Highlights

- Multiple heat stress exposures over two generations modify seed yield and quality in oilseed rape.
- Progeny effects were predominant over Mother effects.
- The most negative heat stress sequences matched the longest stress duration over the two generations.
- The most impacted variables were seed weight, oil, storage capacity and seed dormancy.
- Thermopriming protocols are challenging acclimation strategies due to their fine tuning in terms of stress features.

Original article

Effects of two-generational heat stress exposure at the onset of seed maturation on seed yield and quality in *Brassica napus* L.

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Abstract

Many studies point out the deleterious effect of high temperatures during the crop reproductive stages on seed yield and quality. However, the response of plants to repeated stressing events across plant generations remains poorly investigated, especially in a context of climate change characterized by increased interseasonal variability frequency in terms of spring and summer heat waves. In our study, we attempted to gain a better insight on the effects of repeated heat stresses over two plant generations (i.e. Mother and Progeny plants) on yield components, seed nutritional and physiological quality criteria, under two contrasting sulphur supplies. Results measured in seeds that were at the onset of maturation when the temperature stress was applied indicated that (i) Progeny effects were predominant over Mother effects on most measured variables, thus indicating no intergenerational effects, (ii) the extent of the Progeny effects was modulated by the Mother effects e.g. amplified or attenuated differences on the desiccation tolerance proxy, according to the Mother plant origin, and (iii) the longer the cumulated duration of the temperature stress over the two plant cycles, the more negatively impacted the quality criteria with decreased fatty acids concentration, increased ω6:ω3 ratio, lower desiccation tolerance and increased seed dormancy. Sulphur limitation had little effect on the Progeny responses to heat stress, thus maintaining similar trends to those observed under well-supplied plants. This work provides insights to define thermopriming protocols over multiple plant generations to stabilize or even improve yield and seed quality in a context of stress exposure fluctuations.

Key words: intergenerational effect, oilseed rape, seed quality, high temperature, repeated stresses, priming, stress memory, thermotolerance, sulphur.

Main abbreviations: S: sulphur; C: carbon; N: nitrogen; FA: fatty acid; UFA: unsaturated FA; SFA: saturated FA; ABA: abscisic acid; GA3: gibberellic acid; TSW: thousand seed weight; DW: dry weight.

1. Introduction

Crop adaptation to ever-changing environment has become one of the major issues for agriculture to ensure food safety and maintain or improve the quality of harvested products. The Intergovernmental Panel on Climate Change report (Hoegh-Guldberg et al., 2018) indicates that extreme events, such as heat waves, are expected to become more frequent, to last longer and to increase in intensity (Christidis et al., 2015; Trnka et al., 2014). Therefore the ability to acclimate to repeated and fluctuating environmental stressful conditions has become a challenging objective in crop breeding, not only at the crop cycle but also across generations (Delgado et al., 2011; Henry, 2020; Janni et al., 2020; Wang and Liiang, 2017; Kakoulidou et al. 2021). Nevertheless, in contrast to single-long lasting or single-short extreme/mild environmental stresses (Hassan et al., 2021; Janni et al., 2020; Kotak et al., 2007; Ohama et al., 2016; Wahid et al., 2007; Zhao et al., 2021), repeated stresses remained much less documented from molecular to the whole plant levels.

Within reasonable limits, the ability to resist to a lethal temperature stress can be conferred by exposure to a previous mild nonlethal temperature stress, which induce the ability to survive. This response is known as acquired thermotolerance in the case of temperature stress (Saidi et al., 2011; Sung et al., 2003). It underlies that plants are able to store and retrieve information that they have acquired upon an initial exposure to the stress, which defines stress memory (Crisp et al., 2016; Hilker and Schmülling, 2019). This information acts as a priming process with beneficial effects when the stress recurs and it can lead to earlier, more rapid, intense and sensitive responses that help plants to acclimate in changing environments (Kinoshita and Seki, 2014). This concept was originally developed to describe plant defense mechanisms against biotic stresses (Dowen et al., 2012; Frost et al., 2008; Pastor et al., 2013), such that plants undergoing a second attack by pathogens or herbivores have faster and more robust responses than upon the first exposure. Then, this concept has been extended to abiotic stresses in view of other studies which pointed out that repeated mild stressing events, such as drought, cold, heat or salt stresses, could help stimulating stress memory and alleviating negative effects of a stressing event alone. A large body of literature described the mechanisms that contribute to stress memory, which act at different levels of regulation e.g. epigenetic marks, transcriptional priming, primed conformation of proteins, and/or specific hormonal or metabolic signatures that are maintained after the stress exposure (Bokszczanin et al., 2013; Crisp et al., 2017; Groot et al., 2016; Hatzig et al., 2018; Wang et al., 2016). In the case of heat, it has been found that plants exposed to short periods of mild heat early in their development can later tolerate normally-lethal high temperatures, which is likely to be explained by epigenetic based regulatory mechanism (Sung et al., 2003).

At the plant cycle level, heat stress memory (i.e. intra-generational or somatic memory) has been observed in several crops. In bread wheat, pre-anthesis high temperatures improved carbohydrates remobilization and grain starch accumulation when they faced post-anthesis intense heat stress (Wang et al., 2014) or heat shock pre-treatment during germination reduced seed yield losses in post-anthesis heat stressed plants (Zhang et al., 2016). In maize, exposure to high temperature at the seedling stage was demonstrated to prevent from permanent damage on plants later subjected to a short heat shock (Sinsawat et al., 2004). In winter oilseed rape, effects of heat priming applied at the onset of seed maturation led to higher seed nitrogen content and seed desiccation tolerance for plants later exposed to heat peaks (Magno et al., 2021).

Beneficial effects of priming have also been investigated across generations to assess whether positive effects of a pre-acclimation treatment applied on plants could pass on to the offspring (i.e. inter-generational memory when the memory effect is observed only in the first stress-free generation, or transgenerational memory when observed in at least two stress-free generations, Lämke and Bäurle, 2017). To date, few studies have demonstrated the effects of abiotic stresses across several generations and how stress memory can be transmitted to offspring (Crisp et al., 2016; Hatzig et al., 2018; Kinoshita and Seki, 2014; Kumar, 2018; Molinier et al., 2006; Wang et al., 2016). Most of them characterized responses of *Arabidopsis thaliana* as in Whittle et al. (2009), where high temperature on parental and F1 generations resulted in accelerated flowering and in increased seed yield for plants exposed to heat in the F3 generation. Likewise, accelerated flowering was also observed in F5 *Arabidopsis* plants after two stress-free generations (Suter and Widmer, 2013) and transgenerational effects of mild heat stress were observed over two generations of heat-stressed *Arabidopsis* plants on seed yield (Groot et al., 2017). However, studies of multi-generational heat stress exposure were much less investigated in valuable crops. In bread wheat, heat priming applied at the pre and post-anthesis stages to the first generation of plants resulted in greater grain yield and photosynthesis related parameters in the next generation of plants challenged with post-anthesis stress (Wang et al., 2016). More transgenerational studies on crops were designed to assess the effects of other abiotic stresses, such as heavy metals in rice (enhanced tolerance to mercury of Progenies from stressed Mother plants, Ou et al., 2012) and drought in oilseed rape (positive effects on seedling vigor of seeds from stressed Mother plants, Hatzig et al., 2018), or biotic stresses such as herbivory attacks in tomato plants (decreased caterpillars growth on the subsequent generations from parents subjected to caterpillar feeding, Rasmann et al., 2012).

Because oilseed rape plants display indeterminate growth, different processes will be impacted by heat stress according to its earliness of occurrence during the reproductive stage. While heat stress at flowering limits pollination (Sage et al., 2015) and/or induces early pod abortion resulting in yield losses (Morrison and Stewart, 2002; Young et al., 2004), heat stress that occurs during seed filling and maturation affects seed storage compounds qualitatively and quantitatively , leading to seed quality alteration (Baux et al., 2013; Brunel-Muguet et al., 2015; Magno et al., 2021). In addition, sulphur (S) nutrition impacts yield components and seed quality in Brassica species because of their high S requirements (Scherer, 2001; Postma et al., 1999). Sulphur not only contributes to the synthesis and the signaling of stress tolerance-controlling phytohormones (Hasanuzzaman et al., 2018), but it might also be associated to epigenetic regulatory mechanisms of thermotolerance (Bokszczanin et al., 2013), such as DNA methylation which implies S-adenosylmethionine as a donor of methyl groups (Meng et al., 2018).

In the present study, we analyzed the effects of heat stress on the quality of seeds collected from the second generation of stressed plants (F2, Progeny), also exposed to the same stressing events as the ones applied to the first generation (F1, Mother). The underlying hypothesis is that the history of stressful events experienced by the Mother plants would determine the performances of future generations. Consequently, the monitoring of temperature sequences in terms of stress intensity, duration and frequency, could be a promising strategy to design transmissible thermotolerance protocols, along with adequate S supply in Brassica species.

2. Materials and Methods

2.1. Experimental treatments and growth conditions

Mother plants (cv. Aviso) were grown under greenhouse conditions in 2016-2017 as described in Magno et al. (2021), . They were subjected to five different temperature modalities (Mother-modalities) for 17 days from stage GS72. F1 seeds were provided by the Center of Biological Resources BrACySol (Brassica-Allium-Cynara-Solanum), supervised by the INRAE Center of Rennes (Brittany, France). For the purpose of our intergenerational study, Progeny plants (from F1 seeds) were grown under similar conditions as the ones applied to the Mother plants from October 2018 until July 2019, which included two N applications at the end of vernalization and flowering (100 kg N/ha and 50 kg N/ha respectively) and a single S application at the end of vernalization (75 kg SO_3/ha for the High Sulphur treatment and 25 kg SO_3/ha for the Low Sulphur treatment). In the same way as for the Mother plants (F1), Progeny plants (F2) were subjected to five different temperature modalities (Progeny-modalities) as illustrated in Figure 1a. The complete design resulted in 16 Mother x Progeny stress combinations (Figure 1b), that is, 64 plants for each Sulphur treatment. Because the F1 seeds from the 4LHP Mother-modality were the most negatively impacted, they were not used as a Mother-modality in the Mother x Progeny combinations.

Briefly the different temperature modalities aimed at highlighting any beneficial priming effect of an early mild stress prior to later heat peaks over the crop cycle (Magno et al., 2021). Therefore, we applied similar modalities on the Progeny plants as follows: (i) the Control modality with natural thermoperiod conditions, (ii) the Early Mild Stress (EMS) modality that included 5 mild stress days [i.e. 25.8°C ±1.4 (day, 16h)/22.2°C ± 1.2 (night, 8h)] followed by 12 days under natural thermoperiod (mean, maximum and minimum temperatures being 16.6°C ±2.4, 20.8°C and 12.4°C, respectively), (iii) the 3 Late Heat Peaks (3LHP) modality that included 14 days under natural thermoperiod followed by 3 mild stress days with one daily heat peak applied for 5 hours (i.e. 31.1°C ±2.6 between 10 am until 3 pm), (iv) the 4 Late Heat Peaks (4LHP) modality that included 10 days under natural thermoperiod followed by 7 mild stress days with one daily heat peak on the last 4 days, and (v) the Priming modality which is the combination of the EMS and the 4LHP modalities.

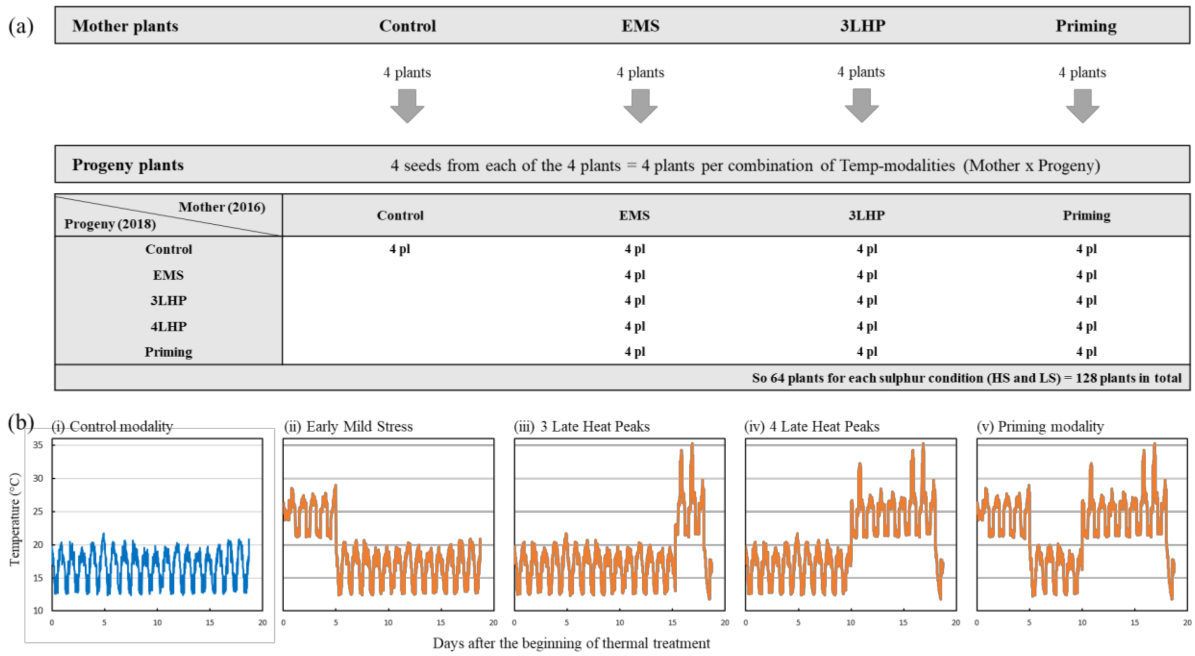

Figure 1: Experimental design which illustrates the 16 Mother x Progeny stress combinations from Mother plants grown in 2016-2017 to the Progeny plants grown in 2018-2019 (Figure 1a). Figure 1b shows the recordings of hourly temperatures in the greenhouse for each temperature sequence applied in 2018-2019. As applied on the Mother plants, two contrasting S conditions (High Sulphur and Low Sulphur) were applied on the Progeny plants (128 plants). Graphs are displayed by level of sequence complexity: (i) Control modality, (ii) Early Mild Stress modality (EMS), (iii) 3 Late Heat Peaks (3LHP), (iv) 4 Late Heat Peaks (4LHP) and (v) Priming modality.

As described in Magno et al. (2021), we distinguished two categories of pods according to their developmental stage at the beginning of the temperature modalities exposure. Indeed, due to their indeterminate growth, oilseed rape plants displayed mixed-aged pods over the 17 day-temperature treatments. Therefore, to avoid any age effect over temperature effect, we collected two categories of pods separately: pods whose length was greater than 5 cm (pods$_{L\geq 5cm}$), and pods whose length was shorter than 5 cm (pods$_{L<5cm}$) following the protocol set up in Magno et al. (2021). When the pods started desiccating, each branch was wrapped with plastic pouches to avoid the mixing of seeds between the different pod categories.

2.2. Seed yield and components

After freeze-drying, the seeds from the two categories of pods were weighed for dry weight (DW) measurements. To determine the Thousand Seed Weight (TSW), we photographed the seeds so as to score their number using image analysis algorithms (ImageJ Software, Schindelin et al., 2012).

2.3. Biochemical characteristics of seeds from pods$_{L\geq5cm}$

In the following sections, biochemical characteristics of each individual plant were measured solely on seeds from pods$_{L\geq5cm}$. Three homogeneous plants out of the four replicates were harvested and used to perform the analyses (*n*=3). After harvest, seeds from pods$_{L\geq5cm}$ of each individual plant were pooled and lyophilized for 48h. Then, seeds were manually ground and the resulting powder was used to perform the biochemical analyses, in different amounts as indicated in the following detailed protocols.

2.3.1. Seed C/N/S concentrations and protein content

Seed powder (around 3 mg per plant) was placed into tin capsules for elemental analysis. The percentage of carbon, nitrogen and sulphur was determined with a C/N/S analyzer (EA3000, Euro Vector, Milan, Italy) linked to a continuous flow isotope mass spectrometer (IRMS, Isoprime, GV Instrument, Manchester, UK). Protein content was calculated using the nitrogen-to-protein conversion factor of 5.5 (Ezeagu et al., 2002).

2.3.2. Seed fatty acid concentrations and profiles

Oil content and fatty acids profiles were determined as described in Marchand et al. (2016). Approximately 30 mg of seeds powder from each plant were suspended in 1 mL of methanol/toluene/H_2SO_4 solution (100:20:2.5; v/v) containing C17:0 as internal standard (25 µg mL^{-1}), remaining 1h at 80°C for transmethylation. After cooling, 750 µL of hexane and 1.5 mL of water were added, and the hexane phase containing the resulting Fatty Acid Methyl Esters (FAMES) was recovered for gas chromatography analysis combined with flame ionization detection (GC-FID). The FAMES (1 µL) were injected into an Agilent 7890A gas chromatograph equipped with a DB-WAX column (15m x 1µm, 0.53 mm, Agilent) and FID system. FAMES were identified by comparing their retention times with those of commercial standards (Sigma, St. Louis, MO) and quantified using ChemStation (Agilent) to calculate the peak areas.

2.3.3. Seed soluble sugar concentrations

Soluble sugars were extracted from 50 mg of seeds powder under water, with three stages of water bath and centrifugation. A solution with 40 µL melicitose was used as the internal standard. Sucrose, raffinose and stachyose contents were identified using High Performance Liquid Chromatography (HPLC), and quantified using a refractive index detector (2410 Differential Refractometer, Millipore Waters, Waters Corporation, MA, USA) as described in Magno et al. (2021).

2.3.4. Seed dormancy-related phytohormones

For each treatment, 50 mg of seeds powder were mixed with 500 µL of extraction solvent [2-propanol/H_2O/ concentrated HCl (2:1:0.002, v/v/v)], and then analyzed by HPLC-MS as described in Pan et al. (2010).

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2.4. Germination time courses at sub (5°C) and optimal (20°C) temperatures

Because the requirements for the germination tests (2 temperatures x 50 seeds per replicate) were substantial, we used the seeds from $\text{pods}_{L<5cm}$ which could provide enough material. Their developmental stage during the 17 day-stress period (younger stage compared to seeds from $\text{pods}_{L\geq5cm}$) has been considered for the results interpretation. For each tested temperature, 3 replicates of 50 seeds of each Progeny plants were disposed homogeneously in a Petri dish, on a double layer of blotting paper and with 10 mL of osmotic water (Ling et al., 2015). The Petri dishes were sealed with parafilm to maintain constant moisture. They were put in a growth chamber (Binder KBWF 720, BINDER GmbH, Tuttlingen, Allemagne), monitored at 5 or 25°C and 80% of humidity. Both temperatures were chosen to track the germination time courses under a sub-optimal temperature (i.e. 5°C) and an optimal temperature (i.e. 25°C). Observations and scoring of germinated seeds were regularly carried out in the light, as prior tests were performed to ensure that the seeds were not photosensitive (data not shown). The Gompertz function was fitted to the germination rates for each replicate batch of 50 seeds:

$$G(t) = a \exp(-\frac{b}{c}. \exp -ct)$$

Were *G(t)* is the germination percentage at t time (in hour) after sowing. *a*, *b* and *c* are three parameters of the model: *a* refers to the maximum germination rate, *b* and *c* are shape parameters of the simulated curve (Brunel et al., 2009). This function is an adjustment model usually used to simulate the growth of living organisms (Brunel et al., 2009; Dantigny et al., 2007). Then germination times were deduced and used to compare Progeny-plants germination behavior. Statistical analyses were performed with Rstudio (R version 4.0.3 Rstudio, PBC) for the final germination rates and for 3 germination times (i.e. T25, T50 and T75, which correspond to the time to observe 25, 50 and 75% of germinated seeds respectively).

2.5. Statistical analysis

Both sulphur conditions (i.e. High Sulphur and Low Sulphur) results were analyzed separately. To highlight the intergenerational effects, two-way ANOVAs to test the Mother and Progeny temperature stress (i.e. the Mother-modalities and the Progeny-modalities) effects were performed on the measured variables, both considered as independent factors. Residues independence and normality, and homogeneity of variances were tested before performing the ANOVAs (using Dubin-Watson, Shapiro-Wilk and Bartlett tests respectively). Mother-modalities, Progeny-modalities and Mother x Progeny interactions effects were analyzed using R software (version 4.0.2, R Core Team, 2020), following a Type III two-way ANOVAs. To have a complete and balanced model, the [Mother-control x Progeny-control] combination has been left out, as the Mother-control was only combined to Progeny-control. As interaction effects were not observed in this experiment, Tukey tests were performed for the significant factor (i.e. Mother and/or Progeny temperature stress effect) among the 5 Progeny-modalities or among the 4 Mother-modalities (Tables 1, 2, 3 and 4), also taking in account the [Mother-control x

Progeny-control] treatment. In addition, we performed mean comparison tests among Progeny-modalities for a given Mother-modality (Supplemental Data, Tables 1 and 2). Different Progeny-modalities rankings mean that the Mother-modality modulates the effect of Progeny-modality.

3. Results

Because S supply is a secondary factor in this study, the results under the Low Sulphur condition are commented after the results under High Sulphur condition, and the tables and graphs are given in Supplemental Data. In doing so, we meant to simply observe to what extent S limitation modifies the plant performances under heat stress.

3.1. Yield components

Table 1 displays total yield and yield components (i.e. seed number and TSW) for the whole plant seeds and for both categories of seeds collected separately (from $pods_{L\geq5cm}$ and $pods_{L<5cm}$) under the High Sulphur condition. No significant Progeny x Mother interaction effect was observed for any of the yield-related variables. Total seed yield among the Progeny-modalities ranged from 5.53 g $plant^{-1}$ (Progeny-priming) to 7.92 g $plant^{-1}$ (Progeny-control) with a significant Progeny effect ($p<0.001$). The decrease in the total seed yield was only significant for the Progeny-priming (-30% relative to Progeny-control) as a consequence of the seed yield penalties for $pods_{L<5cm}$ which was important in this treatment (-43% relative to Progeny-control) and globally affected by all the Progeny-modalities ($p<0.001$). A significant Progeny effect was also observed on TSW for total pods and both pod categories. Decreases in the TSW were observed for all the Progeny-modalities compared to the Progeny-control, with the Progeny-priming modality being the most penalizing modality (-29%, -32% and -27% for total pods, $pods_{L<5cm}$ and $pods_{L\geq5cm}$ respectively from the Progeny-control value). No Mother effect was observed except for TSW from $pods_{L\geq5cm}$ ($p<0.05$), with the Mother-EMS being 22% lower than the Mother-control. Regarding the Progeny-modalities ranking for a given Mother-modality under High Sulphur condition (Supplemental Data, Table 1), no difference in rankings were observed for total seed yield nor total TSW, as for every Mother-modality the Progeny-priming and the Progeny-control were respectively the most and the less penalizing modalities. For seeds from $pods_{L\geq5cm}$ differences can be observed for Mother-3LHP, with Progeny-control having the lowest values (along with Progeny-priming and Progeny-EMS for seed yield from $pods_{L\geq5cm}$). For seeds from $pods_{L<5cm}$, different rankings among Progeny-modalities were observed for seed yield and TSW i.e. lowest seed yield for Progeny-priming under Mother-EMS and Mother-priming only, and lowest TSW for Progeny-priming under Mother-3LHP and Mother-priming only.

As observed under the High Sulphur condition, no significant Progeny x Mother interaction effect was observed for any of the yield-related variables under Low Sulphur condition (Supplemental Data, Table 3). Total seed yield among the Progeny-modalities ranged from 5.60 g $plant^{-1}$ (Progeny-priming) to 7.25 g $plant^{-1}$ (Progeny-control) with a significant Progeny effect (*$p<0.001$*). The decrease of seed yield under the temperature modalities resulted from decreased yield of seeds from $pods_{L<5cm}$ (*$p<0.01$*). Seed number (i.e. total, $pods_{L<5cm}$ and $pods_{L\geq5cm}$) were not impacted by the Progeny nor by the Mother modalities, although the total seed number displayed a significant Mother effect (*$p<0.01$*) with the Mother-priming having the highest value (+30% from the Progeny-control value). A significant Progeny effect was observed on TSW for total pods and both pod categories (*$p<0.001$*) and a Mother effect was also observed for total pods and $pods_{L<5cm}$ (*$p<0.001$*, Supplemental Data, Figure 1). Both Progeny and Mother effects led to decreased TSW with the Mother-priming and Progeny-priming being the most penalizing sequences (Supplemental Data, Table 3). Regarding the Progeny-modalities ranking for a given Mother-modality under Low Sulphur condition (Supplemental Data, Table 2), differences in rankings were observed for total seed yield under Mother-EMS and Mother-3LHP, with the Progeny-priming being the most penalizing modality. The Progeny-priming modality was also the most impacting on TSW whatever the Mother-modalities. As for the total seed number, Progeny rankings were different only under the Mother-priming modality, with Progeny-EMS having the lowest value. For both pods categories, the Mother-priming and Mother-3LHP modalities impacted the Progenies ranking for TWS while the Mother-EMS modality only affected TSW of seeds from $pods_{L\geq5cm}$.

3.2. Nutritional seed quality criteria

3.2.1. Seed C and S concentrations

Under High Sulphur condition, the seed C and S concentrations (Table 2) were slightly impacted by Progeny-modalities (Table 2, $p<0.05$ and $p<0.01$, respectively), but neither Mother nor Progeny x Mother interaction effects were not observed. The Progeny-priming modality displayed the lowest C concentration (5% lower than the Progeny-control), whereas the Progeny-EMS modality displayed the highest value. Adversely, the seeds from the Progeny-priming modality plants displayed the highest seed S concentration (32% higher than the Progeny-control). Regarding the Progeny-modalities ranking for a given Mother-modality under High Sulphur (Supplemental Data, Table 1), rankings among Progeny-modalities differed under Mother-3LHP and Mother-EMS modalities. For C concentration, while Progeny-control displayed the highest value among the Progeny-modalities for the Mother-3LHP, its value was the lowest among the Progeny-modalities for the Mother-EMS.

As observed under the High Sulphur condition, only a Progeny effect was observed on C and S concentrations under the Low Sulphur condition (Supplemental Data, Table 4). Similarly, the Progeny-priming displayed the lowest C concentration (-7% from the Progeny-control). By contrast, while the Progeny-priming significantly displayed the highest value in S under High Sulphur condition, the Low Sulphur condition alleviated the increase under the Progeny-priming and negatively impacted the value under the Progeny-EMS (-19% from the Progeny-control). Regarding the Progeny-modalities ranking for a given Mother-modality under Low Sulphur (Supplemental Data, Table 2), the Progeny-priming was the most negatively impacted modality regarding the C concentration under the Mother-EMS and Mother-3LHP modalities, while it was the Progeny-EMS under the Mother-priming modality. For S concentration, the Progeny-modalities were significantly ranked only under Mother-EMS, with the Progeny-EMS displaying the lowest value (-36% from the Progeny-control).

3.2.2. Seed N and total protein concentrations

Table 2 displays seed N and protein concentrations. Similar to C and S, the seed N and protein concentrations were only affected by Progeny-modalities ($p<0.001$). The highest N and protein concentration values were observed for the Progeny-priming modality (+24% compared to Progeny-control). These observations lead to conclude that the early mild stress had a positive effect over the late heat peaks, which suggested an alleviating effect. Rankings among the Progeny-modalities under High Sulphur condition (Supplemental Data, Table 1) for N concentrations were not changed whether the Mother-modality, with Progeny-priming and Progeny-control seeds having the highest and the lowest values respectively.

As observed under the High Sulphur condition, only a Progeny effect was observed to N and protein concentrations under the Low Sulphur condition (Supplemental Data, Table 4), with the Progeny-priming modality displaying the highest values (+12% compared to Progeny-control). Rankings among the Progeny-modalities under Low Sulphur condition (Supplemental Data, Table 2) for N concentrations were not changed for the Mother-EMS, while Progeny-priming displayed the highest values under Mother-priming and Mother-3LHP.

3.2.3. Fatty acids concentration and profiles

Total FAs, saturated FAs (SFAs) and unsaturated FAs (UFAs) concentrations under High Sulphur condition are displayed in Table 2. The 3 variables were strongly impacted by the Progeny-modalities ($p<0.001$), but no Mother nor Progeny x Mother interaction effects were observed. Total FAs ranged from 11.00 %DW (Progeny-priming) to 28.67 %DW (Progeny-control) among the Progeny-modalities and decreases in total FAs concentration were observed on seeds from all the heat stressed plants compared to Progeny-control, although only the Progeny-priming mean was significantly lower (-61% compared to Progeny-control). The SFAs (including C16:0, C18:0, C20:0, C22:0) concentrations ranged from 1.90 %DW (Progeny-priming) to 2.51 %DW (Progeny-3LHP) and the UFAs (including C16:1, C18:1, C18:2, C18:3, C20:1) concentrations ranged from 15.11 %DW (Progeny-priming) to 26.26 %DW (Progeny-control). Overall, the Progeny-priming modality had the greatest impact on decreasing FAs concentration, as a result of both SFAs and UFAs decreased concentrations, meaning that the early mild stress event did not alleviate the negative effects of the later heat peaks. These decreases are in line with increased proteins concentration as usually observed in oilseed crops for which oil and protein contents are inversely proportional. The ω6:ω3 ratio (i.e. C18:2/C18:3 ratio) is commonly used as an indicator of the health quality of vegetable oils. This ratio was significantly impacted by the Progeny and Mother-modalities (*p<0.001* and *p<0.05*, respectively), but no significant Progeny x Mother interaction effects were observed (Table 2 and Figure 2). The lowest ratios were observed on both Mother-control and Progeny-control modalities. The highest ω6:ω3 ratios were observed for the Progeny-priming (+43% compared to Progeny-control) and for the Mother-EMS (+18% compared to Mother-control). These results indicated that the number of desaturations decreased with greater duration of stress exposure. Whatever the Mother-modality, ranking among Progeny-modalities under High Sulphur (Supplemental Data, Table 1) remained unchanged for the total FAs, SFAs and UFAs concentrations, with the Progeny-priming being the most impacting modality (lowest values). Similarly, extreme rankings among Progeny-modalities for the ω6:ω3 ratio remained unchanged with Progeny-priming and Progeny-control having the highest and the lowest values whatever the Mother-modality.

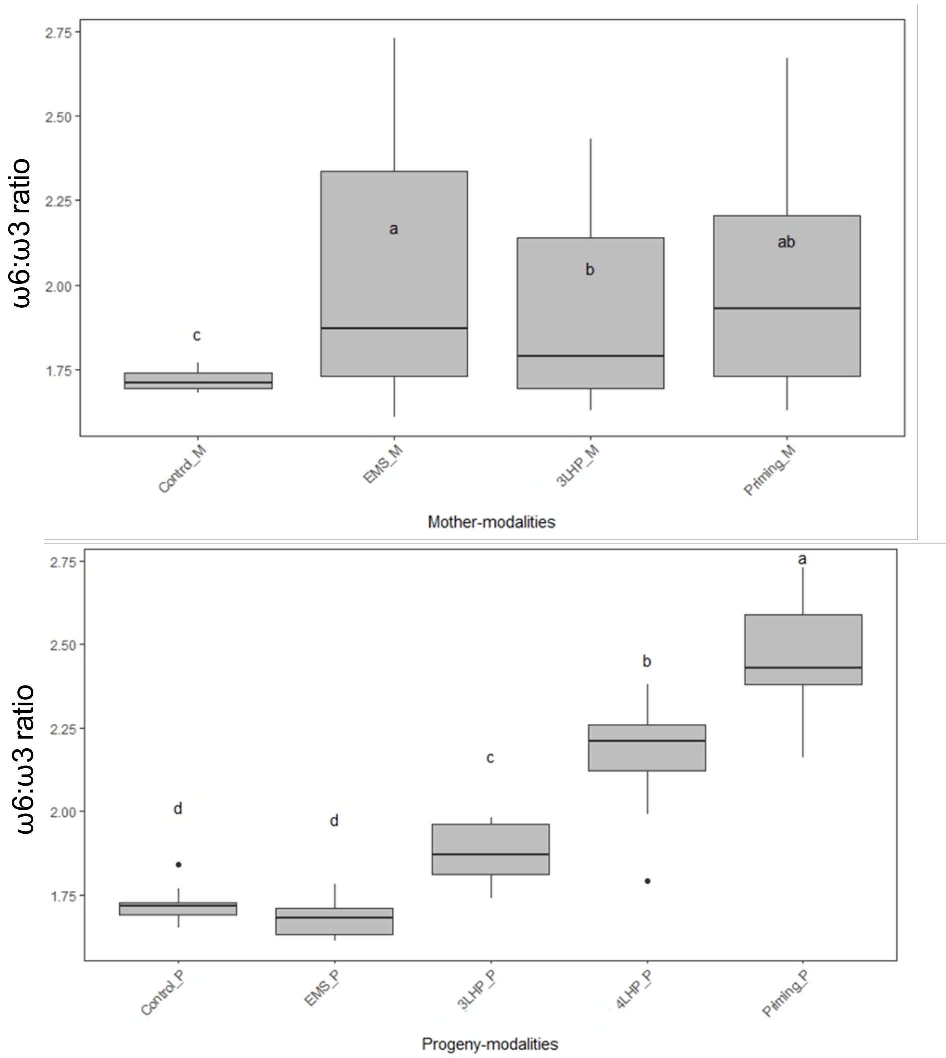


Figure 2: Boxplots of the ω6:ω3 ratio measured in seeds from pods$_{L\geq 5cm}$ under High Sulphur condition among mother modalities (top) and progeny modalities (bottom). This ratio is the main quality criteria impacted significantly by both Mother and Progeny modalities. Letters indicate the ranking among all the treatments (including the control). P-values and levels of significance are given on Table 2. EMS: Early Mil Stress modality, 3LHP: 3 late heat peaks modality, 4LHP: 4 late heat peaks modality.

As observed under the High Sulphur condition, only a Progeny effect was observed on FA, SFA and UFA concentrations under the Low Sulphur condition (Supplemental Data, Table 4), with the Progeny-priming displaying the lowest values (-44%, -24% and -46% compared to Progeny-control, respectively). In addition, the Progeny-EMS also led to significant decrease (-18%, -12% and -19% compared to the Progeny-control, respectively). Under Low Sulphur, the ω6:ω3 ratio was only affected by Progeny-modalities (unlikely to the High Sulphur condition, for which a significant Mother effect was observed). As under High Sulphur condition, the Progeny-control displayed the lowest values and the Progeny-priming displayed the highest values (+49% compared to Progeny-control). Rankings among the Progeny-modalities under Low Sulphur condition (Supplemental Data, Table 2) for FA, SFA and UFA were not changed whatever the Mother-modality, with Progeny-priming having the lowest values, and the Progeny-control and the Progeny-3LHP modalities having the highest values.

3.3. Physiological seed quality-related criteria

3.3.1. Soluble sugar ratio as indicator of desiccation tolerance

Table 3 gives the concentrations of three soluble sugars found in seeds of oilseed rape (i.e. sucrose, raffinose and stachyose) under High Sulphur condition. For all of them, only Progeny effects were observed ($p<0.01$ for sucrose and raffinose, and $p<0.001$ for stachyose). Sucrose concentrations ranged from 2.83 %DW (Progeny-priming) to 3.98 %DW (Progeny-4LHP). The late heat peaks tended to increase the sucrose concentration, as the Progeny-3LHP and Progeny-4LHP modalities displayed the highest values. The lowest sucrose concentrations were observed in the Progeny-priming modality (-12% than in the Progeny-control). Raffinose and stachyose concentrations were the highest in the

Progeny-control and Progeny-EMS modalities. Under the Progeny-priming modality, raffinose and stachyose concentrations were respectively 25% and 53% lower than under the Progeny-control. The [raffinose+stachyose]:sucrose ratio was used as an indicator of drying tolerance i.e. the higher the value, the more tolerant to desiccation (Bailly et al., 2001). Results ranged from 0.21 (Progeny-priming) to 0.39 (Progeny-EMS) (Table 3), thus indicating indicate that whatever the Mother-modality, the Progeny-priming modality was the most negatively impacted for the acquisition of seed desiccation tolerance. When comparing the Progeny-modalities for a given Mother-modality under High Sulphur (Supplemental Data, Table 1), the lowest [raffinose+stachyose]:sucrose ratio was obtained under the Progeny-priming for all the Mother-modalities, but the highest ratio was not obtained for the same Progeny-modality i.e. the 4 other Progeny-modalities (including Progeny-control) for the Mother-priming but only the Progeny-EMS for the Mother-EMS and the Mother-3LHP.

As observed under the High Sulphur condition, only a Progeny effect ($p<0.001$) was observed to the three soluble sugars and the [raffinose+stachyose]:sucrose ratio under Low Sulphur condition (Supplemental Data, Table 5), with the Progeny-priming modality displaying the lowest values. Similarly, the late heat peaks tended to increase the sucrose concentration, as the Progeny-3LHP and Progeny-4LHP modalities displayed the highest values. The rankings among the Progeny-modalities under the Low Sulphur condition were almost similar to those observed under the High Sulphur condition, thus leading to conclude that the thermal stress is far more impacting on soluble sugars than sulphur limitation as applied in our experimental conditions (Supplemental Data, Table 5). When comparing the Progeny-modalities for a given Mother-modality under Low Sulphur (Supplemental Data, Table 2), the lowest [raffinose+stachyose]:sucrose ratio was obtained under the Progeny-priming for all the Mother-modalities.

3.3.2. ABA:GA3 ratio as indicator of temperature-induced seed dormancy

Because the dynamics of ABA and GA3 controls the balance between dormancy and germination, the ABA:GA3 ratio was used as a proxy for seed dormancy under stress condition i.e. a high ABA:GA3 ratio indicates increased secondary seed dormancy, which is induced by thermo-inhibition (Debeaujon and Koornneef, 2000; Finkelstein, 2013). Table 3 displays the values of the ABA:GA3 ratio under High Sulphur condition. As expected, under high temperature this ratio increased, but only Progeny effects were observed ($p<0.01$). The ABA:GA3 ratio ranged from 0.44 (Progeny-3LHP) to 1.66 (Progeny-priming), thus indicating that the most negatively impacting modality (highest ratio) corresponded to the longest cumulated heat days during the Progenies generation. Rankings were different according to the Mother-modality under High Sulphur (Supplemental Data, Table 1). Significant lower values of the ratio were observed for Progeny-4LHP and Progeny-3LHP under Mother-priming, and for Progeny-EMS and Progeny-control under Mother-EMS.

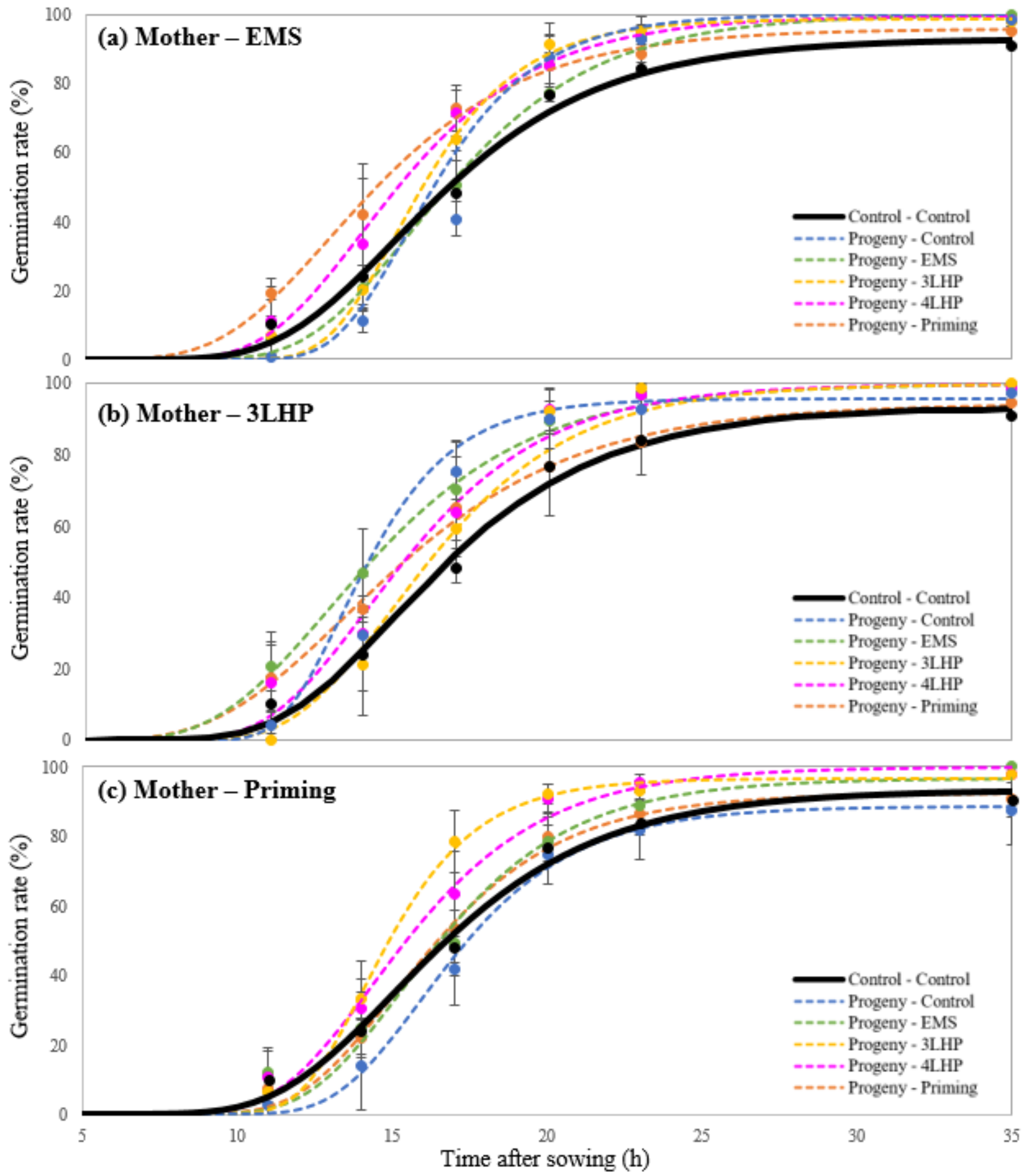
Similar to the High Sulphur condition, only a Progeny effect ($p<0.01$) was observed to the ABA:GA3 ratio under Low Sulphur condition (Supplemental Data, Table 5). When comparing the Progeny-modalities for a given Mother-modality under Low Sulphur (Supplemental Data, Table 2), different rankings can be observed. While under Mother-priming the highest and lowest values were observed on Progeny-3LHP and Progeny-priming respectively, under Mother-EMS the highest and lowest values were observed to Progeny-control and Progeny-EMS respectively, and no differences among the Progenies were observed under the Mother-3LHP.

3.3.3. Germination capacity as indicator of seed vigor

The germination time courses (T25, T50 and T75) and the final rates obtained at optimum (25 °C) and suboptimum (5 °C) temperatures under the High Sulphur condition are presented in Table 4. The final germination percentages were high (over 90%) under both temperatures and only the Progeny effect was significant ($p<0.01$). The short duration temperature stress (early mild stress or late heat peaks) have not affected the final rate, as the Progeny-EMS, Progeny-3LHP and Progeny-4LHP modalities displayed the highest values. However, under both temperatures (i.e. 25 °C and 5 °C) the Progeny-priming displayed the lowest final rates, thus suggesting that the longer the stress exposure, the more negative the impacts on final germination rates. Regarding the time courses, only T25 was significantly affected by the Progeny-modalities at both germination temperatures ($p<0.05$), with the Progeny-priming displaying the lowest values (faster germination). When comparing the Progeny-modalities for a given Mother-modality under High Sulphur (Supplemental Data, Table 6), different Mother effect on Progeny rankings were observed according to the germination temperature. At 25 °C, the Progeny-priming modality displayed the lowest final rates whatever the Mother-modality, followed by the Progeny-control. Differences among the Progeny T25 values were only observed under Mother-EMS, with the Progeny-control and the Progeny-priming displaying the highest and lowest values respectively. For T50, differences among the Progeny-modalities were observed under Mother-priming and Mother-EMS, with the Progeny-control displaying the highest values. The differences in T75 among the Progeny-modalities were only observed under Mother-priming, with the Progeny-control and the Progeny-3LHP displaying the highest and lowest values respectively. In contrast to the results under optimal temperature, the time courses under 5 °C were only affected under Mother-3LHP, with Progeny-EMS displaying the lowest T25, T50 and T75 values. Globally, heat stress under High Sulphur condition accelerated germination time (Figure 3). Although the thermal modalities applied on the Progeny plants were more impacting than those applied on the Mother plants, the history of the Mother plants modified the Progeny-modality effects on the germination time courses and final rates of the seeds from the Progeny plants.

Under Low Sulphur condition, no significant Progeny, Mother nor interaction effects were observed for any germination variables at 25 °C, except for T75 which was impacted by the Mother modalities ($p<0.05$) (Supplemental Data, Table 7). By contrast, under 5 °C T25, T50 and T75 were affected by the Progeny-modalities ($p<0.01$) and a slight Mother effect ($p<0.05$) can be observed on T75. The Progeny-EMS modality displayed the lowest values of T25, T50 and T75, thus suggesting a faster germination. The final rates were only impacted by a Progeny x Mother interaction effect, thus suggesting that the final germination rate was the result of the stress exposure of both generations. When comparing the Progeny-modalities for a given Mother-modality under Low Sulphur (Supplemental Data, Table 8), different Mother effects were observed. At 25 °C, differences in the final rate among the Progenies were observed under Mother-priming and Mother-3LHP, with Progeny-EMS displaying the highest values in both Mother-modalities. Differences in germination times were observed only under Mother-priming, with the Progeny-priming displaying the lowest values to T25, T50 and T75 (faster germination). At 5

°C, the final rates were different under Mother-priming and Mother-3LHP, with lower values for the Progeny-3LHP and the Progeny-control respectively. Differences in T25 and T50 were observed only under the Mother-priming, with Progeny-EMS displayed the lowest values (faster germination). The slowest germination rates (i.e. higher T75) were observed for Progeny late heat peaks modalities irrespective of the Mother-modalities. Globally, heat stress under the Low Sulphur condition accelerated germination to some extent according to the Mother-modalities (Supplemental Data, Figure 8), but these observations were less obvious than under the High Sulphur condition.



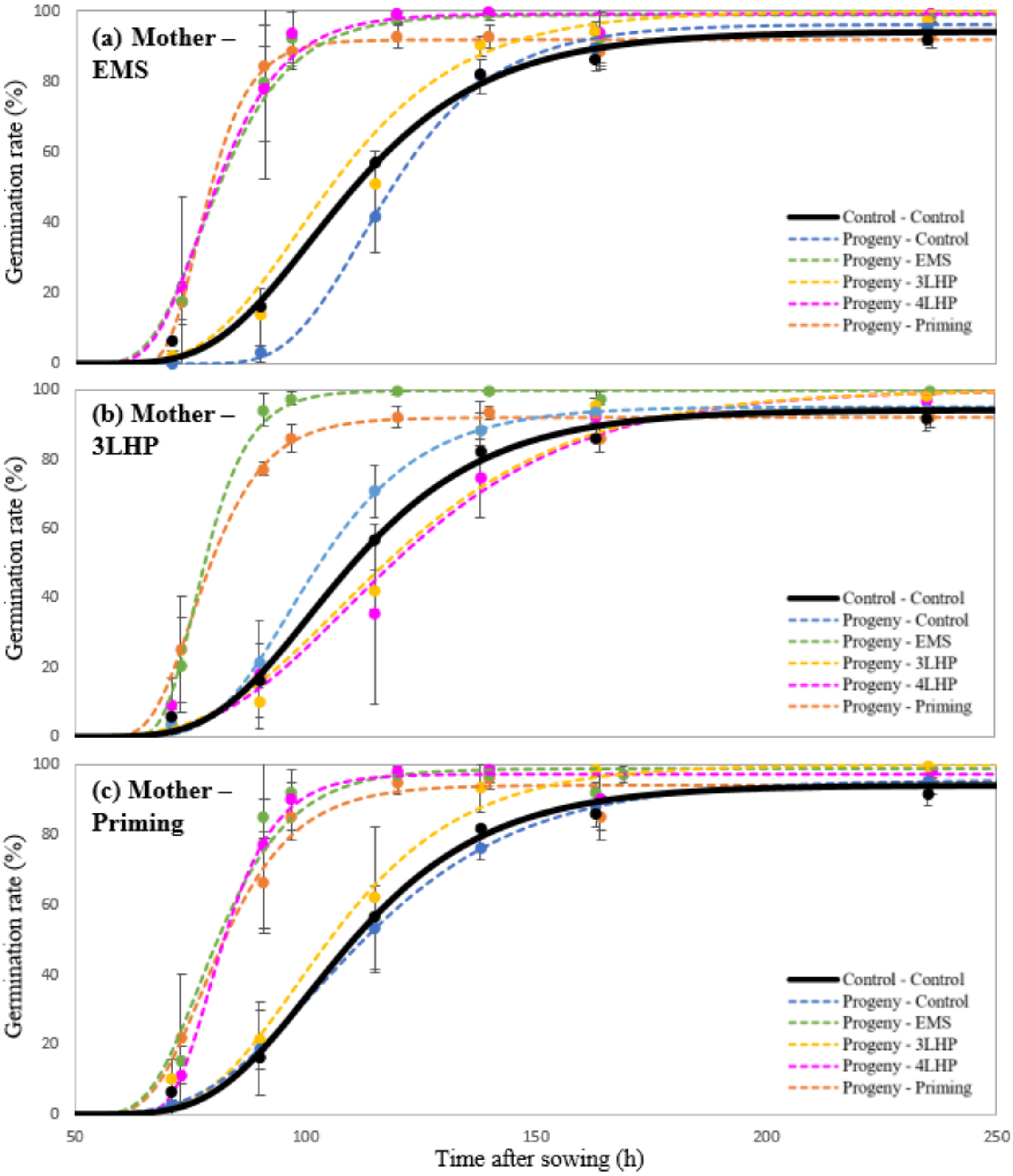


Figure 3: Germination time courses at 25◦C (3 top graphs) and 5◦C (3 bottom graphs) under High Sulphur condition. Each graph illustrates the germination time courses for the 5 Progeny-modalities (including the [Mother-Control x Progeny-Control] combination for reference) for a given Mother modality.. Dots are germination observations and lines are fitted Gompertz functions. Vertical bars denote standard errors. P-values of the germination time courses (T25, T50 and T75) and final germination rates are given in Table 4. EMS: Early Mild Stress modality, 3LHP: 3 late heat peaks modality, 4LHP: 4 late heat peaks modality.

4. Discussion

4.1. Progeny effects overrule Mother effects in most seed quality criteria

Under both sulphur conditions, Progeny effects were predominant over Mother effects on most variables. Overall, our findings suggest that the effects of Mother-modalities (as described in Magno et al., 2021) did not pass on to the next generation, which supports that the stress-triggered modifications at the Mother level were erased. In consistence with Herman and Sultan (2011), Mother effects (i.e. herein transgenerational plasticity) do not entail negative impacts of the history of the Mother plants, such that they could be erased/reset when passed on to the next generation. This was not observed in Groot et al. (2017), as seed size was positively affected by Mother effect and to a much lesser extent Progeny effect. Because of contrasting findings in the literature, fine description of stress sequences i.e. in terms of stress intensity, duration, frequency and synchrony with phenological stages, are required to interpret resetting vs. memory strategies from an adaptive perspective at the light of the metabolic costs that sustain individual and species survival.

Regarding yield components, the Progeny effects were observed on yield and TSW from total seeds and seeds from $pods_{L<5cm}$, and TSW of seeds from $pods_{L\geq5cm}$, with the Progeny-priming being the most penalizing modality under both S conditions. Heat stress during flowering was shown to reduce seed yield as a consequence of alterations of gametogenesis, fertilization and damage on post-fertilization structures in canola (Elferjani and Soolanayakanahally, 2018). Other studies described the trade-off between grains number and grain weight and highlighted a hierarchy in the plasticities of these components such that the grain number was the only component able to adjust to massive stressful changes in contrast with grain weight (wheat, Slafer et al. 2014). By contrast, our results indicated that the number of seeds could not be adjusted anymore at the time of stress exposure whatever the pod categories and that the weight of seeds from $pods_{L\leq5cm}$ were the most impacted by stresses, in line with our previous conclusions (Magno et al., 2021). Under Low Sulphur, the other Progeny-modalities also impacted these seed yield components, but to a lesser extent (Table 1 and Supplemental Data, Table 3). The one-off Mother effects were only observed on TSW of seeds from $pods_{L\geq5cm}$ under High Sulphur, and total TSW and TSW from $pods_{L<5cm}$ under Low Sulphur. These observations suggest that the Low

Sulphur condition applied on the Progeny plants might have delayed flowering and further seed development and maturation, thus leading to Mother effects observed on younger seeds (from $pods_{L<5cm}$) at the timing of heat stress. They also indicate that two consecutive generations of plants growing in a S limiting environment are more susceptible to high temperature stress, thus leading to lower thermotolerance compared to well-supplied plants. In addition, although Mother effects were weak, Mother-modalities impacted the Progenies performances in response to the Priming treatment in different directions (Supplemental Data, Tables 1 and 2). For instance, under the High Sulphur condition, while the Mother-3LHP induced contrasting progeny rankings for seed yield and seed number from $pods_{L\geq5cm}$, the Mother-EMS alleviated the differences among the Progeny-modalities for TSW of seeds from $pods_{L\geq5cm}$ (as the Progeny-priming did not differ anymore from the other Progeny-modalities). Under the Low Sulphur condition, the effects of the Mother-modalities on the Progeny rankings were also observed although distinct Mother-modalities alleviated or exacerbated the Progeny effects among the Progeny-modalities. The lowest total seed yield was also observed for seeds from two generations of plants challenged with the Priming treatment i.e. which underwent the longest cumulated duration of stressing days, as previously shown at the plant cycle level in Magno et al. (2021).

Similar to the yield-related variables, Progeny effects also prevailed over Mother effects on the concentrations of FAs-related variables and N under both sulphur conditions, as a one-off Mother effect was observed on the ω6:ω3 ratio under the High Sulphur condition (Table 2 and Supplemental Data, Table 4). As observed in Magno et al. (2021), the Progeny-priming (i.e. the longest stress duration) was also the most negative to FAs, SFAs, UFAs concentrations (low values) and to ω6:ω3 ratio (high value), which resulted in opposite effects on seed N and protein concentrations which is a well-known negative relationships in oil crops (Si et al., 2003 and references herein; Hammac et al., 2017). These observations confirm that high temperature throughout seed filling induced negative impacts on FAs contents and quality i.e. decreases in poly-UFAs in favor of SFAs and mono-UFAs, thus leading to high ω6:ω3 ratio as a result of temperature-triggered impairment of desaturase enzyme activity (Aksouh-Harradj et al., 2006; Baux et al., 2013; Brunel-Muguet et al., 2015; Gauthier et al., 2017).

In consistence with the observations on yield components and nutritional quality criteria, seed desiccation tolerance, seed dormancy and germination behavior were only impacted by Progeny-modalities. For both sulphur conditions, Progeny values pointed out that the longest the cumulated stress duration (over the two generations), the more negative the effects i.e. low [raffinose+stachyose]:sucrose ratio suggesting low desiccation tolerance, and high ABA:GA3 ratio indicating high seed dormancy. Nevertheless, under Low Sulphur Mother effects were observed on T75 at both germination temperatures along with interaction effects on the final rate at 5°C (Supplemental Data, Table 7). A slight positive effect of short heat stress applied on the Progeny plants (EMS, 3LHP and 4LHP) was observed on the final rate at both temperatures under High Sulphur in contrast to the sulphur limiting conditions, for which the germination times courses were slowed down (Supplemental Data, Table 8, Figure 2), in line with prior studies (Brunel-Muguet et al., 2015; D'Hooghe et al., 2019). Consistently with most prior results, the lowest final germination rates at the optimal temperature were observed for seeds from the modalities combination that matched the longest stress duration over the two plant generations (Supplemental Data, Table 6).

In terms of features of the stress events, the shortest duration combination over the two generations, which also matched with the latest events [Mother-3LHP x Progeny-3LHP] tended to be the least penalizing scenario for the yield and FAs- related variables (except for the ω6:ω3 ratio), which was less remarkable for N concentration, soluble sugars and the ABA:GA3 ratio under High Sulphur. As expected, the longest stress duration over the two generations which also matched with the earliness of stress application [Mother-priming x Progeny-priming] tended to be the most negative, except on N concentration. Under the sulphur limiting conditions, these observations slightly differed as a consequence of a delay in the phenology which impacted younger pods than under the non-limiting sulphur condition (e.g. total seed yield).

4.2. Intergenerational thermopriming protocols as climate acclimation strategy

One outcome of our study deals with the identification of Mother x Progeny stress combinations that penalize or improve yield and quality of seeds from two stressed generations, hence guiding towards thermotolerance acquisition breeding schemes. Figure 4 displays the values of yield components and nutritional and physiological quality criteria for the 15 Mother x Progeny modalities combinations, expressed as the relative difference to the [Mother-control x Progeny-control] combination (i.e. both Mother and Progeny plants were grown under natural conditions). Similar patterns of responses to these combinations were observed for both sulphur conditions, such that the results will be discussed regardless of the S conditions and specifications will be provided when differences are observed.

Regarding yield components, slight increases in total yield were only observed under Progeny-control that derived from Mother-priming or Mother-EMS, which also demonstrate the overruling effect of the Progeny-modalities. The most negative combinations implied either the longest stress duration over the two generations or late stress events which impaired the filling phase of seeds from lately-fertilized flowers. In contrast, the number of seeds increased at the expense of averaged seed weight as usually observed for R-selected species such as the wild relative *Arabidopsis* that favors the dispersal of a large number of seeds which is not predetermined before the reproductive stage as a consequence of adjustments to later stresses (Bennett et al., 2011). This trade-off between seed size and number was particularly exacerbated under our stressing conditions as it was also observed under drought stress (sesame, Najafabadi and Ehsanzadeh, 2017; wheat, Slafer et al. 2014). As previously mentioned, grain number appears to be a coarse-tuning stress-adjustment variable where grain weight might to act as a fine-tuning mechanism.

Under Low Sulphur, the features of the combinations that led to decreased yield and decreased seed weight gathered late events over the two generations which also matched the most intense events. This was likely explained by the delay and slowdown in the phenology induced by sulphur limitation (although moderate) which extended the seed development period until the late heat peaks event, thus resulting in longer heat stress sensitive window and penalties on later-formed pods and seeds.

The total FA concentration was negatively impacted under almost all combinations, with the most pronounced effects being induced by a high cumulated duration (i.e. for Progeny-priming modalities regardless of the history of the Mother plants). Indeed, two combinations under High Sulphur, led to unchanged concentrations i.e. [Mother-EMS x Progeny-EMS] and [Mother-3LHP x Progeny-3LHP] which indicates that the shortest the duration over the two generations, the less impacting. The little impact of an early stress is consistent with the mismatch with timing of seed storage compounds biosynthesis which starts by lipids along with starch (Baud et al., 2002; Borisjuk et al., 2013). As expected, the concentrations in SFA benefited from high temperature as a consequence of the impairment in the desaturase enzyme activity (Menard et al., 2017) with logically more pronounced effects upon late events during the Progeny cycle, regardless of the history of the Mother plants. Therefore, increased values of the ω6:ω3 ratio, which is a negative diet characteristic (Simopoulos, 2006), were more prone to be observed under the late heat peaks modalities. By contrast, an early mild stress on the Progenies, regardless of the history of the Mother plants, slightly decreased the ω6:ω3 ratio, thus improving this targeted characteristic. As expected, due to the extension of the heat stress sensitive window resulting from sulphur limitation, these negative effects on FAs concentrations were exacerbated especially when late heat peaks were applied over the two generations. By contrast with FA concentrations, the Progeny-priming displayed expectedly the highest N concentrations regardless of the history of the Mother plants, under both sulphur conditions. This effect was more pronounced under High Sulphur. The effective priming effect observed on N concentration on the first stressed plants generation (Magno et al., 2021) was not reset under the temperature modalities applied to the offspring, as the values of the different Progeny-modalities from the Mother-priming plants were higher than the [Mother-control x Progeny-control] combination except for seeds from Progeny-control. There might be a slight dilution of the beneficial priming effect, as the value of the seeds from the Priming treatment at the first generation (Magno et al., 2021) was higher than the values of the seeds from the [Mother-priming x Progeny-control] combination. This dilution effect over successive generations was also observed in Groot et al. (2017), where transgenerational effects were usually weaker and less commonly observed than effects observed of the stress applied on the last generation of plants (offspring).

The priming effect observed on the first stressed plants generation for the [raffinose+stachyose]:sucrose ratio used as a proxy of seed desiccation tolerance (Magno et al., 2021) was not observed on the offspring as lower values were observed for all the combinations compared to the [Mother-control x Progeny-control] combination, except for seeds from two generations of plants challenged with early mild stress only under High Sulphur. These results also suggested that the late heat peaks were the most detrimental events to the acquisition of desiccation tolerance under both sulphur supplies, whether they occurred at the first or the second plant generation, as the soluble sugars biosynthesis was likely to overlap the timing of the soluble sugars biosynthesis and/or maturation.

Regarding seed S concentrations under High Sulphur, higher values were mostly observed, meaning that heat stress did not impair sulphur allocation to the seeds while the seed weight decreased. Whether the amount of S in the seeds was assimilated or stored under mineral forms remains to be investigated so as to claim the benefits of heat stress on S-containing proteins (including napins sand cruciferins) which are of interest in high protein-oil cakes used for cattle feeding. Seed dormancy was the most impacted by heat stress at both sulphur conditions although in contrasting ways, since relative increased ABA:GA3 ratios were mainly observed under High Sulphur unlike under Low Sulphur. However, several combinations led to reduce the relative ABA:GA3 ratio, thus breaking seed dormancy, but no straightforward features (in terms of timing, duration nor intensity) could be identified. As observed in Magno et al. (2021), the Progeny-priming led to the highest increases regardless of the Mother-modalities under High Sulphur. Low Sulphur had negative impact on the ratio, which was also observed when comparing the [Mother-control x Progeny-control] modalities under both sulphur supplies. However, heat sequences tended to alleviate the negative impact of S limitation as for most combination, the relative ABA:GA3 ratios increased. This might be another consequence of the delay in the phenology thus making the developing seeds more prone to germinate as already observed under heat stress and eventually to undergo pre-harvest sprouting (Brunel-Muguet et al., 2015).

Finally, the final germination rate remained almost unchanged under both sulphur conditions. The early mild stress applied on the Mother plants led to a slight decrease in final rates at optimal temperature under High Sulphur. Although little effects on relative final rates were observed, germination times courses were much more affected (Supplemental Data, Tables 6 and 7) which is important for base temperature calculations, thus meaning that repeated stresses over several plant generations can lead to changes in the base temperature value usually defined at the species, and sometimes, genotype levels. Overall, increases times courses were observed under heat stress meaning that the germination behavior is tightly related to the history with the seed's ancestors and that their environmental conditions could help interpreting the germination behavior of seedlots produced in the fields.

These results clearly pointed out the complexity of priming effects which are criteria-dependent in addition to stress features-determined. Indeed, as observed in other inter/transgenerational studies, the beneficial effects of prior stress exposures are observed on specific behavior and stage on the progeny. Yadav et al. (2020) demonstrated positive impacts of multigenerational exposure to heat stress at the mature plant-stage (i.e. survival rate under heat stress at bolting) but not at the germination stage in *Arabidopsis*. In addition, although we observed that the offspring performances were mainly driven by the stress sequence applied on the Progeny, the history of the Mother plants leads to different extent of the Progeny responses to heat stress. For some variables, the accumulation of stress days over the two generations leads to amplified negative impact (e.g. seed weight, ω6: ω3 ratio), or to a negative impact while the stress sequence at the Mother plant level had a priming effect (e.g. [raffinose+stachyose]: sucrose ratio). For other variables, the negative effect of the stress sequence applied on the Mother plants disappeared when the Progenies were not stressed (e.g. total fatty acids), which might indicate that the effects were not memorized and rather reset when passed on to the next generation. However, although positive effects of heat treatment across multiple generations could be highlighted on reproductive outputs (e.g. seed production) in *Arabidopsis* (Whittle et al., 2009), in our conditions, no priming effect of a stress sequence applied on the Mother plants, could be observed on the offspring performances when challenged with heat stress.

5. Conclusion

The underlying objective of this work was to pinpoint, in a crop species, whether the temperature stress history experienced by the Mother plants impacted the Progeny responses to heat stress. Our results highlighted several significant results: (i) the Progeny effects overruled the Mother effects on most criteria, but the history of the Mother plants had an impact on the extent of the offspring responses to heat stress; (ii) the effects of heat stress sequences were strongly correlated with the duration of high temperature events over the two generations and they should be interpreted regarding the synchrony between the timing of the stress event and the dynamics of the seed storage compound synthesis; (iii) priming effects observed on the first stressed-plant generation for specific quality criteria (seed N concentrations and soluble sugar ratio used as a proxy of desiccation tolerance) were not systematically maintained (for the proxy of desiccation tolerance only) as a result of negative accumulated effects of late heat peaks or a reset of the stress induced modifications; and (iv) sulphur limitation had little effect on the Progeny responses to heat stress, and when the effects were amplified, it was due to the delay and slowdown in the phenology which resulted in a longer heat stress sensitive window. Interestingly, S restriction led to lower heat stress-induced seed dormancy.

Eventually, these highlights should be bear in mind in the perspective to define multiple generations priming protocols in oilseed rape, and more widely demonstrated the challenge of defining priming-based acclimation strategies due to trade-offs between criteria and the fine tuning (i.e. in terms of heat sequence features and adequate S-fertilization supply to target the desired seed characteristics). In conclusion, our results suggest different processes that support intergenerational memory, as their extent, persistence and impact (positive or negative) vary according to seed characteristics.

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Tables

Factor\Variables	Total pods			Pods L < 5cm			Pods L ≥ 5cm		
	Yield (g.pl^{-1})	Seed number	TSW (g)	Yield (g.pl^{-1})	Seed number	TSW (g)	Yield (g.pl^{-1})	Seed number	TSW (g)
Progeny-modalities									
Control	7.92 a	1838 ns	4.34 a	5.67 a	1347 ns	4.25 a	2.25 ns	491 ns	4.76 a
Early mild stress	6.84 a	1711 ns	4.04 a	4.50 ab	1150 ns	3.90 a	2.34 ns	560 ns	4.31 a
3 late heat peaks	7.53 a	1911 ns	3.98 a	4.70 ab	1239 ns	3.79 a	2.83 ns	672 ns	4.42 a
4 late heat peaks	6.91 a	1813 ns	3.91 a	3.51 b	970 ns	3.64 ab	3.39 ns	842 ns	4.30 a
Priming	5.53 b	1843 ns	3.10 b	3.24 b	1155 ns	2.91 b	2.29 ns	687 ns	3.48 b
se	*0.29*	*116*	*0.14*	*0.34*	*85*	*0.17*	*0.39*	*82*	*0.17*
Mother-modalities									
Control	7.92 ns	1741 ns	4.55 ns	6.15 ns	1394 ns	4.42 ns	1.77 ns	347 ns	5.10 a
Early mild stress	7.17 ns	1951 ns	3.72 ns	4.21 ns	1164 ns	3.61 ns	2.96 ns	786 ns	3.95 b
3 late heat peaks	6.92 ns	1761 ns	3.95 ns	4.14 ns	1100 ns	3.75 ns	2.78 ns	660 ns	4.25 ab
Priming	6.76 ns	1777 ns	3.91 ns	4.54 ns	1243 ns	3.70 ns	2.21 ns	534 ns	4.50 a
se	*0.32*	*91*	*0.15*	*0.40*	*95*	*0.16*	*0.32*	*113*	*0.14*
Progeny effect	**0.000** ***	0.856	**0.000** ***	**0.001** **	0.169	**0.001** **	0.309	0.187	**0.002** **
Mother effect	0.533	0.359	0.375	0.589	0.449	0.797	0.251	0.430	**0.044** *
Progeny x Mother	0.604	0.900	0.600	0.283	0.663	0.544	0.529	0.752	0.815

Table 1: Yield components distinguishing the two pools of pods (i.e. pods$_{L< 5cm}$ and pods$_{L\geq 5cm}$ at the beginning of the temperature stress application). Results are presented by factor (Progeny-modality and Mother-modality) under High Sulphur condition. For a given variable, different letters (Tukey multiple comparisons test) indicate the ranking among Progeny or Mother modalities (including the control). P-values and levels of significance are given for Progeny, Mother and Progeny x Mother interaction effects. Levels of significance: ns non-significant, *p<0.05**, *p<0.01***, *p<0.001****. SE: standard error, TSW: Thousand Seed Weight.

	Seed C, S, N and protein concentrations (%DW)				Oil content and fatty acids (%DW)			
Factor\Variables	**Carbon**	**Sulphur**	**Nitrogen**	**Protein ($mg.g^{-1}$)**	**FA**	**SFA**	**UFA**	**ω6:ω3 ratio**
Progeny-modalities								
Control	47.53 ab	0.34 b	3.30 b	181.47 b	28.67 a	2.40 a	26.26 a	1.72 d
Early mild stress	48.14 a	0.37 b	3.52 b	193.99 b	26.67 a	2.28 a	24.39 a	1.68 d
3 late heat peaks	47.74 ab	0.35 b	3.39 b	186.53 b	27.99 a	2.51 a	25.48 a	1.87 c
4 late heat peaks	46.89 ab	0.37 b	3.56 b	195.87 b	26.05 a	2.49 a	23.56 a	2.16 b
Priming	45.00 b	0.45 a	4.09 a	225.35 a	17.00 b	1.90 b	15.11 b	2.46 a
se	*0.54*	*0.02*	*0.25*	*5.86*	*0.89*	*0.07*	*0.83*	*0.04*
Mother-modalities								
Control	48.53 ns	0.35 ns	3.21 ns	176.71 ns	29.15 ns	2.41 ns	26.74 ns	1.72 c
Early mild stress	46.41 ns	0.36 ns	3.57 ns	196.33 ns	25.00 ns	2.33 ns	22.67 ns	2.03 a
3 late heat peaks	47.52 ns	0.37 ns	3.60 ns	198.25 ns	25.77 ns	2.32 ns	23.45 ns	1.91 b
Priming	47.06 ns	0.39 ns	3.57 ns	196.29 ns	24.96 ns	2.30 ns	22.67 ns	1.99 ab
se	*0.82*	*0.02*	*0.12*	*6.63*	*1.15*	*0.08*	*1.08*	*0.07*
Progeny effect	**0.011** *	**0.008** **	**0.000** ***	**0.000** ***	**0.000** ***	**0.000** ***	**0.000** ***	**0.000** ***
Mother effect	0.269	0.465	0.945	0.943	0.664	0.881	0.631	**0.029** *
Progeny x Mother	0.109	0.941	0.132	0.133	0.256	0.286	0.259	0.589

Table 2: Nutritional seed quality criteria measured in seeds from pods$_{L\geq 5cm}$. Results are presented by factor (Progeny-modality and Mother-modality) under High Sulphur condition. For a given measured variable, different letters (Tukey multiple comparisons test) indicate the ranking among Progeny or Mother modalities (including the control). P-values and levels of significance are given for Progeny, Mother and Progeny x Mother interaction effects. Levels of significance: ns non-significant, *p<0.05*, p<0.01**, p<0.001****. SE: standard error, DW: dry weight, FA: fatty acid, UFA: unsaturated FA, SFA: saturated FA.

Factor\Variables	Soluble sugars: Sucrose (%DW)	Soluble sugars: Raffinose (%DW)	Soluble sugars: Stachyose (%DW)	Soluble sugars: [Raffinose+stachyose] : sucrose ratio	Hormones: ABA:GA3 ratio
Progeny-modalities					
Control	3.23 b	0.20 a	0.99 a	0.37 a	0.58 b
Early mild stress	3.10 b	0.23 a	0.98 a	0.39 a	0.75 b
3 late heat peaks	3.55 ab	0.18 ab	0.97 a	0.32 ab	0.44 b
4 late heat peaks	3.98 a	0.19 ab	0.94 a	0.29 bc	0.65 b
Priming	2.83 b	0.15 b	0.46 b	0.21 c	1.66 a
se	*0.17*	*0.01*	*0.06*	*0.01*	*0.18*
Mother-modalities					
Control	3.18 ns	0.23 ns	1.09 ns	0.41 ns	0.67 ns
Early mild stress	3.31 ns	0.20 ns	0.86 ns	0.32 ns	0.85 ns
3 late heat peaks	3.23 ns	0.18 ns	0.82 ns	0.31 ns	0.55 ns
Priming	3.47 ns	0.19 ns	0.91 ns	0.32 ns	1.00 ns
se	*0.14*	*0.01*	*0.06*	*0.02*	*0.25*
Progeny effect	**0.003** **	**0.008** **	**0.000** ***	**0.000** ***	**0.001** **
Mother effect	0.526	0.493	0.430	0.775	0.212
Progeny x Mother	0.777	0.959	0.547	0.058	0.459

Table 3: Seed physiological quality values measured in seeds from pods$_{L\geq 5cm}$. Results are presented by factor (Progeny-modality and Mother-modality) under High Sulphur condition. For a given measured variable, different letters (Tukey multiple comparisons test) indicate the ranking among Progeny or Mother modalities (including the control). P-values and levels of significance are given for Progeny, Mother and Progeny x Mother interaction effects. Levels of significance: ns non-significant, $p<0.05$*, $p<0.01$**, $p<0.001$***. SE: standard error, DW: dry weight, ABA: abscisic acid, GA3: gibberellic acid.

	Germination at 25°C				Germination at 5°C			
Factor\Variables	**T25 (h)**	**T50 (h)**	**T75 (h)**	**Final rate (%)**	**T25 (h)**	**T50 (h)**	**T75 (h)**	**Final rate (%)**
Progeny-modalities								
Control	14.7 a	16.6 ns	19.6 ns	93 b	97.3 a	111.5 ns	131.1 ns	98 a
Early mild stress	13.4 a	15.8 ns	18.9 ns	99 a	81.9 a	98.9 ns	120.8 ns	98 a
3 late heat peaks	14.2 a	15.6 ns	17.5 ns	98 ab	99.7 a	111.4 ns	126.4 ns	99 a
4 late heat peaks	13.2 a	15.1 ns	17.7 ns	98 ab	100.4 a	112.3 ns	127.7 ns	98 a
Priming	12.8 a	15.5 ns	19.3 ns	93 b	79.3 b	104.7 ns	139.3 ns	94 b
se	*0.5*	*0.4*	*0.5*	*1.0*	*5.1*	*4.7*	*5.3*	
Mother-modalities								
Control	14.1 ns	16.8 ns	20.6 ns	97 ns	95.6 ns	111.4 ns	133.4 ns	96 ns
Early mild stress	13.9 ns	15.9 ns	18.4 ns	95 ns	93.9 ns	109.1 ns	129.1 ns	98 ns
3 late heat peaks	13.1 ns	15.2 ns	18.0 ns	98 ns	90.3 ns	105.1 ns	125.1 ns	97 ns
Priming	14.0 ns	16.1 ns	19.2 ns	95 ns	91.2 ns	109.1 ns	132.6 ns	98 ns
se	*0.4*	*0.4*	*0.5*	*1.4*	*4.3*	*4.5*	*6.1*	
Progeny effect	**0.037** *	0.131	0.063	**0.000** ***	**0.046** *	0.405	0.302	**0.007** **
Mother effect	0.168	0.111	0.178	0.147	0.859	0.765	0.533	0.810
Progeny x Mother	0.226	0.056	0.141	0.254	0.856	0.808	0.687	0.797

Table 4: Germination variables measured at optimal (25◦C) and suboptimal (5◦C) temperatures. Results are presented by factor (Progeny-modality and Mother-modality) under High Sulphur condition. T25, T50 and T75 represent respectively the time (in hours) for 25%, 50% and 75% of germinated seeds, as adjusted from the observations with the Gompertz functions. Final rate is the observed final rate (in percentage). For a given measured variable, different letters (Tukey multiple comparisons test) indicate the ranking Progeny or Mother modalities (including the control). P-values and levels of significance are given for Progeny, Mother and Progeny x Mother interaction effects. Levels of significance: ns non-significant, *p<0.05**, *p<0.01***, *p<0.001****. SE: standard error.

(a)

Temp-modalities		Yield components			Seed nutritional quality criteria								Seed physiological quality indicators			
Mother	Progeny	Total yield	Total SN	Total TSW	Total FA	UFA	SFA	ω6:ω3	C	N	S	Protein	ABA:GA3	SS ratio	Final rate at 25°C	Final rate at 5°C
Control	Control	7,9	1741,7	4,6	29,2	26,7	2,4	1,7	48,5	3,2	0,35	176,7	0,7	0,41	97	96
Priming	Control	+1%	0%	+2%	-1%	-1%	+1%	0%	-3%	-3%	-6%	-3%	+81%	-5%	-6%	+2%
	Priming	-35%	+12%	-40%	-43%	-44%	-23%	+46%	-7%	+25%	+40%	+25%	+212%	-51%	-6%	0%
	EMS	-21%	-11%	-11%	-14%	-14%	-11%	-1%	-2%	+16%	+9%	+15%	+30%	-15%	+3%	+2%
	4LHP	-11%	+2%	-10%	-7%	-8%	+7%	+24%	-3%	+9%	+11%	+9%	-6%	-22%	+1%	+3%
	3LHP	-8%	+8%	-13%	-8%	-9%	+2%	+11%	-1%	+9%	+6%	+9%	-64%	-20%	+1%	+3%
EMS	Control	+10%	+26%	-13%	-2%	-2%	+3%	+2%	-9%	+2%	-6%	+1%	-54%	-10%	-10%	+3%
	Priming	-31%	+3%	-31%	-50%	-52%	-27%	+49%	-8%	+36%	+23%	+36%	+151%	-51%	-2%	-3%
	EMS	-5%	+12%	-14%	0%	0%	+4%	-2%	+1%	-1%	-3%	-1%	-1%	+12%	+3%	+3%
	4LHP	-16%	+10%	-21%	-16%	-18%	-1%	+33%	-5%	+12%	+3%	+12%	+18%	-37%	2%	+4%
	3LHP	-6%	+9%	-13%	-4%	-4%	+6%	+9%	-2%	+7%	+3%	+7%	+19%	-22%	+1%	+4%
3LHP	Control	-10%	-3%	-8%	-5%	-5%	-4%	-2%	+3%	+13%	0%	+13%	-61%	-24%	+2%	+2%
	Priming	-25%	+3%	-25%	-33%	-34%	-14%	+35%	-7%	+22%	+23%	+22%	+48%	-39%	-2%	-2%
	EMS	-15%	-7%	-9%	-12%	-12%	-8%	-2%	-2%	+15%	+9%	+15%	+12%	-10%	+2%	+3%
	4LHP	-12%	0%	-11%	-9%	-10%	+4%	+20%	-3%	+12%	+3%	+12%	-21%	-32%	+2%	+1%
	3LHP	-1%	+13%	-12%	0%	-1%	+5%	+7%	-2%	0%	-9%	0%	-58%	-20%	+3%	+3%

(b)

Temp-modalities		Yield components			Seed nutritional quality criteria								Seed physiological quality indicators			
Mother	Progeny	Total yield	Total SN	Total TSW	Total FA	UFA	SFA	ω6:ω3	C	N	S	Protein	ABA:GA3	SS ratio	Final rate at 25°C	Final rate at 5°C
Control	Control	7,1	1552,3	4,6	29,8	27,4	2,4	1,7	45,4	3,5	0,34	191,1	1,2	0,33	96	98
Priming	Control	+8%	+21%	-11%	-1%	-2%	+2%	+3%	+1%	-8%	-18%	-8%	-28%	-3%	-1%	+2%
	Priming	-14%	+45%	-41%	-41%	-43%	-21%	+51%	-4%	+4%	-18%	+4%	-78%	-36%	-1%	+1%
	EMS	-7%	+13%	-19%	-14%	-15%	-8%	+3%	-6%	+3%	-24%	+3%	-82%	+3%	+4%	+2%
	4LHP	-2%	+28%	-23%	-12%	-14%	+7%	+36%	0%	+2%	-15%	+2%	-79%	-27%	+3%	+1%
	3LHP	+6%	+42%	-26%	+3%	+2%	+15%	+11%	0%	-10%	-24%	-10%	+1%	-18%	+3%	0%
EMS	Control	0%	+17%	-15%	+1%	+1%	+3%	+1%	0%	-1%	+6%	-1%	-26%	+6%	+4%	+2%
	Priming	-27%	+4%	-29%	-43%	-45%	-23%	+53%	-7%	+8%	-15%	+8%	-74%	-42%	+1%	+1%
	EMS	-20%	+1%	-20%	-21%	-22%	-11%	+7%	0%	+3%	-32%	+3%	-78%	-21%	+1%	0%
	4LHP	-2%	+27%	-22%	-19%	-21%	0%	+40%	-2%	+4%	-9%	+4%	-76%	-33%	+1%	-1%
	3LHP	+2%	+33%	-23%	-9%	-9%	-1%	+12%	+1%	0%	-6%	0%	-70%	-18%	+3%	+2%
3LHP	Control	+2%	+8%	-6%	+6%	+5%	+9%	+4%	0%	-3%	-21%	-3%	-61%	-12%	-6%	-1%
	Priming	-21%	+11%	-29%	-46%	-48%	-20%	+51%	-9%	+12%	-9%	+12%	-75%	-45%	0%	+2%
	EMS	-12%	+5%	-16%	-16%	-17%	-9%	+2%	-3%	+5%	-24%	+6%	-84%	+3%	+3%	+2%
	4LHP	-12%	+10%	-20%	-20%	-22%	0%	+43%	-3%	+9%	-6%	+9%	-80%	-30%	+2%	+2%
	3LHP	-13%	-6%	-7%	-3%	-4%	+8%	+18%	0%	-1%	-21%	-1%	-7%	-18%	+1%	+2%

Decrease (-) ← -10% 0 +10% → Increase (+)

Figure 4: Summary of the effects of the combined temperature sequences (Mother x Progeny) in yield components, nutritional and physiological quality criteria under both High Sulphur (a) and Low Sulphur (b) conditions. Numbers in boxes display the relative difference between the stressed modality and the [Mother-control x Progeny-control] modality. As illustrated in the legend, colors indicate the trends by level of increase or decrease. SN: seed number, TSW: Thousand Seed Weight, FA: fatty acid, UFA: unsaturated FA, SFA: saturated FA, C: carbon, N: nitrogen, S: sulphur, ABA: abscisic acid, GA3: gibberellic acid, SS: soluble sugars.

Supplemental Data

Mother-modalities	Control	Priming					EMS					3LHP				
Progeny-modalities	**Control**	**Control**	**Priming**	**EMS**	**4LHP**	**3LHP**	**Control**	**Priming**	**EMS**	**4LHP**	**3LHP**	**Control**	**Priming**	**EMS**	**4LHP**	**3LHP**
Seed yield - pods $L_{\geq 5cm}$ (g/pl)	1.77	2.05 a	2.69 a	2.02 a	2.98 a	1.35 a	3.07 a	2.21 a	2.73 a	3.72 a	3.07 a	2.11 b	1.97 b	2.27 b	3.49 ab	4.06 a
Seed number - pods $L_{\geq 5cm}$ (per pl)	347	402 a	791 a	467 a	701 a	312 a	751 a	704 a	691 a	1017 a	770 a	464 b	568 ab	525 ab	810 ab	935 a
TSW - pods $L_{\geq 5cm}$ (g)	5.10	5.16 a	3.54 b	4.46 ab	4.82 a	4.50 ab	4.20 a	3.28 a	4.17 a	3.72 a	4.40 a	4.58 a	3.64 b	4.31 a	4.38 a	4.37 a
Seed yield - pods $L_{<5cm}$ (g/pl)	6.15	5.90 a	2.47 b	4.26 ab	4.09 ab	5.95 a	5.62 a	3.28 b	4.79 ab	2.98 b	4.37 ab	5.0 a	3.97 a	4.45 a	3.48 a	3.79 a
Seed number - pods $L_{<5cm}$ (per pl)	1394	1333 a	1155 a	1086 a	1079 a	1562 a	1438 a	1088 a	1265 a	904 a	1126 a	1222 a	1224 a	1100 a	927 a	1029 a
TSW - pods $L_{<5cm}$ (g)	4.42	4.57 a	2.38 b	3.90 a	3.74 ab	3.91 a	3.93 a	3.08 a	3.76 a	3.44 a	3.85 a	4.07 a	3.29 b	4.05 a	3.76 ab	3.60 ab
Total seed yield (g/pl)	7.92	7.98 a	5.16 b	6.28 ab	7.07 ab	7.29 a	8.69 a	5.49 c	7.52 ab	6.69 bc	7.45 ab	7.11 ab	5.93 b	6.72 ab	6.97 ab	7.85 a
Total seed number (per pl)	1741	1735 a	1946 a	1552 a	1780 a	1875 a	2190 a	1792 a	1956 a	1921 a	1896 a	1686 a	1792 a	1625 a	1738 a	1965 a
Total TSW (g)	4.55	4.66 a	2.75 b	4.07 a	4.09 a	3.97 a	3.97 a	3.16 b	3.91 ab	3.59 ab	3.97 a	4.19 a	3.39 b	4.14 a	4.05 a	3.99 a
Fatty acids (%DW)	29.15	28.99 a	16.73 b	25.11 a	27.25 a	26.75 a	28.71 a	14.68 b	29.14 a	24.36 a	28.11 a	27.83 a	19.61 b	25.77 a	26.55 a	29.12 a
UFA (%DW)	26.74	26.55 a	14.87 b	22.97 a	24.66 a	24.29 a	26.23 a	12.92 b	26.64 a	21.97 a	25.56 a	25.52 a	17.54 b	23.55 a	24.05 a	26.60 a
SFA (%DW)	2.41	2.43 ab	1.86 c	2.14 bc	2.59 a	2.46 ab	2.48 a	1.76 b	2.50 a	2.39 a	2.55 a	2.31 ab	2.07 b	2.21 ab	2.50 a	2.52 a
ω6:ω3 ratio	1.72	1.72 b	2.51 a	1.70 b	2.13 b	1.91 b	1.76 cd	2.57 a	1.68 d	2.29 b	1.88 c	1.68 c	2.32 a	1.68 c	2.06 b	1.84 bc
C concentration (%DW)	48.5	47.2 a	45.2 a	47.6 a	47.3 a	47.9 a	44.2 c	44.8 bc	49.0 a	46.2 abc	47.8 ab	50.2 a	44.9 b	47.8 ab	47.2 ab	47.5 ab
N concentration (%DW)	3.21	3.11 b	4.00 a	3.71 ab	3.51 ab	3.51 ab	3.26 b	4.37 a	3.18 b	3.59 b	3.45 b	3.62 ab	3.91 a	3.69 ab	3.59 ab	3.21 b
S concentration (%DW)	0.35	0.33 a	0.49 a	0.38 a	0.39 a	0.37 a	0.33 b	0.43 a	0.34 b	0.36 b	0.36 b	0.35 ab	0.43 a	0.38 ab	0.36 ab	0.32 b
Protein (mg/g DW)	176.7	170.8 b	220.3 a	204.1 ab	192.9 ab	193.3 ab	179.3 b	240.5 a	174.9 b	197.3 ab	189.6 ab	199.0 ab	215.2 a	203.0 ab	197.4 ab	176.6 b
[Raff+stach] : suc ratio	0.41	0.39 a	0.2 b	0.35 a	0.32 a	0.33 a	0.37 b	0.2 d	0.46 a	0.26 cd	0.31 bc	0.31 ab	0.25 b	0.37 a	0.28 ab	0.33 ab
ABA:GA3 ratio	0.67	1.21 ab	2.09 a	0.87 ab	0.63 b	0.24 b	0.31 b	1.68 a	0.66 b	0.79 ab	0.79 ab	0.25 a	0.99 a	0.75 a	0.53 a	0.28 a

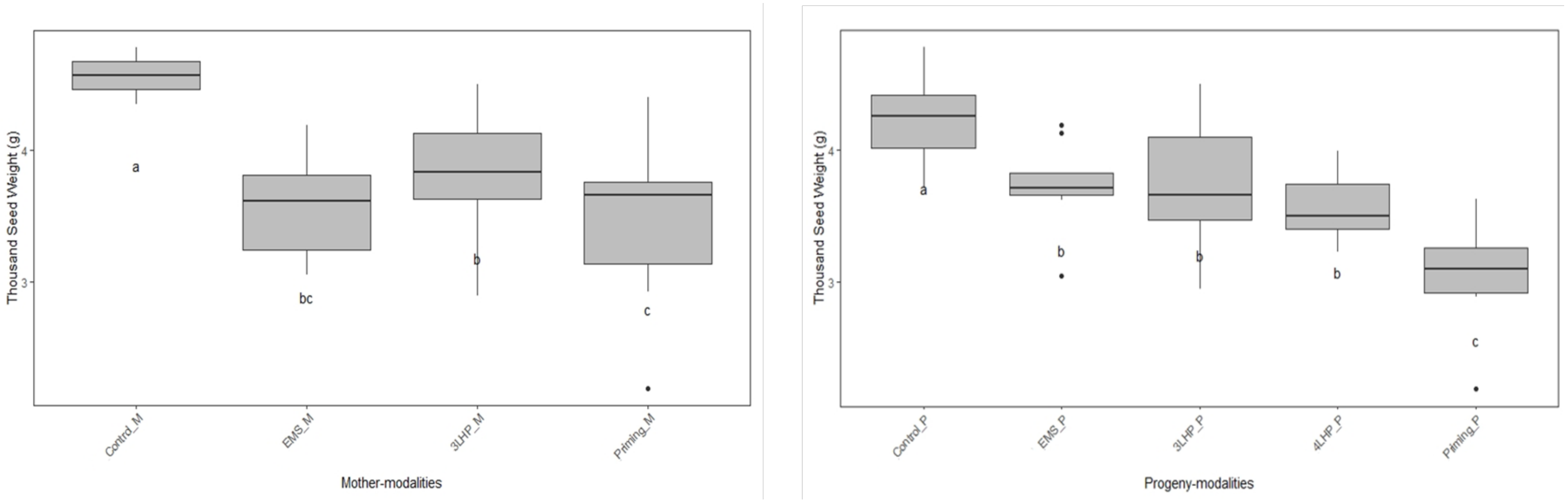
SD, Table 1: Values of each Mother-modality x Progeny-modality combination for the measured variables in the High Sulphur condition. Letters indicate the ranking among Progeny-modalities for a given Mother-modality (mean pairwise comparison test, LSD test). TSW: Thousand Seed Weight, DW: dry weight, FA: fatty acid, UFA: unsaturated FA, SFA: saturated FA, C: carbon, N: nitrogen, S: Sulphur, ABA: abscisic acid, GA3: gibberellic acid.

Mother-modalities	Control	Priming					EMS					3LHP				
Progeny-modalities	**Control**	**Control**	**Priming**	**EMS**	**4LHP**	**3LHP**	**Control**	**Priming**	**EMS**	**4LHP**	**3LHP**	**Control**	**Priming**	**EMS**	**4LHP**	**3LHP**
Seed yield - pods $L_{\geq 5cm}$ (g/pl)	2.29	2.48 a	2.88 a	3.54 a	3.01 a	3.17 a	2.50 abc	1.32 c	2.36 bc	3.02 ab	3.69 a	2.02 a	2.22 a	2.72 a	2.90 a	2.84 a
Seed number - pods $L_{\geq 5cm}$ (per pl)	465	574 a	862 a	871 a	818 a	844 a	593 bc	400 c	629 bc	805 ab	1063 a	434 a	689 a	696 a	771 a	631 a
TSW - pods $L_{\geq 5cm}$ (g)	4.92	4.60 a	3.40 b	4.06 ab	3.77 ab	3.96 ab	4.30 a	3.32 b	3.79 ab	3.94 ab	3.49 ab	4.80 a	3.35 b	4.10 ab	3.89 ab	4.57 a
Seed yield - pods $L_{<5cm}$ (g/pl)	4.78	5.16 a	3.16 a	3.01 a	3.9 a	4.29 a	4.58 a	3.87 a	3.33 a	3.88 a	3.48 a	5.16 a	3.48 a	3.35 a	3.30 a	3.32 a
Seed number - pods $L_{<5cm}$ (per pl)	1087	1310 a	1392 a	887 a	1165 a	1364 a	1222 a	1206 a	940 a	1161 a	1000 a	1238 a	1037 a	928 a	930 a	827 a
TSW - pods $L_{<5cm}$ (g)	4.40	3.93 a	2.35 c	3.39 ab	3.37 ab	2.98 bc	3.73 a	3.21 a	3.57 a	3.36 a	3.53 a	4.16 a	3.23 d	3.07 bc	3.48 cd	4.03 ab
Total seed yield (g/pl)	7.07	7.65 a	6.05 a	6.55 a	6.92 a	7.46 a	7.08 a	5.19 c	5.69 bc	6.90 ab	7.18 a	7.18 a	5.56 b	6.20 ab	6.20 ab	6.17 ab
Total seed number (per pl)	1552	1884 ab	2255 a	1759 b	1983 ab	2208 a	1815 a	1607 a	1570 a	1966 a	2063 a	1672 a	1727 a	1624 a	1701 a	1459 a
Total TSW (g)	4.57	4.09 a	2.70 c	3.72 ab	3.52 ab	3.36 b	3.90 a	3.24 b	3.65 ab	3.56 ab	3.50 ab	4.29 a	3.25 c	3.86 ab	3.66 bc	4.24 a
Fatty acids (%DW)	29.83	29.40 a	17.64 b	25.58 a	26.14 a	30.70 a	30.12 a	16.87 c	23.60 b	24.16 b	27.22 ab	31.51 a	16.12 c	25.09 b	23.80 b	28.91 ab
UFA (%DW)	27.39	26.91 a	15.71 b	23.33 a	23.54 a	27.90 a	27.61 a	15.00 c	21.45 b	21.72 b	24.80 ab	28.86 a	14.17 c	22.87 b	21.36 b	26.28 ab
SFA (%DW)	2.44	2.49 ab	1.93 c	2.25 bc	2.60 ab	2.80 a	2.51 a	1.87 c	2.16 bc	2.43 ab	2.42 ab	2.65 a	1.95 b	2.22 ab	2.44 a	2.63 a
ω6:ω3 ratio	1.73	1.78 b	2.61 a	1.78 b	2.36 a	1.92 b	1.75 c	2.65 a	1.85 c	2.42 b	1.93 c	1.80 b	2.62 a	1.76 b	2.47 a	2.04 a
C concentration (%DW)	45.43	45.99 a	43.41 ab	42.60 b	45.37 ab	45.41 ab	45.43 a	42.34 b	45.42 a	44.54 ab	45.82 a	45.59 a	41.52 b	44.29 a	44.09 a	45.59 a
N concentration (%DW)	3.48	3.19 ab	3.61 a	3.60 a	3.54 ab	3.13 b	3.46 a	3.77 a	3.59 a	3.61 a	3.48 a	3.36 c	3.90 a	3.67 abc	3.79 ab	3.44 bc
S concentration (%DW)	0.34	0.28 a	0.28 a	0.26 a	0.29 a	0.26 a	0.36 a	0.29 ab	0.23 b	0.31 a	0.32 a	0.27 a	0.31 a	0.26 a	0.32 a	0.27 a
Protein (mg/g DW)	191.1	175.4 ab	198.3 a	197.8 a	194.7 ab	171.8 b	190.0 a	207.2 a	197.4 a	198.8 a	191.2 a	184.6 c	214.8 a	201.9 abc	208.2 ab	189.2 bc
[Raff+stach] : suc ratio	0.33	0.32 ab	0.21 d	0.34 a	0.24 cd	0.27 bc	0.35 a	0.19 c	0.26 b	0.22 bc	0.27 b	0.29 ab	0.18 c	0.34 a	0.23 bc	0.27 ab
ABA:GA3 ratio	1.21	0.88 ab	0.27 b	0.22 b	0.26 b	1.23 a	0.90 a	0.32 ab	0.27 b	0.29 b	0.36 ab	0.47 a	0.31 a	0.19 a	0.24 a	1.13 a

SD, Table 2: Values of each Mother-modality x Progeny-modality combination for the measured variables in the Low Sulphur condition. Letters indicate the ranking among Progeny-modalities for a given Mother-modality (mean pairwise comparison test, LSD test). TSW: Thousand Seed Weight, DW: dry weight, FA: fatty acid, UFA: unsaturated FA, SFA: saturated FA, C: carbon, N: nitrogen, S: Sulphur, ABA: abscisic acid, GA3: gibberellic acid.

Factor\Variables	Total pods			Pods L < 5cm			Pods L ≥ 5cm		
	Yield (g.pl^{-1})	**Seed number**	**TSW (g)**	**Yield (g.pl^{-1})**	**Seed number**	**TSW (g)**	**Yield (g.pl^{-1})**	**Seed number**	**TSW (g)**
Progeny-modalities									
Control	7.25 a	1731 ns	4.21 a	4.92 a	1214 ns	4.06 a	2.32 ns	516 ns	4.65 a
Early mild stress	6.15 bc	1651 ns	3.74 b	3.27 b	918 ns	3.56 b	2.87 ns	732 ns	3.98 b
3 late heat peaks	6.93 ab	1909 ns	3.70 b	3.70 ab	1063 ns	3.52 b	3.23 ns	845 ns	4.00 b
4 late heat peaks	6.67 abc	1883 ns	3.58 b	3.70 ab	1085 ns	3.40 bc	2.98 ns	798 ns	3.87 b
Priming	5.60 c	1862 ns	3.06 c	3.46 b	1212 ns	2.93 c	2.14 ns	650 ns	3.35 b
se	*0.26*	*103*	*0.11*	*0.31*	*91*	*0.13*	*0.30*	*96*	*0.16*
Mother-modalities									
Control	7.07 ns	1552 b	4.57 a	4.78 ns	1087 ns	4.42 a	2.29 ns	465 ns	4.92 ns
Early mild stress	6.41 ns	1804 ab	3.57 bc	3.83 ns	1106 ns	3.48 bc	2.58 ns	697 ns	3.77 ns
3 late heat peaks	6.26 ns	1636 b	3.86 b	3.72 ns	992 ns	3.72 b	2.54 ns	644 ns	4.14 ns
Priming	6.93 ns	2017 a	3.47 c	3.91 ns	1223 ns	3.21 c	3.02 ns	793 ns	3.96 ns
se	*0.39*	*100*	*0.11*	*0.47*	*116*	*0.11*	*0.30*	*82*	*0.13*
Progeny effect	**0.001** **	0.268	**0.000** ***	**0.014** *	0.113	**0.000** ***	0.143	0.254	**0.001** **
Mother effect	0.081	**0.002** **	**0.006** **	0.886	0.094	**0.001** **	0.366	0.426	0.177
Progeny x Mother	0.807	0.206	0.251	0.877	0.787	0.154	0.722	0.659	0.793

SD, Table 3: Yield components distinguishing the two pools of pods (i.e. pods$_{L< 5cm}$ and pods$_{L\geq 5cm}$ at the beginning of the temperature stress application). Results are presented by factor (Progeny-modality and Mother-modality) under Low Sulphur condition. For a given variable, different letters (Tukey multiple comparisons test) indicate the ranking among Progeny or Mother modalities (including the control). P-values and levels of significance are given for Progeny, Mother and Progeny x Mother interaction effects. Levels of significance: ns non-significant, *p<0.05**, *p<0.01***, *p<0.001****. SE: standard error, TSW: Thousand Seed Weight.



SD, Figure 1: Boxplot to the Thousand Seed Weight (TSW) criteria measured in seeds from total pods under Low Sulphur condition. The TSW is the most important yield component impacted significantly by both Mother and Progeny modalities. Letters indicate the ranking amongst all the treatments (including the control). P-values and levels of significance are given on Supplemental Data (SD, Table 3). EMS: Early Mil Stress modality, 3LHP: 3 late heat peaks modality, 4LHP: 4 late heat peaks modality.

Factor\Variables	Seed C, S, N and protein concentrations (%DW)				Oil content and fatty acids (%DW)			
	Carbon	**Sulphur**	**Nitrogen**	**Protein (mg.g^{-1})**	**FA**	**SFA**	**UFA**	**ω6:ω3 ratio**
Progeny-modalities								
Control	45.61 a	0.31 a	3.37 b	185.29 b	30.22 a	2.52 a	27.69 a	1.76 d
Early mild stress	44.10 ab	0.25 b	3.62 ab	199.04 ab	24.76 b	2.21 b	22.55 b	1.79 cd
3 late heat peaks	45.61 a	0.28 ab	3.35 b	184.06 b	28.94 a	2.62 a	26.33 a	1.96 c
4 late heat peaks	44.65 a	0.31 a	3.65 ab	200.57 ab	24.70 b	2.49 a	22.21 b	2.41 b
Priming	42.43 b	0.29 ab	3.76 a	206.78 a	16.88 c	1.91 c	14.96 c	2.62 a
se	*0.40*	*0.01*	*0.08*	*4.57*	*0.85*	*0.06*	*0.78*	*0.04*
Mother-modalities								
Control	45.43 ns	0.34 ns	3.48 ns	191.12 ns	29.83 ns	2.44 ns	27.39 ns	1.73 ns
Early mild stress	44.71 ns	0.30 ns	3.58 ns	196.94 ns	24.40 ns	2.28 ns	22.12 ns	2.12 ns
3 late heat peaks	44.22 ns	0.28 ns	3.62 ns	199.74 ns	25.08 ns	2.38 ns	22.71 ns	2.14 ns
Priming	44.56 ns	0.27 ns	3.41 ns	187.60 ns	25.89 ns	2.41 ns	23.48 ns	2.09 ns
se	*0.57*	*0.01*	*0.11*	*6.04*	*1.33*	*0.08*	*1.25*	*0.08*
Progeny effect	**0.000** ***	**0.017** *	**0.004** **	**0.004** ***	**0.000** ***	**0.000** ***	**0.000** ***	**0.000** ***
Mother effect	0.584	0.170	0.069	0.067	0.394	0.243	0.407	0.703
Progeny x Mother	0.262	0.231	0.950	0.950	0.915	0.848	0.915	0.983

SD, Table 4: Nutritional seed quality criteria measured in seeds from pods$_{L\geq 5cm}$. Results are presented by factor (Progeny-modality and Mother-modality) under Low Sulphur condition. For a given measured variable, different letters (Tukey multiple comparisons test) indicate the ranking among Progeny or Mother modalities (including the control). P-values and levels of significance are given for Progeny, Mother and Progeny x Mother interaction effects. Levels of significance: ns non-significant, *p<0.05*, p<0.01**, p<0.001****. SE: standard error, DW: dry weight, FA: fatty acid, UFA: unsaturated FA, SFA: saturated FA.

Factor\Variables	Soluble sugars: Sucrose (%DW)	Raffinose (%DW)	Stachyose (%DW)	[Raffinose+stachyose] : sucrose ratio	Hormones: ABA:GA3 ratio
Progeny-modalities					
Control	3.75 b	0.16 a	1.03 a	0.32 a	0.87 a
Early mild stress	3.59 b	0.17 a	0.64 a	0.31 a	0.24 a
3 late heat peaks	4.78 a	0.18 a	1.12 a	0.27 ab	0.91 a
4 late heat peaks	4.65 a	0.15 ab	0.93 a	0.23 bc	0.27 a
Priming	3.35 b	0.13 b	0.52 b	0.19 c	0.30 a
se	*0.16*	*0.01*	*0.05*	*0.01*	*0.10*
Mother-modalities					
Control	3.69 ns	0.17 ns	1.05 ns	0.33 ns	1.21 ns
Early mild stress	3.89 ns	0.15 ns	0.84 ns	0.25 ns	0.43 ns
3 late heat peaks	4.17 ns	0.16 ns	0.92 ns	0.26 ns	0.53 ns
Priming	4.03 ns	0.15 ns	0.96 ns	0.28 ns	0.60 ns
se	*0.20*	*0.01*	*0.08*	*0.02*	*0.29*
Progeny effect	**0.000** ***	**0.000** ***	**0.000** ***	**0.000** ***	**0.005** **
Mother effect	0.334	0.388	0.133	0.212	0.596
Progeny x Mother	0.068	0.477	0.853	0.129	0.362

SD, Table 5: Seed physiological quality values measured in seeds from pods$_{L\geq 5cm}$. Results are presented by factor (Progeny-modality and Mother-modality) under Low Sulphur condition. For a given measured variable, different letters (Tukey multiple comparisons test) indicate the ranking Progeny or Mother modalities (including the control). P-values and levels of significance are given for Progeny, Mother and Progeny x Mother interaction effects. Levels of significance: ns non-significant, *p<0.05**, *p<0.01***, *p<0.001****. SE: standard error, DW: dry weight, ABA: abscisic acid, GA3: gibberellic acid.

Mother-modalities		Control	Priming					EMS					3LHP				
Progeny-modalities		**Control**	**Control**	**Priming**	**EMS**	**4LHP**	**3LHP**	**Control**	**Priming**	**EMS**	**4LHP**	**3LHP**	**Control**	**Priming**	**EMS**	**4LHP**	**3LHP**
25°C	T25 (h)	14.1	15.3 a	14.3 a	13.8 a	13.4 a	13.4 a	15.8 a	12.0 c	14.5 ab	13.0 bc	14.3 ab	13.6 a	12.1 a	11.8 a	13.1 a	14.8 a
	T50 (h)	16.8	17.3 a	16.5 ab	16.5 ab	15.4 ab	14.9 b	17.3 a	14.5 c	16.7 ab	15.0 bc	15.9 abc	15.2 a	15.6 a	14.2 a	15.1 a	16.1 a
	T75 (h)	20.6	21.2 a	19.8 ab	20.0 ab	17.9 bc	16.9 c	19.2 a	17.9 a	19.6 a	17.5 a	17.9 a	15.2 a	15.6 a	14.2 a	15.1 a	16.1 a
	Final rate (%)	97	91 b	91 b	100 a	98 a	98 a	87 b	95 ab	100 a	99 a	98 a	99 a	95 b	99 a	99 a	100 a
5°C	T25 (h)	95.6	95.5 a	84.6 a	84.3 a	98.6 a	93.1 a	107.0 a	82.9 a	85.4 a	94.9 a	99.5 a	91.0 ab	70.3 b	76.0 b	107.7 a	106.5 a
	T50 (h)	111.4	111.8 a	111.7 a	102.6 a	113.6 a	105.7 a	118.9 a	103.4 a	105.4 a	105.9 a	111.9 a	103.7 ab	99.0 ab	88.7 b	117.5 a	116.6 a
	T75 (h)	133.4	134.6 a	147.3 a	126.6 a	132.7 a	121.8 a	134.9 a	131.8 a	130.8 a	119.8 a	128.0 a	121.6 ab	138.9 a	105.0 b	130.7 a	129.4 a
	Final rate (%)	96	98 a	96 a	98 a	99 a	99 a	99 a	93 b	99 a	100 a	100 a	98 a	94 a	99 a	97 a	99 a

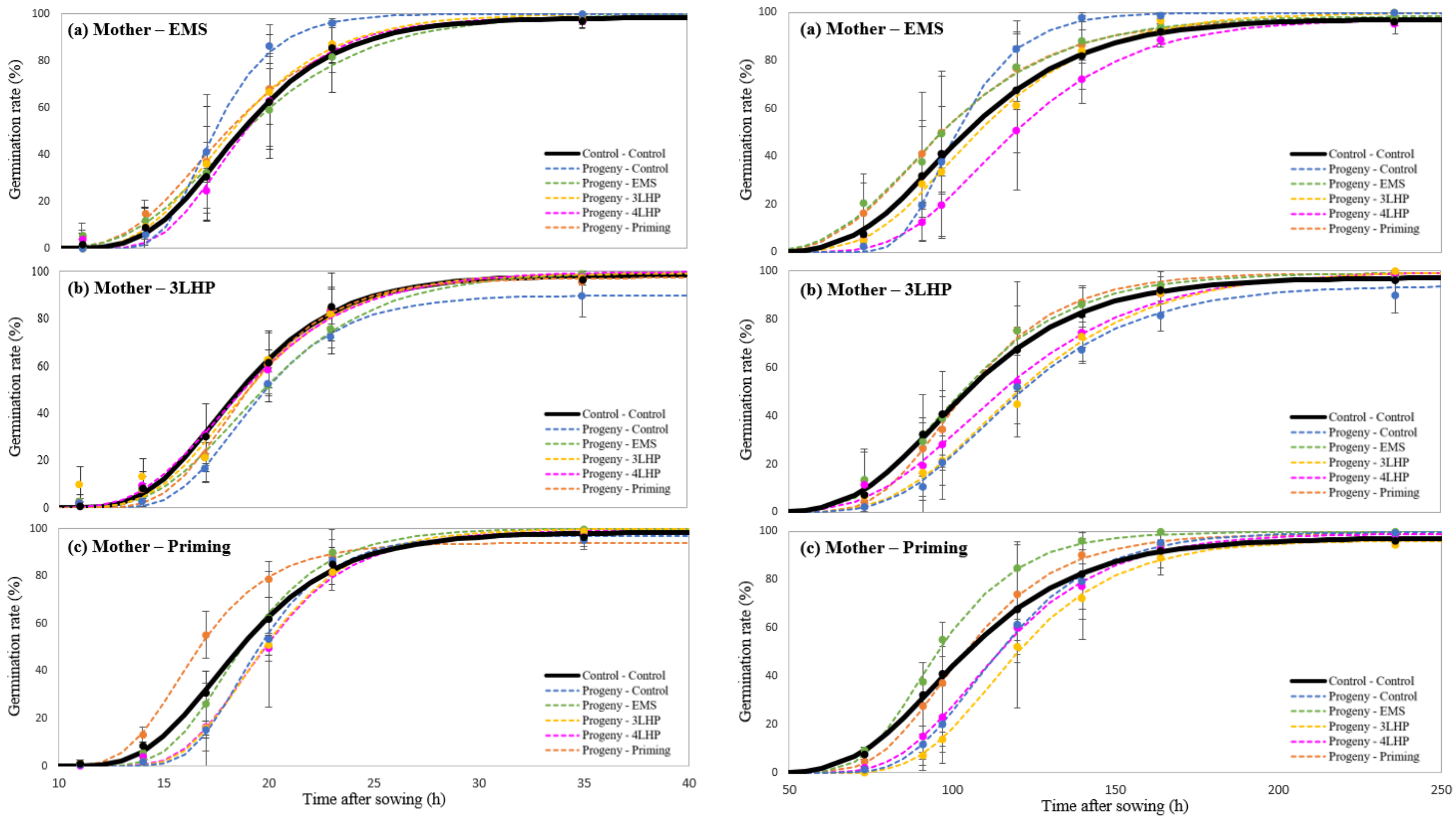
SD, Table 6: Values of each Mother-modality x Progeny-modality combination for the germination variables in the High Sulphur condition. Letters indicate the ranking among Progeny-modalities for a given Mother-modality (mean pairwise comparison test, LSD test). T25, T50 and T75 represent respectively the time (in hours) for 25%, 50% and 75% of germinated seeds, as adjusted from the observations with the Gompertz functions. Final rate is the observed final rate (in percentage). EMS: Early Mil Stress modality, 3LHP: 3 late heat peaks modality, 4LHP: 4 late heat peaks modality.

Factor\Variables	Germination at 25°C T25 (h)	T50 (h)	T75 (h)	Final rate (%)	Germination at 5°C T25 (h)	T50 (h)	T75 (h)	Final rate (%)
Progeny-modalities								
Control	17.2 ns	18.8 ns	21.1 ns	95 ns	97.2 ab	110.6 ab	128.7 abc	98 ns
Early mild stress	16.8 ns	19.1 ns	22.1 ns	98 ns	83.7 b	98.3 b	117.1 c	99 ns
3 late heat peaks	16.8 ns	19.1 ns	21.8 ns	98 ns	100.3 a	116.3 a	136.9 ab	99 ns
4 late heat peaks	16.9 ns	19.1 ns	21.9 ns	97 ns	98.8 a	115.9 a	138.2 a	98 ns
Priming	15.8 ns	18.0 ns	20.8 ns	96 ns	88.2 ab	101.7 ab	119.3 bc	99 ns
se	*0.4*	*0.4*	*0.6*	*0.9*	*3.5*	*3.6*	*4.3*	*0.5*
Mother-modalities								
Control	16.7 ns	18.5 ns	20.8 a	96 ns	89.8 ns	104.3 ns	123.8 a	98 ns
Early mild stress	16.2 ns	18.3 ns	20.9 a	98 ns	90.7 ns	104.7 ns	123.1 a	98 ns
3 late heat peaks	16.9 ns	19.4 ns	22.6 a	96 ns	95.1 ns	112.4 ns	135.0 a	99 ns
Priming	17.1 ns	18.9 ns	21.3 a	97 ns	96.6 ns	109.8 ns	127.1 a	99 ns
se	*0.6*	*0.6*	*0.7*	*1.2*	*4.3*	*2.5*	*6.1*	*0.5*
Progeny effect	0.172	0.261	0.577	0.075	**0.009** **	**0.003** **	**0.002** **	0.936
Mother effect	0.116	0.060	**0.047** *	0.262	0.346	0.182	**0.046** *	0.379
Progeny x Mother	0.250	0.166	0.333	0.114	0.959	0.772	0.259	**0.016** *

SD, Table 7: Germination variables measured at optimal (25◦C) and suboptimal (5◦C) temperatures. Results are presented by factor (Progeny-modality and Mother-modality) under Low Sulphur condition. T25, T50 and T75 represent respectively the time (in hours) for 25%, 50% and 75% of germinated seeds, as adjusted from the observations with the Gompertz functions. Final rate is the observed final rate (in percentage). For a given measured variable, different letters (Tukey multiple comparisons test) indicate the ranking Progeny or Mother modalities (including the control). P-values and levels of significance are given for Progeny, Mother and Progeny x Mother interaction effects. Levels of significance: ns non-significant, *p<0.05*, p<0.01**, p<0.001****. SE: standard error.

Mother-modalities		Control	Priming					EMS					3LHP				
Progeny-modalities		**Control**	**Control**	**Priming**	**EMS**	**4LHP**	**3LHP**	**Control**	**Priming**	**EMS**	**4LHP**	**3LHP**	**Control**	**Priming**	**EMS**	**4LHP**	**3LHP**
25°C	T25 (h)	16.7	18.2 a	14.9 b	16.9 ab	17.7 a	17.3 a	16.1 a	15.2 a	16.4 a	16.7 a	16.3 a	17.7 a	17.2 a	17.1 a	16.3 a	16.5 a
	T50 (h)	18.5	19.5 a	16.7 b	18.7 a	19.8 a	19.8 a	17.3 a	18.1 a	18.9 a	18.7 a	18.1 a	19.8 a	19.1 a	19.7 a	18.8 a	19.2 a
	T75 (h)	20.8	21.3 ab	19.3 b	21.0 ab	22.4 a	22.3 a	18.9 a	21.5 a	22.2 a	21.4 a	20.5 a	23.3 a	21.7 a	23.0 a	22.1 a	22.7 a
	Final rate (%)	96	95 b	95 b	100 a	99 ab	99 ab	100 a	97 a	97 a	97 a	99 a	90 b	96 ab	99 a	98 ab	97 ab
5°C	T25 (h)	89.9	104.9 a	90.5 ab	83.9 b	99.1 ab	104.5 a	92.8 a	82.2 a	80.8 a	101.5 a	96.1 a	101.2 a	91.9 a	86.5 a	95.9 a	100.2 a
	T50 (h)	104.3	115.3 a	103.8 ab	95.8 b	114.3 ab	120.3 a	101.7 a	96.6 a	97.2 a	119.2 a	108.9 a	121.2 a	104.9 a	101.9 a	114.5 a	119.7 a
	T75 (h)	123.8	128.6 ab	120.9 ab	110.8 b	133.8 a	141.3 a	113.1 b	115.7 b	118.8 ab	142.7 a	125.2 ab	149.4 a	121.4 b	121.8 b	138.1 ab	144.4 a
	Final rate (%)	98	100 a	99 a	100 a	99 a	98 b	100 a	99 a	98 a	97 a	100 a	97 b	100 a	100 a	100 a	100 a

SD, Table 8: Values of each Mother-modality x Progeny-modality combination for the germination variables in the Low Sulphur condition. Letters indicate the ranking among Progeny-modalities for a given Mother-modality (mean pairwise comparison test, LSD test). T25, T50 and T75 represent respectively the time (in hours) for 25%, 50% and 75% of germinated seeds, as adjusted from the observations with the Gompertz functions. Final rate is the observed final rate (in percentage). EMS: Early Mil Stress modality, 3LHP: 3 late heat peaks modality, 4LHP: 4 late heat peaks modality.



SD, Figure 2: Germination time courses at 25◦C (left) and 5◦C (right) under Low Sulphur condition. Each graph represents one Mother-modality and each curb represents one Progeny-modality. Lines are fittings to a Gompertz function. Vertical bars denote standard errors. P-values of the germination time courses (T25, T50 and T75) and final germination rates are given in Supplemental Data (SD, Table 7 and 8). EMS: Early Mil Stress modality, 3LHP: 3 late heat peaks modality, 4LHP: 4 late heat peaks modality.