DOI: 10.32615/ps.2019.099 PHOTOSYNTHETICA 57 (4): 1165-1175, 2019

Ecophysiological study of the impact of SiK® fertilization on *Castanea sativa* Mill. seedling tolerance to high temperature

A. CARNEIRO-CARVALHO[*,+], R. ANJOS[*], A. AIRES[*], T. MARQUES[**], T. PINTO[*], and J. GOMES-LARANJO[*]

Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Tras-os-Montes and Alto Douro (UTAD), Vila Real, Portugal[*]
University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal[**]

Abstract

The aim of this work was to evaluate the impact of Si fertilization on the resilience capacity of chestnut plants growing under high air temperatures and its recovery capacity after returning to optimal temperatures. *Castanea sativa* plants were supplied with 0, 5, 7.5, and 10 mM potassium silicate (SiK®) and exposed for a month at each temperature, 25, 32, and 25°C. The results demonstrated that phytoliths were accumulated in the leaf tissues, both on the cell wall and xylem vessels, suggesting their role in the plant's tolerance to heat. Under high temperature, Si fertilization in chestnut plants increased the gas exchange and the photochemical efficiency of the PSII as the increase of 50% on performance index suggests. The presence of Si also induced higher contents of photosynthetic pigments and promoted a better adaptation of chloroplasts to high temperatures. The present study suggests that the application of Si may be used to enhance the high temperature tolerance of chestnut plants.

Additional key words: chloroplast activity; fluorescence transient; heat stress; lipids; silicon.

Introduction

Agriculture is one of the main activities affected by climate change, namely by the increase in temperature, changes in precipitation, and increase in the occurrence of heat and drought waves (Das *et al*. 2016). In Portugal, eight of the ten warmest years of the last 100 years occurred during the last 20 years, with Portugal being considered a climate change hotspot (Carvalho *et al*. 2014). Until the end of the 21st century, an increase in the mean summer season temperature of about 3–7°C is expected, affecting the northern and central part of Portugal in particular (APA 2009).

Castanea sativa is classified as a thermophilic species, well adapted to ecosystems with an annual mean temperature ranging between 8–15°C and monthly mean temperatures for six months, corresponding to the vegetative cycle, over 6–8°C (Gomes-Laranjo *et al*. 2008a). It is classified as a mesophyll species of the Mediterranean temperate climates, growing preferentially in the coastal area at sea level or in mountainous area of the interior regions. In these regions, crops are found between 400 and 1,000 m (Gomes-Laranjo *et al*. 2007), where typical rainfall must be between 600–1,200 mm without a long dry season (Fernández-López and Alía 2003). This dim-light species prefers the cool north-facing slope localities with mean annual temperatures between 8 and 15°C, and daily maximum values during the vegetative cycle temperature around 27°C (Gomes-Laranjo *et al*. 2007, 2008b). In such regions, during the summer time, a combination of heat stress and water stress is more and more frequent, causing many problems in chestnut crops, which leads to a reduction in plant vigour, plant health, and consequently the reduction of chestnut production (Gomes-Laranjo *et al*. 2004, 2008b; Mota *et al*. 2014). High air temperatures may constitute one of the main restrictive factors affecting the

Received 20 March 2019, *accepted* 7 June 2019.
[+]Corresponding author; e-mail: andreiamcc_500@hotmail.com

Abbreviations: ABS/RC – light absorption flux (for PSII antenna chlorophylls) per reaction center; BSA – bovine serum albumin; Car – carotenoids; Chl – chlorophyll; Chl/Car – chlorophylls to carotenoids ratio; CS – excited cross section; DI_0/RC – dissipation energy flux per PSII reaction center; *E* – transpiration rate; EDTA – ethylenediamine tetraacetic acid; ET_0/CS – electron transport flux per cross section; ET_0/RC – maximum electron transport flux (further than Q_A^-) per PSII reaction center; Fd – ferredoxin; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; OEC – oxygen-evolving complex; OJIP – fast chlorophyll fluorescence transients; Pht – phytoliths; PI – performance index; P_N – net photosynthetic rate; PQ – plastoquinone; PVP – polyvinylpyrrolidone; Q_A – primary quinone acceptor of PSII; Q_B – secondary quinone acceptor of PSII; RC – reaction center; RC/ABS – density of reaction centers per PSII antenna chlorophyll; SiK® – potassium silicate; SM – (Area)/($F_m - F_0$), representing energy necessary for the closure of all reaction centers; TR_0/RC – flux of energy trapping per reaction center of PSII; UI – unsaturated index; WUE – water-use efficiency (= P_N/E).

Acknowledgements: The authors thanks to Mrs. Ana Fraga and Mrs. Cesaltina Carvalho for technical assistance. This work was supported by National Funds by FCT – Portuguese Foundation for Science and Technology, under the project UID/AGR/04033/2019.

photosynthetic apparatus.

The photosynthesis occurs in the chloroplasts where the photosynthetic membranes, thylakoids, are located. These membranes are responsible for the light reaction whereby light is captured and its energy converted to chemical energy in the form of ATP and NADPH concomitant with the development of oxygen. The thylakoids are composed of many membrane proteins that together with pigments, chlorophyll (Chl) and carotenoids (Car) and membrane lipids, mainly galactolipids, form a highly complex membrane system that carries out electron transport; ATP synthesis absorbs the light through the help of the pigments in a very efficient way. In addition, the thylakoids have the ability to repair and promote antioxidant protection against damage caused by light and oxidative stress (Albertsson and Âkerlund 2015).

High temperatures induce high transpiration rates, resulting in a premature stomatal closure, reducing the leaf thermoregulation capacity and affecting the metabolic activity, namely causing a strong reduction in photosynthetic rate (Gomes-Laranjo 2007). Moreover, plant tissues and organs are damaged in different ways (Larkindale *et al*. 2005a). The reduction of fruit setting was also referred by Conedera *et al*. (2016), who reported that the European chestnut species shows high sensitivity to the combination of high temperatures and scarce precipitation. Concerning diseases, the spread of ink disease (caused by *Phytophthora cinnamomi* Rands) is one of most important consequences (Ahanger *et al*. 2013).

Silicon is absorbed by root hairs through a passive uptake by a transpiration stream, being later transported as monosilic acid to the aerial part, where it is polymerized as solid amorphous silica bodies ($SiO_2.nH_2O$) called phytoliths. These bodies are not remobilized anymore (Reynolds *et al*. 2016). The phytoliths play an important structural and protective role in the plant's defence, with low energy costs, due to the increase in the rigidity and abrasiveness of the vegetal tissues, thereby forming a mechanical barrier against abiotic stresses. Similar conclusions were obtained by Carnelli *et al*. (2001) in alpine plant species and other plant communities. Moreover, phytoliths contribute to an increase in the resistance of xylem vessels to drought and heat, by increasing the water volume assimilation of plants under abiotic stress (da Silva Lobato *et al*. 2013).

Silicon (Si) is recognized as a fertilizer, biostimulating plant protection under environmental stress (Savvas and Ntatsi 2015, Latef and Tran 2016). The beneficial effect of silicon is associated with the enhanced activity of superoxide dismutase and ascorbate peroxidase (Soundararajan *et al*. 2014). On the other hand, Agarie *et al*. (1998) reported that silicon is involved in the improvement of the photosynthetic activity in plants under heat stress, avoiding the photooxidation of Chl. In addition, the amorphous bodies of Si, the phytoliths, induce anatomical changes on the cell walls of leaves, increase the stability of the membranes, reduce the oxidative damage of functional molecules, and improve the antioxidative defence ability (Kim *et al*. 2017).

The aim of the present study was to evaluate the impact of Si fertilization on the resilience capacity of chestnut growing under high air temperatures and its recovery capacity after returning to adequate temperatures.

Materials and methods

Experimental design and treatments: The experiment was performed in the growth chamber located in University of Trás-os-Montes and Alto Douro, Vila Real, Portugal. *Castanea sativa* seedlings, 2 months old, from Sousã variety, were planted in 2-L pot filled with 70% of turf and 30% of perlite and regularly watered with the same water volume. The pots were randomly distributed in four groups, with twenty replications per treatment. Subsequently, the fertilization of chestnut plants was done with 50 mL of potassium silicate (SiK®) at the concentrations of 0, 5, 7.5, and 10 mM. The solution was applied directly to the soil. The concentrations of SiK® chosen for the present study were selected according to the results of the previous study on chestnut plants published by Zhang *et al*. (2013).

The plants were left in the growth chamber at 25°C (A25) with a photoperiod of 12 h (8:00–20:00 h) for one month. After that period, the air temperature was increased to 32°C (B32) for one month. Then, the temperature was reduced back to 25°C (C25) for one month in order to study the recovery capacity of the plants. The watering of the chestnut plants was done twice a week, manually applying 150 ml of water in each pot. Leaf samples were collected for study after the month of exposure to 25°C (A25), then after the period to exposition to 32°C (B32), and finally at 25°C (C25).

Light microscopy: The cross-transversal sections of chestnut leaves were obtained from 10 mM SiK® treatments. The fragments were then placed in FAA solution (5% formaldehyde, 5% glacial acetic acid, 90% ethanol) during 48 h, and subsequently dehydrated through an ethanol series (70, 80, 90, 95, and 100%) for 1 h in each ethanol solution. Later, the samples were placed in 100% xylol for 1 h and, finally, embedded in paraffin at 60°C for 24 h. The cross sections of leaves were 5 µm thick obtained using a rotary microtome (*RMC Power Tome XL*, *Boeckeler Instruments*, *Inc*., Arizona, USA) and placed on histological slides. The samples were stained with a solution of 0.1% toluidine blue-O solution in citrate buffer (pH 0.5) developed by Ruzin (1999) for staining vegetal tissues and specifically to identify the presence of phytoliths. An *Olympus IX 51* inverted microscopy (*Olympus Optical Co.*, *GmbH*, Hamburg, Germany) connected to a camera *Olympus BX50* was used to visualize all these structures. For the analysis of the phytoliths of chestnut leaves, five replicates per treatment were made ($n = 5$).

Determination of Si content in leaves: The content of Si was analysed according to the method described by Korndörfer *et al*. (2004), through a colorimetric analysis of the alkaline digestion of 0.1 g of dry leaf tissue. The reading was performed using spectrophotometer (*Cary 100 Bio*, *Varian*, Australia) at a wavelength of 410 nm. The measurement was replicated three times per treatment ($n = 3$).

Gas-exchange measurements: For the gas-exchange measurements, a portable open system infrared gas analyser (IRGA, *LCpro+*, *Analytical Development Co®*, Hoddesdon, UK) was used to evaluate the fully expanded leaves of six plants per treatment. The gas-exchange parameters analysed were the net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E). The water-use efficiency (WUE) was determined by the ratio P_N/E according to Zhang *et al*. (2013). The measurements were made between 10:00 and 12:00 h, with a PPFD of 1,600 µmol(photon) m^{-2} s^{-1}.

Chlorophyll (Chl) fluorescence was analysed using a pulse-amplitude modulated fluorometer (*OS-30p*, *Opti-Sciences*, *Inc*., Hudson, USA) by the OJIP test protocol. In order to do so, leaf clips (*Hansatech*, UK) were applied on the plant to keep the samples in the dark prior to the measurement (30 min), according to Zhori *et al*. (2015). For high spatial resolution leaves were detached and fixed in leaf clips. Fluorescence induction kinetics was measured with high temporal resolution during a saturation pulse. Excitation intensity was 3,500 µmol(photon) m^{-2} s^{-1} with red light of 650 nm for 3 s. From the fluorescence induction signal from 10 µs to 3 s the instrument determines initial (F_0) and maximum (F_m) fluorescence and the variable fluorescence (F_v) at specified time intervals.

The OJIP is considered an important tool to evaluate the effect of stress on photosynthesis and to investigate the function of PSII and its reactions to changes in the environment (Zhang *et al*. 2013). Specific parameters were evaluated: O, J, I, P, F_v/F_m (maximum efficiency of PSII photochemistry), SM (energy necessary for the closure of all reaction centers), ABS/RC [light absorption flux (for PSII antenna chlorophylls) per reaction center], TR_0/RC (flux of energy trapping per reaction center of PSII), DI_0/RC (dissipation energy flux initial per PSII reaction center), ET_0/RC [maximum electron transport flux initial (further than Q_A^-) per PSII reaction center], TR_0/CS_0 [trapping at time zero, per cross section (CS)], TR_0/CS_m [trapped (maximum) energy flux (leading to Q_A reduction) per CS], DI_0/CS (dissipation at time zero, per CS), ET_0/CS (electron transport at time zero, per CS), RC/ABS (light absorption flux (density of reaction centers per PSII antenna chlorophyll), and PI_{abs} (the performance index).

Photosynthetic pigments were spectrophotometrically (*Cary 100 Bio*, *Varian*, Australia) determined according to Šesták *et al.* (1971) and Lichtenthaler (1987). Four discs (8-mm diameter) were used from each leaf and the extraction was done using 10 mL of 80% acetone (v/v). Using 5 mL of this extract, Chl *a*, *b*, total (*a*+*b*), and carotenoids (Car) were quantified using absorbance at 663.6, 646.6, and 470 nm.

Chloroplast isolation and measurements of oxygen evolution: The isolation of chestnut chloroplasts was performed using fresh leaves, according to Gomes-Laranjo *et al*. (2006) and Zhang *et al*. (2013). The isolation buffer was composed of 20 mM sorbitol, 10 mM Tricine-NaOH (pH 8.0), 30 mM KCl, 5 mM $MgCl_2$, 0.75mM ethylenediamine tetraacetic acid (EDTA), 0.1% (w/v) bovine serum albumin (BSA), 1% (w/v) ascorbic acid, and 0.4% polyvinylpyrrolidone (PVP). The storage medium was composed of 165 mM sorbitol, 10 mM Tricine-NaOH (pH 8.4), 5 mM $MgCl_2$, and 1% ascorbic acid.

According to Zhang *et al*. (2013), the oxygen evolution was measured using an *Oxylab* oxygen electrode control unit (*Hansatech Instruments Ltd.*, Norfolk, UK). The reaction buffer contained 200 mM sorbitol, 2 mM Tricine-NaOH (pH 8.4), 4 mM $MgCl_2$, and 100 mM KCl. A red light with a PPFD of 1,000 µmol(photon) m^{-2} s^{-1} was used for 2 min as excitation light for chloroplasts.

Extraction and chromatography of lipids: Using the chloroplast extracts, lipids were determined according to the Bligh and Dyer (1959) procedure, with same modifications. A volume of 100 µl of chloroplast extract was incubated with 3 mL of methanol solution 2:0.8 (v/v) and 1 mL of chloroform and agitated in a vortex during 5 min. Subsequently, the samples were centrifuged at 2,000 × *g* for 5 min. The liquid phase was removed to a new tube, with 1 mL of chloroform, 1 mL of distilled water, and a few drops of 100 mM KCl added. A new centrifugation of 2,000 × *g* was carried out for 5 min. With a Pasteur pipette, the pellet was transferred to new tubes under gas nitrogen atmosphere to remove the chloroform from the samples. Subsequently, the samples were degassed by the addition of 5 ml of a 5% (w/v) solution of sulfuric acid with methanol to the lipid residue and were placed in a water bath for 2 h at 70°C. After cooling to room temperature, 5 mL of hexane were added to each sample and later centrifuged at 1,000 × *g* for 5 min. The supernatant was transferred to other tubes and saved for later. The pellet was resuspended with 5 ml of hexane, followed by a new centrifugation. The supernatant was mixed with the previous supernatant after which 3 mL of distilled water was added and stirred for 2 min. Subsequently the organic phase was transferred and the anhydrous sodium sulphate was added to remove any water present and the samples were filtered. Finally, the tubes were closed under the atmosphere of gaseous nitrogen and stored at –20°C for lipid analysis. The unsaturated index (UI) was calculated as: monoenoic acids _1 [%] + dienoic acids _2 [%] + trienoic acids _3 [%], according to Pamplona *et al*. (1999) and to Rosa and Catalá (1998). All the reactants used in the experiments were of an analytical degree.

Statistical analysis: All the data were subjected to statistical analysis using *StatView* (*BrainPower Inc*., California) and *Statistic 8.0* (*StatSoft Europe*, Germany). Analysis of variance (*ANOVA*) was also performed among the data treatments, using the *Tukey*'s test (P<0.05).

Results

Identification of phytoliths in leaves: The observation of leaf histology revealed the presence of phytoliths (Fig. 1) in treated plants, inserted in sieve vessels.

Determination of Si content in leaves: Determination

was done at the end of each phase. At the end of A25 (2 months after fertilization with SiK®), results indicate an increase of Si leaf content in treated plants. While in control plants (0 mM SiK®) leaves had 0.35 mg(Si) kg^{-1}, in treated ones with 10 mM SiK®, the Si content was 1.68 mg kg^{-1} (Fig. 2) representing an increase of 380% (Fig. 2). The Si content significantly increased in B32 (3 months after SiK® fertilization), while maintaining the contents in C25. The Si content increased 39% in 10 mM SiK®-treated plants (Fig. 2). Results also indicate that 3 months were needed to achieve the stabilization of Si content after application. On the other hand, there was a proportionality between the content of SiK® in fertilization and the content in leaves, demonstrating that the chestnut species is a siliceous species as also indicated by Carneiro-Carvalho *et al*. (2017).

Gas-exchange measurements: The SiK® fertilization influenced net photosynthetic rate (P_N) (Fig. 3*A*). The highest concentration, or the highest P_N, was observed in three phases, with the rates under 25°C being higher than those obtained under 32°C. Comparing both periods, A25 and C25, results indicate that SiK® (at 7.5 and 10 mM treatments) positively influenced the recovery capacity of P_N after the heat stress period (B32), contrary to control

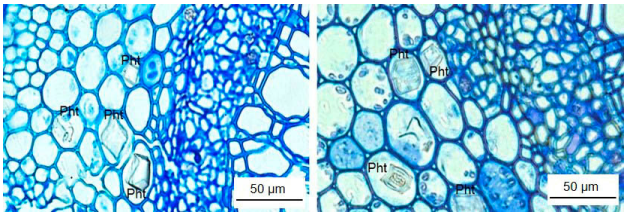

Fig. 1. Magnification of phytoliths (Pht) present in sieve vessels of chestnut leaves treated with 10 mM SiK®. Chestnut leaves were harvested 120 d after SiK® fertilization. Bars 50 µm.

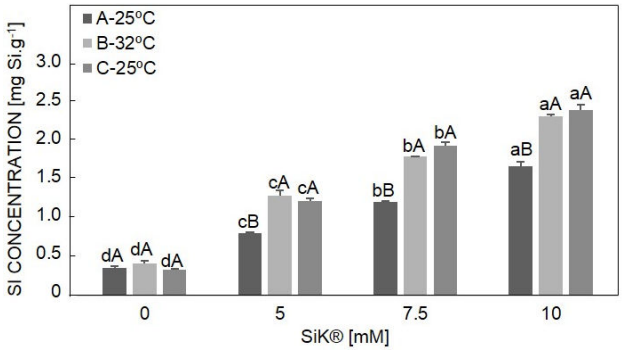

Fig. 2. The Si content in chestnut leaves from plants treated with different concentration of Si (0, 5, 7.5, and 10 mM SiK®) and exposed to 25°C (A-25°C), heat stress of 32°C (B-32°C), and recovery at 25°C (C-25°C). *Identical small letters* mean nonsignificant differences between treatments under the same temperature and *identical capital letters* mean nonsignificant differences between different temperatures of the same treatment according to the *Tukey*'s test ($P \leq 0.05$). Vertical bars represent standard error (SE) ($n = 12$).

plants and the plants treated with 5 mM SiK®. Maximal rates were obtained at C25, 3.7 µmol(CO_2) m^{-2} s^{-1} (10 mM SiK®). The impact of the temperature increase to 32°C (B32) was stronger in nonfertilized plants than that in fertilized ones. Reduction on P_N was 52% in Si-free plants, whilst in fertilized ones (10 mM SiK®), it was 19%. When the temperature was newly reduced to 25°C, to analyze the recovery period, an increase of P_N was observed in Si-treated plants, 39, 76, and 82% in 5, 7.5, and 10 mM SiK® treatments, respectively, while in control plants, P_N continued to be similar. The comparison between the initial period (A25) and the recovery period (C25) after the heat stress period (B32), indicates a reestablishment of the A25 rates at C25 for Si-plants but not for the control plants, which presented lower values in comparison to the beginning of the study.

With reference to the transpiration rate (*E*, Fig. 3*B*), an increase in temperature induced a high *E* in all treatments. Despite the fact that under initial conditions no differences were observed between treatments, they were observed during the heat stress phase, at B32. The highest increase of *E* was measured in the control plants, 72%, while after 7.5 and 10 mM SiK® treatments, only a slight increase was observed (near 28%). In relation to the recovery period, C25, a decrease in *E* was observed in all plants, though it was insignificant in the Si-treated plants. Comparing A25 with C25, the recovery of *E* in the Si-treated plants (7.5 and 10 mM) was complete, while in control plants and 5 mM SiK® plants, *E* continued to be significantly higher than that in B25 and in A25, suggesting an incomplete recovery.

Si treatments also positively influenced the recovery capacity of the stomatal conductance (g_s). No significant differences were observed between A25 and C25, contrary to the increase measured in control plants during the last period. In terms of heat stress tolerance, when comparing A25 and B32, heat induced a high increase in control plants (66%), while only a slight increase was measured in the Si-treated plants (Fig. 3*C*).

The water-use efficiency (WUE) quantitatively describes the behavior of gas exchanges in leaves, indicating the ability of the plant to use the water resource (Tankari *et al.* 2019). The initial exposure of chestnut plants at 25°C (A25) showed an increase in WUE from 3.2 to 4.7 mol(CO_2) mol(H_2O)$^{-1}$ (Fig. 3*D*) between 0 and 10 mM SiK® treatments. However, under the warm period (B32), a significant decrease in WUE was observed, though it was lower in the Si-fertilized plants (44 and 53% in 7.5 and 10 mM SiK® treatments, respectively) than that in control plants (140%) (Fig. 3*D*). It is noteworthy that the return to initial values occurred once again at C25 for the Si-treated plants, while for the control plants, it was only partial (56%) (Fig. 3*D*). According the analysis of the factor interaction (Table 1S, *supplement*), Si treatment was the most important factor to explain the variation of all the parameters, representing 45.9% for P_N, 50.2% for *E*, 28.1% for g_s, and 56.4% for WUE.

Chl fluorescence, OJIP test: Chl fluorescence provides information about the photosynthetic mechanism of plants,

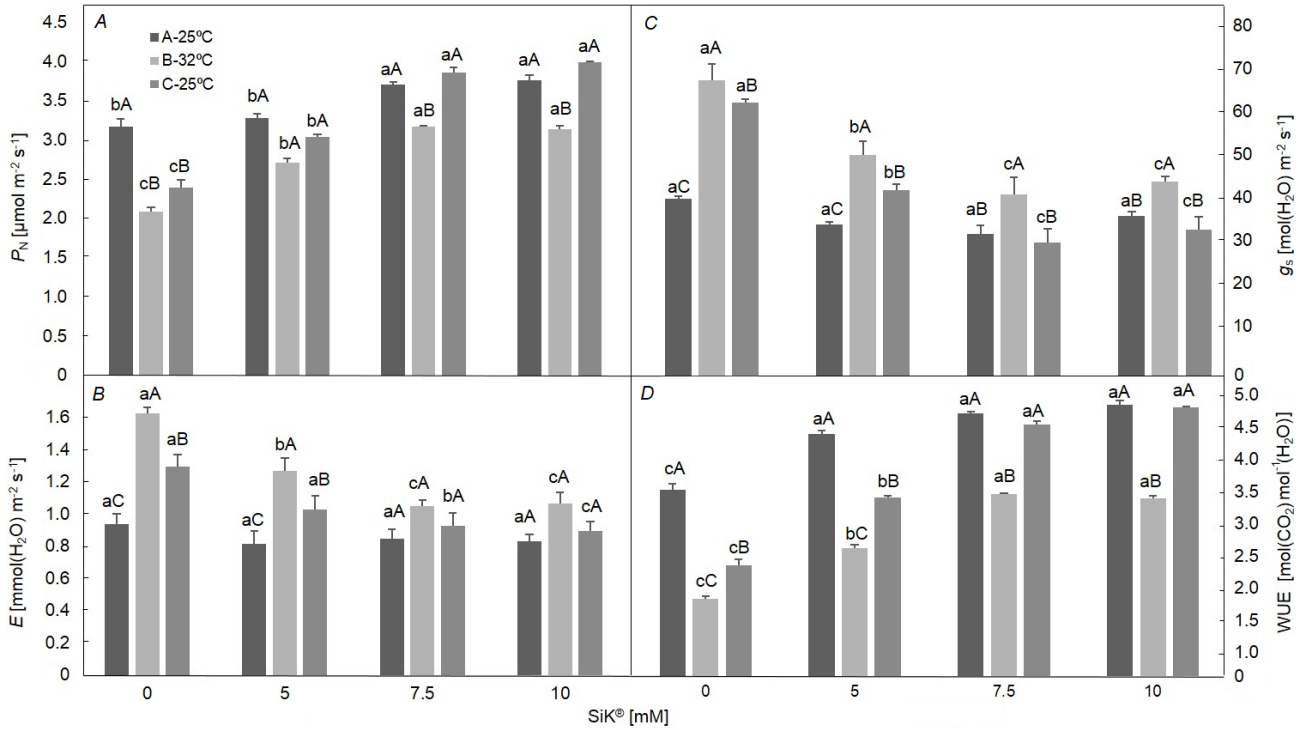

Fig. 3. Effect of Si application (0, 5, 7.5, and 10 mM SiK®) on gas-exchange parameters of chestnut plants exposed to 25°C (A-25°C), heat stress of 32°C (B-32°C), and recovery at 25°C (C-25°C). Net photosynthetic rate (P_N) (*A*), transpiration rate (*E*) (*B*), stomatal conductance (g_s) (*C*), and water-use efficiency (WUE) (*D*). *Identical small letters* mean nonsignificant differences between treatments under the same temperature and *identical capital letters* mean nonsignificant differences between different temperatures of the same treatment according to the *Tukey*'s test ($P \leq 0.05$). Vertical bars represent standard errors (SE) ($n = 12$).

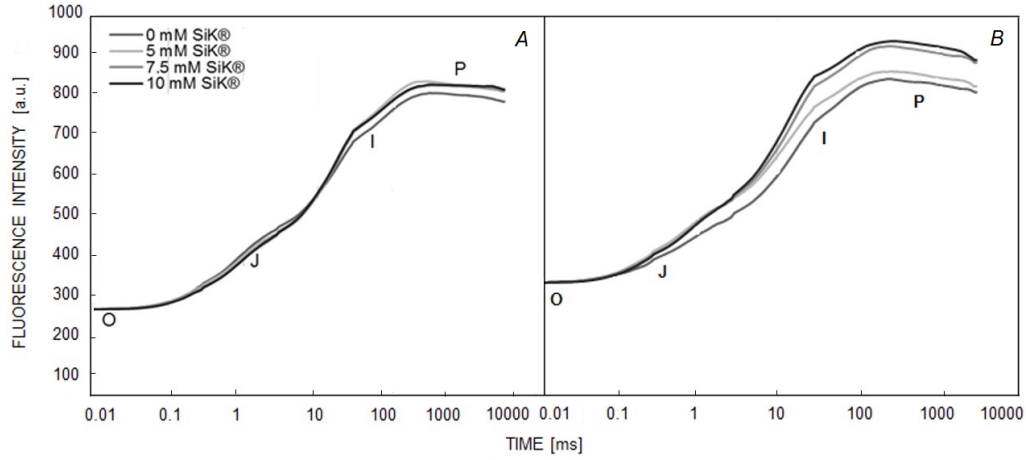

Fig. 4. OJIP standard curves measured on chestnut plants fertilized with 0, 5, 7.5, and 10 mM SiK® at the end of the heat stress of 32°C (*A*) and recovery phase at 25°C (*B*), ($n = 6$).

as well as the structure and function of the electron transport chain. Therefore, it has been used to detect changes in the PSII, which can occur under environmental stress conditions (Strasser *et al*. 2004, Martinazzo *et al*. 2012).

Fig. 4 shows the OJIP curves measured at the end of B32 and C25. Under 32°C, the main differences were observed during the P phase, where the fluorescence of control plants was lower compared with the Si-treated plants.

At the end of C25, the differences between treatments were improved. They appeared right from the start, from J to P. In the control plants, the maximal fluorescence during the P phase was around 840, while for the 10 mM-treated plants, it was 940 (Fig. 4). The higher fluorescence values in phase I–P reflect the increase in the final electron acceptor of the PSI.

The antenna size and reduced side heterogeneity were also analyzed (Fig. 1S, *supplement*). At the two highest SiK

concentrations, the number of inactive centers increased less (about 5%) than that in the control plants. Concerning the maximal rate by which an exciton is trapped by the RC, resulting in the reduction of the primary quinone acceptor of PSII (Q_A), stress can increase the flux of energy trapping per reaction center of PSII (TR_0/RC) since it indicates that the Q_A has been reduced, but it is not able to oxidize back due to temperature stress (*i.e.*, the reoxidation of the Q_A is inhibited, so that the Q_A cannot transfer electrons efficiently to the secondary quinone acceptor of PSII (Q_B) and maximum energy is also lost in dissipation). For this parameter, only a slight difference was observed between treatments. The same tendency was also observed for DI_0/RC (Fig. 1S), which represents the ratio of the total dissipation of untrapped excitation energy from all the RCs with respect to the number of active reaction centers (RCs). Dissipation results from the excess absorption of photons that occurs as the heat, fluorescence, and energy transfer to other systems. As the inactive centers increased, the dissipation energy flux per PSII reaction center (DI_0/RC) also increased because the inactive centers were unable to trap the photon. This increase also reflects the loss of connectivity between the PSII heterogeneous units (Mathur *et al*. 2011). In relation to maximum electron transport flux (further than Q_A^-) per PSII reaction center (ET_0/RC), which reflects the reoxidation of reduced Q_A *via* electron transport in an active RC, the differences were not significant. The density of reaction centers per PSII antenna Chl (RC/ABS) also was 15% lower for the 7.5 and 10 mM SiK® treatments (Fig. 1S).

Analyzing plants during the recovery period (C25), PI_{ABS} (Fig. 1S) in fertilized plants continued to be higher (30 to 50%, according to Si concentrations) than that in control plants. However, the difference was lower, indicating that heat stress affected plants at higher degrees. In relation to the others, for the energy necessary for the closure of all reaction centers (SM), light absorption flux (for PSII antenna chlorophylls) per reaction center (ABS/RC), flux of energy trapping per reaction center of PSII (TR_0/RC), electron transport flux per cross section (ET_0/CS), and RC/ABS, the values from three Si-treatments were similar, but different when comparing with control plants, which suggests a positive impact of Si in protecting the photosynthetic apparatus.

No significant differences were observed on PI_{ABS} between SiK®-fertilized and control plants at the end of A25 (Fig. 5). However, the warm period (B32) induced a significant decrease in the PI of control plants (53%) and only a slight decrease in treated plants (*e.g.*, 7% at 10 mM SiK® treatment). Many authors consider the performance index (PI) included in the OJIP test to be a powerful nondestructive and fast method that offers information about the functionality of PSII and allows researchers to detect modifications in the photosynthetic activity of the plants influenced by changes in the surrounding environment, as well as to provide information about the functionality of the PSII (Živčák *et al*. 2008, Giannakoula and Ilias 2013, Rykaczewska and Mankowski 2015). Concerning the recovery period (C25), once again the recovery was almost complete in the Si-treated plants, while that was not the case in the control plants (around 69% of A25 value) (Fig. 5).

Photosynthetic pigments: As shown in Table 1, under heat stress conditions (B32) Chl (*a*+*b*) content was significantly higher in the Si-fertilized plants (121% in 10 mM SiK® treatment) than that in the control plants. When the temperature rose from 25 to 32°C, the Chl (*a*+*b*) amount in the control plants decreased by 33%, while in the Si-fertilized plants the increase of 41% (at 10 mM SiK® treatment) was observed (Table 2). Concerning carotenoids (Car), SiK® induced the Car synthesis, since at A25, the Si-treated plant had 32% higher content than that of the control plants. Moreover, in the end of B32, the warm period, SiK® also induced the synthesis of more Car, while in control plants, its content decreased by 30% (Table 2). Consequently, Chl/Car was lower in SiK®-fertilized plants than that in the control. Relatively to the Chl *a*/*b* ratio, no significant differences were observed at the first exposure of chestnut plants to 25°C. However, the warm period (B32) led to a strong decrease in the control plants, from 2.2 (A25) to 1.3 (B32) mg g^{-1}(DM), while in Si-treated plants the Chl *a*/*b* was preserved. These results are corroborated by those from the *ANOVA* analysis, which suggest that the SiK® treatment was the main factor for the reported variations.

Chloroplast isolation and measurements of oxygen evolution: Heat stress causes damages at the level of chloroplasts, on their photochemical components and on the thylakoid membranes (Bita and Gerats 2013). As shown in Fig. 5, fertilization of SiK® increased the oxygen evolution. When air temperature increased to 32°C (B32), the decay of the oxygen evolution of fertilized plants was lower than that in the control plants (92%) (Fig. 6) and the recovery capacity at C25 was complete for treated plants, while it was not observed for the control plants.

These findings can be explained by the fact that Si application in chestnut plants resulted in well preserved

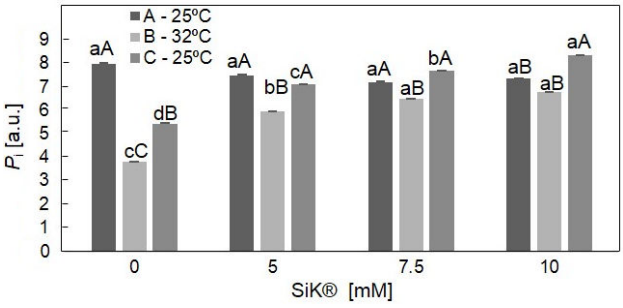

Fig. 5. Effect of different concentrations of Si (0, 5, 7.5, and 10 mM SiK®) applied to chestnut plants subjected to 25°C (A-25°C), heat stress of 32°C (B-32°C), and recovery at 25°C (C-25°C) on chlorophyll fluorescence parameter, performance index (PI). *Identical small letters* mean nonsignificant differences between treatments under the same temperature and *identical capital letters* mean nonsignificant differences between different temperatures of the same treatment according to the *Tukey*'s test ($P \leq 0.05$). Vertical bars represent standard error (SE) ($n = 6$).

Table 1. Effect of silicon treatment (0, 5, 7.5 and 10 mM SiK®) on total chlorophyll (Chl) content, carotenoid (Car) content, Chl *a*/*b*, and Chl/Car of chestnut plants exposed to 25°C, heat stress of 32°C, and recovery at 25°C. Data are means ± SE (n = 6). Values within rows, followed by *the same letter(s)*, are not significantly different according to *Tukey*'s test ($P \leq 0.05$). *Identical small letters* mean nonsignificant differences between treatments under the same temperature and *identical capital letters* mean nonsignificant differences between different temperatures of the same treatment.

SiK® [mM]	Temp.[°C]	Chl (*a*+*b*) [mg g^{-1}(DM)]	Car [mg g^{-1}(DM)]	Chl *a*/*b*	Chl/Car
0	25	20.5 ± 0.24^{aA}	3.25 ± 0.04^{cA}	2.20 ± 0.05^{aA}	6.31 ± 0.06^{aB}
5		19.1 ± 0.18^{aB}	3.67 ± 0.04^{bA}	2.05 ± 0.04^{aA}	5.20 ± 0.06^{bA}
7.5		20.9 ± 0.19^{aC}	4.12 ± 0.05^{aB}	2.33 ± 0.05^{aB}	5.07 ± 0.04^{bA}
10		21.5 ± 0.20^{aC}	4.29 ± 0.06^{aB}	2.16 ± 0.04^{aB}	5.01 ± 0.03^{bA}
0	32	13.7 ± 0.17^{cB}	2.29 ± 0.03^{cB}	1.30 ± 0.01^{cB}	5.98 ± 0.06^{aB}
5		22.5 ± 0.24^{bA}	3.96 ± 0.04^{bA}	2.34 ± 0.03^{bA}	5.68 ± 0.06^{aA}
7.5		26.8 ± 0.30^{abB}	5.00 ± 0.05^{aA}	2.75 ± 0.03^{abA}	5.36 ± 0.05^{aA}
10		30.3 ± 0.32^{aB}	5.75 ± 0.06^{aA}	3.03 ± 0.03^{aA}	5.27 ± 0.05^{aA}
0	25	20.3 ± 0.18^{bA}	2.61 ± 0.04^{cB}	1.60 ± 0.02^{bB}	7.77 ± 0.08^{aA}
5		24.4 ± 0.21^{bA}	4.34 ± 0.03^{bA}	2.40 ± 0.03^{abA}	5.61 ± 0.07^{bA}
7.5		31.8 ± 0.35^{aA}	5.37 ± 0.05^{aA}	3.05 ± 0.03^{aA}	5.93 ± 0.07^{bA}
10		35.5 ± 0.37^{aA}	6.00 ± 0.05^{aA}	3.11 ± 0.04^{aA}	5.92 ± 0.07^{bA}
Variation factor					
Treatment		48.8	76.8	61.6	55.6
Temperature		30.2	9.53	6.88	29.7
Treatment × Temperature		20.5	13.2	30.8	14.1
Residual		0.494	0.443	0.800	0.553

Table 2. Lipid composition of chestnut leaves from plants treated with 0, 5, 7.5 and 10 mM SiK® and exposed to 25°C, heat stress of 32°C, and recovery at 25°C. Total saturation (Total sat.), total unsaturation (Total unsat.), unsaturation/saturation ratio (Unsat./sat.), unsaturated index (UI), peroxidation index (PI), fatty acids with 16-carbon chain (C_{16}), and fatty acids with 18-carbon chain (C_{18}), (n = 3). *Identical small letters* mean nonsignificant differences between treatments under the same temperature and *identical capital letters* mean nonsignificant differences between different temperatures of the same treatment according to *Tukey*'s test ($P \leq 0.05$).

SiK® [mM]	Temp. [°C]	Total sat. [%]	Total unsat. [%]	Unsat./sat.	PI	UI	Total C_{16} [%]	UI C_{16}	Total C_{18} [%]	UI C_{18}
0	25	16.8^{aA}	83.2^{bA}	5.00^{bA}	100.4^{cB}	183.0^{cB}	22.8^{bB}	7.91^{aB}	61.0^{cB}	135.2^{cB}
5		17.8^{aA}	82.2^{bA}	4.60^{bA}	125.8^{bA}	207.6^{bA}	25.1^{aB}	8.28^{aB}	66.0^{bB}	161.9^{bB}
7.5		15.0^{bB}	85.0^{aA}	5.70^{aA}	137.9^{aA}	222.5^{aA}	21.8^{bA}	7.45^{aA}	64.0^{bB}	158.8^{bB}
10		15.3^{bB}	84.7^{aA}	5.50^{aA}	139.2^{aA}	223.6^{aA}	20.8^{bB}	6.24^{bA}	72.0^{aA}	180.4^{aA}
0	32	19.1^{bA}	80.9^{aA}	4.20^{aB}	127.0^{aA}	207.6^{aA}	23.8^{bB}	6.50^{aB}	73.6^{aA}	190.3^{bA}
5		20.1^{bA}	79.9^{aA}	4.00^{aB}	130.0^{aA}	209.7^{aA}	22.2^{bB}	4.07^{bC}	74.9^{aA}	194.0^{aA}
7.5		20.5^{bA}	79.5^{aB}	3.90^{aB}	125.2^{aB}	204.4^{aB}	24.7^{bA}	6.08^{aA}	73.0^{aA}	188.2^{bA}
10		24.9^{aA}	75.1^{bB}	3.00^{bB}	113.8^{bB}	188.6^{bC}	28.7^{aA}	7.07^{aA}	65.8^{bB}	163.0^{cC}
0	25	16.6^{bA}	83.4^{aA}	5.00^{aA}	94.9^{cB}	177.6^{cB}	35.1^{aA}	20.00^{aA}	63.3^{bB}	139.2^{dB}
5		19.0^{aA}	81.0^{bA}	4.30^{bA}	106.8^{bB}	187.3^{bB}	30.3^{bA}	11.20^{bA}	69.0^{aB}	156.9^{cC}
7.5		20.5^{aA}	79.5^{bB}	3.90^{cB}	109.7^{bC}	188.8^{bB}	26.6^{cA}	7.61^{cA}	66.0^{aB}	161.7^{bB}
10		20.8^{aA}	79.2^{bB}	3.80^{cB}	115.0^{aB}	193.8^{aB}	26.7^{cA}	6.85^{cA}	67.7^{aB}	171.3^{aB}

and stable chloroplast membranes under heat stress conditions.

Extraction and chromatography of lipids: Fertilization with SiK seemed to induce a slight increase of the fatty acid unsaturation degree, 83 (control plants) to 85% (7.5 and 10 mM SiK®) when plants were growing under favourable temperature (A25). However, when plants faced heat stress, unsaturation decreased by 3 and 11% for the control and treated chloroplasts, respectively (Table 2). Consequently, the saturation level increased in a similar way. Therefore, the presence of SiK® seemed to promote the adaptation of unsaturated/saturated chloroplast fatty acids against the air temperature conditions, inducing a reduction under heat stress conditions.

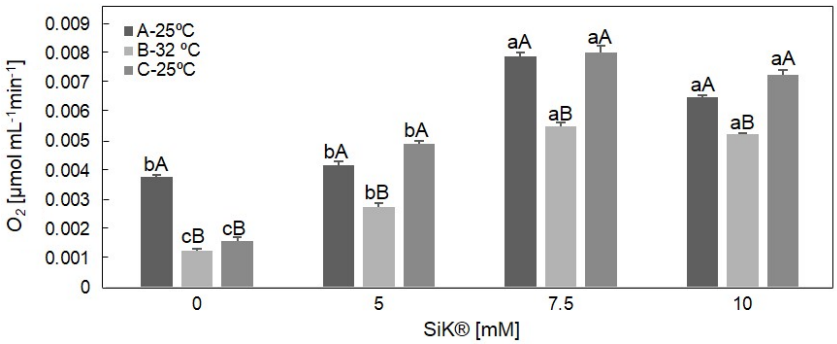

Fig. 6. Effect of Si application (0, 5, 7.5, and 10 mM SiK®) on the chloroplasts activity of chestnut plants exposed to 25°C (A-25°C), heat stress of 32°C (B-32°C), and recovery at 25°C (C-25°C). *Identical small letters* mean nonsignificant differences between treatments under the same temperature and *identical capital letters* mean nonsignificant differences between different temperatures of the same treatment according to the *Tukey*'s test ($P \leq 0.05$). Vertical bars represent standard error (SE) ($n = 4$).

Discussion

As discussed above, the chestnut tree is a thermophilic species presenting low tolerance to high temperatures. The results analysed here suggest that silicon can improve the chestnut's tolerance through anatomical, structural, and ecophysiological mechanisms.

Concerning anatomical and structural issues, this study demonstrated that fertilization with SiK® improved the deposition of phytoliths in the leaves in wide amounts, namely in the conducting vessels (Fig. 1). This suggests that the chestnut might be a siliceous plant according to Mattson and Leatherwood (2010) and Cooke and Leishman (2011). Their presence is attributed to an increase in the stability of the vessels, indicating the existence of a passive transport of Si over the plants (Shakoor *et al*. 2015). According to Quigley and Anderson (2014), the presence of phytoliths also promotes the plant protection mechanisms against heat stress. Otherwise, due to the increase in heat-stress tolerance, which in turn promotes higher rates of photosynthesis and consequently high transpiration rates, Si-fertilized chestnut leaves can accumulate more and more Si as opposed to the absence found in Si-deficient leaves (Fig. 2), as was also referred by Agarie *et al*. (1998).

Chestnut plants under high temperatures (above 32°C) can suffer more than 50% reduction in P_N (Fig. 3*A*) (Gomes-Laranjo *et al.* 2006, 2008b). Consistently, the results of this study demonstrated the benefits of Si fertilization. In Si-fertilized plants, only a slight decrease was observed when the temperature rose from 25 to 32°C, contrary to that observed in non-Si-supplemented plants, leading to a cascade of inhibitions in the leaf photochemistry, as well as metabolic impairments, some of them studied here. These findings were also confirmed by other studies carried out by Habibi and Hajiboland (2013), Perdono (2017), and Lipiec *et al*. (2013) in pistachio, rice, and maize plants, respectively.

It is now widely accepted that Chl fluorescence parameters provide useful information concerning PSII activity and photosynthetic metabolism in stressed plants (Habibi 2016). The OJIP test is widely used to appraise stress-induced impairments in the photosynthetic apparatus (Maghsoudi *et al*. 2015). Kavitha and Murugan (2016) observed a decrease of H_2O_2 production and an improvement in the photochemical efficiency of the PSII in tomato plants under abiotic stress, in Si-treated plants compared with the control plants. The cause of the fluorescence decline at high temperatures has been linked to the decline in the functioning of primary photochemical reactions, primarily involving inhibition of the PSII, located in the thylakoid membrane system (Mathur *et al*. 2011).

Many authors (Strasser *et al*. 2004, Bacarin *et al*. 2011, Kalaji *et al*. 2011) have characterized the vitality of plants in response to different environmental stresses by the PI_{ABS}. In the present study, Si fertilization generally promoted the increase in the vitality of chestnut plants submitted to high temperatures in the B32 phase (Fig. 5). The increase of the PI_{ABS} was attributed by Strasser *et al*. (2000) to an increase of the primary photochemistry and photochemical efficiency in the photosynthetic electron transport, which can also be associated to a TR_0/RC decrease and ET_0/CS increase as concluded by Bacarin *et al*. (2011) for *Brassica napus* plants. The same was observed in this study.

Heat stress frequently causes a decrease in the amount of photosynthetic pigments, because of their susceptibility to oxidative damage. The accumulation of phytoliths in the leaves favoured the increase of Chl content (Fig. 1, Table 1), mainly Chl *a* as the increase in Chl *a*/*b* suggests, as well as Car content by increasing the protection of the photosynthetic apparatus against heat damage and avoiding the degradation of Chl by oxidative stress (Table 2). These results are in line with Agarie *et al*. (1998), Al-aghabary *et al*. (2005), Rubinowska *et al*. (2014), and Sivanesan *et al*. (2014), who demonstrated that Si application in rice, tomato, *Polygonatum multiflorum*, and *Nephrolepsis exaltata* plants increase the photochemical efficiency of PSII, by stimulating the synthesis of photosynthetic pigments through the detoxification of H_2O_2 under heat stress.

The presence of phytoliths in chestnut leaves (Fig. 1) might be involved in the regulation of the stomatal movement and in the increase of cuticle thickness by a thick layer of silica gel associated with the cellulose in the walls of epidermal cells. This can lead to a decrease in water loss by the cuticle and/or stomata, as detected in this experiment, where lower values of E and g_s were recorded in the Si-supplied plants (Fig. 3*B*,*C*). The presence of phytoliths can act as a solar screen (Shen *et al*. 2010), absorbing heat and induced biosynthesis of protective pigments (Car and anthocyanin), promoting the protection of the PSII from the photodamage, which consequently favoured the higher rates of photosynthesis and indirectly promoted the increase of WUE (Fig. 3*D*). The present findings are in accordance with Zanetti

et al. (2016) and Sattar *et al*. (2017), who proved that Si fertilization in cacao and wheat plants interfered in stomatal dynamics and photochemical reactions, therefore regulating photosynthesis.

Chloroplasts are considered good biosensors of heat stress because they are the key site for photosynthesis, a process which is highly susceptible to damage caused by high temperatures (Gomes-Laranjo *et al*. 2006). When exposed to high temperatures, they can exhibit two opposite effects at the level of the electron transport chain (Tóth *et al*. 2007). On the other hand, PSI is stimulated by heat (as measured by the rate of $P700^+$ reduction), due to a greater reduction of the plastoquinone (PQ) pool by ferredoxin (Fd) at high temperatures (Tóth *et al*. 2007). In contrast, PSII, particularly the oxygen-evolving complex (OEC), is deactivated even at slightly elevated temperatures (Yamane *et al*. 1998), demonstrating that this process is especially sensitive to temperature stress (Pushpalatha *et al*. 2008). Heat stress causes severe damages to the PSII, the most thermosensitive component of thylakoid membranes. According to Balakhnina and Borkowska (2013), the Si application induces the stability of the thylakoid membrane under heat stress, and consequently of the chloroplast structure. This promotes a high chloroplast activity and high photosynthetic rate, influencing Chl content and photochemical quantum yields of photosynthesis. According to our results, the oxygen-evolution rate in chloroplasts from SiK®-treated plants was higher than that in control plants, both under 25 or 32°C. At the B32 phase, oxygen evolution decreased in Si-deprived plants by 60%, while in the Si-treated leaves the reduction was of 20% (Fig. 6).

High temperatures alter lipid properties, causing membranes to become more fluid and then disrupting membrane process (Larkindale *et al*. 2005b). In regard to the fatty acids composition in chestnut leaves, heat tolerance was observed after Si treatments through the increasing of the saturation of fatty acids (Table 3). Furthermore, the Si-fertilized plants present a decrease in unsaturation of fatty acids at 32°C, a strategy that allows plants to adapt to high temperatures, as suggested by Gombos *et al*. (1994) and Zheng *et al*. (2011). Several studies reported that plants under heat stress can reduce the degree of unsaturation of fatty acids thereby increasing the heat tolerance in *Arabidopsis thaliana*, chestnut, and tobacco plants (Murakami *et al*. 2000, Falcone *et al*. 2004, Gomes-Laranjo *et al*. 2006, Zheng *et al*. 2011). Moreover, the silicon fertilization in chestnut plants decreased the degree of membrane lipid unsaturation in response to the rise of temperature (B32) (Table 2), reducing membrane injury by high accumulation of saturated fatty acids, enhancing heat stability of lipids through maintaining the integrity, stability, and function of cell membrane (Liu and Huang 2004, Ahmed *et al*. 2012). Similar results were found by Hajiboland *et al*. (2012) and Liang *et al*. (2015).

Heat can influence the fluidity of thylakoid membranes, leading to the disintegration of the lipid bilayer, which affects namely the PQ pool and adenosine triphosphate (ATP) synthesis (Pshybytko *et al*. 2008, Ashraf and Harris 2013).

Conclusions: The present study demonstrated that fertilization of chestnut plants with SiK® increased their tolerance to high temperature, with 7.5 to 10 mM SiK® being the adequate range of concentration. The improvement of heat tolerance might be associated with the presence of Si as phytoliths in the leaf tissues, which helps improve their resilience at the morphological, physiological, and biochemical levels.

References

Agarie S., Hanaoka N., Ueno O. *et al*.: Effect of silicon on tolerance to water deficit and heat stress in rice plants (*Oryza sativa* L.), monitored by electrolyte leakage. – Plant Prod. Sci. **1**: 96-103, 1998.

Ahanger R.A., Bhat H.A., Bhat T.A. *et al.*: Impact of climate change on plant diseases. – Int. J. Mod. Plant Animal Sci. **1**: 105-115, 2013.

Ahmed M., Asif M., Goyal A.: Silicon the non-essential beneficial plant nutrient to enhanced drought tolerance in wheat. – In: Goyal A. (ed.): Crop Plant. Pp. 31-48. InTech, 2012.

Al-aghabary K., Zhu Z., Shi Q.: Influence of silicon supply on chlorophyll content, chlorophyll fluorescence, and antioxidative enzyme activities in tomato plants under salt stress. – J. Plant Nutr. **27**: 2101-2115, 2005.

Albertson P. A., Âkerlund, H.E.: Structure and function of the photosynthetic membrane. Center for Molecular Protein Science, Lund University. http://www.cmps.lu.se/biostruct/people/per-aake-albertsson/structure-and-function-of-the-photosynthetic-membrane/, 2015. (*Accessed* 20 May 2019).

APA: Portuguese Environment Agency with the cooperation of Ecoprogresso. https://www.climatechangepost.com/portugal/climate-change/, 2009. (*Accessed* 10 September 2017).

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

Bacarin M.A, Deuner S., da Silva F.S.P. *et al.*: Chlorophyll *a* fluorescence as indicative of the salt stress on *Brassica napus* L. – Braz. J. Plant Physiol. **23**: 245-253, 2011.

Balakhnina T., Borkowska A.: Effects of silicon on plant resistance to environmental stresses: review. – Int. Agrophys. **27**: 225-232, 2013.

Bita C.E., Gerats T.: Plant tolerance to high temperature in a changing environment: Scientific fundamentals and production of heat stress-tolerant crops. – Front. Plant Sci. **4**: 273, 2013.

Bligh E.G., Dyer W.J.: A rapid method of total lipid extraction and purification. – Can. J. Biochem. Phys. **37**: 912-919, 1959.

Carneiro-Carvalho A., Pereira C., Marques T. *et al*.: Potential of silicon fertilization in the resistance of chestnut plants to ink disease (*Phytophthora cinnamomi*). – Int. J. Environ. Agr. Biotech. **2**: 2740-2753, 2017.

Carnelli A.L., Madella M., Theurillat J.P.: Biogenic silica production in selected alpine plant species and plant communities. – Ann. Bot.-London **87**: 425-434, 2001.

Carvalho A., Schmidt L., Santos F.D., Delicado A.: Climate change research and policy in Portugal. – Wires Clim. Change **5**: 199-217, 2014.

Conedera M., Tinner W., Krebs P. *et al.*: *Castanea sativa* in Europe: Distribution, habitat, usage and threats. – In: San-Miguel-Ayanz J., De Rigo D., Caudullo G. *et al*. (ed.): European Atlas of Forest Tree Species. Pp. 78-79. European Union, Luxembourg 2016.

Cooke J., Leishman M.R.: Is plant ecology more siliceous than

we realise? – Trends Plant Sci. **16**: 61-68, 2011.
da Silva Lobato A.K., Guedes E.M.S., Marques D.J., de Oliveira Neto C.F.: Silicon: A benefic element to improve tolerance in plants exposed to water deficiency. – In: Akinci S. (ed.): Responses of Organisms to Water Stress. Pp. 95-113. IntechOpen, 2013.
Das M., Hajong M., Majumdar D. *et al.*: Climate change impacts on plant diseases. – SAARC J. Agr. **14**: 200-209, 2016.
Falcone D.L., Ogas J.P., Somerville C.R..: Regulation of membrane fatty acid composition by temperature in mutants of *Arabidopsis* with alterations in membrane lipid composition. – BMC Plant Biol. **4**: 17, 2004.
Fernández-López J., Alía R.: Chestnut (*Castanea sativa*). Technical Guidelines for Genetic Conservation and Use. Pp. 6. EUFORGEN, Rome 2003.
Giannakoula A.E., Ilias I.E.: The effect of water stress and salinity on growth and physiology of tomato (*Lycopersicon esculentum* Mill.). – Arch. Biol. Sci. **65**: 611-620, 2013.
Gombos Z., Wada H., Hideg E., Murata N.: The unsaturation of membrane lipids stabilizes photosynthesis against heat stress. – Plant Physiol. **104**: 563-567, 1994.
Gomes-Laranjo J., Araújo-Alves J., Ferreira-Cardoso J. *et al.*: Effect of chestnut ink disease on photosynthetic performance. – J. Phytopathol. **152**: 138-144, 2004.
Gomes-Laranjo J., Coutinho J.P., Galhano V., Ferreira-Cardoso J.: Differences in photosynthetic apparatus of leaves from different sides of the chestnut canopy. – Photosynthetica **46**: 63-72, 2008a.
Gomes-Laranjo J., Coutinho J.P., Peixoto F. *et al.*: [Ecology of chestnut.] – In: Gomes-Laranjo J., Cardoso J.F., Portela E., Abreu C.G. (ed.): [Chestnut.] Pp. 109-149. University of Trás-os-Montes and Alto Douro, Vila Real 2007. [In Portuguese]
Gomes-Laranjo J., Coutinho J.P., Peixoto F.: Ecophysiological characterization of *C. sativa* Mill. – Acta Hortic. **784**: 99-106, 2008b.
Gomes-Laranjo J.C.E., Peixoto F., Wong Fong Sang H.W., Torres-Pereira J.: Study of the temperature effect in three chestnut (*Castanea sativa* Mill.) cultivars' behaviour. – J. Plant Physiol. **163**: 945-955, 2006.
Habibi G., Hajiboland R.: Alleviation of drought stress by silicon supplementation in pistachio (*Pistacia vera* L.) plants. – Folia Hortic. **25**: 21-29, 2013.
Habibi G.: Effect of foliar-applied silicon on photochemistry, antioxidant capacity and growth in maize plants subjected to chilling stress. – Acta Agr. Slov. **107**: 33-43, 2016.
Hajiboland R.: Effect of micronutrient deficiencies on plants stress responses. – In: Ahmad P., Prasad M.N.V. (ed.): Abiotic Stress Responses in Plants. Pp. 283-329. Springer, New York 2012.
Kalaji H.M., Govindjee, Bosa K. *et al.*: Effects of salt stress on photosystem II efficiency and CO_2 assimilation in two syrian barley landraces. – Environ. Exp. Bot. **73**: 64-72, 2011.
Kavitha C.H., Murugan K.: Photochemical efficacy analysis using chlorophyll fluorescence of *Dicranopteris linearis* in response to dessiccation and rehydration stress. – Biosci. Biotech. Res. Commun. **9**: 439-444, 2016.
Kim Y.H, Khan A.L., Waqas M., Lee I.J.: Silicon regulates antioxidant activities of crop plants under abiotic-induced oxidative stress: A review. – Front. Plant Sci. **8**: 510, 2017.
Korndörfer G.H., Pereira H.S., Nolla A.: [Silicon analysis: soil, plant and fertilizer.] – UFU-ICIAGGPSi. Bol. Tec. **2**: 1-34, 2004. [In Portuguese]
Larkindale J., Hall J.D., Knight M.R., Vierling E.: Heat stress phenotypes of *Arabidopsis* mutants implicate multiple signaling pathways in the acquisition of thermotolerance. – Plant. Physiol. **138**: 882-897, 2005b.
Larkindale J., Mishkind M., Vierling E.: Plant responses to high temperature. – In: Jenks M.A., Hasegawa P.M. (ed.): Plant Abiotic Stress. Pp. 103-143. Blackwell Publishing, New Jersey, 2005a.
Latef A.A.H.A., Tran L.S.P.: Impacts of priming with silicon on the growth and tolerance of maize to alkaline stress. – Front. Plant Sci. **7**: 243, 2016.
Liang Y., Nikolic M., Bélanger R. *et al.*: Silicon in Agriculture: From Theory to Practice. Pp. 235. Springer, New York 2015.
Lichtenthaler H.K.: Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. – Method. Enzymol. **148**: 350-382, 1987.
Lipiec J., Doussan C., Nosalewicz A., Kondracka K.: Effect of drought and heat stresses on plant growth and yield: A review. – Int. Agrophys. **27**: 463-477, 2013.
Liu X., Huang B.: Changes in fatty acid composition and saturation in leaves and roots of creeping bentgrass exposed to high soil temperature. – J. Am. Soc. Hortic. Sci. 129: 795-801, 2004.
Maghsoudi K., Emam Y., Ashraf M.: Influence of foliar application of silicon on chlorophyll fluorescence, photosynthetic pigments, and growth in water-stressed wheat cultivars differing in drought tolerance. – Turk. J. Bot. **39**: 625-634, 2015.
Martinazzo E.G., Ramm A., Bacarin M.A.: The chlorophyll *a* fluorescence as an indicator of the temperature stress in the leaves of *Prunus persica*. – Braz. J. Plant Physiol. **24**: 237-246, 2012.
Mathur S., Allakhverdiev S.I., Jajoo A.: Analysis of high temperature stress on the dynamics of antenna size and reducing side heterogeneity of photosystem II in wheat leaves (*Triticum aestivum*). – BBA-Bioenergetics **1807**: 22-29, 2011.
Mattson N.S., Leatherwood W.R.: Potassium silicates drenches increase leaf silicon content and affect morphological traits of several floriculture crops grown in a peat-based substrate. – HortScience **45**: 43-47, 2010.
Mota M., Marques T., Pinto T.: [Effect of watering on chestnut tree photosynthetic productivity.] – Simp. Nac. Frut. **1**: 166-173, 2014. [In Portuguese]
Murakami Y., Tsuyama M., Kobayashi Y. *et al.*: Trienoic fatty acids and plant tolerance of high temperature. – Science **287**: 476-479, 2000.
Pamplona R., Portero-Otín M.J.R., Requena J.R.: A low degree of fatty acid unsaturation leads to lower lipid peroxidation and lipoxidation-derived protein modification in heart mitochondria of the longevous pigeon than in the short-lived rat. – Mech. Ageing Dev. **106**: 283-296, 1999.
Perdono J.A., Capó-Bauçà S., Carmo-Silva E.: 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, 2017.
Pshybytko N., Kruk J., Kabashnikova L.F., Strzalka K.: Function of plastoquinone in heat stress reactions of plants. – BBA-Bioenergetics **1777**: 1393-1399, 2008.
Pushpalatha P., Sharma-Natu P., Ghildiyal M.C.: Photosynthetic response of wheat cultivar to long-term exposure to elevated temperature. – Photosynthetica **46**: 552-556, 2008.
Quigley K.M., Anderson T.M.: Leaf silica concentration in Serengeti grasses increases with watering but not clipping: Insights from a common garden study and literature review. – Front. Plant Sci. **5**: 568, 2014.
Reynolds O.L., Padula M.P., Zeng R., Gurr G.M.: Silicon: Potential to promote direct and indirect effects on plant defense against arthropod pests in agriculture. – Front. Plant Sci. **7**: 744, 2016.

Rosa D., Catalá A.: Fatty acid profiles and non enzymatic lipid peroxidation of microsomes and mitochondria from bovine liver, kidney, lung and heart. – Arch. Physiol. Biochem. **106**: 33-37, 1998.

Rubinowska K., Pogroszewska E., Laskowska H. *et al*.: The subsequent effect of silicon on physiological and biochemical parameters of *Polygonatum multiflorum* (L.) All. 'Variegatum' cut shoots. – Acta Sci. Pol.-Hortoru. **13**: 167-178, 2014.

Ruzin S.E.: Plant Microtechnique. Pp. 127-129. The Iowa State College Press, Iowa 1999.

Rykaczewska K., Mankowski D.: The effect of physiological age of potato plants on chosen chlorophyll fluorescence parameters. – Plant Soil Environ. **10**: 462-467, 2015.

Sattar A., Cheema M.A., Abbas T. *et al.*: Physiological response of late sown wheat to exogenous application of silicon. – Cereal Res. Commun. **45**: 202-213, 2017.

Savvas D., Ntatsi G.: Biostimulant activity of silicon in horticulture. – Sci. Hortic.-Amsterdam **196**: 66-81, 2015.

Šesták Z., Čatský J., Jarvis P.G.: Plant Photosynthetic Production: Manual of Methods. Pp. 819. Dr. W. Junk N. V., The Hague 1971.

Shakoor S.A., Bhat M.A., Mir S.H.: Phytoliths in plants: A review. – J. Bot. Sci. **10**: 10-24, 2015.

Shen X., Zhou Y., Duan L. *et al.*: Silicon effects on photosynthesis and antioxidant parameters of soybean seedlings under drought and ultraviolet-B radiation. – J. Plant Physiol. **167**: 1248-1252, 2010.

Sivanesan I., Moon Sook S., Soundararajan P., Byoung Ryong J.: Effect of silicon on growth and temperature stress tolerance of *Nephrolepsis exaltata* 'Corditas'. – Korean J. Hortic. Sci. **32**: 142-148, 2014.

Soundararajan P., Sivanesan I., Jana S., Jeong B.R.: Influence of silicon supplementation on the growth and tolerance to high temperature in *Salvia splendens*. – Hortic. Environ. Biote. **55**: 271-279, 2014.

Strasser R.J., Srivastava A., Tsimilli-Michael M.: The fluorescence transient as a tool to characterize and screen photosynthetic samples. – In: Yunus M., Pathre U., Mohanty P. (ed.): Probing Photosynthesis: Mechanism, Regulation and Adaptation. Pp. 443-480. Taylor and Francis, London 2000.

Strasser R.J., Tsimilli-Michael M., Srivastava A.: Analysis of the chlorophyll *a* fluorescence transient. – In: Papageorgiou G.C., Govindjee (ed.): Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Advances in Photosynthesis and Respiration. Pp. 321-362. Springer, Dordrecht 2004.

Tankari M., Wang C., Zhang X. *et al.*: Leaf gas exchange, plant water relations and water use efficiency of *Vigna unguiculata* L. Walp inoculated with rhizobia under different soil water regimes. – Water **11**: 498, 2019.

Tóth S.Z., Schansker G., Garab G., Strasser R.J.: Photosynthetic electron transport activity in heat-treated barley leaves: The role of internal alternative electron donors to photosystem II. – BBA-Bioenergetics **1767**: 295-305, 2007.

Yamane Y., Kashino Y., Koike H., Satoh K.: Effects of high temperatures on the photosynthetic systems in spinach: Oxygen-evolving activities, fluorescence characteristics and the denaturation process. – Photosynth. Res. **57**: 51-59, 1998.

Zanetti L.V., Milanez C.R.D., Gama V.N. *et al.*: Leaf application of silicon in young cacao plants subjected to water deficit. – Pesqui. Agropecu. Bras. **51**: 215-223, 2016.

Zhang C., Moutinho-Pereira J.M., Correia C. *et al.*: Foliar application of Sili-K® increases chestnut (*Castanea* spp.) growth and photosynthesis, simultaneously increasing susceptibility to water deficit. – Plant Soil **365**: 211-225, 2013.

Zheng G., Tian B., Zhang F. *et al.*: Plant adaptation to frequent alterations between high and low temperatures: Remodeling of membrane lipids and maintenance of unsaturation levels. – Plant Cell. Environ. **34**: 1431-1442, 2011.

Zhori A., Meco M., Brandl H., Bachofen R.: *In situ* chlorophyll fluorescence kinetics as a tool to quantify effects on photosynthesis in *Euphorbia cyparissias* by a parasitic infection of the rust fungus *Uromyces pisi*. – BMC Res. Notes **8**: 698, 2015.

Živčák M., Brestič M., Olšovská K., Slamka P.: Performance index as a sensitive indicator of water stress in *Triticum aestivum* L. – Plant Soil Environ. **54**: 133-139, 2008.