Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
Wydział Rolniczo – Ekonomiczny

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Nr albumu: 1090

Rozwojowe i fizjologiczne mechanizmy aborcji kwiatów kształtujące plon nasion gryki zwyczajnej (*Fagopyrum esculentum* Moench.)

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Kraków, styczeń 2022

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Tytuł pracy w języku polskim

Rozwojowe i fizjologiczne mechanizmy aborcji kwiatów kształtujące plon nasion gryki zwyczajnej (*Fagopyrum esculentum* Moench)

Słowa kluczowe

białka szoku cieplnego, gryka zwyczajna, plon nasion, profil hormonalny, proteomika, rozwój embriologiczny, stres termiczny, stres troficzny

Streszczenie pracy

Gryka zwyczajna (*Fagopyrum esculentum* Moench) należy do rodziny rdestowatych (*Polygonaceae)*. Jest dwuliścienną rośliną jednoroczną uprawianą głównie na cele spożywcze ze względu na dobrze zbilansowany skład chemiczny nasion. Gryka stosowana jest jako plon główny oraz jako poplon ze względu na właściwości fitosanitarne. Charakteryzuje ją niski i nierównomierny plon, na który wpływ mają m.in. krótka żywotność pojedynczego kwiatu, samoniezgodność wynikająca z heterostylii kwiatów, nieprawidłowości w rozwoju woreczków zalążkowych i wrażliwość gryki na stresy środowiskowe np. wysoką temperaturę w okresie kwitnienia. Jest rośliną niesamokończącą tj. kwitnie przez cały okres wegetacji, co powoduje występowanie konkurencji o asymilaty pomiędzy zawiązanymi nasionami a wciąż produkowanymi kwiatami. Czynniki te determinują przedwczesne opadanie kwiatów i niedojrzałych nasion gryki zwyczajnej.

Celem niniejszej rozprawy doktorskiej, obejmującej cykl pięciu oryginalnych publikacji, była analiza rozwojowych i fizjologicznych mechanizmów aborcji kwiatów kształtujących plon nasion gryki zwyczajnej. Hipoteza badawcza zakładała, że głównymi przyczynami tych procesów jest stres wysokotemperaturowy i troficzny. Do weryfikacji postawionych hipotez wykorzystano dwa genotypy gryki zwyczajnej tj. odmiany „Panda" i ród PA15 (obecnie odmiana „Korona"), które wykazują różnice w stopniu degeneracji woreczków zalążkowych i aborcji kwiatów oraz plonem nasion. W badaniach prowadzonych na roślinach traktowanych wysoką temperaturą (30 °C) lub w stresie troficznym *in planta*, modyfikowanym poprzez częściowe usuwanie kwiatów lub pędów bocznych, przeprowadzono szereg analiz rozwoju embriologicznego, zmian hormonalnych, zachodzących w różnych stadiach rozwojowych kwiatów oraz w liściach. Ponadto, oceniano stopień aborcji kwiatów i elementy struktury plonu tj. liczbę nasion pełnych i pustych wyprodukowanych przez jedną roślinę, ich masę oraz masę tysiąca nasion. Dodatkowo, w stresie termicznym wykonano analizę wydajności fotochemicznej i fotosyntetycznej, oraz zmiany proteomiczne i akumulację białek szoku cieplnego (HSP) w różnych stadiach rozwojowych kwiatów i w liściach. Stres troficzny badano

dodatkowo w warunkach *in vitro* poprzez wykładanie pąków kwiatowych na pożywki o zmniejszonym składzie substancji odżywczych.

Przeprowadzone analizy wykazały, że wysoka temperatura (30°C), która jest optymalna dla rozwoju wegetatywnego gryki zwyczajnej, zwiększa stopień degeneracji woreczków zalążkowych w okresie rozwoju embriologicznego. W stresie wysokotemperaturowym białko HSP-70 pełni funkcję ochronną w rozwoju zalążków i mikrospor, oraz w procesach fotosyntetycznych. Badania proteomiczne wykazały, że izoforma X2 syntazy indolilo-3-glicerolo-fosforanu może stanowić marker wrażliwości na stres termiczny roślin gryki zwyczajnej.

Wykazano też, że stres troficzny jest główną przyczyną słabego plonowania gryki zwyczajnej. Nawet, gdyby wzrosła liczba prawidłowo rozwiniętych woreczków zalążkowych, roślina nie byłaby zdolna wypełnić wszystkich nasion. Usuwanie częściowe kwiatów prowadziło do kompensacyjnej ich produkcji. Wzrost liczby kwiatów korelował ze zwiększoną aborcją kwiatów i zarodków. Analiza plonowania w warunkach *in planta* wykazała jednak, że usuwanie kwiatów (50 lub 75%) u odmiany „Panda" spowodowało zwiększenie liczby nasion. W przypadku obu badanych odmian, rośliny z jednym pędem głównym produkowały najmniej dojrzałych i pustych nasion w stosunku do kontroli, odznaczały się jednak najkrótszą wegetacją i najszybszym dojrzewaniem nasion. Ponadto, ród PA15 w traktowaniu jednopędowym wykazywał najwyższą masę pojedynczego nasiona. Należy podkreślić, że momentem krytycznym decydującym o wysokości plonu nasion jest już moment kwitnienia i zapłodnienia. Nadprodukcja kwiatów oraz tworzenie zarodków jest procesem wyczerpującym asymilaty. Niewypełnianie wszystkich nasion, jako swoista strategia rośliny, nie zrekompensuje już tych strat. Z kolei badania w warunkach *in vitro* dowiodły, że indukowany stres troficzny, zwłaszcza na pożywkach o trzykrotnie uboższym składzie substancji odżywczych, zwiększa procent degeneracji woreczków zalążkowych. Do najczęściej występujących zaburzeń rozwojowych woreczków zalążkowych należały: rozrost komórek integumentów, wadliwa wakuolizacja komórek jajowych, zapadanie światła woreczka zalążkowego czy brak synergid.

Jak dotąd, klasyczne programy hodowlane nie doprowadziły do wytworzenia odmiany gryki zwyczajnej o wysokim plonowaniu, tak jak to uzyskano w przypadku innych wiodących gatunków roślin uprawnych. Wyhodowanie formy gryki zwyczajnej samoistnie kończącej kwitnienie prawdopodobnie zmniejszyłoby stopień degeneracji zalążków oraz aborcji kwiatów i nasion, a tym samym zwiększyłoby plon nasion.

Tytul pracy w języku angielskim

Developmental and physiological mechanisms of flower abortion affecting the seed yield of common buckwheat (*Fagopyrum esculentum* Moench)

Slowa kluczowe

common buckwheat, embryological development, heat shock proteins, hormonal profile, nutritional stress, seed yield, thermal stress

Common buckwheat (*Fagopyrum esculentum* Moench) belongs to the knotweed family (*Polygonaceae*). It is a dicotyledonous, annual plant grown mainly for food purposes due to the well-balanced chemical composition of the seeds. Buckwheat is used as the main crop and as an aftercrop due to its phytosanitary properties. It is characterized by a low and unstable yield, which is influenced, among others, by the short life of a single flower, self-incompatibility due to flower heterostyly, abnormalities in the development of ovule sac, and buckwheat sensitivity to environmental stresses, for example high temperature during the flowering period. It is a self-finishing plant, it blooms throughout the growing season, which causes competition for assimilates between the seeds and the flowers still being produced. These factors determine premature flower and unripe seed fall.

The aim of the doctoral dissertation, including a series of five original publications, was to analyze the developmental and physiological mechanisms of flower abortion affecting the seed yield of common buckwheat. The research hypothesis assumed that the main causes of these processes are high-temperature and nutritional stress. To verify the hypotheses, two common buckwheat genotypes were studied, 'Panda' cultivar and the PA15 strain (currently the 'Korona' cultivar), which show differences in the degree of degeneration of ovule sacs, flower abortion, and seed yield. In the studies conducted on the plants treated with high temperature (30°C) or cultivated in nutritional stress *in planta*, induced by partial removal of flowers or side shoots, a number of analyzes of embryological development, hormonal changes occurring in the flowers at various stages of development and in the leaves were performed. Moreover, the degree of flower abortion and the elements of the yield structure were measured, i.e., the number of full and empty seeds produced by one plant, their weight, and the weight of a thousand seeds. Additionally, under thermal stress, an analysis of the photochemical and photosynthetic efficiency, as well as proteomic changes and the accumulation of heat shock proteins (HSP) in the flowers at various stages of development and in the leaves, were performed. Nutritional stress *in vitro* was investigated additionally by exposing flower buds to a nutrient-reduced medium.

The analyzes showed that high temperature (30°C), which is optimal for the vegetative development of buckwheat, increases the degree of degeneration of the ovules during embryological development. In high-temperature stress, the HSP-70 protein probably plays a protective role in the development of ovules and microspores, and in photosynthetic processes. Proteomic studies showed that the indole-3-glycerol phosphate synthase chloroplastic-like isoform X2 may be a marker of heat sensitivity of buckwheat plants.

It was also shown that nutritional stress is the main cause of the poor seed yield of common buckwheat. Even if the number of properly developed embryo sacs increased, the plant would not be able to full fill all the seeds. Partial removal of flowers led to their compensatory production. The increase in the number of flowers correlated with the increased abortion of flowers and embryos. The analyses of yielding *in planta*, showed that removal of flowers (50 or 75%) in the cv. 'Panda' resulted in an increase in seed number. In the case of both studied cultivars, plants

with one main shoot produced the least mature and empty seeds as compared to the control, but they were characterized by the shortest vegetation and the fastest seed maturation. Moreover, the PA15 plants cultivated with the single shoot had the highest weight of a single seed. It should be emphasized that the critical moment that determines the amount of the seed yield is the flowering and fertilization phase. The overproduction of flowers and the formation of embryos is a process that exhausts nutritional assimilates. These losses can no longer be compensated for by not filling the seeds, which is a specific plant strategy. In turn, studies *in vitro* conditions showed that the induced nutritional stress, especially on media with three times poorer nutrient composition, increased the percentage of degeneration of the ovules. To the most common developmental disorders of the embryo sacs belonged: integument cell proliferation, defective ovule vacuolization, the collapse of the embryo sacs, and lack of synergids.

So far, classical breeding programs do not result in high-yielding common buckwheat cultivar, as has been achieved with other leading crop species. Obtaining a buckwheat form that terminates flowering would probably reduce the degree of ovule degeneration and abortion of flowers and seeds, and thus increase the seed yield.

A. Przen

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Rozwojowe i fizjologiczne mechanizmy aborcji kwiatów kształtujące plon nasion gryki zwyczajnej (*Fagopyrum esculentum* Moench.)
/ Tytul pracy /

Student/-ka Uniwersytetu Rolniczego im. Hugona Kołłątaja w Krakowie

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a Studentką: Martą Hornyák

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zrealizowanej w Katedrze Fizjologii, Hodowli Roślin i Nasiennictwa

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zrealizowanej w Katedrze Fizjologii, Hodowli Roślin i Nasiennictwa

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Prof. dr hab. inż. Agnieszce Płażek

Promotorowi niniejszej rozprawy doktorskiej, za daną mi szansę powrotu do „świata nauki”, życzliwość i otwartość oraz wszechstronną możliwość rozwoju. Dziękuję za wsparcie merytoryczne i duchowe

Współautorom publikacji
Za cenne rady, naukową dyskusję i owocną współpracę

Kubie, Ani i Przemkowi
Za wspólne chwile spędzone w pokoju 203, śmiech i łzy, wsparcie w badaniach, i słowa otuchy, gdy były potrzebne. Za charakter nadany studiom doktoranckim w Fitotronie

Pracownikom Katedry Fizjologii, Hodowli Roślin i Nasiennictwa
Za życzliwość i rodzinną atmosferę

To my beloved Husband
Thank you for your great faith in me, unlimited support, and an unwavering motivation

Kochanym Rodzicom
Za troskę, motywację w dążeniu do celu i wiarę we mnie. Dziękuję za lata edukacji i okazywanego wsparcia

Rozprawę doktorską dedykuję ***Frankowi i Florze***

„Cel, do którego człowiek dąży, jest zawsze niejasny.” M. Kundera

Spis treści

Wykaz używanych skrótów i terminów

Wykaz używanych skrótów i terminów

1S (ang. *a plant with the single main shoot*) – roślina w uprawie jednopędowej

2-DE (ang. *two-dimensional gel electrophoresis*) – dwukierunkowa elektroforeza żelowa

50% (ang. *plant with 50% of flowers removed*) – roślina z usuniętymi 50% kwiatów

6PGDH (ang. *6-phosphogluconate dehydrogenase*) – dehydrogenaza 6-fosfoglukonianowa

75% (ang. *plant with 75% of flowers removed*) – roślina z usuniętymi 75% kwiatów

ABA (ang. *abscisic acid*) – kwas abscysynowy

ABA-free (ang. *active forms of abscisic acid*) – aktywne formy kwasu abscysynowego

ABA-glc (ang. *abscisic acid glucosyl ester, non-active abscisic acid*) – ester glukozowy kwasu abscysynowego, nieaktywny kwas abscysynowy

ABS/CS$_m$ (ang. *energy absorbed by antennas*) – energia zaabsorbowana przez anteny

ACP (ang. *acyl-[acyl-carrier-protein]*) – acylo-[acyl-białko-nośnikowe]

BA (ang. *benzoic acid*) – kwas benzoesowy

CHAPS (ang. *3-[(cholamidopropyl)dimethyl-ammonio]-1-propane sulfonate*) – sulfonian 3-[(cholamidopropylo)dimetylo-amonio]-1-propanu

ChlF (ang. *chlorophyll fluorescence*) – fluorescencja chlorofilu

***C*$_i$** (ang. *intercellular CO_2 concentration*) – międzykomórkowe stężenie CO_2

CS$_m$ (ang. *excited cross-section of the leaf*) – wzbudzana powierzchnia fotosyntetyzującej próbki

CYT (ang. *cytokinins*) – cytokininy

DI$_o$/CS$_m$ (ang. *energy dissipation from PSII*) – rozpraszanie energii z fotosystemu II

DREB2A (ang. *dehydration-responsive element-binding protein 2A*) – czynnik transkrypcyjny DREB2A

DTT (ang. *dithiothreitol*) – ditiotreitol

DW (ang. *dry weight*) – sucha masa

E (ang. *transpiration rate*) – współczynnik transpiracji

E1 (ang. *initial electrolyte leakage*) – przewodność początkowa elektrolitów

E2 (ang. *final conductivity*) – przewodność końcowa elektrolitów

EL (ang. *electrolyte leakage*) – wypływ elektrolitów

ET$_o$/CS$_m$ (ang. *the energy used for electron transport*) – energia wykorzystywana do transportu elektronów

F$_0$ (ang. *minimal fluorescence yield of the dark-adapted state*) – fluorecencja minimialna próby adaptowanej do ciemności

FBA (ang. *fructose-bisphosphate aldolase*) – aldolaza fruktozobisfosforanowa

FBP (ang. *fructose-1,6-bisphosphate*) – fruktozo-1,6-bisfosforan

$\mathbf{F_m}$ (ang. *maximal fluorescence yield of the dark-adapted state*) – maksymalna wydajność fluorescencji w liściach zaadaptowanych do ciemności

$\mathbf{F_v}$ (ang. *variable fluorescence*) – zmienna fluorescencja

$\mathbf{F_v/F_m}$ (ang. *maximal quantum yield of PSII photochemistry*) – maksymalna fotochemiczna wydajność kwantowa PSII

FW (ang. *fresh weight*) – świeża masa

GA (ang. *gibberellin*) – gibereliny

GAs (ang. *active gibberellins*) – aktywne gibereliny (GA_1, GA_3, GA_4, GA_5, GA_6, GA_7)

GLR (ang. *glutamate receptor-like channel*) – receptor glutaminianu

$\boldsymbol{g_s}$ (ang. *stomatal conductance*) – przewodnictwo szparkowe

HSF (ang. *heat-shock transcription factor*) – czynnik transkrypcyjny szoku cieplnego

HsfA1 (ang. *heat-shock transcription factor A1*) – A1 czynnik transkrypcyjny szoku cieplnego

HSP (ang. *heat-shock proteins*) – białka szoku cieplnego

IAA (ang. *indole-3-acetic acid*) – kwas indolilo-3-octowy

IEF (ang. *isoelectric focusing*) – ogniskowanie izoelektryczne

IGP (ang. *indole-3-glycerol phosphate*) – fosforan indolilo-3-glicerolu

IGPS (ang. *indole-3-glycerol phosphate synthase*) – syntaza fosforanu indolilo-3-glicerolu

JA (ang. *jasmonic acid*) – kwas jasmonowy

JA-Met (ang. *jasmonic acid methyl ester*) – ester metylowy kwasu jasmonowego

Jas (ang. *jasmonates*) – jasmoniany (JA + JA-Met)

MTS (ang. *the mass of thousand seeds*) – masa tysiąca nasion

OPPP (ang. *oxidative pentose phosphate pathway*) – oksydacyjny szlak pentozofosforanowy

PGK (ang. *phosphoglycerate kinase*) – kinaza fosfoglicerynianowa

PI (ang. *performance index of PSII*) – wskaźnik wydajności PSII

PMF (ang. *peptide mass fingerprinting*) – peptydowy odcisk palca

$\boldsymbol{P_N}$ (ang. *net photosynthesis rate*) – wydajność fotosyntezy netto

PPFD (ang. *photosynthetic photon flux density*) – gęstość strumienia fotonów fotosyntetycznie czynnych

PS (ang. *photosystem*) – fotosystem

$\mathbf{Q_A}$ i $\mathbf{Q_B}$ (ang. *plastoquinone*) – cząsteczki plastochinonu związane z białkami PSII

$\mathbf{RC/CS_m}$ (ang. *number of active reaction centers*) – liczba aktywnych centrów reakcji

ROS (ang. *reactive oxygen species*) – reaktywne formy tlenu

SA (ang. *salicylic acid*) – kwas salicylowy

SAM (ang. *S-adenosylmethionine*) – *S*-adenozylometionina

SAMS (ang. *S-adenosylmethionine synthetase*) – syntetaza *S*-adenozylometioniny

SC (ang. *soluble carbohydrates*) – węglowodany rozpuszczalne

SDS (ang. *sodium dodecyl sulfate*) – laurylosiarczan sodu

SE (ang. *standard error*) – błąd standardowy

SFBA (ang. *sedoheptulose/fructose-bisphosphate aldolase*) – sedoheptuloza/aldolaza fruktozo-bisfosforanowa

SuBP (ang. *sedoheptulose-1,7-bisphosphate*) – sedoheptulozo-1,7-bisfosforan

Stres termiczny (ang. *thermal stress*) – stres wywołany wysoką temperaturą

Stres troficzny (ang. *nutritional stress*) – stres pokarmowy, polegający na zaburzonej dystrybucji składników pokarmowych do kwiatów i nasion, konkurencja o asymilaty

TF (ang. *transcription factor*) – czynnik transkrypcyjny

TR_o/CS_m (ang. *excitation energy trapped in PSII*) – energia związana w PSII

UFA (ang. *unsaturated fatty acids*) – nienasycone kwasy tłuszczowe

V-ATPase (ang. *V-type proton ATPase*) – ATPaza protonowa typu V

ZEA (ang. *zeatin*) – zeatyna

δRo (ang. *the efficiency with which an electron can move from the reduced intersystem of electron acceptors to the PSI and electron acceptors*) – wydajność, z jaką elektron może przemieszczać się ze zredukowanego systemu akceptorów elektronów do PSI i akceptorów elektronów

φRo (ang. *the quantum yield of electron transport from Q_A^- to the PSI and electron acceptors*) – wydajność kwantowa transportu elektronów z Q_A^- do PSI i akceptorów elektronów

ψRo (ang. *probability, at time 0, of the electron transport chain beyond Q_A^-)* – prawdopodobieństwo, w czasie 0, transportu elektronów poza Q_A^-

1. Wykaz publikacji stanowiących rozprawę doktorską

I. Płażek A., Słomka A., Kopeć P., Dziurka M.*, **Hornyák M**., Sychta K., Pastuszak J. (2019). Effects of high temperature on embryological development and hormone profile in flowers and leaves of common buckwheat (*Fagopyrum esculentum* Moench). *International Journal of Molecular Sciences*, 20(7), 1705.
DOI: 10.3390/ijms20071705

IF_{2019}: 4,602
5-letni IF: 6,132

II. **Hornyák M**.*, Płażek A., Kopeć P., Dziurka M., Pastuszak J., Szczerba A., Hura T. (2020). Photosynthetic activity of common buckwheat (*Fagopyrum esculentum* Moench) exposed to thermal stress. *Photosynthetica*, 58(1), 45–53.
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III. Płażek A., Hura K., Hura T.*, Słomka A., **Hornyák M.**, Sychta K. (2020). Synthesis of heat-shock proteins HSP-70 and HSP-90 in flowers of common buckwheat (*Fagopyrum esculentum*) under thermal stress. *Crop and Pasture Science*, 71(8), 760–767.
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V. Kopeć P., **Hornyák M.,** Pastuszak J., Szczerba A., Rapacz M.*, Waga J., Płażek A. (2021). Changes in the flower and leaf proteome of common buckwheat (*Fagopyrum esculentum* Moench) under high temperature. *International Journal of Molecular Sciences*, 22(5), 2678.
DOI: 10.3390/ijms22052678

IF_{2021}: 5,542
5-letni IF: 6,132

*autor korespondencyjny

Sumaryczny IF: 18,832
Sumaryczny 5-letni IF: 23,701

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Niniejsza praca doktorska została wykonana w ramach badań realizowanych w projekcie finansowanym przez Narodowe Centrum Nauki pt. „Badanie mechanizmu degeneracji woreczków zalążkowych i aborcji kwiatów jako przyczyny słabego zawiązywania nasion gryki zwyczajnej (*Fagopyrum esculentum* Moench)".
Nr grantu: 2017/25/B/NZ9/00148
Projekt w ramach konkursu OPUS 13 realizowanego w latach 2018 – 2021
Kierownik projektu: prof. dr hab. inż. Agnieszka Płażek

W ramach projektu byłam zatrudniona jako doktorant-stypendysta.

2. Streszczenie

Rozwojowe i fizjologiczne mechanizmy aborcji kwiatów kształtujące plon nasion gryki zwyczajnej (*Fagopyrum esculentum* Moench)

Słowa kluczowe: białka szoku cieplnego, gryka zwyczajna, plon nasion, profil hormonalny, proteomika, rozwój embriologiczny, stres termiczny, stres troficzny

Gryka zwyczajna (*Fagopyrum esculentum* Moench) należy do rodziny rdestowatych (*Polygonaceae)*. Jest dwuliścienną rośliną jednoroczną uprawianą głównie na cele spożywcze ze względu na dobrze zbilansowany skład chemiczny nasion. Gryka stosowana jest jako plon główny oraz jako poplon ze względu na właściwości fitosanitarne. Charakteryzuje ją niski i nierównomierny plon, na który wpływ mają m.in. krótka żywotność pojedynczego kwiatu, samoniezgodność wynikająca z heterostylii kwiatów, nieprawidłowości w rozwoju woreczków zalążkowych i wrażliwość gryki na stresy środowiskowe np. wysoką temperaturę w okresie kwitnienia. Jest rośliną niesamokończącą tj. kwitnie przez cały okres wegetacji, co powoduje występowanie konkurencji o asymilaty pomiędzy zawiązanymi nasionami a wciąż produkowanymi kwiatami. Czynniki te determinują przedwczesne opadanie kwiatów i niedojrzałych nasion gryki zwyczajnej.

Celem niniejszej rozprawy doktorskiej, obejmującej cykl pięciu oryginalnych publikacji, była analiza rozwojowych i fizjologicznych mechanizmów aborcji kwiatów kształtujących plon nasion gryki zwyczajnej. Hipoteza badawcza zakładała, że głównymi przyczynami tych procesów jest stres wysokotemperaturowy i troficzny. Do weryfikacji postawionych hipotez wykorzystano dwa genotypy gryki zwyczajnej tj. odmiany „Panda" i ród PA15 (obecnie odmiana „Korona"), które wykazują różnice w stopniu degeneracji woreczków zalążkowych i aborcji kwiatów oraz w plonowaniu nasion. W badaniach prowadzonych na roślinach traktowanych wysoką temperaturą (30 ºC) lub w stresie troficznym *in planta,* modyfikowanym poprzez częściowe usuwanie kwiatów lub pędów bocznych, przeprowadzono szereg analiz rozwoju embriologicznego, zmian hormonalnych, zachodzących w różnych stadiach rozwojowych kwiatów oraz w liściach. Ponadto, oceniano stopień aborcji kwiatów i elementy struktury plonu tj. liczbę nasion pełnych i pustych wyprodukowanych przez jedną roślinę, ich masę oraz masę tysiąca nasion. Dodatkowo, w stresie termicznym wykonano analizę wydajności fotochemicznej i fotosyntetycznej, oraz zmiany proteomiczne i akumulację

białek szoku cieplnego (HSP) w różnych stadiach rozwojowych kwiatów i w liściach. Stres troficzny badano dodatkowo w warunkach *in vitro* poprzez wykładanie pąków kwiatowych na pożywki o zmniejszonym składzie substancji odżywczych.

Przeprowadzone analizy wykazały, że wysoka temperatura (30°C), która jest optymalna dla rozwoju wegetatywnego gryki zwyczajnej, zwiększa stopień degeneracji woreczków zalążkowych w okresie rozwoju embriologicznego. W stresie wysokotemperaturowym białko HSP-70 pełni funkcję ochronną w rozwoju zalążków i mikrospor, i w procesach fotosyntetycznych. Badania proteomiczne wykazały, że zwiększona akumulacja izoformy X2 syntazy indolilo-3-glicerolo-fosforanu może stanowić marker wrażliwości na stres termiczny roślin gryki zwyczajnej.

Wykazano też, że stres troficzny jest główną przyczyną słabego plonowania gryki zwyczajnej. Nawet, gdyby wzrosła liczba prawidłowo rozwiniętych woreczków zalążkowych, roślina nie byłaby zdolna wypełnić wszystkich nasion. Usuwanie częściowe kwiatów prowadziło do kompensacyjnej ich produkcji. Wzrost liczby kwiatów korelował ze zwiększoną aborcją kwiatów i zarodków. Analiza plonowania w warunkach *in planta* wykazała jednak, że usuwanie kwiatów (50 lub 75%) u odmiany „Panda" spowodowało zwiększenie liczby nasion. W przypadku obu badanych odmian, rośliny z jednym pędem głównym produkowały najmniej dojrzałych i pustych nasion w stosunku do kontroli, odznaczały się jednak najkrótszą wegetacją i najszybszym dojrzewaniem nasion. Ponadto, ród PA15 w traktowaniu jednopędowym wykazywał najwyższą masę pojedynczego nasiona. Należy podkreślić, że momentem krytycznym decydującym o wysokości plonu nasion jest już moment kwitnienia i zapłodnienia. Nadprodukcja kwiatów oraz tworzenie zarodków jest procesem wyczerpującym asymilaty. Niewypełnianie wszystkich nasion, jako swoista strategia rośliny, nie zrekompensuje już tych strat. Z kolei badania w warunkach *in vitro* dowiodły, że indukowany stres troficzny, zwłaszcza na pożywkach o trzykrotnie uboższym składzie substancji odżywczych, zwiększa procent degeneracji woreczków zalążkowych. Do najczęściej występujących zaburzeń rozwojowych woreczków zalążkowych należały: rozrost komórek integumentów, wadliwa wakuolizacja komórek jajowych, zapadanie światła woreczka zalążkowego czy brak synergid.

Jak dotąd, klasyczne programy hodowlane nie doprowadziły do wytworzenia odmiany gryki zwyczajnej o wysokim plonowaniu, tak jak to uzyskano w przypadku innych wiodących gatunków roślin uprawnych. Wyhodowanie formy gryki zwyczajnej samoistnie kończącej kwitnienie prawdopodobnie zmniejszyłoby stopień degeneracji zalążków oraz aborcji kwiatów i nasion, a tym samym zwiększyłoby plon nasion.

3. Summary

Developmental and physiological mechanisms of flower abortion affecting the seed yield of common buckwheat (*Fagopyrum esculentum* Moench)

Keywords: common buckwheat, embryological development, heat shock proteins, hormonal profile, nutritional stress, seed yield, thermal stress

Common buckwheat (*Fagopyrum esculentum* Moench) belongs to the knotweed family (*Polygonaceae*). It is a dicotyledonous, annual plant grown mainly for food purposes due to the well-balanced chemical composition of the seeds. Buckwheat is used as the main crop and as an aftercrop due to its phytosanitary properties. It is characterized by a low and unstable yield, which is influenced, among others, by the short life of a single flower, self-incompatibility due to flower heterostyly, abnormalities in the development of ovule sac, and buckwheat sensitivity to environmental stresses, for example high temperature during the flowering period. It is a self-finishing plant, it blooms throughout the growing season, which causes competition for assimilates between the seeds and the flowers still being produced. These factors determine premature flower and unripe seed fall.

The aim of the doctoral dissertation, including a series of five original publications, was to analyze the developmental and physiological mechanisms of flower abortion affecting the seed yield of common buckwheat. The research hypothesis assumed that the main causes of these processes are high-temperature and nutritional stress. To verify the hypotheses, two common buckwheat genotypes were studied, 'Panda' cultivar and the PA15 strain (currently the 'Korona' cultivar), which show differences in the degree of degeneration of ovule sacs, flower abortion, and seed yield. In the studies conducted on the plants treated with high temperature (30 °C) or cultivated in nutritional stress *in planta*, induced by partial removal of flowers or side shoots, a number of analyzes of embryological development, hormonal changes occurring in the flowers at various stages of development and in the leaves were performed. Moreover, the degree of flower abortion and the elements of the yield structure were measured, i.e., the number of ripe and empty seeds produced by one plant, their weight, and the weight of a thousand seeds. Additionally, under thermal stress, an analysis of the photochemical and photosynthetic efficiency, as well as proteomic changes and the accumulation of heat shock proteins (HSP) in the flowers at various stages of development and in the leaves, were

performed. Nutritional stress *in vitro* was investigated additionally by exposing flower buds to a nutrient-reduced medium.

The analyzes showed that high temperature (30 °C), which is optimal for the vegetative development of buckwheat, increases the degree of degeneration of the ovules during embryological development. In high-temperature stress, the HSP-70 protein probably plays a protective role in the development of ovules and microspores, and in photosynthetic processes. Proteomic studies showed that the greater accumulation of indole-3-glycerol phosphate synthase chloroplastic-like isoform X2 may be recognized as a marker of heat sensitivity of buckwheat plants.

It was also shown that nutritional stress is the main cause of the poor seed yield of common buckwheat. Even if the number of properly developed embryo sacs increased, the plant would not be able to full fill all the seeds. Partial removal of flowers led to their compensatory production. The increase in the number of flowers correlated with the increased abortion of flowers and embryos. The analyses of yielding *in planta*, showed that removal of flowers (50 or 75%) in the cv. 'Panda' resulted in an increase in seed number. In the case of both studied cultivars, plants with one main shoot produced the least mature and empty seeds as compared to the control, but they were characterized by the shortest vegetation and the fastest seed maturation. Moreover, the PA15 plants cultivated with the single shoot had the highest weight of a single seed. It should be emphasized that the critical moment that determines the amount of the seed yield is the flowering and fertilization phase. The overproduction of flowers and the formation of embryos is a process that exhausts nutritional assimilates. These losses can no longer be compensated for by not filling the seeds, which is a specific plant strategy. In turn, studies *in vitro* conditions showed that the induced nutritional stress, especially on media with three times poorer nutrient composition, increased the percentage of degeneration of the ovules. To the most common developmental disorders of the embryo sacs belonged: integument cell proliferation, defective ovule vacuolization, the collapse of the embryo sacs, and lack of synergids.

So far, classical breeding programs do not result in high-yielding common buckwheat cultivar, as has been achieved with other leading crop species. Obtaining a buckwheat form that terminates flowering would probably reduce the degree of ovule degeneration and abortion of flowers and seeds, and thus increase the seed yield.

4. Wstęp

4.1. Opis gatunku

Gryka zwyczajna (*Fagopyrum esculentum* Moench) należy do rodziny rdestowatych (*Polygonaceae*). Zalicza się ją do tzw. pseudozbóż ze względu na podobny sposób uprawy i użytkowania oraz skład chemiczny nasion. Jest rośliną jednoroczną o krótkim okresie wegetacyjnym trwającym od 10 do 12 tygodni. Zaliczana jest do grupy tzw. roślin wtórnych, wyodrębnionych z chwastów występujących w innych roślinach uprawnych (Zarzecka i in. 2014). Obecnie, gryka jest rozprzestrzeniona na niemal wszystkie kontynenty. Do rodzaju *Fagopyrum* zalicza się obecnie dwa gatunki uprawne: *F. esculentum* (Moench) i *F. tataricum* (L.) Gaertn. oraz ponad 25 dzikich gatunków gryki, w większości endemicznych dla obszaru południowo-zachodnich Chin (Ohnishi 1990; Ohsako i Li 2020; Zhang i in. 2021). Przyjmuje się, że gryka zwyczajna wywodzi się z *Fagopyrum esculentum* subsp. *ancestrale* Ohnishi, formy dzikiej pochodzącej z prowincji Yunnan w południowych Chinach (Lityńska-Zając i Wasilikowa 2005). Wraz z gryką tatarką stanowią główne źródło rutyny i kwercetyny, cennych flawonoidów. Nasiona gryki zwyczajnej są bogate w składniki odżywcze takie, jak żelazo, błonnik, aminokwasy egzogenne, metabolity wtórne o właściwościach farmaceutycznych, zaś miód gryczany posiada właściwości bakteriostatyczne. Ponadto, nasiona gryki nie zawierają glutenu, co w połączeniu z wysoką wartością odżywczą stanowi doskonałe źródło pokarmu, zwłaszcza dla osób chorych na celiakię. Nasiona gryki zwyczajnej spożywa się w postaci kaszy, a słoma i plewy służą jako pasza dla zwierząt (Songin 2003). W Japonii bardzo popularna jest mąka gryczana, z której produkuje się makaron Soba. Zielone części roślin zawierają fagopirynę, która stosowana jest obecnie w terapii fotodynamicznej komórek nowotworowych (Sytar i in. 2013).

4.2. Charakterystyka biologiczna

Gryka zwyczajna posiada palowy system korzeniowy sięgający do jednego metra w głąb gleby oraz korzenie II i III rzędu. Część korzeni zaczyna brunatnieć już 18 dni po wschodach, co jest objawem ich starzenia. Łodyga gryki o antocyjanowym zabarwieniu osiąga od 60 do 100 cm wysokości i jest rozgałęziona. Liście dolne ogonkowe mają kształt sercowato-strzałkowaty, górne zaś są typu siedzącego. Gryka posiada wonne białe, bladoróżowe lub

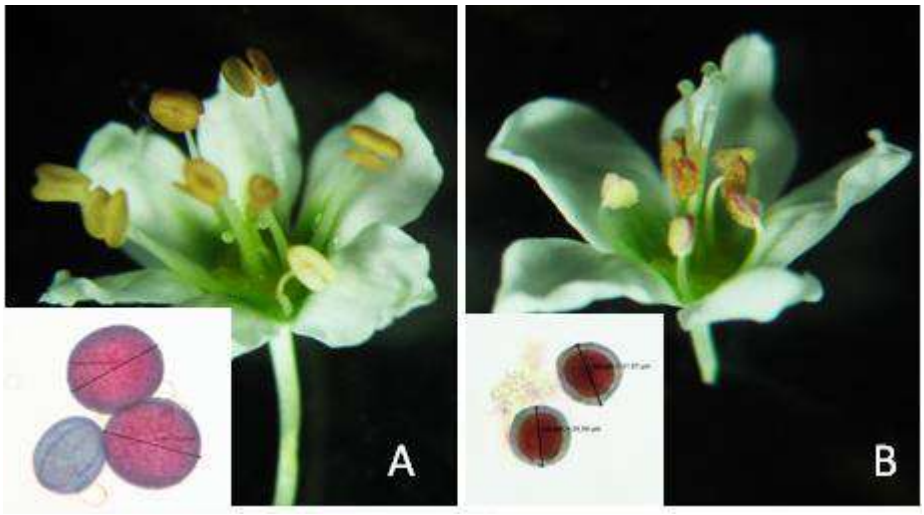
czerwone kwiaty, które zebrane są na zakończeniach rozgałęzień łodyg w luźne grona lub baldachy (Ryc. 1). Roślina zakwita 15-25 dni od siewu. Okres kwitnienia jest stosunkowo długi i trwa od 44 do 59 dni. Jedna roślina produkuje od 500 do 2000 kwiatów, z czego tylko 4-10% wykształca nasiona. Plonowanie w dużej mierze zależy od zastosowanej agrotechniki, odmiany gryki oraz od warunków pogodowych panujących w okresie kwitnienia. Owocem gryki jest ciemnobrunatny, trójgraniasty orzeszek. Masa tysiąca nasion mieści się w przedziale od 18 do 32 g (Songin 2003; Zarzecka i in. 2014).

Ryc. 1. Pokrój rośliny gryki zwyczajnej (*Fagopyrum esculentum* Moench)
(Rys. M. Hornyák)

4.3. Biologia kwitnienia

Gryka zwyczajna jest rośliną samoniezgodną, co wynika z występowania heterostylii, tj. kwiatów typu Pin i Thrum, o zróżnicowanej długości słupków i pręcików (Fot. 1). Do zapłodnienia może dojść tylko wtedy, gdy nastąpi zapylenie krzyżowe pomiędzy tymi dwoma morfami kwiatów (Cawoy i in. 2009). Pojedynczy kwiat gryki cechuje bardzo krótka żywotność, ponieważ jest zdolny do zapylenia tylko przez jeden dzień. Do najważniejszych

zapylaczy gryki należą owady błonkoskrzydłe (*Hymenoptera*), wśród nich głównie pszczoły i trzmiele oraz dwuskrzydłe (*Diptera*). Orzeszki dojrzewają nierównomiernie (Songin 2003). Gryka jest rośliną niesamokończącą i kwitnie przez cały okres wegetacji (70-90 dni), co powoduje występowanie silnej konkurencji o asymilaty pomiędzy zawiązanymi już nasionami a wciąż produkowanymi kwiatami (Fot. 2).



Fot. 1. Kwiaty gryki zwyczajnej typu Thrum (A) i typu Pin (B) wraz z ziarnami pyłku produkowanymi przez określony typ kwiatu (Fot. A. Słomka)

Fot. 2. Kwiatostan gryki zwyczajnej z kwiatami w różnych fazach rozwojowych oraz z zielonymi i dojrzałymi nasionami (Fot. A. Płażek)

4.4. Wymagania klimatyczno-glebowe

Gryka jest rośliną dnia długiego, ciepłolubną, wrażliwą na przymrozki. Jest rośliną jarą, do skiełkowania wymaga wyższej temperatury gleby, wynoszącej co najmniej 10 ºC. W klimacie Polski wysiewa się ją po ostatnich przymrozkach, w drugiej połowie maja. Ma również duże wymagania wodne, największe od siewu do zakończenia kwitnienia – 70 mm, najmniejsze podczas dojrzewania nasion – 15 mm. Gryka reaguje niekorzystnie na silne wiatry powodujące jej wyleganie, oraz na długotrwałe susze, które wysuszają znamię słupka i ograniczają produkcję nektaru niezbędnego do odwiedzania roślin przez owady zapylające. Grykę uprawia się zazwyczaj na glebach lekkich i mało urodzajnych, najlepiej jednak plonuje na glebach kompleksu pszennego bardzo dobrego, o uregulowanych stosunkach powietrzno-wodnych i o pH 5,6-7,0. Gryka może być uprawiana na terenie całej Polski z wyjątkiem terenów nadmorskich i podgórskich (Songin 2003).

4.5. Agrotechnika

Przedplonem dla gryki zwyczajnej są najczęściej rośliny zbożowe. Optymalna obsada roślin gryki wynosi od 2,5 do 3,5 mln roślin na 1 ha. Oznacza to, że należy wysiać od 60 do 125 kg nasion na 1 ha. Grykę wysiewa się na głębokość 2-3 cm w glebie wilgotnej i 4-5 cm w glebie przesuszonej. Siew z szeroką rozstawą rzędów (40 cm) zapewnia korzystniejsze warunki dla plonowania niż siew wąskorzędowy (13-15 cm), wymaga jednak mechanicznej pielęgnacji międzyrzędów. Gryka jest rośliną raczej odporną na choroby i szkodniki. Do najczęstszych chorób należy szara pleśń i mączniak rzekomy, zaś do najgroźniejszych pasożytów należy mszyca chmielowa. W uprawie gryki największym problemem jest zachwaszczenie, dlatego szczególnie ważne jest, aby przed siewem pole było dokładnie odchwaszczone. W 2021 roku w krajowym rejestrze Centralnego Ośrodka Badania Odmian Roślin Uprawnych wpisane były cztery odmiany gryki zwyczajnej: „Panda”, „Kora”, „Smuga” i „Korona” (poprzednio nazywana jako ród PA15) Małopolskiej Hodowli Roślin (Songin 2003; Zawojski 2019; www.coboru.gov.pl).

4.6. Znaczenie gospodarcze

W 2018 roku, według danych Organizacji Narodów Zjednoczonych do spraw Wyżywienia i Rolnictwa (ang. *Food and Agriculture Organization of the United Nations,* FAO), powierzchnia uprawy gryki na świecie wynosiła 3 mln ha. Według danych FAO do dziesięciu największych producentów gryki w latach 2014-2019 należały: Rosja, Chiny, Francja, Ukraina, Polska, USA, Kazachstan, Brazylia, Litwa i Japonia. W 2018 roku w Polsce powierzchnia uprawy gryki wynosiła 78 tys. ha. Plony gryki średnio wahają się w granicach od 0,6 do 1,5 t/ha. Z zastosowaniem odpowiedniej agrotechniki plony mogą osiągać 3 t/ha (Songin 2003). Według Głównego Urzędu Statystycznego (www.stat.gov.pl) w 2018 roku w Polsce pod względem procentowego udziału powierzchni uprawy gryki przodowały województwa lubelskie (22%), zachodniopomorskie (16%) i pomorskie (10%). W województwach: kujawsko-pomorskim, małopolskim i opolskim nie obserwuje się upraw tego gatunku.

Gryka zwyczajna wykazuje właściwości agroekologiczne dzięki zaletom takim, jak: wysoka miododajność (140-220 kg z 1 ha), odporność na choroby i szkodniki, działanie fitosanitarne – przeciwdziałanie rozwojowi nicieni, ochrona gleby przed erozją, walory estetyczne. Nie wymaga również specjalnego nawożenia mineralnego i zaliczana jest do żywności funkcjonalnej o cennych właściwościach odżywczych i prozdrowotnych (Songin 2003; Chłopicka 2008). W rolnictwie produkty uboczne z przerobu gryki wykorzystywane są jako pasza dla trzody chlewnej i ptactwa, a słoma gryczana w połączeniu ze słomą innych zbóż stanowi pożywienie dla bydła. Łuski gryczane służą jako wypełnienie poduszek, mat, materacy i wkładek do butów. Ponadto z produktów ubocznych przerobu gryki można produkować pelet i ekologiczne opakowania o dużej wytrzymałości mechanicznej (Borkowska i Robaszewska 2012).

4.7. Właściwości odżywcze i zdrowotne

Gryka zwyczajna może być wszechstronnie wykorzystywana w przemyśle spożywczym, farmaceutycznym i ziołolecznictwie. W przemyśle spożywczym orzeszki gryki przerabia się na kaszę, a mąka gryczana służy m.in. do wyrobu pieczywa bezglutenowego, makaronów, naleśników i herbat (Zarzecka i in. 2014). Nasiona są bogate w składniki odżywcze, zawierają dużo białka (8,5 – 19% suchej masy) o dobrze zbilansowanym składzie

aminokwasowym, skrobię (59 – 70% s.m.), lipidy (2 – 4% s.m.) z wysoką zawartością nienasyconych kwasów tłuszczowych i błonnik (5 – 11% s.m.). Nasiona stanowią również źródło wielu składników mineralnych takich, jak magnez, cynk, miedź, mangan, żelazo, potas i fosfor. Nasiona gryki zawierają witaminy z grupy B (tiaminę, ryboflawinę, pirydoksynę), niacynę i witaminę E. Wysoka zawartość polifenoli zapewnia właściwości przeciwutleniające u gryki. W różnych częściach gryki zidentyfikowano m.in. flawonoidy (rutynę, kwercetynę, orientynaę izoorientynę, witeksynę, izowiteksynę), flawony, kwasy fenolowe, taniny, fitosterole i fagopiryny. Ze względu na zawartość substancji biologicznie czynnych gryka wykorzystywana jest m.in. w profilaktyce chorób nowotworowych, chorób układu krążenia i leczeniu stanów zapalnych (Christa i Soral-Śmietana 2007; Zarzecka i in. 2014). Fagopiryny występujące w liściach i zielonych częściach gryki (Kreft i in. 2013) mają działanie przeciwgrzybiczne i przeciwwirusowe (Karioti i Bilia 2010; Sytar i in. 2016), a ze względu na właściwości fototoksyczne są wykorzystywane w terapii fotodynamicznej komórek nowotworowych (Sytar i in. 2016). Miód gryczany zawiera cukry proste i cholinę (Christa i Soral-Śmietana 2007), i odznacza się silnymi właściwościami bakteriostatycznymi (Songin 2003).

4.8. Problematyka badawcza

W porównaniu z innymi roślinami uprawnymi, grykę zwyczajną cechuje duża zmienność plonowania (Songin 2003). W klimacie Polski roślina ta słabo plonuje. Do głównych przyczyn niskiego plonu nasion należy krótka żywotność pojedynczego kwiatu oraz nieprawidłowy rozwój woreczków zalążkowych (Słomka i in. 2015, 2016, 2017). Gatunek ten cechuje samoniezgodność, wynikająca z występowania heterostylii, tj. o zróżnicowanej długości słupków i pręcików (Cawoy i in. 2009). Do zapłodnienia dochodzi tylko wtedy, gdy nastąpi zapylenie krzyżowe pomiędzy tymi dwoma typami kwiatów. Gryka jest rośliną niesamokończącą i kwitnie przez cały okres wegetacji, co powoduje występowanie silnej konkurencji o asymilaty pomiędzy zawiązanymi już nasionami a wciąż produkowanymi kwiatami. Dodatkowo, gryka jest wrażliwa na stresy środowiskowe m.in. na przymrozki, upały i suszę występujące w okresie letnim. Czynniki te powodują przedwczesne opadanie kwiatów i zawiązków (Ruszkowska i Ruszkowski 1981). Według Cawoy i in. (2007) zdolność do powstawania nasion jest inicjowana podczas lub zaraz po zapłodnieniu. Proces ten jest bardzo

wrażliwy na czynniki biotyczne i abiotyczne, które mogą przyczyniać się do aborcji nasion i owoców.

Wyniki projektu finansowanego przez Ministerstwo Rolnictwa i Rozwoju Wsi w ramach badań podstawowych wspierających postęp biologiczny w produkcji roślinnej (w latach 2014-2016), dotyczącego biologii kwitnienia i plonowania polskich odmian i rodów gryki, wykazały, że im więcej kwiatów produkuje roślina gryki, tym większa jest ich aborcja. Liczba kwiatów koreluje ujemnie z liczbą dojrzałych nasion. Zwiększanie liczby kwiatów na roślinie nie poprawia zatem ich plonu nasion. Żywotność ziaren pyłku jest bardzo wysoka (od 97 do 100%), co świadczy, że proces mikrogametofitogenezy przebiega prawidłowo i nie jest to przyczyną słabego zawiązywania nasion. Liczba zdegenerowanych woreczków zalążkowych natomiast w tych roślinach jest wysoka i waha się od 10 do 30%. Degeneracja woreczków zalążkowych i zarodków może wynikać z cech genotypowych, wysokiej temperatury podczas kwitnienia oraz z niedoboru asymilatów. Kwiaty wykazują zdolność do zapłodnienia w ciągu zaledwie jednego dnia (Cawoy i in. 2009). Zawiązywanie nasion, jest niewystarczające, zależne od genotypu oraz warunków uprawy i wynosi 15-53% (Cawoy i in. 2009; Słomka i in. 2015, 2016).

5. Hipotezy badawcze i cel prowadzonych badań

Na podstawie aktualnego stanu wiedzy na temat czynników determinujących plonowanie nasion gryki zwyczajnej oraz badań własnych postawiono następujące **hipotezy badawcze**:

1. Wysoka temperatura wpływa negatywnie na przebieg procesów embriologicznych ze szczególnym uwzględnieniem rozwoju gametofitu żeńskiego gryki zwyczajnej.
2. Zaburzenia w rozwoju woreczków zalążkowych gryki zwyczajnej wynikają z niedoboru składników pokarmowych lub konkurencji o asymilaty pomiędzy licznie produkowanymi kwiatami a zawiązywanymi nasionami w trakcie całego cyklu wegetacyjnego.

Celem pracy było wskazanie głównej przyczyny wadliwego rozwoju woreczków zalążkowych oraz silnej aborcji kwiatów i niedojrzałych nasion, co ma bezpośredni wpływ na niskie plonowanie nasion gryki zwyczajnej.

6. Materiał i metody

Badania przeprowadzono w latach 2018-2021 w Katedrze Fizjologii, Hodowli Roślin i Nasiennictwa Uniwersytetu Rolniczego w Krakowie. Doświadczenia przeprowadzono w komorach fitotronowych w warunkach ściśle kontrolowanych (stres termiczny), pokojach hodowlanych (stres troficzny indukowany *in vitro*), oraz tunelu foliowym (stres troficzny indukowany *in planta*). Do badań prowadzonych w ramach rozprawy doktorskiej wykorzystano rośliny gryki zwyczajnej polskiej odmiany „Panda" i rodu PA15 (odmiana „Korona", zarejestrowana w roku 2019; ze względu na to, że w publikacjach wchodzących w skład rozprawy doktorskiej omawiane są wyniki na roślinach PA15, także w autoreferacie stosowane są te dwie nazwy zamiennie), pochodzących z Małopolskiej Hodowli Roślin Sp. z o.o., Zakładu Produkcyjnego w Palikijach. Obie odmiany różnią się istotnie pod względem stopnia degeneracji zalążków i zarodków oraz wysokością plonowania nasion. W kwiatach odmiany „Panda" degeneruje 30% woreczków zalążkowych, co przekłada się na niskie plonowanie, natomiast w przypadku rodu PA15 tylko 10% woreczków wykazuje zaburzenia rozwojowe (Słomka i in. 2015, 2016).

Do oceny rozwojowych i fizjologicznych mechanizmów aborcji kwiatów kształtujących plon nasion gryki zwyczajnej zastosowano poniższe metody, osobno dla rodzaju stresu.

I. Stres termiczny:
   - ocena wybranych parametrów kinetyki fluorescencji chlorofilu *a* w liściach gryki zwyczajnej (ChlF),
   - badania embriologiczne wykonane standardową metodą parafinową z hematoksyliną żelazistą wg Heidenhaina w połączeniu z błękitem alcjanowym (Filutowicz i Kużdowicz 1951), ziarna pyłku barwiono odczynnikiem Alexandra (Singh 2003),
   - oznaczanie białek szoku cieplnego HSP-70 i HSP-90 metodą Western Blot,
   - badania proteomu pąków kwiatowych, kwiatów w pełni rozwiniętych i przekwitniętych oraz liści donorowych, wykorzystujące kombinację elektroforezy 2-D z techniką LC-MS/MS (Klubicová i in. 2011),
   - badanie profilu hormonalnego w kwiatach, pąkach i liściach wg protokołu Dziurki i in. (2016),
   - ocena parametrów wymiany gazowej w liściach donorowych przy użyciu przenośnego analizatora dwutlenku węgla w podczerwieni (Analytical Development Co. Ltd., UK),

- ocena przepuszczalności membran cytoplazmatycznych metodą konduktometryczną, analizującą wypływ elektrolitów (EL),
- ocena całkowitej zawartości węglowodanów rozpuszczalnych metodą antronową (Yemm i Willis 1954).

II. Stres troficzny:
   - analiza embriologiczna w stresie troficznym wywołanym w warunkach *in vitro* i *in vivo*,
   - kultury *in vitro* pąków kwiatowych gryki zwyczajnej w kontrolowanych warunkach stresu troficznego; pąki kwiatowe gryki sterylizowano i wykładano na pożywki kontrolne: 1) wg Slawinska i Obendorf (2001) i 2) wg Asaduzzmann i in. (2009) oraz ich warianty o zmniejszonym składzie składników odżywczych o połowę lub dwie trzecie,
   - badanie profilu hormonalnego pąków, kwiatów i liści donorowych w stresie troficznym *in vivo*.

Szczegółowy opis materiału roślinnego i zastosowanych metod wykorzystywanych w poszczególnych częściach prowadzonych badań opisano w rozdziałach *Materials & Methods*, w każdej z pięciu publikacji wchodzących w skład rozprawy doktorskiej.

7. Najważniejsze wyniki przeprowadzonych badań

7.1. Stres termiczny

7.1.1. Przebieg mikro- i megasporogenezy oraz mikro- i megagametofitogenezy w temperaturach: 20°C i 30°C (artykuł I: Płażek i in. 2019).

U gryki zwyczajnej występuje silna aborcja kwiatów. Samoistne zrzucanie kwiatów jest często obserwowane u roślin najczęściej w wyniku braku zapylenia. Proces odrzucania kwiatów jest inicjowany podczas wczesnej fazy wzrostu pędów i różnicowania zawiązków kwiatów (Moe 1971). W tym wrażliwym stadium rozwojowym temperatura w przedziale 12–15°C silnie wpływa na powstawanie tzw. "ślepych" pędów. Zdolność do powstawania nasion jest inicjowana podczas lub zaraz po zapłodnieniu, jak również w tym czasie jest określana ich liczba, wielkość, a zatem ostateczny plon (Cawoy i in. 2007). Proces ten jest bardzo wrażliwy

zwłaszcza na czynniki abiotyczne, które mogą przyczyniać się do aborcji nasion. Gryka jest wrażliwa m.in. na przymrozki, upały i suszę występujące w okresie letnim. Czynniki te powodują przedwczesne opadanie kwiatów i zawiązków (Ruszkowska i Ruszkowski 1981). W rozprawie doktorskiej skupiono się na poszerzeniu wiedzy na temat procesów embriologicznych zachodzących pod wpływem działania wysokiej temperatury. Rozwój embrionalny analizowano w pąkach kwiatowych oraz w kwiatach w pełni rozwiniętych, zdolnych do zapłodnienia. Ponadto, analizowano zmiany w profilu hormonalnym w pąkach, kwiatach w pełni rozwiniętych i przekwitłych, oraz w liściach. Taki układ eksperymentalny miał dostarczyć informacji, czy i w jakim stopniu degeneracja woreczków zalążkowych oraz ziaren pyłku jest kontrolowana przez poszczególne fitohormony. W przypadku odmiany „Panda", wysoka temperatura (30°C) nie obniżyła żywotności ziaren pyłku, natomiast zmniejszyła nieznacznie ich żywotność w roślinach rodu PA15. W warunkach temperatury kontrolnej (20°C) żywotność ziaren pyłku w otwartych kwiatach u odmiany „Panda" była istotnie mniejsza niż u rodu PA15, co jest cechą warunkowaną raczej genotypowo. Poziom większości analizowanych hormonów w tym stadium rozwojowym kwiatów u obu odmian był podobny, z wyjątkiem wolnego kwasu abscysynowego i kwasu salicylowego, których poziom był istotnie wyższy w kwiatach PA15. Stwierdzono, że proces megasporofitogenezy i megagametogenezy jest bardziej wrażliwy na wpływ wysokiej temperatury w porównaniu do mikrosporofitogenezy i mikrogametogenezy. W temperaturze 30°C w pąkach kwiatowych u odmiany „Panda" zaobserwowano prawie o 40% mniej prawidłowo rozwiniętych woreczków zalążkowych w porównaniu do roślin kontrolnych. Efekt termiczny w przypadku rodu PA15 na rozwój woreczków zalążkowych w pąkach był nieznaczny. Z tego względu uznano większą wrażliwość odmiany „Panda" na stres wysokiej temperatury niż rodu PA15. W pełni rozwiniętych kwiatach u obu genotypów obserwowano dwa razy mniej prawidłowo wykształconych zalążków w 30°C niż w 20°C, natomiast w kwiatach przekwitniętych stopień degeneracji woreczków zalążkowych był porównywalny. Zaburzenia rozwojowe polegały między innymi na rozroście komórek integumentów, wadliwej wakuolizacji komórek jajowych, zapadaniu się światła woreczka zalążkowego oraz braku synergid. W przypadku odmiany „Panda" w temperaturze 30°C proces silnego rozrostu komórek woreczka zalążkowego można łączyć z istotnym wzrostem poziomu form aktywnych giberelin (GAs), cytokinin (CYT) oraz estru metylowego kwasu jasmonowego (JA-Met).

7.1.2. Profil fitohormonów w różnych fazach rozwojowych kwiatów oraz liści donorowych (leżących u podstawy badanych kwiatów) w stresie termicznym (artykuł I: Płażek i in. 2019).

Na przebieg procesów embriogenezy oraz na aborcję kwiatów i owoców mają wpływ fitohormony i regulatory wzrostu m.in. w różnicujących się merystemach. W procesie zawiązywania kwiatów i determinacji powstawania pręcikowia i słupkowia biorą udział auksyny, gibereliny, cytokininy, kwas abscysynowy, poliaminy, kwas salicylowy i jasmonowy (Bernier i in. 1993) . Badania opisane w artykule Płażek i in. (2019) można uznać za pionierskie, bowiem po raz pierwszy przeanalizowano tak dokładnie profil zmian hormonalnych zachodzących w trakcie rozwoju kwiatów gryki zwyczajnej i ich przekwitania, oraz w liściach donorowych, leżących najbliżej kwiatostanów. Ponadto, zbadano zmiany hormonalne zachodzące w kolejnych stadiach rozwojowych kwiatów i w liściach donorowych pod wpływem działania wysokiej temperatury. Zmiany te zależały głównie od odmiany i organu, a istotne różnice w poziomie hormonów w różnych organach obserwowano w roślinach kontrolnych i pod wpływem stresu termicznego. U roślin kontrolnych najmniejsza ilość cytokinin występowała w liściach w porównaniu do pąków, otwartych i przekwitniętych kwiatów. Gibereliny występowały przede wszystkim w otwartych i przekwitniętych kwiatach. Najwyższy poziom auksyny (kwasu indolilo-3-octwoego – IAA) wykryto w przekwitniętych kwiatach, a najniższy w liściach. Większe ilości kwasu abscysynowego (ABA) stwierdzono w otwartych kwiatach w porównaniu do innych organów. We wszystkich stadiach rozwojowych kwiatów wykazano wyższy poziom kwasu salicylowego (SA) niż w liściach, w przeciwieństwie do JA i JA-Met. Stres termiczny zwiększył istotnie zawartość CYT w kwiatach otwartych i przekwitniętych u odmiany „Panda", zwiększył poziom IAA w przekwitniętych kwiatach u rodu PA15 oraz zawartość giberelin w kwiatach odmiany „Panda". W temperaturze 30°C stwierdzono obniżenie poziomu ABA w kwiatach PA15 oraz JA w pąkach obu odmian. Pod wpływem wysokiej temperatury zwiększyła się zawartość JA-Met w pąkach i kwiatach odmiany „Panda", natomiast zawartość SA nie uległa zmianie. W 30°C w pełni rozwiniętych kwiatach u odmiany „Panda" zaobserwowano istotny wzrost poziomu kwasu abscysynowego (ABA), zaś u rodu PA15 następowało jego obniżenie. Kwas abscysynowy pełni funkcję sygnalną w starzeniu się różnych organów, w tym także kwiatów, z tego względu w tej pracy istotny wzrost poziomu ABA w pełni rozwiniętych kwiatach uznano jako wcześniejszy sygnał prowadzący do obumierania kwiatów przekwitniętych, w których poziom ABA istotnie się już

zmniejsza. Warto też zwrócić uwagę na fakt, że sygnał ABA występuje już w pełni rozwiniętych kwiatach, które są zdolne do zapłodnienia u gryki tylko przez jeden dzień.

7.1.3. Wydajność aparatu fotosyntetycznego w stresie termicznym (artykuł II: Hornyák i in. 2020).

Fotosynteza jest najbardziej wrażliwym procesem komórek roślinnych na wysoką temperaturę (Sharkey i Schrader 2006). W przypadku obu badanych odmian w temperaturze 30°C wartości większości parametrów wydajności fotochemicznej fotosystemu II (PSII) (PI, F_v/F_m, F_v/F_0, ABS/CS_m, TR_o/CS_m, ET_o/CS_m, RC/CS_m) były wyższe niż w temperaturze 20°C. Świadczy to o tym, że zastosowana wysoka temperatura nie była czynnikiem stresowym, lecz była korzystniejsza dla procesów fazy jasnej fotosyntezy. Badane odmiany różniły się wydajnością fotosyntetyczną w wysokiej temperaturze. Odmiana „Panda" w 30°C asymilowała dwukrotnie więcej dwutlenku węgla niż w 20°C, natomiast u PA15 wydajność fotosyntezy netto była taka sama w obu temperaturach i była istotnie wyższa niż u odmiany „Panda". Rośliny obu badanych genotypów rosły szybciej w temperaturze 30°C niż w 20°C. Tą reakcją można tłumaczyć istotnie mniejszą zawartość węglowodanów rozpuszczalnych w liściach donorowych wszystkich roślin rosnących w 30°C w porównaniu do roślin z 20°C. Cukry były prawdopodobnie zużywane do budowy tkanek wegetatywnych. Należy zaznaczyć, że w liściach roślin rodu PA15 było istotnie mniej węglowodanów rozpuszczalnych w obu temperaturach w porównaniu do roślin odmiany „Panda". Ilość cukrów w 30ºC w liściach odmiany „Panda" zmniejszyła się 1,6 razy w porównaniu do liści kontrolnych, a w liściach PA15 zawartość cukrów była aż czterokrotnie mniejsza. Analiza specyficznych parametrów kinetyki fluorescencji chlorofilu *a*, tj. ψR0 oraz φR0, (φRo – wydajność kwantowa transportu elektronów z Q_A^- do PSI i akceptorów elektronów; ψRo – prawdopodobieństwo, w czasie 0, transportu elektronów poza Q_A^-) sugeruje, że u PA15 część energii w postaci ATP została uzyskana w 30°C w procesie fosforylacji cyklicznej, bez syntezy drugiej siły asymilacyjnej – NADPH, potrzebnej w fazie ciemnej fotosyntezy. Skutkowałoby to zmniejszeniem syntezy węglowodanów. Tego zjawiska nie zaobserwowano u odmiany „Panda". Wyniki te potwierdzają różnicę w reakcji obu odmian na stres termiczny. Rośliny rodu PA15 wydają się lepiej „radzić sobie" z tym stresem niż rośliny odmiany „Panda". Wyższa temperatura, choć sprzyja rozwojowi wegetatywnemu gryki zwyczajnej, to jednak jest czynnikiem stresowym w trakcie rozwoju woreczków zalążkowych, ale u PA15 efekt ten jest dużo mniejszy.

7.1.4. Białka szoku cieplnego HSP-70 i HSP-90 w pąkach, kwiatach w różnych fazach rozwojowych oraz w liściach donorowych (artykuł III: Płażek i in. 2020).

Podstawową reakcją roślin na stres wysokiej temperatury jest ekspresja białek szoku cieplnego (ang. *heat shock proteins*), o masie cząsteczkowej od 10 do 200 kDa. HSP-70 spełniają istotne funkcje w zapobieganiu agregacji oraz wspieraniu ponownego zwijania innych białek zarówno w warunkach optymalnych, jak i podczas stresu (Wang i in. 2004) . Białka HSP-90 odpowiadają za fałdowanie białek oraz odgrywają kluczową rolę w transdukcji sygnału, regulacji cyklu komórkowego, transporcie białek i ich degradacji. W tej pracy analizowano zawartość HSP-70 i HSP-90 w pąkach, kwiatach w pełni rozkwitniętych i przekwitniętych oraz liściach donorowych, usytuowanych najbliżej kwiatostanów gryki zwyczajnej poddanej działaniu wysokiej temperatury. Analiza poziomu białek metodą Western Blot wykazała u obu odmian uprawianych w 20°C i 30°C zdecydowanie większą akumulację białka HSP-70 niż HSP-90. Dotyczyło to wszystkich badanych organów u obu odmian. W przypadku odmiany „Panda", wyraźny wzrost HSP-70 pod wpływem stresu termicznego wykazano w pąkach i kwiatach przekwitniętych w stosunku do roślin kontrolnych, natomiast w 20°C (kontroli) zawartość tego białka była wyższa w kwiatach otwartych. W liściach donorowych tej odmiany poziom HSP-70 był niższy w 30°C niż w liściach kontrolnychi. W 20°C u PA15 wyraźnie wyższy poziom HSP-70 zaobserwowano w kwiatach otwartych. W pąkach i liściach zawartość tego białka zwiększyła się pod wpływem 30°C. Zawartość HSP-90 była mało zróżnicowana u obu odmian, jedynie w liściach kontrolnych odmiany „Panda" obserwowano więcej tego białka niż w liściach roślin uprawianych w 30°C, zaś w liściach PA15 reakcja była odwrotna. Również te analizy dowiodły zróżnicowanej reakcji obu odmian na stres termiczny. Rośliny rodu PA15 (obecnie odmiany „Korona") akumulują w tym stresie więcej białka HSP-70 niż odmiana „Panda", co potwierdza wcześniejsze spostrzeżenia, że „Korona" jest bardziej odporna na wyższą temperaturę. Uzyskane wyniki sugerują, że w stresie termicznym białko HSP-70 odgrywa ochronną rolę w rozwoju kwiatów gryki zwyczajnej.

7.1.4. Profil białkowy w pąkach, kwiatach w różnych fazach rozwojowych oraz w liściach donorowych (artykuł V: Kopeć i in. 2021).

Wysoka temperatura wypływa negatywnie m.in. na procesy metaboliczne i strukturę białek (Timperio i in. 2008). W opisanym eksperymencie zbadano profil białkowy w kwiatach w różnych stadiach rozwojowych oraz liściach donorowych metodą elektroforezy dwukierunkowej i spektrometrii masowej. Zbadane odmiany gryki zwyczajnej różnią się tolerancją na wysoką temperaturę, co potwierdza stopień degeneracji woreczka zalążkowego pod wpływem tego stresu. Różne reakcje na stres cieplny znalazły odzwierciedlenie także w profilach białkowych tych odmian. W analizach elektroforezy 2-D wykryto różnice ilościowe i jakościowe białek w zależności od organu rośliny i odmiany. Na mapach 2-D białek w pąkach kwiatowych „Pandy" wykryto 1189 plamek białkowych, zaś w przypadku rodu PA15 – 1159. W kwiatach otwartych obserwowano 900 plamek dla „Pandy" i 977 dla PA15. Z kolei w kwiatach przekwitłych, niezapylonych, odmiany „Panda" było 1117 plamek białkowych i 1097 w kwiatach PA15. Najmniej plamek białkowych wykryto w próbkach liści donorowych, a mianowicie tylko 701 w przypadku „Pandy" i 826 u PA15.

Do identyfikacji białek wytypowano z żeli poliakrylamidowych 200 białek, których zawartość była co najmniej dwukrotnie większa w roślinach rosnących w wyższej temperaturze, w stosunku do rosnących w warunkach kontrolnych. Białka te pełnią różne funkcje w komórce: sygnałową, w hydrolizie ATP, w procesach fotosyntezy, syntezy białek, rozkładzie proteolitycznym białek, metabolizmie węglowodanów i azotu, oraz organizacji komórki. Udało się zidentyfikować tylko część białek. Wysoka temperatura nie wpłynęła na liczbę plamek białkowych w badanych odmianach, ale wpłynęła na ilość niektórych białek. W pąkach kwiatowych podwyższona temperatura wpłynęła na ekspresję 17 białek u „Pandy" i 34 u PA15. W wyniku reakcji na stres cieplny w otwartych kwiatach stwierdzono zwiększoną ilość 13 białek w przypadku odmiany „Panda" i 21 u PA15. Wysoka temperatura silnie zwiększyła ekspresję białek w kwiatach przekwitniętych: 28 białek u odmiany „Panda" i 63 u PA15. W liściach donorowych stres cieplny wpłynął na ekspresję 11 białek w przypadku odmiany "Panda" i 20 białek w liściach rodu PA15. Zawartość 15 białek w roślinach rosnących w wysokiej temperaturze była podobna dla wszystkich badanych roślin. Ekspresja izoformy X2 syntazy indolo-3-glicerolo- fosforanu, podobna do tej zawartej w chloroplastach, wzrosła znacząco w otwartych kwiatach odmiany „Panda" pod wpływem stresu termicznego. Odmiana ta została uznana za mniej odporną na stres wysokotemperaturowy, stąd wytypowano wzrost

ilościowy izoformy X2 syntazy indolo-3-glicerolo- fosforanu jako marker wrażliwości gryki zwyczajnej na ten stres.

7.2. Stres troficzny

Wpływ indukowanego stresu troficznego w warunkach *in vitro* i *in planta* na rozwój embriologiczny, profil fitohormonów oraz strukturę plonowania gryki zwyczajnej (artykuł IV: Hornyák i in. 2020).

Gryka jest rośliną niesamokończącą i kwitnie przez cały okres wegetacji, co powoduje występowanie silnej konkurencji o asymilaty pomiędzy zawiązanymi nasionami a wciąż produkowanymi, licznymi kwiatami. W pracy tej analizowano wpływ warunków troficznych na przebieg procesów embriologicznych tego gatunku w warunkach *in vitro*, oraz dodatkowo zmiany hormonalne i elementy plonowania w warunkach *in planta*.

7.2.1. Badania *in vitro*

Zielone pąki kwiatowe pobierano z roślin uprawianych w 20°C i wykładano na pożywki agarowe zawierające składniki niezbędne dla prawidłowego rozwoju zalążków i mikrospor. Indukcja stresu troficznego polegała na hodowli pąków na pożywkach agarowych zawierających połowę lub 1/3 składu pożywek kontrolnych, z wyjątkiem hormonów, których skład był w każdym wariancie taki sam.

Skład pożywek:

Pożywka 1, kontrola – pożywka wyjściowa: ½ MS (Murashige i Skoog 1962) + 3% sacharozy + 1 mg·dm^{-3} BA + 0.1 mg·dm^{-3} NAA.

Pożywka 1A – zawierała połowę stężeń składników odżywczych pożywki 1.

Pożywka 1B – ⅓ stężeń składników odżywczych pożywki 1.

Pożywka 2, kontrola – pożywka wyjściowa: makro- i mikroelementy MS + 2 mg·dm^{-3} zestawu witamin jak w pożywce B5 (Gamborg i in. 1968) + 2,5 g·dm^{-3} sacharozy + 0,1 g·dm^{-3} glutaminy + 1 mg·dm^{-3} kinetyny + 1 mg·dm^{-3} gibereliny GA3.

Pożywka 2A – zawierała połowę stężeń składników odżywczych pożywki 2.

Pożywka 3, kontrola – pożywka wyjściowa: MS + 3% sacharoza + 2 mg·dm^{-3} zeatyny.
Pożywka 3A – zawierała połowę zawartości składników odżywczych pożywki 3.

Na poszczególnych pożywkach kontrolnych po 10. dniach hodowli zaobserwowano zaburzenia w rozwoju woreczków zalążkowych. Należały do nich przede wszystkim: rozrost komórek integumentów, rozrost komórek ośrodka zalążka, dodatkowe podziały w woreczkach zalążkowych, rozwój struktur zarodkopodobnych. Zaburzenia w megasporofitogenezie oraz megagametogenezie obserwowano na wszystkich pożywkach kontrolnych oraz ich odpowiednikach o zmniejszonej zawartości składników odżywczych, ale najwięcej deformacji zanotowano na pożywce 1B, czyli zawierającej tylko jedną trzecią składników odżywczych. Dużo więcej przypadków degeneracji woreczków zalążkowych (zarówno w kontroli, jak i ich wariantach o obniżonej zawartości składników odżywczych) obserwowano w przypadku rodu PA15 niż u odmiany „Panda". Zaburzenia rozwojowe woreczków zalążkowych na pożywkach kontrolnych o pełnym składzie substancji odżywczych należy uznać za cechę warunkowaną genetycznie i związaną z danym genotypem.

7.2.2. Badania *in planta*

Pomiędzy liczbą kwiatów a ich aborcją wykazano dodatnią korelację. Oznacza to, że im więcej roślina produkuje kwiatów, tym więcej kwiatów ulegało aborcji. Jednym ze sposobów sprawdzenia, czy zmniejszenie konkurencji o asymilaty wpływa na zwiększenie plonu nasion, jest usunięcie części kwiatostanów. W przypadku gryki można usuwać pędy boczne wraz z nowymi kwiatostanami lub redukować liczbę kwiatów. Tego typu eksperymenty były prowadzone przez grupy badawcze Halbrecq'a i in. (2005) oraz Cawoy i in. (2007). Wykazały one brak istotnego wpływu usuwania pędów bocznych i kwiatów na ostateczny plon nasion, jednak nie analizowały procesów i zaburzeń zachodzących w rozwoju embrionalnym i hormonalnym pod wpływem tego traktowania, co analizowano w niniejszych badaniach (Hornyák i in. 2020).

Rośliny uprawiano w latach 2019 i 2020 od maja do września w doniczkach w tunelu foliowym otwartym umożliwiającym oblot owadów zapylających. Część roślin prowadzono w uprawie jednopędowej (boczne pędy były sukcesywnie usuwane), na części roślin usuwano 50 lub 75% kwiatów. Na roślinach kontrolnych pozostawiano pędy boczne i wszystkie kwiaty. W pozostawionych na roślinach analizowano przebieg procesów embriologicznych oraz wyznaczano profil fitohormonów, w pąkach kwiatowych, kwiatach otwartych i

przekwitających. Ponadto, oceniano produkcję kwiatów przez pojedynczą roślinę oraz skuteczność zawiązywania nasion. Zbiór nasion nastąpił pod koniec sierpnia. Zanalizowano również parametry plonowania.

W roślinach rodu PA15, z których usunięto 50% kwiatów obserwowano najmniejszy procent zdegenerowanych woreczków zalążkowych (o 20% mniej niż w roślinach kontrolnych). W pąkach kwiatowych tych roślin odnotowano istotnie mniej giberelin, cytokinin i ABA, zaś istotnie więcej JA, i SA w stosunku do roślin kontrolnych, jak i roślin z pozostałych traktowań. U roślin z 50% pozostawionych kwiatów, w otwartych kwiatach zdolnych do zapłodnienia zanotowano istotnie więcej ABA, JA, SA i kwasu benzoesowego (BA), prekursora kwasu salicylowego, niż u roślin kontrolnych. W tych kwiatach stężenia SA i BA były dużo wyższe. Wykryto w nich również więcej IAA. Najprawdopodobniej, auksyna ta podtrzymuje rozwój zarodków, co mogło przekładać się na większy plon nasion z tych roślin. Kwas salicylowy i jasmonowy pełnią istotną rolę w procesie kwitnienia i przywabiania owadów. Ponadto kwas jasmonowy jest niezbędny do powstawania komórki jajowej, zaś jego brak jest charakterystyczny dla sterylnych kwiatów. Uważa się, że ABA, jako antagonista giberelin, hamuje proces kwitnienia, dlatego też zaskakujący jest fakt, że w kwiatach, które najlepiej zawiązywały nasiona, tego hormonu było więcej niż w kwiatach odznaczających się większym stopniem degeneracji woreczków zalążkowych. Wysokie stężenie SA wskazuje na jego cenną rolę w procesie kwitnienia roślin. Prekursorem kwasu salicylowego jest kwas benzoesowy, dlatego też stężenie BA w kwiatach było wysokie. Wszystkie powyższe obserwacje dotyczyły jedynie profilu fitohormonów w kwiatach roślin rodu PA15 po usunięciu 50% kwiatów, natomiast w tak traktowanych roślinach odmiany „Panda" tego typu zmian nie zaobserwowano. W pąkach roślin PA15, z których usunięto 75% kwiatów, wykazano najwięcej IAA i ABA. W otwartych kwiatach stwierdzono najmniej IAA i jasmonianów oraz najwięcej giberelin. Pomimo wyższego procentu nieprawidłowo wykształconych woreczków zalążkowych u PA15 po usunięciu 75% kwiatów, wykazano większą efektywność zawiązywania nasion niż u roślin po usunięciu 50% kwiatów. W roślinach odmiany „Panda" po usunięciu 75% kwiatów stwierdzono dużo więcej nieprawidłowo rozwiniętych woreczków zalążkowych niż u roślin z innych traktowań, natomiast efektywność zawiązywania nasion była podobna do roślin, z których usunięto 50% kwiatów. Efekt ten był prawdopodobnie spowodowany większym stężeniem giberelin oraz tym, że hormony te wykazują większą niż auksyny efektywność w podtrzymywaniu przy życiu powstających po zapłodnieniu zarodków.

Usuwanie kwiatów powodowało nadprodukcję kwiatów. Zwłaszcza u roślin odmiany „Panda", usunięcie 50% kwiatów wywołało zwiększenie ich liczby o 130%, a w roślinach, z

których usunięto 75% kwiatów wzrost ich liczebności nastąpił o 260%. Wzrost liczby kwiatów korelował ze zwiększoną aborcją kwiatów i zarodków. Usuwanie 75% kwiatów oraz uprawa jednopędowa u obu odmian znacznie zmniejszyły liczbę kwiatów wyprodukowanych przez jedną roślinę w porównaniu z kontrolą. Po usunięciu 50% lub 75% kwiatów, u odmiany „Panda" zwiększyła się liczba nasion. Rośliny w uprawie jednopędowej, w przypadku obu badanych odmian produkowały najmniej dojrzałych i pustych nasion w porównaniu z kontrolą, jednakże u PA15, masa pojedynczego nasiona była największa. Mniejsza liczba dojrzałych nasion przełożyła się na lepsze ich wypełnienie, a w konsekwencji zwiększenie masy tysiąca nasion. Rośliny w uprawie jednopędowej wcześniej kończyły wegetację, a ich nasiona dojrzewały szybciej, co jest cechą korzystną w przypadku gatunku rośliny niesamokończącej. Pojedyncze nasiona rodu PA15 (odmiana „Korona") wykazały istotnie większą masę niż nasiona odmiany „Panda". Usuwanie kwiatów, a zwłaszcza w uprawie jednopędowej istotnie zwiększyło procent pustych nasion u obu badanych odmian. Pomimo, że procent pustych nasion nie korelował istotnie z liczbą pełnych nasion, to korelował ujemnie z masą jednego dojrzałego nasiona Wyniki te jednoznacznie wskazują na fakt, że zawiązywanie nasion niewypełnionych, obniża masę nasion dojrzałych. Można zatem sądzić, że punktem krytycznym dla plonu nasion jest sam moment kwitnienia i zapłodnienia. Nadprodukcja kwiatów oraz tworzenie zarodków jest procesem wyczerpującym zapasy asymilatów rośliny. Niewypełnianie wszystkich nasion już nie zrekompensuje tych strat.

8. Podsumowanie i wnioski

W ramach prezentowanego cyklu pięciu publikacji wykazano, że:

1. Wysoka temperatura (30ºC) jest optymalna dla rozwoju wegetatywnego gryki zwyczajnej. Ta sama temperatura występująca w okresie rozwoju embrionalnego oraz w okresie kwitnienia zwiększa stopień degeneracji woreczków zalążkowych, nie zmniejsza jednak żywotności ziaren pyłku.

2. W wysokiej temperaturze białko HSP-70 pełni funkcję ochronną w procesach embriologicznych i fotosyntetycznych u gryki zwyczajnej.

3. Markerem wrażliwości roślin gryki zwyczajnej na stres wysokiej temperatury może być zwiększona ekspresja izoformyX2 syntazy indolo-3-glicerolo-fosforanu.

4. Wysoki poziom kwasu abscysynowego w otwartych kwiatach gotowych do zapłodnienia można interpretować jako sygnał indukujący obumieranie niezapylonych kwiatów, obserwowane w kolejnych dniach.

5. Wzrost liczby kwiatów produkowanych przez rośliny gryki zwyczajnej zawsze pociąga za sobą zwiększenie ich aborcji. Punktem krytycznym dla plonu nasion jest moment kwitnienia i zapłodnienia.

6. Główną przyczyną niskiego plonowania gryki jest stres troficzny. Nawet gdyby wzrosła liczba prawidłowo wykształconych woreczków zalążkowych, roślina nie byłaby zdolna do wypełnienia wszystkich nasion.

7. Wytworzenie formy samokończącej kwitnienie zmniejszyłoby stopień degeneracji zalążków i aborcji kwiatów.

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10. Publikacje stanowiące pracę doktorską

International Journal of
Molecular Sciences

MDPI

Article

Effects of High Temperature on Embryological Development and Hormone Profile in Flowers and Leaves of Common Buckwheat (*Fagopyrum esculentum* Moench)

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Received: 1 March 2019; Accepted: 2 April 2019; Published: 5 April 2019

Abstract: Common buckwheat is a valuable crop, mainly due to the beneficial chemical composition of its seeds. However, buckwheat cultivation is limited because of unstable seed yield. The most important reasons for the low yield include embryo and flower abortion. The aim of this work is to verify whether high temperature affects embryological development in this plant species. The experiment was conducted on plants of a Polish cultivar 'Panda' and strain PA15, in which the percentage of degenerating embryo sacs was previously determined and amounted to 32% and 10%, respectively. The plants were cultivated in phytotronic conditions at 20 °C (control), and 30 °C (thermal stress). The embryological processes and hormonal profiles in flowers at various developmental stages (buds, open flowers, and wilted flowers) and in donor leaves were analyzed in two-month-old plants. Significant effects of thermal stress on the defective development of female gametophytes and hormone content in flowers and leaves were observed. Ovules were much more sensitive to high temperature than pollen grains in both genotypes. Pollen viability remained unaffected at 30 °C in both genotypes. The effect of temperature on female gametophyte development was visible in cv. Panda but not in PA15 buds. A drastic reduction in the number of properly developed embryo sacs was clear in open flowers at 30 °C in both genotypes. A considerable increase in abscisic acid in open flowers ready for fertilization may serve as a signal inducing flower senescence observed in the next few days. Based on embryological analyses and hormone profiles in flowers, we conclude that cv. 'Panda' is more sensitive to thermal stress than strain PA15, mainly due to a much earlier response to thermal stress involving impairment of embryological processes already in the flower buds.

Keywords: abscisic acid; auxin; common buckwheat; cytokinins; embryo sacs; gibberellins; jasmonic acid; pollen grains; thermal stress; salicylic acid

1. Introduction

Common buckwheat (*Fagopyrum esculentum* Moench) belongs to the *Polygonaceae* family, but it is classified as a "pseudocereal" crop due to the cereal-like chemical composition of its seeds. The seeds are gluten-free; have high dietary fiber, high content of rutin and protein, and a well-balanced

amino acid composition; and are especially rich in lysine [1,2]. Buckwheat is also used as a fodder crop. The species shows numerous desired properties: it protects soil from erosion, exerts some health effects, efficiently absorbs nitrogen and phosphorus from the soil, is resistant to pests and diseases, and tolerates variable soil conditions. Buckwheat is pollinated primarily by bees and is a source of prized nectar. Common buckwheat seeds are produced mainly in Russia, China, Ukraine, Canada, Poland, Kazakhstan, and the United States of America (USA) (FAOSTAT, 2016). However, the crop has been in a decline for a long time in all countries as a result of low seed production and unstable yield [3]. The cultivation area of buckwheat is limited not only due to low seed yield, as compared with cereals, but also because of the heterogeneity of its maturation, which makes the harvest time difficult to determine [2]. The biggest drawback is the short life of its single flowers, which are only open for one day [4]. Buckwheat is also sensitive to ground frost, high temperatures, and drought, which may cause extensive flower and embryo abortion. It forms dimorphic plants with flowers harboring pistils and stamens of different lengths (pin and thrum types) and shows self-incompatibility [4,5]. Fertilization requires cross-pollination [6,7]. The seed set is insufficient, and it amounts to 15%–53%, depending on the genotype and growth conditions [4,8]. The most important reasons for the low yield include self-incompatibility, insufficient fertilization, embryo abortion, sensitivity to heat, and drought stress, as well as deficiency of assimilates in aging plants [6,9,10]. Slawinska and Obendorf [9] showed that the cultivation of buckwheat plants at a temperature below 25 °C can increase seed formation by up to 40%, while Kreft [11] reported that temperature above 30 °C is deleterious to buckwheat pollen and flowers. The optimal temperature for buckwheat growth is 18–23 °C [4]. Taylor and Obendorf [6] stated that aging plants are less capable of fertilization. The pollen germinates, and the pollen tube is visible, but there is no fusion of gametes. At 35 °C, Guan and Adachi [12] observed ultrastructural changes in embryo sacs of common buckwheat, e.g., accumulation of osmophilic deposits in the egg cell and synergids, collapsed synergids, incomplete membrane systems, and enlarged ribosome/endoplasmic reticulum in the egg cell. Our earlier studies showed that the key problem of the low seed yield in buckwheat is defective development of female gametophytes [8]. The formation of female gametophytes (megasoporogenesis and megagametophytogenesis) was shifted in time as compared with pollen development and occurred in older flowers and at higher temperature. Pollen viability was high, and it effectively germinated on the stigma. Long pollen tubes reaching the ovules were present in buckwheat flowers; thus, pollen was not the reason for the seed formation issues [8]. In other plant species, heat-related elimination of stigma receptivity, inhibition of pollen germination, and pollen tube growth are well known symptoms of temperature stress [13–15].

Another problem in buckwheat cultivation is the high degree of abortion of unfertilized flowers. Buckwheat blooming takes a long time and lasts from 30 to 60 days. One plant develops from 500 to 2000 flowers, but only some of them develop into seeds [2,9]. A positive correlation was noticed between the number of flowers and their abortion in all studied cultivars and strains, confirming that the more flowers the plant produced, the more were aborted [8].

The course of embryogenesis and flower abortion is under genetic control, but plant hormones and growth regulators, and particularly their concentrations, also play an important role. Auxins, gibberellins, cytokinins, polyamines, abscisic acid, salicylic acid, and jasmonic acid determine the growth and condition of stamens and pistils [16]. Bernier et al. [16] argued that flowering is affected by both plant hormones and sugars—mainly sucrose levels in the leaves that supply nutrients to the apical meristems.

The aim of this work is to investigate the hormonal profile during flower development associated with the embryological development of common buckwheat exposed to thermal stress (30 °C). A Polish cultivar 'Panda' and strain PA15 were chosen based on our earlier experiments [8]. The cultivar, 'Panda', showed 32% degenerated embryo sacs, while in strain PA15, disturbances occurred in 10% of flowers. We compared the changes in hormonal profiles at three developmental stages of flower—buds, open flowers, and wilted flowers—with the embryological development of pollen grains and embryo sacs.

2. Results

2.1. Embryological Analyses

2.1.1. Pollen Viability and Development

Pollen viability was generally high (>82%) in cv. 'Panda' and strain PA15, independent of temperature, suggesting regular meiosis. Once released from the tetrad (after meiosis), the microspores enlarged, forming vacuoles and thick sporodermis (Figure 1a). Then, they developed into pollen grains. Mature (three-celled) viable pollen exhibited dense cytoplasm (Figure 1b–e) and a positive purple-red or green stain in Alexander and fluorescein diacetate (FDA) tests, respectively (Figure 1b,c). 'Panda's' flowers showed significantly lower pollen viability at 20 °C (82%) than that of strain PA15 (96.3%) (compare Figure 1b vs. Figure 1e). We found no significant influence of thermal stress on pollen viability in either genotype compared with that of the control (compare Figure 1c vs. Figure 1d).

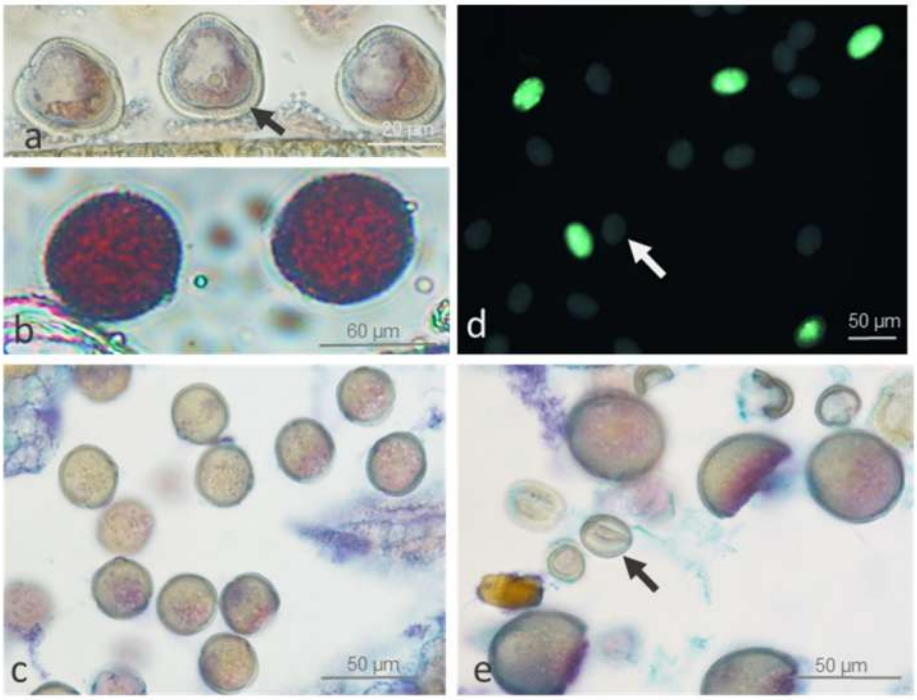

Figure 1. Viable and non-viable microspores and pollen grains of common buckwheat cv. 'Panda' and strain PA15 under control (20 °C) and thermal stress (30 °C) conditions. Vacuolated microspores of 'Panda' at 20 °C with thick sporodermis (**a**, arrow); viable pollen grains of PA15 at 20 °C stained with Alexander dye (**b**) and at 30 °C stained with Ehrlich's hematoxylin combined with Alcian blue (**c**); and viable and non-viable (arrows) pollen grains of 'Panda' at 30 °C stained with fluorescein diacetate (**d**) and at 20 °C stained with Ehrlich's hematoxylin combined with Alcian blue (**e**). At least 7900 pollen grains per treatment were analyzed in two replicates.

2.1.2. Ovule Development

We observed regular megasporogenesis and different stages of female gametophyte development in buds, open flowers, and wilted flowers of both genotypes (Figure 2). In buds, we noticed clear influence of temperature in cv. 'Panda' but not in strain PA15, in which embryological processes were slightly impaired at both 20 °C and 30 °C (83.3% and 79.5%, respectively) (Table 1). In open flowers, the number of properly developed embryo sacs at the control temperature slightly decreased in both genotypes (88.2% in 'Panda' and 77.8% in PA15), and a strong impact of high temperature on female gametophyte development was evident in both genotypes. In wilted flowers, this effect was not so clear, probably because flowers with degenerated embryo sacs had been earlier aborted (Table 1).

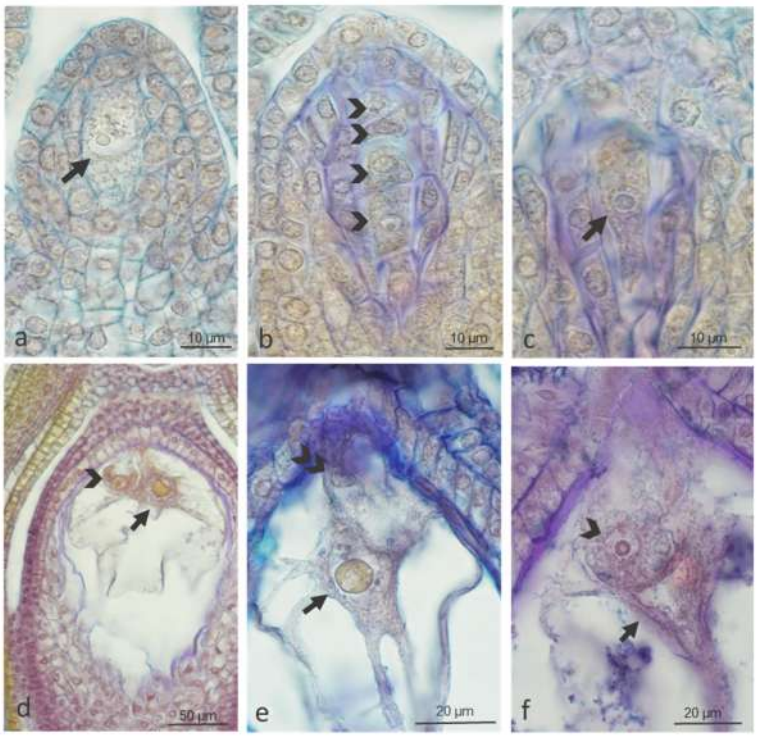

Figure 2. Proper megasporogenesis and female gametophyte development (megagametophytogenesis) at three stages of flower development in 'Panda' and PA15 genotypes of common buckwheat under control (20 °C) and thermal stress (30 °C) conditions. Megaspore mother cell in prophase I in 'Panda' bud at 20 °C (arrow, **a**), tetrad of megaspores in 'Panda' bud at 20 °C (each of four cells marked with arrowhead, **b**), 1-nucleate embryo sac of PA15 at 20 °C (arrow, **c**), central cell (arrows), egg cell (arrowheads), one of the two synergids (double arrowhead) of 7-celled embryo sac in open and wilted flowers (**d**–**f**) of 'Panda' at 30 °C (**d**), 'Panda' at 20 °C (**e**), and PA15 at 30 °C (**f**). Paraffin sections stained with Ehrlich's hematoxylin combined with Alcian blue. The number of analyzed flowers per treatment at each stage of development was 15–32 in two replicates.

Table 1. Percentage [%] of properly developed embryo sacs of cv. 'Panda' and strain PA15 at two temperatures.

Organ	cv. 'Panda'		PA15	
	20 °C	**30 °C**	**20 °C**	**30 °C**
Buds	100.0	57.7 *	83.3	79.5
Open flowers	88.2	44.4 *	77.8	26.7 *
Wilted flowers	83.3	80.0	81.3	78.9

* statistically significant embryo sac development dependence on temperature (n = 10) (Chi-squared test; $p < 0.05$).

Of the abnormal development patterns of female gametophyte observed under both temperatures, improper position of the nucleus in relation to the vacuole in the egg cell, degeneration of the entire embryo sac, and degeneration of the cells of the embryo sac and of entire ovules were the most frequent (Figure 3).

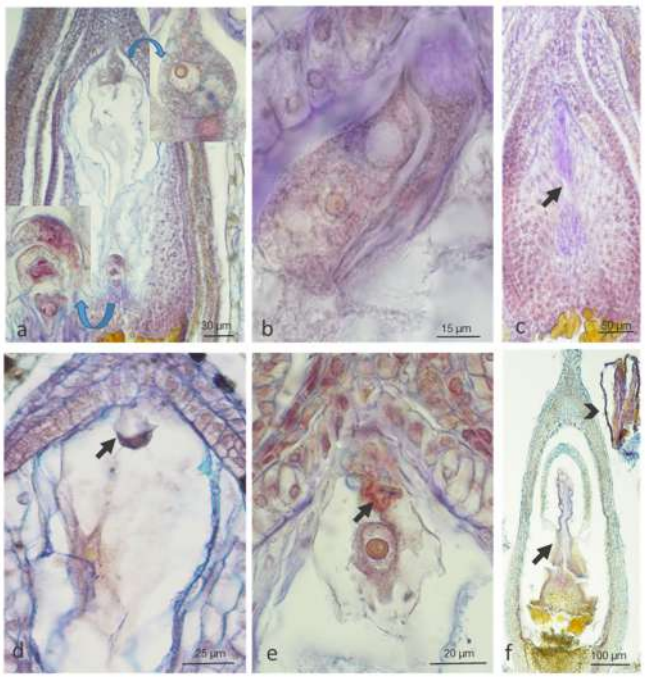

Figure 3. Abnormal female gametophyte development (megagametophytogenesis) at three stages of flower development in 'Panda' and PA15 genotypes of common buckwheat under control (20 °C) and thermal stress (30 °C) conditions. Abnormal vacuolization of egg cells of 'Panda' (upper insert in (**a**)) and of PA15 open flowers at 30 °C (**b**), at the chalazal pole three antipodal cells visible (lower insert in **a**); degeneration of 2–4-nucleate embryo sac of 'Panda' bud at 20 °C (arrow, **c**), of egg cell in PA15 open flowers at 20 °C (arrow, **d**), and of egg apparatus (arrow, **e**), ovule (arrow, **f**), and stamens (arrowhead, **f**) of 'Panda' open flowers at 30 °C. Paraffin sections stained with Ehrlich's hematoxylin combined with Alcian blue. The number of analyzed flowers per treatment at each stage of development was 15–32 in two replicates.

2.2. Hormonal Profile

2.2.1. Total Hormone Content

We determined the hormone content in the flower buds, open flowers, and wilted flowers and in the donor leaves. Table 2 presents the total contents of the studied hormones in the plant organs for strain PA15 and cv. 'Panda' at both temperatures. We decided to show the differences between hormone amounts in particular organs independently of growth conditions, as such data are lacking in the literature.

Leaves accumulated the lowest amounts of cytokinins (zeatin and kinetin) as compared with buds and open and wilted flowers. Active forms of gibberellin (GA) and non-active GA_8 occurred mainly in the open and wilted flowers. Among non-active forms of gibberellins, GA_9 was the most abundant, and it was detected mainly in the open flowers. This gibberellin also constituted the greatest share in the total pool of gibberellins. Non-active GA_{20} was present in scarce amounts in the flowers but reached its highest level in the leaves. The greatest amount of indole-3-acetic acid (IAA) was found in the wilted flowers, whereas the leaves contained only minute amounts of this hormone. Open flowers accumulated greater amounts of active abscisic acid (ABA-free) than the other organs. In contrast, leaves contained the highest levels of ABA glucosyl ester (ABA-glc). All the developmental stages of flowers showed higher levels of salicylic acid (SA) than leaves, whereas leaves were the richest in jasmonates. The results described above prompted us to conclude that it is difficult to state how or if hormone contents in the donor leaves affect hormone contents in the flowers.

Table 2. Total amount of individual hormones [μmol g^{-1} dry weight (DW)] in the studied organs of common buckwheat. Mean (n = 12) ± SE.

Hormone	Buds	Open Flowers	Wilted Flowers	Leaves
CYT	0.98 ± 0.08 ^{b}	1.20 ± 0.11 ^{a}	1.27 ± 0.12 ^{a}	0.59 ± 0.06 ^{c}
IAA	9.63 ± 1.72 ^{b}	10.07 ± 1.51 ^{b}	16.63 ± 2.50 ^{a}	0.49 ± 0.07 ^{c}
GAs-active	11.08 ± 0.99 ^{c}	22.10 ± 2.32 ^{a}	18.74 ± 2.06 ^{b}	10.76 ± 1.61 ^{c}
GA_8	2.39 ± 1.15 ^{b}	6.45 ± 0.97 ^{a}	5.22 ± 0.78 ^{a}	1.16 ± 0.17 ^{c}
GA_9	6.50 ± 0.98 ^{c}	13.86 ± 1.52 ^{a}	10.27 ± 1.02 ^{b}	6.73 ± 0.84 ^{c}
GA_{20}	0.49 ± 0.06 ^{a}	0.42 ± 0.06 ^{a}	0.31 ± 0.05 ^{b}	3.91 ± 0.99 ^{ab}
ABA-free	3.71 ± 0.53 ^{c}	9.27 ± 1.39 ^{a}	6.60 ± 0.99 ^{b}	1.66 ± 0.25 ^{d}
ABA-glc	23.51 ± 3.90 ^{c}	24.97 ± 3.75 ^{c}	36.27 ± 5.44 ^{b}	57.91 ± 8.69 ^{a}
SA	59.60 ± 6.88 ^{a}	46.04 ± 6.91 ^{a}	55.34 ± 8.30 ^{a}	23.06 ± 3.46 ^{b}
JA	19.21 ± 2.89 ^{b}	9.41 ± 1.41 ^{c}	11.30 ± 1.70 ^{c}	57.76 ± 8.70 ^{a}
JA-Met	0.16 ± 0.01 ^{b}	0.16 ± 0.02 ^{b}	0.15 ± 0.02 ^{b}	7.11 ± 1.07 ^{a}

CYT—cytokinins (zeatin and kinetin), IAA—indole-3-acetic acid, GAs—active gibberellins: GA_1, GA_3, GA_4, GA_5, GA_6, and GA_7, non-active gibberellins: GA_8 and GA_{20}, ABA-free—active abscisic acid; ABA-glc—non-active abscisic acid, ABA glucosyl ester; SA—salicylic acid; JA—jasmonic acid; JA-Met—methyl jasmonate. Values represent means (n = 12) ± SE. Different superscript letters (a, b, c, ...) within rows for each hormone indicate significant differences between means (Duncan's multiple range test; $p < 0.05$).

GA_6 and GA_1 were the most common in the total pool of active gibberellins in all studied organs, whereas GA_7 level was the lowest (Table 3). No significant differences in percentage content of particular gibberellins in cv. 'Panda' and strain PA15 were observed.

Table 3. Content of individual active gibberellins in relation to the total pool of active gibberellin forms in the buds, open flowers, wilted flowers, and leaves of the cv. 'Panda' and PA15 strain plants grown at 20 °C and 30 °C. Means for the buds and flowers (n = 9) and means for the leaves (n = 3) ± SE.

Active Gibberellin	Buds and Flowers		Leaves	
	cv. 'Panda'	Strain PA15	cv. 'Panda'	Strain PA15
GA_1	30.6 ± 4.7 ^{a}	24.3 ± 3.6 ^{a}	16.7 ± 2.0 ^{b}	18.7 ± 2.0 ^{b}
GA_3	18.3 ± 2.7 ^{a}	17.1 ± 2.6 ^{a}	20.7 ± 2.4 ^{a}	17.2 ± 2.0 ^{a}
GA_4	5.1 ± 0.5 ^{a}	4.5 ± 0.5 ^{b}	5.7 ± 0.7 ^{a}	4.7 ± 0.5 ^{b}
GA_5	8.6 ± 1.0 ^{c}	8.3 ± 1.0 ^{c}	11.3 ± 1.3 ^{bc}	15.1 ± 1.8 ^{a}
GA_6	36.6 ± 5.3 ^{b}	44.4 ± 7.7 ^{a}	43.6 ± 5.2 ^{a}	42.5 ± 5.1 ^{a}
GA_7	0.9 ± 0.1 ^{b}	1.5 ± 0.1 ^{b}	2.1 ± 0.2 ^{ab}	1.8 ± 0.2 ^{a}

Different superscript letters (a, b, c, ...)) within rows for each hormone indicate significant differences between means (Duncan's multiple range test; $p < 0.05$).

2.2.2. Hormone Content in the Flowers and Leaves

The content of cytokinins (CYT) in the buds, open flowers, and wilted flowers was similar in the PA15 plants at both temperatures and 'Panda' plants grown at 20 °C (Figure 4a). Only 'Panda' flowers at 30 °C accumulated considerably greater amounts of CYT, whereas CYT in the buds was still similar to the other objects. Leaves of all the plants showed similar CYT levels at both temperatures (Figure 4b).

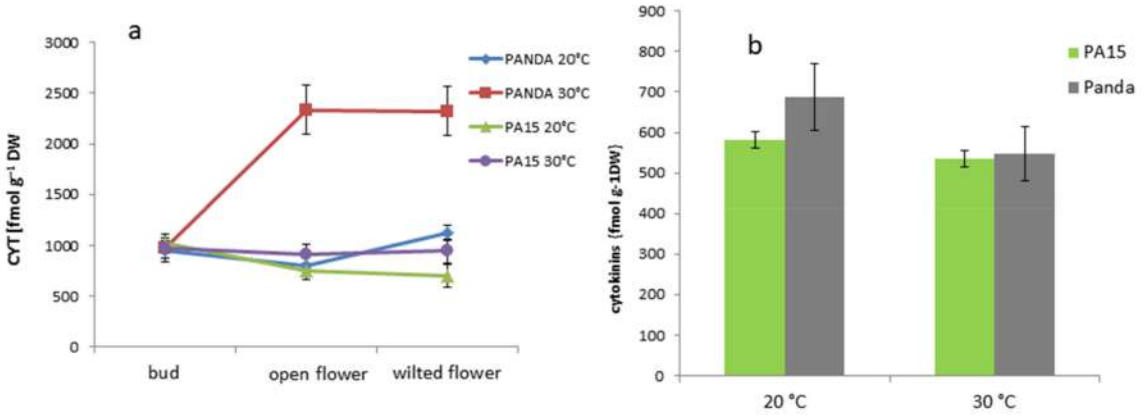

Figure 4. Content of cytokinins (CYT; zeatin and kinetin) [fmol g^{-1} DW] in the flower buds, open flowers, and wilted flowers (**a**) and the leaves (**b**) of the 'Panda' and PA15 plants grown at 20 °C (control) and 30 °C (thermal stress). Means (n = 3) ± SE.

The IAA level increased drastically in the wilted flowers of the PA15 plants exposed to thermal stress (Figure 5a), but in the other cases, high temperature did not change the amount of this hormone. The IAA level in flower buds of cv. 'Panda' at both temperatures was significantly higher than that in the PA15 plants. We found no effects of temperature on IAA amounts in the leaves of any of the studied plants (Figure 5b).

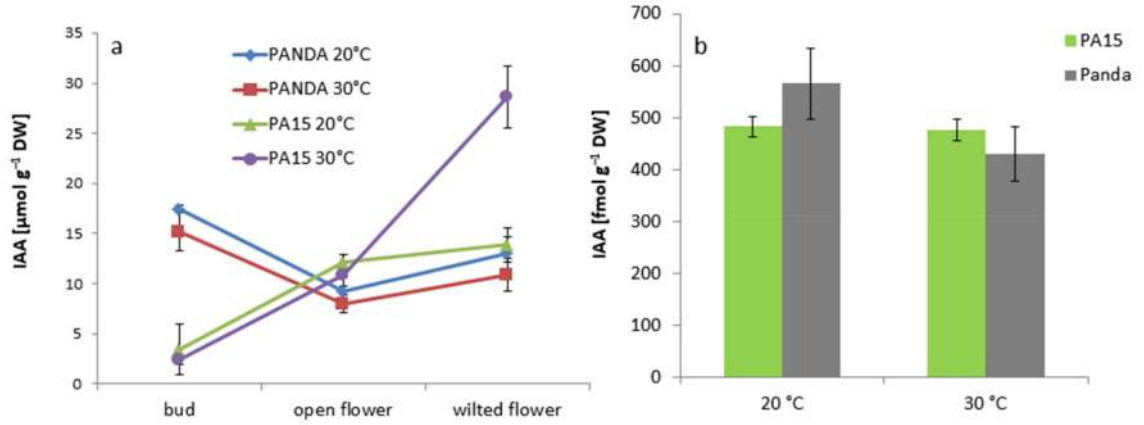

Figure 5. IAA [µmol g^{-1} DW] in the flower buds, open flowers, and wilted flowers (**a**) and the leaves (**b**) of the 'Panda' and PA15 plants grown at 20 °C (control) and 30 °C (thermal stress). Means (n = 3) ± SE.

The amount of active gibberellins—GA_1, GA_3, GA_4, GA_5, GA_6, and GA_7 (GAs active)—was greater in the well-developed and wilted flowers of PA15 and 'Panda' than in the flower buds (Figure 6a). The thermal stress caused a drastic increase in GA levels in the open flowers of 'Panda', and a significant decline in the wilted flowers.

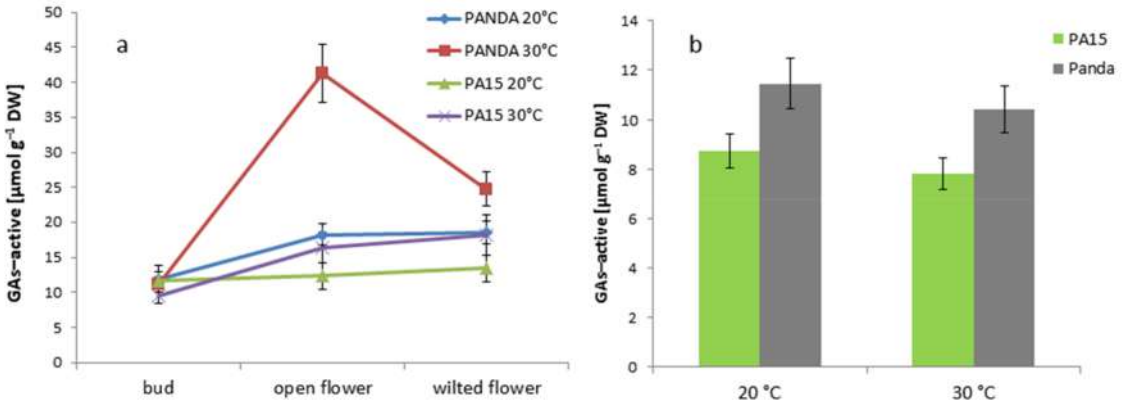

Figure 6. GAs [µmol g^{-1} DW] in the flower buds, open flowers, and wilted flowers (**a**) and the leaves (**b**) of the 'Panda' and PA15 plants grown at 20 °C (control) and 30 °C (thermal stress). Means (n = 3) ± SE.

The GA level in the leaves of cv. 'Panda' was higher at both temperatures than in the PA15 leaves, but thermal stress did not affect the GA amounts in either genotype (Figure 6b).

Generally, greater amounts of non-active GA_8 were detected in the buds than in the flowers, except for in the PA15 plants grown at 20 °C (Figure 7a). Only in cv. 'Panda' did high temperature significantly increase this GA form in the buds and open flowers. The leaves of cv. 'Panda' accumulated less GA_8 than did the PA15 leaves, but we noticed no changes in the hormone levels following the exposure of all the plants to 30 °C (Figure 7b).

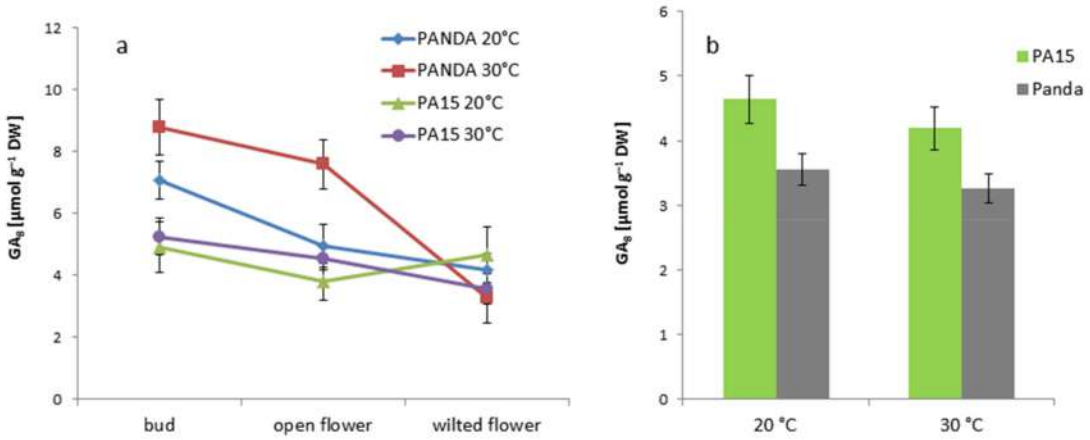

Figure 7. Content of non-active gibberellin GA_8 [µmol g^{-1} DW] in the flower buds, open flowers, and wilted flowers (**a**) and the leaves (**b**) of the 'Panda' and PA15 plants grown at 20 °C (control) and 30 °C (thermal stress). Means (n = 3) ± SE.

Another non-active gibberellin, GA_{20}, occurred in the flowers in lower amounts than GA_8 (Figure 8a).

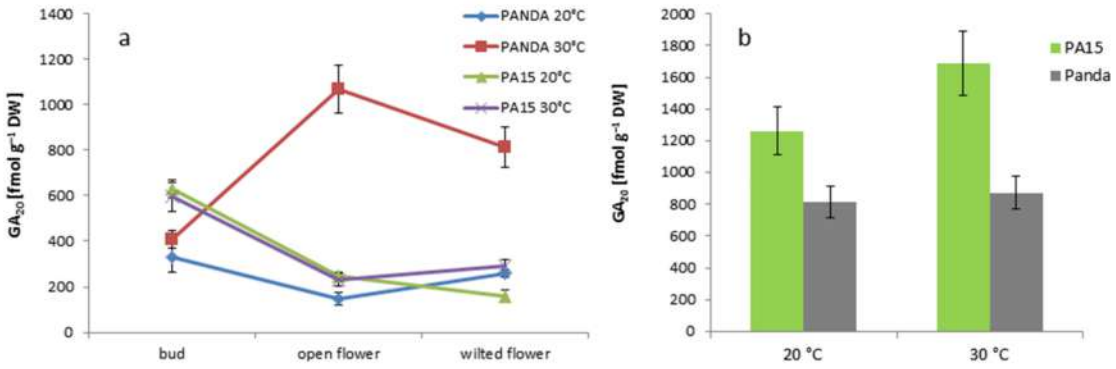

Figure 8. Content of non-active gibberellin GA_{20} [fmol g^{-1} DW] in the flower buds, open flowers, and wilted flowers (**a**) and the leaves (**b**) of the 'Panda' and PA15 plants grown at 20 °C (control) and 30 °C (thermal stress). Means (n = 3) ± SE.

The PA15 plants accumulated more GA_{20} in the buds than in the flowers, and high temperature increased the amount of this hormone only in the wilted flowers. In cv. 'Panda', thermal stress triggered more than a five-fold increase in GA_{20} in the well-developed flowers and a two-fold increase in the wilted flowers as compared with the buds. Contrary to cv. 'Panda', the leaves of PA15 demonstrated an increase in GA_{20} at 30 °C.

Changes in the ABA-free content in the buds, open flowers, and wilted flowers followed a similar pattern in both PA15 and cv. 'Panda' plants (Figure 9a).

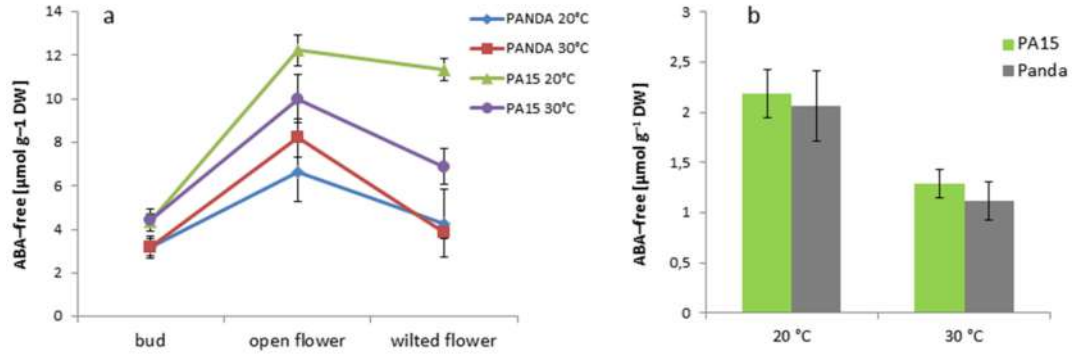

Figure 9. Content of ABA-free [µmol g^{-1} DW] in the flower buds, open flowers, and wilted flowers (**a**) and the leaves (**b**) of the 'Panda' and PA15 plants grown at 20 °C (control) and 30 °C (thermal stress). Means (n = 3) ± SE.

The buds of all the plants accumulated the lowest amount of the active form of ABA at both temperatures. Then, the amount increased significantly in the open flowers and decreased in the wilted flowers, except for in the PA15 plants grown at 20 °C. ABA-free was the most abundant in the open and wilted flowers of strain PA15 at 20 °C. Only in the PA15 plants did thermal stress reduce the ABA-free content in the open and wilted flowers. The leaves of all the studied plants grown at 30 °C showed significantly lower amounts of this hormone than those grown at 20 °C (Figure 9b). The amount of ABA-free was similar in the leaves of all the plants regardless of the temperature. Changes in the content of the non-active form of ABA (ABA-glc) in the flowers showed a reverse pattern as compared with that of ABA-free (Figure 10a).

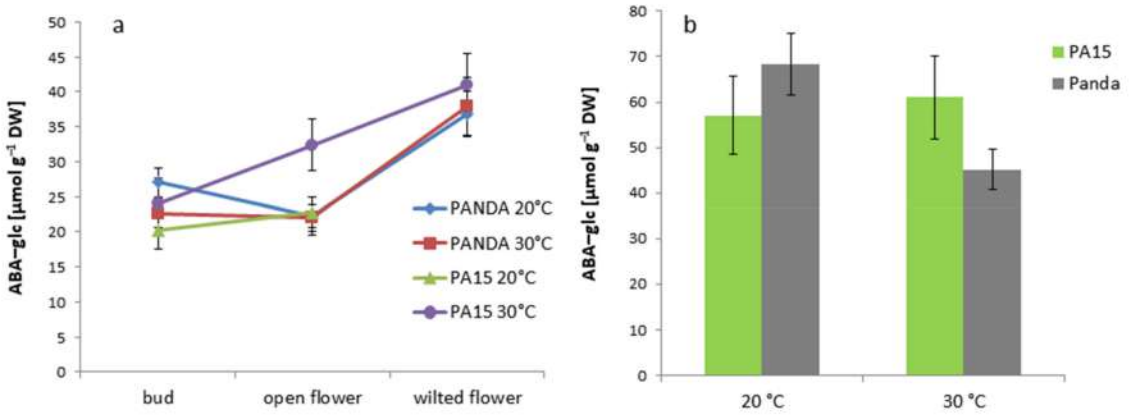

Figure 10. Content of ABA-glc [µmol g^{-1} DW] in the flower buds, open flowers, and wilted flowers (**a**) and the leaves (**b**) of the 'Panda' and PA15 plants grown at 20 °C (control) and 30 °C (thermal stress). Means (n = 3) ± SE.

High temperature considerably enhanced the ABA-glc level only in the open flowers of PA15, and the wilted flowers accumulated more ABA-glc than the buds and open flowers. In the leaves of cv. 'Panda', thermal stress reduced the ABA-glc level, whereas no temperature effect was visible in the PA15 leaves (Figure 10b).

The salicylic acid profiles in the buds and flowers at both temperatures were similar in the PA15 and 'Panda' plants (Figure 11a).

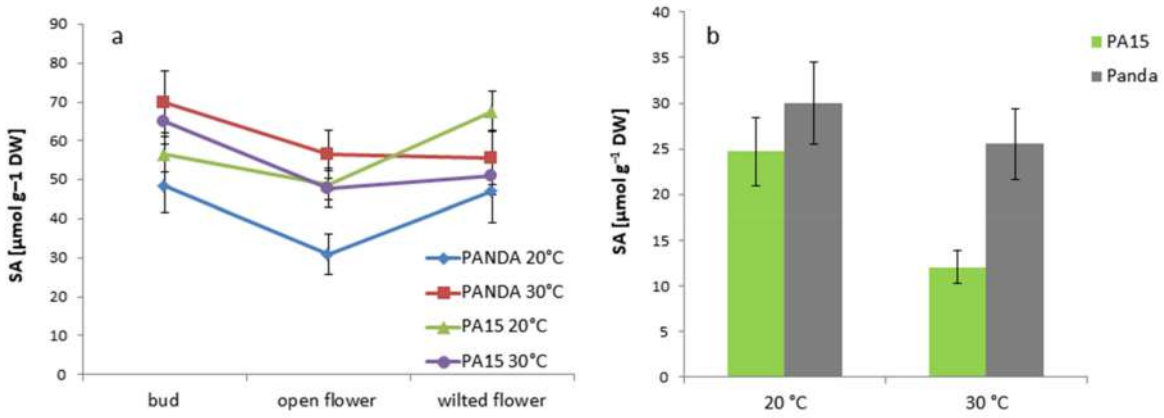

Figure 11. Content of SA [µmol g^{-1} DW] in the flower buds, open flowers, and wilted flowers (**a**) and the leaves (**b**) of the 'Panda' and PA15 plants grown at 20 °C (control) and 30 °C (thermal stress). Means (n = 3) ± SE.

Its lowest amount was detected in the open flowers of the 'Panda' plants grown at 20 °C. High temperature increased the SA content mainly in the open flowers of all the studied plants. In the leaves of the PA15 plants, thermal stress reduced the SA content, contrary to that in the leaves of cv. 'Panda', where no effect of high temperature was observed (Figure 11b).

The jasmonic acid (JA) level was much higher in the buds of the PA15 and 'Panda' plants at both temperatures than those at the other stages of flower development. However, the buds at 30 °C contained less JA than the control buds (Figure 12a).

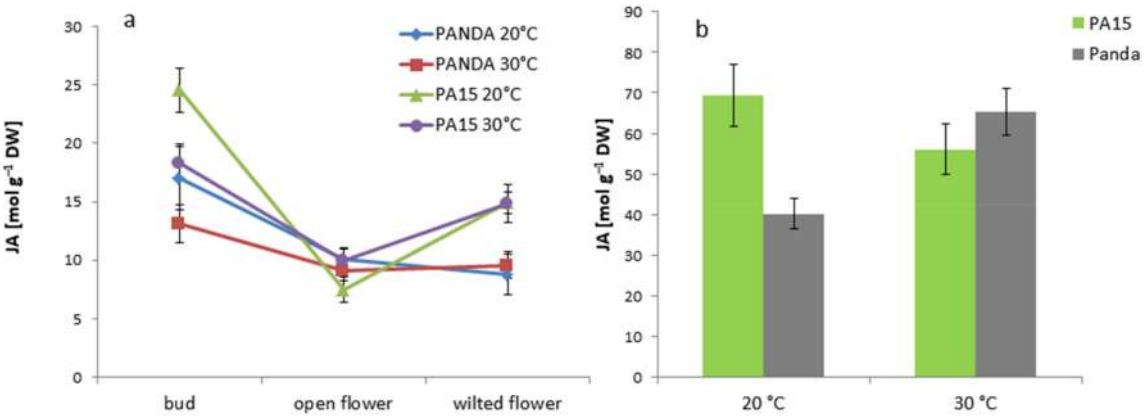

Figure 12. Jasmonic acid (JA) [µmol g^{-1} DW] in the flower buds, open flowers, and wilted flowers (**a**) and the leaves (**b**) of the 'Panda' and PA15 plants grown at 20 °C (control) and 30 °C (thermal stress). Means (n = 3) ± SE.

The lowest JA amount was detected in the open flowers of all the plants at 20 °C and 30 °C. In contrast with cv. 'Panda', the amount of JA in the wilted flowers of the PA15 plants grown at both temperatures was greater than that in the open flowers. Additionally, the leaves of PA15 and cv. 'Panda' responded differently to high temperature: in the PA15 leaves, the JA amount declined, but in the 'Panda' leaves, it was higher than that at 20 °C (Figure 12b). In general, JA was more abundant in the leaves than in the flowers of all the plants.

The amounts of methyl ester of JA (JA-Met) detected in the flowers (expressed as fmol g^{-1} dry weight (DW)) were much lower than those in the leaves (expressed as µmol g^{-1} DW) (Figure 13). We noticed huge differences in the accumulation of this JA form at different temperatures. In the PA15 flowers of all the developmental stages, no significant temperature effect on JA-Met amount was visible, whereas in the 'Panda' flowers, high temperature increased its level as compared with that of the control. The highest amount of JA-Met was detected in the 'Panda' open flowers exposed to high temperature. In the leaves of all the studied plants, thermal stress drastically lowered the JA-Met amount.

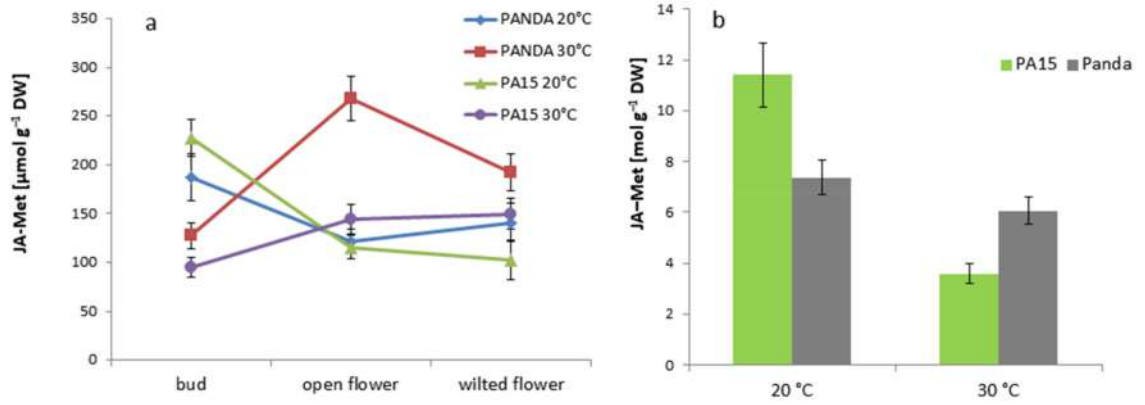

Figure 13. JA [fmol g^{-1} DW] in the flower buds, open flowers, and wilted flowers (**a**) and the leaves [µmol g^{-1} DW] (**b**) of the 'Panda' and PA15 plants grown at 20 °C (control) and 30 °C (thermal stress). Means (n = 3) ± SE.

2.2.3. Quantitative Ratio of the Studied Hormones

Taking into account the role of individual hormones in the generative development of plants at the applied temperatures, we calculated the ratio of active GAs to ABA-free, IAA to ABA-free, and IAA to CYT (Table 4).

Table 4. Ratios of GAs* to ABA-free, IAA to ABA-free, and IAA to total cytokinins (CYT) in buds, well-developed flowers, wilted flowers, and leaves of common buckwheat plants grown at 20 °C (control) and 30 °C (thermal stress).

Strain/Cultivar	Temp.	Organ			
		Buds	Open Flowers	Wilted Flowers	Leaves
GAs/ABA-free					
PA15	20	2.77 ± 0.33^{bB}	1.12 ± 1.00^{cC}	1.19 ± 0.09^{dC}	4.57 ± 0.51^{bA}
	30	3.56 ± 0.42^{aC}	3.08 ± 0.27^{bC}	4.44 ± 0.49^{bB}	9.19 ± 1.09^{aA}
'Panda'	20	2.15 ± 0.25^{cB}	1.64 ± 0.15^{cC}	2.69 ± 0.24^{cB}	5.86 ± 0.65^{bA}
	30	3.77 ± 0.45^{aC}	6.68 ± 0.62^{aB}	6.52 ± 0.71^{aB}	10.82 ± 1.18^{aA}
IAA/ABA-free					
PA15	20	0.82 ± 0.09^{bB}	1.10 ± 0.08^{bB}	2.52 ± 0.28^{cA}	0.22 ± 0.02^{bA}
	30	5.55 ± 0.66^{aA}	1.56 ± 0.09^{aC}	3.07 ± 0.39^{bB}	0.45 ± 0.05^{aD}
'Panda'	20	0.59 ± 0.07^{cC}	1.08 ± 0.08^{bB}	3.94 ± 0.41^{aA}	0.28 ± 0.03^{bD}
	30	5.05 ± 0.49^{aA}	1.07 ± 0.08^{bD}	3.03 ± 0.29^{bC}	0.41 ± 0.05^{aB}
IAA/CYT					
PA15	20	3.29 ± 0.34^{cB}	17.19 ± 1.72^{aA}	19.89 ± 1.97^{bA}	0.87 ± 0.08^{aC}
	30	18.02 ± 2.1^{aA}	10.29 ± 1.08^{cC}	13.94 ± 1.45^{cB}	0.92 ± 0.09^{aD}
'Panda'	20	2.65 ± 2.58^{dC}	13.91 ± 1.35^{bB}	24.77 ± 2.67^{aA}	0.87 ± 0.09^{aD}
	30	15.75 ± 1.74^{bA}	4.94 ± 0.52^{dC}	6.92 ± 0.68^{dB}	0.92 ± 0.09^{aD}

Values represent means ($n = 3$) ± SE. Different superscript letters (a, b, c, ...) within each column for each parameter and organ and different capital letters within each row indicate significant differences between means of each temperature and organ (Duncan's multiple range test; $p < 0.05$).* Sum of active gibberellins involved: GA_1, GA_3, GA_4, GA_5, GA_6, and GA_7.

Gibberellins were more abundant than ABA-free in all the organs of the PA15 and 'Panda' plants at both temperatures. In all the organs, thermal stress significantly increased the amount of active gibberellins in relation to ABA-free, and thus, the GAs/ABA-free ratio increased. The ratio was the highest in the 'Panda' open flowers at 30 °C. High temperature significantly boosted the IAA/ABA ratio mainly in buds (six times for PA15 and 10 times for 'Panda') and almost doubled it in the leaves. In all the studied plants, CYT levels were much lower than those of IAA. A significant advantage of auxin over cytokinins was observed at 30 °C in the buds of PA15 and cv. 'Panda', whereas in the open and wilted flowers, IAA prevailed over CYT at 20 °C. In the buds of all the studied plants at 20 °C, the IAA/CYT ratio was much lower than those in the open and wilted flowers, whereas at 30 °C this proportion was reversed. In the 'Panda' flowers, the IAA/CYT ratio at higher temperature decreased to a greater extent than that in the PA15 flowers. High temperature did not affect the IAA/CYT ratio in the leaves.

3. Discussion

Our earlier studies showed that the percentage of developmental disturbances occurring in mature female gametophytes resulting from premature degeneration of synergids or the egg apparatus in flowers of plants grown in natural conditions amounts to 40%–55% and depends mainly on the genotype [8]. It seems, therefore, that the key reason for the low yield is the defective development of female gametophytes, taking into account that the anthers of buckwheat produce viable pollen (>90%) [8]. Pollen is formed earlier than the female gametophytes, when plants still have abundant assimilates and air temperature is not high. Therefore, these factors do not affect microsporogenesis and microgametophytogenesis (data not shown). In contrast, the developmental processes in megasoporogenesis and megagametophytogenesis happen later and occur in older flowers and at higher temperatures; therefore, they may be affected by the availability of assimilates and temperature.

McGregor [17] revealed an approximate flower abortion rate of 45% in *Brassicaceae*. The cause of this phenomenon may be the genetic background or the influence of abiotic factors, such as temperature. For example, abortion causes smaller yields, especially in legumes [18]. Patric [19] suggests that in *Vicia faba* L., minor apical auxin probably regulates the disintegration of generative organs as a result of competition for assimilates rather than through polar and basal transport to the lower parts of the main shoot. Some plant species have the ability to abort low-quality embryos selectively, which raises the average quality of the surviving offspring [20].

According to Moe [21], the process of floral abortion is initiated during the early stages of shoot growth before the differentiation of floral parts is completed. Low temperatures (12–15 °C) at this critical stage of development strongly promote blind shoot formation but do not affect stamen and pistil primordia formation in the apical flower bud. Apart from genetic control, the course of embryogenesis and the abortion of flowers and fruit is also influenced by plant hormones and growth regulators, and in particular the relationship between their concentrations [22].

In this experiment, we analyzed the changes in individual hormone content during buckwheat flower development from buds to wilting. Our attempt to explain the disturbances in the embryological development of buckwheat by changes in the hormonal profile of the flowers is an innovative approach and may account for poor seed yielding of buckwheat. The hormone content in individual organs of common buckwheat has not been reported yet.

We found numerous differences in hormone accumulation in common buckwheat organs. Contrary to ABA-free, ABA-glc was present in greater amounts in the leaves than in the flowers. Similarly, as reported by Wang and Irving [22], a higher level of jasmonates accumulated in the donor leaves than in the flowers. According to these authors, the insufficiency of jasmonic acid affects anther or ovule development and results in sterile flower organs. This is probably the reason for embryo sac degeneration in buckwheat plants grown under thermal stress.

Our results showed that both studied genotypes differ in their response to thermal stress. Cultivar 'Panda' seems to be more sensitive to high temperature than strain PA15, as its response to thermal stress was more rapid. Only in the 'Panda' buds did high temperature drastically increase the percentage of embryo sac degeneration. In the open flowers of 'Panda' at 30 °C, the number of degenerated embryo sacs doubled, and in the open flowers of PA15, the percentage of properly developed embryo sacs was 2.7 times lower than that of the control. These processes were accompanied by an increase in the content of cytokinins, active gibberellins, and JA-Met. Cytokinins control cell division and organ differentiation, for example, while gibberellins stimulate plant elongation and promote flowering. In *Prunus avium*, endogenous GA induced early embryo sac development, which resulted in a low seed set under high temperature [23]. Methyl jasmonate and jasmonic acid are important cellular regulators mediating diverse developmental processes, such as seed germination, flower and fruit development, leaf abscission, and senescence [24]. Interestingly, particularly in the 'Panda' ovules at 30 °C, female gametophytes appeared to be extremely luxuriant, but detailed analysis revealed their abnormal vacuolization. Abnormal vacuolization of common buckwheat embryos was observed at 32 °C in Japanese cultivars, displaying only 30% seed set [25,26].

Buckwheat flowers and leaves contain active and non-active forms of gibberellins. GA_9 occurred in the largest quantities of all the determined gibberellins. This gibberellin is a precursor in the GA_1 biosynthesis pathway [27]. Gibberellins GA_6, GA_1, and GA_3 occurred in larger quantities than other active gibberellins. The profiles of individual active gibberellins in buds, open flowers, and wilted flowers at both temperatures were similar. For this reason, we presented the changes in total sum of active GAs. Halińska and Lewak [28] and Chien et al. [29] reported that a combination of some gibberellins had a greater effect on plant growth and development than individual gibberellin levels.

ABA is a phytohormone affecting many physiological processes. Its role in flowering promotion is ambiguous. There are data showing negative [30], as well as positive [31], regulation of flowering in *Arabidopsis*. In our study, the pattern of changes in ABA content during flower development was concurrent with earlier reports that ABA plays a signaling role in flower senescence [32,33].

According to Aneja et al. [32] and Panavas et al. [34], the levels of endogenous ABA had increased markedly before any signs of senescence became visible and kept rising during petal senescence in such plant species as cocoa and daylily. Flower senescence in some plant species depends on another signal hormone—ethylene [35]. Zhong and Ciafré [36] described *Iris* as a species whose flowers are ethylene-independent and whose senescence is regulated by ABA. In our opinion, common buckwheat could also belong to this group, because a single flower of buckwheat lives very briefly. In the plants we investigated, ABA-free content was significantly greater in all open flowers than in the flower buds and wilted flowers. Only in the PA15 wilted flowers at 20 °C was the ABA-free level still high, and in the other variants, the wilted flowers demonstrated a considerable decrease in this hormone. In the 'Panda' open flowers, high temperature increased ABA-free content, which decreased in the open flowers of PA15.

The GAs/ABA-free ratio in open flowers of cv. 'Panda' demonstrated that at a high ABA level at 30 °C, the content of active gibberellins was even higher. Gibberellins and ABA can act antagonistically [37], and their role is very well known, especially in seed dormancy and germination [38]. We also observed changes in the IAA/ABA and IAA/CYT ratios. The IAA/ABA ratio increased drastically at 30 °C in the 'Panda' and PA15 flower buds. This could explain the lack of embryological disturbances under thermal stress but only in the PA15 plants. In the 'Panda' buds, only 58% of embryo sacs developed properly as compared with the control. In the buds of all the plants, the IAA/CYT ratio increased six times under thermal stress, but in the open flowers, it was lower at 30 °C than at 20 °C. This result seems to be in accordance with data reported for the effects of high temperature in *Arabidopsis*. These conditions trigger an activation of PHYTOCHROME-INTERATING FACTOR 4 (PIF4). This protein regulates auxin biosynthesis at higher temperatures [30,39]. PIF4 genes stimulate GAs accumulation, increase the transcription level of enzymes synthesizing GAs, and lower the transcription level of GAs inactivating enzymes [40]. Auxins and cytokinins also interact on metabolic levels, and auxins rapidly suppress the cytokinin pool [41]. These considerations could be supported by future experiments with developing flower explants treated with exogenous phytohormones.

4. Materials and Methods

4.1. Plant Material

The study was carried out in common buckwheat plants of a Polish cultivar 'Panda' and strain PA15 in phytotronic conditions. Seeds were supplied by Plant Production Facility in Palikije (Małopolska Plant Breeding Station, Polanowice, Poland).

4.2. Experimental Treatments

The experiment was carried out in phytotronic chambers. Plants were cultivated in pots (20 × 20 × 25 cm; 9 plants per pot), containing commercial soil substrate (pH = 5.8) mixed 1:1 with perlite (*v*:*v*). Plants were grown for 3 weeks at the control temperature (20 °C) at a humidity of 50%–60% under 16 h photoperiod and 300 μmol m^{-2} s^{-1} of PPFD (photosynthetic photon flux density). Then, half of the plants (all at the vegetative stage) were transferred into a chamber with a temperature of 30 °C (heat stress) and the same humidity and light conditions. Then, from two-month-old plants, the flowers at three developmental phases (buds, open developed flowers, and wilted flowers) were collected, and their embryological development (embryo sacs) and hormonal profiles were analyzed. Pollen viability was determined in open flowers. Additionally, donor leaves (fully developed young leaves, closest to the flower cluster) were collected for hormone analysis.

4.3. Measurements

4.3.1. Embryological Analyses

Pollen Viability

Initially, several randomly chosen flowers per treatment were taken for pollen viability screening via a FDA test (fluorescein diacetate). FDA dye was prepared according to Dafni and Firmage [42], as follows: 2 cm^3 20% sucrose in H_2O with several drops of stock solution of FDA (2 mg FDA/1 cm^3 acetone). Freshly stained pollen was kept in a humid chamber for 30 min at 24 °C and afterwards observed with a Nikon E80i microscope with a UV-2A filter. Viable pollen emits yellow-green fluorescence. No counts were performed for the stained pollen. For the pollen viability count using Alexander's test, 20 open flowers per treatment were randomly collected and fixed in FAA (10 cm^3 96% ethanol, 7 cm^3 H_2O, 2 cm^3 37% formaldehyde solution, 1 cm^3 glacial acetic acid) solution. Alexander's dye is a mixture of malachite green staining the cellulose of pollen walls green and acid fuchsin staining the pollen protoplast red [43]. Viable pollen grains appear purple-red, and non-viable pollen grains stain green. At least 7900 (altogether, viable and non-viable) pollen grains per treatment were counted under a Nikon E80i microscope (Tokyo, Japan) in two replicates.

Ovule Development

Paraffin sections of ovules were obtained by fixing flowers at three stages of development (buds, fully developed, and wilted flowers) in FAA solution, dehydrating them in increasing series of ethanol and saturating them with chloroform (in increasing proportion—1:3, 1:1, 3:1, 1:0—with absolute ethanol, each for 2 h at room temperature (RT)) and with paraffin dissolved in chloroform (at 57° for several days, until chloroform evaporated). Flowers prepared this way were embedded in paraffin blocks, sliced into 11–15 µm sections on a rotary microtome (Adamas Instrumenten BV, HM 340E, Leersum, Netherlands), and double stained with Ehrlich's hematoxylin and Alcian blue [44]. Finally, the slides were mounted in Entellane (Sigma-Aldrich, St Louis, MO, USA) and analyzed under a Nikon E80i microscope. The number of analyzed flowers per treatment at each stage of development was 15–32 in two replicates.

4.3.2. Hormone Content Analysis

Selected phytohormones (auxin, active and non-active forms of gibberellins and abscisic acid, kinetin acid, salicylic acid, and jasmonic acid) were assessed according to the procedure described by Płażek et al. [45]. Freeze-dried and pulverized samples of buds, well-developed and wilted flowers, and leaves were extracted (5 min, 30 Hz, MM400, Retch, Haan, Germany) in 1 cm^3 of an extraction buffer (methanol/water/formic acid, MeOH/H_2O/HCOOH, 15/4/1 v/v/v) after the addition of an internal standard solution. Samples were centrifuged (3 min 22,000 × *g*, R32, Hettich, Tuttlingen, Germany), supernatant was collected, and the extraction step was repeated twice. The pooled supernatant was evaporated under N_2, resuspended in 5% MeOHin 1 M HCOOH, and cleaned up on mixed-mode SPE cartridges (BondElutPlexa PCX, Agilent, Santa Clara, CA, USA), as reported by Dziurka et al. [46]. Phytohormones (auxins, cytokinins, gibberellins, abscisic acid, and jasmonates) were analyzed by ultrahigh performance liquid chromatography (UHPLC) using an Agilent Infinity 1260 device coupled with 6410 Triple Quad LC/MS with an electrospray interface (ESI) ion source (Agilent Technologies, USA). Separation was achieved on an AscentisExpres RP-Amide analytical column (2.7 µm, 2.1 mm × 150 mm; Supelco, Bellefonte, PA, USA) at a linear gradient of H_2O vs. acetonitril with 0.01% of HCOOH. The stable isotope-labeled internal standard of phytohormones consisted of the following: [$^{15}N_4$]dihydrozeatin, [$^{15}N_4$]kinetin, [$^2H_2$]gibberellin A_1, [$^2H_2$]gibberellin A_4, [$^2H_2$]gibberellin A_6, [$^2H_2$]gibberellin A_5 [$^2H_5$]indole-3-acetic acid, and [$^2H_6$]cis,trans-abscisic acid (OlChemIm, Olomouc, Czech Republic). Targeted profiling of phytohormones was conducted using multiple reaction monitoring (MRM) with a comparison with data obtained for pure and

stable isotope-labeled standards of investigated compounds. The following hormone forms were determined: IAA, kinetin (KIN), zeatin (ZEA), active gibberellins (GA_1, GA_3, GA_4, GA_5, GA_6, GA_7), non-active gibberellins (GA_8, GA_9, and GA_{20}), active abscisic acid (ABA-free) (±)-cis,trans-abscisic acid, non-active (±)-cis,trans-abscisic acid glucosyl ester (ABA-glc), SA, JA, and JA-Met. Detailed descriptions are given in Płażek et al. [45]. The data were presented as fmol (femtomol) or μmol g^{-1} DW. Analyses were performed in three replicates.

4.3.3. Statistical Analyses

Two-way analysis of variance (ANOVA) and Duncan's multiple range test (at $p < 0.05$) were performed using the statistical package STATISTICA 13.0 (Stat-Soft, Inc., Tulusa, OK, USA). Data were presented as means ± SE (standard error). Non-normal distribution data were analyzed using Chi-squared test (χ^2, $p < 0.05$).

5. Conclusions

Ovules are much more sensitive to thermal stress than stamens in both genotypes. A drastic reduction in the number of properly developed ovules in open flowers is visible at 30 °C, but this temperature does not affect pollen development and wilted flowers. A considerable increase in ABA in open flowers ready for fertilization may serve as a signal inducing flower senescence observed in the next days. Based on the embryological analyses and hormone profiles in flowers, we conclude that cv. 'Panda' is more sensitive to thermal stress than strain PA15, mainly due to a much earlier response to this stress involving the disturbances in embryological processes already in the flower buds.

Author Contributions: A.P. conceived of and designed the experiments; A.P., A.S., F.D., P.K., M.H., K.S., M.D., and J.P. performed the experiments and analyses; A.P., A.S., and M.D. analyzed the data; and A.P. wrote the manuscript.

Funding: This study was supported by funding of the National Centre of Knowledge of Poland (No. 2017/25/B/NZ9/00148).

Acknowledgments: We thank Mrs. Teresa Stokłosa from the Department of Plant Cytology and Embryology of the Jagiellonian University in Cracow for her technical assistance.

Conflicts of Interest: The authors declare no conflicts of interest. The funding sponsors had no role in the design of the study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

Abbreviations

ABA	abscisic acid
ABA-free	active form of abscisic acid
ABA-glc	abscisic acid glucosyl ester, non-active abscisic acid
CYT	cytokinins
GA	gibberellin
IAA	indole-3-acetic acid
JA	jasmonic acid
JA-Met	jasmonic acid methyl ester
SA	salicylic acid
ZEA	zeatin

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DOI: 10.32615/ps.2019.140 PHOTOSYNTHETICA 58 (1): 45-53, 2020

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

Received 20 June 2019, *accepted* 22 October 2019.
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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^-.
Acknowledgements: This study was supported by funding of the National Science Centre in Poland (2017/25/B/NZ9/00148).

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

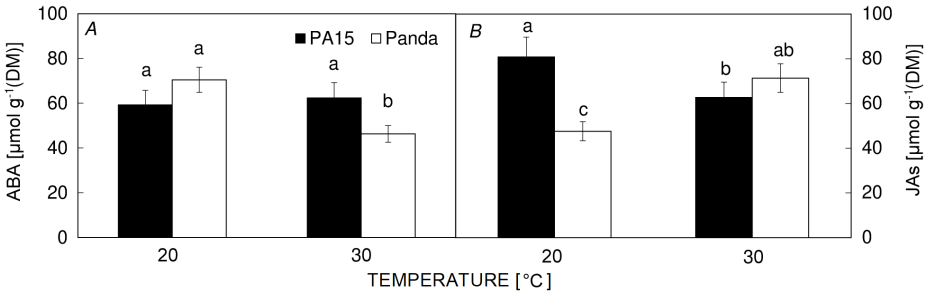

Fig. 1. Abscisic acid (ABA) (*A*) and jasmonates (JAs) (*B*) in common buckwheat donor leaves of cv. 'Panda' and PA15 strain grown at 20 and 30°C. Analyses included leaves collected from eight-week-old plants grown under thermal stress for five weeks. The means (n = 3) ± SE marked with *the same letter* 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.

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CSIRO PUBLISHING
Crop & Pasture Science
https://doi.org/10.1071/CP20011

Synthesis of heat-shock proteins HSP-70 and HSP-90 in flowers of common buckwheat (*Fagopyrum esculentum*) under thermal stress

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Abstract. Common buckwheat (*Fagopyrum esculentum* Moench) is a valuable crop plant with cereal-like seed chemical composition; however, it is susceptible to thermal stress. The aim of the study was to determine whether heat-shock proteins HSP-90 and HSP-70 can protect common buckwheat against thermal stress during development of microspores and embryo sacs. The study was performed on two accessions of common buckwheat, Panda and PA15, which differed in their tolerance to thermal stress. Accumulation of these proteins was determined in buds, open and wilted flowers, and donor leaves of plants grown at 20°C (control) and 30°C (thermal stress). Photochemical efficiency of donor leaves, closest to the inflorescences, based on chlorophyll *a* fluorescence (ChlF) was also analysed. All plants demonstrated higher values of ChlF at 30°C than at 20°C, which suggests that this 30°C temperature is more conducive to their vegetative growth. Pollen grains of both accessions demonstrated normal development at 30°C, whereas embryo sacs showed many developmental disturbances. Panda was more sensitive to thermal stress than PA15, as manifested in a higher percentage of degenerated embryo sacs at the flower bud phase. Moreover, a decrease in both HSPs in the studied organs of Panda was found relative to the control. At 30°C, both accessions accumulated more HSP-70 than HSP-90. These results suggest that, under heat stress, HSP-70 plays a protective role for flowers of common buckwheat. The analyses indicated that the donor leaf closest to the flower cluster may be a reliable indicator of temperature sensitivity in buckwheat flowers.

Additional keywords: chlorophyll fluorescence, embryological analyses, stress tolerance, western-blot.

Received 14 January 2020, accepted 19 June 2020, published online 16 July 2020

Introduction

Common buckwheat (*Fagopyrum esculentum* Moench) is a valuable crop plant of Polygonaceae family, considered a cereal crop owing to its cereal-like seed chemical composition. Its seeds do not contain gluten, they are rich in dietary fibre and rutin, and they have high protein content and well-balanced amino acid composition. Buckwheat is pollinated mainly by bees and is a source of prized nectar. Despite its many beneficial properties, global cultivation area of buckwheat is decreasing because of its unstable seed yield.

According to Halbrecq *et al*. (2005), the cultivation area of buckwheat is limited not only by its low seed yield compared with cereals but also by the heterogeneity of its maturation, which makes harvest time difficult to determine. The biggest challenge is the short lifespan (1 day) of single flowers (Cawoy *et al*. 2009). Buckwheat forms dimorphic plants with flowers harbouring pistils and stamens of different length; pin (long styles) and thrum (short styles) types show self-incompatibility (Adachi 1990; Cawoy *et al*. 2009). Fertilisation occurs after cross-pollination between these flower types (Taylor and Obendorf 2001; Cawoy *et al*. 2006). Seedset is insufficient and amounts to 15–53%, depending on the genotype and growth conditions (Cawoy *et al*. 2009). Insufficient setting of seed is associated with a low number of seeds in relation to the number of flowers produced by plants during the entire growing season. The developmental disturbance occurring in mature female gametophytes resulting from premature degeneration of synergids or the egg apparatus in flowers amounts to 40–55%, depending on the genotype (Słomka *et al*. 2017). Our previous study indicated that the key reason for the low seed yield is not pollen viability but defective development of female gametophytes (Słomka *et al*. 2017).

Another challenge of buckwheat cultivation is the frequent abortion of flowers and very young embryos. Buckwheat

blooming takes a long time and lasts 30–60 days. One plant develops 500–2000 flowers, but only a small percentage of flowers produce seeds. Massive abortion of flowers and fruit is observed in many other plant species (Stephenson 1981). Overproduction of flowers is explained by various hypotheses such as unpredictable mortality or selective abortion of offspring of relatively low quality (Melser and Klinkhamer 2001). McGregor (1981) reported ~45% aborted flowers in Brassicaceae plants. The cause of this phenomenon may be associated with plant genetic background or the effects of abiotic factors such as high temperature.

Although common buckwheat prefers higher temperatures, as evidenced by the fact that it is sown in the Polish climate only after 20 May, when the soil temperature rises above 10°C, its embryogenesis is exceptionally sensitive to thermal stress, which causes intense embryo and flower abortion (Halbrecq *et al*. 2005). Slawinska and Obendorf (2001) showed that buckwheat cultivation at a temperature <25°C can increase seed-setting by up to 40%. Heat stress is detrimental to many plant species in terms of their growth and productivity. In addition, global warming will probably further intensify the effects of heat stress on economically important crops (Huang and Xu 2008; Fischer and Knutti 2015).

A fundamental heat-stress response in plants involves expression of heat-shock proteins (HSPs), known as elements of an important adaptive strategy of heat-stress tolerance. The HSPs, with molecular mass ranging from 10 to 200 kDa, participate in signal transduction during heat stress and act as chaperones, folding and unfolding cellular proteins and protecting functional sites from adverse effects of high temperature (Wang *et al*. 2005). HSP-70 is essential for preventing aggregation and assisting in refolding of non-native proteins under both normal and stress conditions. These molecules are often involved in folding of *de novo* synthesised polypeptides and translocation of protein precursors. Other family members are expressed only under environmental stress, which indicates that they are involved in refolding and proteolytic degradation of proteins. A major role of HSP-90 is to manage protein folding. It also plays a key role in signal transduction, cell-cycle control, and protein degradation (Huang and Xu 2008). HSP-90 is a part of a multi-chaperone mechanism together with HSP-70 and it works closely with other chaperones (Imai *et al*. 2003).

The aim of the work was to investigate whether HSPs such as HSP-90 and HSP-70 play a protective role in buckwheat exposed to thermal stress during development of microspores, pollen grains, and embryo sacs. According to our observations, many buckwheat embryos die of starvation. This effect may suggest that the factor limiting embryo development and seed filling is insufficient photosynthetic efficiency of the donor leaf. For this reason, we investigated photochemical efficiency and HSP accumulation in the donor leaves. Measurement of chlorophyll *a* fluorescence (ChlF) is a non-invasive method for determining photosynthesis efficiency under various environmental conditions. The ChlF signal is highly sensitive to changes in photosynthetic apparatus (Kalaji *et al*. 2014). Based on our earlier results (Płażek *et al*. 2019), two accessions of buckwheat differing in thermotolerance, Panda and PA15, were chosen for the study. Under heat stress, Panda demonstrated a 30% embryo-degeneration rate, whereas in PA15, the disturbances in megasporogenesis and megagametophytogenesis were detected in only 10% of flowers.

Material and methods

Plant material

Seeds of Panda and PA15 were supplied by Plant Production Facility in Palikije (Małopolska Plant Breeding Station in Polanowice, Poland).

Experimental treatments

The experiment was conducted in phytotronic chambers as described by Płażek *et al*. (2019). The plants were cultivated in pots (8 L, 10 pots per accession per treatment, 9 plants per pot, total 90 plants per accession per treatment) containing a commercial soil substrate (pH 5.8), mixed 1 : 1 with perlite (v : v). Plants were grown for 3 weeks at a control temperature (20°C), with 50–60% humidity, a 16-h photoperiod and photosynthetic photon flux density of 300 µmol m^{-2} s^{-1}. After 3 weeks, a half of the plants were transferred to a chamber with a temperature of 30°C (heat stress) and the same humidity and light conditions. Flowers at three developmental stages (buds, open developed flowers, wilted flowers) were collected from 2-month-old plants (since sowing), and their embryological development (embryo sacs and pollen), as well as HSP-70 and HSP-90 content, were analysed. HSPs were also determined in donor leaves (i.e. those closest to the inflorescence). Additionally, kinetics of ChlF in donor leaves was measured.

Embryological analyses

Paraffin slides of buds and open flowers were prepared, stained and observed as described by Płażek *et al*. (2019). Approximately 30 stamens and the corresponding 30 ovules (from the same flower) per accession, treatment and developmental stage were analysed in order to find possible correlations between regularity of pollen and ovule development.

Paraffin sections of ovules and anthers were obtained by fixing flowers in FAA (formalin-aceto-alcohol) solution, dehydrating them in increasing concentrations of ethanol, saturating with chloroform–absolute ethanol mixture of increasing proportion (1 : 3, 1 : 1, 3 : 1, 1 : 0; 2 h each), and with paraffin dissolved in chloroform (at 57°C for several days, until chloroform evaporation). The flowers were embedded in paraffin blocks, sectioned 11–15 µm thick on a rotary microtome (HM 340E; Adamas Instrumenten, Leersum, The Netherlands), and double-stained with Ehrlich's hematoxylin and Alcian blue (Gerlach 1972). The slides were mounted in Entellan (Sigma-Aldrich, St. Louis, MO, USA) and analysed under Nikon E80i microscope (Nikon, Tokyo, Japan).

Analysis of HSP-70 and HSP-90

Heat-shock proteins were analysed by using a western-blot method described by Crosatti *et al*. (1999). The flower samples were lyophilised in a FreeZone 4.5 freeze-dry system (Labconco, Kansas City, MO, USA) and stored at –70°C until analysis.

Total flower proteins were precipitated with 10% (w/v) trichloroacetic acid and 0.07% (w/v) β-mercaptoethanol dissolved in acetone. Proteins were separated by SDS-PAGE, using the buffer system of Laemmli (1970). Proteins (5 mg) were dissolved in loading buffer (280 µL: 4% w/v SDS, 12% w/v glycerol, 2% w/v β-mercaptoethanol, 0.01% w/v bromophenol blue in 50 mM Tris, pH 6.8), boiled for 2 min, and centrifuged (7 min, 12 000*g*). Supernatant (30 µL) was loaded into a 12% polyacrylamide gel with a 5% stacking gel. Electrophoresis was run in Mini-Protean III apparatus (Bio-Rad, Hercules, CA, USA) at 195 V for 45 min. The polypeptides from gels were transferred (Semi-Dry Electrophoretic Transfer Cell; Bio-Rad) to ImmunBlot PVDF membranes (pore size 0.2 µm; Bio-Rad) by applying changing voltage (10 V, 15 min; 13 V, 15 min; 15 V, 15 min) and non-limiting current in a transfer buffer (Tris-glycine, pH 9.0) (Towbin *et al*. 1979; Kurien and Scofield 2003). After overnight blocking of the membrane with 2% (w/v) fat-free milk powder in Tris-buffered saline (TBS) and washing in TBS, the membrane was incubated with a primary antibody (Agrisera, Vännäs, Sweden) for 1 h. Polypeptide–primary-antibody complexes were incubated for 1 h with a secondary antibody conjugated with alkaline phosphatase and washed in TBS and alkaline phosphate buffer. The complexes were visualised using SIGMAFAST BCIP/NBT alkaline phosphatase activity kit (Sigma-Aldrich). Membranes were photographed with fixed settings (Canon EOS 300D camera; Canon, Tokyo, Japan). Analyses were performed in three replicates for each flower stage and leaves per each temperature treatment per cultivar or strain.

Chlorophyll fluorescence measurement

Measurements of photochemical efficiency were performed in donor leaves of the flower cluster with a Handy PEA (Hansatech, Kings Lynn, UK) after 20 min of leaf adaptation to darkness. Maximum quantum yield of photosystem II (PSII; F_v/F_m) was calculated according to van Kooten and Snel (1990). The excitation irradiance was 3000 µmol (quanta) m^{-2} s^{-1} (peak at 650 nm). Fluorescence was recorded during irradiation between 10 µs and 1 s. During the initial 2 ms, data were scored every 10 µs with 12-bit resolution. The following parameters were calculated per excited leaf cross-section (CS_m): ABS/CS_m, energy absorption by antennas; PI, overall performance index of PSII photochemistry; DI_o/CS_m, energy amount dissipated from PSII; RC/CS_m, number of active reaction centres; ET_o/CS_m, amount of energy used for the electron transport; TR_o/CS_m, amount of excitation energy trapped in PSII reaction centres; δR_o, the efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI (photosystem I) end electron acceptors; φR_o, the quantum yield of electron transport from the first stable electron quinone acceptor in PSII (Q_A^-) to the PSI end electron acceptors; ψR_o, probability at time 0 that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-. The calculations were based on the theory of energy flow in PSII and the JIP test (Strasser *et al*. 2000; Strasser and Tsimilli-Michael 2001). Measurements were done in 10 replicates for each accession and temperature treatment.

Statistical analyses

Statistical analysis was performed using Statistica 13.0 (StatSoft, Tulsa, OK, USA). Two-way analysis of variance (ANOVA) and Duncan's multiple range test at $P = 0.05$ were used to determine the significance of differences among treatment means. Data with non-normal distribution were analysed by using a nonparametric chi-square test ($P < 0.05$). Data are presented as means ± standard error (s.e.).

Results

Pollen and embryo sac development

Microsporogenesis and microgametophytogenesis in PA15 and Panda under both temperatures followed the same developmental pattern and were completely regular (Fig. 1). We observed all of their stages, starting from the microspore mother cell, meiosis and tetrad formation (Fig. 1*a–d*), through to microspore development into mature three-celled pollen grains (Fig. 1*e–h*).

Contrary to normal pollen development, ovule development under thermal stress was disturbed in both accessions, particularly in Panda plants at 30°C, with embryo-sac degeneration already occurring at the bud stage

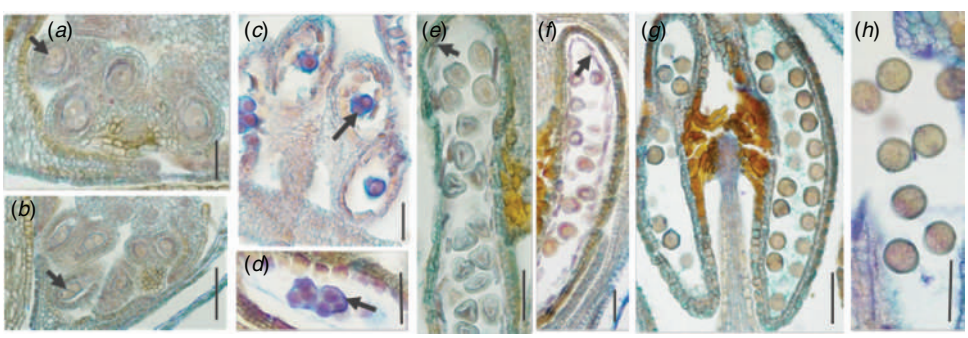

Fig. 1. Successive stages of normal (regular) pollen development in (*a*, *c*, *e*, *g*) Panda and (*b*, *d*, *f*, *h*) PA15 at 20°C (*a*, *c*, *e*) and 30°C (*b*, *d*, *f–h*). Prophase I at microspore mother cells (*a*, *b*, arrows) in anther locules; tetrads of microspores formed after male meiosis (microsporogenesis) surrounded by callose sheaths (*c*, *d*, arrows); 1-nucleate microspores (*e*, *f*), tapetum is still visible (arrows); mature pollen grains with dense cytoplasm (*g*, *h*). Bars are 50 µm in *a*, *c–f*, *h*; 100 µm in *b*, *g*.

(Table 1). At this temperature, Panda buds comprised 42.3% degenerated embryo sacs, whereas in PA15, the percentage of abnormally developed embryo sacs was similar at 20°C and 30°C. Thermal stress increased the number of abnormal embryo sacs in open flowers of Panda by 4.7 times, and of PA15 by 3.2 times. Disturbances involved young and mature female gametophytes, cells of the nucellus and integument degeneration (Fig. 2). In wilted flowers of all studied plants, no differences were observed in the number of degenerated embryo sacs at both temperatures (Table 1). This result was probably due to earlier abortion of flowers with abnormally developed ovules.

HSP-70 and HSP-90 accumulation

All flowers of PA15 and Panda plants grown under control and thermal-stress conditions contained higher amounts of HSP-70 than HSP-90 (Fig. 3). Enhanced accumulation of HSP-70 was observed at 30°C in flower buds and donor leaves of PA15 and in buds and wilted flowers of Panda. Under thermal stress, this protein was accumulated in greater amounts in Panda buds than in PA15 buds. Reduction in HSP-70 levels was observed in wilted flowers of PA15 and in open flowers and donor leaves of Panda.

The highest amount of HSP-90 was found at 30°C in PA15 donor leaves—higher than that of the control. Under thermal stress, PA15 flowers at all developmental stages also accumulated slightly higher amounts of HSP-90 than at the control temperature. High temperature did not induce accumulation of this protein in Panda flowers or leaves.

Table 1. Percentage of degenerated embryo sacs of common buckwheat PA15 and Panda at high temperature

Significant differences between means (n = 10) at 30°C (thermal stress) and 20°C (control) are indicated for each accession: ***P < 0.001 (chi-square test)

Organs	PA15		Panda	
	20°C	30°C	20°C	30°C
Buds	15.9	20.5	0	42.3***
Open flowers	22.9	73.3***	11.8	55.6***
Wilted flowers	18.7	21.1	16.7	20.0

Chlorophyll fluorescence in donor leaves

Both PA15 and Panda plants grown at 30°C showed an increase in photosynthetic apparatus efficiency compared with plants grown at 20°C (Table 2). Values of chlorophyll fluorescence parameters F_v/F_m, PI, ET_o/CS_m, ABS/CS_m, TR_o/CS_m, ET_o/CS_m and RC/CS_m were higher, whereas δR_o and ψR_o dropped at 30°C. Only in Panda plants at 30°C were an increase of the quantum yield of electron transport from Q_A^- to the PSI end electron acceptors (φR_o) and a decrease in energy amount dissipated from PSII (DI_o/CS_m) noted. These results indicate more effective use of energy excitation and its transfer to PSI induced by high temperature. Simultaneously, this effective mechanism of energy transmission to the PSI end electron acceptors at 30°C could be a consequence of a higher amount of energy used for electron transport (ET_o/CS_m), a rise in the amount of excitation energy trapped in PSII reaction

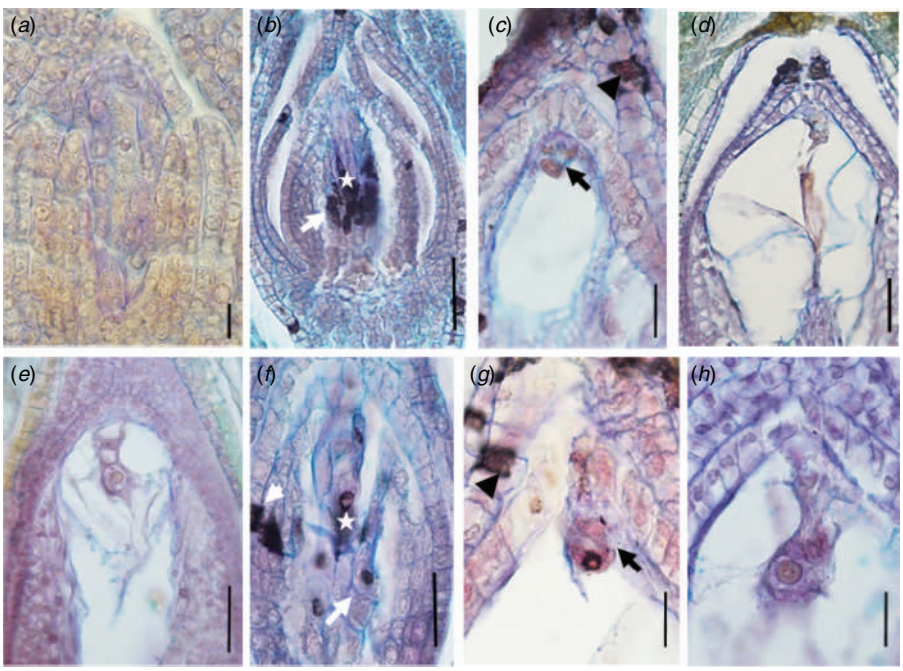

Fig. 2. Ovule degeneration in (*b–d*) Panda and (*f–h*) PA15 at 30°C. For comparison, normal ovules in (*a*) buds of Panda and (*e*) open flowers of PA15 at 20°C are depicted. Degeneration of nucellus (arrows), young embryo sac (stars) and cells of integuments (arrowhead) in buds (*b*, *f*); degeneration of egg apparatus (arrows) and integument cells (arrowheads) in open flowers (*c*, *g*); degeneration of embryo sacs in open flowers (*d*, *h*). Bars are 10 µm in *a*, *g*, *h*; 50 µm in *b–f*.

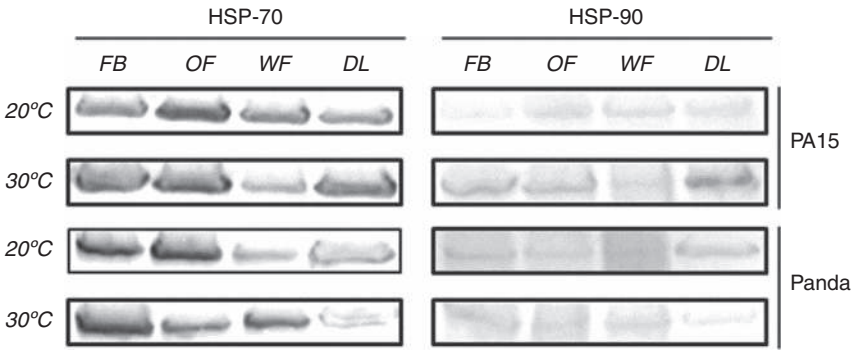

Fig. 3. Western blot detection of HSP-70 and HSP-90 in the flower buds (FB), open flowers (OF), wilted flowers (WF) and donor leaves (DL) of PA15 and Panda plants grown at 20°C (control) and 30°C (thermal stress).

Table 2. Kinetics of chlorophyll *a* fluorescence measured in donor leaves of common buckwheat PA15 and Panda grown at 20°C (control) and 30°C (thermal stress)

Values are means ± s.e. (n = 10). Within rows, means followed by the same letter are not significantly different (Duncan's multiple range test at P = 0.05). F_v/F_m, Potential maximal quantum yield of PSII; ABS/CS_m, energy absorption by antennas; TR_o/CS_m, amount of excitation energy trapped in PSII reaction centres; ET_o/CS_m, amount of energy used for the electron transport; DI_o/CS_m, energy amount dissipated from PSII; RC/CS_m, number of active reaction centers; PI, overall performance index of PSII photochemistry; δR_o, efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors; φR_o, the quantum yield of electron transport from Q_A^- to PSI end electron acceptors; ψR_o, probability, at time 0, that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-

Parameter	PA15		Panda	
	20°C	30°C	20°C	30°C
F_v/F_m	0.670 ± 0.013b	0.753 ± 0.014a	0.639 ± 0.023b	0.751 ± 0.014a
ABS/CS_m	1179 ± 25c	1515 ± 24a	1259 ± 40b	1542 ± 47a
TR_o/CS_m	794 ± 29b	1142 ± 31a	810 ± 42b	1163 ± 47a
ET_o/CS_m	217 ± 22b	410 ± 36a	201 ± 21b	427 ± 45a
DI_o/CS_m	385 ± 12b	373 ± 21b	449 ± 26a	378 ± 18b
RC/CS_m	435 ± 23b	596 ± 26a	415 ± 24b	619 ± 25a
PI	0.352 ± 0.066b	0.853 ± 0.168a	0.257 ± 0.049c	0.933 ± 0.188a
δR_o	3.71 ± 0.31a	2.14 ± 0.24b	4.46 ± 0.48a	2.35 ± 0.30b
φR_o	0.158 ± 0.013b	0.178 ± 0.013a	0.143 ± 0.009b	0.182 ± 0.016a
ψR_o	0.589 ± 0.010a	0.507 ± 0.012b	0.593 ± 0.020a	0.520 ± 0.011b

centres (TR_o/CS_m), and higher number of active reaction centres (RC/CS_m).

Discussion

In our previous study (Płażek *et al*. 2019), we reported reduced pollen viability in Panda plants grown at 20°C compared with PA15 plants grown in the same conditions (82% vs 96.3%). The present study clearly demonstrated that this was not caused by irregularities in microsporogenesis or microgametophytogenesis, because they were completely regular at both temperatures. Therefore, pollen degeneration must occur at the final stage, just before its release from pollen sacs. Panda is much more sensitive to thermal stress than PA15 in terms of ovule development, especially at the bud stage, but its pollen development is temperature-insensitive (82% at 20°C and 89.5% at 30°C, not significantly different) (Płażek *et al*. 2019). A decrease in pollen viability in Panda plants (~80% instead of >90%) may result from other factors such as temporary soil dryness. Indeed, Kadrylova and Mukhametshina (2015) showed that pollen of common buckwheat is very sensitive to drought. An induction of HSP genes in developing anthers, microspores and pollen, as well as accumulation of different types of HPSs in anthers and pollen after a short period of high-temperature stress, may activate thermotolerance mechanisms (Rieu *et al*. 2017). It seems that HSP-70, the concentration of which under thermal stress increased to a greater degree in Panda than in PA15 plants, may protect pollen but not ovules. In Panda plants, the degenerated embryo sacs were already observed in flower buds, whereas in the PA15 plants, abnormal development of embryo sacs occurred only in open and wilted flowers.

Heat-shock proteins act as molecular chaperones and are mobilised to protect cellular proteins against irreversible high-temperature-induced damage (Boston *et al*. 1996; Li *et al*. 2014). HSP-70 and HSP-90 in PA15 donor leaves at 30°C were potentially involved in photoprotection and repair of

PSII. Similar results were obtained by Li *et al*. (2016) in their study on HSP-70 accumulation during heat stress in cucumber (*Cucumis sativus* L.) leaves. Other studies also indicate that HSPs maintain PSII activity and reduce oxidative stress under heat stress (Murata *et al*. 2007; Takahashi and Murata 2008).

The role of HSP-90 in the mechanism of acclimation of photosynthetic apparatus to heat stress is poorly understood. In our study, high-temperature-induced accumulation of this protein occurred only in PA15 donor leaves. Other authors reported on a protective nature of HSP-90 in relation to signalling proteins such as steroid hormone receptor, kinases, nitric oxide synthase and calcineurin (Picard *et al*. 1990; Garcia-Cardeña *et al*. 1998; Imai and Yahara 2000; Richter and Buchner 2001). It is also emphasised that the activity of HSP-90 requires the supportive presence of other co-chaperone proteins (Cao *et al*. 2003).

Our results on HSP-70 and HSP-90 accumulation corroborate those from earlier studies that showed greater sensitivity of Panda than PA15 to high temperature (Płażek *et al*. 2019).

Plant photosynthetic apparatus is sensitive to an increase in temperature (Huang *et al*. 2019). Our experiments conducted in two accessions of common buckwheat demonstrated their higher photochemical efficiency at 30°C than at 20°C. The same response to temperature stress was observed in tomato (*Solanum lycopersicum* L.) plants (Abdelmageed and Gruda 2009). It is believed that electron transport is highly heat-sensitive and is also the main factor limiting plant photosynthetic activity at high temperatures (Hüve *et al*. 2006). In Panda plants at 30°C, an increase was noted in the quantum yield of electron transport from Q_A^- to the PSI end electron acceptors (φR_o), which probably indicated adaptation of the photosynthetic apparatus to the higher temperature. These changes, and the reduction of energy amount dissipated from PSII (DI_o/CS_m), may suggest PSII sensitivity to heat stress and the resulting effective energy transfer to the heat-resistant PSI.

The increase in φR_o values in Panda plants may indicate electron transfer from plastoquinone Q_A and/or Q_B (the second electron quinone acceptor in PSII) to PSI rather than to oxygen molecules. Some studies have indicated possible electron leakage in PSII in the places of plastoquinone activity. Overloading of the electron transport chain redirects some electrons from Q_A^-/Q_B^- to O_2, forming superoxide radical that is then dismutated to H_2O_2 by chloroplastic Cu/Zn-SOD (Takahashi *et al*. 1988; Polle 1996; Edreva 2005; Hura *et al*. 2015, 2018, 2019). However, Wiese *et al*. (1998) showed that effective electron transfer to PSI was insufficient to protect photosystems I and II against photoinactivation. An important mechanism preventing overloading of PSII with excitation energy during environmental stresses is effective electron transfer to PSI and then excitation energy dissipation in the form of far-red fluorescence (Baker 1991; Huner *et al*. 1998; Agati *et al*. 2000; Hura *et al*. 2015).

In PA15 plants, both photosystems appear to be resistant to 30°C (no differences at the studied temperatures for DI_o/CS_m and φR_o), the activity of the photosynthetic apparatus being supported by the accumulation of chaperones (HSP-70 and HSP-90) in the donor leaves.

Many studies have confirmed higher resistance of PSII to temperature stress than of PSI (Mihailova *et al*. 2011; Essemine *et al*. 2012; Yan *et al*. 2013; Li *et al*. 2016). Allakhverdiev *et al*. (2008) identified PSII donor as the most heat-sensitive component. Detailed studies have demonstrated temperature-stress-induced changes in the structure of D_1 and D_2 proteins, thus triggering abnormalities in Q_A fixation and loss of PSII stability (Sinsawat *et al*. 2004; Camejo *et al*. 2005).

In conclusion, our analyses identified two different responses of buckwheat to heat stress. The accumulation of HSP-70 and HSP-90 dominated in the donor leaves of PA15 strain, and was accompanied by an increase in the activity of the photosynthetic apparatus. However, in PA15 plants, the efficiency of using excitation energy remained unchanged. In Panda donor leaves, a clear decrease in the level of both proteins was noted, combined with the increase in the quantum yield of electron transport from Q_A^- to the PSI end electron acceptors and the reduction of energy amount dissipated from PSII. Panda seems more susceptible to heat stress, which can be associated with low levels of HSPs and PSII sensitivity. At the same time, the analyses indicated that the donor leaf closest to the flower cluster may be as reliable an indicator of temperature sensitivity in buckwheat as flowers. Further studies are needed to determine both the changes in HSP transcripts and different abundance of HSP-70 and HSP-90 in anthers and pistils under heat stress. It would be also interesting to investigate *in vitro* or *in vivo* germination to verify whether the ability of pollen tube to grow in a medium and in stigma is affected by heat stress, which is vital for grain production.

Conclusions

The common buckwheat accessions in this study differ in terms of sensitivity to thermal stress. We suggest that Panda is more sensitive than PA15 owing to higher percentage of degenerated embryo sacs in buds and open flowers and a decrease in accumulation of the studied HSPs. Under thermal stress, buckwheat plants accumulate considerably more HSP-70 than HSP-90. At high temperature, HSP-70 plays a protective role towards flowers of common buckwheat. Common buckwheat demonstrates higher photochemical efficiency at 30°C than at 20°C. However, higher temperature is unfavourable for embryo-sac development. Pollen grains of both accessions demonstrated tolerance toward high temperature.

Conflicts of interest

The authors declare no conflict of interest.

Author contributions

A.P.: conceptualisation, funding acquisition, writing of original draft, writing after review and editing. K.H.: formal analysis of HSP content, methodology of HSP analysis, visualisation. T.H.: formal analysis of chlorophyll fluorescence, writing of original draft. A.S.: formal embryological analysis, visualisation, writing after review and editing. M.H.: PhD student, plant cultivation, formal analysis of chlorophyll fluorescence and embryological

development. K.S.: PhD student, formal embryological analysis.

Funding

This work was supported by The National Science Centre, Poland (grant number 2017/25/B/NZ9/00148).

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Handling Editor: Youhong Song

International Journal of
Molecular Sciences

MDPI

Article

Reducing Flower Competition for Assimilates by Half Results in Higher Yield of *Fagopyrum esculentum*

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Received: 22 October 2020; Accepted: 23 November 2020; Published: 25 November 2020

Abstract: Despite abundant flowering throughout the season, common buckwheat develops a very low number of kernels probably due to competition for assimilates. We hypothesized that plants with a shorter flowering period may give a higher seed yield. To verify the hypothesis, we studied nutrient stress in vitro and in planta and analyzed different embryological and yield parameters, including hormone profile in the flowers. In vitro cultivated flowers on media with strongly reduced nutrient content demonstrated a drastic increase in degenerated embryo sacs. In in planta experiments, where 50% or 75% of flowers or all lateral ramifications were removed, the reduction of the flower competition by half turned out to be the most promising treatment for improving yield. This treatment increased the frequency of properly developed embryo sacs, the average number of mature seeds per plant, and their mass. Strong seed compensation under 50% inflorescence removal could result from increased production of salicylic and jasmonic acid that both favor more effective pollinator attraction. Plants in single-shoot cultivation finished their vegetation earlier, and they demonstrated greater single seed mass per plant than in control. This result suggests that plants of common buckwheat with shorter blooming period could deliver higher seed yield.

Keywords: common buckwheat; embryo sacs; nutrient stress; phytohormones; pollen grains; yield parameters

1. Introduction

Common buckwheat (*Fagopyrum esculentum* Moench) is a valuable plant of *Polygonaceae* family, grown mainly for human food due to favorable chemical composition of its fruits (commonly termed seeds), especially substantial content of lysine and other amino acids. Seeds are also gluten free [1]. Nectar of common buckwheat is a source of valued honey, while grain and straw are used as animal feed. The presence of two morphs of flower, Pin and Thrum, is a manifestation of heteromorphic self-incompatibility (heterostyly). Both types of flowers exhibit differences in pistil and stamen lengths [2]. Fertilization occurs only following cross-pollination between two different types of flowers.

Factors such as low resistance to excessive water, lodging, and pre-harvest sprouting occurring before flowering affect buckwheat yield, which is low and variable [3].

Plant yield is also affected by the short life span (1–2 days) of individual flowers and disturbances in female but not in male line development. The number of degenerated ovule sacs in plants is high and

ranges from 10% to 30%, depending on the strain and cultivar [4]. Additionally, buckwheat flowering is sensitive to many environmental stresses, including frost, heat, and drought occurring in the spring and summer despite stronger vegetative growth at 30 °C than at 20 °C [5]. A drastic reduction in the number of properly developed embryo sacs was clear in open flowers at 30 °C in cultivars 'Panda' and 'Korona' (= strain PA15) [5,6]. Similarly, sensitivity to the thermal stress was shown by Slawinska and Obendorf [1]; plants grown at 18 °C had by 40% increased seed set. They set seeds over a longer period and produced by 40% more dry mass per seed than plants grown at 25 °C. Several authors [7–9] described ultrastructural changes in reproductive cells under thermal stress and suggested that premature synergid collapse may prevent a pollen tube from reaching the embryo sac. It was recently shown that radiation restriction resulting from plant growth could have increased floret mortality and thereby decrease the number of achenes (fruits) per raceme (type of inflorescence in common buckwheat) [10]. High temperature and other environmental factors cause premature flower and immature seed fall. A considerable increase in abscisic acid in open flowers ready for fertilization under thermal stress may serve as a signal inducing flower senescence observed in the next few days [6]. The results of a project carried out during 2014–2016 ('Increasing the vitality and degree of pollination of buckwheat flowers in order to obtain a higher seed yield'), concerning flowering and yielding biology of Polish buckwheat accessions, indicate that the more flowers a plant produces, the greater their abortion, and the number of flowers negatively correlates with the number of mature seeds. Therefore, increasing the number of flowers per plant may not improve the yield It is, however, opposite to a selection index, which was constructed based on seven agro-morphological traits measurable in a single plant, showing that seed yield is positively correlated with the number of flower clusters in common buckwheat [11].

The frequency of aborted embryos in controlled conditions after hand-pollination is rather low (<10%) [12] or moderate (9.6–19.1%) [2,7–9], depending on the genotype, thus it seems that selective embryo abortion resulting from better or worse embryo fitness is not the case of low yield in common buckwheat. Halbrecq et al. [13] and Cawoy et al. [2] suggested that embryo abortion in common buckwheat is fixed by an internal mechanism at a relatively constant level and is not the result of insufficient nutrient supply from leaves. In contrast, studies in four Polish cultivars based on observation of embryo development with free access of pollinators showed that more, up to 28%, of embryos could have degenerated, some of them showing a typical hallmark of starvation [4]. This is in accordance with earlier observations of Inoue and Hagiwara [14] and Inoue et al. [15], who proposed that the percentages of flower fertilization and seed set are influenced by the degree of competition for nutrients between source and sink organs. Buckwheat blooms throughout the whole growing season, which may result in a strong competition for assimilates between the already set seeds and flowers still in production. To resolve these inconsistencies in the above-mentioned results, we analyzed various reproductive and yield parameters and the hormone profile of the flowers of cv. 'Panda' and 'Korona' of *F. esculentum* under in vitro and in planta conditions. Our aim was to investigate whether the seed limitation is associated with a strong sink restriction and linked to internal flower structure and fertilization. We hypothesized that plants with a shorter flowering period may give a higher seed yield.

2. Results

2.1. Embryological Analysis

It was evident that in most cases floral buds of both cultivars kept on media with reduced content of sugar, vitamins, and macro- and microelements showed deterioration in growth and development of ovules and embryo sacs. The cells of embryo sac degenerated, and the ovules narrowed (Table 1 and Figure 1). In the case of cv. 'Panda' on Medium 1, only drastic reduction of nutrients increased dramatically the percentage of degenerated embryo sacs as compared with that of the control. On Medium 2, there was no effect of nutrients content reduction and on Medium 3 even an increase of the number properly developing ovules was noticed. The development of ovules and embryo sacs was

much worse in full content Medium 1 in 'Korona' than in 'Panda', and further (caused by a reduction in the content of ingredients) deterioration of the ovules quality, although statistically significant, was not as drastic as in Panda. On Media 2 and 3, 50% reduction in nutrients increased the number of defective embryo sacs in 'Korona' cv. These findings from in vitro studies supported further in planta experiments. In vitro nutrients' content reduction decreased the number of properly developed ovules and embryo sacs similarly as in planta depletion of assimilates resulting from flower overproduction in plants without flower removal. They clearly demonstrated that the removal of 75% of flowers had the most negative effect on the frequency of properly formed embryo sacs and ovules (Table 1 and Figure 2). Single-shoot cultivation (1S) also decreased the percentage of properly developed ovules and embryo sacs but to a lesser extent than removing 75% of flowers (Table 1). Removal of 50% of flowers significantly increased or did not change the frequency of properly developed ovules and embryo sacs or pollen viability (Table 1 and Figure 3). Overall, pollen viability in both cultivars and all treatment was high (Table 1). In 97% of cv. 'Korona' and in 85% of cv. 'Panda' ovules, normal microgametophytogenesis (Figure 3a–e) and megametophytogenesis (Figure 3f,g) were observed, leading to the formation of seven-cell embryo sac of *Polygonum* type (Figure 3h–l). In some ovules, embryos were also observed (Figure 3m).

Table 1. The influence of medium content on in vitro cultured floral buds, and of flower removal on embryological parameters in cv. 'Panda' and 'Korona' of *Fagopyrum esculentum*.

In Vitro			
Medium	**Content of Compounds (%)**	**Frequency (%) of Degenerating Embryo Sacs and Ovules**	
		'Panda' cv.	**'Korona' cv.**
1	100	0	38
	50	0	42 *
	30	68 *	50 *
2	100	15	36
	50	14	50 *
3	100	29	13
	50	0 *	38 *

In Planta				
Treatment	**'Panda' cv.**		**'Korona' cv.**	
	Frequency (%) of Degenerated			
	Pollen Grains	**Embryo Sacs and Ovules**	**Pollen Grains**	**Embryo Sacs and Ovules**
Control	3.2	10	1.3	23
1S	3.1	50 *	1.6	40 *
50%	2.2 *	15	0.9	3 *
75%	1.1*	66 *	1.2	85 *

Chi-square test ($p < 0.05$) was performed separately for in vitro and in planta treatments. Means marked with asterisks (*) differ significantly from control. In vitro: Analysis performed on 25–30 ovules/treatment. For media content, see Section 4. Media with 100% composition were controls. In planta: Control, plants with all flowers and lateral ramifications; 1S, plants with only one main shoot; 50% and 75%, percentages of flowers removed.

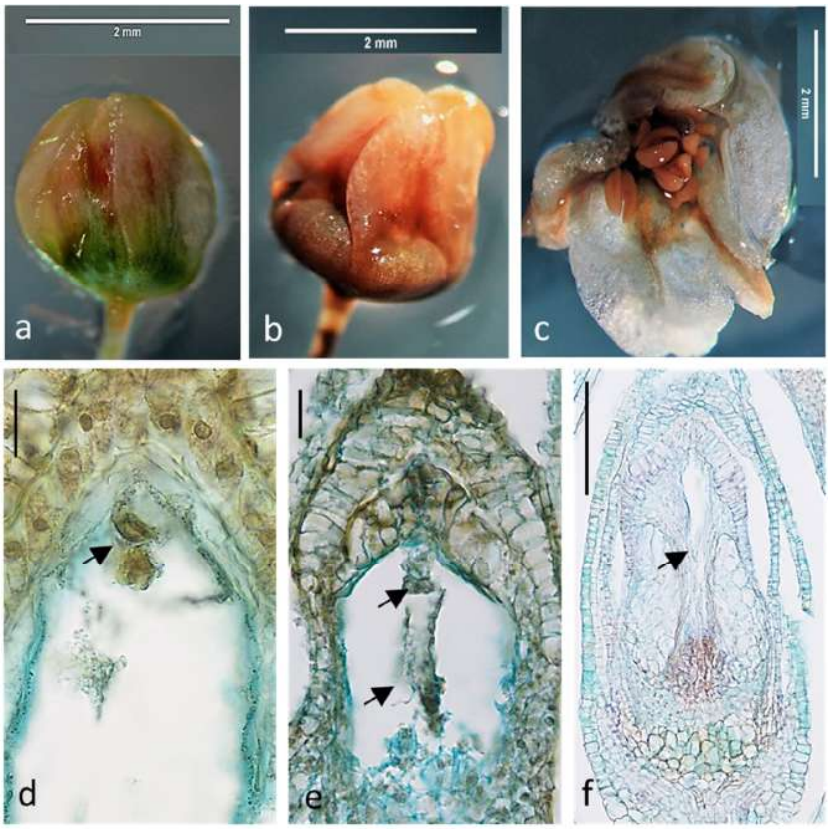

Figure 1. *Fagopyrum esculentum* (cv. 'Korona') floral buds and their internal ovule and embryo sac development impairment cultured in vitro on media with different content of sugar, vitamins, and macro- and microelements. The same features were observed in cv. 'Panda': (**a**) bud laid out on Medium 1 with full content of nutrients at the time 0; (**b**) bud after 10 days of culture on Medium 1 with 30% content of nutrients; (**c**) bud after 10 days of culture on Medium 1 with full content of nutrients; (**d**,**e**) degeneration of the cells of embryo sacs (arrows); and (**e**) shrunken embryo sac (arrow). Bars: (**d**,**e**) 20 μm; and (**f**) 200 μm. For media content, see Section 4.

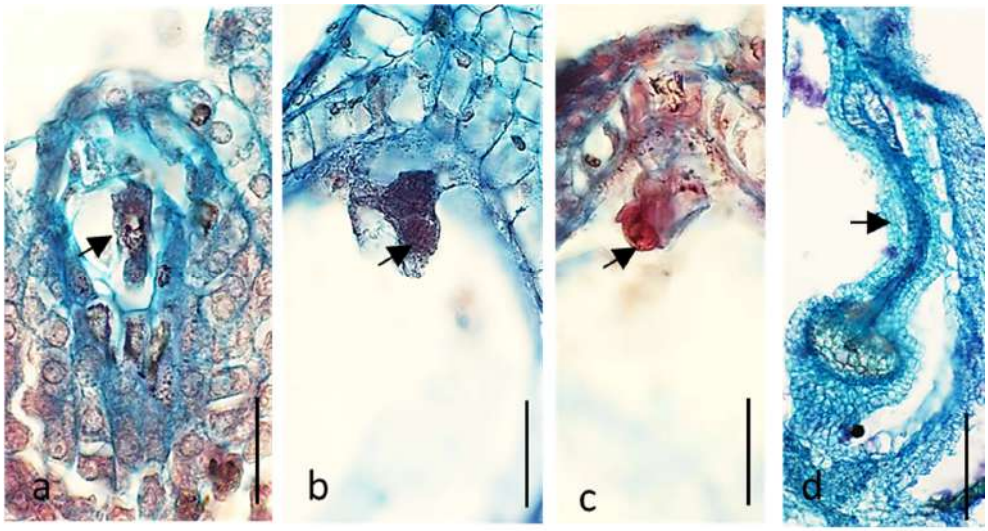

Figure 2. In planta degeneration of the embryo sacs cells and ovules after removal of 75% of flowers in *Fagopyrum esculentum* (cv. 'Korona'). The same features were observed in cv. 'Panda': (**a**) 1-nucleate embryo sac (arrow); (**b**,**c**) egg apparatus of seven-cell embryo sacs (arrows); and (**d**) the whole ovule (arrow). Bars (**a**–**c**) 20 μm; and (**d**) 100 μm.

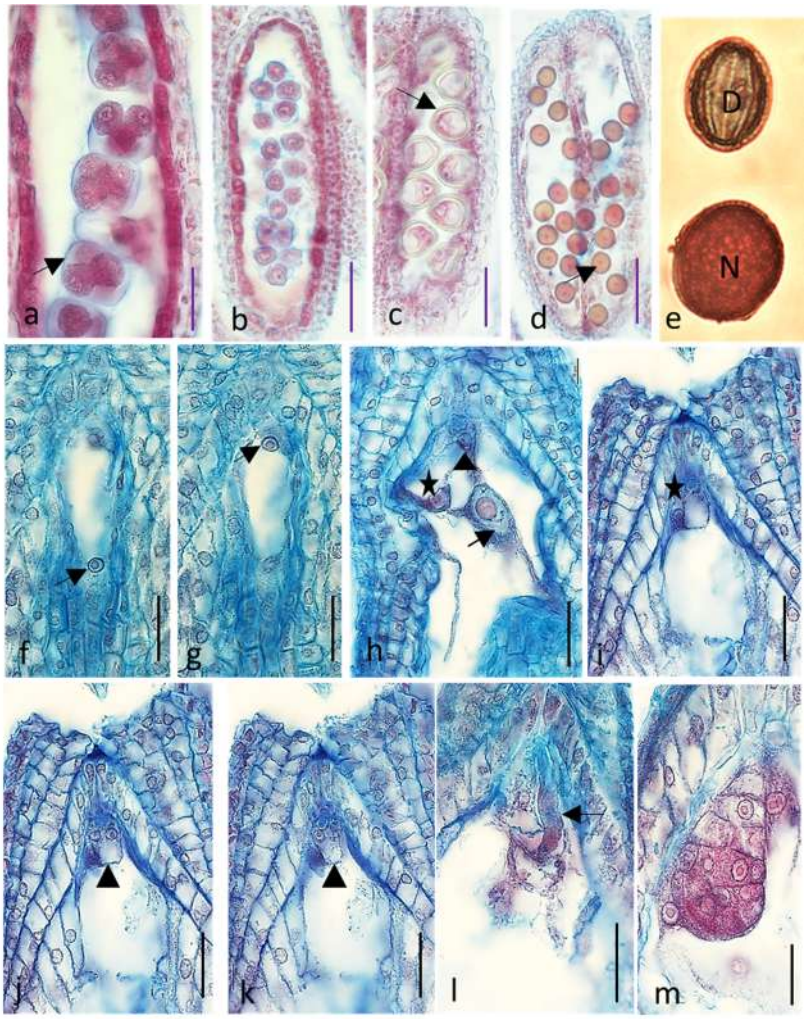

Figure 3. Normal pollen, female gametophyte and embryo development in *Fagopyrum esculentum* (cv. 'Korona') after removal of 50% of flowers. The same features were observed in cv. 'Panda': (**a**) tetrads of microspores in blue callose sheath (arrow); (**b**) microspores released from the callose sheath; (**c**) vacuolated microspores with thick sporodermis (arrow); (**d**) 1-nucleate pollen grains, nuclei visible (arrow); (**e**) degenerated (D) and normal (N) pollen grains stained with Alexander dye; (**f**,**g**) 2-nucleate embryo sac-successive stages, nuclei marked with arrows; (**h**–**k**) cells of two seven-cell embryo sacs (antipodal cells not shown) with secondary nucleus (arrow), egg cell (stars), and synergids (triangles); (**i**–**k**) successive stages of the same embryo sac; (**l**) pollen tube penetrating one of the two synergids (arrow); and (**m**) globular proembryo. Bars: (**a**,**f**–**m**) 20 µm; (**b**,**c**) 50 µm; and (**d**) 100 µm.

2.2. Phytohormones

The studied cultivars differed significantly in changes of the level of growth regulators in response to the removal of organs competing for assimilates. The most significant changes were observed in cv. 'Korona' (Table 2). In buds of these plants in the main-shoot cultivation (1S), significant increases in IAA (indole-3-acetic acid), CYT (sum of cytokinins), JA (jasmonic acid), SA (salicylic acid), and BA (benzoic acid) were noted as compared with the buds of the control (non-treated) plants. IAA, JA, SA, and BA levels increased in open flowers which were capable of fertilization. The wilted flowers (unattractive for pollinators) were additionally richer in GAs (sum of active giberellins).

Table 2. Phytohormone content (μmol g^{-1} DW) in floral, and open and wilted flowers of common buckwheat plants of cv. 'Korona' cultivated with only one main shoot (1S) or after removal of 50% or 75% of flowers. Control, plants with all lateral ramifications and flowers. Analyses were done in the phase of full blooming.

Hormones	Buds				Open Flowers				Wilted Flowers			
	Control	1S	50%	75%	Control	1S	50%	75%	Control	1S	50%	75%
IAA	73.5 ± 7.1 ^{d}	124 ± 11 ^{b}	102 ± 10 ^{c}	149 ± 13 ^{a}	67.9 ± 6.2 ^{b}	81.7 ± 8.0 ^{a}	64.3 ± 6.1 ^{b}	47.2 ± 4.3 ^{c}	157 ± 15 ^{c}	189 ± 17 ^{b}	253 ± 21 ^{a}	66.1 ± 6.1 ^{d}
GAs	8.29 ± 0.79 ^{a}	8.14 ± 0.72 ^{a}	3.51 ± 0.30 ^{b}	8.50 ± 0.81 ^{a}	7.40 ± 0.72 ^{a,b}	6.45 ± 0.61 ^{b}	4.59 ± 0.47 ^{c}	8.67 ± 0.81 ^{a}	7.40 ± 0.73 ^{c}	11.1 ± 0.11 ^{b}	6.87 ± 0.65 ^{c}	15.1 ± 0.12 ^{a}
CYT	2.88 ± 0.25 ^{c}	8.93 ± 0.83 ^{a}	2.55 ± 0.25 ^{c}	3.91 ± 0.36 ^{b}	3.78 ± 0.35 ^{a}	3.92 ± 0.32 ^{a}	2.10 ± 0.19 ^{b}	3.36 ± 0.32 ^{a}	4.09 ± 0.37 ^{a}	4.43 ± 0.42 ^{a}	4.49 ± 0.41 ^{a}	2.40 ± 0.21 ^{b}
ABA	2.16 ± 0.19 ^{c}	1.55 ± 0.12 ^{d}	2.92 ± 0.25 ^{b}	3.50 ± 0.31 ^{a}	4.04 ± 0.36 ^{b}	2.69 ± 0.25 ^{d}	5.63 ± 0.52 ^{a}	3.53 ± 0.29 ^{c}	3.45 ± 0.33 ^{b}	1.80 ± 0.17 ^{d}	4.09 ± 0.39 ^{a}	2.52 ± 0.26 ^{c}
JA	51.42 ± 5.07 ^{c}	94.61 ± 9.11 ^{b}	151 ± 14 ^{a}	30.82 ± 3.98 ^{d}	24.12 ± 2.95 ^{c}	54.14 ± 5.12 ^{a}	30.71 ± 2.76 ^{b}	6.46 ± 0.68 ^{d}	34.94 ± 3.32 ^{b}	56.22 ± 5.21 ^{a}	58.82 ± 5.76 ^{a}	59.15 ± 5.78 ^{a}
SA	22.6 ± 2.1 ^{c}	77.8 ± 7.2 ^{a}	86.8 ± 8.3 ^{a}	41.9 ± 3.9 ^{c}	18.8 ± 1.8 ^{c}	62.4 ± 6.1 ^{b}	105 ± 9.5 ^{a}	65.1 ± 5.8 ^{b}	33.2 ± 3.2 ^{c}	96.7 ± 9.3 ^{b}	124 ± 10.2 ^{a}	31.7 ± 2.8 ^{c}
BA *	31.3 ± 2.8 ^{b}	68.4 ± 5.5 ^{a}	62.7 ± 5.2 ^{a}	28.6 ± 1.9 ^{b}	21.2 ± 1.6 ^{c}	33.2 ± 2.8 ^{b}	41.8 ± 3.8 ^{a}	27.6 ± 2.4 ^{c}	16.2 ± 1.1 ^{c}	25.9 ± 2.3 ^{b}	38.4 ± 2.9 ^{a}	36.4 ± 1.8 ^{a}

Values represent means (n = 5 in each experiment) of two experiments performed during 2019–2020 ± SE (standard error). Different superscript letters (a–d) within rows for each hormone and organ indicate significant differences between means (Duncan's multiple range test; $p < 0.05$). IAA, indole-3-acetic acid; active GAs, sum of active gibberellins (GA_1, GA_3, GA_4, GA_5, GA_6, and GA_7); CYT, sum of cytokinins (kinetin, zeatin, *N*6-izopentenyladenine, and *N*6-izopentenyladenozine); ABA, abscisic acid; JA, jasmonic acid; SA, salicylic acid; * BA, benzoic acid, a precursor of salicylic acid.

Interestingly, cv. 'Korona' plants after removal of 50% of flowers demonstrated a significant increase in the number of properly developed embryo sacs described above (Table 1). In these buds, significantly higher levels of IAA, ABA (abscisic acid), JA, SA, and BA were recorded as compared with the control plants, and the amounts of JA, SA, and BA were also much higher than in the buds of plants from the other treatments. In open flowers capable of fertilization in this group of plants, less GAs and CYT and significantly more ABA, JA, SA, and BA were detected than in the control plants (Table 2), with particularly growth of SA and BA. Similar differences between control and removal of 50% of flowers in SA and BA content were observed in wilted flowers. Additionally, in these flowers, higher content of IAA was detected. The ratio of GAs to ABA in plants from which 50% of flowers were removed was 1.2 in floralbuds, 0.82 in open flowers, and 1.68 in wilted flowers. On the other hand, in control plants of cv. 'Korona', this ratio was much higher (3.8, 1.8, and 2.1, respectively), proving a significant predominance of gibberellins over ABA.

In the buds of cv. 'Korona' plants, from which 75% of the flowers were removed, the most significant increase was detected for IAA and ABA. The open flowers demonstrated the highest decrease in IAA and JA and an increase in GAs. The wilted flowers also accumulated less IAA and CYT and more GAs than the control ones (Table 2).

In cv. 'Panda', the changes in hormonal profile in flowers under removal were slight (Table 3). In almost all treatments, the greatest fluctuations occurred in the amount of BA, which is a precursor of SA. Increase in SA was recorded in open and wilted flowers of plants from which 75% of flowers were removed. Additionally, in wilted flowers of all treatments, a rise in GAs content was noted (Table 3).

Table 3. Phytohormone content (μmol g^{-1} DW) in buds, open and wilted flowers of common buckwheat plants of cv. 'Panda' cultivated with only one main shoot (1S) and after removal of 50% or 75% of flowers. Control, plants with all lateral ramifications and flowers. Analyses were done in the phase of full blooming.

Hormones	Buds				Open Flowers				Wilted Flowers			
	Control	1S	50%	75%	Control	1S	50%	75%	Control	1S	50%	75%
IAA	173 ± 18 ^{a}	77 ± 6 ^{c}	110 ± 10 ^{b}	110 ± 9 ^{b}	91 ± 8 ^{a}	73 ± 7 ^{b}	69 ± 6 ^{b}	71 ± 6 ^{b}	287 ± 26 ^{a}	267 ± 24 ^{a}	133 ± 12 ^{b}	134 ± 12 ^{b}
GAs	5.84 ± 0.42 ^{a}	4.47 ± 0.40 ^{b}	6.22 ± 0.52 ^{a}	4.63 ± 0.39 ^{b}	6.22 ± 0.59 ^{a}	6.87 ± 0.58 ^{a}	5.65 ± 0.57 ^{a}	5.60 ± 0.48 ^{a}	6.15 ± 0.57 ^{c}	10.85 ± 0.99 ^{a}	8.67 ± 0.82 ^{b}	10.26 ± 0.11 ^{a}
CYT	3.58 ± 0.31 ^{b}	2.53 ± 0.21 ^{c}	5.09 ± 0.45 ^{a}	3.29 ± 0.31 ^{b}	3.72 ± 0.34 ^{a,b}	3.46 ± 0.32 ^{b}	4.16 ± 0.41 ^{a}	3.83 ± 0.32 ^{a}	3.22 ± 0.35 ^{b}	4.39 ± 0.38 ^{a}	5.01 ± 0.46 ^{a}	3.27 ± 0.32 ^{b}
ABA	2.74 ± 0.21 ^{a}	1.88 ± 0.19 ^{b}	1.51 ± 0.14 ^{b}	1.30 ± 0.14 ^{c}	5.36 ± 0.49 ^{a}	3.99 ± 0.32 ^{b}	3.75 ± 0.33 ^{b}	2.78 ± 0.27 ^{c}	3.31 ± 0.29 ^{a}	2.54 ± 0.26 ^{b}	1.92 ± 0.17 ^{c}	3.43 ± 0.34 ^{a}
JA	80.42 ± 7.01 ^{a}	80.40 ± 0.76 ^{a}	34.61 ± 3.20 ^{c}	53.36 ± 5.01 ^{b}	14.43 ± 1.42 ^{b}	20.37 ± 2.04 ^{a}	9.67 ± 0.91 ^{c}	9.04 ± 0.90 ^{c}	46.91 ± 4.05 ^{a}	48.99 ± 4.12 ^{a}	15.50 ± 1.40 ^{c}	25.53 ± 2.61 ^{b}
SA	56.93 ± 6.02 ^{a}	25.62 ± 2.56 ^{c}	33.07 ± 3.01 ^{b}	59.37 ± 6.02 ^{a}	44.59 ± 4.8 ^{b}	40.49 ± 4.06 ^{b}	40.08 ± 3.89 ^{b}	71.24 ± 7.05 ^{a}	65.29 ± 6.21 ^{b}	48.02 ± 4.78 ^{c}	45.0 ± 4.31 ^{c}	139 ± 12 ^{a}
BA *	29.80 ± 2.01 ^{c}	66.00 ± 6.26 ^{a}	43.10 ± 4.11 ^{b}	45.31 ± 4.23 ^{b}	21.55 ± 2.01 ^{b}	27.43 ± 2.36 ^{a}	30.05 ± 3.01 ^{a}	26.90 ± 2.48 ^{a}	24.68 ± 2.48 ^{b}	36.72 ± 3.76 ^{a}	34.91 ± 3.02 ^{a}	22.43 ± 2.01 ^{b}

Values represent means (n = 5 in each experiment) of two experiments performed during 2019–2020 ± SE (standard error). Different superscript letters (a–d) within rows for each hormone and organ indicate significant differences between means (Duncan's multiple range test; $p < 0.05$). * Benzoic acid is not a hormone but a precursor of salicylic acid. IAA, indole-3-acetic acid; active GAs, sum of active gibberellins (GA_1, GA_3, GA_4, GA_5, GA_6, and GA_7); CYT, sum of cytokinins (kinetin, zeatin, *N*6-izopentenyladenine, and *N*6-izopentenyladenozine); ABA, abscisic acid; JA, jasmonic acid; SA, salicylic acid; * BA, benzoic acid, a precursor of salicylic acid.

2.3. Yield

Statistical analysis showed that the number of flowers produced by the plant was influenced by the cultivation method, i.e., treatment by removing part of the flowers or all lateral ramifications (Table 4). The number of mature seeds and their weight depended on the genotype and treatment. The percentage of flower and fruit (seed) abortion depended only on the genotype, while the efficiency of seed setting was influenced by the interaction of both factors (Table 4).

Table 4. Analysis of variance of the impact of plant treatment (main shoot only, removal of 50% or 75% of flowers, and control) on flowering and fruiting in common buckwheat cv. 'Panda' and 'Korona'. Efficiency of seed setting, seed mass, and mass of thousand seeds (MTS) were calculated per individual.

Effects	No. of Flowers	No. of Mature Seeds	Empty Seeds (%)	Abortion of Flowers and Fruits (%)	Efficiency of Seed Setting	Mature Seed Mass	MTS
Cultivar	ns	***	*	***	ns	***	***
Treatment	***	**	***	ns	ns	***	***
Cultivar x Treatment	ns	***	ns	ns	*	***	***

*, **, and *** indicate statistically significant effect of treatment at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; ns, not significant.

Removing 50% of flowers increased their production in cv. 'Panda' (Figure 4a,b and Table 5). This effect was visible in general but particularly in individual cases when the number of flowers even doubled or tripled in relation to the control. This effect was not noted in cv. 'Korona'. Removing 75% of flowers and single-shoot cultivation in both cultivars significantly reduced the number of flowers produced by one plant as compared with that of the control (Table 5). However, the number of flowers did not affect the number of mature seeds. Only when 50% or 75% of flowers were removed, cv. 'Panda' increased the number of seeds. 'Panda' and 'Korona' plants with one main shoot left produced the lowest number of mature and empty seeds in comparison with those of the control; however, in cv. 'Korona', the mass of a single seed was the highest and amounted to 0.0334 g. Smaller number of mature seeds translated into better seed filling and consequently increased mass of thousand seeds (Table 5). Plants in single-shoot cultivation finished vegetation earlier and their seeds matured more quickly than the control plants and plants with partial removal of flowers (Figure 4c–f). Generally, cv. 'Korona' showed significantly greater mass of single seed than cv. 'Panda' (Table 5). No correlation was found between the number of empty seeds and the number of flowers; however, a positive correlation was found between flower production and abortion of flowers and fruits ($r = 0.62$; $p < 0.05$). The percentage of empty seeds did not correlate with the number of mature seeds, but it correlated negatively with the mass of a single mature seed ($r = -0.857$; $p < 0.05$). Flower production also had an impact on seed setting efficiency—the fewer flowers the plant produced, the more seeds were set. The strongest effect was observed for cv. 'Panda', in which the removal of 75% of flowers doubled the seed setting efficiency. The correlation between flower and fruit abortion and seed setting efficiency was very high ($r = -0.998$; $p < 0.05$), similarly as between the number of flowers per plant and seed setting efficiency ($r = -0.833$; $p < 0.05$). The removal of 50% of flowers increased the mass of seeds collected per plant, and MTS increased in cv. 'Panda', while, in cv. 'Korona', it was similar to that of the control and plants in the remaining treatments (Table 5).

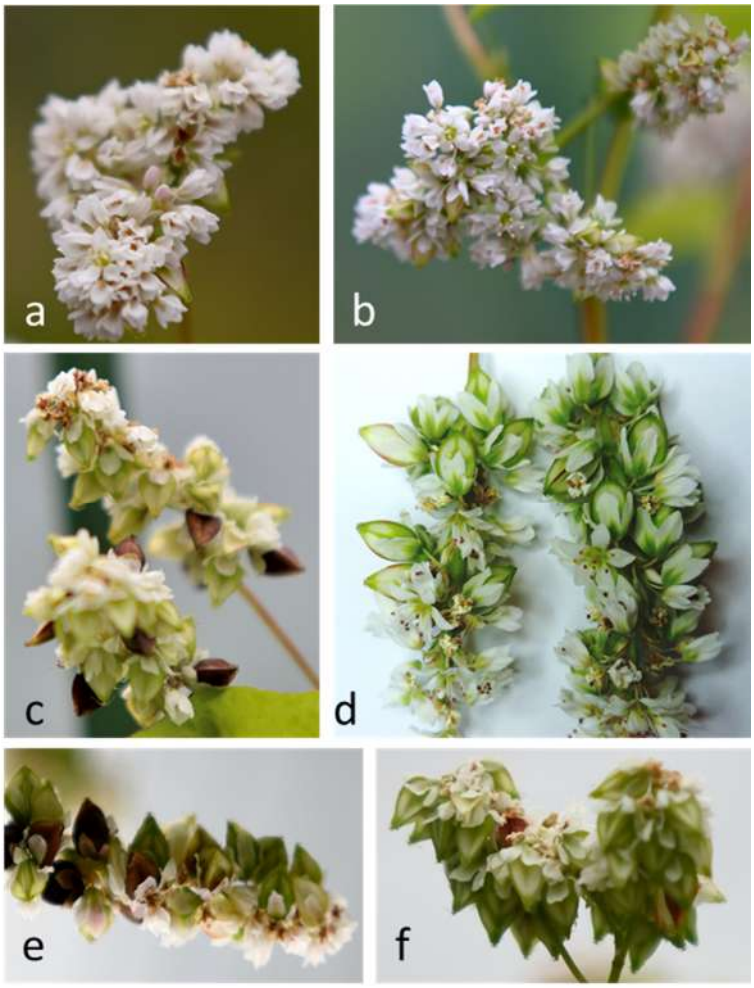

Figure 4. Macroscopic images of flower production (**a**,**b**) and seed setting (**c**–**f**) in cv. 'Panda' (**a**–**d**) and cv. 'Korona' (**e**,**f**) of *Fagopyrum esculentum*. Please compare control plants in bloom with plants from which 50% of flowers were removed:(**a**) vs. (**b**) and control plants in fruiting with plants with single shoot plants: (**c**) vs. (**d**,**e**) vs. (**f**).

Table 5. Effect of removal of all lateral ramifications (1S) or 50% or 75% of flowers on flowering and fruiting parameters of two cultivars of common buckwheat. Control, plants with all flowers and lateral ramifications.

Cultivar	Treatment	No. of Flowers per Plant	No. of Mature Seeds per Plant	No. of Empty Seeds per Plant	Abortion of Flowers and Seeds (%)	Efficiency of Fertilization (%)	Mass of One Seed	Seed Mass per Plant (g)	MTS (g)
'Panda'	Control	669 ± 55 ^{b}	128 ± 9 ^{a,b}	29 ± 8 ^{a}	81	19	0.0267 ± 0.003 ^{e}	3.42 ± 0.07 ^{c}	26.72 ± 1.41 ^{b}
	50%	823 ± 74 ^{a}	136 ± 11 ^{a}	25 ± 5 ^{a}	84	17	0.0292 ± 0.003 ^{d}	3.97 ± 0.09 ^{a}	29.19 ± 1.55 ^{a}
	75%	321 ± 29 ^{e}	130 ± 12 ^{a}	26 ± 4 ^{a}	59	40	0.0252 ± 0.002 ^{f}	3.27 ± 0.08 ^{d}	25.15 ± 1.89 ^{b}
	1 S	357 ± 32 ^{ed}	107 ± 9 ^{b}	22 ± 5 ^{a}	70	30	0.0266 ± 0.003 ^{e}	2.85 ± 0.05 ^{f}	26.63 ± 1.99 ^{b}
'Korona'	Control	778 ± 69 ^{a}	105 ± 10 ^{b,c}	14 ± 2 ^{b}	87	13	0.0311 ± 0.003 ^{b}	3.27 ± 0.08 ^{d}	31.14 ± 2.05 ^{a}
	50%	558 ± 52 ^{c}	125 ± 11 ^{b}	16 ± 4 ^{b}	78	22	0.0303 ± 0.002 ^{c}	3.79 ± 0.07 ^{b}	30.32 ± 2.03 ^{a}
	75%	442 ± 45 ^{d}	100 ± 8 ^{b,c}	22 ± 4 ^{a}	77	23	0.0297 ± 0.003 ^{c,d}	2.97 ± 0.06 ^{e}	29.70 ± 2.07 ^{a,b}
	1 S	427 ± 38 ^{d}	91 ± 7 ^{c}	6 ± 2 ^{c}	79	21	0.0334±0.003 ^{a}	3.04 ± 0.08 ^{e}	33.40 ± 2.27 ^{a}

Values represent means (n = 20 in each experiment) of two experiments performed during 2019–2020 ± SE (standard error). Different superscript letters (a–f) within columns for each treatment and cultivar indicate significant differences between means (Duncan's multiple range test; $p < 0.05$). Percentage of flower and embryo abortion was calculated as: (1—No. of mature seeds/No. of flowers) × 100. Efficiency of fertilization was calculated as: (No. of mature seeds/No. of flowers) × 100.

3. Discussion

Due to the challenges of common buckwheat cultivation described in the Introduction, especially regarding its low yield, breeding and genetic studies have been carried out for decades (for review, see Matsui and Yasui [16]). Genomic Selection in Mass Selection Breeding program for common buckwheat is a powerful program enhancing buckwheat yield by almost 21% [11]. However, classical breeding treatments such as the one described in this study shed a light on the mechanisms involved in seed production of *F. esculentum*. Key genes involved in seed development are already recognized, and they are genes responsible for Ca^{2+} signal transduction pathway, hormone signal transduction pathways, and coding transcription factors (TFs), as well as starch biosynthesis-related genes [17]. Regarding seed size, AP2 and bZIP transcription factors, BR-signal, and ABA are considered the most important regulators [18].

Our in vitro experiment showed that depriving flowers of nutrients leads to deterioration of their quality and to the abortion of ovule sacs, therefore we decided to start reducing the number of inflorescences in common buckwheat. We were also inspired for the further studies by the never-ending flower overproduction of common buckwheat throughout the whole season.

In our in planta experiment, we partly followed the suggestions of Guglielmini et al. [10], not knowing their outcome while conducting our study in 2019. They recommended to determine the causes of reduction in the number of achenes per raceme (= fruits per inflorescence) during the critical period (the period when grain number is determined and it is crucial to obtain higher yields), as they showed that radiation restriction and subsequent assimilate limitation could increase floret mortality and thereby the number of achenes per raceme.

Yabe et al. [11], who used 92FE1-F4, a population produced by bulk crossing of 'Tempest', 'Kitawasesoba', 'Natsusoba', and 'Shinanonatsusoba' cultivars, showed that the number of clusters positively correlated with seed yield. We did not find such a correlation in any of the investigated cultivars. On the contrary, we demonstrated a positive correlation between flower production and the abortion of flowers and fruits.

'Korona' plants after removal of 50% of flowers showed significantly lower number of degenerated embryo sacs and higher number of mature seeds, higher efficiency of seed setting than the control plants and plants from the other variants. Since we detected more IAA in these flowers, it is possible that the auxin supports development of embryos, which could be important for higher seed yield in these plants. Although only 3% of degenerated embryo sacs were found, the percentage of aborted flowers did not drop. This may be due to limited pollination or impaired embryo development, which in cv. 'Panda' flowers were at 9% and 13% depending on the type of flower (Pin or Thrum) [4]. Despite higher percentage of abnormally formed embryo sacs in cv. 'Korona', higher effectiveness of seed setting, calculated as the number of seeds divided by the number of flowers, was found after removing 75% of flowers vs. 50% of flowers. 'Panda' plants with 75% flowers removed also showed a much higher percentage of degenerated embryo sacs than the plants treated differently, but the seed setting efficiency was similar to plants with 50% of flowers removed. It is possible that this effect was caused by a higher concentration of gibberellins and that these hormones are more effective than auxin in keeping the embryos alive, similarly as in *Arabidopsis thaliana* [19].

Salicylic acid plays a crucial role in flowering and luring insects, similarly to jasmonic acid. Moreover, both hormones are involved in defense responses of plants in the event of a pathogen attack [20]. Jasmonic acid is also necessary for the formation of an ovulum, and its absence is characteristic of sterile flowers [21]. Such hormones as gibberellins, brassinosteroids, and abscisic acid are of great importance during flowering. It is usually believed that ABA, as an antagonist of gibberellins, inhibits flowering [22]. It was therefore unexpected that the flowers that set seeds the most efficiently accumulated higher levels of this hormone than those of plants with a greater degree of embryo sac degeneration. GA to ABA ratio in plants from which 50% of flowers were removed was much lower than in the control plants. These results mean that the proportion between gibberellins and abscisic acid is more important than absolute concentrations of these hormones. On the other

hand, in the flowers of control plants, this ratio was much higher, proving a significant predominance of gibberellins over ABA, and at the same time these plants were characterized by a greater degree of ovule sac degeneration. These findings thus indicate that a direct reason for embryo sac abnormal development is probably independent of these two classes of hormones.

Salicylic acid controls, similar to JA, the process of attracting insects during the flowering phase. However, while JA confers flowers their attractive fragrance, SA can increase flower temperature to release volatile compounds [23]. Benzoic acid (BA) is a precursor of salicylic acid, so it was not surprising that BA concentration in flowers was high and translated directly into high content of SA. Our results indicate that the two cultivars studied also differed in terms of producing the hormones responsible for luring insects. 'Panda' flowers accumulated much greater amounts of salicylic acid, while 'Korona' ones produced mainly jasmonic acid. It should also be remembered that majority of hormones stimulate flower formation in the vegetative phase, so the presence of hormones in flowers may not give a complete picture of the role of individual hormones in embryological development.

Taylor and Obendorf [12] argued that poor seed yielding in common buckwheat results from problems with ovule development and fertilization. They also claimed that the lack of fertilization is influenced by variable viability and quality of microspores. Our research shows that pollen viability in both studied genotypes is high, and even enhanced by flower removal in cv. 'Panda'. This research further confirmed our long-term observations of a strong positive correlation between the number of flowers and their abortion, which means that the more flowers a plant produces, the more of them are rejected. According to ourresults, flower abortion is primarily influenced by plant genotype, and this barrier would be very difficult to break. In our opinion, buckwheat plants have a certain limit of seeds, and, above this limit, the plant will not allocate its 'resources' (assimilates) to their filling. A very high negative correlation between flower abortion and efficiency of seed setting was found, which proves that a decrease in assimilate competition significantly increases seed setting. As we noticed, the plant seems to be reaching a certain limit of flower production, and it is difficult to change this limit. Halbrecq et al. [13] reported that the abortion mainly affects flowers from the upper floors, that is, those arising in the later stage of flowering, in relation to the flowers located lower, i.e., previously produced. Sugawara [24] and Asako et al. [25] found that flowers formed earlier are more likely to set seeds than flowers formed later. Our previous unpublished research showed that the nectar is richer in sugars in earlier flowers than in later ones. This factor likely has a significant impact on the flight of pollinating insects, which directly translates into seed setting. In addition, Taylor and Obendorf [12] reported that the embryo sacs are better developed in the earlier formed flowers. Our observations indicate that flower abortion occurs throughout the flowering period and its degree depends on many factors. In buckwheat, the period of vegetative development overlaps with the period of generative organ formation. The formation of vegetative organs coincides with intense flowering and seed formation, which results in strong competition for assimilates. According to Halbrecq et al. [13], when plants reach their maximum vegetative development, competition for assimilates decreases. Assimilates can therefore be located mainly in the generative organs. These authors claimed that more flowers and seeds are produced during this time, and the degree of abortion decreases along with competition for assimilates. Our research did not confirm these conclusions. We proved that the increased number of flowers did not correlate with the number of seeds, because at the same time the abortion of flowers increased. In previous studies, we observed a certain percentage of 'starved' embryos, so the competition for assimilates between the set seeds and the still emerging flowers continued [4]. The present experiments confirmed a negative correlation between the number of empty seeds and mass of a single seed.

Halbrecq et al. [13] performed an experiment involving defoliation (partial and total) and partial removal of buckwheat inflorescences from the main shoot in order to modify the availability of assimilates and reduce competition between seeds. In all cases, regardless of the procedure, they observed a drastic reduction in the number of grains in relation to the number of flowers formed, with very low seed yield, around 20–30%. The authors found that the critical seed setting stage occurs

shortly after flowering and is not affected by a change in donor-acceptor relationship due to defoliation or removal of part of the inflorescences. The drastic limitation of competition between inflorescences and seeds had only a negligible effect on the final grain yield per plant, which indicated a strong compensation by the remaining grains. In our research, we showed that only the removal of 50% of flowers allowed for increasing the seed yield. In the remaining cases, removing flowers reduced the yield or did not change it as compared with the control. Despite the fact that the percentage of empty seeds did not significantly correlate with the number of whole seeds, it significantly negatively correlated with the weight of individual mature seed. These findings clearly indicate that the setting of seeds, although not filled later on, reduced the mass of mature seeds. It can therefore be assumed that flowering and fertilization are the phases critical for seed yield. Flower overproduction and embryo formation is a process that exhausts the plant reserves. Failure to fill all the seeds will no longer compensate for these losses. Forming empty seeds is therefore, apart from flower abortion, another form of crop regulation when the plant produced too many flowers.

It is worth underlining that plants cultivated as single-shoots finished their blooming period earlier and their seeds matured also earlier than those of the other plants. The study results confirm our hypothesis that plants with a shorter flowering period (self-finishing) may achieve higher seed yield. Considering that the number of flowers, their abortion, and the percent of defective embryos are controlled genetically, breeders are faced with a challenging task of producing new genotypes with amended traits. Other difficulties involve strong self-incompatibility and impossibility of inbreeding. Given these limitations, mutations seem to be the only way to obtain new forms of common buckwheat, and we will explore this approach in the years to come.

4. Materials and Methods

4.1. In Vitro and In Planta Experimental Design

Investigation of trophic (nutrient) stress in in vitro conditions was performed in 2019, while the experiments conducted in planta were performed twice in 2019 and 2020. The presented results are the means of two independent experiments. The seeds of *F. esculentum* of Polish cultivars 'Panda' and 'Korona' used for the experiment were provided by Małopolska Hodowla Roślin in Polanowice Sp. z o.o. (Poland).

4.1.1. In Vitro Experiment

Plants obtained from the seeds were cultivated in pots filled with a commercial soil substrate (pH = 6.0) mixed with perlite 1:1 (*v*:*v*) in a phytotronic chamber at 20 °C and humidity of 50–60%, under 16 h photoperiod and 300 µmol m^{-2} s^{-1} of PPFD (photosynthetic photon flux density). Large buds 2.25–3.50 mm in size were collected from two-month-old plants during full blossom stage and sterilized in 70% ethanol for 1 min and 20% sodium hypochlorite for 7 min. Then, they were washed three times in sterile water for 5 min. After sterilization the buds were transferred onto three different media, containing 100%, 50%, and 30% of sugar, vitamins, and macro- and microelements. The full content media was prepared according to the in vitro cultivation protocol of common buckwheat: (1) $\frac{1}{2}$ MS (Murshige & Skoog) + 30 g dm^{-1} sucrose + BA 1 mg dm^{-1} + NAA 0.1 mg dm^{-1} [25]; (2) MS + vit. B_5 2 mg dm^{-1} + sucrose 25 g dm^{-1} + glutamine 0.1 g dm^{-1} + kinetin 1 mg dm^{-1} + GA_3 1 mg dm^{-1} [1]; and (3) MS + 30 g dm^{-1} sucrose + zeatin 2 mg dm^{-1} [25]. pH of the media was established at 5.6, and 0.8% agar was used for solidification. All compounds were obtained from Sigma-Aldrich (St. Louis, MO, USA). The lowest content (30%) of the medium ingredients was used only in Medium 1. The floral buds extracted from inflorescences were cultivated on the media for ten days at a constant temperature of 20 ± 2 °C, relative humidity of air 50–60%, 16 h photoperiod, and 300 µmol m^{-2} s^{-1} PPFD (AGRO Philips sodium lamps, Philips, Aachen, Germany), and then collected for embryological analyses.

4.1.2. In Planta Experiment

In 2019 and 2020, the influence of in planta assimilate availability on the embryological development and yielding of buckwheat plants was investigated. Plants obtained from the seeds were grown from May to September in pots filled with commercial soil substrate (pH = 6.0) mixed 1:1 (*v*:*v*) with perlite in an open tunnel enabling the flight of insects. The plants were divided into four groups, control (without flower removal), single main-shoot cultivation (successive removal of all inflorescence developing on lateral branches and lateral branches as well, marked 1S), 50% (every second inflorescence, i.e., spike of spikelets, was removed), or 75% (every second, third, and fourth spike of spikelets was removed), the latter two marked 50% or 75%, respectively (Figure 5). The flowers for embryological and hormonal analyses were collected during full flowering phase.

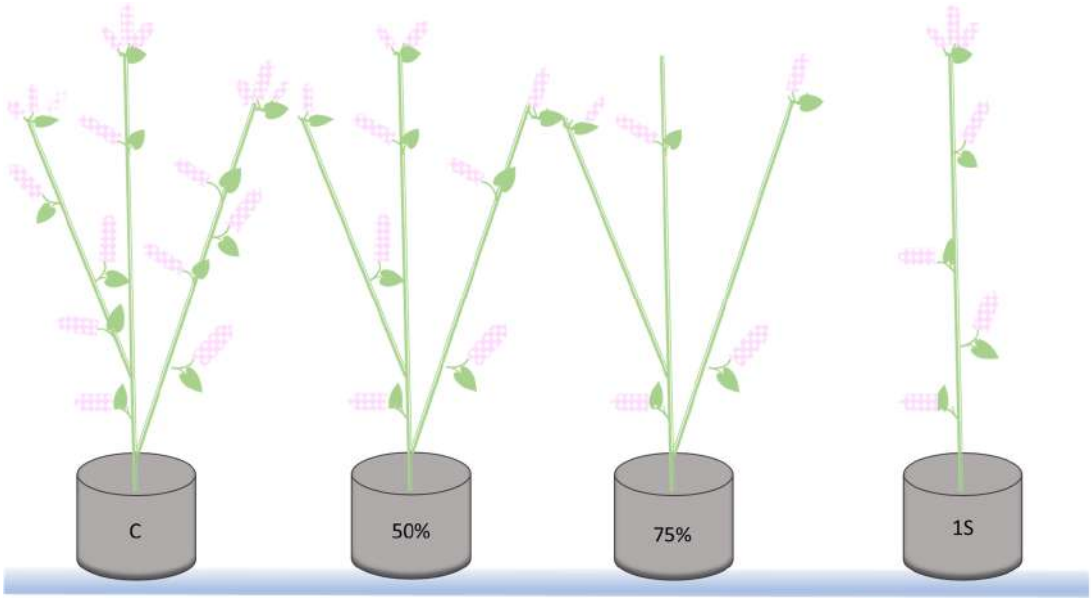

Figure 5. Inflorescences and lateral stem removal performed during in planta experiments on *Fagopyrum esculentum* in 2019 and 2020. C, control plant with all lateral ramifications and with all inflorescences; 50%, plant with half of the spike of spikelets (every second removed); 75%, plant with only 25% of spike of spikelets (every second, third, and fourth removed); 1S, single shoot plant (all lateral ramifications removed).

4.2. Embryological Processes in Flowers in In Vitro and In Planta Experiments

After 10 days of the bud culture on artificial media and at the time of flower number counting (mid-July), enlarged buds (in vitro) and buds and open flowers left on the plants (in planta experiment from 2019) were fixed in a mixture of acetic acid and 96% ethanol (1:3; *v*:*v*) for 24 h at room temperature. Fixed flowers were kept in 70% ethanol for further analyses. Dehydration, paraffin supersaturation, embedding, and slicing were conducted as described in detail for *F. esculentum* by Płażek et al. [6]. Staining was performed as described by Słomka et al. [4]. From 20 to 30 flowers per treatment were analyzed.

Pollen viability (stainability) in the flowers from in planta experiment from 2019 was assessed in 2200–3500 pollen grains per treatment (from at least 20 flowers) by staining with Alexander dye [26]. Isolated pollen grains were stained on a microscopic slide and the number of viable (purple) and non-viable (green or transparent) pollen grains was counted.

4.3. Phytohormonal Profile Analyses in Flowers in In Planta Experiments

Analysis of selected plant hormones was performed as reported previously [6,27–29]. Lyophilized and pulverized plant material (15 mg) was triple extracted in 1 mL of methanol/water/formic acid (15:4:1; *v*:*v*:*v*) solution. At this stage, a heavy-labeled internal standards mixture was added (about 20 pmol of [$^{15}N_4$]dihydrozeatin, [$^{2}H_5$]*trans*-zeatin-riboside, [$^{15}N_4$]kinetin, [$^{2}H_2$]gibberellin A_1, [$^{2}H_2$]gibberellin A_4, [$^{2}H_2$]gibberellin A_6, [$^{2}H_2$]gibberellin A_5 [$^{2}H_5$]indole-3-acetic acid, and [$^{2}H_6$]*cis*,*trans*-abscisic acid, [$^{2}H_4$]salicylic acid, [$^{2}H_5$]benzoic acid (OlChemim, Olomouc, Czech Republic), and [$^{2}H_5$]jasmonic (CND Isotopes, Quebec, QC, Canada)). Samples after evaporation (N_2 stream) were reconstituted (3% methanol in 1 M formic acid) and cleaned up on hybrid SPE columns (BondElut Plexa PCX, Agilent, Santa Clara, CA, USA). Measurements were conducted on ultrahigh performance liquid chromatograph (UHPLC, Agilent Infinity 1260) coupled to 6410 Triple Quad LC/MS with ESI (Electrospray Interface) ion source (Agilent Technologies) in MRM mode. Technical details are provided in references cited. Quantification was based on calibration curves for authentic standards considering recoveries of heavy-labeled internal standards.

The following compounds were determined: indole-3-acetic acid (IAA), kinetin (KIN), gibberellins (GA_1, GA_3, GA_4, GA_5, GA_6, and GA_7), *cis, trans*-abscisic acid (ABA), salicylic acid (SA), benzoic acid (BA), and jasmonic acid (JA). Five plants per treatment were analyzed.

4.4. Yield Related Measurements in In Planta Experiments

The number of flowers produced by control, 1S, 50%, and 75% plants were assessed in mid-July 2019 and 2020 in 20 replicates (20 plants) from each treatment. The seeds were harvested at the end of August 2019 and 2020. The number of mature and empty seeds, as well as the mass of mature seeds per plant were counted as a mean of 20 plants per treatment. The mass of one thousand seeds (MTS) was also calculated. The percentage of flower and embryo abortion was calculated according to the formula: (1 − mature seed number/number of flowers) × 100%. The efficiency of seed setting was calculated according to the formula: (mature seed number/number of flowers) × 100%.

4.5. Statistical Analysis

All results from in planta experiments were analyzed by ANOVA. Differences between means were calculated using Duncan's multiple range test ($p < 0.05$). The values show the means ± SE (standard error). Correlations between the studied parameters (Pearson's coefficient) were tested at $p < 0.05$. In the case of not normal distribution, the non-parametric Chi-square test was used. The Chi-square test of independence was performed for the frequency of ovule and embryo sac disturbances. All statistical analyses were performed in Statistica v. 13 (Statsoft, Kraków, Poland).

5. Conclusions

The conclusions and postulated mechanisms of seed yield regulation in buckwheat plants are as follows:

1. Following the experimental loss of some flowers, a plant initiates compensation processes including: increase in the efficiency of pollen viability by reducing the percentage of degenerated pollen grains; production of additional flowers, but, when the number of flowers turns out to be too high, the plant aborts most of them; increase the percentage of empty seeds if the above-mentioned measures are insufficient; and reduction in the amount of reserve materials accumulated in the seeds, and thereby reduction of seed mass if the other mechanisms prove insufficient.
2. The critical point for seed yield is the moment of flowering and fertilization. Flower overproduction and embryo formation are the processes that exhaust the plant reserves. Failure to fill all the seeds will no longer compensate for these losses. Forming empty seeds is therefore, next to flower abortion, another form of crop regulation when the plant has produced too many flowers.

Our study confirmed the common observation that the greater is the number of seeds, the smaller is the mass of a single seed.

3. Removing 50% of flowers significantly reduces the percentage of defective embryo sacs, which has a direct impact on increasing the yield of mature seeds. However, this relationship was only observed in cv. 'Korona'. In this cultivar, this effect can be attributed to higher concentration of jasmonic acid, salicylic acid (and its precursor - benzoic acid), which play an important role in attraction of pollinators.
4. Plants in single-shoot cultivation finish their vegetation earlier and achieve higher mass of one seed as compared with that of the control. This result confirms our hypothesis that self-finishing plants of common buckwheat, with shorter blooming phase, could deliver higher seed yield.

Author Contributions: A.P. conceived and designed the experiments; M.H., A.S. (Aneta Słomka), K.S., A.P., P.K., M.D., J.P. and A.S. (Anna Szczerba) performed the experiments and analyses; A.P., M.H. and A.S. (Aneta Słomka) analyzed the data; and A.P. and A.S. (Aneta Słomka), wrote the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by funding from the National Science Centre (No. 2017/25/B/NZ9/00148).

Conflicts of Interest: The authors declare no conflict of interest. The founders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Abbreviations

ABA	Abscisic acid
BA	Benzoic acid
CYT	Cytokinins
GAs	Active gibberellins (GA_1, GA_3, GA_4, GA_5, GA_6, GA_7)
IAA	Indole-3-acetic acid
JA	Jasmonic acid
SA	Salicylic acid
MTS	Mass of thousand seeds
1S	Plant with single main shoot
50%	Plant with 50% of flowers removed
75%	Plant with 75% of flowers removed

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International Journal of *Molecular Sciences*

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Changes in the Flower and Leaf Proteome of Common Buckwheat (*Fagopyrum esculentum* Moench) under High Temperature

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Abstract: Common buckwheat (*Fagopyrum esculentum* Moench), a pseudocereal crop, produces a large number of flowers, but this does not guarantee high seed yields. This species demonstrates strong abortion of flowers and embryos. High temperatures during the generative growth phase result in an increase in the degeneration of embryo sacs. The aim of this study was to investigate proteomic changes in flowers and leaves of two common buckwheat accessions with different degrees of heat tolerance, Panda and PA15. Two-dimensional gel electrophoresis and mass spectrometry techniques were used to analyze the proteome profiles. Analyses were conducted for flower buds, open flowers capable of fertilization, and wilted flowers, as well as donor leaves, i.e., those growing closest to the inflorescences. High temperature up-regulated the expression of 182 proteins. The proteomic response to heat stress differed between the accessions and among their organs. In the Panda accession, we observed a change in abundance of 17, 13, 28, and 11 proteins, in buds, open and wilted flowers, and leaves, respectively. However, in the PA15 accession there were 34, 21, 63, and 21 such proteins, respectively. Fifteen heat-affected proteins were common to both accessions. The indole-3-glycerol phosphate synthase chloroplastic-like isoform X2 accumulated in the open flowers of the heat-sensitive cultivar Panda in response to high temperature, and may be a candidate protein as a marker of heat sensitivity in buckwheat plants.

Keywords: common buckwheat; high temperature; proteomics; heat-affected proteins

Citation: Kopeć, P.; Hornyák, M.; Pastuszak, J.; Szczerba, A.; Rapacz, M.; Waga, J. Changes in Flower and Leaf Proteome of Common Buckwheat (*Fagopyrum esculentum* Moench) under High Temperature. *Int. J. Mol. Sci.* **2021**, *22*, 2678. https://doi.org/10.3390/ijms22052678

Academic Editor: P.V. Vara Prasad

Received: 8 February 2021
Accepted: 4 March 2021
Published: 6 March 2021

1. Introduction

Common buckwheat (*Fagopyrum esculentum* Moench), which belongs to the *Polygonaceae* family, is a valuable source of rutin, iron, dietary fiber, and many other microelements. Buckwheat seeds do not contain gluten and have a well-balanced amino acid composition rich in lysine [1,2]. This species does not require good soil; however, it is sensitive to a number of environmental factors, such as frost and cold, high temperature, dry wind, and drought [3]. Its flowering biology is complex, as it is a heterostylic species that produces pin and thrum flowers. The flowers need to be cross-pollinated by insects, mainly bees. This plant is characterized by strong self-incompatibility. A single flower is able to be fertilized for one day only [4]. Buckwheat blooms throughout the growth season, but its abundant flower production (up to 2000 flowers per plant) does not guarantee high seed yields [2,5,6]. Our previous research showed that buckwheat plants have a limited ability to fill seeds, and hence this species shows a strong abortion of flowers and embryos [7].

In earlier investigations, we found that buckwheat plants at the vegetative phase develop much better at a higher temperature (30 °C) than at 20 °C [8]. However, 30 °C is too warm for optimal embryo development [9]. In our previous work [9], we detected a significantly higher degree of embryo sac degeneration in plants grown at 30 °C than in plants grown at 20 °C.

High temperature negatively affects metabolic processes, protein structure, electron transport in cytoplasmic membranes, and the energy status of photosystems, while inducing the formation of reactive oxygen species (ROS) [10]. In addition, heat stress is associated with an enhanced risk of improper protein folding and denaturation of several intracellular and membrane proteins [11]. Heat leads to the increased expression of several proteins, especially those in the large heat-shock protein (HSP) family, which includes high molecular mass HSPs (from 6 to 110 kDa) and small HSPs (from 15 to 45 kDa) [11,12]. Proteomic analyses have shown that many other proteins are also synthesized in plants during adaptation to high temperature; examples include proteins involved in the antioxidant system [13–15], enzymes involved in biosynthesis of UDP-glucose, pyruvate dehydrogenase, transketolases, and enzymes in the Krebs cycle and pentose phosphate pathway that regenerate ribulose-1,5-bisphosphate (RuBP) and activate Rubisco [12].

Previous studies have focused on changes in proteomes during embryogenesis, fertilization, and seed formation [16]. Feng et al. [17] analyzed the proteome of *Arabidopsis thaliana* flowers, and proteins involved in protein synthesis, folding, modification and degradation, as well as a belonging to the regulatory system. Kerim et al. [18] analyzed the proteome of rice at several male gametophyte stages: the pollen mother cell tetrad, early young microspores, the early and late binucleate stages, and the heading stage. The proteins they observed, which played an important role in the development of the male gametophyte, were related to the metabolism of sugars, cell elongation and cell expansion. Although it is more difficult to analyze proteins in the female gametophyte because of technical difficulties, Uchiumi et al. [19] detected some proteins in rice eggs: the cytoplasmic glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase, histone H4, cytoplasmic ascorbate peroxidase, and a member of the HSP 90 subfamily. Feng et al. [17] detected differences in the abundance of pistil-proteins between compatible and self-incompatible *Prunus armeniaca* cultivars. Liu et al. [20] identified more than 40 proteins in aborted seeds, including three cysteine proteases that were possibly involved in programmed cell death. Das et al. [21] detected the differential expression of 44 abiotic stress-responsive proteins in soybean leaves under several abiotic stresses. The results and observations of those studies suggest that many differentially expressed photosynthesis-related proteins disrupt the regulation of Rubisco, electron transport, and the Calvin cycle during exposure to abiotic stresses.

The aim of this study was to investigate proteomic changes in flowers and leaves of two common buckwheat accessions, the Panda cultivar and the PA15 breeding line, which have different degrees of heat tolerance. In our previous study [9], we showed that Panda is more sensitive to heat stress than PA15. Our results showed that there were more degenerated embryo sacs at the flower bud formation stage at 30 °C than at 20 °C in Panda. By contrast, in PA15, the number of degenerated embryo sacs only increased after a longer duration of heat stress, i.e., at the open flower phase. In this study, we explored the proteomic changes during flower development, and the differences in proteomes between high temperature (30 °C) and control (20 °C) conditions. In our study, we mainly wanted to compare the stress-induced changes in the proteome in two accessions with different degrees of tolerance. On this occasion, we wanted to further identify heat-related proteins. To study the proteome we used two-dimensional electrophoresis in combination with liquid chromatography-mass spectrometry (nanoLC-MS/MS) and peptide mass fingerprinting (PMF). Although there have been advances in the methodology of proteome research in recent years, the methods used in our study are still adequate and widely used in studies similar to ours [22–25]. We identified proteins showing differences in abundance in response to heat in the two buckwheat accessions. Our re-

sults shed light on the mechanisms responsible for tolerance to heat stress, as manifested by a lower degree of degraded embryo sacs under high temperature. These analyses were performed for flower buds, open flowers capable of fertilization, and wilted flowers, as well as donor leaves, i.e., those growing closest to the inflorescences.

2. Results

2.1. Protein Profiles in Flowers and Leaves

In the 2-D electrophoresis analyses, different numbers of proteins were identified depending on the plant organ and the accession. Representative 2-D protein patterns are shown in Figure 1a. In the 2-D maps of flower bud proteins, we detected 1189 protein spots in Panda and 1159 in PA15. There were fewer protein spots in the 2-D maps produced from open flowers, namely 900 for Panda and 977 for PA15. In the 2-D maps produced from unpollinated wilted flowers, there were 1117 protein spots for Panda and 1097 for PA15. The smallest numbers of protein spots were detected in donor leaf samples, namely only 701 for Panda and 826 for PA15.

High temperature did not affect the number of protein spots within the accessions, but it affected the abundance of some proteins. In flower buds, high temperature up-regulated the expression of 17 proteins in Panda and 34 in PA15. Fewer proteins were up-regulated by heat in the open flowers: 13 in Panda and 21 in PA15. High temperature strongly affected the proteome of wilted flowers, up-regulating the expression of 28 proteins in Panda and 63 in PA15. In the donor leaves, heat stress affected the expression of 11 proteins in Panda and 20 in PA15. On the other hand, no downregulation of the expression of specific proteins as a result of high temperature was observed in any case.

The proteomic response to heat stress differed among plant organs and between the two accessions, as illustrated by heat maps and the results of hierarchical clustering analysis using Euclidean and Ward's linkage methods (Figure 1b). In Panda, only three proteins were commonly up-regulated by heat stress in different plant organs (in two out of four organs only). In PA15, eight proteins were commonly up-regulated by heat stress in two organs. A few proteins were commonly up-regulated in Panda and PA15 by heat stress, but most of the proteins up-regulated by heat stress differed between the two accessions. Venn diagrams illustrating these results are shown in Figure 1c. We detected four proteins up-regulated in buds of both accessions, one in open flowers, nine in wilted flowers, and one in donor leaves. In each accession, no proteins showed heat-inducible expression at all stages of flower development. In Panda, the abundance of spot 34 changed in the flower buds and wilted flowers in response to heat stress, and spots 57 and 60 were up-regulated by heat stress in open flowers and wilted flowers. In PA15, five protein spots (no. 14, 16, 18, 20, and 21) showed changes in abundance under heat stress in flower buds and open flowers, and three spots (no. 11, 31, and 33) showed changes in abundance under heat stress in flower buds and wilted flowers.

2.2. Identification of Differentially Accumulated Proteins

To qualitatively analyze the protein spots on the two-dimensional gel electrophoresis (2-DE) gels, 182 spots were excised from the gels and analyzed using nanoLC-MS/MS. Only 31 proteins were successfully identified and annotated with the functions of homologous proteins. Next, PMF analysis of protein spots that were not identified by nanoLC-MS/MS identified another 42 proteins. The results are listed in Table 1. For the remaining unidentified spots, searches were performed against the Swiss-Prot database, searching for proteins among all taxa. Based on the identified proteins, we searched for homologous proteins among green plants using the BLASTP program. This procedure allowed us to identify seven additional plant proteins (Table 2).

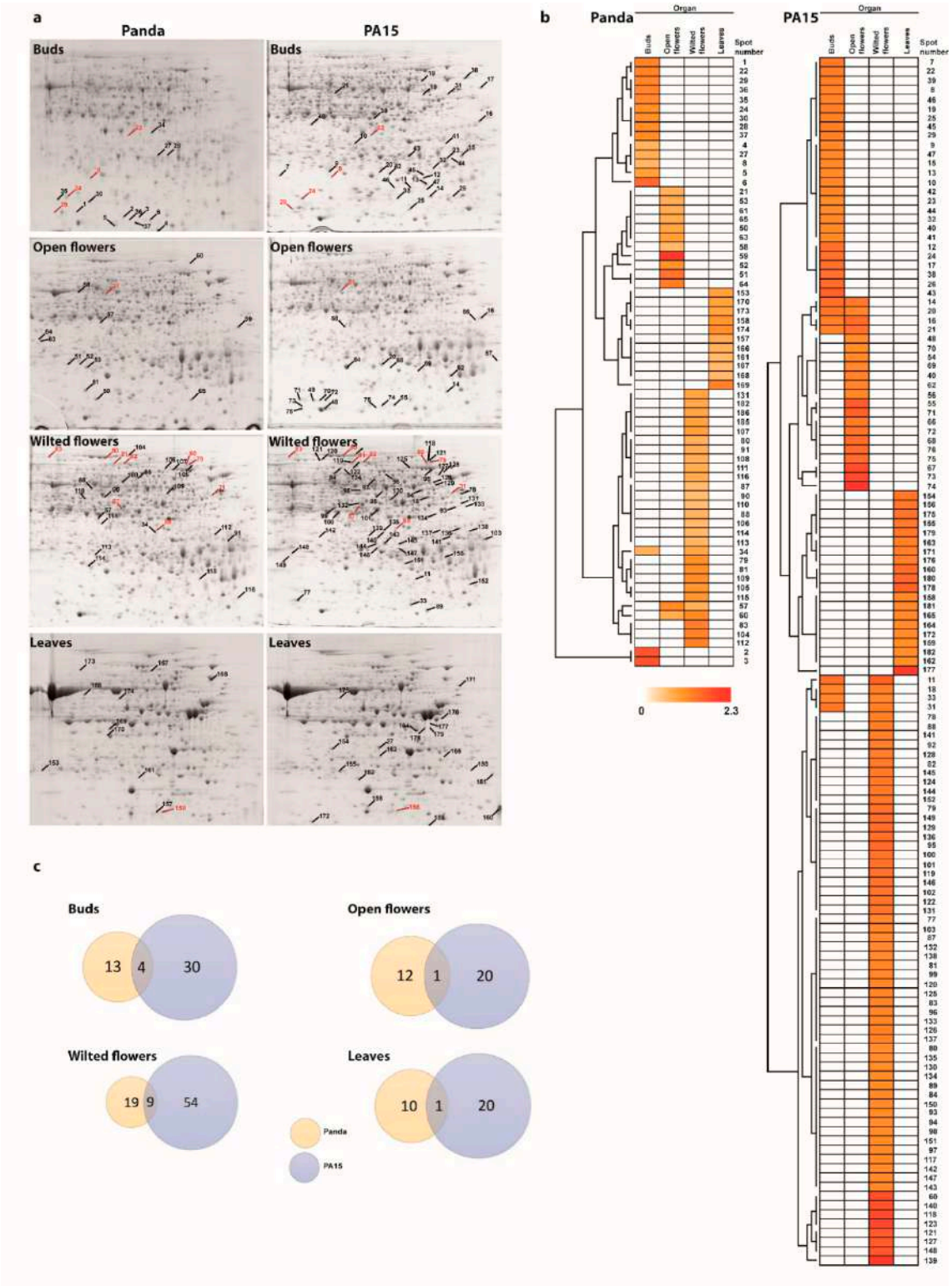

Figure 1. Changes induced by high temperature in proteomes of flower buds, open flowers, wilted flowers, and donor leaves of two common buckwheat accessions, Panda and PA15: (**a**) Representative two-dimensional gel electrophoresis (2-DE) gels of total proteins; proteins were separated by isoelectric focusing on an immobilized pH gradient IPG strip (pH 4–7) followed by sodium dodecyl sulfate (SDS)-PAGE on a 12% acrylamide gel; marked spots are those showing changes in abundance in heat-stressed plants compared with control plants, red color indicate additionally proteins common for both Panda and PA15; (**b**) heatmaps illustrating the results of hierarchical clustering analysis using Euclidean and Ward's linkage methods; cluster analysis was conducted using PermutMatrix software v.1.9.3. (http://www.atgc-montpellier.fr/permutmatrix/); colors correspond to log-transformed values of protein spot fold-change; (**c**) Venn diagrams comparing proteome profiles (up-regulated proteins only) between two buckwheat accessions.

Table 1. Results of protein identification performed on spots showing at least 2 times higher ($p \leq 0.005$) abundance in plants grown at high temperature than in plants grown at control temperature. LC-MS/MS, liquid chromatography-mass spectrometry; PMF, mass fingerprinting.

Spot No. [a]	Organ [b]	Technique [c]	UniProt No. [d]	Protein Name [e]	Reference Organism [f]	M_t [kDa] [g]	pI_t [h]	Protein Score [i]	Peptide Count [j]	Coverage (%) [k]
2	B	LC-MS/MS	RS122_ARATH	40S ribosomal protein S12-2	*Arabidopsis thaliana*	15.3	5.55	169.24	2	12.5
4	B	PMF	RL26_BRACM	60S ribosomal protein L26	*Brassica campestris*	16.9	11.60	135.90	4	8.9
5	B	PMF	EIF3A_MAIZE	Eukaryotic translation initiation factor 3 subunit A	*Zea mays*	111.5	9.80	134.80	4	5.8
6	B	PMF	CLDS_TOBAC	Copal-8-ol diphosphatehydratase, chloroplastic	*Nicotiana tabacum*	93.2	5.50	128.00	2	3.6
7	B	LC-MS/MS	PDRP2_ARATH	Pyruvate, phosphatedikinase regulatory protein 2	*Arabidopsis thaliana*	41.4	9.64	97.92	1	2.1
8	B	PMF	STAD6_ORYSI	Acyl-[acyl-carrier-protein] desaturase 6, chloroplastic	*Oryza sativa subsp. indica*	46.5	7.2	136.80	2	9.0
9	B	LC-MS/MS	PS4_PINST	Putative LRR diseaseresistance protein/transmembrane receptor kinase PS4 (fragment)	*Pinus strobus*	0.90	11.10	95.06	1	100.0
10	B	LC-MS/MS	AB5F_ARATH	ABC transporter F family member 5	*Arabidopsis thaliana*	78	6.49	94.56	1	1.7
13	B	PMF	RHM2_ARATH	Trifunctional UDP-glucose 4,6-dehydratase/UDP-4-keto-6-deoxy-D-glucose 3,5-epimerase/UDP-4-keto-L-rhamnose reductase RHM2	*Arabidopsis thaliana*	75.2	6.00	116.70	1	2.4
15	B	LC-MS/MS	PSA5_ORYSJ	Proteasome subunit alpha type-5	*Oryza sativa subsp. japonica*	26	4.60	557.20	11	37.1
16	B; OF	PMF	UPL1_ARATH	E3 ubiquitin-protein ligase UPL1	*Arabidopsis thaliana*	404.7	4.80	126.20	3	1.3
17	B	LC-MS/MS	PSMD4_ARATH	26S proteasome non-ATPase regulatory subunit 4 homolog	*Arabidopsis thaliana*	40.7	4.30	183.00	2	4.4
18	B; WF	PMF	Y1765_ARATH	Probable LRR receptor-likeserine/threonine-protein kinase At1g07650	*Arabidopsis thaliana*	112.8	9.50	118.90	3	4.0
20	B; OF	PMF	KN12D_ARATH	Kinesin-like protein KIN-12D	*Arabidopsis thaliana*	314.9	5.10	118.00	3	1.3
21	B; OF	LC-MS/MS	6PGD1_SPIOL	6-phosphogluconate dehydrogen-	*Spinacia oleracea*	53.2	6.00	786.00	13	20.1

				ase, decarboxylating 1						
22	B	PMF	RL51_ARATH	60S ribosomal protein L5-1	*Arabidopsis thaliana*	34.3	9.70	130.40	3	11.6
23	B	PMF	QWRF4_ARATH	QWRF motif-containing protein 4	*Arabidopsis thaliana*	66.9	10.20	123.00	3	4.2
24	B	PMF	GTL2_ARATH	Trihelix transcription factor GTL2	*Arabidopsis thaliana*	71.2	6.70	113.50	2	2.7
25	B	PMF	KN12F_ORYSJ	Kinesin-like protein KIN-12F	*Oryza sativa subsp. japonica*	317.1	5.00	137.70	5	2.0
26	B	PMF	MYB98_ARATH	Transcription factor MYB98	*Arabidopsis thaliana*	50.1	6.10	129.70	4	6.8
27	B	LC-MS/MS	TPIC_ARATH	Triosephosphate isomerase, chloroplastic	*Arabidopsis thaliana*	33.3	8.90	355.70	5	16.5
28	B	LC-MS/MS	IPYR2_ARATH	Soluble inorganic pyrophosphatase 2	*Arabidopsis thaliana*	24.7	5.70	125.80	1	5.5
33	B; WF	LC-MS/MS	ADF2_ORYSJ	Actin-depolymerizing factor 2	*Oryza sativa subsp. japonica*	16.8	5.60	102.50	1	8.3
48	OF	LC-MS/MS	AB2C_ARATH	ABC transporter C family member 2	*Arabidopsis thaliana*	182	6.00	103.88	0	0.0
49	OF	PMF	HXK4_ORYSJ	Hexokinase-4, chloroplastic	*Oryza sativa subsp. japonica*	54.7	6.50	122.80	3	6.5
51	OF	PMF	SWTIE_ARATH	Protein SWEETIE	*Arabidopsis thaliana*	244.2	5.10	123.00	3	1.8
52	OF	PMF	CALS2_ARATH	Callosesynthase 2	*Arabidopsis thaliana*	225.9	9.20	132.50	4	1.9
53	OF	LC-MS/MS	PS4_PINST	Putative LRR diseaseresistance protein/transmembrane receptor kinase PS4 (fragment)	*Pinus strobus*	0.90	11.10	90.53	1	100.0
54	OF	LC-MS/MS	ZDHC8_ARATH	Probable protein S-acyltransferase 20	*Arabidopsis thaliana*	76.8	9.60	123.05	2	2.4
57	OF; WF	PMF	MDHC2_ARATH	Malate dehydrogenase 2, cytoplasmic	*Arabidopsis thaliana*	35.7	6.30	466.10	8	26.8
58	OF	PMF	UGDH2_ARATH	UDP-glucose 6-dehydrogenase 2	*Arabidopsis thaliana*	53.1	5.60	162.80	3	7.7
60	OF; WF	LC-MS/MS	HSP70_MAIZE	Heatshock 70 kDa protein	*Zea mays*	70.5	5.10	470.40	8	14.7
61	OF	PMF	AUG8_ARATH	AUGMIN subunit 8	*Arabidopsis thaliana*	69.8	10.70	117.50	4	5.7
77	WF	PMF	RLT2_ARATH	Homeobox-DDT domain protein RLT2	*Arabidopsis thaliana*	190.3	5.30	116.30	4	2.2
78	WF	PMF	RCA_MALDO	Ribulose bisphosphate carboxylase/oxygenase activase, chloroplastic	*Malus domestica*	48	8.20	277.70	5	13.5
79	WF	PMF	HSP7N_ARATH	Heatshock 70 kDa protein 18	*Arabidopsis thaliana*	68.3	5.20	539.30	7	16.4
80	WF	PMF	CLPC_PEA	Chaperone protein ClpC, chloro-	*Pisum sativum*	102.6	6.50	853.70	16	16.3

				plastic						
81	WF	PMF	VATA_BRANA	V-type proton ATPase catalytic subunit A	*Brassica napus*	68.7	5.10	356.60	6	9.0
82	WF	PMF	DEK1_ARATH	Calpain-typecysteineprotease DEK1	*Arabidopsis thaliana*	238.1	6.10	131.20	4	2.4
83	WF	PMF	GLR34_ARATH	Glutamate receptor 3.4	*Arabidopsis thaliana*	107.1	9.10	120.00	4	5.0
84	WF	PMF	C76AD_BETVU	Cytochrome P450 76AD1	*Beta vulgaris*	56.2	8.10	133.30	3	4.6
85	WF	PMF	ILV5_ARATH	Ketol-acid reductoisomerase, chloroplastic	*Arabidopsis thaliana*	63.8	6.40	181.00	2	4.9
86	WF	PMF	Y5537_ARATH	G-typelectin S-receptor-likeserine/threonine-protein kinase At5g35370	*Arabidopsis thaliana*	96	6.60	127.20	3	4.5
87	WF	LC-MS/MS	PGKY_TOBAC	Phosphoglycerate kinase, cytosolic	*Nicotiana tabacum*	42.3	5.60	557.90	10	28.9
88	WF	PMF	ALFP2_ARATH	Fructose-bisphosphatealdolase 2, chloroplastic	*Arabidopsis thaliana*	43	6.80	451.50	8	15.8
89	WF	PMF	SMC3_ARATH	Structural maintenance of chromosomes protein 3	*Arabidopsis thaliana*	139.3	6.10	124.50	4	2.9
91	WF	LC-MS/MS	1433_HELAN	14-3-3-like protein	*Helianthus annuus*	28.9	4.50	356.30	6	19.7
92	WF	LC-MS/MS	HSP7C_PETHY	Heatshock cognate 70 kDa protein	*Petunia hybrida*	71.2	5.00	1145.10	20	30.7
93	WF	LC-MS/MS	SPD1_DATST	Spermidine synthase 1	*Datura stramonium*	34	5.10	246.70	3	8.8
95	WF	LC-MS/MS	GDI_ARATH	Guanosine nucleotide diphosphate dissociation inhibitor	*Arabidopsis thaliana*	49.5	5.00	266.90	5	10.1
96	WF	LC-MS/MS	PMG2_ARATH	Probable 2,3-bisphosphoglycerate-independent phosphoglyceratemutase 2	*Arabidopsis thaliana*	60.7	5.50	267.00	3	6.8
97	WF	LC-MS/MS	PMGI_MESCR	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	*Mesembryanthemum crystallinum*	61.1	5.30	340.60	6	12.7
98	WF	LC-MS/MS	RH15_ARATH	DEAD-box ATP-dependent RNA helicase 15	*Arabidopsis thaliana*	48.3	5.30	369.40	6	12.6
99	WF	LC-MS/MS	ALF_ORYSJ	Fructose-bisphosphate aldolase cytoplasmic isozyme	*Oryza sativa subsp. japonica*	38.8	7.70	254.80	4	7.8
100	WF	LC-MS/MS	GLYG1_SOYBN	Glycinin G1	*Glycine max*	55.7	5.80	394.60	8	21.0
101	WF	LC-MS/MS	UPTG_MAIZE	Alpha-1,4-glucan-protein synthase	*Zea mays*	41.2	5.70	423.00	9	27.7

				[UDP-forming]						
102	WF	LC-MS/MS	PSA1_ORYSJ	Proteasome subunit alpha type-1	*Oryza sativa subsp. japonica*	29.6	5.60	151.70	2	10.0
153	L	LC-MS/MS	CAHC_TOBAC	Carbonic anhydrase, chloroplastic	*Nicotiana tabacum*	34.5	6.46	139.33	1	3.1
154	L	LC-MS/MS	CAHC_TOBAC	Carbonic anhydrase, chloroplastic	*Nicotiana tabacum*	34.5	6.46	110.55	1	5.6
158	L	PMF	METK_CAMSI	S-adenosylmethionine synthase	*Camellia sinensis*	42.8	5.20	150.70	3	6.9
160	L	PMF	PP207_ARATH	Pentatricopeptide repeat-containing protein At3g02330, mitochondrial	*Arabidopsis thaliana*	101.6	6.00	128.60	3	4.9
161	L	PMF	CHR4_ARATH	Protein CHROMATIN REMODELING 4	*Arabidopsis thaliana*	247.8	5.90	128.70	4	3.2
162	L	LC-MS/MS	CAHC_PEA	Carbonic anhydrase, chloroplastic	*Pisum sativum*	35.4	7.74	263.63	1	5.5
163	L	LC-MS/MS	CDSP_ARATH	Thioredoxin-like protein CDSP32, chloroplastic	*Arabidopsis thaliana*	33.7	9.40	214.50	3	7.9
164	L	LC-MS/MS	CYSKP_SPIOL	Cysteine synthase, chloroplastic/chromoplastic	*Spinacia oleracea*	40.6	7.60	211.70	4	12.5
165	L	PMF	CRK20_ARATH	Putativecysteine-rich receptor-like protein kinase 20	*Arabidopsis thaliana*	74	6.60	118.50	3	7.1
166	L	PMF	RUB2_BRANA	RuBisCO large subunit-binding protein subunit alpha, chloroplastic	*Brassica napus*	61.6	5.00	569.20	9	15.8
167	L	PMF	TKTC_SPIOL	Transketolase, chloroplastic	*Spinacia oleracea*	80.2	6.20	262.90	5	6.5
168	L	PMF	HUAL1_ARATH	Protein HUA2-LIKE 1	*Arabidopsis thaliana*	156.5	8.90	113.20	3	2.4
169	L	PMF	GRDP1_ARATH	Glycine-richdomain-containing protein 1	*Arabidopsis thaliana*	89.4	6.60	129.60	5	6.7
170	L	PMF	FENR1_ORYSI	Ferredoxin--NADP reductase, leaf isozyme 1, chloroplastic	*Oryza sativa subsp. indica*	40	8.70	192.90	3	7.7
171	L	PMF	KN12F_ORYSJ	Kinesin-like protein KIN-12F	*Oryza sativa subsp. japonica*	317.1	5.00	123.70	4	1.9
172	L	PMF	GLTB2_ARATH	Ferredoxin-dependent glutamatesynthase 2, chloroplastic	*Arabidopsis thaliana*	177.6	6.60	128.00	4	2.8

[a] Spot number in 2-D gels; [b] buckwheat organs containing identified proteins; B—buds, OF—open flowers, WF—wilted flowers, L—leaves; [c] technique used to identify protein; [d] Uniprot reference number of protein; [e] homologous protein name from the UniProt/NCBI database; [f] organism from which protein is derived; [g] Mt—theoretical mass weight obtained from protein database; [h] pI_t—theoretical isoelectric point obtained from protein database; [i] statistical probability of true positive identification of predicted protein; [j] amino acid sequence coverage of identified protein; [k] percentage of sequence covered by matched peptides.

Table 2. Corresponding plant homologs of non-plant proteins identified in nanoLC-MS/MS analysis. Homologous proteins were found using the protein–protein program (BLASTP) at NCBInr.

Spot No. [a]	Organ [b]	UniProt No.	Protein Name [c]	Reference Organism [d]	M_t [e] [kDa]	pI_t [f]	Protein Score [g]	Peptide Count [h]	Coverage [%] [i]	Homologous Protein Name [j]	NCBI No.	Reference Organism [f]	I [k]	P [l]
3	B	CAPZA_KLULA	F-actin-capping protein subunit alpha	*Kluyveromyces lactis*	29.9	4.54	99.31	1	4.2	F-actin-capping protein subunit alpha-like	XP_023907009.1	*Quercus suber*	33%	53%
11	B; WF	MTNC_GLUDA	Enolase-phosphatase E1	*Gluconacetobacter diazotrophicus*	24.9	4.97	97.57	1	3.0	Probable bifunctional methylthioribulose-1-phosphate dehydratase/enolase-phosphatase E1 1	XP_028794642.1	*Prosopis alba*	39%	53%
19	B	IF2_THEFY	Translation initiation factor IF-2	*Thermobifida fusca*	100.5	9.82	101.74	1	1.2	Translation initiation factor IF-2, chloroplastic	GEZ89434.1	*Tanacetum cinerariifolium*	52%	71%
50	OF	MURA_PSEU5	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	*Pseudomonas stutzer*	44.6	5.62	94.32	1	2.1	Glutamate synthase 1 [NADH], chloroplastic isoform X1	GEU28314.1	*Tanacetum cinerariifolium*	59%	74%
56	OF	LEXA_MYXXD	LexA repressor	*Myxococcus xanthus*	24.7	9.29	99.27	1	5.4	DNA-3-methyladenine glycosylase 1	GEX09398.1	*Tanacetum cinerariifolium*	37%	58%
59	OF	TRPC_ACIF2	Indole-3-glycerol phosphate synthase	*Acidithiobacillus ferrooxidans*	28.7	5.02	101.36	1	3.4	Indole-3-glycerol phosphate synthase, chloroplastic-like isoform X2	XP_026448585.1	*Physcomitrella patens*	48%	63%
159	L	SCP_CHIOP	Sarcoplasmic calcium-binding protein (fragment)	*Chionoecetes opilio*	0.8	11.1	114.44	1	100.0	F-box protein At3g58530 isoform X1	XP_021283280.1	*Herrania umbratica*	87%	100%

[a] Spot number in 2-D gels; [b] buckwheat organs containing identified proteins; B—buds, OF—open flowers, WF—wilted flowers, L—leaves; [c] protein name from the UniProt database; [d] organism from which protein is derived; [e] Mt—theoretical mass weight obtained from protein database; [f] pI_t—theoretical isoelectric point obtained from protein database; [g] statistical probability of true positive identification of the predicted protein; [h] amino acid sequence coverage of identified protein; [i] percentage of sequence covered by matched peptides; [j] homologous protein name from the NCBI database; [k] identity—extent to which two amino acid sequences match; [l] positives—similarities based on scoring matrix.

Using the information from the UniProt database, we assigned the biological function (Figure 2a) and subcellular location (Figure 2b) to the identified proteins. Some proteins were assigned to more than one subcellular location, which was reflected in the plots.

In flower buds, heat stress mainly caused an increase in the abundance of cytosol-localized proteins related to protein synthesis. In Panda, these were mainly ribosomal proteins, whereas in PA15, two out of four proteins were transcription factors. Other heat-affected proteins in PA15 were related to organization of the cytoskeleton. Three proteins were up-regulated by heat stress in both Panda and PA15: two proteins involved in protein synthesis (60S ribosomal protein L5-1 and trihelix transcription factor GTL2) and one involved in lipid metabolism (acyl-[acyl-carrier-protein] desaturase 6).

For open flowers, the majority of the proteins showing changes in abundance under heat stress were related to carbohydrate metabolism in Panda, and protein modification in PA15 (Figure 2a). In Panda, most of the heat-affected proteins were localized to the cytosol, but some were localized to the chloroplast, plasma membrane, and nucleus (Figure 2b). In PA15, more of the heat-affected proteins were localized to the plasma membrane than to the cytosol, cytoskeleton, chloroplasts, mitochondria, and nucleus. Only one protein in open flowers, 6-phosphogluconate dehydrogenase, was up-regulated by heat stress in both buckwheat accessions. This enzyme is involved in the pentose phosphate pathway. Among all of the heat-affected proteins, indole-3-glycerol phosphate synthase showed the largest increase in abundance under heat stress. In Panda, its abundance in open flowers of plants grown at 30 °C was 213-fold that in plants grown at 20 °C. This enzyme is involved in the biosynthesis of the precursor of indole ring-containing compounds. It was identified using the BLASTP program on the basis of its homology to indole-3-glycerol phosphate synthase in *Acidithiobacillus ferrooxidans*.

Only a few of the heat-affected proteins in wilted flowers were identified. The identified proteins were mainly involved in the stress response (Figure 2a). Two 70 kDa heat-shock proteins and the glutamate receptor 3.4 were up-regulated by heat stress in wilted flowers of both buckwheat accessions. The other common proteins were chaperone protein ClpC, V-type proton ATPase catalytic subunit A, calpain-type cysteine protease DEK1, phosphoglycerate kinase, and fructose-bisphosphate aldolase 2. In PA15, several proteins related to carbohydrate metabolism were up-regulated under heat stress. The identified heat-affected proteins in wilted flowers of Panda were mainly localized in chloroplasts, while those in PA15 were mainly localized in the cytosol (Figure 2b).

In the donor leaves, high temperature up-regulated proteins related to photosynthesis in Panda (Figure 2a). Most of the identified heat-affected proteins in the donor leaves of Panda and PA15 were localized in the chloroplast (Figure 2b). Many of the heat-affected proteins in the donor leaves of PA15 were not identified, but most of the identified proteins were involved in amino acid biosynthesis. The cytosol-localized enzyme S-adenosylmethionine synthase showed changes in abundance in donor leaves in both accessions under heat stress.

All results obtained in this experiment are summarized in detail in Table S1.

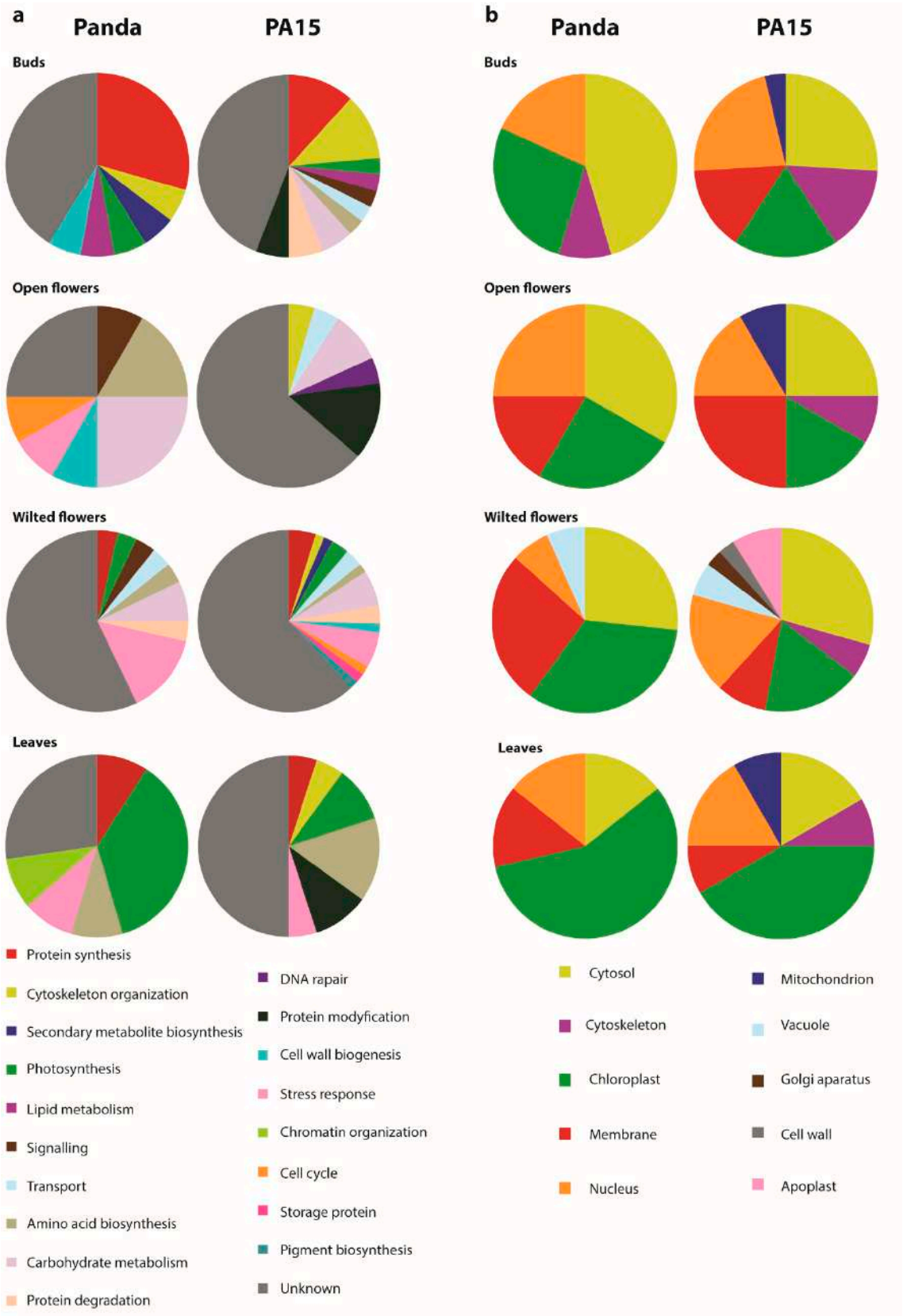

Figure 2. Composite graph showing heat-affected proteins in different organs of Panda and PA15 buckwheat accessions: (**a**) Biological functional categorization of heat-affected proteins; (**b**) distribution of identified proteins according to subcellular location; proteins with two and more localizations in the cell were assigned to all places. Biological function and subcellular localization were assigned based on information in the UniProt database.

3. Discussion

A central role in plant thermotolerance is played by reactive oxygen scavenging enzymes, heat-shock proteins (HSPs), and heat stress-responsive transcription factors (HSFs), which induce expression of HSPs, signal and regulatory proteins, proteins involved in metabolism, and redox homeostasis. The plant's response to heat stress involves the heat-shock transcription factor A1 (HsfA1) that is indispensable in the activation of transcriptional networks. It is responsible for regulating the level of transcription factors expression, including the dehydration-responsive element binding protein 2A (DREB2A). However, the activity of HsfA1 is regulated by interaction with HSPs [26,27].

In this study, we explored the effect of heat stress on protein expression in two accessions of buckwheat, Panda and PA15. The total protein content increased under heat stress in both buckwheat accessions, but none of the proteins were newly expressed in response to heat stress. Up-regulation of protein expression may indicate a positive effect of high temperature on vegetative and reproductive development. However, the two studied accessions responded differently to heat stress, as illustrated by their different proteomes. In all organs, heat stress up-regulated more proteins in PA15 than in Panda. In our previous studies, the different responses of these accessions' to heat stress were also reflected in the content of hormones [9] and HSP-70 and HSP-90 proteins [28] in their flowers and leaves.

Characteristic protein spots for the large Rubisco subunit were observed in the 2-D gels of proteins extracted from buds, open flowers, and wilted flowers. We also detected some chloroplast-localized proteins related to photosynthesis, because the floral tissues that the proteins were extracted from included the green pedicel. To identify proteins from gel spots we used nanoLC-MS/MS. It is the most adequate method used for this purpose, but may not be of use for low-abundant proteins [29]. In many cases, the analyzed sample had insufficient protein concentration; therefore, in the second attempt, the proteins were identified using the PMF technique. The PMF method requires less protein and is also much cheaper, but has many limitations that influenced the number of proteins identified in the experiment. The method fails to identify mixture proteins, low molecular mass proteins, and protein fragments. Additionally, PMF raises problems with the identification of large proteins. The success of the analysis is also determined by the presence of the protein sequences of interested search in the database [30]. In our experiment, among the proteins identified, none were specific to common buckwheat. All of them were homologous to proteins in different species. Similar results were presented by other authors when studying poorly known species [31]. In future studies on the buckwheat proteome in protein identification, a buckwheat genome database should be included [32]. This approach will allow to identify even proteins that we have not been able to identify so far. However, a potential change in the identification methods used would not change the overall picture obtained in our study and thus the conclusions drawn from our research on buckwheat response to heat stress. On the other hand, a change in methodology could be useful if the aim of our work was to identify candidate genes for high temperature stress tolerance.

Four proteins were up-regulated by heat stress in the flower buds of both accessions. These proteins included acyl-[acyl-carrier-protein] (ACP) desaturase 6, 60S ribosomal protein L5-1, and the trihelix transcription factor GTL2. ACP desaturase 6 is localized in the chloroplasts. It is responsible for unsaturated fatty acid biosynthesis, and its role is to introduce a double bond during esterification of the acyl group to the acyl carrier protein. Derivatives of unsaturated fatty acids (UFA) are known to function as signaling molecules in responses to various stresses. High temperature has been shown to increase the UFA content in olive plants [33]. Changes to the UFA content have been shown to affect the stress response, and result in changes in salicylic acid (SA)- and jasmonic acid (JA)-mediated defense responses, especially to biotic stresses [34]. In our previous study, we found that high temperature led to increased SA contents in buds of Panda and PA15, but decreased the contents of JA and its methyl ester (JA-Met) in both accessions [9]. The

other two identified proteins induced by heat stress in the two accessions were related to protein synthesis pathways. The 60S ribosomal protein L5-1 is a component of the ribosome, whereas the trihelix transcription factor GTL2 binds to a specific DNA sequence to regulate gene transcription. Magwanga et al. [35] also found that the trihelix transcription factor GTL2 was highly up-regulated in cotton under drought and salt stress conditions.

In open flowers, only 6-phosphogluconate dehydrogenase (6PGDH) was up-regulated by heat stress in both buckwheat accessions. This enzyme plays a key role in the oxidative pentose phosphate pathway (OPPP), which is critical for maintaining redox balance under stress situations. 6PGDH probably controls the efficiency of this pathway. In rice, expression of the gene encoding 6PGDH was found to be up-regulated by abscisic acid (ABA) treatment [36]. In our previous study, we found that the free ABA content in open flowers increased in Panda and decreased in PA15 under heat stress. However, the concentration of the conjugate ABA-glc increased in PA15 but remained stable in Panda under heat stress [9].

Heat stress resulted in a dramatic increase in the abundance of one protein spot in the open flowers of Panda (to 213 times that in control plants grown at 20 °C). We identified this protein as the indole-3-glycerol phosphate synthase (IGPS) chloroplastic-like isoform X2. This enzyme produces indole-3-glycerol phosphate (IGP) as the precursor of indole ring-containing compounds and participates in the biosynthesis of tryptophan, indole 3-acetic acid, phytoalexin alkaloids, and glucosinolates. Indole plays roles in abiotic and biotic stress responses, but also in flowers, where it is emitted as a scent to attract pollinators [37]. IGP may be a branchpoint compound in the tryptophan-independent and tryptophan-dependent auxin biosynthetic pathways [38]. Auxins are responsible for plant fertility and high temperatures reduce plant fertility through repression of expression of the YUCCA auxin biosynthesis genes [39]. YUCCA (YUC)-type flavin-containing monooxygenases catalyze a reaction whose product is indole 3-acetic acid [40]. Thus, in this context, it can be speculated that the increase in IGPS accumulation in the flowers of the heat-sensitive Panda may be an attempt to counteract the reduction in auxin content in heat-treated flowers observed in this accession in contrast to the tolerant PA15, where this decrease was lower [9]. It is possible that one of the possible heat-tolerance mechanism in PA15 is the lower heat-sensitivity of some elements of auxin biosynthesis pathway.

Few of the proteins showing heat-induced changes in abundance in wilted flowers were identified, especially those in PA15. Many of the protein spots on the 2-D gels of proteins from wilted flowers were probably protein fragments resulting from an increase in proteolytic enzyme activity and limited repair mechanisms. Wilted flowers were aborted due to a lack of pollination. Proteins can be fragmented by reactive oxygen species (ROS) and proteolytic enzymes. Heat stress accelerates the generation and reactions of ROS, and senescing tissues do not have an efficient antioxidant system [41]. In wilted flowers, nine protein spots accumulated under heat stress in both accessions. One of them could not be identified. Two spots were identified as HSP-70s. Members of the HSP-70 family function as chaperones to facilitate protein folding, degradation, complex assembly, and translocation. They play a key role in stabilizing proteins under optimal and stress conditions [42]. Previously, we detected the accumulation of HSP-70 and HSP-90 in buckwheat flowers at various stages of development [28]. Moreover, we identified the chaperone protein ClpC in the HSP-100 family that plays a vital role in chloroplast function [43]. The presence of HSP-70 and HSP-100 proteins suggests that certain defense mechanisms function in wilted flowers, but they may be involved in the proper degeneration of the organ. Another protein expressed under heat stress in both Panda and PA15 was the V-type proton ATPase (V-ATPase) catalytic subunit A, which is a component of the membrane-bound V-ATPase located at the tonoplast and other sites in the endomembrane system of plant cells. The abundance of V-ATPase subunits is known to be modulated by environmental stresses [44]. Another heat-affected protein in wilted flowers was the calpain-type cysteine protease DEK1, the only calpain protein in plants.

This protein is essential for embryo development [45]. The glutamate receptor 3.4 was also commonly up-regulated by heat stress in both accessions. This protein is a component of the glutamate receptor-like channel (GLR). GLRs play a role in calcium signaling during the response to environmental stresses [46]. Two proteins up-regulated by heat stress in wilted flowers were involved in carbohydrate metabolism: cytosolic phosphoglycerate kinase (PGK) and chloroplastic fructose-bisphosphate aldolase (FBA). Cytosolic PGK is involved in glycolysis and gluconeogenesis. Plants also have plastidial isoforms of PGK that may simultaneously participate in the Calvin–Benson cycle and glycolytic/gluconeogenic reactions [47]. Chloroplastic FBA is a bifunctional enzyme involved in the formation of fructose-1,6-bisphosphate (FBP) and sedoheptulose-1,7-bisphosphate (SuBP) in the Calvin cycle. It also functions as a sedoheptulose/fructose-bisphosphate aldolase (SFBA). FBA aldolase activity, but not SuBP activity, is important for glycolytic and gluconeogenetic reactions in the cytoplasm [48].

In donor leaves, only *S*-adenosylmethionine synthetase (SAMS) was up-regulated by heat stress in both buckwheat accessions. This enzyme synthesizes *S*-adenosylmethionine (SAM) from ATP and L-methionine. It is involved in the biosynthesis of ethylene, nicotiamine, and polyamines. It represents the major hub of the methionine metabolism and participates in plant responses to environmental stresses [49].

4. Materials and Methods

4.1. Plant Material and Growth Conditions

Seeds of common buckwheat (*Fagopyrum esculentum* Moench), the Polish cultivar Panda and the PA15 strain, were supplied by Małopolska Plant Breeding (Polanowice, Poland), and were produced at the plant production facility in Palikije. Panda is more sensitive to heat stress than PA15 is, as manifested by the degeneration of a large number of embryo sacs [9]. The plant growth conditions in the phytotron have been described in our previous papers [8,9]. Plants were cultivated in plastic pots of 10 dm^3 capacity (six plants per pot) filled with a mixture of commercial soil substrate (pH = 5.8) and perlite (1:1, *v:v*) under a 16 h photoperiod and 300 μmol m^{-2} s^{-1} (High-Pressure Sodium, HPS lamps, SON-T+ AGRO, Philips, Brussels, Belgium) at a humidity of 50–60%. For the first 3 weeks, all plants were grown at 20 °C, and then half of them were transferred to a chamber at 30 °C (heat stress) with the same humidity and light conditions. When the plants were 2 months old, we collected flowers at three developmental phases (buds, open flowers, and wilted flowers) and donor leaves (fully developed young leaves closest to the flower cluster) from plants in the control and heat stress treatments. The experiment was repeated twice. The samples were immediately frozen in liquid nitrogen and then stored at −80 °C until subsequent analysis. Protein extraction and electrophoretic separation were performed from three aggregate replicates for each development phase of flowers and donor leaves, for both accessions in both temperature treatments.

4.2. Protein Extraction

Total proteins were extracted using a phenol-based procedure [50] (modified by Hajduch et al. [51]). Buckwheat tissues were pulverized into a fine powder in liquid nitrogen with a mortar and pestle. The powder was suspended in 10 cm^3 of a phenol-based extraction buffer (50% [*v*/*v*] phenol, 0.45 M sucrose, 5 mM EDTA, 0.4% [*v*/*v*] 2-mercaptoethanol, 50 mM Tris-HCl pH 8.8). The homogenate was allowed to reach room temperature, transferred to a Falcon tube, and shaken for 30 min. The phenol and aqueous phases were separated by centrifugation (5000 *g*, 15 min, 4 °C). Proteins were precipitated with five volumes of ice-cold 0.1 M ammonium acetate in 100% methanol at −20 °C for 16 h. After centrifugation (5000× *g*, 10 min, 4 °C), the protein pellet was washed twice with the precipitation solution, then with 80% acetone, and then with 70% ethanol. The total protein extracts were dissolved in 200 μL isolectricfocusing (IEF) sample solution (8 M urea, 2 M thiourea, 4% (*w*/*v*) 3-[(3-Cholamidopropyl)dimethylammonio]-1-

propanesulfonate (CHAPS), 50 mM dithiothreitol (DTT)) by shaking for 1 h. The protein concentration was determined using a 2D Quant Kit (GE Healthcare, Piscataway, NJ, USA). Protein extracts in the IEF sample solution were stored at −80 °C until analysis.

4.3. Two-Dimensional Gel Electrophoresis

Two-dimensional gel electrophoresis (2-DE) was based on the procedure recommended by GE Healthcare. The desired amount of protein (700 mg) was mixed with 4.6 µL IPG buffer (pH range 4–7) (GE Healthcare), adjusted to 450 µL with IEF sample solution, and loaded onto a 24 cm immobilized pH gradient strip (Immobiline DryStrip gel; GE Healthcare) with a linear pH range of 4–7. The strips were passively rehydrated in a DryStrip IPGbox (GE Healthcare) for 16 h. The first dimension of isoelectrofocusing (IEF) was carried out using an Amersham Ettan IPGphor II unit (GE Healthcare). The six-step focusing protocol with a current limit of 75 µA per strip was as follows: (a) 45 Vh at 150 V, (b) 375 Vh at 150 V, (c) 500 Vh at 500 V; (d) 800 Vh at 1000 V; (e) 16,500 Vh at 10,000 V; (f) 27,200 Vh at 10,000 V. After IEF, the strips were incubated in an equilibration buffer (1.5 M Tris-HCl pH 8.8, 6 M urea, 30% (*v*/*v*) glycerol, 5% (*w*/*v*) sodium dodecyl sulfate (SDS)) for 15 min with 2% (*w*/*v*) dithiothreitol (DTT) and then for another 15 min with 2.5% (*w*/*v*) iodoacetamide. After equilibration, each strip was placed onto a 12% SDS-polyacrylamide gel and then overlaid with 0.5% (*w*/*v*) agarose in an SDS running buffer with Coomassie Brilliant Blue G-250 as the tracking dye. Separation on the second dimension was performed using an SE900 Large Format Vertical Gel Protein Electrophoresis Unit (Hoefer Scientific Instruments, San Francisco, CA, USA) at 48 W per gel until the dye migrated off the gel. After electrophoresis, each gel was washed three times for 15 min each time in deionized water and then stained overnight in colloidal Coomassie staining solution (20% (*v*/*v*) ethanol, 1.6% (*v*/*v*) phosphoric acid, 8% (*w*/*v*) ammonium sulfate, 0.08% (*w*/*v*) Coomassie Brilliant Blue G-250) using the modified method of Neuhoff et al. [52].

4.4. Gel Image Analysis

Stained 2-DE gels were digitalized using an Epson Perfection V850 Pro scanner at a resolution of 300 dpi and 16-bit gray scale pixel depth. Gel images were analyzed using Delta2D software (Decodon, Greifswald, Germany). The volume of the spots was normalized against the total volume of all spots in the analysis. The *t* test value for each spot was calculated using Delta2D software. Only spots with a *p*-value lower than or equal to 0.05 were considered to be differentially expressed. Spots with at least 2.0-fold differences in protein abundance between two treatments were chosen for further analysis.

4.5. Protein Identification

The protein spots were manually selected and excised from the gels for identification. First, proteins were analyzed by liquid chromatography-mass spectrometry (nanoLC-MS/MS). If proteins could not be identified by nanoLC-MS/MS, peptide mass fingerprinting (PMF) analysis was performed. Mass spectrometry analysis was performed at the Laboratory of Proteomics and Mass Spectrometry, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków (Poland).

The excised protein spots were prepared for mass spectrometry analysis according to the protocol described by Hartman et al. [53]. The gel pieces in tubes were incubated at 40 °C in 100 mM ammonium bicarbonate for 10 min. Then, acetonitrile was added to a final concentration of 50% (*v*/*v*) and the mixture was incubated at 40 °C for 10 min. After incubation, the solution was removed. The washing step was repeated three times to remove all Coomassie dye, until the gels were completely colorless. Finally, the gels were dehydrated in anhydrous acetonitrile. The acetonitrile was removed, and then the dry gel pieces were reswollen in 25 µL 50 mM DTT dissolved in 100 mM ammonium bicarbonate, followed by incubation at 60 °C for 45 min. Then, the DTT solution was replaced

with 25 µL 100 mM iodoacetamide in 100 mM ammonium bicarbonate. The gel pieces were incubated at room temperature in darkness for 20 min, then 25 µL 10 ng µL^{-1} trypsin Gold (Promega, Madison, WI, USA) solution in 100 mM ammonium bicarbonate was added and the gel pieces were incubated at 4 °C for 1 h. Then, 25 µL 50 mM ammonium bicarbonate solution was added and the gel pieces were incubated overnight at 37 °C. The next day, the supernatant containing the digested peptides was collected in a new tube and combined with subsequent fractions. The gel pieces were immersed in 50 mM ammonium bicarbonate and incubated at 40 °C for 10 min before adding acetonitrile to a final concentration of 50% (*v*/*v*). The resulting supernatants were collected. Further extraction of peptides was performed in acidic conditions by incubation with 5% (*v*/*v*) formic acid in 50% (*v*/*v*) acetonitrile, twice. The gel pieces were dehydrated in anhydrous acetonitrile. Combined solutions from one sample were dried and dissolved in 20 µL 0.1% formic acid and then analyzed by nanoLC-MS/MS and PMF.

The nanoLC-MS/MS analyses were performed using an Easy-nLC II nano capillary chromatography system (Bruker Daltonics, Bremen, Germany) as described in Drabik et al. [54]. Peptides were separated on a 3 µm Biosphere C18 column (10 cm long, 75 µm internal diameter; Nanoseparations, Nieuwkoop, The Netherlands). The gradient was formed using two mobile phases: Phase A: 0.1% formic acid in water; phase B: 0.1% formic acid in acetonitrile. The total flow rate was 300 nl min^{-1}. The system was controlled using Hystar software (Bruker Daltonics). The gradient program was as follows: from 2% to 45% Phase B in 30 min, then 90% Phase B for 10 min, decreasing to 2% Phase B until 60 min for column equilibration. Fractions eluted from the column were directly deposited with a w matrix on the MALDI target plate by a Proteineerfc II sample collector (Bruker Daltonics). Fractions were collected at 15 s intervals. Samples made up of 96 fractions were spotted on a 384 MALDI target plate. α-Cyano-4-hydroxycinnamic acid was used as the MALDI matrix. The mass spectrometry analyses were performed on an Ultraflextreme instrument (Bruker Daltonics) in the positive ion mode.

Samples for PMF analysis were bound to C18 resin in ZipTip columns (Supel-Tips C18 PipetteTips, Supelco/Sigma-Aldrich, Bellefonte PA, USA) according to the manufacturer's instructions. The peptides were eluted from the column with saturated α-cyano-4-hydroxycinnamic acid solution in 60% (*v*/*v*) acetonitrile with 0.1% (*v*/*v*) trifluoroacetic acid directly on the MALDI target plate.

The acquired mass spectra and fragment mass spectra (for both LC-MS and PMF) were analyzed using FlexAnalysis software (Version: 3.4, Bruker Daltonics) and ProteinScape (Version: 3.0.0 446, Bruker Daltonics) and were processed using the Mascot algorithm (Engine version: 2.3, Matrix Science) against the SwissProt_2015_04 database. The searches were performed with the following parameters: Cerbamidomethylation of cysteine as a fixed modification; oxidation of methionine as an allowable variable modification; up to 1 missed cleavage allowed; 25 ppm for precursor mass tolerance; 0.6 Da for MS/MS mass tolerance; peptide charge: 1+ for PMF (MALDI-TOF instrument-UltrafleXtreme from Bruker Daltonics) and 0.3 Da for precursor mass tolerance; 0.6 Da for MS/MS mass tolerance; peptide charge: 2+, 3+, 4+ for LC-MS (ESI-IT instrument on an Amazon SL from Bruker Daltonics) analyses. The database search was run against the protein database Viridiplantae (563,552 sequences; 203,007,781 residues; May 2020). If no protein was identified, the database was searched for all taxa. Proteins with a mascot score higher than 30 and with a level of false positives of $p \leq 0.05$ were considered as identified. In cases where the identified protein belonged to an organism other than plants, a search was performed based on the amino acid sequence of the homologous protein among green plants using BLASTP (https://blast.ncbi.nlm.nih.gov, May 2020).

5. Conclusions

Two common buckwheat accessions, Panda and PA15, differ in their tolerance to high temperature, as illustrated by the frequency of embryo sac degradation under heat stress. The different responses of the two accessions to heat stress were reflected in their

protein profiles. All heat-affected proteins showed up-regulated expression in the organs of the two accessions. Many proteins could not be identified. It is possible that some protein spots were protein fragments resulting from proteolysis and inadequate repair mechanisms. There were more heat-affected proteins in PA15, the heat-tolerant accession, than in Panda, the heat-sensitive accession. Surprisingly, only a few proteins were commonly up-regulated by heat in both accessions. Plants' tolerance to heat stress, as to other environmental stresses, is the sum of minor and major changes in the proteome, and cannot be explained by single changes. Proteins common to both accessions characterize the heat-affected protein profile of common buckwheat. The abundance of indole-3-IGPS chloroplastic-like isoform X2 increased markedly in open flowers of Panda under heat stress. This may be a candidate protein to serve as a marker of sensitivity of buckwheat plants to heat stress.

Supplementary Materials: The following are available online at www.mdpi.com/1422-0067/22/5/2678/s1, Table S1: List of heat-affected proteins in buds, open flowers, wilted flowers, and donor leaves of common buckwheat accessions Panda and PA15; comparison of protein abundance between heat-treated plants and those growing in optimal conditions.

Author Contributions: Conceptualization, A.P., P.K., M.H.; methodology, P.K.; performing the experiment, P.K., M.H., J.P., A.S., J.W.; data analysis, P.K.; writing—original draft preparation, P.K., A.P., M.H., M.R.; writing—review and editing, M.R.; project leader, A.P. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by funding from the National Centre of Knowledge of Poland (No. 2017/25/B/NZ9/00148).

Institutional Review Board Statement: Not applicable

Informed Consent Statement: Not applicable

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: We thank the staff at the Laboratory of Proteomics and Mass Spectrometry, Maj Institute of Pharmacology, Polish Academy of Sciences, Krakow, for assistance with protein identification. We thank Przemysław Mielczarek from Maj Institute of Pharmacology PAS for technical support and Jennifer Smith from Edanz Group for editing the English text of draft of this manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

2-DE	Two-dimensional gel electrophoresis
6PGDH	6-phosphogluconate dehydrogenase
ABA	Abscisic acid
ACP	Acyl-[acyl-carrier-protein]
CHAPS	3-[(3-cholamidopropyl)dimethyl-ammonio]-1-propane sulfonate
DREB2A	Dehydration-responsive element binding protein 2A
DTT	Dithiothreitol
FBA	Fructose-bisphosphate aldolase
FBP	Fructose-1,6-bisphosphate
GLR	Glutamate receptor-like channel
HSF	Heat-shock transcription factor
HsfA1	Heat-shock transcription factor A1
HSP	Heat-shock proteins
IEF	Isoelectricfocusing
IGP	Indole-3-glycerol phosphate
IGPS	Indole-3-glycerol phosphate synthase

JA	Jasmonic acid
JA-Met	Methyl ester of jasmonic acid
OPPP	Oxidative pentose phosphate pathway
PGK	Phosphoglycerate kinase
PMF	Peptide mass fingerprinting
ROS	Reactive oxygen species
SA	Salicylic acid
SAM	*S*-adenosylmethionine
SAMS	*S*-adenosylmethionine synthetase
SDS	Sodium dodecyl sulfate
SFBA	Sedoheptulose/fructose-bisphosphate aldolase
SuBP	Sedoheptulose-1,7-bisphosphate
TF	Transcription factor
UFA	Unsaturated fatty acids
V-ATPase	V-type proton ATPase

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11. Oświadczenia współautorów publikacji wchodzących w skład rozprawy doktorskiej

Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
Wydział Rolniczo-Ekonomiczny

Prof. dr hab. inż. Agnieszka Płażek

Katedra Fizjologii, Hodowli Roślin i Nasiennictwa

Wydział Rolniczo-Ekonomiczny

Uniwersytet Rolniczy w Krakowie

Kraków, 18.01.2022r

OŚWIADCZENIE

Oświadczam, że w pracy:

Płażek A., Słomka A., Kopeć P., Dziurka M.*, **Hornyák M.**, Sychta K., Pastuszak J. (2019). Effects of high temperature on embryological development and hormone profile in flowers and leaves of common buckwheat (*Fagopyrum esculentum* Moench). *International Journal of Molecular Sciences*, 20(7), 1705.
DOI: 10.3390/ijms20071705

brałam udział w:

- opracowaniu metodologii doświadczenia,
- przeprowadzeniu doświadczenia w komorze fitotronowej,
- analizie uzyskanych wyników profilu hormonalnego,
- pisaniu wstępnej i ostatecznej wersji manuskryptu.

A. Płażek

.....

Prof. dr hab. inż. Agnieszka Płażek

Uniwersytet Jagielloński w Krakowie
Wydział Biologii

dr hab. Aneta Słomka, prof. UJ
Zakład Cytologii i Embriologii Roślin
Instytut Botaniki

Kraków, 18.01.2022 r.

OŚWIADCZENIE

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brałam udział w:

- opracowaniu metodologii doświadczenia,
- wykonaniu analiz embriologicznych i interpretacji uzyskanych wyników,
- pisaniu wstępnej i ostatecznej wersji manuskryptu.

.....

dr hab. Aneta Słomka, prof. UJ

dr Przemysław Kopeć

Zakład Biologii Komórki

Instytut Fizjologii Roślin

Polska Akademia Nauk

Kraków, 18.01.2022r

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DOI: 10.3390/ijms20071705

- brałem udział w przeprowadzeniu eksperymentu w komorze fitotronowej,
- brałem udział w analizowaniu wyników profilu hormonalnego.

Przemysław Kopeć

dr Przemysław Kopeć

dr Michał Dziurka
Zakład Biologii Rozwoju
Instytut Fizjologii Roślin
im. F. Górskiego PAN

Kraków, 25.01.2022r

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Oświadczam, że w pracy:

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DOI: 10.3390/ijms20071705

- opracowałem metodykę i przeprowadziłem analizy hormonów roślinnych,
- brałem udział w interpretacji wyników.

……..

dr Michał Dziurka

Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
Wydział Rolniczo-Ekonomiczny

mgr Marta Hornyák

Katedra Fizjologii, Hodowli Roślin i Nasiennictwa

Wydział Rolniczo-Ekonomiczny

Uniwersytet Rolniczy w Krakowie

Kraków, 25.01.2022r

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DOI: 10.3390/ijms20071705

- przeprowadziłam doświadczenia w komorze fitotronowej,
- pobierałam materiał roślinny do analiz biochemicznych i embriologicznych,
- brałam udział w wykonywaniu analiz hormonów roślinnych,
- brałam udział w wykonywaniu analiz embriologicznych woreczków zalążkowych,
- wykonałam analizy żywotności ziaren pyłku,
- brałam udział w interpretacji wyników i wykonywaniu wizualizacji danych.

Marta Hornyák

mgr Marta Hornyák

dr Klaudia Sychta
Zakład Cytologii i Embriologii Roślin
Instytut Botaniki
Uniwersytet Jagielloński

Kraków, 18.01.2022 r.

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DOI: 10.3390/ijms20071705

- brałam udział wykonaniu analiz embriologicznych.

Sychta Klaudia
.....

dr Klaudia Sychta

Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
Wydział Rolniczo-Ekonomiczny

mgr inż. Jakub Pastuszak

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DOI: 10.3390/ijms20071705

- brałem udział w przeprowadzeniu eksperymentu w komorze fitotronowej,
- brałem udział w pobieraniu materiału roślinnego do analiz biochemicznych,
- wykonałem część analiz żywotności ziaren pyłku.

Jakub Pastuszak

mgr inż. Jakub Pastuszak

Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
Wydział Rolniczo-Ekonomiczny

mgr Marta Hornyák
Katedra Fizjologii, Hodowli Roślin i Nasiennictwa
Wydział Rolniczo-Ekonomiczny
Uniwersytet Rolniczy w Krakowie

Kraków, 25.01.2022r

OŚWIADCZENIE

Oświadczam, że w pracy:

Hornyák M.*, Płażek A., Kopeć P., Dziurka M., Pastuszak J., Szczerba A., Hura T. (2020). Photosynthetic activity of common buckwheat (*Fagopyrum esculentum* Moench) exposed to thermal stress. *Photosynthetica*, 58(1), 45–53.
DOI: 10.32615/ps.2019.140

- brałam udział w opracowaniu metodologii doświadczenia,
- przeprowadziłam doświadczenia w komorze fitotronowej,
- pobierałam materiał roślinny do analiz biochemicznych,
- wykonywałam analizy cukrów rozpuszczalnych i brałam udział w wykonywaniu analiz hormonów roślinnych,
- wykonywałam pomiary fluorescencji chlorofilu *a*, wymiany gazowej oraz wypływu elektrolitów,
- brałam udział w analizie uzyskanych wyników,
- pisałam wstępną i ostateczną wersję manuskryptu.

.....Marta Hornyák.....

mgr Marta Hornyák

Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
Wydział Rolniczo-Ekonomiczny

Prof. dr hab. inż. Agnieszka Płażek
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Wydział Rolniczo-Ekonomiczny
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brałam udział w:

- opracowaniu metodologii doświadczenia,
- przeprowadzeniu doświadczenia w komorze fitotronowej,
- analizie uzyskanych wyników,
- pisaniu wstępnej i ostatecznej wersji manuskryptu.

.....

Prof. dr hab. inż. Agnieszka Płażek

dr Przemysław Kopeć
Zakład Biologii Komórki
Instytut Fizjologii Roślin
Polska Akademia Nauk

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DOI: 10.32615/ps.2019.140

- brałem udział w przeprowadzeniu eksperymentu w komorze fitotronowej,
- analizowałem wyniki zawartości węglowodanów rozpuszczalnych.

Przemysław Kopeć

dr Przemysław Kopeć

dr Michał Dziurka
Zakład Biologii Rozwoju
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- opracowałem metodykę i przeprowadziłem analizy hormonów roślinnych,
- brałem udział w interpretacji wyników.

……..

dr Michał Dziurka

Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
Wydział Rolniczo-Ekonomiczny

mgr inż. Jakub Pastuszak
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- brałem udział w przygotowaniu eksperymentu w komorze fitotronowej,
- brałem udział w pobieraniu materiału roślinnego do analiz biochemicznych,
- wykonałem część pomiarów fluorescencji chlorofilu *a*.

.....
mgr inż. Jakub Pastuszak

Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
Wydział Rolniczo-Ekonomiczny

mgr inż. Anna Szczerba

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- brałam udział w przeprowadzeniu eksperymentu w komorze fitotronowej,
- brałam udział w pobieraniu materiału roślinnego do analiz biochemicznych.

Szczerba Anna

mgr inż. Anna Szczerba

dr hab. Tomasz Hura

Zakład Ekofizjologii

Instytut Fizjologii Roślin

Polska Akademia Nauk

Kraków, 18.01.2022r

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- analizowałem parametry fluorescencji chlorofilu *a*,
- brałem udział w przygotowaniu wstępnej i ostatecznej wersji manuskryptu.

.....

dr hab. Tomasz Hura

Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
Wydział Rolniczo-Ekonomiczny

Prof. dr hab. inż. Agnieszka Płażek
Katedra Fizjologii, Hodowli Roślin i Nasiennictwa
Wydział Rolniczo-Ekonomiczny
Uniwersytet Rolniczy w Krakowie

Kraków, 18.01.2022r

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Oświadczam, że w pracy:

Płażek A., Hura K., Hura T.*, Słomka A., **Hornyák M.**, Sychta K. (2020). Synthesis of heat-shock proteins HSP-70 and HSP-90 in flowers of common buckwheat (*Fagopyrum esculentum*) under thermal stress. *Crop and Pasture Science*, 71(8), 760–767.
DOI: 10.1071/CP20011

brałam udział w:

- opracowaniu metodologii doświadczenia,
- przeprowadzeniu doświadczenia w komorze fitotronowej,
- pisaniu wstępnej i ostatecznej wersji manuskryptu.

.....

Prof. dr hab. inż. Agnieszka Płażek

Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
Wydział Rolniczo-Ekonomiczny

dr hab. Katarzyna Hura, prof. URK
Katedra Fizjologii, Hodowli Roślin i Nasiennictwa
Wydział Rolniczo-Ekonomiczny
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DOI: 10.1071/CP20011

- wykonałam analizy Western Blot,
- analizowałam zawartość białek szoku cieplnego,
- przygotowałam wizualizację uzyskanych wyników.

Katarzyna Hura

dr hab. Katarzyna Hura, prof. URK

dr hab. Tomasz Hura,
Zakład Ekofizjologii
Instytut Fizjologii Roślin
Polska Akademia Nauk

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- analizowałem parametry fluorescencji chlorofilu *a*,
- brałem udział w przygotowaniu wstępnej i ostatecznej wersji manuskryptu.

.....

dr hab. Tomasz Hura

Uniwersytet Jagielloński w Krakowie
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dr hab. Aneta Słomka, prof. UJ
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brałam udział w:

- wykonaniu analiz embriologicznych i interpretacji uzyskanych wyników.

ASłomka

dr hab. Aneta Słomka, prof. UJ

Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
Wydział Rolniczo-Ekonomiczny

mgr Marta Hornyák

Katedra Fizjologii, Hodowli Roślin i Nasiennictwa

Wydział Rolniczo-Ekonomiczny

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DOI: 10.1071/CP20011

- przeprowadziłam doświadczenia w komorze fitotronowej,
- zebrałam materiał roślinny do analiz białek szoku cieplnego,
- wykonałam pomiary fluorescencji chlorofilu *a* oraz analizę uzyskanych danych,
- uczestniczyłam w wykonywaniu analiz embriologicznych.

Marta Hornyák

mgr Marta Hornyák

dr Klaudia Sychta
Zakład Cytologii i Embriologii Roślin
Instytut Botaniki
Uniwersytet Jagielloński

Kraków, 18.01.2022 r.

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DOI: 10.1071/CP20011

- brałam udział wykonaniu analiz embriologicznych.

Sychta Klaudia
.....

dr Klaudia Sychta

Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
Wydział Rolniczo-Ekonomiczny

mgr Marta Hornyák
Katedra Fizjologii, Hodowli Roślin i Nasiennictwa
Wydział Rolniczo-Ekonomiczny
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Kraków, 25.01.2022r

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Oświadczam, że w pracy:

Hornyák M., Słomka A.*, Sychta K., Dziurka M., Kopeć P., Pastuszak J., Szczerba A., Płażek A. (2020). Reducing flower competition for assimilates by half results in higher yield of *Fagopyrum esculentum*. *International Journal of Molecular Sciences*, 21(23), 8953. DOI: 10.3390/ijms21238953

- przeprowadzałam doświadczenia w warunkach *in planta*,
- przeprowadzałam doświadczenia w warunkach *in vitro*,
- pobierałam materiał roślinny do analiz biochemicznych i embriologicznych,
- brałam udział w wykonywaniu analiz embriologicznych,
- brałam udział w wykonywaniu pomiarów parametrów plonowania,
- brałam udział w analizie uzyskanych wyników,
- brałam udział w przygotowaniu wizualizacji uzyskanych wyników.

.....Marta Hornyák.....

mgr Marta Hornyák

Uniwersytet Jagielloński w Krakowie
Wydział Biologii

dr hab. Aneta Słomka, prof. UJ
Zakład Cytologii i Embriologii Roślin
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brałam udział w:

- opracowaniu metodologii doświadczenia,
- wykonaniu analiz embriologicznych i interpretacji uzyskanych wyników,
- pisaniu wstępnej i ostatecznej wersji manuskryptu.

.....

dr hab. Aneta Słomka, prof. UJ

dr Klaudia Sychta
Zakład Cytologii i Embriologii Roślin
Instytut Botaniki
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OŚWIADCZENIE

Oświadczam, że w pracy:

Hornyák M., Słomka A.*, Sychta K., Dziurka M., Kopeć P., Pastuszak J., Szczerba A., Płażek A. (2020). Reducing flower competition for assimilates by half results in higher yield of *Fagopyrum esculentum*. *International Journal of Molecular Sciences*, 21(23), 8953. DOI: 10.3390/ijms21238953

- brałam udział w przygotowaniu eksperymentu w warunkach *in vitro*,
- brałam udział wykonaniu analiz embriologicznych.

Sychta Klaudia
.....

dr Klaudia Sychta

dr Michał Dziurka
Zakład Biologii Rozwoju
Instytut Fizjologii Roślin
im. F. Górskiego PAN

Kraków, 25.01.2022r

OŚWIADCZENIE

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DOI: 10.3390/ijms21238953

- opracowałem metodykę i przeprowadziłem analizy hormonów roślinnych
- brałem udział w interpretacji wyników.

……..

dr Michał Dziurka

dr Przemysław Kopeć
Zakład Biologii Komórki
Instytut Fizjologii Roślin
Polska Akademia Nauk

Kraków, 18.01.2022r

OŚWIADCZENIE

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- brałem udział w przygotowaniu eksperymentu,
- brałem udział w wykonywaniu pomiarów parametrów plonowania.

Przemysław Kopeć

dr Przemysław Kopeć

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Wydział Rolniczo-Ekonomiczny
Uniwersytet Rolniczy w Krakowie

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- brałem udział w przeprowadzeniu eksperymentów,
- brałem udział w wykonywaniu pomiarów parametrów plonowania.

Jakub Pastuszak
mgr inż. Jakub Pastuszak

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- brałam udział w przeprowadzeniu eksperymentu,
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Szczerba Anna

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brałam udział w:

- opracowaniu metodologii doświadczenia,
- przeprowadzeniu doświadczenia w warunkach *in planta*,
- analizie uzyskanych wyników plonowania,
- pisaniu wstępnej i ostatecznej wersji manuskryptu.

.....

Prof. dr hab. inż. Agnieszka Płażek

dr Przemysław Kopeć
Zakład Biologii Komórki
Instytut Fizjologii Roślin
Polska Akademia Nauk

Kraków, 18.01.2022r

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Oświadczam, że w pracy:

Kopeć P., **Hornyák M.,** Pastuszak J., Szczerba A., Rapacz M., Waga J., Płażek A. (2021). Changes in the flower and leaf proteome of common buckwheat (*Fagopyrum esculentum* Moench) under high temperature. *International Journal of Molecular Sciences*, 22(5), 2678. DOI: 10.3390/ijms22052678

- brałem udział w przeprowadzeniu eksperymentu w komorze fitotronowej,
- przeprowadziłem elektroforezę dwukierunkową,
- analizowałem uzyskane wyniki,
- brałem udział w pisaniu wstępnej i ostatecznej wersji manuskryptu.

Przemysław Kopeć

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- przeprowadziłam doświadczenia w komorze fitotronowej,
- pobierałam materiał roślinny do analiz proteomicznych,
- brałam udział w ekstrakcji białek metodą fenolową,
- brałam udział w wykonywaniu elektroforezy dwukierunkowej,
- brałam udział w analizie uzyskanych danych,
- brałam udział w wykonywaniu wizualizacji uzyskanych danych.

Marta Hornyák

mgr Marta Hornyák

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- brałem udział w przygotowaniu eksperymentu w komorze fitotronowej,
- brałem udział w pobieraniu materiału roślinnego do analiz proteomicznych.

Jakub Pastuszak

mgr inż. Jakub Pastuszak

Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
Wydział Rolniczo-Ekonomiczny

mgr inż. Anna Szczerba
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Szczerba Anna

mgr inż. Anna Szczerba

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- brałem udział w redagowaniu manuskryptu.

.....

Prof. dr hab. Marcin Rapacz

Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
Wydział Rolniczo-Ekonomiczny

dr Jacek Waga

Katedra Fizjologii, Hodowli Roślin i Nasiennictwa

Wydział Rolniczo-Ekonomiczny

Uniwersytet Rolniczy w Krakowie

Kraków, 18.01.2022r

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- brałam udział w analizie danych proteomicznych.

.....

dr Jacek Waga

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Prof. dr hab. inż. Agnieszka Płażek
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brałam udział w:

- opracowaniu metodologii doświadczenia,
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- analizie uzyskanych wyników proteomicznych,
- pisaniu wstępnej i ostatecznej wersji manuskryptu.

.....

Prof. dr hab. inż. Agnieszka Płażek

12. Dorobek naukowy

Wykaz publikacji naukowych niebędących tematem rozprawy doktorskiej:

Gołębiewska M., Kiedrowicz A., Skoracka A. (2013). Host specialization in eriophyoid mites: *Aceria tosichella*, *Abacarus hystrix* and *Aculodes mckenziei*: are these plant parasites generalists or specialists? *Annals of Parasitology*, 59, ISSN 2299-0631

IF$_{2013}$: 0,357
5-letni IF: 0,787

Laska, A., Majer, A., Szydło, W., Karpicka-Ignatowska, K., **Hornyák, M.**, Labrzycka, A., Skoracka, A. (2018). Cryptic diversity within grass associated *Abacarus* species complex (*Acariformes: Eriophyidae*), with the description of a new species, *Abacarus plumiger* n. sp., *Experimental and Applied Acarology*, 76(1): 1-28.

IF$_{2018}$: 1,760
5-letni IF: 1,959

Pastuszak, J., Kopeć, P., Płażek, A., Gondek, K., Szczerba, A., **Hornyák, M.**, & Dubert, F. (2020). Cadmium accumulation in the grain of durum wheat is associated with salinity resistance degree. *Plant, Soil and Environment*, 66(6), 257-263.

IF$_{2020}$: 1,799
5-letni IF: 1,874

Pastuszak, J., Kopeć, P., Płażek, A., Gondek, K., Szczerba, A., **Hornyák, M.**, & Dubert, F. (2020). Antioxidant activity as a response to cadmium pollution in three durum wheat genotypes differing in salt tolerance. *Open Chemistry*, 18(1), 1230-1241.

IF$_{2020}$: 1,216
5-letni IF: 1,536

Szczerba, A., Płażek, A., Pastuszak, J., Kopeć, P., **Hornyák, M**., & Dubert, F. (2021). Effect of low temperature on germination, growth, and seed yield of four soybeans (*Glycine max* L.) cultivars. *Agronomy*, 11(4), 800.

IF$_{2021}$: 3,336
5-letni IF: 3,486

Pastuszak, J., Szczerba, A., Dziurka, M., **Hornyák, M**., Kopeć, P., Szklarczyk, M., & Płażek, A. (2021). Physiological and biochemical response to *Fusarium culmorum* infection in three durum wheat genotypes at seedling and full anthesis stage. *International Journal of Molecular Sciences*, 22(14), 7433.

IF$_{2021}$: 5,542
5-letni IF: 6,132

Freitag, H., **Hornyák, M.** (2021). Additional records and new species of *Hydraena Kugelann*, 1794 (Insecta: *Coleoptera*: *Hydraenidae*) from Balabac and Palawan, Philippines. *Tijdschrift voor Entomologie*, 1, 1-24.

IF$_{2021}$: 0,364
5-letni IF: 0,412

Hornyák, M., Dziurka, M., Kula-Maximenko, M., Pastuszak, J., Szczerba, A., Szklarczyk, M., Płażek, A. (2022). Photosynthetic efficiency, growth, and secondary metabolism of common buckwheat (*Fagopyrum esculentum* Moench) in different controlled-environment production systems. *Scientific Reports*, 12(1), 1-13.

IF$_{2022}$: 4,379
5-letni IF: 4,409

Doniesienia konferencyjne:

11th International Conference "Plant Functioning under environmental stress", 12-15 września 2018, Kraków. Referat: **Hornyák M.,** Słomka A., Kopeć P., Dziurka M., Sychta K., Dubert F., Pastuszak J., Płażek A. Influence of environmental factors on seed yield of common buckwheat (*Fagopyrum esculentum* Moench.).
Referat – pierwsza nagroda za najlepszą prezentację ustną w konkursie dla młodych naukowców.

11th International Conference "Plant Functioning under environmental stress", 12-15 września 2018, Kraków. Poster: Pastuszak J., **Hornyák M.**, Płażek A., Gondek K., Dubert F. The influence of different Cd concentrations in soil on photosynthetic efficiency of three genotypes of *Triticum durum* Desf.

XV Ogólnopolska Konferencja Kultur In Vitro i Biotechnologii Roślin - Biotechnologiczne wykorzystanie zmienności w warunkach kultur *in vitro*", Rogów, 17-20 września 2018. Referat: Płażek A., Słomka A., Dubert F., Kopeć P., **Hornyák M.**, Dziurka M., Pastuszak J. „Wpływ stresu troficznego na rozwój woreczków zalążkowych w słupkach gryki zwyczajnej w warunkach *in vitro*".

58. Zjazd Polskiego Towarzystwa Botanicznego „Botanika bez granic". Kraków, 1–7 lipca 2019. Referat: **Hornyák M.**, Kopeć P., Pastuszak J., Płażek A. „Zmiany proteomu liści i kwiatów gryki zwyczajnej (*Fagopyrum esculentum* Moench) pod wpływem działania wysokiej temperatury.

58. Zjazd Polskiego Towarzystwa Botanicznego „Botanika bez granic". Kraków, 1–7 lipca 2019. Poster: Kopeć P., **Hornyák M**., Płażek A., Dubert F., Pastuszak J., Dziurka M., Słomka A., Hura K. „Różnice w profilu białkowym pomiędzy pąkami kwiatowymi, rozwiniętymi i przekwitłymi kwiatami gryki zwyczajnej (*Fagopyrum esculenthum* Moench).

58. Zjazd Polskiego Towarzystwa Botanicznego „Botanika bez granic". Kraków, 1–7 lipca 2019. Poster: Pastuszak J., Kopeć P., Płażek A., Szczerba A., **Hornyák M.**, Dubert F. „Wpływ jonów kadmu na aktywność enzymów cyklu kwasu glutationowo - askorbinianowego w liściach pszenicy twardej (*Triticum durum* Desf.)".

58. Zjazd Polskiego Towarzystwa Botanicznego „Botanika bez granic”. Kraków, 1–7 lipca 2019 Poster: Szczerba A., Dubert F., Pastuszak J., **Hornyák M.**, Kopeć P. „Wpływ biostymulatorów na plonowanie wybranych gatunków z rodziny bobowatych.

Konferencja „Bioróżnorodność funkcjonalna gleb Polski”. Puławy, 18-19 października 2018 r. Poster: **Hornyák M.**, Pastuszak J., Płażek A. „Ocena wybranych parametrów fluorescencji chlorofilu a u gryki zwyczajnej (*Fagopyrum esculentum* Moench) pod wpływem działania wysokiej temperatury”.

XI Konferencja „Kultury *in vitro* w biotechnologii i fizjologii roślin”. Kraków, 4-6 grudnia 2019 r. Poster: **Hornyák M.,** Płażek A., Słomka A, Szczerba A., Pastuszak J., Kopeć P., Dubert F. „Czy stres troficzny powoduje wadliwy rozwój gametofitu żeńskiego i słaby plon nasion gryki zwyczajnej? – Modele eksperymentów w warunkach *in vivo* i *in vitro*”.