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Photosynthetic activity of common buckwheat (*Fagopyrum esculentum* Moench) exposed to thermal stress

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Abstract

The aim of the work was to investigate thermal stress effect on photosynthetic activity of common buckwheat. Seedlings of common buckwheat were exposed to 20°C (control) and 30°C (thermal stress). The research involved the Polish cultivar 'Panda' and strain PA15 and determined kinetics of chlorophyll *a* fluorescence (Chlf), leaf gas exchange, soluble carbohydrate (SC) content in donor leaves, electrolyte leakage as a parameter of cell membrane permeability, and amount of abscisic acid and jasmonates. In 'Panda' and PA15 plants grown at 30°C, most of Chlf parameters improved. 'Panda' plants grown at 30°C demonstrated a higher increase in net photosynthetic rate, lower transpiration rate, and smaller SC reduction than those of PA15 strain. At this temperature, 'Panda' leaves accumulated greater amounts of jasmonates than that of the control. We concluded that studied genotypes demonstrated disparate responses to thermal stress, but for both, 30°C is more favourable temperature for vegetative growth than 20°C.

Additional key words: heat stress; ion leakage; photochemical efficiency; water-use efficiency.

Introduction

Fagopyrum esculentum Moench known as common buckwheat belongs to Polygonaceae family and is considered a pseudocereal crop because of a similar chemical seed composition. It is one of vital crops with a high medicinal and nutritional value. Its grains contain multiple beneficial compounds, such as lipids, polyphenols, rutin, dietary fibre, polysaccharides, and amino acids, especially lysine. Moreover, the seeds do not contain gluten, which makes buckwheat appropriate for people with celiac disease (Halbrecq *et al.* 2005, Christa and Soral-Śmietana 2008). The species is also used as forage for animals and cover crop with allelopathic potential to suppress weeds (Iqbal *et al.* 2006). In 2017, the largest producers of buckwheat in the world included Russia, China, Ukraine, France, Kazakhstan, Poland, United States, Brazil, Lithuania, and Japan (FAOSTAT 2019). However, due to a low and unstable seed yield, many countries implemented cultivation constraints and limited its production (Farooq *et al.* 2016). Floral biology of this self-incompatible plant and heterogeneity of seed maturation limit harvest time and yield (Halbrecq *et al.* 2005, Cawoy *et al.* 2009). This heterostylous species has two morphologically different flower morphs (pin and thrum), which differ in length of stamens and pistils. Fertilization is possible after cross pollination between pin and thrum flower type. A single plant produces multiple inflorescences, but the biggest issue is that only a few percent of flowers produce seeds (Adachi 1990, Slawinska and Obendorf 2001, Taylor and Obendorf 2001, Cawoy *et al.* 2006, 2009). During the flowering time, temperature conditions affect embryological development that then influences final seed yields. Optimal growth temperature ranges from 18 to 23°C. Temperature below 15°C inhibits flowering and below 10°C flowers wither (Slawinska and Obendorf 2001, Cawoy *et al.* 2009). Our

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Abbreviations: ABA – abscisic acid; ABS/CS_m – energy absorption by antennas; Chlf – chlorophyll fluorescence; C_i – internal CO_2 concentration; CS_m – excited cross section of a leaf; DI_0/CS_m – energy dissipation from PSII; DM – dry mass; E – transpiration rate; EL – electrolyte leakage; EL1 – initial electrolyte leakage; EL2 – final conductivity; ET_0/CS_m – energy used for electron transport; F_0 – minimal fluorescence yield of dark-adapted state; F_m – maximal fluorescence yield of dark-adapted state; F_v – variable fluorescence; F_v/F_0 – maximum efficiency of water-splitting reaction of the donor side of PSII; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; JA – jasmonic acid; JA-Met – jasmonic acid methyl ester; JAs – jasmonates (JA + JA-Met); PI – performance index of PSII; P_N – net photosynthetic rate; P_N/C_i – apparent carboxylation efficiency; RC/CS_m – number of active reaction centres; SC – soluble carbohydrates; TR_0/CS_m – excitation energy trapped in PSII; WUE – water-use efficiency; WUE_i – intrinsic water-use efficiency; δ_{R0} – efficiency with which an electron can move from the reduced intersystem of electron acceptors to PSI end electron acceptors; φ_{R0} – quantum yield of electron transport from Q_A^- to PSI end electron acceptors; ψ_{R0} – probability at time 0 that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-.
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earlier studies confirmed that the anthers of buckwheat produce viable pollen grains (> 90%) (Słomka *et al.* 2017). It seems that a defective development of female gametophytes can be the yield-limiting factor (Cawoy *et al.* 2009, Słomka *et al.* 2017). The development of female gametophytes is shifted in time and appears in older flowers at higher temperatures.

Anthropogenic climate changes observed in 21st century dramatically reduced the buckwheat yield in many regions of the world (Lobell *et al.* 2008). High temperature and drought stresses belong to the most important factors limiting biomass yield. Photosynthesis is a process the most sensitive to high temperature (Sharkey and Schrader 2006). Heat stress changes the reduction-oxidation properties of PSII acceptors and reduces the efficiency of photosynthetic electron transport in both photosystems (Mathur *et al.* 2014). The most rapid plant response to osmotic stress evoked by drought, heat or salinity is a decrease in stomatal aperture (James *et al.* 2002). The reduced photosynthetic rate increases generation of reactive oxygen species (ROS), which disturb photochemical processes in thylakoids and provoke photoinhibition of PSII. The effects of stresses, such as high temperature, drought or salinity, cause cell dehydration, increase plasma membrane permeability, and decrease the photosynthetic rate that together result in yield reduction (Kalaji *et al*. 2016). According to these authors, chlorophyll (Chl) fluorescence measurement is a very powerful, noninvasive tool in agricultural, environmental, and ecological studies.

The course of embryogenesis and flower abortion is under genetic and phytohormone control (Bernier *et al.* 1993). It is argued that flowering is affected by both plant hormones and sugars in the leaves that supply nutrients to the apical meristems. Abscisic acid (ABA) is a phytohormone affecting many physiological processes (Wang and Irving 2011). Its role in flowering promotion is ambiguous. According to Aneja *et al.* (1999) and Ferrante *et al.* (2006), ABA plays a signalling role in flower senescence. Methyl jasmonate (JA-Met) and jasmonic acid (JA) are important cellular regulators in such developmental processes as seed germination, flowering, and fruit development, leaf abscission, and senescence (Seo *et al.* 2001). Synthesis of ABA and JAs can be triggered by strong cell dehydration under drought, salinity or heat shock (Yoshida and Uemura 1990, Bravo *et al.* 1998).

The aim of this research was to understand the effect of thermal stress on photosynthetic apparatus efficiency of common buckwheat that was evaluated by the kinetics of chlorophyll *a* fluorescence (Chlf), leaf gas exchange, cell membrane permeability, and soluble carbohydrate (SC) accumulation in the leaves. Additionally, abscisic acid and jasmonates (jasmonic acid and its methyl ester) synthesized as an effect of thermal stress were determined. The experiment was performed on plants of Polish cv. 'Panda' and PA15 strain that differed significantly in the degree of embryo sac degeneration. Our earlier studies (Słomka *et al.* 2017) showed 32% of degenerating embryo sacs in cv. 'Panda' and only 10% in PA15 strain. Moreover, we demonstrated that megasporogenesis and megagametophytogenesis occurring in flowers of cv. 'Panda' were more sensitive to high temperature (30°C) than those in PA15 (Płażek *et al.* 2019).

Materials and methods

Plant material and growth conditions: Seeds of common buckwheat (*Fagopyrum esculentum* Moench) were supplied by breeders from the Plant Production Facility in Palikije (*Małopolska Plant Breeding*, Polanowice, Poland). Experiments were carried out on common buckwheat plants of Polish cultivar 'Panda' and strain PA15 under controlled conditions in phytotronics chambers. The plants were grown in pots (20 × 20 × 25 cm; six plants per pot) containing commercial soil substrate (pH 5.8) mixed 1:1 (v/v) with perlite. The seedlings were grown for three weeks at the control temperature (20°C) at humidity of 50–60%, under 16-h photoperiod and PPFD of 300 μmol(photon) m^{-2} s^{-1}. Then, a half of them (all plants at the vegetative stage) were transferred to a chamber with a temperature of 30°C (heat stress) and the same humidity and light conditions. The measurements of studied parameters were performed on donor leaves (fully developed young leaves, closest to the top inflorescences) of eight-week-old plants at the stage of full flowering. Buckwheat blooms throughout the growing season. The species still produces new flowers when seeds are already set.

Chl *a* fluorescence (Chlf): Prior to measurements, a LED-light source of a fluorometer (*Hansatech Ltd.*, King's Lynn, UK) was calibrated using an *SQS* light meter (*Hansatech Ltd.*, King's Lynn, UK). Excitation irradiance intensity was 3,000 μmol(photon) m^{-2} s^{-1} (peak at 650 nm). Measurements were taken after 30 min of the leaf adaptation to darkness (clips with a 4-mm diameter hole). Changes in fluorescence were registered during irradiation between 10 μs and 1 s. During the initial 2 ms, the data were collected every 10 μs with 12-bit resolution. After this period, the frequency of measurements was reduced automatically. F_v/F_m (quantum yield of PSII) was calculated according to van Kooten and Snel (1990) as $(F_m - F_0)/F_m$, where F_0 and F_m represent the minimal and maximal Chl fluorescence, respectively. These measurements were used to calculate the following parameters based on the theory of energy flow in PSII and the JIP-test (Lazár 1999, Strasser *et al.* 2000): ABS/CS_m, TR_0/CS_m, ET_0/CS_m, DI_0/CS_m, RC/CS_m, PI, F_v/F_m, F_v/F_0, ψ_{R0}, δ_{R0}, and φ_{R0}. The measurements included ten plants per each treatment.

Leaf gas-exchange parameters: The rate of gas exchange was measured on the fully developed donor leaf, closest to the top inflorescence, using a portable carbon dioxide infrared analyser, model *lCA-2* (*Analytical Development Co. Ltd.*, UK). Two 1,000-W tungsten-halogen light bulbs were used as a source of PAR of 850–900 μmol(photon) m^{-2} s^{-1}. The measurements were made between 9:00 and 11:00 h. The following parameters were determined and calculated: the rate of net photosynthesis (P_N) and transpiration (E), stomatal conductance (g_s), and internal CO_2 concentration (C_i). Apparent carboxylation efficiency (P_N/C_i) was calculated according to Niu *et al.* (2004),

instantaneous (WUE, P_N/E), and intrinsic water-use efficiencies (WUE_i, P_N/g_s) according to Medrano *et al.* (2015). The measurements included ten replicates per each treatment.

Electrolyte leakage (EL): Three leaf discs (1 cm in diameter) cut from three different donor leaves of a single plant (treated as one replicate) were placed into a plastic vial containing 10 cm^3 of ultrapure water. They were shaken (100 rpm) at room temperature and the initial electrolyte leakage (EL1) was measured with a conductivity meter (*CI 317*, *Elmetron*, Poland) after 24 h. The same vials were stored at –70°C overnight, shaken after thawing, and then their final conductivity – total content of ions (EL2) was measured. The permeability of cell membranes was represented as a percentage of total electrolyte leakage (EL = EL1 × 100/EL2). The measurements were made in ten replicates.

Extraction and measurement of soluble carbohydrates (SC): The amount of **s**oluble carbohydrates was determined in the same leaves used for photosynthetic efficiency measurement. SC amount was measured based on the anthrone method (Yemm and Willis 1954) with a slight modification. Leaf samples (0.5 g) were lyophilized and homogenized with 10 cm^3 of ultrapure water. The tissue homogenate was heated in a boiling water bath for 15 min and then centrifuged for 10 min at 3,000 × *g*. To each 0.5 cm^3 of the supernatant, 10 cm^3 of ultrapure water were added. Two cm^3 of 0.2% anthrone reagent (2 g of anthrone dissolved in 1 dm^3 of 95% H_2SO_4) were added to 1 cm^3 of the water extract. The reaction mixture was heated for 3 min and then rapidly cooled on ice. Absorbance of the extract was read at 620 nm using a UV-Vis spectrophotometer (*Ultrospec 2100 Pro*, *Amersham Bioscience*, Cambridge, UK). The SC concentration was finally calculated using a calibration curve (glucose as the calibration standard; *Sigma-Aldrich*) and exhibited as mg g^{-1}(dry mass, DM). Each assay was performed in five replications representing five different leaves.

Hormone content analysis: Abscisic acid (ABA) and jasmonates (JAs) (jasmonic acid and jasmonic acid methyl ester) were assessed according to Płażek *et al.* (2019). Freeze-dried and pulverized samples of donor leaves were extracted (5 min, 30 Hz, *MM400*, *Retch*, Germany) in 1 cm^3 of an extraction buffer ($MeOH/H_2O/HCOOH$, 15/4/1, v/v/v) after addition of an internal standard solution. Samples were centrifuged (3 min; 22,000 × *g*; *R32*, *Hettich*, Germany), the supernatant was collected, and the extraction step was repeated twice. Pooled supernatant was evaporated under N_2 and resuspended in 5% MeOH in 1 M HCOOH, and cleaned up on mixed-mode SPE cartridges (*BondElutPlexa PCX*, *Agilent*, USA), as reported by Dziurka *et al.* (2016). Phytohormones were analyzed by ultrahigh performance liquid chromatography (UHPLC) using *Agilent Infinity 1260* device coupled with *6410 Triple Quad LC/MS* with ESI (electrospray interface) ion source (*Agilent Technologies*, USA). Stable isotope-labelled internal standard of phytohormones consisted of: [$^2H_6$] cis,trans-abscisic acid (*OlChemIm*, Olomouc, Czech Republic) and [$^2H_5$]dinor-12-oxo phytodienoic acid purchased from *Cayman Chemical Company* (*Michigan*, MI, USA). The following hormone forms were determined: active abscisic acid (ABA-free) (±)-cis,trans-abscisic acid, non-active (±)-cis,trans-abscisic acid glucosyl ester (ABA-glc), jasmonic acid (JA), and jasmonic acid methyl ester (JA-Met). The data represent total amounts of all forms of ABA and JAs as μmol g^{-1}(DM). The analyses were performed in three replicates.

Statistical analysis: The data were analysed by two-way analysis of variance (*ANOVA*) using *STATISTICA 13* package (*Statsoft*, Tulsa, OK, USA). Significance of the differences between means was determined at $p<0.05$ by the *Duncan*'s multiple range test. Linear correlation *Pearson*'s coefficients were assumed as statistically significant at $p<0.05$. The data were presented as the means ± standard errors (SE).

Results

Chl *a* fluorescence: The overall performance index of PSII photochemistry (PI) ratio showed a significant increase in both genotypes exposed to the thermal stress (Table 1). The lowest value of PI was found in strain PA15 at 20°C, while the highest value was observed in the same genotype at 30°C. There were no significant differences between genotypes at the same temperature treatment. The value of maximal quantum yield of PSII (F_v/F_m) increased in both genotypes under the higher temperature. The highest values of F_v/F_m were seen at the same time in both genotypes under the thermal stress and the lowest ones in PA15 at 20°C. The values of F_v/F_0 were significantly greater at 30°C in both genotypes, however, in the case of PA15, high temperature increased the value of this parameter 2.7 times, while in cv. 'Panda' plants, it raised only 1.6 times. The lowest value of this parameter was found in strain PA15 at 20°C. Thermal stress significantly enhanced light energy absorption (ABS/CS_m) in both genotypes *vs*. control. The excitation energy trapped in PSII reaction centres (TR_0/CS_m) grew significantly under thermal stress for both genotypes. Energy used for electron transport (ET_0/CS_m) was significantly greater at 30°C in both genotypes. The higher temperature reduced the energy dissipated from PSII (DI_0/CS_m) in both genotypes, which may indicate that high temperature was not perceived by the plants as stress. The lowest value was observed in 'Panda' at 30°C, and the highest in PA15 under control temperature. The number of active reaction centres (RC/CS_m) significantly increased in cv. 'Panda' and PA15 under high temperature treatment. Values of δ_{R0}, denoting the efficiency with which an electron can move from the reduced intersystem of electron acceptors to the PSI end electron acceptors, did not differ in both 'Panda' and PA15 plants at 20 and 30°C. The probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (ψ_{R0}) as well as the quantum yield of electron transport from Q_A^- to the PSI end electron acceptors (φ_{R0}) in plants of PA15 were higher under thermal stress compared to that of the control.

Table 1. Changes of kinetics of chlorophyll *a* fluorescence in common buckwheat cv. 'Panda' and strain PA15 grown at control temperature (20°C) and under thermal stress (30°C). Values represent means (n =10) ± SE. Values within columns for each assay followed by *the same letter* do not differ significantly according to the *Duncan*'s multiple range test (p<0.05). ABS/CS_m – energy absorption by antennas; DI_0/CS_m – energy dissipation from PSII; ET_0/CS_m – energy used for electron transport; F_v/F_0 – maximum efficiency of water-splitting reaction of the donor side of PSII; F_v/F_m – maximal quantum yield of PSII photochemistry; PI – performance index of PSII; RC/CS_m – number of active reaction centres; TR_0/CS_m – excitation energy trapped in PSII; δ_{R0} – efficiency with which an electron can move from the reduced intersystem of electron acceptors to PSI end electron acceptors; φ_{R0} – quantum yield of electron transport from Q_A^- to PSI end electron acceptors; ψ_{R0} – probability at time 0 that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-.

Cv./strain	Temp. [°C]	PI	F_v/F_m	F_v/F_0	ABS/CS_m	TR_0/CS_m	ET_0/CS_m	DI_0/CS_m	RC/CS_m	δ_{R0}	φ_{R0}	ψ_{R0}
'Panda'	20	0.58 ± 0.18^c	0.74 ± 0.02^a	3.05 ± 0.32^b	1,566 ± 93^b	1,169 ± 81^b	385 ± 33^b	396 ± 43^b	556 ± 60^b	0.33 ± 0.05^a	0.09 ± 0. 02^{bc}	0.12 ± 0.03^b
	30	1.55 ± 0.15^a	0.83 ± 0.07^a	4.95 ± 0.05^a	1,762 ± 105^a	1,466 ± 93^a	638 ± 64^a	296 ± 30^c	713 ± 80^a	0.33 ± 0.02^a	0.12 ± 0.01^{ab}	0.14 ± 0.01^b
PA15	20	0.29 ± 0.13^b	0.55 ± 0.02^b	1.83 ± 0.50^c	1,274 ± 80^c	747 ± 44^c	214 ± 22^c	527 ± 67^a	326 ± 48^c	0.29 ± 0.04^a	0.05 ± 0.02^c	0.08 ± 0.02^c
	30	2.05 ± 0.33^a	0.83 ± 0.08^a	4.90 ± 0.26^a	1,749 ± 104^a	1,448 ± 98^a	711 ± 76^a	301 ± 35^{bc}	715 ± 84^a	0.36 ± 0.03^a	0.15 ± 0.01^a	0.18 ± 0.02^a

This result may indicate a cyclic electron transport without NADPH synthesis in PA15 leaves at 30°C.

Leaf gas exchange: The thermal stress significantly increased P_N in cv. 'Panda' compared with that of the control, while did not change P_N in PA15 leaves (Table 2). CO_2 assimilation in PA15 plants was more efficient than that in 'Panda', irrespective of the temperature. Thermal stress significantly reduced transpiration rate (E) in cv. 'Panda' but not in strain PA15. The highest E was observed in strain PA15 under two experimental treatments, while the lowest E was found in cv. 'Panda' at 30°C. The higher temperature reduced stomatal conductance (g_s) in the leaves of 'Panda' and had no effect in PA15. In 'Panda' plants, g_s reduction correlated negatively with P_N (r = –0.643; p<0.05) and positively (r = 0.786; p<0.05) with E. Internal CO_2 concentration did not change under the thermal stress in any of the studied plants. Reassuming, gas exchange processes in cv. 'Panda' demonstrated higher sensitivity to temperature changes than that in PA15 strain. Higher temperature significantly increased WUE and WUE_i in 'Panda' plants but not in PA15 plants (Table 3). The rate of apparent carboxylation efficiency (P_N/C_i) did not change in both genotypes under thermal stress.

Electrolyte leakage (EL) from leaf cells increased under the thermal stress in all studied plants as compared to that of the control (Table 4). Heat doubled EL value in 'Panda' leaf cells *vs*. control ones. The cell membranes of 'Panda' leaves showed considerably higher permeability at 30°C than that of PA15. 'Panda' plants demonstrated also a negative correlation (r = –0.758; p<0.05) between g_s and EL. This result means that the greater the ion efflux was, the lower stomatal opening rate was.

Soluble carbohydrates: In the donor leaves of both genotypes, a considerable decrease in total soluble carbohydrate (SC) content under thermal stress conditions was noted (Table 5). In cv. 'Panda', the amount of SC in the leaves at 30°C was 1.6 times lower than that in the control leaves, while in PA15 plants grown under thermal stress, the SC amount was reduced four times. This observation was unexpected, as in cv. 'Panda', P_N (Table 2), F_v/F_m, and PI were higher at 30°C than that under control conditions (Table 1). Considering Chl fluorescence as ψ_{R0} and φ_{R0} in PA15, we can suppose that such a huge drop in SC was caused by limited NADPH synthesis. In PA15 plants, a correlation between SC content and ψ_{R0} and φ_{R0} was found (r = –0.874 at p<0.05 and r = –0.769 at p<0.05, respectively).

Hormone amount in donor leaves: Leaves of cv. Panda grown at 30°C contained lower amounts of ABA, while in PA15 ones, this temperature did not change ABA amount as compared with that of the control (Fig. 1*A*). At 20°C, PA15 leaves contained considerably more JAs than that of 'Panda', however, high temperature decreased JAs content in PA15 leaves and increased it in 'Panda' (Fig. 1*B*). This result confirmed disparate responses of the studied genotypes to the thermal stress.

Table 2. Changes in the rate of net photosynthesis (P_N) and transpiration (E), values of stomatal conductance (g_s) and internal CO_2 concentration (C_i) in the donor leaves of common buckwheat cv. 'Panda' and strain PA15 grown at control temperature (20°C) and under thermal stress (30°C). Values represent means (n = 10) ± SE. Values within columns for each assay followed by *the same letter* do not differ significantly according to *Duncan*'s multiple range test (p<0.05).

Cv./strain	Temp. [°C]	P_N [μmol(CO_2) m^{-2} s^{-1}]	E [mmol(H_2O) m^{-2} s^{-1}]	g_s [mol(H_2O) m^{-2} s^{-1}]	C_i [μmol mol^{-1}]
'Panda'	20	1.2 ± 0.2^c	0.8 ± 0.1^b	37.0 ± 5.5^b	263 ± 49^a
	30	2.3 ± 0.3^b	0.5 ± 0.1^c	19.9 ± 3.5^c	163 ± 31^a
PA15	20	4.0 ± 0.6^a	1.1 ± 0.1^a	51.0 ± 6.2^a	206 ± 50^a
	30	4.6 ± 0.7^a	1.1 ± 0.1^a	47.3 ± 2.9^{ab}	201 ± 36^a

Table 3. Instantaneous (WUE), intrinsic (WUE_i) water-use efficiencies, and apparent carboxylation efficiency (P_N/C_i) rate in the donor leaves of common buckwheat cv. 'Panda' and strain PA15 plants grown at control temperature (20°C) and under thermal stress (30°C). Values represent means (n = 10) ± SE. Values within columns for each assay followed by *the same letter* do not differ significantly according to *Duncan*'s multiple range test (p<0.05).

Cv./strain	Temp. [°C]	WUE [μmol(CO_2) mol^{-1}(H_2O)]	WUE_i [μmol(CO_2) mol^{-1}(H_2O)]	P_N/C_i [mol m^{-2} s^{-1}]
'Panda'	20	2.6 ± 0.9^b	0.065 ± 0.025^b	0.008 ± 0.003^a
	30	5.5 ± 0.6^a	0.148 ± 0.018^a	0.017 ± 0.004^a
PA15	20	4.2 ± 0.8^a	0.038 ± 0.011^c	0.030 ± 0.011^a
	30	4.1 ± 0.8^a	0.042 ± 0.007^c	0.040 ± 0.024^a

Table 4. Electrolyte leakage [% of total ion content] from leaf cells of common buckwheat cv. 'Panda' and strain PA15 plants grown at control temperature (20°C) and under thermal stress (30°C). Values represent means (n = 10) ± SE. Values within columns for each assay followed by *the same letter* do not differ significantly according to *Duncan*'s multiple range test (p<0.05).

Cv./strain	Temp. [°C]	Electrolyte leakage [%]
'Panda'	20	4.34 ± 0.14^c
	30	9.21 ± 1.88^a
PA15	20	4.90 ± 1.19^c
	30	6.03 ± 0.40^b

Table 5. Changes in the amount of soluble carbohydrates in the leaves of common buckwheat cv. 'Panda' and strain PA15 plants grown at control temperature (20°C) and under thermal stress (30°C). Values represent means (n = 5) ± SE. Values within columns for each assay followed by *the same letter* do not differ significantly according to the *Duncan*'s multiple range test (p<0.05).

Cv./strain	Temp. [°C]	Soluble carbohydrates [mg g^{-1}(DM)]
'Panda'	20	3.26 ± 0.83^a
	30	1.98 ± 0.1^{ab}
PA15	20	1.11 ± 0.03^b
	30	0.28 ± 0.11^c

Discussion

The main effect of higher temperature is usually accelerated plant development. The optimal temperature is generally lower for grain yield than that for photosynthesis (Rawson 1992, Conroy *et al.* 1994). Nevertheless, heat stress is considered one of the most harmful environmental stresses (Jumrani *et al.* 2017). Crop plants respond to temperature depending on the optimum temperature for photosynthesis that is species specific (Downton and Slatyer 1972, Garber 1977). We have previously reported (Płażek *et al.* 2019) that 30°C is a stress factor for generative development of common buckwheat. However, the data presented here regarding photosynthetic efficiency suggest more favourable vegetative growth of this plant species at the higher temperature.

PSII is very sensitive to the effects of various environmental factors. According to Lichtenhaler (1996), altered kinetics of Chl *a* fluorescence informs on damage of proteins building PSII, and simultaneous changes in fluorescence parameters can serve as indicators of plant stress response. In this study, we investigated a response of common buckwheat cv. 'Panda' and strain PA15 to thermal stress. The outcomes showed that almost all analysed parameters (PI, F_v/F_m, F_v/F_0, ABS/CS_m, ET_0/CS_m, TR_0/CS_m, and RC/CS_m) in both cv. 'Panda' and strain PA15 were higher at 30°C than that under the control conditions. We therefore concluded that this temperature is not a stress factor but is in fact optimal for the vegetative development of common buckwheat. The species response also confirmed its thermophilic properties. Energy dissipation evaluated by DI_0/CS_m was lower at 30°C than that at 20°C. Moradi and Ismail (2007) stated that plant stress tolerance may be demonstrated by greater dissipation of excess energy. This phenomenon can minimise photoinhibition damage to PSII (Demmig *et al.* 1987).

Closing of stomata is one of the most often observed mechanisms initiated by plants in response to high

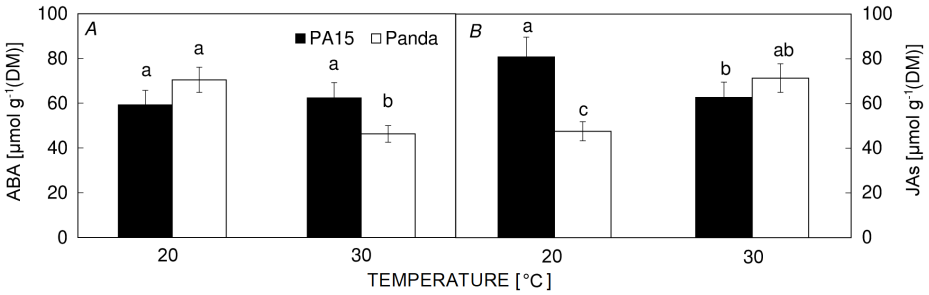

Fig. 1. Abscisic acid (ABA) (*A*) and jasmonates (JAs) (*B*) in common buckwheat donor leaves of cv. 'Panda' and PA15 strain grown at 20 and 30°C. Analyses included leaves collected from eight-week-old plants grown under thermal stress for five weeks. The means (n = 3) ± SE marked with *the same letter* did not differ significantly (*Duncan*'s multiple range test, p<0.05).

temperature (James *et al*. 2002). At 30°C, 'Panda' leaves demonstrated lower stomatal conductance than that of control and significantly reduced transpiration rate. Although PA15 plants slightly limited stomatal opening, transpiration intensity remained unchanged. Closing of stomata in the studied plants seems not to be dependent only on the ABA content. 'Panda' plants demonstrated the lower ABA amount at 30°C than at 20°C, while in PA15, the content of this hormone was similar at both temperatures. This effect might seem strange because it is believed that ABA is responsible for stomata closing (Bravo *et al.* 1998). Thus, in this case, the high temperature did not cause water stress because plants were watered regularly. Under thermal stress, JAs content was higher only in 'Panda' leaves, which may suggest that this might be responsible for the lesser stomatal conductance.

Changes in photosynthesis course are considered important indicators of plant integrity under various environmental conditions (Piao *et al.* 2008, Jumrani *et al.* 2017). The processes involved in photosynthesis showed tolerance to heat stress in the range of 30–35°C in various crop species (Wahid *et al.* 2007). The photosynthesis disruption under high temperature stress could be caused by stomatal or nonstomatal factors (Athar and Ashraf 2005). Cultivar 'Panda' demonstrated a higher net photosynthesis efficiency at 30°C than that under the control temperature (20°C). Contrary to that, strain PA15 did not show any changes in this process at higher temperature, but its P_N at both temperatures was significantly higher than that in 'Panda' leaves. Stomata closure did not correlate with P_N: in cv. 'Panda' at 30°C, g_s was lower than that in control plants, while in PA15 plants, both parameters, P_N and g_s, did not change at 30°C. The higher P_N at lower g_s could be achieved due to remobilization of CO_2 from respiration. We observed this effect in *Miscanthus* × *giganteus* plants grown in saline soil (Płażek *et al.* 2014). Osmotic stress over several weeks may lead to both stomatal and nonstomatal inhibition of photosynthesis. In C_3 plants, stomatal closure is recognized as a major protective mechanism against water loss and it decreases CO_2 availability. C_4 plants cope much better with stomatal closure, since a carboxylating enzyme, phospho*enol*pyruvate carboxylase (PEPC), shows a minimal K_m for its substrate, HCO_3^-. For example, in sorghum, long-term stress resulted in nonstomatal limitations of photosynthesis, probably through an accumulation of malate and following inhibition of PEPC, and through a decreased activity of phosphate and pyruvate dikinase (Beyel and Brüggemann 2005). In our studies on *Fabaceae* plants cultivated under drought, we observed CO_2 remobilization by closed stomata based on the degree of ^{13}C isotope discrimination (unpublished). The findings of this work indicate that the temperature of 30°C is more conducive to vegetative growth of common buckwheat than 20°C. Contrary to that, the higher temperature discourages generative development of this species, as showed in our earlier study (Płażek *et al.* 2019). We claim both these outcomes crucial for researchers who try to explain the mechanism of low seed yielding in common buckwheat.

Plant WUE is commonly measured at different scales, ranging from leaf to plant level, depends on the facilities, capacity, and the specification of the experiment. In this work, instantaneous (WUE) and intrinsic (WUE_i) water-use efficiencies were determined. The values of both parameters significantly increased in donor leaves of 'Panda' under thermal stress, while did not change in PA15. Medrano *et al.* (2015) evaluated WUE in two cultivars of grapevines comparing the conditions of optimal hydration with those of drought. The experiment identified spatial and temporal variation in water and carbon balances at the leaf and whole plant levels. However, WUE measurements on a single leaf provided an efficient method to compare genotypes that were also used in our study. In contrast to WUE and WUE_i, apparent carboxylation efficiency (P_N/C_i) did not change under thermal stress in the studied genotypes. This effect could be explained by a high photosynthetic efficiency of both genotypes at 30°C, however, 'Panda' plants showed significant improvement of P_N at 30°C in comparison to the control temperature. According to Wullschleger (1993), analyses of P_N/C_i curves are very useful for testing mechanistic models of photosynthetic metabolism. Harley and Sharkey (1991) stated that sometimes P_N actually declines at high CO_2. These authors explain this phenomenon by changes in photorespiration and use of glycine and serine in protein synthesis.

In both cv. 'Panda' and PA15 strain, the amount of soluble carbohydrates in donor leaves was lower at 30°C than that in control. This effect could be explained by higher consumption of carbohydrates manifested by greater and faster growth of plants and intensified

respiration. Highly interesting data on SC reduction come also from parameters, such as probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (ψ_{R0}), and the quantum yield of electron transport from Q_A^- to the PSI end electron acceptors (φ_{R0}). In 'Panda' leaves at both temperatures, these parameters did not differ significantly, suggesting that under thermal stress electron transport ran as a noncyclic and NADPH was synthesized in sufficient amounts in the reduction phase of Calvin cycle. In PA15 plants, both ψ_{R0} and φ_{R0} increased considerably at 30°C *vs*. the control temperature. This may indicate cyclic phosphorylation in which only ATP is produced without NADPH synthesis. This outcome could explain such a substantial drop in the carbohydrate content in the leaves of PA15 plants under thermal stress. Hura *et al.* (2015) found a correlation between ψ_{R0} and φ_{R0} and hydrogen peroxide production as an effect of drought stress in triticale leaves.

Cell membrane stability is a vital indicator of plant tolerance to various environmental stresses (Munns and James 2003, Hura *et al.* 2007, Filek *et al.* 2012). Plant stress response involves its ability to rebuild membrane structure, especially under unfavourable temperature. Strong cell dehydration may trigger synthesis of ABA and reactive oxygen species (ROS) that cause oxidation of unsaturated fatty acids and affect membrane selectivity (Skoczowski and Filek 1986, Yoshida and Uemura 1990). ROS may initiate production of JA or ethylene that induce premature leaf senescence or play a key role in signal transduction involved in plant response to stress (Bravo *et al.* 1998). Cell membrane selectivity interplays with all metabolic processes in membranes, for example, light phase of photosynthesis in thylakoids and electron transport chain in mitochondria. Increased cell membrane permeability not only causes peroxidative damage of cell membranes, but also changes their protein conformation and opening of ion channels (Santarius 1980, Havaux *et al.* 1996). In the present study, EL in all plants was significantly higher at 30°C than that at 20°C, but the EL change in 'Panda' was greater than that in PA15**.** Gulen and Eris (2004) stated that strawberry seedlings treated with gradual heat stress from 25°C up to 40°C demonstrated lower EL than plants exposed to shock heat stress. In our experiment, common buckwheat plants were cultivated for a long time under high temperature, so such a growth conditions cannot be considered as a heat shock but rather as a thermal stress. Cell membrane permeability did not correlate with net photosynthetic rate in any of the studied plants.

The reported data clearly showed that more research is needed to understand differential response of photosynthetic apparatus to the thermal stress in various genotypes of common buckwheat. These results are the basis for further research on antioxidant enzyme activity and molecular analyses. It is also necessary to translate the data from single-leaf to whole-plant estimates of WUE to improve our understanding of this process.

Conclusions: Our results indicate disparate responses of the studied cultivar and strain to high temperature. The photosynthetic apparatus of PA15 responded more strongly to high temperature than cultivar 'Panda'; we observed it in changes in chlorophyll fluorescence parameters (PI, F_v/F_m, F_v/F_0, ABS/CS_m, φ_{R0}, ψ_{R0}). We suggest that ψ_{R0} (probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-) and φ_{R0} (a high quantum yield of electron transport from Q_A^- to the PSI end electron acceptors) were responsible for maintaining photosynthetic activity under heat stress in PA15. Results obtained from these experiments suggest that 30°C is a more favourable temperature for vegetative growth of common buckwheat than 20°C.

References

Adachi T.: How to combine the reproductive system with biotechnology in order to overcome the breeding barrier in buckwheat? – Fagopyrum **10**: 7-11, 1990.

Aneja M., Gianfagna T., Ng E.: The roles of abscisic acid and ethylene in the abscission and senescence of cocoa flowers. – Plant Growth Regul. **27**: 149-155, 1999.

Athar H., Ashraf M.Y.: Photosynthesis under drought stress. – In: Pessarakli M. (ed.): Photosynthesis. 2nd Edition. Pp. 795-810. CRC Press, New York 2005.

Bernier G., Havelange A., Houssa C. *et al*.: Physiological signals that induce flowering. – Plant Cell **5**: 1147-1155, 1993.

Beyel V., Brüggemann W.: Differential inhibition of photosynthesis during pre-flowering drought stress in *Sorghum bicolor* (L.) Moench genotypes with different senescence traits. – Physiol. Plantarum **124**: 249-259, 2005.

Bravo L.A., Zúñiga G.A., Alberdi M., Corcuera L.J.: The role of ABA in freezing tolerance and cold acclimation in barley. – Physiol. Plantarum **103**: 17-23, 1998.

Cawoy V., Deblauwe V., Halbrecq B. *et al*.: Morph differences and honeybee morph preference in the distylous species *Fagopyrum esculentum* Moench. – Int. J. Plant Sci. **167**: 853-861, 2006.

Cawoy V., Ledent J.F., Kinet J.M., Jacquemart A.L.: Floral biology of common buckwheat (*Fagopyrum esculentum* Moench). – Eur. J. Plant Sci. Biotech. **3**: 1-9, 2009.

Christa K., Soral-Śmietana M.: Buckwheat grains and buckwheat products – nutritional and prophylactic value of their components – a review. – Czech J. Food Sci. **26**: 153-162, 2008.

Conroy J.P., Seneweera S., Basra A.S. *et al*.: Influence of rising atmospheric CO_2 concentrations and temperature on growth, yield and grain quality of cereal crops. – Aust. J. Plant Physiol. **21**: 741-758, 1994.

Demmig B., Winter K., Krüger A., Czygan F.C.: Photoinhibition and zeaxanthin formation in intact leaves. A possible role of the xanthophyll cycle in the dissipation of excess light energy. – Plant Physiol. **84**: 218-224, 1987.

Downton J.Y., Slatyer R.O.: Temperature dependence of photosynthesis in cotton. – Plant Physiol. **50**: 518-522, 1972.

Dziurka M., Janeczko A., Juhász C. *et al*.: Local and systemic hormonal responses in pepper leaves during compatible and incompatible pepper-tobamovirus interactions. – Plant Physiol. Bioch. **109**: 355-364, 2016.

FAOSTAT: Food and Agriculture Organisation of the United Nations Online Database, 2019. (*Available at*: http://www.fao.org/statistics/en/)

Farooq S., Rehman R.U., Pirzadah T.B. *et al.*: Cultivation, agronomic practices, and growth performance of buckwheat. – In: Meilinag Z., Kreft I., Woo S.-H. *et al*. (ed.): Molecular Breeding and Nutritional Aspects of Buckwheat. Pp. 299-320. Academic Press, Oxford 2016.

Ferrante A., Vernieri P., Tognoni F., Serra G.: Changes in abscisic acid and flower pigments during floral senescence of *Petunia*. – Biol. Plantarum **50**: 581-585, 2006.

Filek M., Walas S., Mrowiec H. *et al*.: Membrane permeability and micro- and macroelement accumulation in spring wheat cultivars during the short-term effect of salinity-and PEG-induced water stress. – Acta Physiol. Plant. **34**: 985-995, 2012.

Garber M.P.: Effect of light and chilling temperatures on chilling-sensitive and chilling-resistant plants. Pre-treatment of cucumber and spinach thylakoids *in vivo* and *in vitro*. – Plant Physiol. **59**: 981-985, 1977.

Gulen H., Eris A.: Effect of heat stress on peroxidase activity and total protein content in strawberry plants. – Plant Sci. **166**: 739-744, 2004.

Halbrecq B., Romedenne P., Ledent J.F.: Evolution of flowering, ripening and seed set in buckwheat (*Fagopyrum esculentum* Moench.): Quantitative analysis. – Eur. J. Agron. **23**: 209-224, 2005.

Harley P.C., Sharkey T.D.: An improved model of C_3 photosynthesis at high CO_2: Reversed O_2 sensitivity explained by lack of glycerate reentry into the chloroplast. – Photosynth. Res. **27**: 169-178, 1991.

Havaux M., Tardy F., Ravenel J. *et al.*: Thylakoid membrane stability to heat stress studied by flash spectroscopic measurements of the electrochromic shift in intact potato leaves: Influence of the xanthophyll content. – Plant Cell Environ. **19**: 1359-1368, 1996.

Hura T., Hura K., Grzesiak M.T., Rzepka A.: Effect of longterm drought stress on leaf gas exchange and fluorescence parameters in C_3 and C_4 plants. – Acta Physiol. Plant. **29**: 103-113, 2007.

Hura T., Hura K., Ostrowska A., Dziurka K.: Rapid plant rehydration initiates permanent and adverse changes in the photosynthetic apparatus of triticale. – Plant Soil **397**: 127-145, 2015.

Iqbal A., Hiradate S., Noda A. *et al*.: Allelopathy of buckwheat: Assessment of allelopathic potential of extract of aerial parts of buckwheat and identification of fagomine and other related alkaloids as allelochemicals. – Weed Biol. Manag. **2**: 110-115, 2006.

James R.A., Rivelli A.R., Munns R., von Caemmerer S.: Factors affecting CO_2assimilation, leaf injury and growth in salt-stressed durum wheat. – Funct. Plant Biol. **29**: 1393-1403, 2002.

Jumrani K., Bhatia V.S., Pandey G.P.: Impact of elevated temperatures on specific leaf weight, stomatal density, photosynthesis and chlorophyll fluorescence in soybean. – Photosynth. Res. **131**: 333-350, 2017.

Kalaji H.M., Jajoo A., Oukarroum A. *et al*.: Chlorophyll *a* fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. – Acta Physiol. Plant. **38**: 102, 2016.

Lazár D.: Chlorophyll *a* fluorescence induction. – BBA-Bioenergetics **1412**: 1-28, 1999.

Lichtenhaler H.K.: Vegetation stress: An introduction to the stress concept in plants. – J. Plant Physiol. **148**: 4-14, 1996.

Lobell D.B., Burke M.B., Tebaldi C. *et al*.: Prioritizing climate change adaptation needs for food security in 2030. – Science **319**: 607-610, 2008.

Mathur S., Agrawal D., Jajoo A.: Photosynthesis: Limitations in response to high temperature stress. – J. Photoch. Photobio. B **137**: 116-126, 2014.

Medrano H., Tomás M., Martorell S. *et al*.: From leaf to whole-plant water use efficiency (WUE) in complex canopies: Limitations of leaf WUE as a selection target. – Crop J. **3**: 220-228, 2015.

Moradi F., Ismail A.M.: Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging system to salt stress during seedling and reproductive stages in rice. – Ann. Bot.-London **99**: 1161-1173, 2007.

Munns R., James R.: Screening methods for salinity tolerance: a case study with tetraploid wheat. – Plant Soil **253**: 201-218, 2003.

Niu S.L., Li L.H., Jiang G.M. *et al*.: Gas exchange and chlorophyll fluorescence response to simulated rainfall in *Hedysarum fruticosum* var. *mongolicum*. – Photosynthetica **42**: 1-6, 2004.

Piao S.P., Ciais P., Friedlingstein P. *et al*.: Net carbon dioxide losses of northern ecosystems in response to autumn warming. – Nature **451**: 49-52, 2008.

Płażek A., Dubert F., Kościelniak J. *et al.*: Tolerance of *Miscanthus* × *giganteus* to salinity depends on initial weight of rhizomes as well as high accumulation of potassium and proline in leaves. – Ind. Crop. Prod. **52**: 278-285, 2014.

Płażek A., Słomka A., Kopeć P. *et al*: Effects of high temperature on embryological development and hormone profile in flowers and leaves of common buckwheat (*Fagopyrum esculentum* Moench). – Int. J. Mol. Sci. **20**: 1705, 2019.

Rawson H.M.: Plant responses to temperature under conditions of elevated CO_2. – Aust. J. Bot. **40**: 473-490, 1992.

Santarius K.A.: Membrane lipids in heat injury of spinach chloroplasts. – Physiol. Plantarum **49**: 1-6, 1980.

Seo H.S., Song J.T., Cheong J.J. *et al*.: Jasmonic acid carboxyl methyltransferase: A key enzyme for jasmonate-regulated plant responses. – P. Natl. Acad. Sci. USA **98**: 4788-4793, 2001.

Sharkey T.D., Schrader S.M.: High temperature stress. – In: Rao K.V.M., Raghavendra A.S., Reddy K.J. (ed.): Physiology and Molecular Biology of Stress Tolerance in Plants. Pp. 101-129. Springer, Berlin 2006.

Skoczowski A., Filek M.: Cold-induced changes in lipids from hypocotyls of winter and spring rape. I. The lipid synthesis and fatty acids composition. – Acta Physiol. Plant. **48**: 203-212, 1986.

Slawinska J., Obendorf R.L.: Buckwheat seed set in planta and during *in vitro* inflorescence culture: evaluation of temperature and water deficit stress. – Seed Sci. Res. **11**: 223-233, 2001.

Słomka A., Michno K., Dubert F. *et al.*: Embryological background of low seed set in distylous common buckwheat (*Fagopyrum esculentum* Moench) with biased morph ratios, and biostimulant-induced improvement of it. – Crop Pasture Sci. **68**: 680-690, 2017.

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. 445-483. CRC Press, New York 2000.

Taylor D.P., Obendorf R.L.: Quantitative assessment of some factors limiting seed set in buckwheat. – Crop Sci. **41**: 1792-1799, 2001.

van Kooten O., Snel J.F.H.: The use of chlorophyll fluorescence nomenclature in plant stress physiology. – Photosynth. Res. **25**: 147-150, 1990.

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

Wang Y.H., Irving H.R.: Developing a model of plant hormone interactions. – Plant Signal. Behav. **6**: 494-500, 2011.

Wullschleger S.D.: Biochemical limitations to carbon assimilation in C_3 plants – A retrospective analysis of the A/C_i curves from 109 species. – J. Exp. Bot. **44**: 907-920, 1993.

Yemm E.W., Willis A.J.: The estimation of carbohydrates in plant extracts by anthrone. – Biochem. J. **57**: 508-514, 1954.

Yoshida S., Uemura M.: Responses of the plasma membrane

to cold acclimation and freezing stress. – In: Larsson C., Møller I.M. (ed.): The Plasma Membrane. Structure, Function and Molecular Biology. Pp. 293-319. Springer Verlag, Berlin 1990.