Author/s:
Lohani, N;Singh, MB;Bhalla, PL

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PROF. PREM BHALLA (Orcid ID : 0000-0002-2910-0393)

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Short term heat stress during flowering results in a decline in Canola seed productivity

Neeta Lohani, Mohan B. Singh, Prem L. Bhalla*

Authors Affiliation

Plant Molecular Biology and Biotechnology Laboratory, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Melbourne, VIC 3010, Australia

*Corresponding author

Prem L Bhalla

Tel: +61-3-8344-5051

E-mail: premlb@unimelb.edu.au

ORCID:

Neeta Lohani (0000-0003-2375-9709)

Mohan B. Singh (0000-0001-9427-8975)

Prem L. Bhalla (0000-0002-2910-0393)

E-mail addresses of other authors

mohan@unimelb.edu.au

nlohani@student.unimelb.edu.au

Short running title: Short term heat stress during flowering in Canola

Abstract

Oilseed rape (*Brassica napus*) or Canola is an important oilseed crop produced and consumed globally. The predicted increase in the frequency of high-temperature events associated with climate change poses a threat to Canola productivity. In the present work, we report the impact of short-term heat stress on the reproductive fitness and yield components of Canola. Short heat stress episodes, especially above 36°C, resulted in diminished reproductive fitness due to reduced pollen viability and germinability. Heat stress exposure led to asynchronous male and female development and suppressed pollen development in developing buds. As the temperature increased above 32°C, the seed production decreased significantly. Temperatures beyond 38°C resulted in >50% reduction in total seed production and seed weight. Short-term heat stress also reduced yield components, seed vigour and post-harvest seed characteristics. Biochemical investigations of the seeds harvested from heat-stressed (40°C for 12h) plants showed decreased oil content along with a variable fatty acid composition. The omega6/omega3 ratios increased in response to heat stress indicated a possible decline in oil nutritional quality. Understanding the impact of short heat stress episodes can provide efficient heat tolerance screening tools and pave the way for developing heat-stress tolerant *B. napus* varieties.

Keywords: *Brassica napus*, heat stress, heat waves, transcriptome, reproduction, pollen, stigma, style

Introduction

Brassica napus L., also known as Canola, oilseed rape or rapeseed, is the third-largest vegetable oil source globally (USDA 2020). The sizeable majority of Canola oil is used in the food industry. Since its introduction in the late 1960s, Canola production has grown significantly, making it the third-largest broadacre crop produced in Australia (GRDC, 2017). Australian Canola production is increasing almost four million metric tonnes annually,

making up 15–20 per cent of the world's export trade (ABARES, 2018). Canola meal, a by-product of oil extraction, is used in feeds for poultry and livestock production.

Agricultural productivity is largely dependent on climate change, with water availability and temperature being the critical factors. Among the climatic variables, the temperature increase is the most significant factor which will negatively affect crop yields (Ortiz-Bobea, Wang, Carrillo, & Ault, 2019). Since 1910, Australia has warmed by about 1.1°C (mean surface air temperature). This warming has brought about significant shifts in the frequency of extreme temperatures, with a general rise in heatwaves and a decline in the number of cold days (Gergis, Ashcroft, & Whetton, 2020). Climate change has reduced Australian broadacre farms' average annual profitability by 22% in the past two decades (Hughes, Galeano, & Hatfield-Dodds, 2019). The frequency and severity of extreme warm daily temperatures and cold extremes will rise worldwide in the 21st century. Extreme temperature events can last a few days, with more than 5°C above-average temperatures. Since climate change will have far-reaching impacts on agriculture, the understanding of environmental impacts, particularly the high-temperature events by policymakers, farmers, and crop breeders, is crucial in ensuring global food protection.

Temperature increases can influence the productivity of Canola in a variety of ways, but the effect of temperature on reproduction is arguably the most significant. Temperatures above the threshold during reproductive development in Canola can adversely affect flower development, pollination, fertilisation, oil synthesis/accumulation, and grain filling (Angadi et al., 2000; Kirkegaard, Lilley, Brill, Ware, & Walela, 2018; Lohani, Singh, & Bhalla, 2020b; M. J. Morrison & Stewart, 2002; Pokharel, Stamm, Hein, & Jagadish, 2021; Polowick & Sawhney, 1988; Singh & Bhalla 2007; S. Singh, Kakani, Brand, Baldwin, & Reddy, 2008; Uppal, Brill, & Bromfield, 2019). Seed yield and oil quality are the two major economic factors for *B. napus*. Thus, a decrease in oil yield per acre and oil quality due to heat stress will impact the global oilseed trade (Lohani, Golicz, Singh & Bhalla, 2019). The intensity, timing and duration of heat stress exposure also govern the sensitivity of sexual reproduction to high temperature. Even a short spell of heat stress at a crucial reproductive stage can be detrimental to seed yield and quality. Previous studies on the impact of heat stress on reproduction in Canola *B. napus* employed prolonged and primarily moderate heat stress regimes, highlighting an urgent need for studies focusing on the impact of short episodes of extreme heat stress in *B. napus* productivity.

In this report, we investigated the effects of various day time temperatures ranging from 30-40℃ lasting only 12h during flowering on reproductive fitness and productivity in Canola using a commercial cultivar AV Garnet that is grown worldwide, including Australia. We measured the impact of short-term heat stress on pollen viability, germination, and tube growth. We quantified silique abortion rate, seed yield, seed size, seed weight, seed composition, post-harvest seed germination and storage capacity to highlight the negative impact of short heat stress episode on Canola productivity. We also observed the pollen development and synchrony of male and female reproductive development in response to a short episode of heat stress. Understanding the potential impacts of temperature spikes on plant growth and development will help develop new varieties that can sustain productivity under stress conditions.

Materials & Methods

Plant growth conditions and temperature treatments

Brassica napus var. Garnet was chosen for this study. Three replicates of the experiments were carried out using a Thermoline growth cabinet (model TPG-2400-TH) at the Plant Growth Facility of the University of Melbourne, Australia. Growth conditions of 23/18℃ day/night; a photoperiod of 16/8 hours light/dark, 200μmol $m^{-2}s^{-1}$ light intensity and 60% humidity were used as control. When the plants were bearing secondary inflorescence (2 weeks after the first flower), they were exposed to different temperatures (30, 32, 34, 36, 38 and 40℃) for 12 hours. A sampling of buds (6-7mm, unopened) and open flowers was done at an interval of 0, 4, 8 and 12 hours (in the case of 40℃, 10 hours sample was also included). The plants (after 12h treatment) were transferred to control growing conditions until desiccation to measure the total number of aborted siliques, seed yield (mg), seed weight (mg), and seed size (mm). Throughout each experiment, plants were randomized weekly and kept well-watered to minimize any effects of drought stress. Fertilizer was applied directly to the soil once a week for each plant.

Pollen viability and germination assay

Pollen viability was evaluated by double staining with Fluorescein Diacetate (FDA) and Propidium Iodide (PI) (Regan & Moffatt, 1990; Zhang & Bhalla, 2004; Zhang, Singh, Swoboda, Bhalla, 2005). Anthers isolated from the buds of appropriate size (6-7mm) were macerated gently to release pollen grains in the staining solution (20μL: 10% sucrose, 1μL: 2mg/mL FDA and 2μL: 1mg/mL PI). The samples were kept in the staining solution in the

dark at room temperature for 20 minutes and observed under a fluorescence microscope (Olympus BX60). The viability was presented as percentage (%) calculated by counting a minimum of 200 pollen grains from each sample.

For pollen germination, pollen grains were collected from flowers with freshly dehisced anthers after each treatment. The pollen grains were allowed to hydrate for 30 mins after which were brushed onto the surface of the freshly prepared solid pollen germination medium (100g sucrose, 25mg boric acid, 90mg calcium chloride, 50mg potassium nitrate and 100mg of Tris dissolved in 500mL of water; 1% agar was used for solidifying it) (S. Singh et al., 2008). The pollen grains were germinated for 4h under high humidity (>70%) and light ($200\mu molm^{-2}s^{-1}$). After 4h, the plates were observed under a microscope for scoring.

Freshly harvested anthers were collected from 6-7mm buds after seven days of heat stress treatments, and Alexander staining was performed as described by Hedhly, Vogler, Eichenberger, and Grossniklaus (2018). The anthers were submerged in Alexander's solution, and the samples were kept under the fume hood for 3h. Then the Alexander's solution was replaced with Herr's 4½ solution and the samples were kept under the fume hood for overnight incubation. The next day the anthers were paced in 20µl Herr's 4½ solution on a slide, and the anthers were squeezed to release pollen. The debris of anthers was removed using forceps, and a coverslip was gently placed on the slide. The slides placed in a slide holder and kept under the fume hood for at least 4h, and then observed under Olympus BX60 microscope.

Seed composition analysis

Seeds from plants grown in control conditions and heat-stressed plants (34℃ and 40℃ for 12h) were used for analysing the impact of heat stress on seed chemical composition. Three biological replicates per treatment were analysed. Fatty acid profile was determined by using gas chromatography analysis combined with flame ionization detection (GC-FID). Oil extraction from 1g of finely ground seeds was performed by using Soxhlet apparatus (Tan et al., 2011). 150mL of n-Hexane was used as the solvent and the total extraction time was 4h. Total carbon and nitrogen were determined with an automated dry combustion method (Dumas method) by Leco TruMac CN- analyser, Leco Corporation, USA.

Seed germination test and electrolyte leakage

The seeds from control and heat-stressed plants (34℃ and 40℃ for 12h) were randomly selected and were surface sterilized. For each treatment, three replicates with 40 seeds per

154 replicate were placed on a filter paper in 100mm diameter Petri dishes (20 seeds/petri dish)
155 containing $3cm^3$ of distilled water. The Petri dishes were sealed using surgical tape and kept
156 in a growth chamber at 20℃ in dark conditions and 60% relative humidity for five days.
157 After five days, the seeds were scored for germination and then the plates were kept in light
158 for two more days and observed again. Seeds were counted as germinated when the radicle
159 extended for at least 2 mm.
160 Measurements of electrolyte leakage were performed on 100 unsprouted individual seeds in
161 three replicates for each treatment. Seeds were weighed and soaked in 40mL of deionized
162 water at 22℃ for 16h. Conductivity was measured with a portable electrical conductivity
163 meter (HI98304 DiST® 3 EC Tester). Results are expressed in $\mu Scm^{-1}mg^{-1}$ of fresh seed
164 weight.

165 **Statistical analysis**

166 All experiments were performed in triplicates. Results are expressed as the mean ± SD of n
167 replicates available per treatment. In pollen tube length, the results are expressed in a Box-
168 Plot highlighting the mean, maximum and minimum pollen tube length for each treatment.
169 The data were analysed statistically using GraphPad Prism 8.2.1 (© 1992-2020 GraphPad
170 Software, Inc.) software. Data analysis was done by performing Welch's t-test to compare a
171 time point at a given temperature with the control conditions. The variance was considered
172 unequal for the comparison. Significant differences among the treatment were considered at p
173 < 0.05.

174

175 ## Results

176 **Pollen viability and germination**

177 To study the impact of short episodes (4-, 8- and 12h) of heat stress on mature pollen, we
178 exposed *Brassica napus* var. Garnet plants bearing secondary inflorescences to 30℃, 32℃,
179 34℃, 36℃, 38℃ and 40℃ during the day. The plants were grown under 16h day length with
180 the heat stress starting 2h after the beginning of the day and finishing 2h before the daylight
181 ends. The mature buds (6-7mm) were sampled after each time-point at a particular
182 temperature to observe pollen viability and germination. A set of control plants were grown
183 at optimum condition (23℃/18℃, light/dark) throughout the experiment. The pollen grains
184 of control plants recorded the maximum pollen viability (~90%) and pollen germination

(~62%). Heat-stressed plants displayed a gradual reduction in pollen fitness as the duration at a temperature or the temperature at a time point increased (Figure 1A, 1B). We recorded a complete loss of pollen viability following exposure to 38℃ for 12h and 40℃ for 10h. Similarly, we observed a complete failure in pollen germination after exposure to 38℃ for 12h and 40℃ for 8h and 12h. The average pollen tube length also followed a similar trend (Figure 1D). Due to low or failed pollen germination, pollen tube lengths for 36℃: 12h, 38˚C: 12h and 40℃: 8h and 12h were not recorded. These observations suggest that the extent of negative effects of heat stress on pollen viability and germination in *B. napus* depends on heat exposure intensity and duration. Even shorter duration exposure to a higher temperature can result in severe damage to pollen fitness.

Growth and yield components

The control and heat-stressed exposed plants were allowed to complete their development under optimum growth conditions until seed filling and maturity. After complete seed filling, we recorded the plant height, branching, number of aborted or shrivelled siliques, seed yield, seed weight, and seed size. The plant height decreased with a short-term increase in temperature at temperatures 34℃ and beyond; however, the difference was not significant. However, the number of branches increased when the plants are exposed to temperatures above 36℃ (Figure 2A). This significant increase in branching was also accompanied by increased total siliques produced by the plant (Figure 2B). As a higher number of siliques are produced in response to heat stress, the rate of silique abortion also increased with increasing temperature (Figure 2C, 3A). The seed yield reduced significantly as the temperature increased above 32℃. ~51% shrivelled siliques observed at 38℃ and ~41% at 40℃ translated into ~69% and ~64% loss in yield, respectively (Figure 2C, 2D). The seed weight also decreased significantly at and above 34℃. As compared to control conditions, seed weight decreased by 27% at 34℃ and 36℃, ~37% at 38℃ and the maximum decline of ~54% at 40℃ heat stress treatments (Figure 2E).

The seeds harvested from plants stressed for 12h at different temperatures showed variations in appearance (Figure 3B). The seed size showed no significant changes after heat stress treatment at 30℃ for 12h. Temperatures beyond 30℃ negatively affected the seed size (Figure 2F). The drastic reduction in seed size was observed at 38℃ and 40℃, which agrees with the severe decrease in seed weight and total seed yield. Heat stress resulted in shrivelled, smaller, and lighter coloured seeds as compared to control seeds. At 30℃, no significant differences were observed in terms of growth and yield parameters. At 32℃, the number of

aborted siliques was slightly higher, and the seed was somewhat smaller than the control, but we did not observe any significant changes in terms of seed yield and seed weight.

Reproductive development

As heat stress resulted in a severe reduction in seed yield and seed quality, we examined the impact of heat stress events on flower and pollen development. For these experiments, we tagged 1-1.5mm buds on the plants exposed to heat stress. 1-1.5mm buds contain the uninucleate stage of microspores. The buds developed for seven days after returning to control conditions, and then we sampled buds of length 6-7mm. We observed the morphological changes in stamen development after heat treatments (Figure 4A, 4B). The stamens had significantly shorter filaments in buds collected from plants heat-stressed at 40°C and 38°C (Figure 4B). The difference between the stamen height and the pistil was significantly higher in heat-stressed plants than control plants. The buds sampled from plants heat-stressed at 40°C, and 38°C also showed a protruding stigma (Figure 4A). This asynchrony between the male and female reproductive organ development interferes with pollen and stigma interactions and reduces fertility.

Furthermore, we performed Alexander staining to observe pollen viability (Zhang & Bhalla, 2004; Zhang et al 2005). The anthers of control plants contained fully developed and viable pollen. On the contrary, the buds collected from plants heat-stressed at 34°C and above showed 100% abortion of pollen (Figure 4C). The pollen development was arrested, indicating that the uninucleate microspore stage was unable to undergo pollen mitosis I to form binucleate pollen, and it collapsed in response to heat stress. Based on these observations, we concluded that exposure to high temperature for even 12h could suppress normal stamen and pollen development in developing buds exposed to heat stress.

Seed biochemical properties

To further explore the impact of heat stress on seed characteristics, seeds harvested from the following three treatments were selected: control, 12h exposure at 34°C (HS34) and 12h exposure at 40°C (HS40). Total- carbon (C), nitrogen (N), oil content, as well as fatty acid (FA) profile from three replicates from each treatment were measured (Table 1). While the seed C and N contents remained stable in each treatment, the total oil content and the FA composition showed variability in seeds from heat stress treatments. We observed the

maximum oil content observed in seeds collected from control plants. At HS34, there was no significant decline in total oil content; however, at 40°C, it declined by ~41%.

In terms of FA composition, no significant differences are observed in the monosaturated FA profiles such as oleic, stearic, and palmitic acids in HS34 seeds. On the contrary, in HS40 seeds, oleic acid levels showed a slight reduction, whereas palmitic and stearic acid levels showed a slight increase. The profiles of two important polyunsaturated FAs, linoleic (omega-6) acid levels increased, and linolenic (omega-3) acid levels decreased in HS34 and HS40 seeds. Our results indicate an increasing trend of ω6/ω3 ratio in response to heat stress, possibly reducing oil nutritional quality (Table 1).

Post-harvest seed quality

We next investigated the germination potential of seeds harvested from control and heat-stressed plants (Figure 5A, 5B). Heat stress resulted in a significant reduction in the germination ability of the seeds. The control seeds showed ~93% germination, which reduced by ~24% and ~57% in seeds harvested from HS34 and HS40 after five days of sowing and germination in the dark (Figure 5B). The germination plates were shifted to optimum light condition for the next two days. We observed no further germination. The seedlings germinated from control seeds were normal and did not show bleaching, whereas we observed abnormal seedlings with bleached cotyledons for HS34 and HS40 (Figure 5A, Day 7). Additionally, the electrolyte leakage increased from ~0.28$\mu Scm^{-1}mg^{-1}$ in control seeds to ~0.57$\mu Scm^{-1}mg^{-1}$ and ~0.96$\mu Scm^{-1}mg^{-1}$ in HS34 and HS40 seeds, respectively (Figure 5C). Heat stress possibly led to a reduction in seed storage capacity, as indicated by an increase in the seed's electrolyte leakage.

Discussion

Over the past decades, studies have elucidated the physiological impacts and molecular mechanisms of *B. napus* responses to prolonged high-temperature treatments or comparatively shorter high-temperature treatments during the reproductive stages (Aksouh-Harradj, Campbell, & Mailer, 2006; Angadi et al., 2000; Kirkegaard et al., 2018; Lohani, Singh, & Bhalla, 2020a; Lohani, Singh, et al., 2020b; Pokharel et al., 2021; Uppal et al., 2019; Singh, Lohani & Bhalla 2021). In the present work, we exposed plants to high temperature lasting only 12h during flowering to study the impact of a short heat stress episode on reproductive fitness and yield in a commercial Canola variety AV Garnet. The threshold temperature during flowering in *B. napus* is 29.5°C, with plants experiencing heat

stress when exposed to temperatures higher than the threshold during a developmental stage (M. J. Morrison & Stewart, 2002). Therefore, we decided to use temperatures ranging from 30℃ to 40℃ for studying the impact of a short heat stress episode on Canola productivity. Short episodes of extreme temperature were chosen in this study to highlight the negative impact of heat stress events mimicking a day of the heatwave on overall crop productivity.

Short episodes of high temperature negatively regulate pollen viability and germination

B. napus heat-stressed plants displayed a gradual reduction in pollen viability, pollen germination, pollen tube length as the duration or intensity of the heat stress increased (Figure 1A, 1B). Previous studies in *B. napus* have suggested that prolonged exposure to temperatures during flowering negatively impacts pollen viability and functionality (Angadi et al., 2000; M. Morrison, 1993; M. J. Morrison & Stewart, 2002; Polowick & Sawhney, 1988; S. Singh et al., 2008; Young, Wilen, & Bonham-Smith, 2004). We report a linear trend between pollen viability and germination (Figure 1C) for all treatments. S. Singh et al. (2008) also reported a high positive correlation between pollen viability and in vitro pollen germination in response to heat stress above 23.6℃ across twelve *B. napus* cultivars. The higher mean percentage of pollen viability than pollen germination in our data also agrees with previous studies (S. Singh et al., 2008; Young et al., 2004).

High variability is evident across heat stress studies in terms of cultivars, developmental stage, duration, and intensity of heat stress; it makes it difficult to compare the extent of heat stress-induced damage to mature pollen. Therefore, there is a need to develop standard high-temperature treatment protocols based on commercial Canola varieties depending on the cultivation region. The short heat stress regime applied in this study offers a potential application for terminal heat stress tolerance screening of Canola varieties cultivated in areas prone to irregular high-temperature events.

A short episode of heat stress suppresses silique development, yield and reproductive development

Day time temperatures ranging from 30-32℃ are reported as the threshold temperature for seed set and seed development in *B. napus* (Polowick & Sawhney, 1988). Accordingly, we observed no significant changes in seed yield and seed weight at 30℃ and 32℃. As the pollen fitness declined with an increase in temperature, the seed yield also declined gradually, similarly, in cotton (Kakani et al., 2005), tomato (Sato, Peet, & Thomas, 2000), rice (Wang et al., 2019), wheat (Bheemanahalli et al., 2019), sorghum (V. Singh et al., 2015), maize (Begcy

et al., 2019) and pea (Jiang, Davis, Vujanovic, & Bueckert, 2019), a reduction in pollen germination across different genotypes and an increase in temperature regimes correlated with a decrease in seed set. (Young et al., 2004) reported in heat-stressed *B. napus* plants that pollen germination was possibly the major contributor resulting in reducing of pollen fitness and total seed set.

Exposure to a short episode of heat stress also significantly reduced seed mass. Increased temperatures led to both the reduction in seed size and seed mass. We observed lighter in colour shrunken and irregularly shaped seeds in heat-stressed plants. Previous studies have also reported that heat stress during flowering in *Brassica* species results in yield loss exclusively due to a reduction in seed mass potentially due to reduced seed filling duration or increased rate of seed filling (Aksouh-Harradj et al., 2006; Chen, Stefanova, Siddique, & Cowling, 2021; Gan et al., 2004; M. J. Morrison & Stewart, 2002; Pokharel et al., 2021; Rashid, Hampton, Rolston, Khan, & Saville, 2018; Young et al., 2004; Yu et al., 2014). Our findings indicate that seeds failed to mature properly in some pods. In contrast, a fraction of pods was aborted due to heat stress resulting in deterioration of overall seed yield properties.

In heat-stressed plants, aborted or shrivelled siliques primarily arose from the flowers or buds present at the time of heat stress. Angadi et al. (2000) also reported that flowers opened during the duration of the 35/15℃ HS in *B. napus* did not produce any fertile pods. Moderate heat stress resulted in flowers that developed into seedless parthenocarpic fruits or aborted on the stem due to the combined effect of heat stress on pollen and pistil development and function (Young et al., 2004). The abnormal/unsuccessful fertilisation event in the HS flowers and buds led to a lower or failed seed set due to loss of pollen and pistil functionality. Asynchronous male and female development is observed in buds at the uninucleate microspore stage during heat stress exposure. The difference in length between stamen and pistil was significant. The pistil was also protruding out of mature unopened buds, indicating possible induction of pistil hyperplasia in response to heat stress. Protruding pistils and asynchrony in male and female reproductive development is reported in *B. napus* in response to heat stress that lasted from few days to weeks (Pokharel et al., 2021). High-temperature exposure for just 12h during flowering impacted reproductive parts at anthesis and earlier reproductive growth and reproductive organ development.

We also report an increase in branching and the total number of siliques produced in plants heat stressed to temperatures above 36℃. An increase in branching upon exposure to long term heat stress in different varieties of *B. napus* was also reported by (Macova et al., 2021). In *B. napus*, a few studies report the induction of a higher number of floral primordia in

response to heat stress (Angadi et al., 2000; McGregor, 1981). According to McGregor (1981), heat stress injury during flowering is compensated by the plant by producing more branches or flowers. In accordance with our results, Angadi et al. (2000) suggested that the floral primordia developed during or after heat stress exposure may not develop into normal flowers or pods. Thus, heat stress negatively regulates pod development. The flowers that developed beyond a particular developmental stage or fertilised during heat stress treatment are the major contributors to the overall yield.

Short heat stress episode alters seed oil content and fatty acid composition

The high temperature is reported to modify the C-N metabolism and gaseous exchange, favouring protein accumulation at the expense of oil and carbohydrates (Lohani, Jain, Singh, & Bhalla, 2020). Contrary to published reports, seed C and N contents remained stable across all treatments in our study. The variations in the results are probably due to the difference in the length of heat stress treatment adopted in our study compared to previous reports. Additionally, C and N resource allocation in the oil crops is generally controlled by genetic and environmental factors. A significant effect of heat on seed oil content is also previously reported in *B. napus* (Brunel-Muguet et al., 2015; Huang et al., 2019). We observed a significant reduction in total oil content in seeds harvested from plants heat-stressed at 40°C. High temperature is suggested to regulate the enzymes involved in the lipid biosynthesis pathways leading to decreased oil content (Baud & Lepiniec, 2010; Iyer et al., 2008).

In terms of fatty acid composition, oleic acid levels slightly reduced, whereas palmitic and stearic acid levels slightly increased in seeds harvested from plants heat-stressed at 40°C. The ω6/ω3 ratio showed an increasing trend i.e., the linoleic acid increased, and linolenic acid decreased under heat stress, indicating a possible reduction in oil nutritional quality (Table 1). In *B. napus* and other oil crops, high temperatures reduce the percentage of polyunsaturated fatty acids (linoleic, linolenic) and increase that of monounsaturated fatty acids (oleic) and saturated fatty acids such as palmitic and stearic (Aksouh-Harradj et al., 2006; Canvin, 1965; Gibson & Mullen, 1996). However, Elferjani and Soolanayakanahally (2018) reported a decrease in the oleic acid content and increased linoleic acid and the saturated fatty acid fraction due to heat stress. Increased saturated (palmitic and stearic) fatty acids without significant changes in unsaturated fatty acids (oleic, linoleic, linolenic) in *B. napus* in response to high night temperature reported by Pokharel et al. (2021). Overall, our results and

previous studies reflect a highly variable and complex FA composition regulation across *B. napus* in response to heat stress.

High-temperature exposure deteriorates post-harvest seed quality

Heat stress during seed development reduces seed vigour in crops (Gibson & Mullen, 1996; Hampton, Boelt, Rolston, & Chastain, 2013). Similar to the present study, lower seed germination and very high rates of abnormal seedlings were also previously observed in *B. napus* in response to high temperature (Brunel-Muguet et al., 2015; Rashid et al., 2018). The electrolyte leakage also increased in heat-stressed seeds. The seed electrolyte leakage test of seeds harvested from heat-stressed plants in the present study is in agreement with earlier reports in *B. napus* (Brunel-Muguet et al., 2015) and other crops such as wheat (Balla, Bencze, Janda, & Veisz, 2009), rice (Sohn & Back, 2007), and peas (Castillo, Hampton, & Coolbear, 1993). Higher electrolyte leakage is also associated with lower germination (Battisti & Naylor, 2009). Poor seed storage ability is also correlated with such increased seed leakage (Brunel-Muguet et al., 2015; Demir, Cebeci, & Guloksuz, 2012; Mavi, Mavi, Demir, & Matthews, 2014).

Conclusions

Our findings highlight the severe effects of short-term heat stress during flowering on the success of reproduction in *B. napus*. We reported that the pollen viability and germination gradually reduced as the intensity and duration of heat stress increased. The short-term heat stress at 34°C and above for a 12h duration induced parthenocarpic silique production and caused a reduction in seed yield, size, weight, and post-harvest seed germination and storage capacity. Heat stress exposure also increased branching and total silique production at temperatures above 36°C. Extreme heat stress decreased oil content and altered fatty acid composition, thus potentially deteriorating the seed nutritional and economic worth. A 12h heat stress also altered stamen morphology and suppressed pollen development in developing buds. Short heat stress episode resulted in asynchronous male and female development and further reduced overall productivity. Our results describing the sensitivity of reproductive processes to short term heat stress pave the way for studies to further explore reproductive heat stress response in response to high-temperature weather events. With global warming, heat stress may become more of a problem in the major Canola growing regions, and to

safeguard future yield, an in-depth understanding of heat stress responses during critical reproductive stages is required.

Authors' contributions

NL designed and performed the experiments, analysed the data and wrote the initial draft of the manuscript. PLB and MBS conceived the research, supervised, and extensively edited the article.

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578

579 **Table 1** Mean values of the measured variable of seed biochemical composition.

Biochemical component	Control		HS_34		HS_40	
	Mean	SD	Mean	SD	Mean	SD
Total oil, %	28.73	0.112	27.68	0.677	16.89*	0.665
Total carbon, %	56.2	0.7	56	0.6	55.4	0.7
Total nitrogen, %	5.41	0.23	4.98	0.53	5.31	0.6
Palmitic acid (16:0)	4.730	0.121	4.837	0.376	5.863*	0.627
Stearic acid (18:0)	2.265	0.091	1.936	0.410	2.632**	0.085
Oleic acid (18:1)	55.540	0.491	55.563	0.828	52.658*	1.241
Erucic acid (22:1)	-	-	-	-	-	-
Linoleic acid (18:2)	15.993	0.215	16.551*	0.302	17.832***	0.386
Linolenic acid (18:3)	12.720	0.128	11.424*** *	0.127	10.953****	0.013

580 The asterisk (*) represents a significant difference between the heat stress treatment and
581 control (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001). SD: standard deviation.

582

583 **Figure Legends**

584 **Figure 1** Effect of short-term heat stress on pollen fitness. (A) pollen viability, %; (B) pollen
585 germination, %; (C) Correlation coefficients between pollen viability and pollen germination
586 in response to short term heat stress; (D) Effect of different heat stress treatment on pollen
587 tube growth, µm. Data are the mean of four replicates ± SEM. The asterisk (*) represents a
588 significant difference between the heat stress treatment and control (*P ≤ 0.05, **P ≤ 0.01,
589 ***P ≤ 0.001, ****P ≤ 0.0001).

590 **Figure 2** Effect of 12h of heat stress treatments on (A) number of branches, (B) the total
591 number of siliques, (C) empty or shrivelled siliques, %; (D) seed yield, g, (E) 1000 seed
592 weight, g, (F) seed size, pixels^2. Data is the mean of 3 replicates ± SEM. The asterisk (*)
593 represents significant difference between the heat stress treatment and control (*P ≤ 0.05, **P
594 ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001).

Figure 3 (A) Effects of short heat stress episode on silique development and seed filling; (B) Effect of short heat stress episode on the appearance of harvested seeds.

Figure 4 Examination of the impact of short heat stress episode on reproductive development (A) bud morphology, (B) stamen and pistil morphology, (C) pollen viability by Alexander staining.

Figure 5 Effect of 12h of heat stress treatments on post-harvest characteristics of seeds harvested from NS, HS34 and HS40 plants: (A) Germination potential; (B) Germination percentage; (C) Electrical conductivity, $\mu Scm^{-1}mg^{-1}$.

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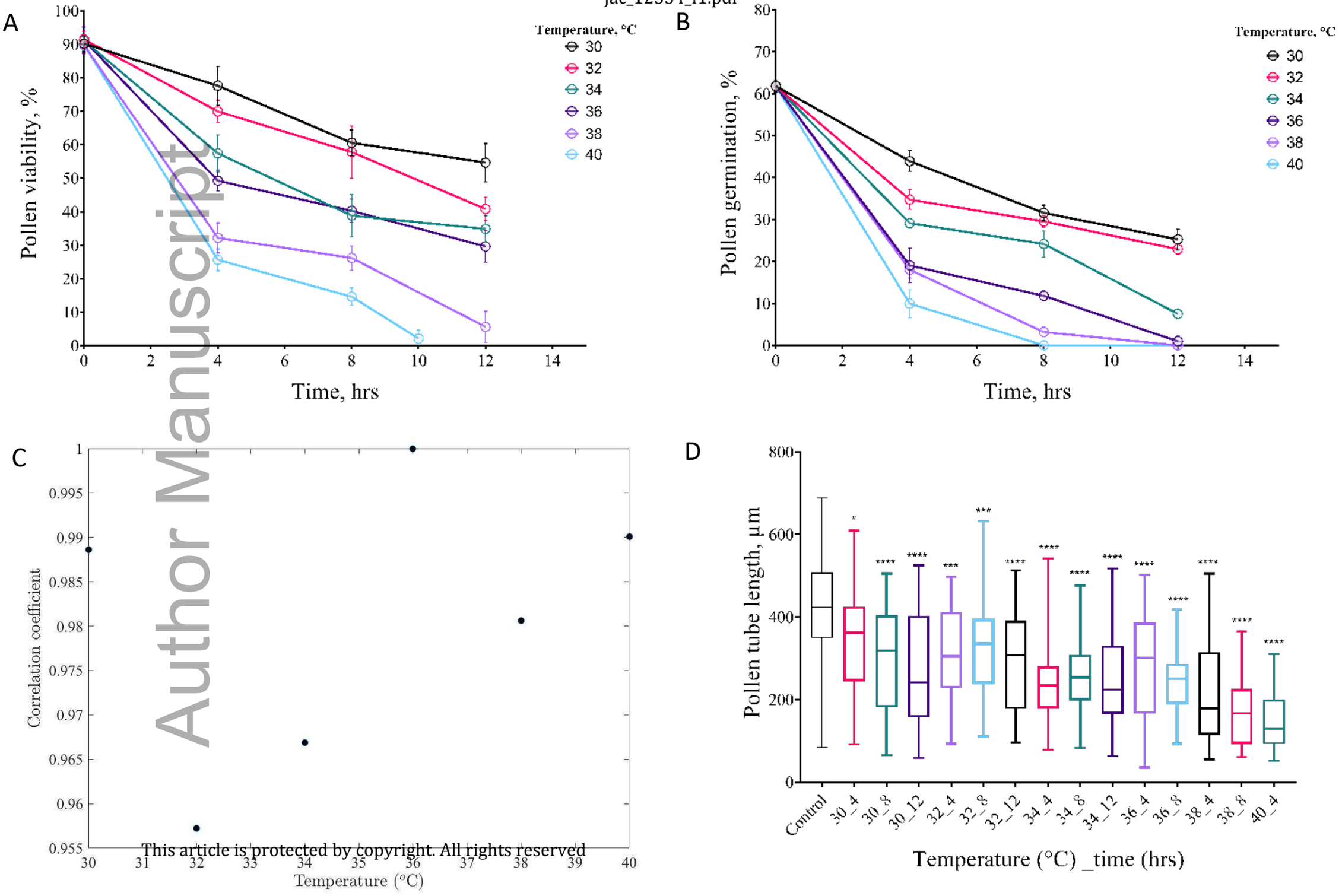

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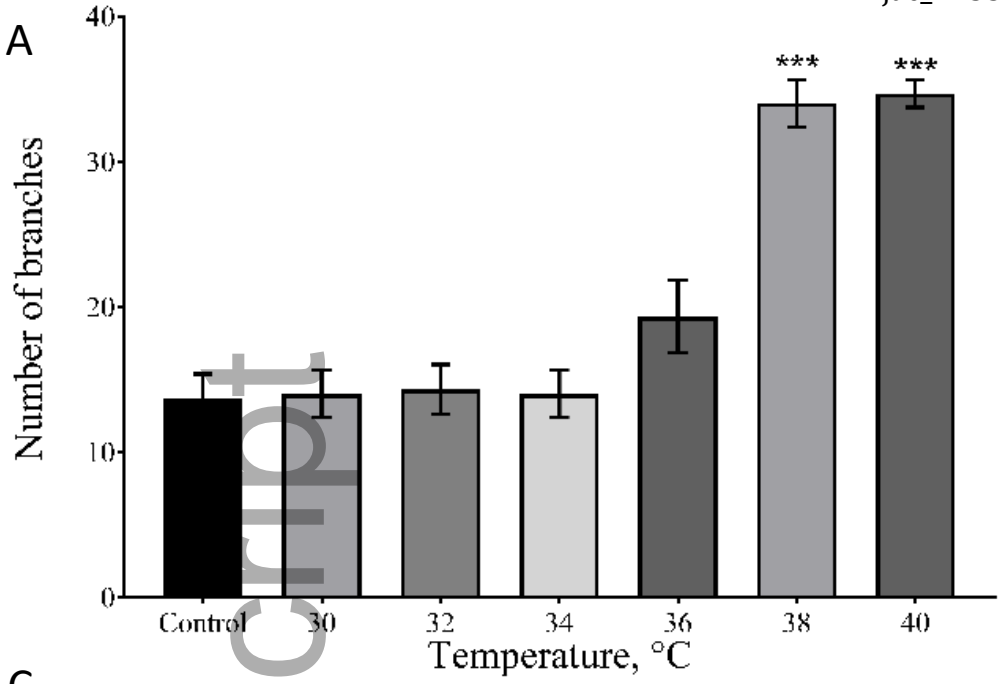

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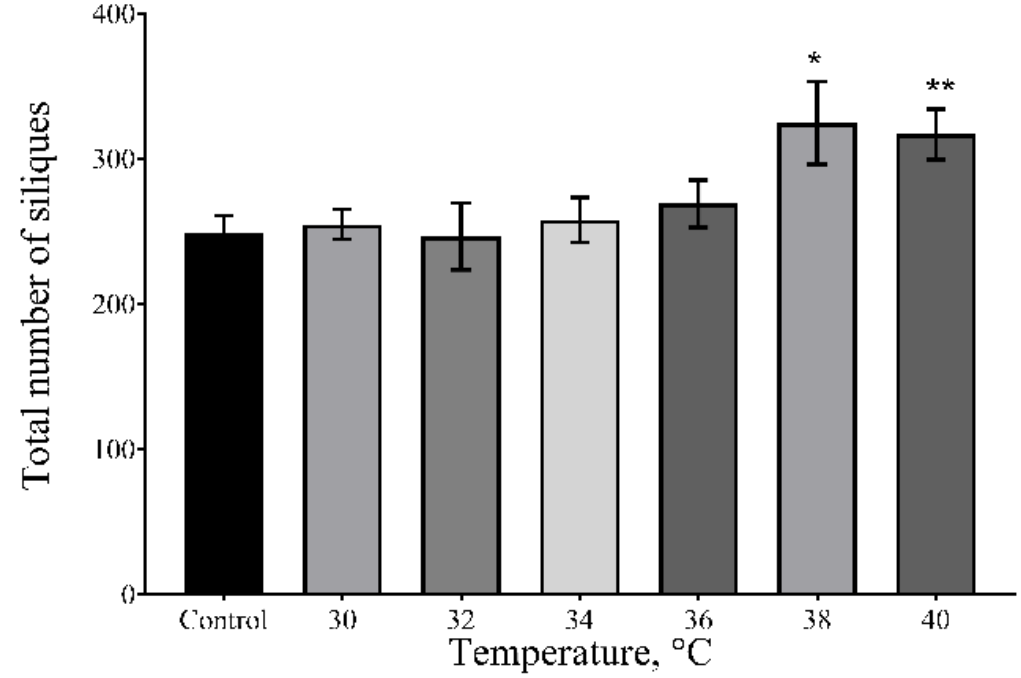

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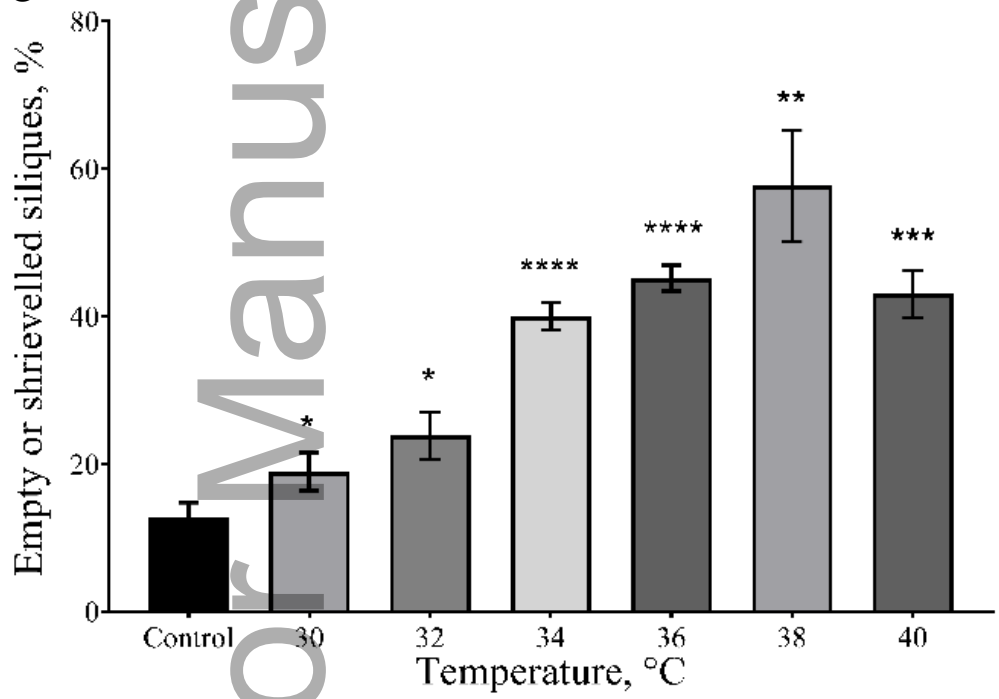

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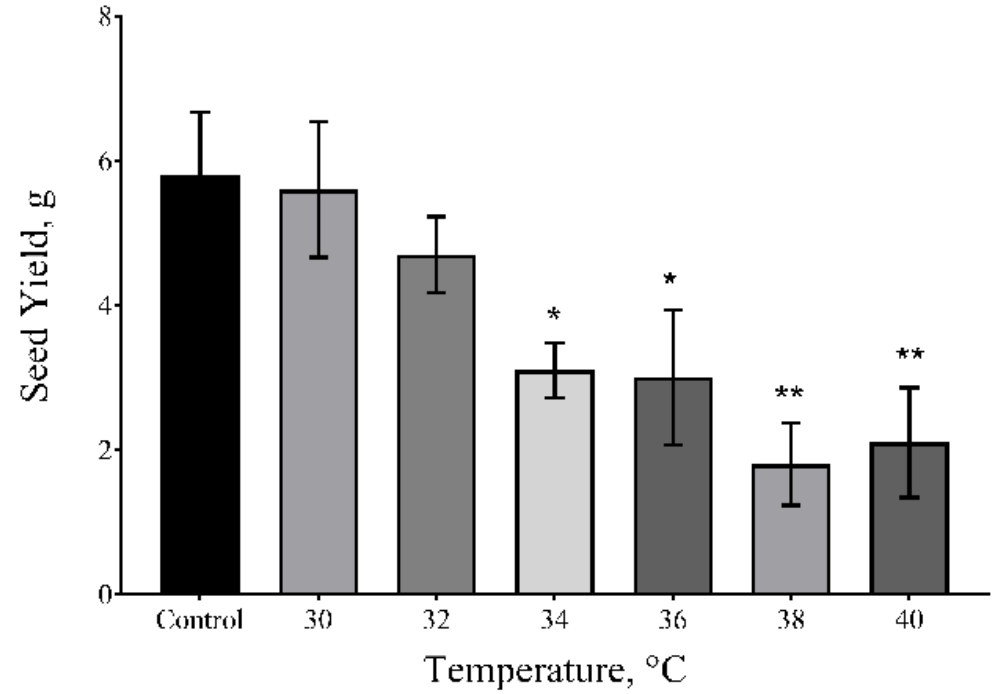

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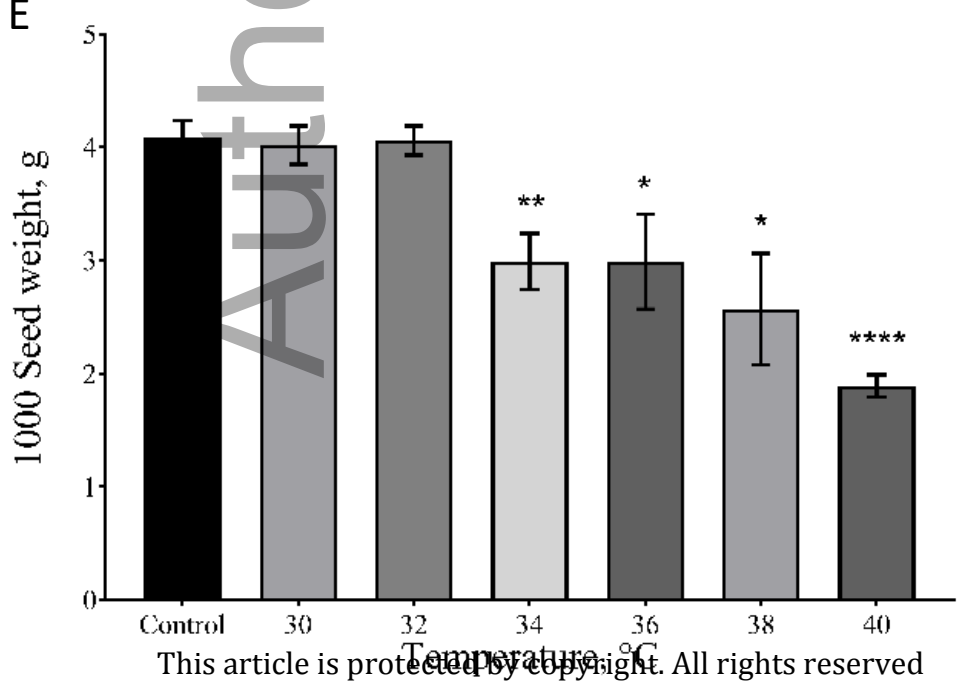

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