, Beijing 100091, P.R. China*
[3]*Research Institute of Economic Forest, Xinjiang Academy of Forestry, Urumqi 830063, P.R. China*
[*]*Corresponding author's email: lkyszy@126.com*

Abstract

Sexual-specific photosynthetic responses to high temperature stress were investigated in the dioecious species, *Salix viminalis*. The males and females of *S. viminalis* were cultivated for 7 weeks in greenhouse, afterwards they were processed to high temperature in climate box. Half of the plants were exposed to 40ºC/30ºC (day/night temperature) which increased stepwise (5ºC every 48 h), while the other half were maintained at 25ºC /15ºC served as a control. Seven days later, photosynthetic physiological parameters in leaves of *S. viminalis* were analysed. The high temperature treatment caused significant reduction of net photosynthetic rate (P_N) in *S. viminalis*, but male plants showed obviously higher P_N than female plants. The F_v/F_m value was relatively stable and no obvious difference was detected between male and female plants, indicating the photosynthetic light reaction was not inhabited and both male and female plants possess functional PS II under high temperature. In photosynthetic carbon cycle, the activity of Rubisco declined and the higher activity of the enzyme in males than females revealed that inhibition of photosysthesis occurred and the photosysthesis in males were less inhibited under high temperature. In addition, Carotenoids (Car) content increased and the content of male plants was higher than that of females, which stated that the high temperature could cause photoprotective response and the photoprotection ability were stronger for males. These findings suggest that male plants of *S. viminalis* could maintain higher photosynthetic capacity under high temperature stress, thus male plants should be selected when breeding heat-resistance species according to photosynthetic parameters.

Key words: Chlorophyll fluorescence, Gas exchange, High temperature, Photosynthetic pigment, Sexual differences.

Introduction

It is essential to have a suitable environment for plant in its life cycle, but they are commonly subjected to diversiform adverse environment in nature, such as extreme temperature, drought, waterlogging, salinity and heavy mental (He *et al*., 2017; Szymańska *et al*., 2017). Among adverse environments, the constantly increasing environment temperature has been regarded as one of the most harmful stress (Hasanuzzaman *et al*., 2013). Because of the greenhouse effect, global temperature shows an uptrend. It is predicted that the global temperature may rise by 0.85ºC over the period 1880-2012, which will lead to the temperature at the end of twenty-first century 1.5ºC higher than the value before industrial revolution (Anon., 2013). Hence, it is likely that plants will experience more high temperature stress.

Negative implications on morphology, physiology, biochemistry and molecular metabolisms would be brought about in plant under high temperature stress, such as perturbation of membrane integrity, protein denaturation and aggregation, increased fluidity of membrance lipids (Wahid *et al*., 2007; Mathur *et al*., 2018). Photosynthesis is the basis of plant growth and development, and it is considered the most sensitive cell function under high temperature which is often suppressed before other cell functions are impaired (Chen *et al*., 2017). The reduction of photosynthetic rate can be caused by several reasons. It is reported that the stomatal closure can repress photosynthesis by stomatal restriction and no-stomatal restriction (Higuchi *et al*., 1999; Chen *et al*., 2014). When both stomatal conductance (g_s) and intracellular CO_2 concentration (C_i) decline, stomatal restriction plays a major role; however, when C_i increases in spite of decreasing g_s, non-stomatal restriction is dominant (Xu, 1997; Yang *et al*., 2011; Neves *et al*., 2019). Moreover, photosystem Ⅱ (PS Ⅱ) located in thylakoid membranes is highly susceptible to high temperature stress and the decreases of photosynthesis could probably be due to the inhibition of photosystem Ⅱ (PSⅡ) (Havaux, 1993; Vivitha *et al*., 2018). It has been confirmed that chlorophyll fluorescence is intricately linked to multiple processes occurring in PS II in the energy transfer process of turning light energy into steady chemical energy, and it has been regarded as a prominent indicator expressing high temperature stress (Mathur *et al*., 2014; Jedmowski & Brüggemann, 2015).

In Calvin cycle, the primary CO_2 fixation enzyme is Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco). The quantity and kinetic properties of the enzyme may be a critical regulatory process inhibiting photosynthesis (Adachi *et al*., 2013; Rotundo *et al*., 2019). Besides, Chlorophyll and carotenoids (Car) are the green photosynthetic pigments in plant's chloroplast. The high temperature usually reduced the amount of photosynthetic pigments and the decrease of them is taken for a premonitor of perceiving stress signals (Liu *et al*., 2013; Evlard *et al*., 2014; He *et al*., 2017). Among the pigments, Car serves for a precursor of signal transduction in both abiotic and biotic stress, and Car is necessary for photoprotection of photosynthesis (Ashraf & Harris, 2013).

Dioecious plants are a significant component of terrestrial ecosystem, and they dominate many ecosystems. They account about 6% angiosperm species (14, 620 of 240, 000) and distribute over 959 genuses of anthophytes (Renner & Ricklefs, 1995). Sexual differences of dioecious plants have been a research hotspot since Darwin's time (Boecklen *et al*., 1990; Correia & Barradas, 2000; Li *et al*., 2013). Because resource requirements vary differently between male and female plants related to reproduction, the two sexes may behave differently in response to biotic stresses (Dawson & Ehleringer, 1993; Chen *et al*., 2010). Past researches revealed that male aspen are more tolerance to drought, salinity and chilling (Chen *et al*., 2010; Zhang *et al*., 2010a, 2010b; Peng *et al*., 2012), but female plants of *Salix myrsinifolia* are more UV-B tolerant (Randriamanana *et al*., 2015). However, sex-specific replies of dioecious plants to high temperature are less well documented.

Salix viminalis L, which is a dioecious species, pertains to the family Salicaceae. Owing to fast initial growth, large biomass, perennial habit and favourable environmental credentials, *S. viminalis* has been bred as energy-oriented species in European (Lee, 2012; Berlin *et al*., 2014). Thus, breeding new varieties with fast growth rate and strong resistance to adversity conditions is of important significance. *S. viminalis* is mainly distributed in cool areas, and high temperature may a reason affecting its survival and distribution. Therefore, clarifying the sexual differences under high temperature is useful to breed new variety and expand the growing area of this species. In this research, male and female plants of *S. viminalis* were subjected to high temperature with the purposes of: (1) elucidating the photosynthetic changes for both male and female plants under high temperature and (2) identifying which sex possess stronger ability of high temperature resistance.

Materials and Methods

Plant material: We applied pair-wise sampling method to collect cuttings of *S. viminalis* from 40 different trees sampled from 20 sites in Saihanwula national nature reserve zone (44°14′ N, 118°20′ E), including 20 males and 20 females. We chose pairs of male and female plants with similar feature and the distance was less than 2 m, so the heterogeneity in soil, light and water content can be reduced. Moreover, each pair was at least 50 m apart in order to ensure they pertain to different genotypes. After the cuttings had been carried back to Beijing, they were propagated in 8 cm × 10 cm nutrition bowls filled with 70% peat and 30% perlite in greenhouse at once. After sprouting and growing for 35 days, ten pairs of male and female plants were chosen and transplanted in 14 cm × 17 cm plastic pots.

Experimental design: Two weeks later, plants were transferred in climate box (RXZ-500D, Ningbo Jiangnan Instrument Factory) to acclimate for a week, with a relative humidity of 70% and a maximum irradiance of 300 μmol m^{-2} s^{-1}. The daytime temperature was 25ºC with 12 h, while the darkness temperature was 15ºC with 12 h. Then temperature rose step by step, 5ºC every 48 h, from 25ºC to 40ºC, and one half of the plants was exposed to 40ºC /30ºC (day/night temperature) for a week. The other half was still maintained at 25ºC /15ºC and served as a control. Three replications were made with ten pairs of male and female *S. viminalis* for treatment and control.

Measurements of photosynthetic gas exchange parameters: The portable photosynthesis equipment Li-Cor 6400XT (Li-Cor Inc. Lincoln, Nebraska, USA) was employed to record parameters of net photosynthesis rate (P_N), g_s, transpiration rate (E), and C_i on health and mature leaves located in upper of plants. The ambient conditions were set as follows: photosynthetic photon flux density (PPFD) 1000 μmol m^{-2} s^{-1}, CO_2 concentration 400 μmol mol^{-1}, leaf temperature 26ºC. Before the measurement, plants should be transported to light conditions to activate for 20 min. In addition, the leaf area was computed manually as the summation of two trapezoids.

Analysis of chlorophyll fluorescence: We inspected chlorophyll fluorescence parameter by modulated chlorophyll fluorescence imaging system (IMAGING-PAM, Heinz Walz, Germany) in the same leaves for gas exchange parameters. The minimum fluorescence yield of the dark adaptive state (F_o) and maximum fluorescence yield of the dark adaptive (F_m) were measured respectively by a weak measuring light and a 0.8 s saturated pulse light after 30 min dark adaptation. We calculated the maximal quantum yield of PSII photochemistry by the ratio of F_v to F_m ($F_v = F_m – F_o$).

Determination of pigment content: The leaves, which were disposed of main vein, were sheared to patches of 0.25 cm^2. Then 0.20 g patches were soaked in test tubes with 15 ml 80% acetone (volume ratio) for one day at 25 ºC in dark condition. We read the absorbance at 663 nm, 645 nm and 470 nm in ultraviolet spectrophotometer, and the concentrations of Chlorophyll a (Chl a), Chlorophyll b (Chl b), and Car were computed based on the absorbance.

Assays of Rubisco activity: About 0.30 g frozen leaves were powered by liquid nitrogen and were extracted in 0.01 mol L^{-1} phosphate buffer (pH 7.4). After centrifugation with a speed of 12, 000 r min^{-1} at 4ºC for 10 min, the supernatant were collected to test Rubisco activities by adding reagents of enzyme-linked immunoassay (ELISA) kit (Beijing Dongge Weiye science and Technology Co., Ltd.). The absorbance at 450 nm was detected to calculate Rubisco activity.

Statistical analysis: The data obtained from the research were statistically processed with statistical software SPSS 18.0 (SPSS Inc., Chicago, IL, USA) and the data were showed as the average ± SD. One-way analysis of variance (ANOVA) was operated to estimate the significant influence of temperature and sex. Significant differences among mean values at the 0.05 level of significance were analyzed by Duncan's multiple range tests. The histogram was drawn in Origin 8.0 (OriginLab Corporation, Northampton, Massachusetts, USA).

Results

Sexual differences in photosynthetic gas exchange parameters: No obvious differences were detected in gas exchange parameters at normal temperature. However, under high temperature stress, P_N, g_s and E decreased by 44.84%, 43.24% and 30.11% in males, and 54.43%, 50.74% and 38.96% in females, respectively. Moreover, males behaved significantly higher values of P_N, g_s and E than females under high temperature, while no significant difference of C_i was found between males and females (Table 1).

Sexual differences in chlorophyll fluorescence: Both male and female plants of *S. viminalis* displayed lower F_v/F_m values under high temperature, but the decreasing rate was not significant. Besides, male and female plants showed no remarkable differences both under normal and high temperature conditions (Fig. 1).

Sexual differences in pigment contents: No obvious differences in pigment contents were discovered among male and female plants of *S. viminalis* under normal condition. Under high temperature stress, the Chl b and Car contents of *S. viminalis* had increased by 22.71% and 27.24% in males and 16.09% and 22.28% in females separately, though Chl a contents had no significant changes. Moreover, the male plants behaved obviously more Car contents than that of female plants in high temperature condition (Table 2).

Sexual differences in Rubisco activity: The Rubisco activities of *S. viminalis* plants were affected by high temperature and it declined by 31.52% in males and 39.71% in females. Furthermore, the Rubisco activities of males were significantly higher than females under both normal and high temperature conditions (Fig. 2).

Table 1. Net photosynthesis rate (P_N), stomatal conductance (g_s), intracellular CO_2 concentration (C_i), and transpiration rate (E) in sexual plants of *S. viminalis* as affected by temperature. Each value is presented as the average (± SD) of three replicates and each repeat contains ten plants.

Temperature (°C / °C)	Sex	P_N (µ mol m^{-2} s^{-1})	g_s (mol(H_2O)m^{-2} s^{-1})	C_i (µmol(CO_2) mol^{-1})	E (mmol(H_2O)m^{-2} s^{-1})
25/15	Male	13.63 ± 0.81^a	0.32 ± 0.06^a	299.26 ± 3.46^a	5.38 ± 0.27^a
	Female	13.47 ± 1.05^a	0.33 ± 0.05^a	301.37 ± 7.96^a	5.39 ± 0.53^a
40/30	Male	7.52 ± 0.76^a	0.18 ± 0.01^a	301.87 ± 7.53^a	3.76 ± 0.35^a
	Female	6.14 ± 0.66^b	0.16 ± 0.01^b	312.71 ± 4.96^a	3.29 ± 0.28^b

The different superscript lowercase letter in the same column indicated obvious differences in male and female plants at normal or high temperature at $p<0.05$ level

Table 2. Chlorophyll a (Chl a), Chlorophyll b (Chl b) and Carotenoids (Car) contents in sexual plants of *S. viminalis* as affected by temperature. Each value is presented as the average (± SD) of three replicates and each repeat contains ten plants.

Temperature(°C / °C)	Sex	Chl a (mg g^{-1})	Chl b (mg g^{-1})	Car(mg g^{-1})
25/15	Male	8.62 ± 0.64^a	1.77 ± 0.17^a	1.75 ± 0.21^a
	Female	8.52 ± 0.77^a	1.77 ± 0.11^a	1.71 ± 0.15^a
40/30	Male	8.90 ± 0.17^a	2.17 ± 0.20^a	2.23 ± 0.26^a
	Female	8.50 ± 0.05^a	2.05 ± 0.21^a	2.09 ± 0.24^b

The different superscript lowercase letter in the same column indicated obvious differences in male and female plants at normal or high temperature at $p<0.05$ level

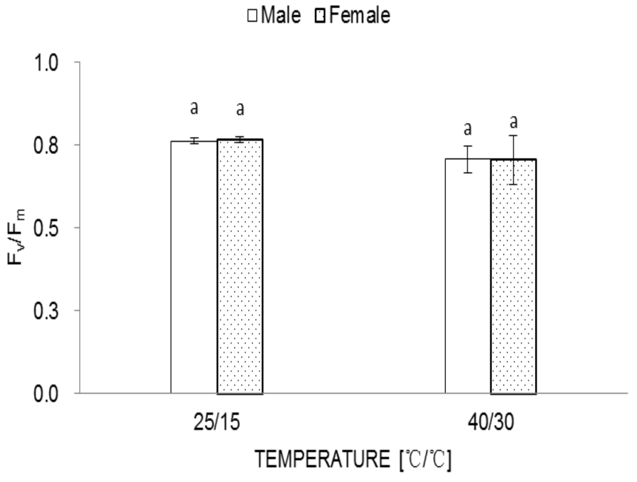

Fig. 1. Maximum photochemical efficiency of PSII (F_v/F_m) in sexual plants of *S. viminalis* under normal and high temperature conditions. Data are the average (± SD) of three replicates and each repeat contains ten plants. The same small letter indicates no obvious difference in the male and female plants under the same temperature at $p>0.05$ level.

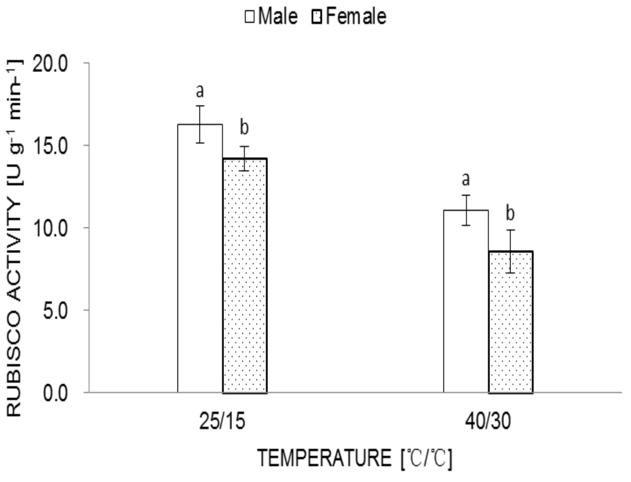

Fig. 2. Rubisco activities in sexual plants of *S. viminalis* under normal and high temperature conditions. Data are the average (± SD) of three replicates and each repeat contains ten plants. The different small letter indicates obvious difference in the male and female plants under the same temperature at $p<0.05$ level.

Discussion

Photosynthesis is one of the susceptive processes to heat force and photosynthetic rate always decline under the stress (Wahid *et al*., 2007). In our study, high temperature led to reduction of P_N for *S. viminalis*, while the males maintained higher P_N than females (Table 1). Meanwhile, the g_s decreased while C_i was stable (Table 1), which implies the occurrence of non-stomatal restriction under high temperature. Moreover, lower P_N of females than males might also be duo to non-stomatal restriction because of no significant differences in C_i between two genders. Our results corresponded with the earlier studies about tomato cultivars (Camejo *et al*., 2005), citrus species (Guo *et al*., 2006), *Vitis amurensis* (Luo *et al*., 2011), *Vaccinium corymbosum* (Hao *et al*., 2019) and *Euterpe oleracea* (Neves *et al*., 2019) that non-stomatal restriction was detected under high temperature. However, stomatal restriction was reported in *Pyrus pyrifolia* (Liu *et al*., 2013) and *Populus simonii* (Song *et al*., 2014) accompanied by decreased g_s and C_i, while the effects of stomatal restriction and non-stomatal restriction were detected simultaneously in *Annona cherimola* (Higuchi *et al*., 1999).

The chlorophyll fluorescence parameter F_v/F_m, which is considered as an effective indicator to assess the photo-inhibitor or other injury caused to the PS II complexes, expresses the maximum photochemical quantum efficiency of PSII (Bertholdsson, 2013; Sharma *et al*., 2015; Vivitha *et al*., 2018). Many studies have shown that chlorophyll fluorescence parameter F_v/F_m decreased under stressful conditions (Prieto *et al*., 2009; Liu *et al*., 2013; Li *et al*., 2015; Sharma *et al*., 2015). But in our study, the F_v/F_m value of *S. viminalis* was relatively stable (Fig. 1), reflecting the stability of thylakoid structure and electron flow through the photosystems, which indicated that photosynthetic light reaction was not inhabited under high temperature. This result is consistent with previous study that willow and poplar genotypes under heavy mental stress possess relatively stable F_v/F_m value (Pajević *et al*., 2009). In addition, parameter F_v/F_m of the two sexes showed no significant difference (Fig. 1), indicating both male and female plants possess functional PS II under high temperature.

Rubisco is a crucial enzyme being responsible for CO_2 fixation in photosynthetic process. The complicated enzyme kinetics and some unusual properties of this enzyme often cause change of P_N to extreme temperature (Mathur *et al*., 2014), while deactivation of Rubisco is caused by loss of rubisco activase activity (Kim & Portis, 2005). The high temperature often decreases the activity of Rubisco which would lead to inhibition of photosysthesis (Xue *et al*., 2011; Shan *et al*., 2015; Perdomo *et al*., 2017). In our research, the activity of Rubisco reduced dramatically whereas the enzyme activity in male plants was significantly higher than the females (Fig. 2). It indicated that inhibition of photosysthesis occurred and the photosysthesis of males were less inhibited under high temperature. In general, Rubisco activity would cause photosynthetic rate decreased and also lead to photosynthetic difference between male and female plants. The results of Rubisco also confirm that non-stomatal restriction plays an important role in reduction of P_N for *S. viminalis*.

Chlorophyll, which includes Chl a, Chl b and Car, is an important pigment involved in photosynthesis in plant chloroplasts (Shanmugam *et al*., 2013). Chl a can turn optical energy into chemical energy, and Chl b is to be pivotal in collecting and transferring optical energy. Car can absorb surplus energy and quench singlet oxygen consequently to prevent membrane lipid peroxidation and take effect in protecting chlorophyll and photosynthetic function. High temperature is known to cause Chlorophyll degradation which might be due to either decreasing synthetic rate or increasing decomposition (Tewari & Tripathy, 1998; Crafts-Brandner & Salvucci, 2000; Djanaguiraman *et al*., 2014). However, Chl a content was little changed and Chl b content increased in *S. viminalis* (Table 2). It was suggested that photosynthetic pigments could continue synthesize under high temperature environment (Mlinarić *et al*., 2016) and the decline of photosynthesis would not cause by pigments. The contents of Car increased under high temperature (Table 2), which indicated that high temperature could cause photoprotective response of *S. viminalis* to avoid photooxidation (Mlinarić *et al*., 2016). This is agreement with previous reports of *Sorghum bicolor* (Gosavi *et al*., 2014) and *Cicer arietinum* (Pareek *et al*., 2019) that tolerant cultivars showed increased or less reduction of Car contents. In addition, the Car content of male *S. viminalis* plants was higher than female plants, stating that male plants processed higher capacity of photoprotection replying to high temperature.

Differences of morphology, physiology and ecology characteristics in the two genders of dioecious plants always exist, which might be attributed to different resources demands or different tolerance to environment stress (Dawson & Ehleringer, 1993; Marshall *et al*., 1993; Ueno *et al*., 2006; Zhang *et al*., 2010b). Researches have showed that sexual differences are species-specific. The male plants of aspen could behave stronger defense capability in drought, chilling, salinity and heavy mental stresses (Zhang *et al*., 2010a, 2010b; Chen *et al*., 2010, 2013). However, the female plants of *Baccharis dracunculifolia* (Espírito-Santo *et al*., 2003), *Hippophae Rhamnoides subsp. Sinensis* (Gao *et al*., 2010) and *Salix myrsinifolia* (Randriamanana *et al*., 2015) presented better physiological regulation ability and adaptability under stressful environment. In this research, we found that males of *S. viminalis* could maintain higher photosynthetic capacity under high temperature in view of higher P_N, Rubisco activity and Car content. It is consistent with previous research of *S. viminalis* that female plants experience more severe *Melampsora* spp. infections both in field experiment and literature survey (Moritz *et al*., 2016). But in our recent study, no significant differences were detected between male and female plants of *S. viminalis* under flooding treatment (Zhai *et al*., 2020). The inconsistent results might be due to that *S. viminalis* was riparian species and the two sexes co-exist in riparian zones, so the differences in flooding resistance between sexes was not obvious. The male and female plants might perform differently to different stress and the mechanisms behind dioecious plants responding to adversity remain unclear, so future studies are needed especially investigation of molecular mechanism.

Conclusion

In this study, we found that the photosynthetic physiological parameters in male and female plants of *S. viminalis* were influenced by high temperature. The high temperature treatment caused significant reduction of net photosynthetic rate (P_N) in *S. viminalis*, but male plants showed obviously higher P_N than female plants. The decreased g_s, stable C_i and declined Rubisco activity indicated that non-stomatal restriction plays an important role in the reduction of P_N. Chl a content was little changed while Chl b and Car content increased, suggesting that photosynthetic pigments could continue synthesize under stressful environment. The relatively stable F_v/F_m value of *S. viminalis* indicated no damage of PS II under high temperature stress. Besides, higher activity of Rubisco and Car content in males revealed less inhibited photosysthesis and stronger photoprotection ability for male plants respectively. These findings suggest that male plants of *S. viminalis* could maintain higher photosynthetic capacity under high temperature stress, thus male plants should be selected when breeding heat-resistance species according to photosynthetic parameters. This study increased understanding of the adaptation mechanism of *S. viminalis* to high temperature, and supplied theoretical basis for breeding excellent varieties.

Acknowledgements

The results of this research presented here were financially supported by the National Natural Science Foundation of China (31700533), the Central Public-interest Scientific Institution Basal Research Fund (CAFYBB2018ZB002), the Key Technologies R & D Program of Henan Province (182102310052 and 192102310307), and the Doctoral Scientific Fund Project of Henan Polytechnic University (B2017-40).

References

Adachi, S., T. Nakae, M. Uchida, K. Soda, T. Takai, T. Oi, T. Yamamoto, T. Ookawa, H. Miyake, M. Yano and T. Hirasawa. 2013. The mesophyll anatomy enhancing CO_2 diffusion is a key trait for improving rice photosynthesis. *J. Exp. Bot.*, 64(4): 1061-1072.

Anonymous. 2013. Intergovernmental Panel on Climate Change (IPCC). Summary for policymakers. Cambridge University Press, Cambridge, UK.

Ashraf, M. and P.J.C. Harris. 2013. Photosynthesis under stressful environments: an overview. *Photosynthetica*, 51(2): 163-190.

Berlin, S., S.O. Trybush, J. Fogelqvist, N. Gyllenstrand, H.R. Hallingbäck, I. Åhman, N-E. Nordh, I. Shield, S.J. Powers, M. Weih, U. Lagercrantz, A-C. Rönnberg- Wästljung, A. Karp and S. Hanley. 2014. Genetic diversity, population structure and phenotypic variation in European *Salix viminalis* L. (Salicaceae). *Tree Genet. Genom.*, 10(6): 1595-1610.

Bertholdsson, N.O. 2013. Screening for barley waterlogging tolerance in nordic barley cultivars (*Hordeum vulgare* L.) using chlorophyll fluorescence on hydroponically- grown plants. *Agron.*, 3(2): 376-390.

Boecklen, W.J., P.W. Price and S. Mopper. 1990. Sex and drugs and herbivores: sex-biased herbivory in arroyo willow (*Salix Lasiolepis*). *Ecology*, 71(2): 581-588.

Camejo, D., P. Rodríguez, M.A. Morales, J.M. Dell'Amico, A. Torrecillas and J.J. Alarcón. 2005. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *J. Plant Physiol.*, 162(3): 281-289.

Chen, F., L. Chen, H. Zhao, H. Korpelainen and C. Li. 2010. Sex-specific responses and tolerances of *Populus cathayana* to salinity. *Physiol. Plantarum*, 140(2): 163-173.

Chen, L., L. Wang, F. Chen, H. Korpelainen and C. Li. 2013. The effects of exogenous putrescine on sex-specific responses of *Populus cathayana* to copper stress. *Ecotox. Environ. Safe.*, 97(1): 94-102.

Chen, W.L., W.J. Yang, H.F. Lo and D.M. Yeh. 2014. Physiology, anatomy, and cell membrane thermostability selection of leafy radish (*Raphanus sativus* var. *oleiformis* Pers.) with different tolerance under heat stress. *Sci. Hort.*, 179: 367-375.

Chen, Y.E., C.M. Zhang, Y.Q. Su, J. Ma, Z.W. Zhang, M.Yuan, H.Y. Zhang and Y. Sun. 2017. Responses of photosystem Ⅱ and antioxidative systems to high light and high temperature co-stress in wheat. *Environ. Exp. Bot.*, 135: 45-55.

Correia, O. and M.C.D. Barradas. 2000. Ecophysiological differences between male and female plants of *Pistacia lentiscus* L. *Plant Ecol.*, 149(2): 131-142.

Crafts-Brandner, S.J. and M.E. Salvucci. 2000. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. *P. Natl. Acad. Sci. USA*, 97(24): 13430-13435.

Dawson, T.E. and J.R. Ehleringer. 1993. Gender-specific physiology, carbon isotope discrimination, and habitat distribution in boxelder, *Acer negundo*. *Ecology*, 74(3): 798-815.

Djanaguiraman, M., P.V.V. Prasad, M. Murugan, R. Perumal and U.K. Reddy. 2014. Physiological differences among sorghum (*Sorghum bicolor* L. Moench) genotypes under high temperature stress. *Environ. Expe. Bot.*, 100: 43-54.

Espírito-Santo, M.M., B.G. Madeira, F.S. Neves, M.L. Faria, M. Fagundes and G.W. Fernandes. 2003. Sexual differences in reproductive phenology and their consequences for the demography of *Baccharis dracunculifolia* (Asteraceae), a dioecious tropical shrub. *Ann. Bot.*, 91(1): 13-19.

Evlard, A., K. Sergeant, B. Printz, C. Guignard, J. Renaut, B. Campanella, R. Paul and J.F. Hausman. 2014. A multiple-level study of metal tolerance in *Salix fragilis* and *Salix aurita* clones. *J. Proteom.*, 101: 113-129.

Gao, L., J. Yang and R. Liu. 2010. Leaf morphological structure and physiological and biochemical characteristics of female and male *Hippophae Rhamnoides* subsp. *sinensis* under different soil moisture condition. *Chin. J. Appl. Ecol.*, 21(9): 2201-2208.

Gosavi G.U., A.S. Jadhav, A.A. Kale, S.R. Gadakh, B.D. Pawar and V.P. Chimote. 2014. Effect of heat stress on proline, chlorophyll content, heat shock proteins and antioxidant enzyme activity in sorghum (*Sorghum bicolor*) at seedlings stage. *Ind. J. Biotechnol*., 13: 356-363.

Guo, Y.P., H.F. Zhou and L.C. Zhang. 2006. Photosynthetic characteristics and protective mechanisms against photooxidation during high temperature stress in two citrus species. *Sci. Hort.*, 108(3): 260-267.

Hao, L., L. Guo, R. Li, Y. Cheng, L. Huang, H. Zhou, M. Xu, F. Li, X. Zhang and Y. Zheng. 2019. Responses of photosynthesis to high temperature stress associated with changes in leaf structure and biochemistry of blueberry (*Vaccinium corymbosum* L.). *Sci. Hort. Amsterdam,* 246: 251-264.

Hasanuzzaman, M., K. Nahar, M.M. Alam, R. Roychowdhury and M. Fujita. 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int. J. Mol. Sci.*, 14(5): 9643-9684.

Havaux, M. 1993. Rapid photosynthetic adaptation to heat stress triggered in potato leaves by moderately elevated temperatures. *Plant Cell Environ.*, 16(4): 461-467.

He, B., T. Guo, H. Huang, W. Xi and X. Chen. 2017. Physiological responses of *Scaevola aemula* seedlings under high temperature stress. *S. Afr. J. Bot.,* 112: 203-209.

Higuchi, H., T. Sakuratani and N. Utsunomiya. 1999. Photosynthesis, leaf morphology, and shoot growth as affected by temperatures in cherimoya (*Annona cherimola* Mill.) trees. *Sci. Hort.*, 80(1): 91-104.

Jedmowski, C. and W. Brüggemann. 2015. Imaging of fast chlorophyll fluorescence induction curve (OJIP) parameters, applied in a screening study with wild barley (*Hordeum spontaneum*) genotypes under heat stress. *J. Photochem. Photobiol*., B, 151: 153-160.

Kim, K. and A.R.Jr. Portis. 2005. Temperature dependence of photosynthesis in *Arabidopsis* plants with modifications in rubisco activase and membrane fluidity. *Plant Cell Physiol.*, 46(3): 522-530.

Lee, S.L. 2012. Phenotypic variation of *Salix viminalis* in well-watered and drought conditions. Master, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Li, H., G.C. Zhang, H.C. Xie, K. Li and S.Y. Zhang. 2015. The effects of the phenol concentrations on photosynthetic parameters of *Salix babylonica* L. *Photosynthetica,* 53(3): 430-435.

Li, L., Y. Zhang, J. Luo, H. Korpelainen and C. Li. 2013. Sex-specific responses of *Populus yunnanensis* exposed to elevated CO_2 and salinity. *Physiol. Plantarum*, 147(4): 477-488.

Liu, D.F., D. Zhang, G.Q. Liu, S. Hussain and Y.W. Teng. 2013. Influence of heat stress on leaf ultrastructure, photosynthetic performance, and ascorbate peroxidase gene expression of two pear cultivars (*Pyrus pyrifolia*). *J. Zhejiang Univ. Sci. B*, 14(12): 1070-1083.

Luo, H.B., L. Ma, H.F. Xi, W. Duan, S.H. Li, W. Loescher, J.F. Wang and L.J. Wang. 2011. Photosynthetic responses to heat treatments at different temperatures and following recovery in grapevine (*Vitis amurensis* L.) leaves. *PLoS One*, 6(8): e23033.

Marshall, J.D., T.E. Dawson and J.R. Ehleringer. 1993. Gender-related differences in gas exchange are not related to host quality in the xylem-tapping mistleoe, *Phoradendron juniperinum* (Viscaceae). *Amer. J. Bot.,* 80(6): 641-645.

Mathur, S., D. Agrawal and A. Jajoo. 2014. Photosynthesis: response to high temperature stress. *J. Photoch. Photobiol. B: Biol.*, 137(8): 116-126.

Mathur, S., M.P. Sharma and A. Jajoo. 2018. Improved photosynthetic efficacy of Maize (*Zea mays*) plants with Arbuscular Mycorrhizal Fungi (AMF) under high temperature stress. *J. Photoch. Photobiol. B.*, 180: 149-154.

Mlinarić, S., J.A. Dunić, I. Štolfa, V. Cesar and H. Lepeduš. 2016. High irradiation and increased temperature induce different strategies for competent photosynthesis in young and mature fig leaves. *S. Afr. J. Bot.*, 103: 25-31.

Moritz, K.K., C. Björkman, A.L. Parachnowitsch and J.A. Stenberg. 2016. Female *Salix viminalis* are more severely infected by *Melampsora* spp. but neither sex experiences associational effects. *Ecol. Evol*., 6(4): 1154-1162.

Neves, L.H., R.I.N. Santos, G.I. dos S. Teixeira, D.G. de. Araujo, W.V.D. Silvestre, H. A. Pinheiro. 2019. Leaf gas exchange, photochemical responses and oxidative damages in assai (*Euterpe oleracea* Mart.) seedlings subjected to high temperature stress. *Sci. Hort. Amsterdam,* 257: 108733.

Pajević, S., M. Borišev, N. Nikolić, B. Krstić, A. Pilipović and S. Orlović. 2009. Phytoremediation capacity of poplar (*Populus* spp.) and willow (*Salix* spp.) clones in relation to photosynthesis. *Arch. Biol. Sci*., 61(2): 239-247.

Pareek, A., D. Rathi, D. Mishra, S. Chakraborty and N. Chakraborty. 2019. Physiological plasticity to high temperature stress in chickpea: adaptive responses and variable tolerance. *Plant Sci.,* 289: 110258.

Peng, S., H. Jiang, S. Zhang, L. Chen, X. Li, H. Korpelainen and C. Li. 2012. Transcriptional profiling reveals sexual differences of the leaf transcriptomes in response to drought stress in *Populus yunnanensis*. *Tree Physiol*., 32(12): 1541-1555.

Perdomo, J.A., S. Capó-Bauçà, E. Carmo-Silva and J. Galmés. 2017. Rubisco and rubisco activase play an important role in the biochemical limitations of Photosynthesis in rice, wheat, and maize under high temperature and water deficit. *Front. Plant Sci.*, 8: 490.

Prieto, P., J. Penuelas, J. Llusia, D. Asensio and M. Estiarte. 2009. Effects of long-term experimental night-time warming and drought on photosynthesis, F_v/F_m and stomatal conductance in the dominant species of a Mediterranean shrubland. *Acta Physiol. Plant*, 31(4): 729-739.

Randriamanana, T.R., K. Nissinen, J. Moilanen, L. Nybakken and R. Julkunen-Tiitto. 2015. Long-term UV-B and temperature enhancements suggest that females of *Salix myrsinifolia* plants are more tolerant to UV-B than males. *Environ. Exp. Bot.*, 109: 296-305.

Renner, S.S. and R.E. Ricklefs. 1995. Dioecy and its correlates in the flowering plants. *Amer. J. Bot.*, 82(5): 596-606.

Rotundo, J. L., T. Tang and C.D. Messina. 2019. Response of maize photosynthesis to high temperature: Implications for modeling the impact of global warming. *Plant Physiol. Bioch.,* 141: 202-205.

Shan, T.B., M.H. Zhao, J.L. Wu and Z.J. Xu. 2015. Study on photosynthetic characteristics and Rubisco activity of rice leaves with different stomatal densities. *J. Nucl. Agri. Sci.*, 29(6): 1142-1148.

Shanmugam, S., K.H. Kjaer, C.O. Ottosen, E. Rosenqvist, S.D. Kumari and B. Wollenweber. 2013. The alleviating effect of elevated CO_2 on heat stress susceptibility of two wheat (*Triticum aestivum* L.) cultivars. *J. Agron. Crop Sci.*, 199(5): 340-350.

Sharma, D.K., S.B. Andersen, C.O. Ottosen and E. Rosenqvist. 2015. Wheat cultivars selected for high F_v/F_m under heat stress maintain high photosynthesis, total chlorophyll, stomatal conductance, transpiration and dry matter. *Physiol. Plantarum*, 153(2): 284-298.

Song, Y., Q. Chen, D. Ci, X. Shao and D. Zhang. 2014. Effects of high temperature on photosynthesis and related gene expression in poplar. *BMC Plant Biol.*, 14(1): 111.

Szymańska, R., I. Ślesak, A. Orzechowska and J. Kruk. 2017. Physiological and biochemical responses to high light and temperature stress in plants. *Environ. Exp. Bot.*, 139: 165-177.

Tewari, A.K. and B.C. Tripathy. 1998. Temperature-stress-induced impairment of chlorophyll biosynthetic reactions in cucumber and wheat. *Plant Physiol.*, 117(3): 851-858.

Ueno, N., H. Kanno and K. Seiwa. 2006. Sexual differences in shoot and leaf dynamics in the dioecious tree *Salix sachalinensis*. *Can. J. Bot.*, 84(12): 1852-1859.

Vivitha, P., M. Raveendran, C. Vijayalakshmi and D. Vijayalakshmi. 2018. Genetic dissection of high temperature stress tolerance using photosynthesis parameters in QTL introgressed lines of rice cv. Improved White Ponni. *Ind. J. Plant Physiol*., 23: 741-747.

Wahid, A., S. Gelani, M. Ashraf and M.R. Foolad. 2007. Heat tolerance in plants: an overview. *Environ. Exp. Bot.*, 61(3): 199-223.

Xu, D.Q. 1997. Some problems in stomatal limitation analysis of photosynthesis. *Plant Physiol. Commun.*, 33(4): 241-244.

Xue, W., X.Y. Li, L.S. Lin, Y.J. Wang and L. Li. 2011. Effects of short time heat stress on photosystem II, Rubisco activities and oxidative radicals in *Alhagi sparsifolia*. *Chin. J. Plant Ecol.*, 35(4): 441-451.

Yang, Y.Z., Y.X. Zhang and F.R. Peng. 2011. Effects of drought stress on photosynthetic characteristics in *Toona sinensis* seedlings from different provenances. *J. B. Fore. Univ.*, 33(1): 44-48.

Zhai, F.F., H.D. Li, S.W. Zhang, Z.J. Li, J.X. Liu, Y.Q. Qian, G.S. Ju, Y.X. Zhang, L. Liu, L. Han and Z.Y. Sun. 2020. Male and female plants of *Salix viminalis* perform similarly to flooding in morphology, anatomy, and physiology. *Forests*, 11(3): 321.

Zhang, S., F. Chen, S. Peng, W. Ma, H. Korpelainen and C. Li. 2010a. Comparative physiological, ultrastructural and proteomic analyses reveal sexual differences in the responses of *Populus cathayana* under drought stress. *Proteomics*, 10(14): 2661-2677.

Zhang, S., H. Jiang, S. Peng, H. Korpelainen and C. Li. 2010b. Sex-related differences in morphological, physiological, and ultrastructural responses of *Populus cathayana* to chilling. *J. Exp. Bot.*, 62(2): 675-686.

(Received for publication 21 December 2018)