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Research article

Heat stress during reproductive stages reduces camelina seed productivity and changes seed composition

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ABSTRACT

Camelina (*Camelina sativa* L. Crantz) is a low-input oilseed crop with great potential in bioenergy and industrial oils. Improving tolerance to high temperatures is essential for camelina agronomic sustainability. Two genotypes, Suneson and Pryzeth, were exposed to a transient 14-day heat stress at 37 °C during the reproductive stages. Four cohorts of pods along the main stem, which were at different stages from fully developed pods (C1), young pods (C2), open flowers (C3) and flowering buds (C4) at the time of heat treatment, were examined for morphological and seed quality traits at maturity. The main stem length was shortened in both genotypes. Pods and seeds in all cohorts were negatively affected by heat, resulting in lower seed yield and reduced oil content. Seed size and seed weight had the greatest reduction in C1, pod size reduction was found the most in C3, and the number of fertile pods that contain at least one seed was reduced in C3 and C4. These results suggest that heat stress effects are developmental stage specific. Heat stress significantly reduced fertility during flowering and inhibited storage product biosynthesis and accumulation during seed filling which resulted in smaller and lighter seeds. Analyzing seed composition indicated that oil content decreased while protein content increased in seeds from heat treated plants. In addition, fatty acid composition was altered with the reduction of omega-3 α-linolenic acid and concomitantly increased omega-6 linoleic acid being the most significantly affected. Our results also revealed the different responses in the two genotypes examined, suggesting genetic variation in camelina germplasm which can be explored to improve heat tolerance. This study provides resources and guidance for future studies to understand genetic and physiological mechanisms of heat stress and to assist in improving the sustainability of camelina production facing climate change.

1. Introduction

Heat stress is among the most impactful stressors for most plant species, including agricultural crops [1]. Between 1984 and 2015 in Europe, heatwaves and droughts reduced cereal yields 9% on average and non-cereal yields 3.8% on average [2]. For cool season crops such as Brassicas, growing seasons may be shortening due to earlier onset of summer-like conditions, and heat stress negatively impacts seed development and yield [3]. Camelina (*Camelina sativa* L. Crantz) is a promising oilseed crop in the Brassicaceae family, characterized by high α-linolenic acid, an omega-3 fatty acid, and relatively low agricultural inputs [4]. Currently, camelina is mainly grown as a feedstock for renewable diesel and jet fuel [5]. Camelina production is greatly influenced by field environments. In a

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multi-year trial, seed yield and oil content were remarkably reduced in Hays, KS compared to Moccasin, MT, corresponding with growing season precipitation and temperatures [6]. However, little research has been conducted in camelina on heat responses. As more frequent episodes of heat waves and drought occur in its production regions [7], understanding the genetic mechanisms of heat tolerance and breeding climate resilient camelina varieties is of the utmost importance.

Heat stress is particularly detrimental during the reproductive and seed filling stages of agricultural crops [8–10]. Fertility and yield are significantly impacted by heat stress as observed in many plant species including Brassicas [11–13]. Heat stress during early flowering, which corresponds with fertilization and early embryogenesis, reduced pollen viability, resulting in lower silicle fruit (pod) number and seed production. Concurrently, seed weight and oil content were reduced in rapeseed (*Brassica napus*) [14,15]. The effects of heat durations have also been studied, varying from a few days to the entire life cycle of the plant [16,17]. The results illustrated the impacts of heat stress on both seed setting and seed filling, but also the strong compensation capacity in indeterminate growth species. Rapeseed plants treated with heat for five days beginning shortly after the onset of flowering displayed increased pollen sterility by a factor of 84.4 and increased number of sterile or aborted pods by a factor of 26.1 when compared to controls [17]. Additionally, the total number of silicles (pods) on main racemes were reduced by 13.8% in heat stressed plants [17]. Prolonged heat stress during the reproductive stage of development may also affect flowering quantity and duration. In Brassica plants grown under a two week heat stress during early reproduction, flowering duration was longer due to a larger number of flowers produced compared to controls [18]. This also coincided with fewer pods forming despite having more flowers. Slightly different findings were obtained when Brassica plants were exposed to 10 days of heat stress beginning at inflorescence emergence. Rather than increasing flowering quantity and duration on the main raceme, only the side shoots were affected, showing fewer flowers while the main stem remained static [19]. It should be noted that in Koscielny et al., 2018 [16], heat stress began after the inflorescence had emerged, while Wu et al., 2020 [17] started the heat stress at inflorescence emergence. Clearly, phenotypic variation from heat depends on the precise reproductive stage when the heat stress begins. Furthering this assertion, flax undergoing a 7- or 14-day heat stress during the reproductive stage showed reduced pod formation and seed set [13]. Interestingly, total pod formation was unaffected, as the plants were able to compensate after the heat stress period via prolonged flowering [13]. This compensatory effect was also noted in rapeseed showing the stage-specific response of yield traits. Seed yield decreased only for the intermediate stage of pod growth, and total seed yield for whole plants was unaffected [3].

Heat stress in oilseed plants impacts oil content and the fatty acid composition in seeds. The enzymes responsible for producing poly-unsaturated acids in seed oils are the microsomal ω-6 and ω-3 fatty acid desaturases FAD2 and FAD3, which act sequentially to convert oleic acid to linoleic acid and then to α-linolenic acid [20]. Under intense heat stress, the efficacy of these proteins may be altered, changing the seed oil composition [16]. *Arabidopsis thaliana* plants grown from 10 °C to 30 °C displayed significant differences in oil composition and total oil content. The quantity of C18:1 more than doubled between 10 °C and 30 °C, while the amount of C18:3 was halved [16]. Also, total oil content was the greatest at 10 °C and progressively declined as the temperature increased to 30 °C. These results are supported by another study in *Brassica napus* when siliques at 20, 30, and 40 DAP (days after pollination) were exposed to a 37 °C heat stress [21]. Seeds at the 20 DAP stage exhibited the greatest reduction in total oil contents while simultaneously having the greatest soluble sugar content [21]. These results indicate that prolonged heat stress damaged the molecular machinery responsible for converting carbohydrates to triacylglycerols. This is especially true at earlier stages of seed filling.

In this research, we aim to describe in detail the response of *Camelina sativa* to heat stress during the reproductive stage. In contrast to other Brassica species, little is known about how high temperatures affect seed yield in camelina. A previous study indicated that camelina has limited capacity for acclimation to heat stress. A daily interval of moderate heat stress (35 °C) greatly impacted photosynthesis in camelina. Failure to acclimate to such heat stress caused a 63% reduction in its seed yield [22]. We hypothesize that not all stages of development are equally sensitive to heat, and that the reproductive stages are the most susceptible to heat stress in camelina. Therefore, our experiments are designed around the early stages of reproduction in camelina. We used two wild type camelina lines with different agronomic traits to compare the yield response of developing seeds and pods at different stages to heat stress.

2. Materials and methods

2.1. Plant material and growth conditions

Two *Camelina sativa* genotypes, Suneson and Pryzeth, were used in this study. Obtained from different geographic regions, and differing in multiple traits such as seed size, seed oil contents, and seeds per pod [23,24], we speculated that these two genotypes may also respond differently to heat stress. Seeds from each line were sown in 13 cm pots filled with soil and Sunshine Mix (Clinton, OK, USA) (1:1) before being placed in a climate-controlled growth room (16h of light, 22 °C/8h of dark, 18 °C, 50% humidity, ~325 μmol). This growing environment constituted the control treatment condition. On the treatment start date, plants were transferred to a growth chamber for heat stress treatment for a desired duration. The heat stress included a temperature ramping from 22 °C to 37 °C over 6h from 6:00 to 12:00, maintenance at 37 °C for 4h from 12:00 to 16:00, ramping down from 37 °C to 22 °C over 6h from 16:00 to 22:00, and maintenance at 22 °C for 8h from 22:00 to 6:00. Light intensity was ~325 μmol. This growing environment constituted the heat treatment condition. After heat treatment, plants were returned to the growth room (control environment). Plants were grown under ideal water and nutrient conditions assuming a plant density of 65 plants. m^{-2}.

2.2. Identification of pod cohorts and heat treatment initiation

For the pilot experiment, the heat treatment began when plants entered the flowering stage, with 1–2 flowers opening on the main stem. All pods and seeds on the main stem were pooled to obtain phenotypic measurements.

In the subsequent experiments, we identified four cohorts of pods (Fig. 1) on the day of heat treatment initiation, similar to the methods described in Ref. [3], with slight modifications. When the main stem of a plant formed n pods, where n equals 16 to 20 pods (pod structure visible), cohorts were identified and labeled. A tag was attached to the nth pod, the (nth/2) pod, and the most recent open flower, delineating four distinct sections consisting of adult pods (C1), youth pods (C2) (Fig. 1A), flowers (C3), and flower buds that will become pods during heat treatment (C4) (Fig. 1B). Once these cohorts were identified and labeled, the heat treatment was initiated.

2.3. Experimental design and statistical analysis

In the pilot experiment, camelina plants were arranged in a completely randomized design (CRD) with a total of 6 replications for each treatment (n = 5) and genotype (n = 2) combination. Once plants were fully matured, three (n = 3) uniform plants were selected for phenotypic evaluation.

Subsequently, two sets of experiments were conducted 18 days apart (Set 1 and Set 2). In each set, camelina plants were arranged in a completely randomized design (CRD) with a total of 8 replications for each treatment (n = 2) and genotype (n = 2) combination. Once plants were fully matured, six (n = 6) uniform plants were selected for phenotypic evaluation. In Set 1, both the control and heat-treated individuals were selected for phenotypic evaluation, designated as Control and Heat 1, respectively. In Set 2, only the heat-treated individuals were selected for phenotypic evaluation, designated as Heat 2. Residuals for each trait and cohort were evaluated for normality and homogeneity of variance (Shapiro-Wilke and Levene's tests, respectively) using the R package *car* [25]. One-way ANOVAs were performed to evaluate the significance of the responses to the control and each of the heat treatments. Follow-up Tukey's tests were performed to determine significance of mean trait values amongst the three treatments using the R package *agricolae* [26].

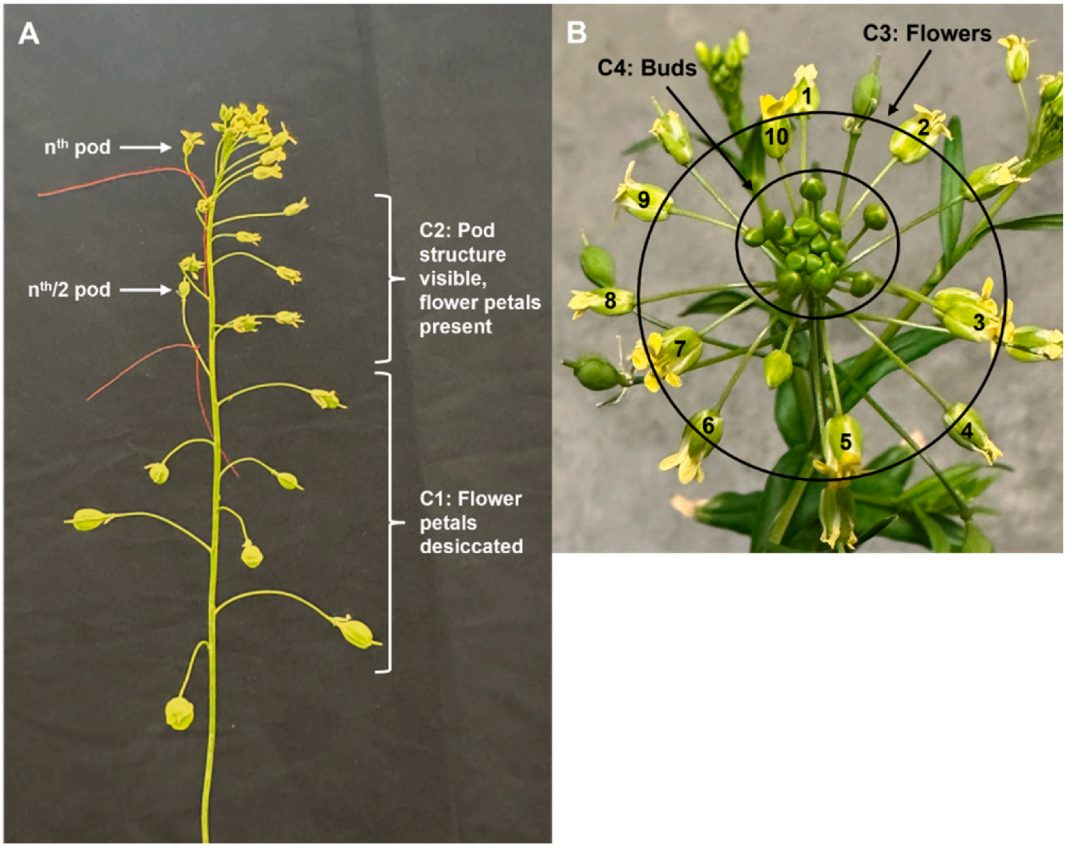


Fig. 1. Pod cohorts on the main stem of a camelina plant. (A) Cohort 1 consists of adult pods and corresponds with the seed filling stage; flower petals desiccated, enlarged pod. Cohort 2 consists of youth pods; pod structure visible, flower petals present. (B) Cohort 3 consists of flowers; petals visible, no pod structure present. Cohort 4 consists of flower buds and all other buds that will form thereafter; flower enclosed, no petals visible.

2.4. Trait measurements

At maturity, stem length was measured as the distance between the node of the first pod to the top of the stem. Pods and seeds were collected from the main stems of each plant and dried at 43 °C for five days. Only the main stem was evaluated in this study. Pods and seeds were scanned with an Epson Perfection V600 Photo Scanner at 600dpi. For image scanning, pod halves, lying flat on the surface, and seeds were spread uniformly so that no individual pods or seeds were in contact. Images for pods and seeds were taken separately and analyzed for morphological traits using the high-throughput seed measurement software SmartGrain [27]. Within the software, a common scale was defined, and the visible surface area of pods and seeds was extracted and used as phenotypic data. All collected seeds from each plant were weighed using a balance (AE100, Mettler).

2.5. Pollen sterility test

Three replications of each genotype were exposed to control and heat stressed environments separately. In the heat environment, flowering plants underwent a 37 °C heat treatment for 3 h. Three to six flower buds prior to opening were collected at the end of the treatment period and immediately stored at −20 °C. Flower buds from the control environment were collected and stored in the same way. For staining pollen grains, buds were removed from storage, and anthers were pressed onto microslides. 1–2 drops of 1% acetocarmine solution were applied to the slides, and cover slips were added for viewing pollen grains under an optical microscope (Axioscope 5, Zeiss). One hundred randomly selected pollen grains were counted, and the percentage of sterile pollen grains was recorded. Sterile pollen grains were un- or lightly stained by the acetocarmine solution, while fertile pollen grains were stained red. Student's t-tests were performed to evaluate significance between mean values.

2.6. Seed oil and protein analysis

Seed oil contents (% dry weight) and fatty acids (% total oil content) were extracted and quantified with GC-MS using an Agilent 7890A GC with C15:0 (pentadecanoic acid) as an internal standard according to Ref. [28]. Total seed oil contents were also quantified using an Oxford Instruments MQC benchtop NRM analyzer. Total nitrogen (TN) and total carbon (TC) from 2 to 3 seeds (~2–4 mg) were measured using a Costech ECS 4010 combustion analyzer. Protein content (% dry weight) in seeds was estimated by multiplying TN by 5.5 [29]. Fatty acid and elemental analysis were conducted with three technical replications.

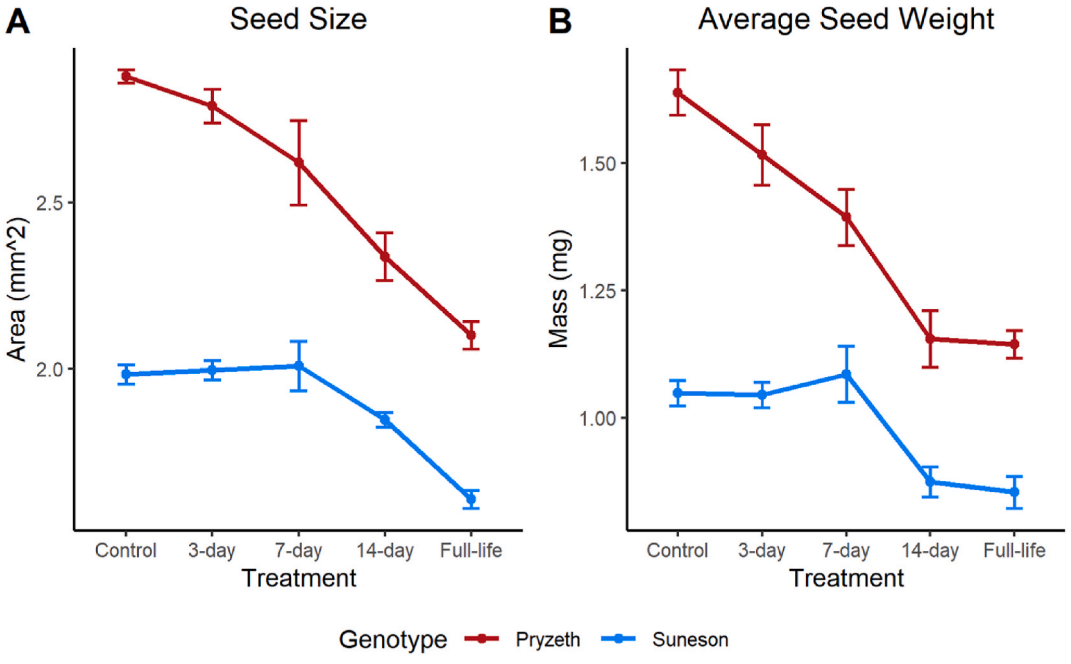


Fig. 2. Treatment duration responses of seed size (A) and average seed weight (B) for Suneson and Pryzeth. Treatments include control, 3-day heat stress, 7-day heat stress, 14-day heat stress, and heat stress through maturity (full-life). Error bars indicate standard error using n = 3 samples.

3. Results

3.1. Effects of heat durations on camelina seed productivity

To evaluate the impacts of high temperature during the reproductive stage on seed productivity, camelina plants entering the flowering stage were moved to the heat chamber for 3, 7, and 14 days before returning to normal growth chamber and growing into maturity. One group of plants were kept in the heat chamber for their remaining growth periods. We examined pod and seed characteristics, e.g., pod and seed sizes, and seed weight. Seed size and weight were among the most affected traits, which is similar to previous studies in Brassicas [15]. Average seed size and weight decreased significantly with the heat periods (Fig. 2). Suneson saw decreased seed size and weight from 14-day heat treated plants, while Pryzeth produced smaller and lighter seeds from 3 days heat treatment and decreased further as the heat durations increased (Fig. 2A and B). This pilot experiment suggested that the effect of heat on seed productivity depends on the heat stress durations, and that the two varieties respond differently with Suneson being more tolerant. We then decided to treat plants for 14 days in the following experiments for detailed analyses, focusing on main stem traits. We selected 14 days, as at this duration, the maximal effect of heat stress on yield traits could be observed without irreversibly damaging the plants.

3.2. Heat stress reduced seed productivity on the main stem

For traits encompassing the entire main stem, we measured stem length (STL), total pod number (TPN), total seed number (TSN), and total seed weight (TSW). Heat stress impacted most traits examined, and the effects were similar between the two separate experiments (Heat 1 and Heat 2). The main stem length was significantly reduced, and the magnitude of this reduction was larger in Pryzeth (25.6%) than in Suneson (19.3%) (Table S1). Other main stem traits in Pryzeth also decreased by a larger magnitude compared to Suneson (Fig. 3A–C). TSW was the most affected of all traits examined in both genotypes (Fig. 3C). In Suneson, TSN and TSW decreased by 8.1% and 22.0%, respectively. In Pryzeth, these same traits decreased by 29.9% and 43.7%, respectively.

TPN also decreased, but not significantly, by 7.2% in Suneson, while TPN decreased significantly by 21.2% in Pryzeth. We also

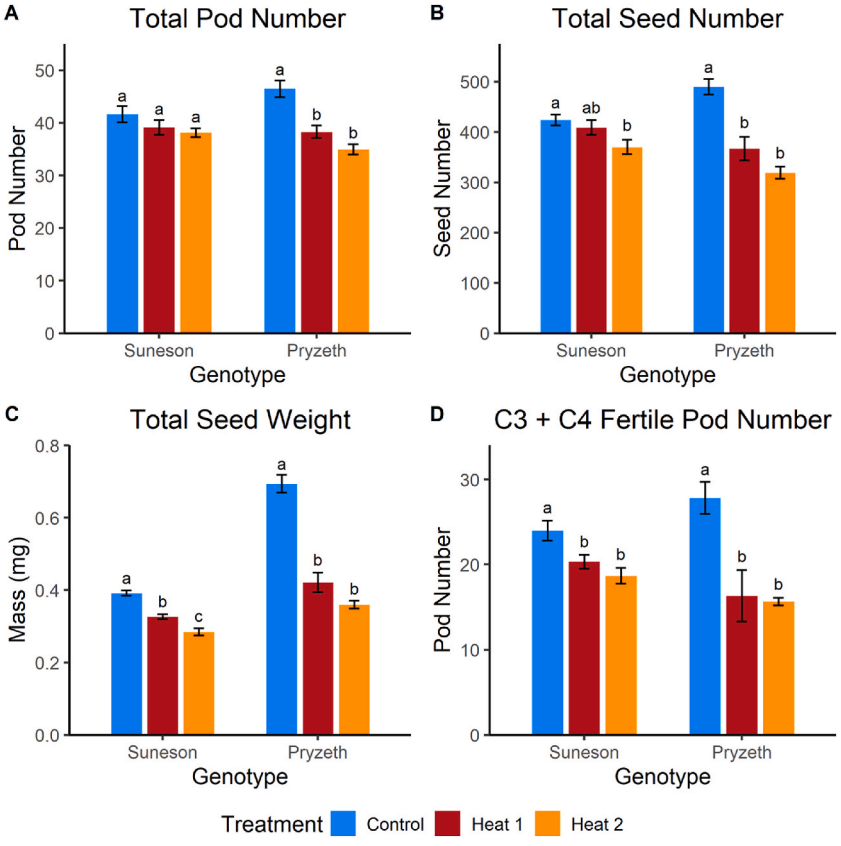


Fig. 3. Whole stem traits in plants in Set 1 (Control and Heat 1) and Set 2 (Heat 2). (A) Total pod number (TPN), (B) total seed number (TSN), (C) total seed weight (TSW), and (D) C3 + C4 fertile pod number (FPN) for Suneson and Pryzeth. Treatments consist of both replications from Set 1 (Control and Heat1) and the heat-stressed replication from Set 2 (Heat2). Error bars indicate standard error using n = 6 samples. Letters indicate group significance determined by Tukey tests.

counted the number of fertile pods (FPN) that contained at least one seed in cohorts 3 and 4, which only contain open flowers or closed buds at the initiation of heat treatment. FPN decreased by 18.8% in Suneson, while in Pryzeth it decreased by 42.5% (Fig. 3D). The formation of fertile pods reflects successful fertilization. Thus, these results suggested that fertility was reduced by heat stress during reproductive development. Pryzeth was more sensitive to heat than Suneson.

3.3. Heat stress increased pollen sterility

The significant decrease in FPN by heat stress prompted us to examine pollen viability. Percentages of sterile pollen grains were calculated (Fig. 4). In Suneson, no significant differences in pollen sterility were detected (*p-value* = 0.43). In Pryzeth, we detected a significant difference in pollen sterility between environments (*p-value* = 0.03). Pollen sterility increased from 1.6% in the control environment to 15.5% in the heat stressed environment. The different pollen sterilities between the two genotypes may explain why Pryzeth produced much fewer fertile pods than Suneson under heat stress.

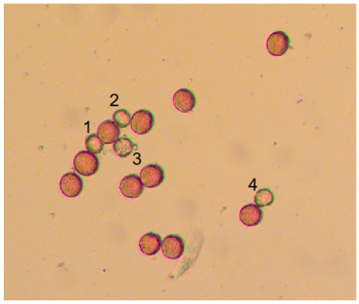
3.4. Heat stress had different effects on seed productivity in each cohort

To further evaluate how heat impacts seed yield in camelina, we measured seed area (SA), average seed weight (ASW), pod area (PA), and seeds per pod (SPP). We collected data for these traits separately between cohorts, as each cohort contained pods and seeds at different developmental stages at the onset of heat treatment. Suneson and Pryzeth both showed variable responses to heat depending on pod cohort for key morphological and yield traits (Fig. 5; Table S2 and Table S3). SA decreased in all cohorts for both genotypes (Fig. 5 A, E). Correspondingly, ASW also decreased significantly in all cohorts for both genotypes (Fig. 5 D, H). C1, consisting of adult pods at the onset of heat treatment, had the largest change in SA and ASW, especially in Pryzeth, decreasing by 11.3% and 25.7% compared to Suneson, decreasing 5.1% and 18.8%, respectively. Reductions of PA (Fig. 5 B, F) and SPP (Fig. 5 C, G) were found to be more significant in C2 and C3 than in C1 and C4. SPP increased in C4 in Suneson (Fig. 5C). The reduction of PA was predominantly caused by fewer SPP rather than by smaller SA (Table S2 and Table S3), consistent with our previous results that PA and SPP are positively correlated traits [24]. Along with FPN, these traits may potentially be indicators of successful fertilization. C3, consisting of flowers at the start of treatment, was the most impacted cohort for these traits (Fig. 5 B, C, F, G). PA and SPP decreased by 33.3% and 33.7%, respectively, in Suneson, while PA and SPP decreased even more by 47.9% and 60.9%, respectively, in Pryzeth.

3.5. Heat stress changed seed composition

Analysis of seed oil and protein was carried out to assess the impact of heat stress on the main seed storage compounds in camelina (Fig. 6). Total oil content (OC), as determined by NMR, decreased in seeds from all heat-treated cohorts in both genotypes. C1 seeds were the most affected, decreasing by 21.1% in Suneson and 31.9% in Pryzeth (Fig. 6 A, D). These results were consistent with the elemental analysis, as the carbon content showed the same trends (Fig. 6 C, F). Conversely, protein content, derived from nitrogen content, showed opposite trends of significant increases in all cohorts but the largest in C1 of both varieties (Fig. 6 B, E). Suneson accumulated less protein than Pryzeth, increasing by 34.3% in C1. In Pryzeth, protein contents increased by 48.4% in C1.

We further analyzed seed fatty acid composition by gas chromatography. Our pilot experiment indicated that heat stress primarily affected the contents of oleic acid (OA) and its derived polyunsaturated linoleic acid (LA) and α-linolenic acid (ALA) in camelina seeds. Heat stress caused decreased ALA and concomitantly increased OA and/or LA (Fig. S1). We therefore examined these fatty acids (Fig. 7). In C1 and C2, ALA (Fig. 7 C, F) was significantly decreased by 9.1% in Suneson and 13.6% in Pryzeth, while OA (Fig. 7 A, D) and LA (Fig. 7 B, E) increased. ALA and OA in C3 and C4 seeds had mixed responses although LA increased significantly in heat treated seeds. ANOVA results indicated significant differences for ALA in C3 and C4 for Suneson and C3 for Pryzeth, but follow up tests confirmed that neither of the heat treatments were significantly different from the control.



Percentage of sterile pollen grains		
	Suneson	Pryzeth
Control	3.44	1.56
sd	*1.08*	*0.86*
Heat	2.70	15.51
sd	*0.98*	*4.32*
p-value	*0.43*	*0.03**

Fig. 4. Heat stress had different effects on pollen sterility in Suneson and Pryzeth. Sterile pollen grains (1–4) are un- or lightly stained with a 1% acetocarmine solution, while viable pollen grains are stained red. Sd indicates standard deviation. p-value indicates significance at α = 0.05 between environment means as determined by student's t-tests.

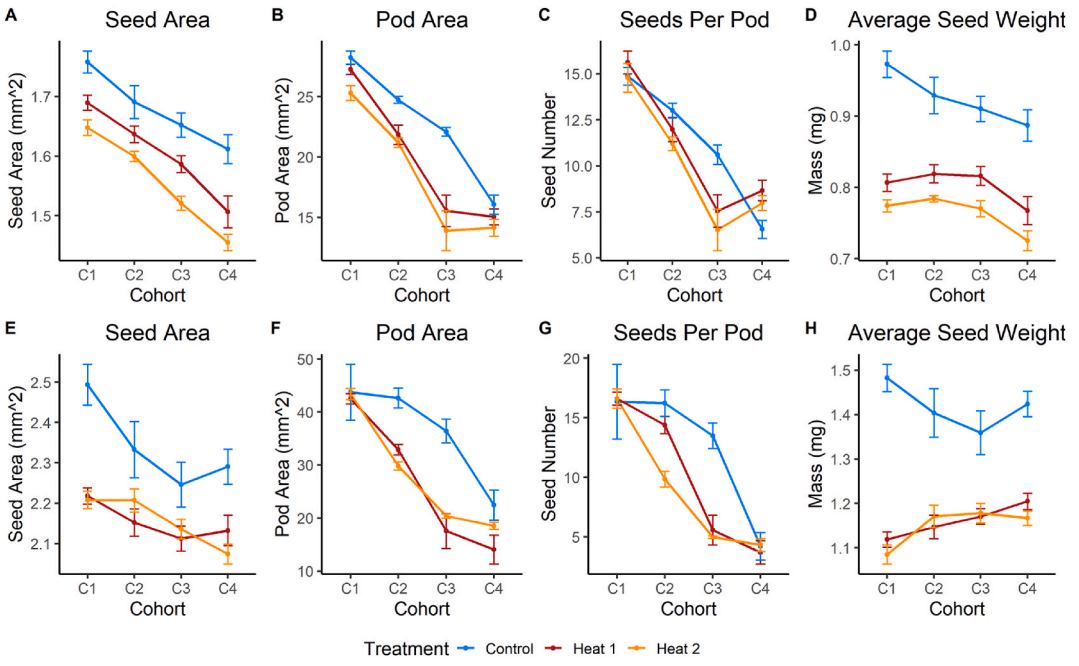


Fig. 5. Cohort responses of morphological and yield components for Suneson (A–D) and Pryzeth (E–H). Treatments consist of both replications from Set 1 (Control and Heat 1) and the heat-stressed replication from Set 2 (Heat 2). Error bars indicate standard error using n = 6 samples.

4. Discussion

Descriptions of the phenotypic response to heat stress have been reported in Brassicas [11,12,16]. However, little is known how camelina responds to heat stress, which presents a serious threat to its sustainable production as global temperature rises. The goal of this research was to evaluate the effects of heat stress during camelina reproductive stages and identify the traits that are most impacted for seed yield. Two wild type camelina genotypes, Suneson and Pryzeth, which have several contrasting phenotypic traits including seed size and oil content [24], were used in this study. Interestingly, the two genotypes also differ in their responses to heat stress. Pryzeth appeared to be more susceptible since its seed size and weight decreased from three days exposure to high temperature while Suneson needed longer duration (14 days) to show significant reductions (Fig. 2). Under the 14-day heat treatment in controlled environments, both genotypes had severely reduced seed production in addition to a range of responses for several agronomic and seed quality traits. The different magnitudes of effects on traits in the two varieties suggested natural genetic variation of heat tolerance in the camelina germplasm.

Collectively, both Suneson and Pryzeth experienced significant reductions for all measured traits on the main stem under 14-day heat stress. This agrees with previous experiments in *Brassica napus* subjected to a seven-day heat stress with a maximum daily temperature of 35 °C [14]. Interestingly, *B. napus* plant height increased under heat treatment [14], but for both camelina varieties Suneson and Pryzeth, main stem length decreased substantially (19.3% and 25.6%, respectively). The difference in response of plant height or main stem height between the two species may indicate two distinct strategies to cope with heat. Heat stress is most impactful during reproduction, and the reproductive strategies for the two species are distinct even under control conditions. In *B. napus,* it is common that only 45–50% of total flowers develop into fertile (seed-bearing) siliques [14,30]. This is in stark contrast to camelina, which exhibits a flower to fertile pod conversion rate of greater than 90% in this experiment. *B. napus* may produce more flowers to offset a low fertilization rate, while camelina produces fewer flowers and relies on a high fertilization rate to maintain high seed yield. In another oilseed crop flax (*Linum usitatissimum*), phenotypic plasticity is observed in heat-treated plants, where number of flowers and pods increases after treatment has ended, appearing to compensate for reduced flower and pod formation during treatment [13]. In camelina, however, this response was not observed. Rather than producing more flowers and pods, the tolerant genotype, Suneson, exhibited increased numbers of seeds per pod in C4 (Table S2). Camelina appears to adopt a conservative strategy in response to heat, diverting resources to existing developing reproductive tissues rather than developing new tissues altogether. In the more susceptible genotype Pryzeth, this strategy does not aid in recovering yield, as SPP is still reduced in C4 (Table S3). The strategy presented by camelina in this study may be partially attributable to the growth environment, which plants were grown in small pots and were unable to produce more flowers following treatment. With less available room for root growth in small pots and hence shoots, total flower formation on the main stem is limited. Additionally, all plants concluded flowering by the end of treatment, so any attempt to increase flower formation may have been masked by the long (14 days) duration of intense (37 °C) heat.

Dissecting the main stem into cohorts offers a more detailed and specific view into the effects of heat on camelina reproduction. The fertility related traits, pod area (PA) and seeds per pod (SPP), saw the greatest reductions in the third pod cohort (C3). C3, consisting of

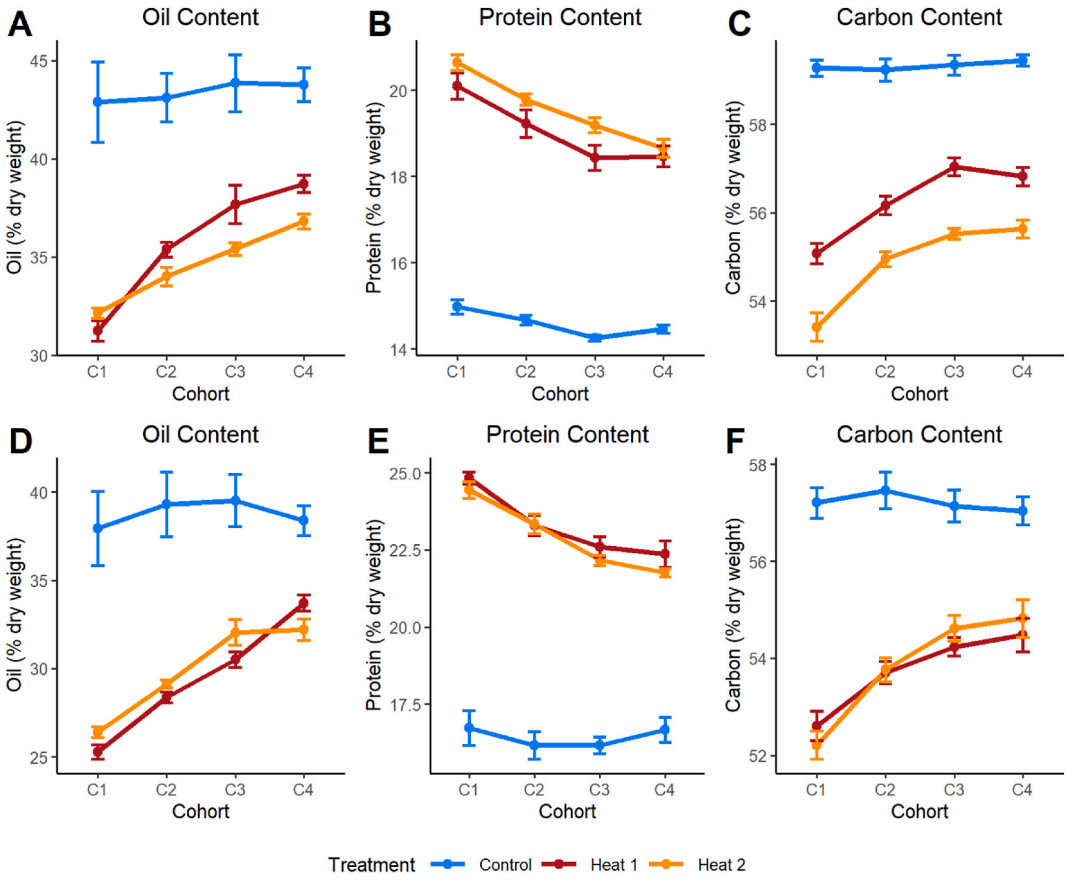


Fig. 6. Cohort responses of oil, protein, and carbon contents for Suneson (A–C) and Pryzeth (D–F). Treatments consist of both replications from Set 1 (Control and Heat 1) and the heat-stressed replication from Set 2 (Heat 2). Error bars indicate standard error.

open flowers at the start of heat treatment, corresponds with fertilization and early embryogenesis. It is well documented that anthesis is a particularly susceptible stage to heat stress. Arabidopsis siliques and seed number were greatly reduced under heat stress [11]. In *Brassica napus*, increased pollen sterility contributed to reduced grain yield and filled pod number in plants exposed to heat at the early flowering stage [19]. Another experiment in the same species highlighted similar findings, where increased pollen sterility corresponded with a higher percentage of sterile pods [17]. The reduced FPN and SPP observed in our experiment indicate that pollen viability or pollen germination may have been impacted by heat. Significant reductions in pollen viability (Fig. 4) in Pryzeth, but not Suneson, align with the phenotypic observations (Fig. 3D). Total pod number, total seed number, and fertile pod number from flowers and buds were more stable across temperature treatments in Suneson, which did not exhibit significant reductions in pollen viability in flower buds. Interestingly, neither genotype was negatively impacted by heat for SPP in C4. This suggests that camelina, if given time to acclimate to the high temperature environment, has some capacity to tolerate heat and maintain pollen viability and successful fertilization.

Seed area (SA) and average seed weight (ASW) decreased in both genotypes under all cohorts, with C1 and C2 being the most affected, indicating that seed filling and storage product synthesis were inhibited by heat stress. This agrees with previous studies where *Brassica napus* plants produced reduced thousand seed weight from heat stress during flowering [31]. The observed decrease of seed weight in *B. napus* may have resulted from inhibited photoassimilate production and translocation to developing seeds [31]. In *Brassica campestris* (Wucai), chlorophyll contents in leaves were reduced after a five-day heat treatment at 35 °C and 40 °C [32]. Similar impacts of heat on photosynthesis were previously shown in camelina, where the Rubisco activation state in leaves decreased from 97% under control conditions to 72% under heat stressed conditions [22]. This reduction in photosynthetic activity was accompanied by reduced individual seed weight and seed number [22]. Camelina pods assimilate up to one-half of seed carbon by proximal photosynthesis, and this latent capacity in the absence of leaves contributes approximately 79% of seed biomass, thus enabling seed filling and maximizing the number of viable seeds [33]. The data evaluated in our experiment agreed with these other findings, suggesting that heat stress inhibits photosynthetic capacity and seed development in camelina. Camelina seed size is determined by both genetics and seed metabolism [34–36]. Under heat stress, reduced photosynthesis and storage oil synthesis may play major roles in seed size and weight determination.

Contrasting the previously discussed seed and pod traits, Oil content appeared to be impacted primarily by the duration of heat

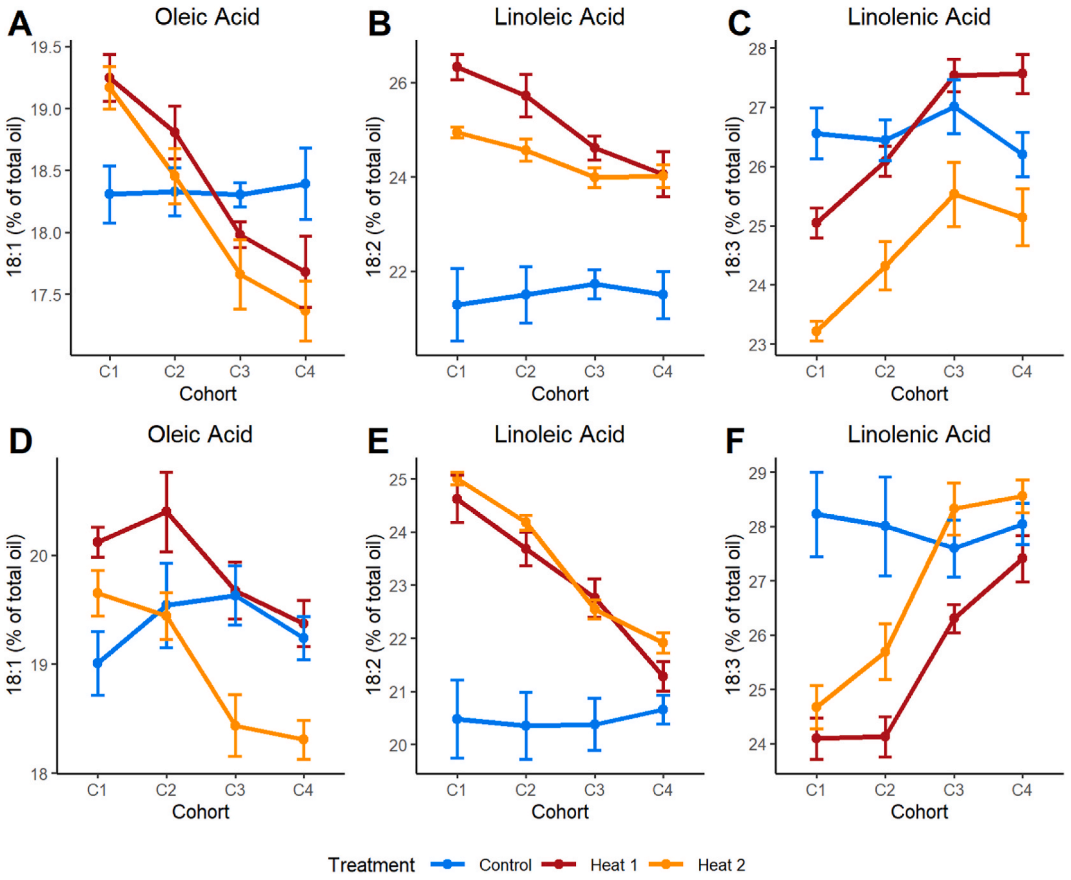


Fig. 7. Composition of the fatty acids oleic acid, linoleic acid, and linolenic acid for Sunseon (A–C) and Pryzeth (D–F). Treatments consist of both replications from Set 1 (Control and Heat 1) and the heat-stressed replication from Set 2 (Heat 2). Error bars indicate standard error using n = 6 samples.

stress during the seed filling stage. Total oil content (OC) was negatively affected most in C1, and to a decreasing degree from C2 to C4 in both genotypes. Protein content (PC), measured from total nitrogen, concomitantly increased from C1 to C4 with decreasing magnitudes (Fig. 6 B, E). The impacts of heat on seed quality components in camelina were consistent with previous findings in related species [3,12,15,16,21,37]. Oil and protein are major storage compounds and their contents in seeds are negatively correlated in Brassica species, indicating a tradeoff between pathways [38]. In camelina, oil and protein content are negatively correlated in both control and heat stressed conditions (Table S2 and Table S3). Under heat, however, more resources may have been diverted to protein synthesis, contributing to reduced OC. The enzymes and pathways for fatty acid and oil biosynthesis were downregulated by heat stress in *B. napus* [21]. Fatty acid composition is greatly influenced by different growth environments with temperature being a major factor. High temperatures inhibited desaturation and reduced polyunsaturated fatty acids (LA and ALA) in oilseeds [16]. Camelina seeds in C1 and C2 saw the greatest reductions in omega-3 α-linolenic acid content and concomitantly increased omega-6 linoleic acid content (Fig. 7). This result reflects the effects of heat on oil synthesis during seed filling and that FAD3 enzymes are thermo-sensitive [39].

5. Conclusions

Camelina sativa is a promising oilseed crop that may provide bioenergy feedstock. As a cool season crop, its sustainable production is threatened by rising atmospheric temperatures. This study is among the first to evaluate the effects of heat stress on seed production in camelina. By exposing plants to high temperatures at the reproductive stages, we measured specific trait responses to heat from morphological, yield, and quality perspectives. We learned that anthesis is a particularly susceptible stage to heat, as fertilization of camelina, measured by lower numbers of fertile pods and seeds per pod, was significantly reduced. Total yield of camelina plants decreased significantly and may be attributed to inhibition of storage product biosynthesis during seed filling, which led to reduced seed size and weight. Seed quality, measured by fatty acid and protein contents, was also changed by heat stress, indicating biochemical acclimation. Importantly, the two genotypes used in this study exhibited different levels of heat responses. This suggests that natural variation is present in the camelina germplasm for heat acclimation. The studies presented here highlight the complexity of camelina responses to heat stress and will assist in designing protocols for future research to understand heat tolerance mechanisms and to improve crop resilience facing climate change.

Data availability statement

Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Brian E. Smith: Writing – original draft, Investigation, Formal analysis. **Chaofu Lu:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26678.

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