

# The second heat wave leads to changes in the efficiency of alfalfa's photosynthetic energy use

Irena Januškaitienė

Vytautas Magnus University,  
K. Donelaičio St. 58,  
Kaunas 44248, Lithuania

Extreme climatic events, which have become more common, more intense, and more frequent, threaten crop productivity and food security. The aim of this study was to investigate the response of chlorophyll *a* fluorescence parameters of *Medicago sativa* L. under regulated environment and recurrent heat waves stress effect. Plants were grown in pots. Two four-day heat waves (35/28°C day/night temperature) were simulated simultaneously with drought (10% soil moisture). Each four-day heat wave was followed by a five-day recovery period. Measurements of chlorophyll *a* fluorescence parameters were taken on the last, fourth, day of the exposure of each heat wave and after each recovery period. Results of this study showed that both the first and second heat waves significantly reduced the quantum yield of PSII photochemistry. During the second heat wave, the negative effects persisted, but were already weaker. The same pattern of change was found for the performance index (PI<sub>ABS</sub>). PI<sub>ABS</sub> decreased by 54.0% and 46.8% during the first and second heat waves, respectively, compared to controls ( $p < 0.05$ ). Despite the increase in absorbed and trapped energy due to the effect of the heat waves, the electron transport rate from Q<sub>A</sub> to Q<sub>B</sub> in the plants exposed to heat waves was not followed by an increase. The above-mentioned changes in the viability of the photosystem may have been caused by a decrease in the density of the active reaction centers (RC/CS<sub>o</sub>) and an increase in the amount of dissociated energy (DIO/CS<sub>o</sub>). After the second recovery period, the RC/CS<sub>o</sub> in heat and drought stressed plants was equal to that of control plants, which resulted in lower energy waste in the form of heat (DIO/CS<sub>o</sub>).

**Keywords:** recurrent heat wave, *Medicago sativa* L., chlorophyll fluorescence, forage crops

## INTRODUCTION

Climate change has had enormous impacts on both human and environmental processes (IPCC, 2021). Extreme heat waves are expected

to have – and may already have – major impacts on crop productivity and natural ecosystems (IPCC, 2007). Regional crop output in Europe, North America, and Asia has dramatically decreased as a result of extreme weather occurrences (Lesk et al., 2016). The heat wave in Europe in summer 2003 reduced total EU agricultural sector

\* Corresponding author. Email: irena.januskaitiene@vdu.lt

production by more than 10% (COPA COGECA, 2003), forest growth by up to 50% in some areas (Bertini et al., 2011), and gross primary output by 30% (Ciais et al., 2005). Over the past two years, the overall EU barley crop has shrunk mostly as a result of extreme weather conditions including heat waves in southern Europe and heavy rains in the Nordic and Baltic nations (European Union, 2018). The frequency and severity of extreme weather events, changes in air temperature, and precipitation climate have an impact on crop yields. Consequently, food security on a local, regional, and global scale is likely to be seriously compromised by climate change and the direct and indirect effects of variability on agricultural production systems. The impact of weather conditions on crop yields during the growing season has been the subject of numerous studies (Zhu et al., 2019; dos Santos et al., 2022).

Global crop production has become severely restricted by heat and drought (Fahad et al., 2017). According to studies, climate change will make cropland more susceptible to drought stress, and strong regional heat waves will become more common in the future. In addition to altering the distribution of rain, rising temperatures will result in a lack of atmospheric water in several regions (Dezsi et al., 2018). Therefore, it is essential to comprehend the response of plants to heat and drought stress in order to maintain agricultural productivity in the face of climatic change (Fahad et al., 2017).

One of the most hazardous plant stressors is heat stress, which has a direct impact on plant metabolism and other concomitant stressful events that often arise and amplify the overall detrimental effects on plant life (Pšidová et al., 2018). Under perfect water availability, heat can have a species-specific effect that is minor or even beneficial; nevertheless, frequent droughts drastically amplify the detrimental effects on C absorption and development (Siebers et al., 2017; Pérez-Jiménez et al., 2019). Therefore, a large leaf water pressure deficit that results in diminished transpirational cooling has a number of direct and indirect impacts on plants. Protein dena-

turation (Kim, Hwang, 2015), impaired thylakoids membrane functions (Schrader et al., 2004), increased photorespiration rate and inhibition of reactions in PSI and cytochrome complex, Rubisco deactivation (Sharkey, 2005; Sharkey, Zhang, 2010), restrictions on RuBP-regeneration (Rashid et al., 2018), and other effects are some of the primary effects of such heat and drought waves. Thus, photosynthesis is one of the physiological processes in plants that is most susceptible to heat, with PSII being the most vulnerable element (Mathur et al., 2014). The following effects of heat stress on photosynthetic processes range from suppression to destruction, such as decrease in CO<sub>2</sub> assimilation due to RuBisCO activase inhibition; disintegration of chlorophyll molecules; increase in the fluidity of thylakoid membranes; disorganization of enzymes; separation of the reaction centers from PSII; and/or damage to the electron transport chain and oxygen-evolving system (Kalaji et al., 2016; Guha et al., 2018). Following these processes, the net photosynthetic rate decreases, reactive oxygen species (ROS) and oxidative damage are synthesised more often, phenological development is hastened, and the growth and productivity of many plant species are reduced (Siebers et al., 2015; Žaltauskaitė et al., 2019). Direct impacts of heat and drought stress usually dominate indirect effects, which are typically small and brought on by extended stomatal closure and a drop in intracellular CO<sub>2</sub> (Macabuhay et al., 2018; Rashid et al., 2018).

The length and size of the initial event and the timing of the subsequent severe event might affect the specific plant and its capacity to recover in the interim and following the two events (Backhaus et al., 2014). Recent research has also shown that plants may develop a stress memory that will help them handle repeated stress better. According to the definition of stress memory, plants under stress undergo genetic, epigenetic, and physiological changes that alter how they react to future stressors, either in the same generation (within-generation priming) or in the generation after that (trans-generation) (Walter et al., 2013; Bej, Basak,

2017; Lukić et al., 2020). Prior stress exposure has been found to increase plants' resistance to subsequent challenges (Wang et al., 2021). Induced higher tolerance to future shocks enhances long-term output, even while acute stress responses may impair plant productivity (i.e., plant performance) (Zamorano et al., 2021). Therefore, the main question raised in this study was whether the recurrent heat wave would have a less significant negative effect on the alfalfa photosystem or whether the effect would just be stronger.

## MATERIALS AND METHODS

The experiments were carried out in two closed controlled environment plant growth chambers located at Vytautas Magnus University (Lithuania), with each chamber volume of 10 m<sup>3</sup>. Alfalfa (*Medicago sativa* L., var. 'Malvina') plants were sown in pots filled with a mixture of field top-soil, perlite, and fine sand (5:3:2, by volume). The alfalfa seeds were provided by the Institute of Agriculture of the Lithuanian Research Centre for Agriculture and Forestry. Before sowing, the commercial fertiliser (NPK 8-19-29) (Achema, Lithuania) was applied at a rate of 310 kg ha<sup>-1</sup> to reach background levels of nitrogen (N), phosphorus (P), and potassium (K) of 25–60–90 kg ha<sup>-1</sup>. Seedlings were thinned, leaving 16 plants per pot at a two-leaf stage, BBCH 12 according to the BBCH growth scale (Meier, 2001). Plants were grown at 21/14±1°C (day/night), the relative air humidity (RH) was 50–60%. The photosynthetic active radiation (PAR ~270 μmol m<sup>-2</sup> s<sup>-1</sup>) was provided by a combination of natural daylight luminescent lamps and one high-pressure sodium lamp and a day length of 14 h with lights on at 8:00 and lights off at 22:00. Plants were watered every second day and volumetric soil water content (SWC) was kept at 30% (Delta-T Devices Ltd., Cambridge, UK). Pots were regularly rotated to minimise potential effects of position on plant performance. Control alfalfa plants were grown repeatedly in different experimental set-ups without significant differences between independent experiments.

The heat waves treatments were imposed when alfalfa plants were at 40 growth stage, according to the BBCH growth scale (Meier, 2001). Two 4-day heat waves (35/28°C day/night temperature) were simulated together with drought (10% soil moisture). The 4-day heat wave was followed by a 5-day recovery period. An analogous heat wave effect and recovery experiment was repeated, i.e., plants were re-exposed to the second heat wave (4-day) and re-evaluated for their recovery potential after five days once again.

**Chlorophyll *a* fluorescence.** The Plant Efficiency Analyser, PEA (Hansatech Instruments, Ltd., King's Lynn, Norfolk, England) was used to measure the parameters of chlorophyll *a* fluorescence on the fourth day after exposure to each heat wave and following each recovery phase. The leaves were pre-darkened with clips for 15 min prior the measurements. The transients of chlorophyll fluorescence were induced by 1 s illumination with an array of three light emitting diodes (LEDs) providing a maximum light intensity of 1800 μmol (photon) m<sup>-2</sup> s<sup>-1</sup> and a homogenous irradiation over a 4-mm diameter of leaf area. The fast fluorescence kinetics ( $f_0$  to  $f_M$ ) were recorded from 10 μs to 1 s. The fluorescence intensity at 50 μs was considered as  $f_0$  (Rasineni et al., 2011).

**Analysis of the fluorescence transients using the JIP-test.** WINPEA 32 software was used to transmit raw fluorescence OJIP transients, which represent the reduction of the photosynthetic electron transport chain (Strasser, Strasser, 1995; Lin et al., 2009; Rasineni et al., 2011). The translation of the measured parameters into JIP-test parameters provided information on the stepwise flow of energy through PSII at different levels such as specific fluxes on the level of the excited leaf cross-section (CS) (absorption (ABS/CS<sub>0</sub>), trapping (TRo/CS<sub>0</sub>), dissipation (DIO/CS<sub>0</sub>) and electron transport (ETo/CS<sub>0</sub>)).  $\Psi_0$ , or the probability that an electron will move further than  $Q_A^-$ , was calculated by the formula:  $(F_p - F_j)/(F_p - F_{50 \mu s})$ , where  $F_p$  = Fluorescence maximum in OJIP transient,  $F_j$  and  $F_{50 \mu s}$  = Fluorescence yield at point *J* and at 50 μs. Normalised total complementary area

above the O–J–I–P transient (reflecting multiple turnover  $Q_A$  reduction events) was calculated as  $S_m [S_m = (\text{Area}) / (F_p - F_{50 \mu s})]$ . Density of RCs (QA-reducing PSII reaction centers) was calculated as  $\text{RC/CSO} = \Phi_{p_0} (V_j/M_0)$  (ABS/CSO) (Lin et al., 2009, Rasinen et al., 2011; Januškaitienė et al., 2021; Januškaitienė et al., 2022).

**Measurements of biomass harvest.** Five randomly selected plants per pot were utilised for the growth measurements after the drought treatments and the recovery period. Biomass from the surface and the roots was dried at 60°C to a consistent dry weight and weighed. The samples' biomasses were converted into dry biomass per plant.

**Statistical analysis.** The least significant differences (LSD) test procedure was applied to estimate the difference between different treatment values in all parameters and  $p < 0.05$  was the threshold for significance.

## RESULTS AND DISCUSSION

Heat stress heightens the risk of water shortage in plants, which can significantly harm photosynthesis and carbon absorption in vegetation (Frank et al., 2015; Sippel et al., 2018). Bilger et al. (1984) and Hueve et al. (2011) discovered that the intensity and length of a heat exposure affected how well the leaves function. Heat stress results in increased photorespiration and mitochondrial respiration as well as reduced photosynthesis and stomatal conductance, all of which are temporary and do not affect the plant over the long term (Doughty, Goulden, 2008; Niinemets, 2018). The results obtained during this study showed that both the first and the second heat stress negatively affected alfalfa plants (Fig. 1–5). Rehydration and a week-long recovery period following each stress helped to neutralise this effect in the plants tested. We used chlorophyll *a* fluorescence signals as an internal plant state sensor to track different steps of excitation energy transformation to determine plant heat tolerance levels. This method is widely used

by other researchers as well (Goltsev et al., 2012; Çiçek et al., 2015; Mlinarić et al., 2017; Stirbet et al., 2018). The OJIP curves include important details regarding the composition and operation of the photosynthetic apparatus (Goltsev et al., 2016, Stirbet et al., 2018). The O–J is a representation of the PSII acceptor side's reduction (and also gives information on the connectivity between the PSII photosynthetic units), the J–I indicates that the PQ pool has been reduced partially, and the I–P indicates that the acceptor side of PSI has been reduced (Yusuf et al., 2010; Ripoll et al., 2016). OJIP transients in Fig. 1 demonstrated that both heat waves had cumulative effect over the photosynthetic electron transport on thylakoid membranes. Already during the first step, a sudden rise in the fluorescence flux of heat-affected plants is clearly visible compared to the control. An increase in the K bands was visible at the beginning of the fluorescence intensity; these changes may indicate impaired energy transfer during light harvesting and inactivation of the oxygen-released complex (Yusuf et al., 2010, Mlinarić et al., 2017). The incompatibility of the acceptor and donor sides of PSII could have been caused by a greater inactivation of the oxygen-evolving complex, as previously reported by Oukarroum et al. (2007). Meanwhile, at the last stage of the fluorescence transit, different patterns of fluorescence intensity were observed than at the beginning. The I–P amplitude of heat-stressed alfalfa plants decreased compared to control plants. Additionally, the J and I steps started 1 and 10 ms later respectively, than they should have at 2 and 30 ms, respectively. Fluorescence intensity variations were seen in both the lengthening of transit amplitudes and the height of JIP steps. Fluorescence intensity of alfalfa plants decreased during heat waves. This trend was evident in both the J and P steps, where statistically significant differences between control and heat-stressed plants were discovered. The reduced I and P amplitudes that resulted from the heat wave may have limited the electron transport on the PSI

acceptor side (Çiçek et al., 2015). Both the first and the second recovery periods led to the recovery of the mentioned indicators almost to the control values for all heat-stressed plants (Fig. 1). In addition, a stronger recovery was evident after the second heat wave, when differences in fluorescence between control and heat-stressed plants decreased in the P step.

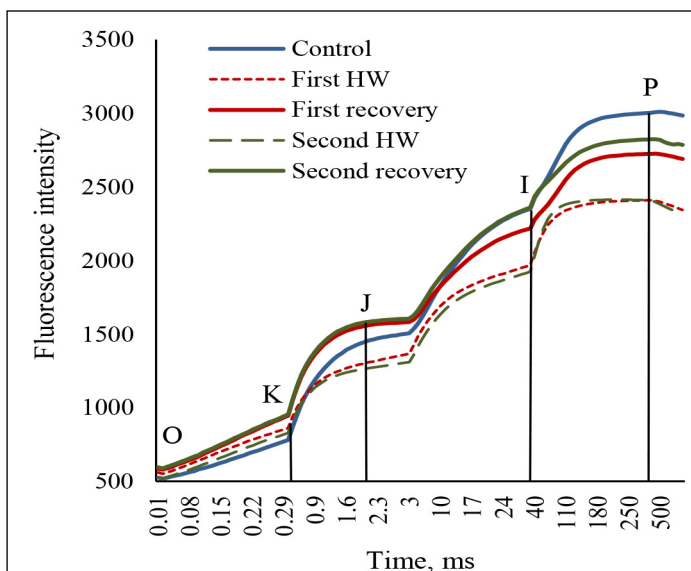
An early sign of severe heat stress is a reduction in photosynthetic electron transfer capability brought on by degradation of thylakoid membrane integrity and inactivation of the water decomposition complex of photosystem II (PSII) (Teskey et al., 2015; Yamamoto, 2016; Ninemets, 2018). Inflicting irreversible (or at least less reversible) damage to photosynthetic biochemistry and leaf tissue when heat tolerance thresholds are exceeded for extended periods of time and/or at high temperatures (Hüve et al., 2011) has a long-term impact on carbon absorption throughout the plant (Tarvainen et al., 2022). According to the findings of this study, both the first and the second heat waves lowered considerably the quantum yield of PSII photochemistry ( $Fv/Fm$ ) (Fig. 2). The largest change in the maximum efficiency of PSII activity was found during the first heat wave, a significant difference remained after the recovery period. During the second heat wave, the maximum efficiency of the second photosystem decreased compared to the control, but the change was

already somewhat smaller than during the first wave, and after the second recovery period, there were no significant differences between the heat wave affected and control plants.

The same pattern of change during the first wave was also found for the performance index ( $PI_{ABS}$ ) parameter (indicator of the relative viability of photosynthesis) but during the second heat wave exposure; the negative effect on this indicator decreased to statistically insignificant compared to the control plants.

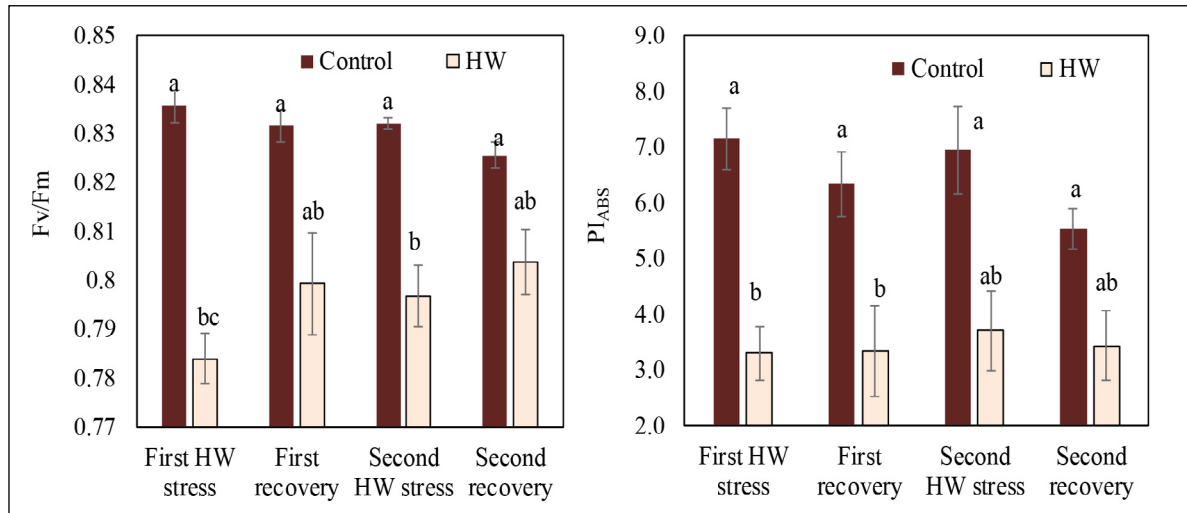
As in the case of the PSII photochemistry  $Fv/Fm$  indicator, after the second recovery period, the photosynthetic viability did not differ statistically significantly between the plants exposed to the two heat waves and the control plants. The performance index ( $PI_{ABS}$ ) combines the response of PSII to photochemical and non-photochemical properties and the density of active RCs per chlorophyll. The  $PI_{ABS}$  may be the most sensitive experimentally-derived parameter to stress (Lou et al., 2016). Figure 2 shows that photosynthesis viability decreased by 54.0% and 46.8% during the first and second heat waves, respectively, compared to controls ( $p < 0.05$ ). Again, 9.56% lower  $PI_{ABS}$  losses were found after the second heat wave recovery period.

Compared to controls, the absorbed energy flux per CS (excited cross section) ( $ABS/CS_0$ ) increased both during heat waves and after



**Fig. 1.** The OJIP transients of alfalfa (*Medicago sativa* L.) after two heat wave events and recovery periods. Control – reference treatment. HW – heat wave treatment

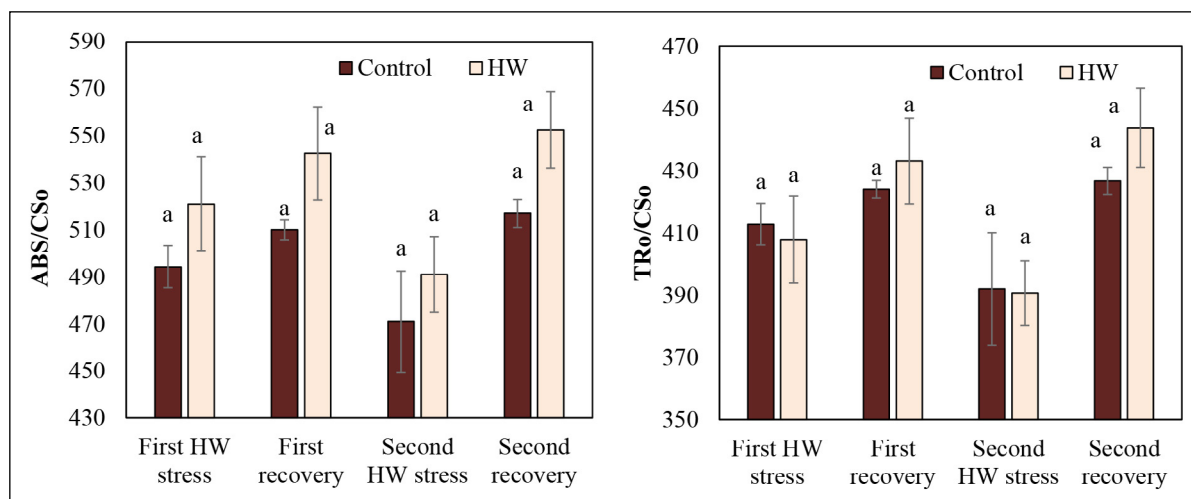




**Fig. 2.**  $F_v/F_m$  ratio and performance index (PI<sub>abs</sub>) on absorption basis of alfalfa (*Medicago sativa* L.) after two heat wave events and recovery periods. Control – reference treatment. HW – heat wave treatment. The statistically significant differences among the treatments are labelled with different lowercase letters at  $p < 0.05$  (Fisher's LSD). The error bars represent standard errors of means ( $n = 5$ )

regeneration periods (Fig. 3). Also, there was a slight increase in trapped energy flux per CS (TRo/CSo) during all phases of the experiment. However, changes in these indicators due to the effects of both heat waves were insignificant. Other scientists have also noted a comparable rise in absorbed energy flow (ABS/CSo) under the effect of heat (Feijão et al., 2018). The electron transport rate from  $Q_A$  to

$Q_B$  (ETo/CSo) in heat-exposed plants was not detected (Fig. 4), when the energy used for electron transport (ETo/CSo) has decreased ( $p > 0.05$ ), despite the rise in absorbed (ABS/CSo) and trapped energy (TRo/CSo) brought on by the effects of the heat wave. The efficiency of the photosystem II was reduced, and the photoprotective mechanisms were activated as was previously reported for higher plants

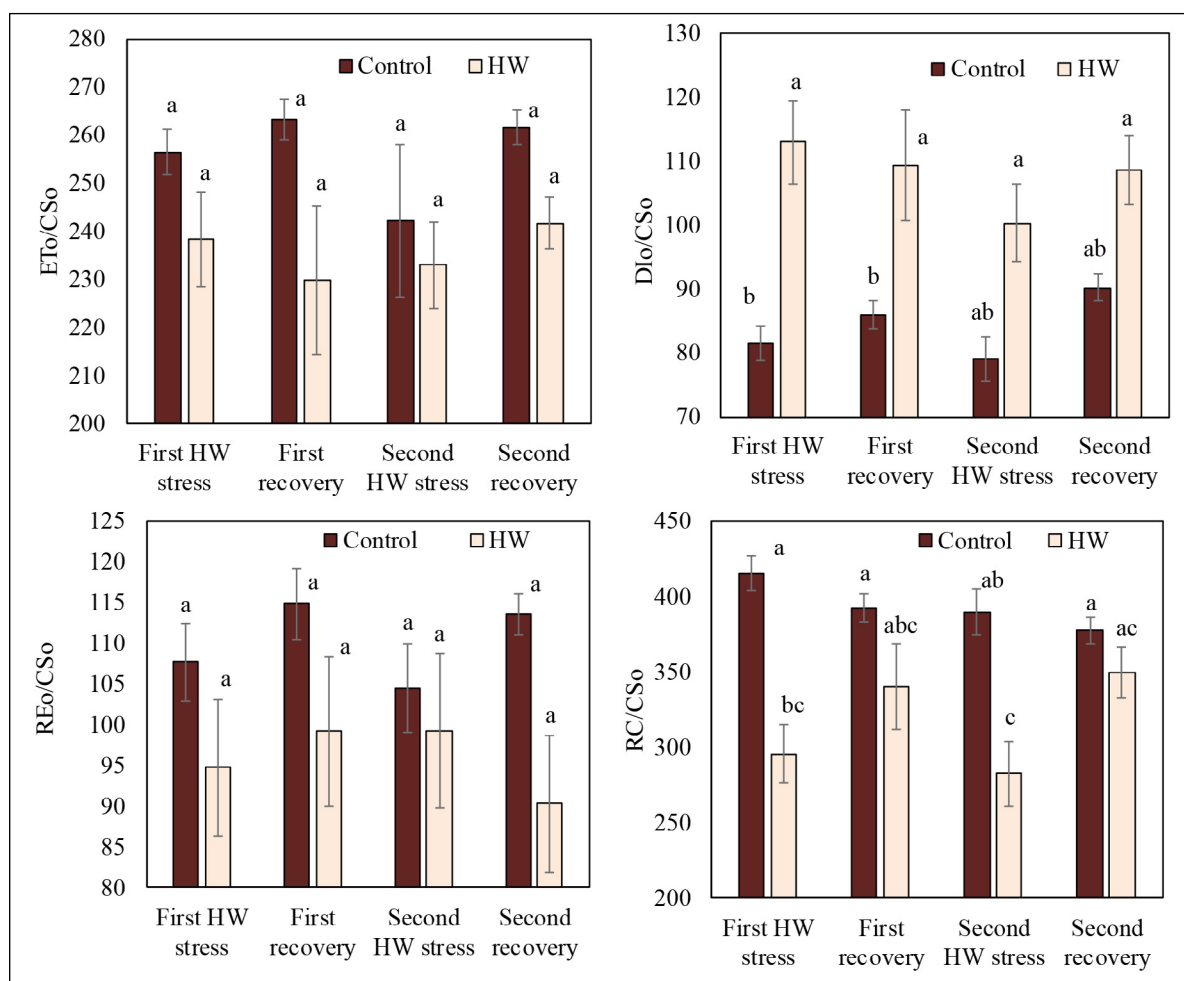


**Fig. 3.** Absorption flux per CS (ABS/CSo) and trapped energy flux per CS (TRo/CSo) of alfalfa (*Medicago sativa* L.) after two heat wave events and recovery periods. Other designations as in Fig. 2

as shown in Fig. 2. As a result, the excess energy that could not be quickly transported had to be dissipated as heat (Duarte et al., 2015). The variation in the ABS/CS<sub>o</sub> might be due to variations in the ratio of inactive RCs or variations in the number of LHC complexes per cross section (Çiçek et al., 2019).

The above-mentioned changes in the viability of the photosystem (Fig. 2) may have been due to an increase in the amounts of dissociated energy DIo/CS<sub>o</sub> in plants during the first and second heat waves (Fig. 4). For instance, during the first heat wave and first recovery period, DIo/CS<sub>o</sub> of alfalfa increased by 38.9% and 27.3% respectively, compared to control

plants ( $p < 0.05$ ). During the second heat wave, the dissociated energy DIo/CS<sub>o</sub> in heat stressed plants also increased, but the change was smaller (27.0%) and statistically insignificant ( $p > 0.05$ ). After the second recovery period, the differences between heatwave-exposed and control plants decreased even more to 20.4% ( $p > 0.05$ ). However, it should be noted that the amount of dissociated energy after the second regeneration period was slightly lower than after the first one. Meanwhile, the energy used for the regeneration of reaction centers (REo/CS<sub>o</sub>) and amount of active PSII RCs per CS (RC/CS<sub>o</sub>) have decreased both during and after the recovery periods of both heat waves.

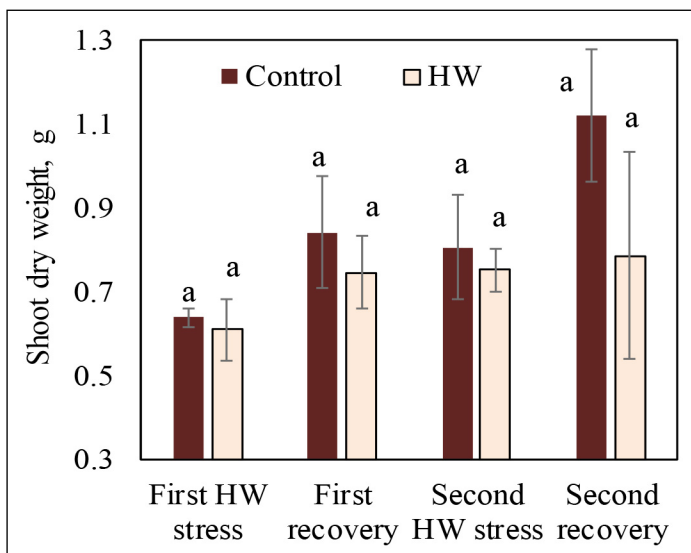


**Fig. 4.** Electron transport flux per CS (ETo/CS<sub>o</sub>), dissipated energy flux per CS (DIo/CS<sub>o</sub>), reduction of end acceptors at PSI electron acceptor side per CS (REo/CS<sub>o</sub>), and density of RCs (QA-reducing PSII reaction centers) (RC/CS<sub>o</sub>) of alfalfa (*Medicago sativa* L.) after two heat wave events and recovery periods. Other designations as in Fig. 2

Changes in the amount of energy allocated to the regeneration of reaction centers (REo/CSo) due to the effects of heat waves were statistically insignificant and remained so even after recovery periods. On the contrary, changes in the amount of active reaction centers (RC/CSo) during both the first and the second heat wave were significant. As in the case of the indicators discussed above, the first heat wave had a greater negative impact on the activity of reaction centers, when the amount of active reaction centers decreased by 30% and 26% during the first and second heat waves respectively, compared to control plants ( $p < 0.05$ ). At the end of recovery periods, the amount of active reaction centers (RC/CSo) of both heat waves did not differ statistically significantly from the control. Other studies have also shown that the effectiveness of PSII is reduced in stressed plants. When high

levels of energy dissipation (D<sub>Io</sub>/CS<sub>o</sub>) and low electron transfer (E<sub>To</sub>/CS<sub>o</sub>), and large number inactive reaction centers compared to the respective controls were detected (Çiçek et al., 2019). Kalaji et al. (2011) suggested that the accumulation of inactive reaction centers might be associated with the increase in heat dissipation of excitation energy under stress. In the present study, heat wave stress also caused the inactivation of reaction centers in affected plants and thus the absorbed energy was dissipated as heat (higher values of D<sub>Io</sub>/CS<sub>o</sub>) (Fig. 4).

As the photochemical properties of PSII changed, the loss of dry biomass during the first and second heat waves increased by 4.5% and 6.8%, respectively ( $p > 0.05$ ) (Fig. 5). Thus, the effect of the studied heat waves on alfalfa biomass was not significant, and it persisted even after the recovery periods when the biomass of



**Fig. 5.** Shoot dry weight of alfalfa (*Medicago sativa* L.) after two heat wave events and recovery periods. Other designations as in Fig. 1

alfalfa shoots affected by the heat wave did not differ statistically significantly from the control plants. The literature provides very different information on the effects of heat waves: even different genotypes of the same agricultural species may respond differently to droughts or hot weather situations (Feller, 2016). The most common effects of heat are reduced leaf area, effects on vegetative and reproductive growth, and a reduced number, mass, and growth of roots (Iqbal et al., 2023). Information can also

be found that some plants are less affected by heat stress: for example, higher heat tolerance of plants, due to various resistance mechanisms, helps them to cope with heat conditions (Kumari et al., 2020). And discussed above, the response of different plants to heat waves can be observed in the activity of photosystem II. In this study, the effect of the first heat wave was stronger compared to the second, when changes in the energy distribution of photosystem II showed that alfalfa used energy more efficiently



during the second stress. Positive changes can also be seen when comparing the first and second recovery periods, while after the second recovery period, the density of alfalfa active reaction centers (RC/CSo) affected by heat and drought stress plants was equal to the density of control plants. Undoubtedly, this led to a statistically significantly lower energy waste in the form of heat (DIO/CSo) and a more intensive recovery after stress, which was indicated by an increased amount of absorbed (ABS/CSo) and trapped energy (TRo/CSo).

## CONCLUSIONS

Both the first and second heat waves significantly reduced the quantum yield of PSII photochemistry ( $F_v/F_m$ ), but the negative effects of the second heat wave were already weaker. The same pattern of change was found for the performance index ( $PI_{ABS}$ ). Photosynthesis viability  $PI_{ABS}$  decreased by 54.0% and 46.8% during the first and second heat waves, respectively, compared to controls ( $p < 0.05$ ).

Despite the increase in absorbed (ABS/CSo) and trapped energy (TRo/CSo) due to heat wave exposure, the electron transport rate from  $Q_A$  to  $Q_B$  (ETo/CSo) was not observed in plants exposed to heat wave.

The changes in the viability of the photosystem may have been caused by a decrease in the density of the active reaction centers RC/CSo and an increase in the amount of dissociated energy DIO/CSo.

After the second recovery period, the density of alfalfa active reaction centers (RC/CSo) after heat and drought stress was equal to that of control plants, and the amount of dissociated energy DIO/CSo was slightly lower.

## ACKNOWLEDGEMENTS

This research received funding from the Research Council of Lithuania (grant number: S-SIT-20-4) as part of the research project 'Sustainable forage crops productivity under climate extremes: resilience, nutritional quality and implications for future management' with-

in the National Research Programme 'Sustainability of Agro, Forest and Water Ecosystems'.

Received 24 March 2023

Accepted 4 April 2023

## References

1. Backhaus S, Kreyling J, Grant K, Beierkuhnlein C, Walter J, Jentsch A. Recurrent mild drought events increase resistance toward extreme drought stress. *Ecosystems*. 2014; 17: 1068–81.
2. Bej S, Basak J. Abiotic stress induced epigenetic modifications in plants: How much do we know? In: Rajewsky N, Jurga S, Barciszewski J, eds. *Plant epigenetics*. Cham: Springer, 493–512; 2017. p. 493–512.
3. Bertini G, Amoriello T, Fabbio G, Piovosi M. Forest growth and climate change: Evidences from the ICP-Forests intensive monitoring in Italy. *iForest*. 2011; 4: 262–7.
4. Bilger HW, Schreiber U, Lange OL. Determination of leaf heat resistance: comparative investigation of chlorophyll fluorescence changes and tissue necrosis methods. *Oecologia*. 1984; 63: 256–62.
5. Ciais P, Reichstein M, Viovy N, Granier A, Ogee J, Allard V, Valentini R. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature*. 2005; 437: 529–33.
6. Çiçek N, Pekcan V, Arslan Ö, Çulha Erdal Ş, Balkan Naçaiyi AS, Çil AN, et al. Assessing drought tolerance in field-grown sunflower hybrids by chlorophyll fluorescence kinetics. *Braz J Bot*. 2019; 42: 249–60.
7. COPA COGECA. Assessment of the impact of the heat wave and drought of the summer 2003 on agriculture and forestry. Fact sheets of the Committee of Agricultural Organisations in the European Union and the General Committee for Agricultural Cooperation in the European Union. 2003.

8. Dezsi Ş, Míndrescu M, Petrea D, Rai PK, Hamann A, Nistor MM. High-resolution projections of evapotranspiration and water availability for Europe under climate change. *Int J Climatol*. 2018; 38: 3832–41.
9. dos Santos CAC, Neale CMU, Mekonnen MM et al. Trends of extreme air temperature and precipitation and their impact on corn and soybean yields in Nebraska, USA. *Theor Appl Climatol*. 2022; 147: 1379–99.
10. Are tropical forests near a high temperature threshold? *J Geophys Res Biogeosci*. 2008; 113: 1–12.
11. Duarte B, Goessling JW, Marques JC, Caçador I. Ecophysiological constraints of *Aster tripolium* under extreme thermal events impacts: Merging biophysical, biochemical and genetic insights. *Plant Physiol Biochem*. 2015; 97: 217–28.
12. European Union. Short-term outlook for EU agricultural markets in 2018 and 2019. 2018. ([http://ec.europa.eu/agriculture/markets-and-prices/short-term-outlook/index\\_en.htm](http://ec.europa.eu/agriculture/markets-and-prices/short-term-outlook/index_en.htm))
13. Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Sadia S, et al. Crop production under drought and heat stress: plant responses and management options. *Front Plant Sci*. 2017; 8: 1147.
14. Feijão E, Gameiro C, Franzitta M, Duarte B, Caçador I, Cabrita MT, Matos AR. Heat wave impacts on the model diatom *Phaeodactylum tricornutum*: Searching for photochemical and fatty acid biomarkers of thermal stress. *Ecol Indic*. 2018; 9: 1026–37.
15. Feller U. Drought stress and carbon assimilation in a warming climate: Reversible and irreversible impacts. *J Plant Physiol*. 2016; 203: 84–94.
16. Frank D, Reichstein M, Bahn M, Thonicke K, Frank D, Mahecha MD, et al. Effects of climate extremes on the terrestrial carbon cycle: concepts, processes and potential future impacts. *Glob Chang Biol*. 2015; 21: 2861–80.
17. Goltsev V, Zaharieva I, Chernev P, Kouzmanova M, Kalaji HM, Yordanov I, et al. Drought-induced modifications of photosynthetic electron transport in intact leaves: analysis and use of neural networks as a tool for a rapid non-invasive estimation. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*. 2012; 1817: 1490–8.
18. Goltsev VN, Kalaji HM, Paunov M et al. Variable chlorophyll fluorescence and its use for assessing physiological condition of plant photosynthetic apparatus. *Russ J Plant Physiol*. 2016; 63: 869–93.
19. Guha A, Han J, Cummings C, McLennan D, Warren JM. Differential ecophysiological responses and resilience to heat wave events in four co-occurring temperate tree species. *Environ Res Lett*. 2018; 13: 065008.
20. Hueve K, Bichele I, Rasulov B, Niinemets ÜLO. When it is too hot for photosynthesis: heat-induced instability of photosynthesis in relation to respiratory burst, cell permeability changes and H<sub>2</sub>O<sub>2</sub> formation. *Plant Cell Environ*. 2011; 34: 113–26.
21. IPCC, 2007: Intergovernmental Panel on Climate Change. *Climatic Change 2007: the Physical Science Basis*, Cambridge University Press, Cambridge, UK and New York, USA. 2007.
22. IPCC, 2021: Summary for policymakers. In: *Climate change 2021: the physical science basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* [Masson-Delmotte VP, Zhai A, Pirani SL, Connors C, Péan S, Berger N, Caud Y, Chen L, Goldfarb MI, Gomis M, Huang K, Leitzell E, Lonnoy JBR, Matthews TK, Maycock T, Waterfield O, Yelekçi R Yu, Zhou B. (eds.)]. Cambridge University Press. In Press.
23. Iqbal S, Iqbal MA, Li C, Iqbal A, Abbas RN. Overlooking Drought and Heat Stress Amelioration—From Plant Responses to Microbe-Mediated Mitigation. *Sustainability*. 2023; 15: 1671.

24. Januškaitienė I, Kacienė G, Dikšaitytė A, Žaltauskaitė J, Miškelytė D, Sujetovienė G, Juknys R. Nitrogen supplement attenuates drought stress for non-leguminous hybrid plant fescue and does not affect nitrogen-fixing alfalfa. *J Agron Crop Sci.* 2022; 208: 283–94.
25. Januškaitienė I, Dikšaitytė A, Kunigiškytė J. Organic fertilizers reduce negative effect of drought in barely (C3) and millet (C4) under warmed climate conditions. *Arch Agron Soil Sci.* 2021; 1–16.
26. Kalaji HM, Jajoo A, Oukarroum A, Bresic M, Zivcak M, Samborska IA, Cetner MD, Łukasik I, Goltsev V, Ladle RJ. Chlorophyll *a* fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. *Acta Physiol Plant.* 2016; 38: 102.
27. Kalaji HM, Bosa K, Kościelniak J, Żuk-Golaszewska K. Effects of salt stress on photosystem II efficiency and CO<sub>2</sub> assimilation of two Syrian barley landraces. *Environ Exp Bot.* 2011; 73: 64–72.
28. Kim NH, Hwang BK. Pepper heat shock protein 70a interacts with the type III effector AvrBsT and triggers plant cell death and immunity. *Plant Physiol.* 2015; 167: 307–22.
29. Kumari P, Rastogi A, Yadav S. Effects of Heat stress and molecular mitigation approaches in orphan legume, Chickpea. *Mol Biol Rep.* 2020; 47: 4659–70.
30. Lesk C, Rowhani P, Ramankutty N. Influence of extreme weather disasters on global crop production. *Nature.* 2016; 529: 84–8.
31. Lin ZH, Chen LS, Chen RB, Zhang FZ, Jiang HX, Tang N. CO<sub>2</sub> assimilation, ribulose-1,5-bisphosphate carboxylase/oxygenase, carbohydrates and photosynthetic electron transport probed by the JIP-test, of tea leaves in response to phosphorus supply. *BMC Plant Biol.* 2009; 9: 43.
32. Lukić N, Kukavica B, Davidović-Plavšić B, Hasanagić D, Walter J. Plant stress memory is linked to high levels of anti-oxidative enzymes over several weeks. *Environ Exp Bot.* 2020; 178: 104166.
33. Luo HH, Merope TM, Zhang YL, Zhang WF. Combining gas exchange and chlorophyll *a* fluorescence measurements to analyze the photosynthetic activity of drip-irrigated cotton under different soil water deficits. *J Integ Agricul.* 2016; 15(6): 1256–66.
34. Macabuhay A, Houshmandfar A, Nuttall J, Fitzgerald GJ, Tausz M, Tausz-Posch S. Can elevated CO<sub>2</sub> buffer the effects of heat waves on wheat in a dryland cropping system? *Environ Exp Bot.* 2018; 155: 578–88.
35. Mathur S, Agrawal D, Jajoo A. Photosynthesis: Response to high temperature stress. *J Photochem Photobiol.* 2014; 137: 116–26.
36. Meier U. Growth stages of mono- and dicotyledonous plants, BBCH monograph. Blackwell Wissenschafts-Verlag. 2001, pp. 204.
37. Mlinarić S, Antunović, Dunić J, Skendrović Babojelić M. et al. Differential accumulation of photosynthetic proteins regulates diurnal photochemical adjustments of PSII in common fig (*Ficus carica* L.) leaves. *J Plant Physiol.* 2017; 209: 1–10.
38. Niinemets Ü. When leaves go over the thermal edge. *Plant Cell Environ.* 2018; 41: 1247–50.
39. Oukarroum A, Madidi SE, Schansker G, Strasser RJ. Probing the responses of barley cultivars (*Hordeum vulgare* L.) by chlorophyll *a* fluorescence OLKJIP under drought stress and re-watering. *Environ Exp Bot.* 2007; 60: 438–46.
40. Pérez-Jiménez M, Piñero MC, del Amor FM. Heat shock, high CO<sub>2</sub> and nitrogen fertilization effects in pepper plants submitted to elevated temperatures. *Sci Hortic.* 2019; 244: 322–9.
41. Pšidová E, Živčák M, Stojnić S, Orlović S, Gömöry D, Kučerová J, et al. Altitude of origin influences the responses of PSII photochemistry to heat waves in European beech (*Fagus sylvatica* L.). *Environ Exp Bot.* 2018; 152: 97–106.
42. Rashid MA, Andersen MN, Wollenweber B, Kørup K, Zhang X, Olesen JE. Impact of heat-wave at high and low VPD on photosynthetic

- components of wheat and their recovery. *Environ Exp Bot.* 2018; 147: 138–46.
43. Rasineni GK, Guha A, Reddy AR. Elevated atmospheric CO<sub>2</sub> mitigated photoinhibition in a tropical tree species, *Gmelina arborea*. *J Photochem Photobiol B.* 2011; 103: 159–65.
  44. Schrader SM, Wise RR, Wacholtz WF, Ort DR, Sharkey TD. Thylakoid membrane responses to moderately high leaf temperature in Pima cotton. *Plant Cell Environ.* 2004; 27: 725–35.
  45. Sharkey TD, Zhang R. High temperature effects on electron and proton circuits of photosynthesis. *J Integr Plant Biol.* 2010; 52: 712–22.
  46. Sharkey TD. Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. *Plant Cell Environ.* 2005; 28: 269–77.
  47. Siebers MH, Slattery RA, Yendrek CR, Locke AM, Drag D, Ainsworth EA, et al. Simulated heat waves during maize reproductive stages alter reproductive growth but have no lasting effect when applied during vegetative stages. *Agric Ecosyst Environ.* 2017; 240: 162–70.
  48. Siebers MH, Yendrek CR, Drag D, Locke AM, Rios Acosta L, Leakey AD, et al. Heat waves imposed during early pod development in soybean (*Glycine max*) cause significant yield loss despite a rapid recovery from oxidative stress. *Glob Chang Biol.* 2015; 21: 3114–25.
  49. Sippel S, Reichstein M, Ma X, Mahecha MD, Lange H, Flach M, Frank D. Drought, heat, and the carbon cycle: a review. *Curr Clim Change Rep.* 2018; 4: 266–86.
  50. Stirbet A, Lazá D, Kromdijk J, Govindjee. Chlorophyll *a* fluorescence induction: Can just a one-second measurement be used to quantify abiotic stress responses? *Photosynthetica.* 2018; 56: 86–104.
  51. Strasser BJ, Strasser RJ. Measuring fast fluorescence transients to address environment questions: the JIP-test P. Mathis (Ed.), *Photosynthesis: From Light to Biosphere*, Kluwer Academic, Dordrecht-Boston, London. 1995; 977–80.
  52. Tarvainen L, Wittemann M, Mujawamariya M, Manishimwe A, Zibera E, Ntirugulirwa B, et al. Handling the heat–photosynthetic thermal stress in tropical trees. *New Phytol.* 2022; 233: 236–50.
  53. Teskey R, Wertin T, Bauweraerts I, Ameye M, McGuire MA, Steppe K. Responses of tree species to heat waves and extreme heat events. *Plant Cell Environ.* 2015; 38: 1699–712.
  54. Walter J, Jentsch A, Beierkuhnlein C, Kreyling J. Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. *Environ Exp Bot.* 2013; 94: 3–8.
  55. Wang X, Chen J, Ge J, Huang M, Cai J, Zhou Q, et al. The different root apex zones contribute to drought priming induced tolerance to a reoccurring drought stress in wheat. *Crop J.* 2021; 9: 1088–97.
  56. Yamamoto Y. Quality control of photosystem II: the mechanisms for avoidance and tolerance of light and heat stresses are closely linked to membrane fluidity of the thylakoids. *Front Plant Sci.* 2016; 7: 1136.
  57. Yusuf MA, Kumar D, Rajwanshi R, Strasser RJ, Tsimilli-Michael M, Govindjee, Sarin NB. Overexpression of  $\gamma$ -tocopherol methyl transferase gene in transgenic *Brassica juncea* plants alleviates abiotic stress: Physiological and chlorophyll *a* fluorescence measurements. *Biochim Biophys Acta.* 2010; 1797: 1428–38.
  58. Žaltauskaitė J, Dikšaitytė A, Miškelytė D, Kacienė G, Sujetovienė G, Januškaitienė I, et al. Does interspecific competition change the barley's response and recovery from heat wave? *J Agron Crop Sci.* 205; 4: 401–13.
  59. Zamorano D, Franck N, Pastenes C, Wallberg B, Garrido M, Silva H. Improved physiological performance in grapevine (*Vitis vinifera* L.) cv. Cabernet Sauvignon facing recurrent drought stress. *Aust J Grape and Wine Res.* 2021; 27: 258–68.
  60. Zhu X, Troy TJ, Devineni N. Stochastically modeling the projected impacts of climate change on rainfed and irrigated US crop yields. *Environ Res Lett.* 2019; 14: 074021.

Irena Januškaitienė

## ANTROJI KARŠČIO BANGA KEIČIA LIUCER- NOS FOTOSINTETINĖS ENERGIJOS NAUDO- JIMO EFEKTYVUMĄ

### *Santrauka*

Dažnesni ir intensyvesni ekstremalūs klimato reiškiniai kelia grėsmę pasėlių produktyvumui. Šio tyrimo tikslas – ištirti *Medicago sativa* L. chlorofilo *a* fluorescencijos rodiklių reakciją reguliuojamoje aplinkoje į pasikartojančių karščio bangų sukeltą stresą. Augalai buvo auginami vegetaciniuose induose. Buvo imituojamos dvi 4 dienų karščio bangos (35 °C dienos ir 28 °C nakties temperatūra) kartu su sausra (10 % dirvožemio drėgmės). Po kiekvienos 4 dienų karščio bangos buvo daroma 5 dienų pertrauka. Chlorofilo *a* fluorescencijos rodikliai matuoti paskutinę (4-ąją) karščio bangos dieną ir po atsistatymo laikotarpio.

Tyrimo rezultatai rodo, kad tiek pirmoji, tiek antroji karščio banga gerokai sumažino FSII fotochemijos kvantinį pajėgumą. Nors neigiamas poveikis išliko ir per antrąją karščio bangą, jis buvo silpnesnis. Toks pat pokyčių dėsningumas buvo būdingas ir fotosintezės našumo indeksui ( $PI_{ABS}$ ). Per pirmą ir antrą karščio bangas  $PI_{ABS}$  sumažėjo atitinkamai 54,0 % ir 46,8 %, palyginti su kontrole ( $p < 0,05$ ). Nors dėl karščio bangų padidėjusi energija buvo sugerta ir sulaikyta, elektronų pernešimo greitis iš  $Q_A$  į  $Q_B$  karščio bangų veikiamuose augaluose nepadidėjo. Minėtus fotosintezės gyvybingumo pokyčius galėjo lemti aktyviųjų reakcijos centrų sumažėjęs tankis (RC/CSo) ir padidėjęs disocijuotos energijos kiekis (DIO/CSo). Po antrosios pertraukos karščio bangą ir sausrą patyrusių augalų RC/CSo buvo toks pat kaip ir kontrolinių augalų, todėl energijos išekvojimas šilumos pavidalu buvo mažesnis (DIO/CSo).

**Raktažodžiai:** pasikartojanti karščio banga, *Medicago sativa* L., chlorofilo fluorescencija, pašariniai augalai