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Ministry of Agriculture

ADF Project Final Report Format

1. **Project title and ADF file number.**
   Impact of drought and heat during flowering on Canola yield
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4. **Abstract/ Summary**: An outline on overall project objectives, methods, key findings and conclusions for use in publications and in the Ministry database (Maximum of 500 words or one page).

   Crops face a unique combination of abiotic stresses due to climate change. Although we know a lot about how crops respond to each of these individual stresses, we know less about how they respond to the combination of multiple stress factors occurring simultaneously (e.g., drought and heat). Several studies have shown that crop quality and productivity decline significantly when multiple multifactorial stress factors co-occur. In the project, we investigated the impacts of drought, heat and their combination on canola flowering, yield and quality parameters using controlled greenhouse and field studies. The study made use of AAFC's canola Nested Association Mapping (NAM) population founder lines (FLs) and recombinant inbred lines (RILs) developed by Drs. Parkin, Vail & Robinson. Results from the greenhouse showed reduced net photosynthetic assimilation rates caused by combinations of heat and drought (heat + drought), drought alone, and to a lesser extent by heat alone. A decrease in stomatal conductance was observed under drought and heat + drought treatments but not under heat treatments. On the other hand, plants exposed to heat or heat and drought had significantly lower mesophyll conductances than plants exposed to drought alone. In response to the heat treatment, carboxylation efficiency and electron transport rates were reduced. Under the heat treatment, seed yield was reduced by 85%, while under the drought treatment, it was reduced by 31%. Seed oil content decreased by 52% in the plants exposed to heat; the protein content increased under all the stress treatments. In order to create a canola germplasm that is adapted to rapid climate change, a better understanding of heat tolerance mechanisms in crops is necessary. We found a noticeable response of leaf cysteine to drought exposure in both drought-tolerant and drought-sensitive canola cultivars and significant correlations between cysteine, ABA and stomatal conductance, suggesting a role of cysteine and sulfur in modulating the stomata movement as a response to drought. This is the first demonstration of ABA levels of NAM FLs under various stresses. Long-term ABA accumulation has a negative impact on growth and yield, so NAM FLs with high ABA accumulation after long-term stress are likely to be stress susceptible. Other lines with low ABA accumulation after long-term stress requires further analysis on early stages of stress. Lastly, the carbon and oxygen stable isotopes (proxies for water-use efficiency and transpiration efficiency, respectively) could be used as physiological markers for selecting high yielding canola lines since they integrate growing season signals.

5. **Extension Messages:** Key outcomes and their importance for producers/processors and the relevant industry sector (3-5 bullet points in lay language).

   - The heat treatment had a more detrimental effect on the seed oil composition than drought, resulting in increased saturated fats and, therefore, decreased oil frying ability.

- As part of the cysteine pathway, sulfur contributes to stress resilience in canola, which is further linked to ABA, which leads to stomatal closure. Due to the redirected sulfur, oil content is adversely affected.
- Based on the ABA profiling in this project, we were able to identify canola NAM FLs that produce less ABA and are able to cope with drought stress.
- Carbon and oxygen stable isotopes (proxies for water-use efficiency and transpiration efficiency, respectively) could be used as physiological markers for selecting high yielding canola lines since they integrate growing season signals.

6. **Introduction:** Brief project background and rationale (Maximum of 1500 words or 1.5-3 pages).

Water availability is perhaps the most crucial factor influencing plant growth and crop yield (Rosenzweig et al. 2014, Awasthi et al. 2014). Therefore, in agricultural regions affected by water scarcity, yields can be reduced by 50% or more as a result of current climate variability. Seasonal droughts in 2001, 2008 and 2021 adversely affected Saskatchewan's crop production, particularly in the central and northern canola-growing regions of the province, resulting in billions in revenue losses for the Canadian economy. Developing crops that can endure prolonged moisture deficits and tolerate heat will become essential for improving and stabilizing productivity. In order to cope with stress conditions, crops have evolved anatomical (epicuticular waxes), biochemical (organic solutes), physiological (stomatal closure, improved water use-efficiency, altered root:shoot ratio) and molecular adaptations. Plant resilience and stress tolerance can be improved by assessing critical stages of plant growth that are sensitive to moisture deficits and heat stress.

The third most common oil used in food, canola oil, is rich in polyunsaturated fatty acids. Seeds from canola have about 40% oil content, 22% protein, and 39% protein in the meal. Additionally, it contains about 60% oleic acid, 20% linoleic acid, and 10% α-linolenic acid (Barthet 2015). As canola moves from vegetative to flowering (and pod development), its evapotranspiration remains high and available soil moisture results in higher yields by 3 to 4 bushels per acre. The canola plant's resilience to heat and drought largely depends on when these stresses occur – stress during the vegetative stage might be tolerable, whereas similar stress during the transition to flowering or during reproductive development can have a devastating impact on yield (Gan et al. 2004). These co-occurring abiotic stresses, in turn, combine to cause altered flowering phenology, reproductive failure and accelerated senescence. Abiotic stress can cause reduced pod fill due to problems with pollen germination, pollen tube growth, flower production, pod development, and photosynthetic partitioning to oil seed yield.

Adapting to co-occurring drought and heat, plants use a variety of strategies, including economical water use. In theory, drought and heat tolerant lines could fill the gap left in canola production created by reduced water availability. However, we will have to harness the vast genomic resources now available to us in order to close the phenotype gap for *Brassica napus*. Thus, understanding the physiological mechanisms of plant growth reduction under abiotic stress offers the promise of developing better canola that could sustain high yields under optimal and suboptimal conditions. Passioura's productivity framework (1977) relates drought tolerance with water-use efficiency (WUE) and factors that maintain metabolic and hormonal functions under water-limited conditions contribute to improved WUE.

This study builds on past research conducted at AAFC Saskatoon Research Center in collaboration with several industry consortium partners (Cargill, Dow AgroSciences, CPS, DL Seeds). Drs. Parkin, Robinson and Vail's past work exploited *B. napus* diversity collections to develop a structured resource for dissecting complex traits, the AAFCs NAM population (51 diverse genotypes of spring type were used as the FLs for the development of the ~2500 RILs from multiple crosses between diverse inbred FLs and a single reference line). Western Canada producers invest a substantial amount of capital (seed, fertilizer, labor, land, etc.) into growing a canola crop with weather variability creating moisture deficits and excessive heat as one of the major uncontrollable

factors. By targeting physiological traits that sustain yields, the results of this study will decipher the knowledge on inbred canola lines suited for future climates.

7. Objectives and the progress towards meeting each objective.

Objectives (Please list the original objectives and/or revised objectives if Ministry-approved revisions have been made to the original objectives. A justification is needed for any deviation from original objectives).	**Status** (e.g. completed/not completed)
a) Evaluate spring canola Nested Association Mapping population founder lines for drought, heat and combined stresses in the greenhouse.	Completed
b) Comprehensive analyses of plant metabolites exhibiting enhanced stress-responsive roles leading to the discovery of biomarkers.	Completed
c) Selection of stress-tolerant canola varieties based on the abscisic acid (ABA) level.	Completed
d) Evaluation of abiotic stress tolerance under field conditions.	Completed
e) Explore new rapid and non-destructive biomolecular imaging techniques to predict stress tolerance using Canadian Light Source.	Completed

8. Methodology: Specify project activities undertaken during the entire project period (without referring to previous progress report). Include approaches, experimental design, methodology, materials, sites, etc. (Maximum of 5 pages).

Greenhouse study #1: ***Dissection of key morpho-physiological and biochemical traits among canola NAM founder lines to accelerate genetic gains***.

Plant material and experimental setup

The spring canola diversity panel used in this study was assembled by the canola breeding group at AAFC, Saskatoon Research and Development Centre, Saskatchewan, Canada. Fifty founder lines of spring canola nested association mapping population and a common parent (reference line) were used in this study. The geographical origins of 51 lines are provided in Figure 1. Three seeds were sown in a randomized complete block design with four replications for each line and thinned to a single plant per pot six days after germination. Seedlings of each line were grown in 30 cm deep pots (11 litres) with peat moss soil mix recipe in a greenhouse. The growth conditions were set at 21/15 °C (day/night) temperature with 16 h day length. The controlled greenhouse chamber was illuminated with Philips CERAMALUX SON AGRO 430W fluorescent lights providing photosynthetically active radiation of 400 µmol m^{-2} s^{-1} at least. The plants were fertilized with water-soluble nitrogen fertilizer (28-14-14) periodically and were irrigated as required. The pots were rotated and randomized every week within replications to minimize positional effects. Standard greenhouse IPM practises were followed throughout the experimental duration. Plants were harvested between 101 and 121 days after sowing depending on their days to maturity (N = 204).

Characterization of lines for morphology, phenology and physiology traits

A total of 14 morphological, 13 physiological, four flowering phenology and three yield- related traits were measured during the course of the experiment (Table 1). The morphological and phenological traits measured in this study were based on descriptors for Brassica and Raphanus from the international board of plant genetic resources (IBPGR, 1990). Days to bolting (DTB), days to flowering (DTF) and days to maturity (DTM) were determined for each plant replicate as follows; DTB: when at least 3 plants replicates out of 4 showed the first flower bud bolting, DTF: when 3 three plants replicates out of 4 four showed the first fully open flower and DTM: in 3 three plants replicates out of four, all pods turned yellow. Flowering duration (FD) is calculated from DTF to until the time 3 plants replicates out 4

show no complete flower structurebeginning of pods development. Pod and root- related traits were measured after harvesting individual plants. Roots from individual pots were washed carefully to remove any adhering growth medium particles before measuring root related traits.

Forty days after emergence, gas exchange measurements were performed for ten days using a Li-COR 6400 XT portable infra-red gas exchange system from Li-COR Biosciences (Lincoln, NE, USA). On any given day, gas exchange measurements were recorded on the 4th leaf of each plant between 8:00 and 11:30 am, with the measured plant randomized among NAM lines and days of measurement. Inside the leaf chamber, the following conditions were maintained: reference CO2 concentration set to 400 ppm using CO2 cartridges; flow rate 500 µmol s^{-1}; block temperature set at 23 °C; relative humidity of incoming air adjusted to ~50−55%; photosynthetic active radiation (PAR) 1000 µmol m^{-2} s^{-1}. Maximum photosynthetic assimilation rate (A; µmol CO2 m^{-2} s^{-1}), stomatal conductance (gs; mol H2O m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}) and intercellular CO_2 concentration (Ci) were measured. The instantaneous water-use efficiency (WUEi) was determined by calculating photosynthetic rate over transpiration rate (µmol CO_2 $mmol^{-1}$ H_2O).

Following gas exchange measurements, chlorophyll fluorescence was recorded using Opti-Sciences OS-30p chlorophyll fluorometer (Hudson, NH, USA). The leaves were dark adapted using clips for 20 min, and Fv/Fm value was measured on the adaxial surfaces of the leaves. The values presented for Fv/Fm are mean values of two dark- adapted leaves (4th and 5th leaves) from the same plant. Chlorophyll content index (CCI) was measured on three fully expanded leaves (4th, 5th and 6th leaves) per plant using an Opti-Sciences CCM-200 meter (Hudson, NH, USA) and averaged for statistical analyses. Later, ten leaf tissue discs were sampled from the 4th leaf using a hand-held paper punch and oven dried at 50°C for 48 h. Leaf mass area (LMA) was calculated for 3 discs and the same used for carbon (C) and nitrogen (N) content and stable isotopes ratios (δ13C and δ15N; ‰) analyzed at the UC Davis Stable Isotope Facility (Davis, CA, USA).

The values for leaf discrimination against the heavy isotope Δ13C were calculated, following Farquhar et al. (1989), as:
Δ^13 C=(〖δ^13 C〗_a- 〖δ^13 C〗_leaf)/(1+ 〖δ^13 C〗_leaf)
where δ13Ca and δ13Cleaf are, respectively, the carbon isotope composition of the ambient air (−8.3‰) and the leaf sample with, as a reference, the Vienna PeeDee Belemnite (VPDB) carbonate standard, according to the following formula:
δ^13 C=(〖(^13 C/^12 C)〗_sample- 〖(^13 C/^12 C)〗_VPDB)/(〖(^13 C/^12 C)〗_VPDB.1000)
where (13C/12C)sample and (13C/12C)VPDB are the ratios of 13C and 12C in the sample and the VPDB, respectively.
In the same way, δ15N was calculated as:
δ^15 N=(〖(^15 N/^14 N)〗_sample- 〖(^15 N/^14 N)〗_AIR)/(〖(^15 N/^14 N)〗_AIR.1000)
The atmospheric nitrogen was the standard used to calculate δ15N. All of the isotopic values were expressed in per mil (‰), and the error of the repeated measurements was ≤ 0.1‰.

At physiological maturity, the siliques were harvested and stored in a dryer at 23 °C for 4–5 days before threshing. The seeds were then collected, counted and thousand seeds weight (TSW) determined.

Untargeted metabolites analysis (Krista Thompson to provide details on LC-MC/GC methods)
Upon completion of gas exchange measurements, the 3rd leaf from all four replications of the 51 lines

were collected, immediately frozen in liquid nitrogen and stored at -80°C until further use. Leaves were sampled between 10.00-11.00 a.m. on a single day. Frozen leaves were ground in a mill with beads to obtain a fine powder for non-targeted metabolites analysis. The untargeted metabolite analysis was performed by following the sampling and extraction protocols explained in Arbona et al. 2009 and Hochberg et al. (2013). Masslynx version 4.1 software (Waters, Milford, MA, USA) was used to acquire data from LC/MS runs. The obtained peak intensities of each metabolite were then annotated using the XCMS (Tautenhahn et al. 2012) online software.

Field experiment

In parallel to the greenhouse experiment, seed yield and a thousand seed weight (TSW) were collected at 5 locations in the Canadian prairies region, Saskatoon, SA [latitude(LAT), 52.1332° N; longitude(LON), 106.6700° W, elevation(ELV), 482 m], Outlook, OU (LAT, 51.4873° N; LON, 107.0578° W, ELV, 540 m), Melfort, ME (LAT, 52.8608° N; LON, 104.6143° W, ELV, 462 m), Scott, SC (LAT, 52.2933° N; LON, 108.9513° W, ELV, 656 m) in Saskatchewan and Beaverlodge, BE (LAT, 55.2140° N; LON, 119.4233° W, ELV, 733 m) in Alberta, Canada. The diversity panel lines were grown in 2 replications in a randomized complete block design (6 rows: 2m × 1m) at the above-mentioned field sites. At Saskatoon site, we also recorded plant height, root width, number of lateral roots, CCI, Fv/Fm, (DTB), (DTM) and TSW. Pearson's correlation coefficient values were calculated between yield traits recorded at the five sites and those collected at Saskatoon field traits versus results obtained in the greenhouse.

Greenhouse study #2: ***Canola Responses to Drought, Heat, and Combined Stress: Shared and Specific Effects on Carbon Assimilation, Seed Yield, and Oil Composition***.

Plant growth environment

A Canadian elite *Brassica napus* L. cultivar (N99-508), widely used in Agriculture and Agri-Food Canada's (AAFC) breeding program, was chosen for the study. To simulate field conditions, topsoil was collected from agriculture fields close to Saskatoon, Canada (52.15 °N, 106.58 °W), and mixed with 10% sand to provide better drainage. The soil type was chernozemic dark brown, and the texture was sandy loam with an average pH of 7.9 and 9, 23, and 295 ppm of nitrogen, phosphorus and potassium nutrients, respectively. The details of the soil characteristics are summarized in Table S1. Eight 60-liter plastic bins were filled with 50 kg of dry topsoil and slow-releasing fertilizer (Osmocote®, Everris, U.S.A.) at a rate of 10.7 g/l to avoid nutrient deficiency effects on plant growth and development. The bases and bottoms of the tubs were perforated for water drainage. In each bin, eight seeds were sown equidistantly and watered to field capacity.

The bins were divided equally between two adjacent greenhouses where the ambient day and night temperatures were 23 ± 0.5 °C and 18 ± 0.5 °C, respectively. The relative humidity was 45−65%. The photoperiod was set as a 16 h day and an 8 h night, and the minimum photosynthetic photon flux density (PPFD) was 400 µmol m^{-2} s^{-1} during the day. After a week of emergence, the plants were thinned, and the number was reduced to four per bin. The soil moisture was kept at field capacity by regular and light watering from emergence to bolting.

Stress treatments

In the first greenhouse (GH-1), the daytime temperature was raised gradually and maintained at 29 ± 0.5 °C from the 38th day after sowing, and the night-time temperature was maintained at 18 ± 0.5 °C. Two plastic bins (8 plants) were maintained at 90% water-holding capacity (heat, H), and the remaining two plastic bins were allowed to reach 30% field capacity (heat+drought, HD) and maintained at that level until silique/pod maturation. In the second greenhouse (GH-2), the daytime temperature was maintained at 23 ± 0.5 °C, and the night-time temperature was maintained at 18 ± 0.5 °C, with two bins (8 plants) at 90% water-holding capacity (well-watered, WW) and the remaining bins at 30% (drought, D). From bolting to final harvest, the soil moisture and the temperature regimes were monitored in both greenhouses using a WATERMARK soil moisture monitor (Model

900M, IRROMETER CA, USA) at 1 h intervals over the experiment. The sensors were set up in two randomly assigned tubs for each treatment. The water-holding capacity of the soil was determined as follows:

$$WHC(\%) = \frac{W_{sat+72} - W_{dry}}{W_{dry}} \times 100$$

where W_{sat+72} is the weight after 72 h of drainage of 20 L of water-saturated soil, and W_{dry} is the weight of 20 L of dry soil.

Gas exchange measurements and isotopic discrimination

The gas exchange measurements were performed using a Li-COR 6400XT portable photosynthesis system equipped with a 6400-08 chamber attached to a 6400-02B LED light source (LI-COR Inc., Lincoln, NE, U.S.A.) on days 9 to 11 after imposing the stress treatments. Measurements were made on the 4th fully developed leaf from the top (N = 32; 4 stress treatments × 8 plants) between 8:30 and 11:30 a.m. The response of the net photosynthesis (A, µmol m^{-2} s^{-1}) to the changing C_i was measured under saturated photosynthetic active radiation, PAR = 1000 µmol m^{-2} s^{-1}. The leaf was first exposed to a chamber CO_2 concentration (C_a = 400 µmol CO_2 mol^{-1}) using CO_2 cartridges to reach a steady state. Next, the C_a was changed in the following order: 400, 300, 200, 100, 50, 400, 500, 600, 800, 1,000 and 1,200 µmol mol^{-1}. This was done while ensuring that the net photosynthetic assimilation rate (A), water vapor, and CO_2 fractions reached steady values at each step before moving to the next. During the measurement periods, the leaf chamber temperatures were kept at 23 °C and 29 °C depending on the greenhouse conditions: air flow at 500 µmol s^{-1}, relative humidity at 55–65%, and VPD at 1.2 ± 0.1 KPa. The order of the measurements was randomized among the treatments and the days and along the measuring period. The A (µmol CO_2 m^{-2} s^{-1}) and the g_s (mol CO_2 m^{-2} s^{-1}) values were extracted from the A–C_i response measurements for C_a = 400 µmol CO_2 mol^{-1} (atmospheric ambient CO_2 concentration). The intrinsic water-use efficiency (WUE_i) was then deduced ($WUE_i = A/g_s$).

The maximum rate of RuBisCO carboxylation (V_{cmax}, µmol m^{-2} s^{-1}), the rate of photochemical electron transport (J, µmol e^- m^{-2} s^{-1}), and the rate of CO_2 diffusion from the C_i to the C_c carboxylation site or g_m (g_m, µmol m^{-2} s^{-1}) were estimated by A-C_i curve fitting, according to Ethier and Livingston (2004) and Ethier et al. (2006), with the biochemical model of C_3 photosynthesis developed by Farquhar et al. (1980).

The final rate, A, was:

A = min (Ac, Aj)

The RuBisCO-limited rate of CO_2 assimilation (A_c) was given by:

$$A_c = \frac{(C_i - \Gamma^*)V_{cmax}}{C_i + K_c(1 + O/K_o)} - R_d$$

where V_{cmax} is the maximum rate of carboxylation, C_c is the chloroplast concentration of CO_2, C_i is the intercellular concentration of CO_2, Γ^* is the CO_2 compensation point, and K_c and K_o, are the Michaelis-Menten constants of RuBisCO for CO_2 and O_2, respectively.

The RuBP-limited rate of CO_2 assimilation (A_j) was given by:

$$A_j = \frac{(C_i - \Gamma^*)J/4}{C_i + 2\Gamma^*} - R_d$$

where J is the rate of electron transport. The rate of electron transport was given by:

$$J = \frac{4(A_j + R_d)(C_i - \frac{A_j}{g_i} + 2\Gamma^*)}{(C_i - \frac{A_j}{g_i} - \Gamma^*)}$$

The g_m was calculated from:

$$\frac{1}{g_m} = \frac{1}{g_i} + \frac{1}{K_c}$$

where

$$g_i = \frac{A}{C_i - C_c}$$

and K_c is the carboxylation efficiency (the initial slope of the A-C_c curve)

$$K_c = \frac{dA}{dC_c}$$

Once the gas exchange measurements were recorded from the very same 4th leaf, 10 leaf punches (5 mm diameter) were collected, using a single-hole punch, on either side of the midrib towards the stable isotopic composition of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and oxygen ($\delta^{18}O$), as well as the carbon:nitrogen (C:N) ratio. Later, the leaf punches were oven-dried at 60 °C for 72 hours at constant mass. Four (4) of the leaf discs were weighed and individually packed in tin ($\delta^{13}C$ and $\delta^{15}N$) and silver ($\delta^{18}O$) capsules. They were then sent to the University of California at Davis Stable Isotope Facility to be combusted and analyzed by an online continuous flow dual analyzer coupled to an isotope ratio mass spectrometer (Europa Scientific Integra, Cheshire, England, UK). The values for leaf discrimination against the heavy isotope $\Delta^{13}C$ were calculated, following Farquhar et al. (1989), as:

$$\Delta^{13}C = \frac{\delta^{13}C_a - \delta^{13}C_{leaf}}{1 + \delta^{13}C_{leaf}}$$

where $\delta^{13}C_a$ and $\delta^{13}C_{leaf}$ are, respectively, the carbon isotope composition of the ambient air (−8.3‰) and the leaf sample with, as reference, the Vienna PeeDee Belemnite (VPDB) carbonate standard, according to the following formula:

$$\delta^{13}C = \frac{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{VPDB}}{(^{13}C/^{12}C)_{VPDB}.1000}$$

where $(^{13}C/^{12}C)_{sample}$ and $(^{13}C/^{12}C)_{VPDB}$ are the ratios of ^{13}C and ^{12}C in the sample and the VPDB, respectively.

In the same way, $\delta^{15}N$ and $\delta^{18}O$ were calculated as:

$$\delta^{15}N = \frac{(^{15}N/^{14}N)_{sample} - (^{15}N/^{14}N)_{AIR}}{(^{15}N/^{14}N)_{AIR}.1000}$$

$$\delta^{18}O = \frac{(^{18}O/^{16}O)_{sample} - (^{18}O/^{16}O)_{VSMOW}}{(^{18}O/^{16}O)_{VSMOW}.1000}$$

The Vienna standard mean ocean water (VSMOW) and atmospheric nitrogen were the standards used to calculate the $\delta^{18}O$ and $\delta^{15}N$, respectively. All of the isotopic values were expressed in per mil (‰), and the error of the repeated measurements did not exceed 0.1‰.

Abscisic acid content

A fully expanded leaf (5th leaf from the top) was sampled and immediately packed in Eppendorf tubes and frozen in liquid nitrogen before being stored in a −80°C freezer until processed for analysis. The ABA content was determined as described in Yan et al. (2016b). The samples were centrifuged to remove debris, and the pellet was washed twice. The supernatant was evaporated in a SpeedVac, reconstituted in 1 ml of 1% (v/v) acetic acid, and purified by solid phase extraction using Oasis HLB, MCX, and WAX cartridge columns (Waters Limited, Mississauga ON, Canada). The solvent was removed under vacuum and subjected to the LC-ESI-MS/MS analysis (Agilent 6,410

TripleQuad LC/MS system). A Liquid Chromatography (Agilent 1200 series) equipped with a 50 × 2.1 mm, 1.8 µm Zorbax SB-Phenyl column (Agilent) was used with a binary solvent system comprising 0.01% (v/v) acetic acid in water (Solvent A) and 0.05% (v/v) acetic acid in acetonitrile (Solvent B). The separations were performed using a gradient of increasing acetonitrile content with a flow rate of 0.2 ml min^{-1}. The gradient was increased linearly from 3% B to 50% B over 15 min. The retention time for the ABA was 14.0 minutes.

Growth

The normalized difference vegetation index (NDVI) was measured using a GreenSeeker handheld crop sensor (Trimble, Westminster CO, USA). The sensor was held 80 cm above the plant canopy, as recommended by the manufacturer. The measurements were taken between 9 and 11 a.m. just before flowering started. Plant height was measured at physiological maturity.

Harvest

Upon reaching physiological maturity, the siliques were harvested, counted, and stored in a dryer at 23 °C for 4−5 days before threshing. The seeds were then collected, counted, and weighed. Later, the seeds were sent to AAFC's oil chemistry lab in Saskatoon for oil and protein analyses. To qualify the effects of the combined *vs*. the single stressors on the yield traits and the photosynthetic *A*, the effect weights of drought (D), heat (H), and heat+drought (HD), compared to the well-watered (WW) treatment were calculated using the following formula:

$$T_e = \frac{X_t - \bar{X}_{WW}}{\bar{X}_{WW}}$$

where T_e is the treatment effect weight, X_t is the trait "X" value for the treatment T, and $\overline{X}_{WW}$ is the corresponding mean value for the well-watered plants. The heat+drought effect weight obtained with the above formula (HD_e) was compared to the calculated heat drought+effect (HD_{calc}) using the following formula:

$$HD_{calc} = \bar{H}_e + \bar{D}_e - \bar{H}_e \times \bar{D}_e$$

where $\overline{H}_e$ and $\overline{D}_e$ are the means of the heat and drought effect weights, respectively (Darling et al., 2010; Bansal et al., 2013).

Fatty acid and protein content of seeds

The seeds were pooled from each stress treatment and further divided into 3 sub-samples for analyses of the total oil content, fatty acid composition, and total protein content. The seed oil fatty acyl composition was analyzed using gas chromatography (GC) following the preparation of the fatty acid methyl esters by base-catalyzed methanolysis (Thies, 1971) and according to the protocol detailed in Heydarian et al. (2016). The individual fatty acids were reported as a percentage of the total fatty acid methyl esters by mass. The total oil content was calculated as the sum of the content of the individual triglycerides. The seed protein content was determined by the American Oil Chemists' Society's generic combustion method for crude protein (Official Method Ba 4e-93). Combustion at a high temperature in pure oxygen frees nitrogen, which is measured by thermal conductivity detection and then converted to the equivalent protein by an appropriate numerical factor (AOAC, 2003). A LECO FP-528 protein analyzer was used, and the results were reported as a percentage, N × 6.25, calculated on a whole-seed dry matter (zero moisture) basis. Subsequently, the ω-3 desaturation efficiency (DE) and the ω-6 DE were deduced from the profile of the fatty acids and calculated according to Menard et al. (2017):

$$\omega - 3\ DE = \frac{18{:}3}{18{:}2 + 18{:}3}$$

$$\omega - 6\ DE = \frac{18{:}2 + 18{:}3}{18{:}1 + 18{:}2 + 18{:}3}$$

Greenhouse study #3: ***Sulfate feeding into cysteine indirectly triggers ABA induced stomata closure to impart drought tolerance in Brassica napus L.***

Growth environment

Two spring canola cultivars (*Brassica napus* L.) previously screened for drought tolerance were selected from a diversity panel: "Czyzowska" originating from Poland, a drought-resistant and "BN-1" a drought-sensitive cultivar from India.

Tubs of 60L each were filled with peat moss soil mix and watered to field capacity (6 tubs per cultivar). Their side base and bottom were perforated to permit water drainage. A slow-releasing fertilizer (Osmocote, Everris, U.S.A.) was added at 10.7 $g.l^{-1}$ to avoid nutrient deficiencies. Canola seeds (9 per tub) were sown at equal distant and thinned after emergence to have five seedlings per tub. Plants were grown under a day/night temperature of 23/18°C, respectively and relative humidity was 45−65%. The day/night photoperiod was set at 16/8h with a photosynthetic photon flux density (PPFD) of at least 400 µmol m^{-2} s^{-1} during the day period (4 a.m-8 p.m.). Plants were regularly watered when required until bolting.
Once plants started bolting, tubs were randomly assigned to one of the two treatments (N = 12 = two treatments × two cultivars × three replications):

- Control treatment (W): To mimic field conditions in summer during bolting/flowering stages, plants were watered at 90% of maximum water holding capacity (WHC); at 9:30 temperature was switched to increase from 23°C to 28°C at around 10:30 a.m. until 3:30 p.m. At this time, the temperature was set back to 23°C and gradually decreased to stabilize around at 23°C p.m one hour later.
- Drought (D): Plants are exposed to the same temperature regime of the control treatment but watered at 40% of WHC. Water holding capacity (%) of the growth medium was determined as in Elferjani and Soolanayakanahally (2018).

Photosynthetic activity

Portable photosynthesis LI-6400XT system equipped with a 6400-08 chamber attached to a 6400-02B LED light source (LICOR Inc., Lincoln, NE, U.S.A.) was used to measure gas exchanges on 12th to 15th days after the beginning of the stress treatment. Measurements were made on the 4th fully developed leaf from the top (N = 12 = two treatments × two cultivars × three replicates) between 10:30 and 12:30 a.m. The response of the net photosynthesis (A, µmol m^{-2} s^{-1}) to the changing C_i was measured under saturated photon photon flux density, PPFD = 1,000 µmol m^{-2} s^{-1}. The leaf was first exposed to a chamber CO_2 concentration (C_a = 400 µmol CO_2 mol^{-1}) using CO_2 cartridges to reach a steady state. Next, the C_a was changed in the following order: 400, 300, 200, 100, 50, 400, 500, 600, 800, 1,000 and 1,200 µmol mol^{-1}. At each step, we ascertained that the net photosynthetic assimilation rate (A), water vapour, and CO_2 fractions reached steady values at each step before moving to the next step. During the measurement periods, the leaf chamber temperature was set to the ambient temperature (28°C), air flow at 500 µmol s^{-1}, relative humidity at 55–65%, and VPD at 1.4 ± 0.2 KPa. The order of the measurements was randomized among the treatments and the cultivars and along the measuring period. The A (µmol CO_2 m^{-2} s^{-1}) and the g_s (mol CO_2 m^{-2} s^{-1}) values were extracted from the A–C_i response measurements for C_a = 400 µmol CO_2 mol^{-1} (atmospheric ambient CO_2 concentration). The maximum rate of RuBisCO carboxylation (V_{cmax}, µmol m^{-2} s^{-1}), the rate of photochemical electron transport (J, µmol e^- m^{-2} s^{-1}), and the rate of CO_2 diffusion from the C_i to the C_c carboxylation site or g_m (g_m, µmol m^{-2} s^{-1}) were estimated by A-C_i curve fitting, according to Ethier and Livingston (2004) and Ethier et al. (2006), with the biochemical model of C_3 photosynthesis developed by Farquhar et al. (1980) as detailed in Elferjani and Soolanayakanahally (2018).

Night gas exchanges

The same equipment used for measuring the photosynthetic activity was used for assessing gas exchanges on the same leaves during night time 16 days after the beginning of the stress. Cuvette parameters that were changed are: PAR = 0 µmol m^{-2} s^{-1}, temperature = 18°C and air flow = 200 µmol s^{-1}. Chamber CO_2 concentration (C_a) was maintained at 400 µmol CO_2 mol^{-1} and A, water vapour and CO_2 fractions were allowed to stabilize before records could be taken. Gas exchanges were monitored twice during night: 1st measurement- night: between 9:45 - 10:45 p.m.; 2nd measurement: Pre-dawn: between 3 and 4 a.m. Gas exchanges were also measured early in the morning, an hour after the beginning of the day time period between 5 - 6 a.m. during which PPFD =1000 µmol m^{-2} s^{-1}, air flow = 500 µmol s^{-1} and cuvette temperature = 23°C

Leaf waxes

One leaf was sampled from two plants per replicate between 10:30 and 11 am (N= 24 = three replicates × two plants × two treatments × two cultivar). Freshly cut leaves were kept in a cool and dry container and stored in a refrigerator at 4°C before being analyzed the next day at the Canadian Light Source facility (Saskatoon SK, Canada). Mid-infrared (mid-IR) spectroscopy was used to determine the total load and to identify the different functional groups of the epicuticular wax in canola leaves according to the protocol of Willick et al. 2017). Briefly, the mid-IR Attenuated Total internal Reflection (ATR) spectra of fresh leaves were collected using the Cary 600 series FTIR spectrometer (Agilent Technologies, Santa Clara, CA USA). ATR crystal used was germanium (45 degrees). Mid-IR data in the spectral range between 4000 and 600 cm^{-1} wavenumbers at a resolution of 4 cm^{-1} were recorded at 256 scans per sample on average.

Abscisic acid content

Young fully expanded leaves (3rd or 4th leaf from the top) were sampled and immediately packed in plastic tubes and frozen in liquid nitrogen before being stored in a -80°C freezer until processed for analyses. Abscisic acid content was determined as described in Yan et al., 2016. Briefly, samples were centrifuged to remove debris, and the pellet was washed twice. The supernatant was evaporated in a SpeedVac, reconstituted in 1 ml of 1% (v/v) acetic acid. ABA was purified by solid phase extraction using Oasis HLB, MCX and WAX cartridge columns (Waters). The solvent was removed under vacuum and subjected to the LC-ESI-MS/MS analysis (Agilent 6,410 TripleQuad LC/MS system). A LC (Agilent 1200 series) equipped with a 50 × 2.1 mm, 1.8-µm Zorbax SB-Phenyl column (Agilent) was used with a binary solvent system comprising 0.01% (v/v) acetic acid in water (Solvent A) and 0.05% (v/v) acetic acid in acetonitrile (Solvent B). Separations were performed using a gradient of increasing acetonitrile content with a flow rate of 0.2 ml min−1. The gradient was increased linearly from 3% B to 50% B over 15 min. The retention time of ABA was 14.0 min.

Cysteine and sucrose

Cysteine was extracted from 10mg powder of freeze dried tissue samples following Inaba et al. (1994) with some modifications. Briefly, 1 ml of 80% (v/v) ethanol solution was added to each sample, vortexed for 10 seconds and the supernatant was recovered by centrifugation (4000 rpm for 10 min) at 4 °C. The pellet were re-extracted under same conditions with additional 500ul of 80%(v/v) ethanol solution . The supernatants were combined and stored at −20 °C. Cysteine were derivatized following Waters AccQTag Reagent Kit (Waters, Milford, Massachusetts, USA; Cohen and Michaud, 1993). Briefly; 10ul aliquot of sample was mixed with 70ul Borate Buffer and 20ul AccQFluor Reagent which was reconstituted in Acetonitrile. AccQFluor reagent was reconstituted as follows: One mL of AccQ Fluor Reagent diluent was transferred to a vial containing AccQ-Fluor reagent powder and vortexed for 10 sec before heating at 55°C for a maximum of 10 minutes or until dissolved. The derivatized mixture was transferred to autosampler vial and incubated at 55°C for 10 min. HPLC was conducted, as described in Waters AccQTag Chemistry Package Instruction manual, with excitation wavelength of 285nm and emission wavelength of 320nm on a Waters Amino Acid Column – 3.9x150mm using 2475 scanning fluorescence detector (Waters, Milford, Massachusetts, USA). The column was set at 37°C with a 5uL of injection volume. Waters AccQTag buffer (100ml AccQTag Buffer concentrate + 1000 ml Super Q water), Acetonitrile and Super Q Water were used as mobile phase A, mobile phase B and mobile phase C respectively. Concentration of cysteine (pmol/uL) from a sample was calculated using peak area values of the chromatogram against the calibration curve of serial dilution (10, 25, 50, 100, 150 pmol/uL) of known standards. The values were converted to umol/gm using the extraction volume and weight of initial sample. (The cysteine standard was kindly gifted by Dr. Wanasundhara's Lab, AAFC, Saskatoon).

Total sugars were extracted from 10 mg powder of freeze-dried leaf tissue samples. One ml of 75% (v/v) methanol solution containing 0.1% formic acid was added to each sample and mixed properly by vortexing for 10 seconds followed by sonication in water bath at room temperature for 15 minutes. The supernatant was obtained by centrifugation (20 000 rpm for 15 min) at room temperature. The resulting supernatants was filtered through 0.2-μm PVDF filter syringe onto HPLC slit vials (Waters) and stored at −20 °C until use. Sucrose standard was prepared in water with concentrations. An evaporative light scattering detector (ELSD) detector was used in conjunction with HPLC.

The carbohydrates were identified and quantified by comparison with known standards. Peaks were quantified using calibration standards of HPLC grade sugars including glucose, fructose, sucrose.

Growth and yield
Leaf temperature was monitored with a thermal imaging camera (FLIR T530, FLIR Systems. Wilsonville, Oregon, USA) by taking images of the canopy at 8 a.m., 12 a.m., 2 p.m., 4 p.m and 6 p.m. Simultaneously, Chlorophyll content was recorded using a chlorophyll content meter (CCM-200- apogee INSTRUMENTS, Logan, UT USA) and plant height measured. After harvest at complete maturity, pods were stored in paper bags under the ambient temperature for 3-5 days until threshing. Seeds were collected and weighted and sent for analyses of oil content. The seed oil fatty acyl composition was analyzed using gas chromatography (GC) following preparation of fatty acid methyl esters by base-catalyzed methanolysis (Thies, 1971) and according to the protocol detailed in Heydarian et al., (2016). The total oil content was calculated as the sum of the content of the individual triglycerides.

9. **Results and discussion:** Describe and discuss the results accomplished during the entire project period under each objective listed under section 7. The results need to be accompanied with pertinent tables, figures and/or other illustrations. Provide discussion necessary to the full understanding of the results. Where applicable, results should be discussed in the context of existing knowledge and relevant literature. Detail any major concerns or project setbacks. (Maximum of 30 pages of text not including figures or tables).

Greenhouse study #1: ***Dissection of key morpho-physiological and biochemical traits among canola NAM founder lines to accelerate genetic gains***.

Trait variation and heritability estimates
A total of 34 traits related to morphology, flowering phenology and yield were analyzed among the NAM FLs. All measured traits showed a wide range of phenotypic variation (Table 1). Among morphology traits, pod length ranged between 2.2-8.9 cm, having the highest percentage genetic variation (PGV, 119.01) and a high heritability estimate (h^2=0.56), followed by beak length which ranged between 0.48-1.67cm (PGV=112.26) and had a heritability of 0.43. On the contrary, lamina width which ranged between 13.37-21.15 cm has the lowest variation among the lines panel (PGV=45.31) and a relatively low heritability (h^2=0.21). For growth physiology, the variation of stomatal conductance (g_s) and transpiration (E) among the FLs was noticeable, ranging between 0.11-0.72 mol m^{-2} s^{-1} and 1.35-5.92 mmol H_2O m^{-2} s^{-1} and having a PGV of 190.63 and 141.61 respectively. While the heritability of these two traits was 0.15. The carbon isotope discrimination ($\Delta^{13}C_{leaf}$) was much more stable among the growth physiology traits with 21.17 - 25.62 $^0/_{00}$ range and PGV=18.71. The most stable trait in this group was chlorophyll fluorescence (F_v/F_m) with PGV=6.02.

Flowering phenology traits were quite variable among the FLs, particularly days to bolting (DTB) and days to flowering (DTF) which ranged between 41-88 and 47-98 days respectively giving a respective PGV of 77.05 and 76.12. Their h^2 was relatively high and similar (0.57 and 0.53). Yield traits were characterized by a pod weight ranging between 0.05-4.87 g, resulting in a PGV of 2095.65, the highest among all traits, followed by the number of seeds by pod which ranged between 6-36 and with a PGV of 130.43. TSW among the FLs lines also exhibited high variation with a PGV = 117.76 (1.06 g ≤TSW≤ 3.58 g).

Phenotypic variation associated within population groups and genotypes
The phenotypic variations associated within population structure groups and genotypes that were statistically significant are shown in Table 2. The highest proportion of variance explained by the population group (POVP) was related to flowering phenology (19.19%, 19.08% and 13.77% for DTB, DTF and DTM respectively), followed by petiole length (11.46%) and root diameter (9.73%) which were all highly significant (P<0.001). The lowest POVP (non-significant) were related to WUE_i and leaf number (0.03%) and beak length (0.02%). The proportions of variance explained by the founder lines that were nested with the population groups (POVG) were higher than POGP and were significant for all the studied traits, except stomatal ratio which has the lowest POVG (14.91%). The highest POVG was associated with morphology traits, particularly, pod length (60.83%), plant

height (57.11%) and root diameter (53.94%), followed by seed yield traits (45.91% and 48.47% for the number of seeds by pod and TSW respectively).

Correlation between phenotypic traits

All 34 phenotypic traits obtained from the greenhouse experiment were tested for correlation between each of them which was represented with the heat map of Pearson's correlation coefficients as shown in Figure 1. Among the morphological traits, root diameter was noticeably correlated (positively) to plant height (r=0.7), stem width (r=0.68) and the number of branches (r=0.62). These traits were also positively correlated between them. The number of lateral roots was positively correlated to root diameter (r=0.7) and shoot morphology traits. As for physiology traits, photosynthetic assimilation rates (*A*) were positively correlated to g_s and E (r=0.68 and 0.77 respectively). Simultaneously, g_s and E were positively correlated to $\Delta^{13}C_{leaf}$ (r=0.54) but negatively correlated to nitrogen isotopic composition, $\delta^{15}N_{leaf}$ (r = -0.45 and -0.41 respectively). DTB, DTF and DTM were positively correlated among them but not correlated to flowering duration (FD) and were negatively correlated to TSW (r=0.47). Plant Height and root diameter both were positively correlated to DTB, DTF and DTM (r ranged between 0.56 and 0.57) but not to FD. However, those three phenology traits were negatively correlated to pod width (r=-0.58, -0.57 and -0.5 respectively).

Correlation between greenhouse and field traits

***Saskatoon field versus greenhouse*:** Plant height (r=0.58) and root width (r=0.46) collected from Saskatoon field trial, correlated positively with the greenhouse data. The same trend and correlation range were obtained for chlorophyll content index (CCI) and Fv/Fm measurements. Positive and stronger correlations were obtained for phenology traits with coefficients ranging between 0.71 (DTM) and 0.85 (DTB and DTF). TSW from the two environments was positively correlated but the correlation coefficient was lower than those with phenology traits. (Fig. 2a).

***Multi-location vs. greenhouse trials*:** Greenhouse yield plotted against the field yield from the five locations (Saskatoon [SA], Outlook [OU], Melfort [ME], Scott [SC] and Beaverlodge [BE]) showed positive correlations. The Pearson's correlation coefficients for yield were significant between greenhouse and SA, OU and ME at $P \leq 0.001$ and SC, ME and BE at $P \leq 0.01$ (Fig. 2b). Similarly, the TSW from the greenhouse experiment showed a higher correlation to TSW from the field locations OU, SC and ME ($P \leq 0.001$) and SA and BE ($P \leq 0.01$) (Fig. 2c).

Table 1. Phenotypic mean and range for morphology, growth physiology, flowering phenology and yield-related traits among the NAM founder lines.

Trait	Mean value (±SD)	Range	PGV	h^2
Morphology				
No. of leaves	19 ± 4	8 – 27	100.00	0.25
Lamina length	24.16 ± 2.90	17.73 – 32.2	59.89	0.21
Petiole length	11.05 ± 2.19	6.00 – 15.93	89.86	0.27
Lamina width	17.17 ± 2.01	13.37 – 21.15	45.31	0.21
Stomatal ratio	0.74 ± 0.14	0.49 – 1.12	85.14	0.11

Plant height	70.20 ± 13.57	34.38 – 99.25	92.41	0.60
Stem width	14.83 ± 2.66	8.90 – 22.06	88.74	0.41
No. of branches	14 ± 4	5 – 21	114.29	0.33
Pod length	5.63 ± 1.02	2.20 – 8.90	119.01	0.56
Pod width	3.65 ± 0.56	2.10 – 4.83	74.79	0.34
Beak length	1.06 ± 0.22	0.48 – 1.67	112.26	0.43
Root length	112.29 ± 25.69	58.33 – 167.75	97.44	0.10
Root diameter	10.91 ± 2.59	5.66 – 17.08	104.67	0.59
No. of lateral roots	15 ± 3	8 – 20	80.00	0.17
Growth physiology				
A	19.28 ± 2.49	12.26 – 23.98	60.79	0.15
g_s	0.32 ± 0.13	0.11 – 0.72	190.63	0.15
E	3.22 ± 0.9	1.36 – 5.92	141.61	0.14
WUE_i	6.92 ± 1.37	4.08 – 12.12	116.81	0.10
PNUE	0.29 ± 0.04	0.18 – 0.40	75.86	0.10
Ci/Ca	0.62 ± 0.09	0.31 – 0.79	77.42	0.13
LMA	7.32 ± 0.66	6.10 – 8.95	38.93	0.14
F_v/F_m	0.83 ± 0.01	0.79 – 0.84	6.02	0.13
CCI	45.11 ± 8.19	28.87 – 65.55	81.31	0.23
Leaf N	67.41 ± 6.89	47.64 – 89.63	62.29	0.15
Leaf C:N	4.77 ± 0.49	4.27 – 7.7	71.91	0.43
$\Delta^{13}C_{leaf}$ ($^0/_{00}$)	23.78 ± 0.9	21.17 – 25.62	18.71	0.18
$\delta^{15}N_{leaf}$ ($^0/_{00}$)	-4.06 ± 0.5	-5.47 – -3.04	NA	NA
Flowering phenology				
DTB	61 ± 11	41 – 88	77.05	0.57
DTF	67 ± 11	47 – 98	76.12	0.53
FD		17 – 29	52.17	NA
DTM	127 ± 11	104 – 151	37.01	0.47
Yield				
Pod weight	0.23 ± 0.66	0.05 – 4.87	2095.65	NA
No. of seeds/pod	23 ± 6	6 – 36	130.43	0.43
TSW	2.14 ± 0.58	1.06 – 3.58	117.76	0.46

Table 2. Phenotypic variation associated within population structure and genotypes among NAM founder lines as analyzed by nested ANOVA.

Trait	Population			Genotype		
	F value	P<	Proportion of variance (%)	F value	P<	Proportion of variance ((%)
Leaf number	1.05	3.54×10^{-01}	0.03	2.42	2.99×10^{-05}	31.19
Petiole length	16.39	3.87×10^{-07}	11.46	1.89	2.07×10^{-03}	21.15
Stomatal ratio	2.98	5.39×10^{-02}	1.89	1.36	8.57×10^{-02}	14.91

Plant height	11.76	1.84×10^{-05}	4.51	6.38	$< 2.2 \times 10^{-16}$	57.11
No. of branches	5.14	6.92×10^{-03}	2.93	2.62	5.21×10^{-06}	32.45
Pod length	1.52	2.22×10^{-01}	0.24	6.34	$< 2.0 \times 10^{-16}$	60.83
Pod width	10.92	3.98×10^{-05}	6.87	2.71	3.39×10^{-06}	33.27
Beak length	1.03	3.60×10^{-01}	0.02	4.19	2.88×10^{-11}	49.14
Root diameter	23.50	1.90×10^{-09}	9.73	5.90	3.34×10^{-16}	53.94
No. of lateral roots	5.39	5.67×10^{-03}	3.97	1.67	1.21×10^{-02}	20.91
A	1.23	2.95×10^{-01}	0.20	1.75	5.76×10^{-03}	21.49
WUEi	1.03	3.59×10^{-01}	0.03	1.46	4.51×10^{-02}	16.48
Ci/Ca	1.25	2.90×10^{-01}	0.22	1.56	2.40×10^{-02}	18.14
LMA	2.17	1.17×10^{-01}	1.01	1.63	1.43×10^{-02}	19.07
CCI	4.19	1.69×10^{-02}	2.46	2.10	3.60×10^{-04}	25.84
Leaf N	1.86	1.59×10^{-01}	0.74	1.65	1.26×10^{-02}	19.41
DTB	43.71	1.15×10^{-15}	19.19	4.33	3.68×10^{-12}	39.03
DTF	40.39	1.09×10^{-14}	19.08	3.86	1.92×10^{-10}	36.60
DTM	26.45	1.58×10^{-10}	13.77	3.54	2.54×10^{-09}	36.74
No. of seeds/pod	5.72	4.10×10^{-03}	2.80	3.93	2.03×10^{-10}	45.91
TSW	8.34	4.01×10^{-04}	4.30	4.24	6.85×10^{-11}	48.47

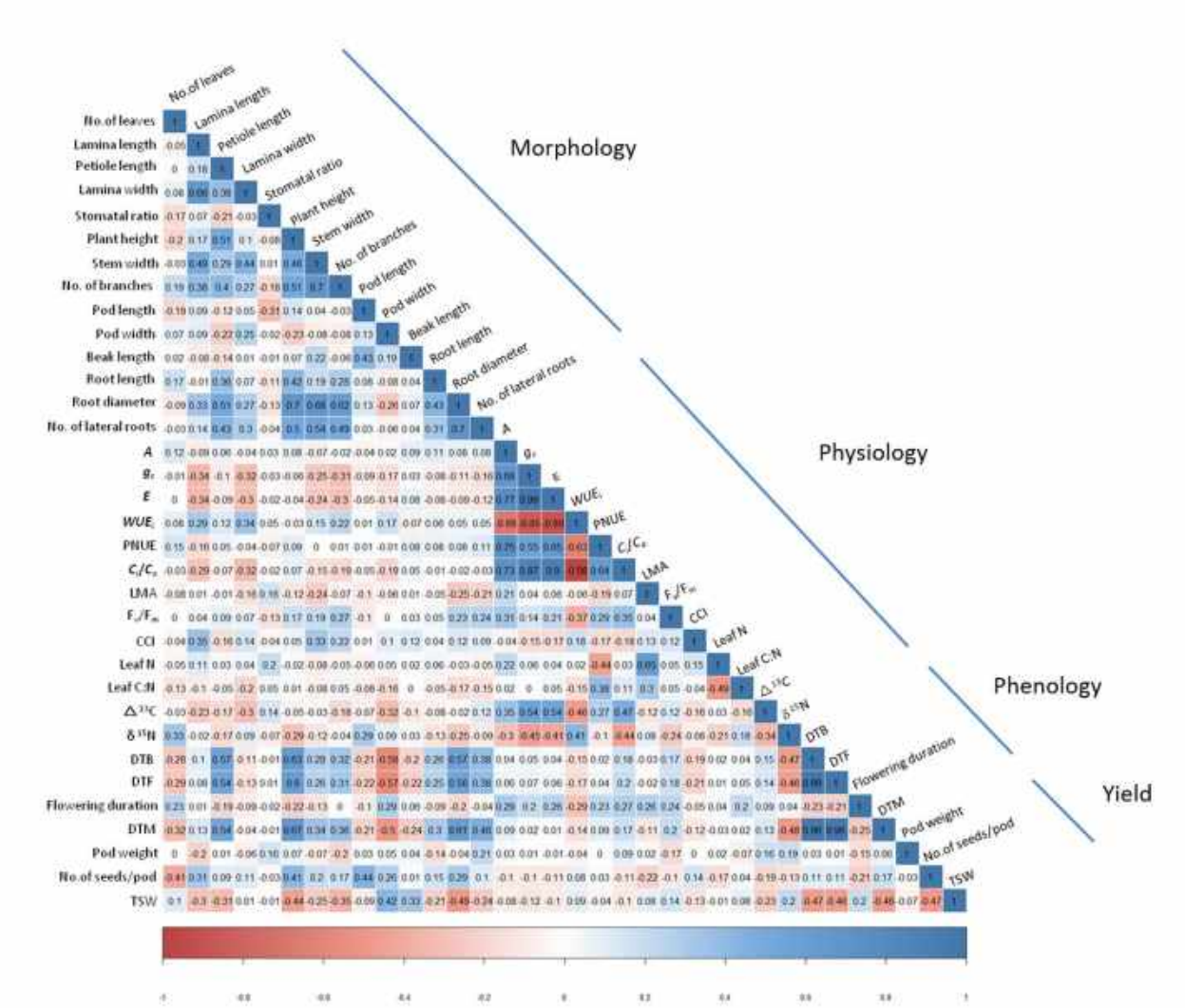


Figure 1. Pearson's correlation coefficient for 34 growth and developmental traits among 51 NAM founder lines of spring *Brassica napus*. Morphology, physiology, phenology and yield-related traits are marked by blue lines on the right side of the figure. The scale bar beneath the heat map denotes the direction/magnitude of correlation between the traits, 1 indicated by dark blue being positive and -1 indicated by dark red being negative.

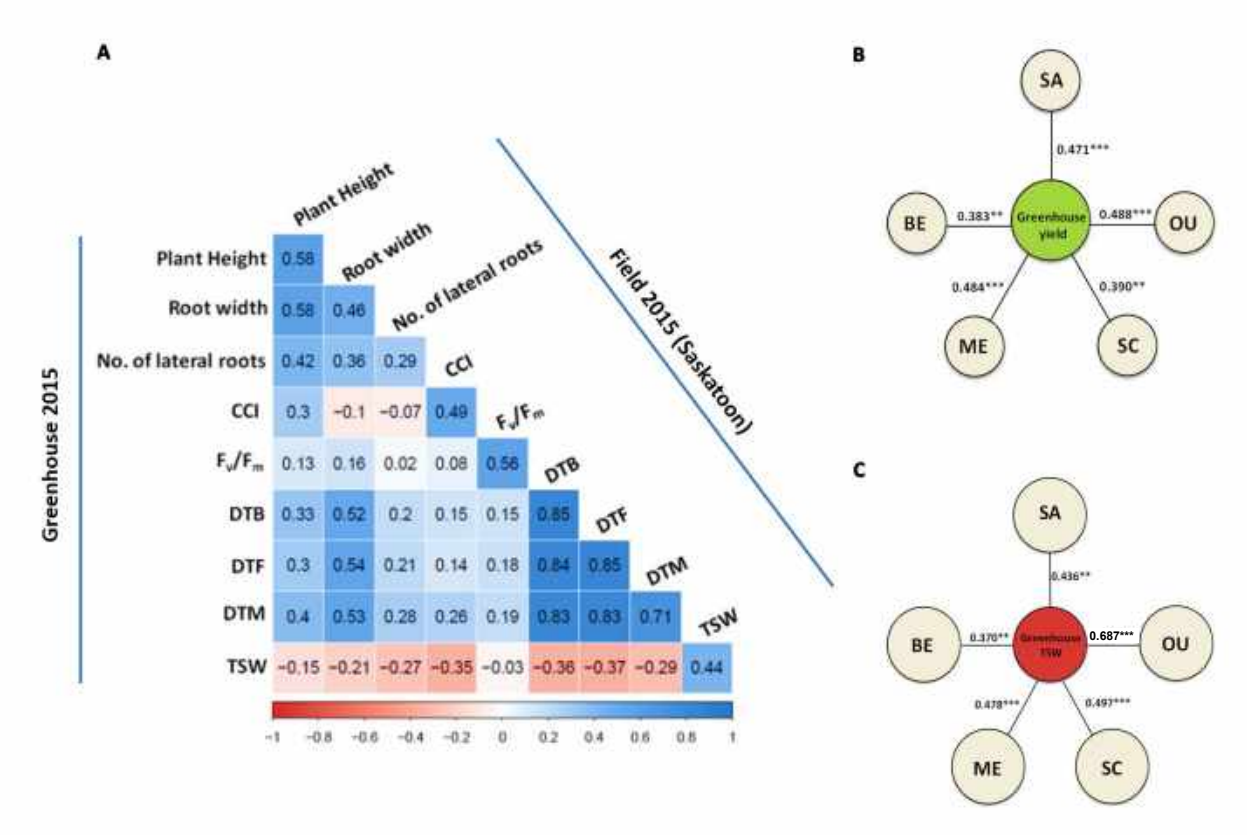


Figure 2. Correlation between greenhouse and field experiment data of NAM founder lines. A) Pearson's correlation coefficient for morphology, physiology, phenology and yield-related traits between greenhouse and Saskatoon field location 2015. The scale bar beneath the heat map denotes the direction/magnitude of the correlation between the traits, 1 indicated by dark blue being positive and -1 indicated by dark red being negative; B) correlation circles explaining the relationship between greenhouse yield and per plot yield from five different field locations; SA-Saskatoon, OU-Outlook, SC-Scott, ME-Melfort and BE-Beaverlodge; C) correlation circles explaining the relationship between greenhouse TSW and per plot TSW from the same five field locations. Stars above the correlation coefficient denote the level of significance (***, P ≤ 0.001; **, P ≤ 0.01).

Discussion: Our work demonstrated high variability in traits related to carbon assimilation, flowering phenology and seed yield with a high correlation between greenhouse and field environments. Metabolic profiling was less discriminating within the diversity panel but showed noticeable high content in metabolites like proline for few lines. These variations can be exploited in developing canola varieties with improved crop performances. As listed in Table 1, the founder lines of the NAM panel possess a wide range of variations with respect to the morphological traits measured. Plant height, pod length and root diameter are notable traits among the founder lines with high broad-sense heritability estimates. A part of the FIGS approach is to identify key functional traits from germplasm that were derived from different geographical centers. This can address the phenotypic plasticity of the germplasm collection. The large variation in morphological responses could be a result of the environmental changes the germplasm has experienced in its natural growth habitat (Valladares et al. 2007).

Morphological trait variations among the lines

Numerous studies have evaluated various morphological traits in plants to explore their potential for use in selection for yield. Qaderi et al. (2006) subjected *B. napus* to drought and heat stress and to an elevated CO_2 environment to study its response. The combination of these stresses imposed a shift in dry matter accumulation resulting in changes in stem height and diameter, leaf number and leaf area. A QTL-based study in multiple

populations of *B. rapa* conducted by Lou et al. (2007), explained the co-localization of phenotypic traits and suggested their effects on seed, growth and flowering phenology related traits. To identify key variations among diverse germplasm collections of *B. oleracea*, 44 morphological traits were studied by El-Esawi et al. (2012). The authors addressed the redundancy in selection traits among genotypes of the same species by showing the lower percentage of contribution of morphological traits such as leaf blade shape and color and petiole morphology. In our study, there is a significant amount of variation in leaf number and petiole length among the genotypes. The variation exhibited by traits such as pod width and beak length is high and positively correlated to TSW. Using a doubled haploid population of 254 *B. napus* varieties, Cai et al. (2016) showed that shoot architecture traits (of branches and inflorescence) could predict yield. Similarly, traits of the root system architecture (RSA) are determinants for nutrients and water absorption, particularly under drought (Polania et al. 2017). What once was considered a difficult system to study, has now become comparatively easier with the introduction of newer root phenotyping techniques such as 'shovelomics" (Trachsel et al. 2011) and automated high-throughput plant phenotyping platforms (Clark et al. 2011). Our study examined the extent of phenotypic variation for root length, diameter and number of lateral roots among diversity panel and all three root traits showed significant variation. In addition, they correlated positively with shoot morphological traits such as plant height, stem width and the number of branches and flowering phenology traits DTB and DTF. Similarly, Rahman and McClean (2013) observed a strong positive correlation between DTF and root length and weight in *B. napus* cultivars. The root diameter trait among the founder lines showed a correlation with the number of seeds/pod. Though substantial progress has been made in developing non-invasive root phenotyping methods and many root ideotypes have been explained, the right combination of root and shoot traits to increase crop productivity remains unidentified, but certainly depends on species and growth conditions (Environment and Management) (Meister et al. 2014).

Physiological trait variations among the lines

Physiological studies on *B. napus* species had been conducted since the late 1970s, for example, Allen et al. 1971, Thurling 1974a and 1974b, Clarke and Simpson 1978 and King and Kondra 1986 to name a few. Germplasms that were selected for increased *A* were expected to exhibit increased yield parameters. But, this is not the case in many crops, as yield is determined by multiple metabolic processes within a plant system (Nelson 1988 and Reynolds and Pfeiffer 2000). Hence it is not surprising that in our study, the physiological parameters (*A*, g_s and *E*) measured were not correlated strongly with any yield-related traits such as pod weight, number of seeds/pod or TSW. Alternately, Qu et al. (2017) assessed the leaf photosynthesis traits correlated to canopy photosynthesis, "proxied" with plant biomass in rice. They found that the photosynthetic assimilation rate under low light was highly related to biomass accumulation. The WUE of plants has become more relevant in today's context of climate change and the resultant water shortage. Here in our study, we have measured the WUE_i of the founder lines and found an obvious negative correlation to *A*, g_s, *E*, PNUE, *Ci/Ca*, LMA, F_v/F_m, leaf C:N and $\Delta^{13}C$. Medrano et al. 2015 investigated the use of WUE as a selection target by comparing WUE measurements at the leaf, plant and canopy level in vine. They found significant discrepancies between the three levels which have been attributed to leaf position in the canopy and significant carbon losses by dark respiration. We also used isotopic discrimination carbon $\Delta^{13}C$, an effective estimate of integrated WUE in leaves, and results showed a low variation of $\Delta^{13}C_{leaf}$ ($^0/_{00}$) (PGV = 18.7%). Pater et al. (2017) reported similar results for a panel of 147 lines of spring and semi-winter canola (*B. napus*). The same trends were observed by Rao et al. (1995) for $\Delta^{13}C$ ranging between 17.5 and 20.9 $^0/_{00}$ among six groundnut cultivars. This low variation under optimal conditions should not hide the evidenced WUE differences between cultivars (measured at the whole plant level) and the potential genetic enhancement of WUE in crops although little increase was achieved so far by breeding programs (Zhou et al. 2014). Flexas (2016) argued that this limited success is due to the "single trait-single gene" approach and suggested that genetic regulation of the different diffusional and biochemical limitations of photosynthesis should be addressed together.

Phenology and yield-related traits variation among the lines

In short-season agro-climates such as the Canadian prairies, the plant must flower early to complete its lifecycle. The flowering phenology related traits we measured in our study, DTB, DTF, FD and DTM showed a wide variation which is attributed to their climatic origin. FD among the founder lines was negatively correlated to plant height. As the plant continued vegetative growth to increase its height, it had less time to remain in the reproductive

phase. But interestingly, the TSW was positively correlated to FD among the founder lines. The lines have instead compensated on pod weight and the number of seeds/pod which were negatively correlated to FD. The large variations identified in yield-related traits among the founder lines and their high heritability indicate the diversity panel's potential to address yield improvement in *B. napus*. The no. of seeds/pod and TSW traits exhibited larger proportions of variance among the genotypes than when grouped according to population structure. The distribution of pods on the raceme can impact the canopy structure, thus impacting the final yield. A study comparing new and old cultivars of *B. napus* explained the differences in canopy architecture and its influence on pod-related traits (Al-Barzinjy et al. 2003). Recently released *B. napus* cultivars possessed denser canopy with smaller leaves which aided better light interception resulting in higher yields in contrast to the old cultivars with larger leaves and less dense canopy. Our results on shoot morphology showed a wide range in plant height and number of branches, as well as the number of leaves and length of the lamina. A detailed study involving pod length, breadth, thickness, volume and density, seed number per pod and TSW in 348 doubled haploid population of *B. napus* showed a positive correlation among the traits measured and identified 25 candidate genes associated with pod-related traits (Wang et al. 2016). Similarly, Jahn et al. (2011) screened 20 lines of rice (*Oryza spp*.) that included landraces and showed that biomass as a trait is influenced by a group of other traits such as tiller number, girth, leaf length, tissue weights and days to maturity.

Correlation between greenhouse and field traits

Physiological and yield traits measured in the greenhouse and field showed a significant correlation between the two environments for the 51 lines tested in our study. This shows that the field performance of our plant material could be predicted with high confidence by the greenhouse trial which is operationally more advantageous in terms of time, labour and pest management. Passioura (2006) argued that some precautions should be considered, particularly regarding the medium drainage and porosity and Poorter et al. (2012) reported that growth, resources use efficiency and photosynthesis can be all affected by inappropriate pot experiments. Other studies found a high correlation in plant traits measured in greenhouse and fields for staple crops like maize and soybean including seed yield. In addition to yield, Bahrani and McVetty (2008) reported that the quality attributes of *B. napus* seeds (proteins, erucic acid, glucosinolate) grown in a greenhouse were conserved in the field.

Biochemical trait variations among the founder lines

Metabolites, like other traits in plants, are variable among species and populations and their variation is driven by the environment through local adaptation (Suomela et al. 1995, Moore et al. 2013). The estimated number of metabolites in plants exceeds 100,000 and the functions and interactions between them mostly remain unclear (Wink et al. 1988 and Keurentjes et al. 2006). In our study, we followed an untargeted metabolite analysis using LC-QTOF/MS (liquid chromatography-quad time of flight/mass spectrometry) analytics. The amino acids and sugars identified in the leaves of the founder lines showed a wide range of distribution indicating the diversity of metabolites present in them. These metabolites are often used as biochemical markers during biotic and abiotic stresses in plants. The amino acid proline, for example, gets induced during abiotic stresses and plays a key role in redox balance and cues the cell to activate stress-adaptive strategies (Fan et al. 2015). In our study, proline content was specifically high for 4 lines even though plants were not stressed which might be an indicator of a high tolerance to stress. One line (29) has a high content in arginine, an amino acid also involved in response to stress as a precursor of polyamines and the mobilization of N storage (Winter et al. 2015). Sugars profile showed a wide range among lines and a high content of fructose for line 9, a sugar involved in the tolerance to cold-induced oxidative stress as reported by Bogdanović et al. (2018) and in response to nitrogen deficit (Krapp et al. 2011). Each class of metabolites acts differently when they undergo drought stress. In wheat, there was an increase in amino acids in one of the tolerant cultivars during the stress and the organic acids content changed only during wilting of the plants (Bowne et al. 2012). Four of the founder lines (34, 12, 9, and 48) had higher proline content than the rest and one founder line (29) was richer in arginine. Unlike the amino acids, there was no distinct cluster formation among the lines for sugar content (glucose, sucrose and fructose). Fructose content was very high in just one line (9) whereas the glucose content was distributed across a wide range among lines.

Metabolomics-based approaches have been used in the past to discriminate genetically modified silent phenotypes that did not show any visible differences in morpho-physiological parameters (Weckworth et al. 2003). The use of untargeted metabolite analysis in addition to the identification of a wide range of compounds provides a platform for the identification of novel metabolites that may regulate complex biochemical mechanisms in plants. This approach has proven to identify biochemical markers associated with post-harvest quality traits in potato breeding (Steinfath et al. 2010). In the case of rice, untargeted metabolite analysis revealed distinctive seed metabolomes for *Oryza japonica* and *O. indica* sub-species though they have the same origins (Hu et al. 2014). A detailed map of the phytochemical composition of *B. napus* leaves, stems, roots, inflorescence and seeds were presented by Farag et al. (2012). The study observed significant differences in flavonoid content among the organs analyzed and demonstrated the variation in secondary metabolism between the organs of the same species. In sorghum, non-targeted metabolic profiling showed that morpho-physiological traits, particularly biomass and photosynthetic rate, were correlated with metabolites (Turner et al. 2016). From our untargeted leaf metabolite analysis, a comprehensive catalog of metabolites that are identified in the diversity panel lines can be designed. These metabolites can then be used as either a biochemical or a molecular marker and find key developmental traits associated with them. Screening differences among accessions of metabolite profile might be used in metabolites marker-based breeding and when combined with the morphological traits and genomic data, genes characterization could be accelerated (Price et al. 2017).

Breeding implications

Phenotyping the potential parent lines for crop breeding and assessing the variability of the trait among them is necessary to achieve the targeted genetic gain in progenies. A noticeable benefit of this approach is the selection of parents having complementary traits that would enhance a gain like tolerance to drought and heat or yield potential (Reynolds and Langridge, 2016). Screening multiple physiology traits might also be useful for a precise selection of lines particularly when the performance of lines is fluctuating between environments (because of the G×E and G×E×M interaction) making a verdict based on a single performance trait (yield for example) uncertain. Physiological trait-based breeding gave better results in wheat by increasing yield under optimal and stressful conditions, compared to conventional breeding (Richards et al. 2010; Bustos et al. 2013; Pask et al. 2014). In addition, the considerable progress made in measuring physiological traits with recent high throughput phenotyping techniques and protocols should accurately identify the traits of interest and accelerate the release of performant cultivars. Given the high variation in several traits measured in the lines of our diversity panel, including yield traits, we believe that breeding based on physiological traits would enhance genetic gain in canola. A subset of the most relevant traits can be teased apart and used in later steps of a breeding program to generate superior canola variants.

Conclusions

Our results showed a remarkable high variation in yield traits among the lines, particularly pod weight. Stomatal conductance and transpiration rate were among the most variable traits while the variation of the other physiological traits was moderate or low. Pod length, root dimeter and phenology traits (DTB, DTF and DTM) had a noticeable variation and high heritability estimate as well. Among all traits, the proportion of variance explained by the genotype was higher than that explained by the population, suggesting that the selection of founder lines should be based on each genotype's performance apart and not on sampling lines among populations. Traits measured in the field showed a positive correlation with those measured in the greenhouse particularly for shoot and root morphology and flowering phenology, and to a lesser extent for TSW. This was supported by a positive correlation between yields obtained in the greenhouse versus 5 field locations showing the robustness of results obtained under controlled conditions. Metabolites profiling was less discriminatory between lines than the above-mentioned traits although 4 lines showed a noticeable high content of proline and 1 line a high content in arginine. The extensive screening of the diversity panel suggests great potential for genetic gain that can be brought from the 51 lines and should give valuable information for the selection of founder lines. Exposure of the selected lines to stressors, particularly drought and heat, can highlight performant lines under adverse and optimal conditions.

Greenhouse study #2: ***Canola Responses to Drought, Heat, and Combined Stress: Shared and Specific Effects on Carbon Assimilation, Seed Yield, and Oil Composition***.

Results

Heat had a significant effect on the photosynthetic-related variables (*A, V_{cmax}*, *J*, and g_m) but not on g_s, WUE_i and $\Delta^{13}C$ (Table 1). Heat also affected the growth attributes (plant height and NDVI) and all of the measured yield and oil quality attributes. In addition, the $\delta^{15}N$ levels were significantly affected by heat alone. The available soil moisture status (drought) had a significant effect on all of the photosynthesis variables except g_m, the growth and yield attributes, and the ABA and seed protein content (Table 1). The heat + drought interaction did not significantly affect the measured variables except for the $\Delta^{13}C$ and the oil content (Table 1). It had a marginally significant effect (p = 0.06) on the NDVI and the seed weight.

Table 1. ANOVA of photosynthetic capacity attributes, yield, and seed composition of an elite canola cultivar in response to drought, heat, and heat + drought.

	A	g_s	WUE_i	V_{cmax}	*J*	g_m	C:N	$\Delta^{13}C$	$\delta^{15}N$	$\delta^{18}O$	Ht	NDVI	ABA	Silique number	Seed weight	Seed number	Oil content (% DM)	ω-3 DE	ω-6 DE	Seed protein
SOURCE OF VARIATION																				
H	***	ns	ns	***	***	***	ns	ns	***	ns	**	***	ns	***	***	***	***	**	**	***
D	***	***	***	***	***	ns	***	***	ns	ns	**	***	***	**	*	***	***	ns	ns	***
H × D	ns	ns	ns	ns	ns	ns	ns	***	ns	ns	ns	ns±	ns	ns	ns±	ns	***	ns	ns	ns

A, Net photosynthetic assimilation rate (μmol CO_2 m^{-2} s^{-1}); g_s, stomatal conductance (mol CO_2 m^{-2} s^{-1}); WUE_i, intrinsic water-use efficiency (μmol CO_2 mol^{-1} H_2O); V_{cmax}, maximal carboxylation rate of CO_2 (μmol CO_2 m^{-2} s^{-1}); *J*, photosynthetic electron transport rate (μmol CO_2 m^{-2} s^{-1}); g_m, mesophyll conductance (mol CO_2 m^{-2} s^{-1}); C:N, carbon to nitrogen ratio; $\Delta^{13}C$, isotopic composition of leaf carbon (‰); $\delta^{15}N$, isotopic composition of leaf nitrogen (‰); $\delta^{18}O$, isotopic composition of leaf oxygen (‰); Ht, plant height (cm); NDVI, normalized difference vegetation index; ABA, abscisic acid leaf content (ng g^{-1} of dry weight); ω-3 DE, desaturation efficiency of ω-3 fatty acids; ω-6 DE, desaturation efficiency of ω-6 fatty acids. ***$p < 0.001$; **$0.001 \leq p < 0.01$; *$0.01 \leq p < 0.05$; ns, non-significant; ns±, marginally significant ($p = 0.06$).

Photosynthetic Carbon Fixation Capacity and Growth

Although heat and drought significantly reduced the net photosynthetic *A*, the heat + drought treatment had the greatest effect: the *A* was ~55% less than the *A* for the well-watered plants (Table 2). In addition, the drought treatment had a greater effect on the *A* (19.4 μmol CO_2 m^{-2} s^{-1}) than did the heat treatment (22.45 μmol CO_2 m^{-2} s^{-1}); however, the well-watered plants maintained the highest photosynthetic carbon fixation capacity (26.74 μmol CO_2 m^{-2} s^{-1}; see Figure 1A). Compared to the g_s under the well-watered treatment (0.52 mol CO_2 m^{-2} s^{-1}), the g_s for the plants exposed to the heat treatment did not change significantly (0.48 mol CO_2 m^{-2} s^{-1}); however, it dropped sharply in the plants exposed to drought (0.14 mol CO_2 m^{-2} s^{-1}; see Figure 1B). The WUE_i was similar between the treatments (well-watered and heat) when water was supplied, averaging 30.75 μmol CO_2 mol^{-1} H_2O (Figure 1C). In contrast, the WUE_i rose significantly, by ~173%, in the plants subjected to a water deficit (drought and heat + drought treatments). The leaf ABA content, being low under the well-watered and heat treatments (219.1 and 198.1 ng g^{-1} DM, respectively), mirrored the WUE_i patterns (Figure 2) and increased noticeably when the plants were exposed to a soil water deficit under drought and heat + drought (2,195 and 2,209 ng g^{-1} DM, respectively).

Table 2. Effect weight of net photosynthetic assimilation rate (*A*) and yield traits as the deviation of the trait value under single and combined stressors on the value under the control conditions (well-watered plants).

	D_e	H_e	HD_e	$H + D_{calc}$	Result of combined stressors
A	0.28	0.16	0.55	0.39	Synergistic
Silique number	0.49	0.59	0.82	0.80	Cumulative
Seed weight	0.31	0.85	0.89	0.90	Heat dominant
Seeds number	0.36	0.70	0.85	0.79	Cumulative
Oil content (%DM)	Null	0.53	0.19	0.53	Antagonistic

D_e, effect weight of drought treatment (vs. well-watered plants); H_e, effect weight of drought treatment; HD_e, effect weight of heat and drought combination; $H + D_{calc}$, calculated effect weight of heat and drought combination.

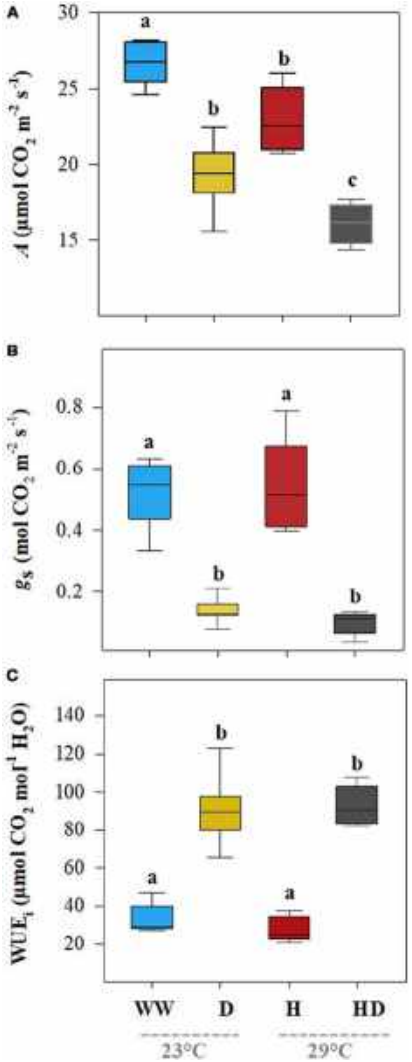


Figure 1. The leaf net photosynthetic assimilation rate (A, A), stomatal conductance of CO2 (gs, B), and intrinsic water use efficiency (WUEi = A/gs, C) of the plants grown under the well-watered (WW), drought (D), heat (H), and heat + drought (HD) treatments. The statistically significant differences among the treatments are labeled with different letters at $p < 0.05$ (Tukey's HSD). The box ends indicate the upper (3rd) to lower (1st) quartiles of the

value ranges, and the whiskers indicate the highest and lowest observations. The horizontal line inside the box marks the median for the observations.

Unlike the result for the g_s, the g_m decreased significantly on exposure to heat (25%) but did not change under the drought treatment (0.13 mol CO_2 m^{-2} s^{-1}; see Figure 3A). The heat + drought combination decreased g_s and g_m significantly; however, the effect of the single stressors was not significantly greater. Compared to those for the well-watered plants, the maximal V_{cmax} and J were reduced under the heat + drought treatment and, to a lesser extent, under the heat but not the drought treatment, following a similar trend as that for g_m (Figures 3B,C). The V_{cmax} was 84.4 µmol m^{-2} s^{-1} under the well-watered treatment and dropped to 63.2 and 43.9 µmol m^{-2} s^{-1} in the plants exposed to the heat and heat + drought treatments, respectively (Figure 3B). The J followed a similar pattern as that for the V_{cmax}, going from 178.2 µmol e^- m^{-2} s^{-1} for the well-watered plants to 133.2 and 95.4 µmol e^- m^{-2} s^{-1} for the plants exposed to the heat and heat + drought treatments, respectively (Figure 3C). Plant height averaged 145.8 cm under the control conditions and decreased under all the stress treatments. Height was the most affected by the heat + drought treatment, decreasing by 35.8% as compared to the well-watered plants (data not shown). The NDVI was 0.76 for the well-watered plants. It decreased under the drought and heat + drought treatments to 0.69 and 0.60, respectively. However, the NDVI was not affected by the heat treatment.

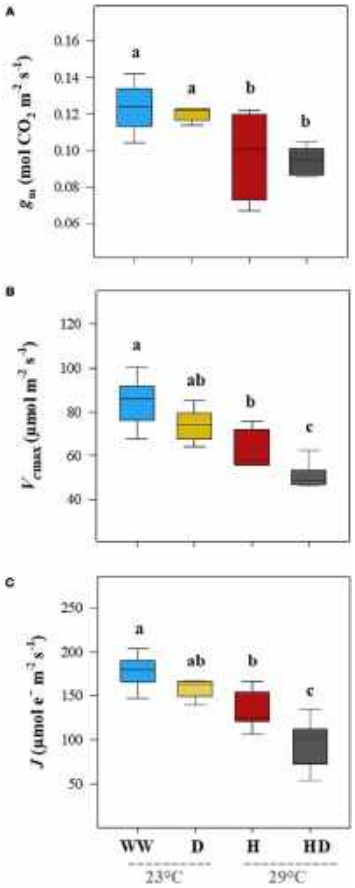


Figure 3. The leaf mesophyll conductance for CO_2 (g_m, A), the maximum carboxylation rate of ribulose-1,5-bisphosphate carboxylase/oxygenase (Vc_{max}, B), and the photosynthetic rate of the electron transport (J, C) for the plants exposed to the well-watered (WW), drought (D), heat (H), and heat +drought (HD) treatments. The statistically significant differences among the treatments are labeled with different letters at $p < 0.05$ (Tukey's

HSD). The box ends indicate the upper (3rd) to lower (1st) quartiles of the value ranges, and the whiskers indicate the highest and lowest observations. The horizontal line inside the box marks the median for the observations.

Resource-Use Efficiencies

The isotopic composition of the leaf carbon, $\Delta^{13}C$ (‰), was sensitive to the stress treatments, and discrimination reduced on exposure to heat + drought and drought (Figure 4A). The observed pattern in the $\Delta^{13}C$ was similar to that in the g_s, where a significant correlation was found between g_s and the $\Delta^{13}C$ ($R^2 = 0.50$, $p < 0.01$; see Figure 5A), but no correlation was found between the g_m and the $\Delta^{13}C$ ($R^2 = 0.03$, $p = 0.36$; see Figure 5B). This result suggests that carbon discrimination is driven mainly by stomatal closure. The treatment differences in the $\delta^{15}N$ were more noticeable, and the values ranged from 0.59‰ (well-watered) to 2.72‰ (heat + drought; see Figure 4B). The $\delta^{15}N$ plotted against the g_m showed a significant negative correlation ($R^2 = 0.34$, $p < 0.01$; see Figure 5D), while no significant correlation was found between $\delta^{15}N$ and g_s (Figure 5C). Similarly, both the V_{cmax} and the J were negatively correlated with the $\delta^{15}N$ ($R^2 = 0.32$ and 0.27, $p < 0.01$, respectively; see Figure S1). The observed $\delta^{18}O$ was not significantly different for the treatments, and the average value was 17.82‰ (Table 1).

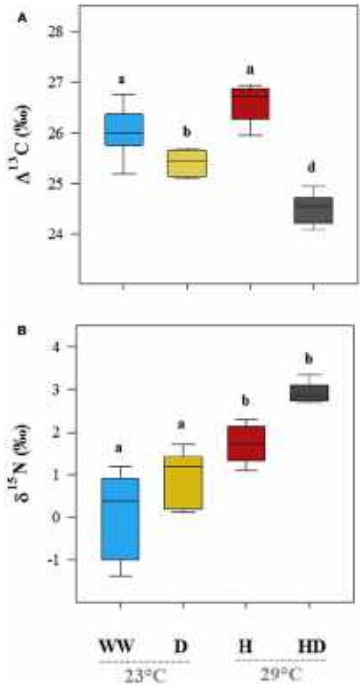


Figure 4. The effects of the well-watered (WW), drought (D), heat (H), and heat + drought (HD) treatments on leaf carbon ($\Delta^{13}C$, A) and the nitrogen ($\delta^{15}N$, B) isotopic composition. Different letters denote significantly different treatments at $p < 0.05$ (Tukey's HSD). The box ends indicate the upper (3rd) to lower (1st) quartiles of the value ranges, and the whiskers indicate the highest and lowest observations. The horizontal line inside the box marks the median for the observations.

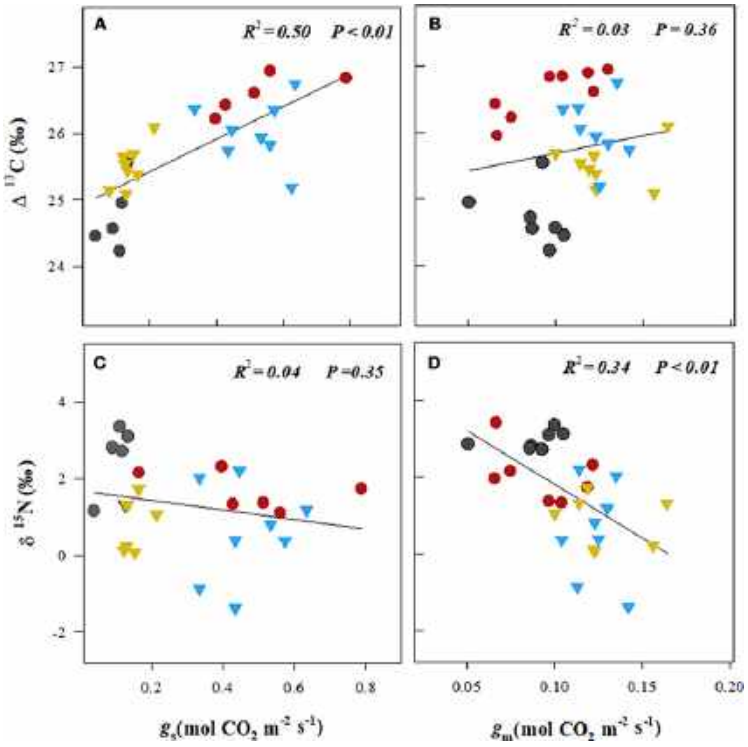


Figure 5. The relationships between leaf stomatal conductance (g_s, A,C) and mesophyll conductance (g_m, B,D) of CO_2 and the leaf isotopic composition of carbon ($\Delta^{13}C$) and nitrogen ($\delta^{15}N$). ▼, well-watered plants (WW, 23°C); ▼, drought (D, 23°C); ●, heat (H, 29°C); ●, heat + drought (HD, 29°C). The lines were fitted by regression using all the points in a plot.

Seed Yield and Total Oil and Protein Content

The number of siliques decreased noticeably by exposure to heat + drought (76%) and less by heat (43%) compared to the well-watered plants, which had 178 siliques/plant on average (Figure 6). The plants exposed to drought were less affected, with 125 siliques per plant. The seed yield was highest for the well-watered plants (6.46 g/plant). It diminished by 85% and 89% for the plants exposed to heat and heat + drought, respectively, and by 31% for the plants subjected to drought (Table 2).

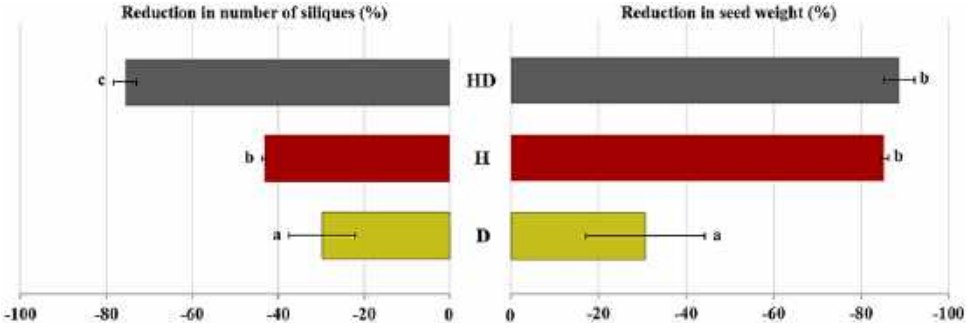


Figure 6. The percentage reduction in the seed weight and number of silique for the plants subjected to the drought (D), heat (H), and heat + drought (HD) treatments compared to the plants subjected to the control treatment. The treatments that were significantly different at $p < 0.05$ are labeled with different letters (Tukey's HSD).

On average, the oil content (% of seed dry matter) of the plants under the heat treatment was particularly low (17.2%) compared to 36.1% in the seeds of the well-watered plants (Figure 7A). The oil content (35.7%) was not significantly affected by drought, but when heat and drought were combined, it dropped to 29.2%. The total protein content (% dry matter) was 30% in the seeds of the well-watered plants. It increased under all of the stress treatments (drought 32.8%, heat 37.2%, and heat + drought 39%; see Figure 7B). The DE of the ω-6 fatty acids increased significantly as compared to the DE in the well-watered plants (17.3) when the plants were exposed to the heat treatment (25.5), but it did not change significantly when the plants were exposed to the drought and the heat + drought treatments (Figure 8A). The drought and heat + drought stress treatments increased the unsaturated fatty acid fraction. The heat treatment increased the saturated fatty acid fraction, but the drought treatment lowered it (Figure S2). The heat application resulted in a decrease in the oleic acid (18:1) content and an increase in the linoleic acid (18:2; see Figure S2). In contrast, the plants exposed to heat and heat + drought had a lower ω-3 DE (0.24 on average) than the well-watered plants (0.33; see Figure 8B) as the α-Linolenic acid (18:3) content decreased and the linoleic (18:2) increased (Figure S2).

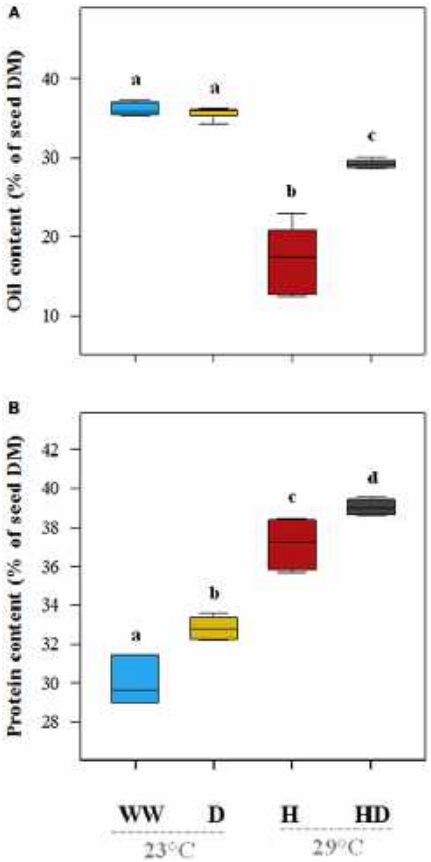


Figure 7. The total oil (A) and total protein (B) content of the seeds under the different treatments expressed as a percentage of dry matter (% DM). The treatments that were significantly different at $p < 0.05$ are labeled with different letters (Tukey's HSD). The box ends indicate the upper (3rd) to lower (1st) quartiles of the value ranges, and the whiskers indicate the highest and lowest observations. The horizontal line inside the box marks the median for the observations.

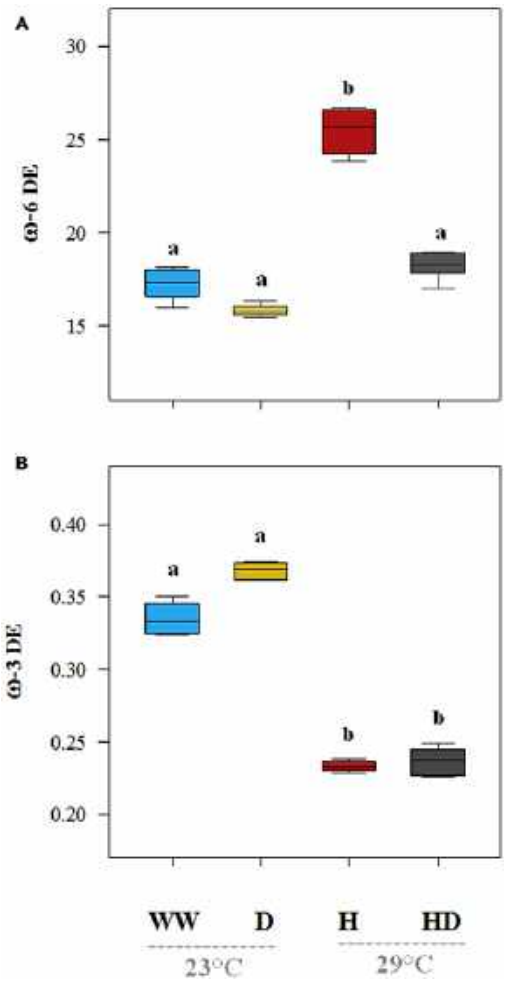


Figure 8. The desaturation efficiency (DE) of the Omega 6 (ω-6) (A) and Omega 3 (ω-3) (B) of the seed fatty acids under the different treatments. The DE values that were significantly different at $p < 0.05$ are labeled with different letters (Tukey's HSD). The box ends indicate the upper (3rd) to lower (1st) quartiles of the value ranges, and the whiskers indicate the highest and lowest observations. The horizontal line inside the box marks the median for the observations.

Relationships Among Photosynthesis, Resource-Use Efficiency, and Yield

The biochemical limitations of photosynthesis (V_{cmax}, J, and g_m) and yield attributes (silique number, seed number, and seed weight) were negatively correlated with the $\delta^{15}N$, but a positive correlation was observed between the $\delta^{15}N$ and the seed protein content (Figure 9). As for the seed composition, the oil content was positively correlated with the physiological variables (A, V_{cmax}, J, and g_m) and with the ω-3 DE ($R^2 = 0.63$) but negatively related to the ω-6 DE ($R^2 = 0.88$).

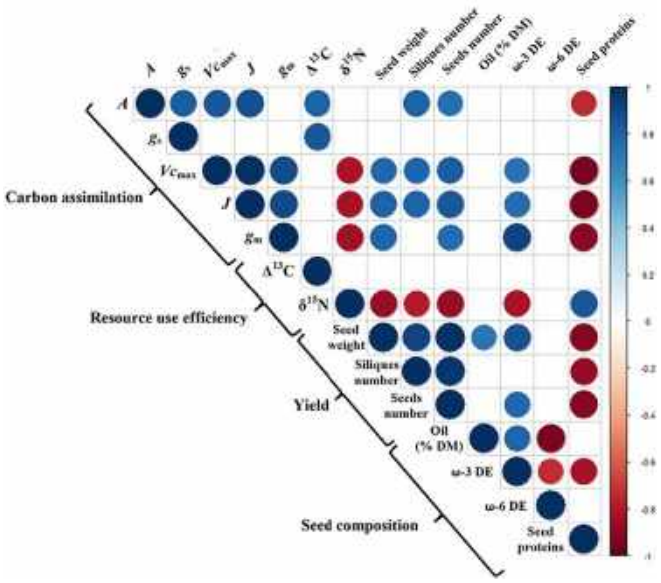


Figure 9. The Pearson's correlation coefficients for the measured traits across all the treatments. The blue and red circles denote significant positive and negative correlations, respectively (p = 0.05), and the empty cases refer to non-significant correlations. The color gradient is proportional to the correlation coefficient.

Discussion

The results of this work emphasizes an exacerbating effect of combined heat and drought on spring canola growth and yield, compared to single applications of stressors as reported for other crops (Nankishore and Farrell, 2016; Mahrookashani et al., 2017; Sehgal et al., 2017). In addition, we noticed a prevailing effect of heat over drought on photosynthetic capacity, yield and seed composition traits, most likely due to the deleterious effect of heat on the enzymes involved in carbon assimilation and metabolism. However, drought specifically affected stomatal conductance of CO_2 and related traits (ABA content, $\Delta^{13}C$ and net assimilation rate).

Response of CO_2 Diffusion and Photosynthetic Capacity to Heat and Drought

Photosynthetic activity is sensitive to both drought and heat, particularly for C_3 metabolic pathway crops, and the degree of tolerance to stressors is determinant for their survival (Feller and Vaseva, 2014). The rapid acclimation of photosynthesis to abiotic stressors has been reported for many species, particularly in response to drought and, to a lesser extent, heat. Acclimation, also termed phenotypic plasticity, consists of adjustments of physiological traits, resulting in a limited decline in growth performance (Sadras et al., 2009).

In this experiment, g_s was affected when plants were subjected to a water deficit (drought and heat + drought treatments). Under the drought conditions and the optimal temperature (23°C), the ABA leaf content increased dramatically leading to stomatal closure. This suggests that the ABA signaling pathway triggered stomatal closure to reduce the loss of tissue turgor (Wilkinson and Davies, 2010; Pantin et al., 2013). However, keeping the stomata open at a high temperature and in increased leaf-to-air VPD conditions ensures leaf transpirational cooling, but this is conditional to soil moisture availability in the root zone (Crawford et al., 2012). The findings of this study are consistent with those of previous studies. High g_s was seen in plants subjected to the heat (29°C) and the well-watered (23°C) conditions, and low g_s was observed under the drought and the heat + drought conditions. Overall, the response of the canola plants seemed to be a "conservational" strategy driven by water economy via stomatal closing rather than leaf cooling. This resulted in greater water-use efficiency but a lower A.

The range and the response of g_m to temperature were reported to be markedly different among species (von Caemmerer and Evans, 2015). The g_m increased linearly in response to a temperature gradient ranging from 15 to

40°C for *Gossypium, Niocotiana*, and *Glycine*, culminating at 0.75 to 1 mol m^{-2} s^{-1} bar^{-1} (Bernacchi et al., 2002). However, for *Arabidopsis thaliana*, which is closely related to canola, the g_m remained unchanged over a temperature gradient, reaching a maximum of 0.22 mol m^{-2} s^{-1} bar^{-1} at 25°C and then decreasing. Similarly, reduced g_m was found under a high temperature, but there was no significant effect under drought. Previous studies have reported that g_m frequently, but not always, decreased in response to a water deficit (Flexas et al., 2008; Warren, 2008; Barbour and Kaiser, 2016). There is a strong relationship between g_s and g_m as the amount of CO_2 in the sub-stomatal cavity should affect the fraction of CO_2 reaching the chloroplast stroma through the C_i, cell membranes, and cytoplasm (Olsovska et al., 2016). In general, drought triggers stomatal closure; consequently, mesophyll conductance would decrease. However, recent studies have shown that g_s and g_m conductance's can be uncoupled although environmental conditions might alter them in the same way (Barbour et al., 2016; Gago et al., 2016). Théroux-Rancourt et al. (2015) found that the g_m of hybrid poplar cuttings exposed to drought declined, but this response was delayed when compared to that for g_s. In contrast, Barbour and Kaiser (2016) observed no effect on g_m under drought conditions when an adequate nitrogen supply was made available. The unchanged g_m under the moisture deficit in the current study might have been caused by a physiological acclimation in response to the stomatal closure and the decline of the C_i, facilitating CO_2 diffusion to the carboxylation site and preventing a shortage of substrate (Flexas et al., 2010). Such adjustment mechanisms are still unclear. They are more likely anatomical and morphological (i.e., the chloroplast position, leaf mass area; Milla-Moreno et al., 2016).

Also, Momayyezi and Guy (2017, 2018) reported a substantial role for carbonic anhydrase in influencing g_m. Apart from the CO_2 diffusion limitations, photosynthetic biochemical limitations have been reported to be remarkably heat-sensitive; however, drought has had a lesser effect (Flexas et al., 2006; Galmés et al., 2007). Indeed, previous studies have found that a severe water deficit has a limited effect or no effect on biochemical limitations rates (V_{cmax} and J) compared to its effect on stomatal limitations (Demirevska et al., 2009; Killi and Haworth, 2017). These findings are in agreement with the trends of non-significant effects of drought treatments on the V_{cmax} and the J but significant decreases to both under heat treatments. Under the heat + drought treatment, the V_{cmax} and the J decreased further, showing the cumulative effects of the combined stressors; however, the J/V_{cmax} ratio did not change over the treatments (data not shown). Changes in the J/V_{cmax} ratio have been observed under adverse conditions. This could be the result of resource allocation, particularly nitrogen, to RuBisCO carboxylation or the electron transfer to optimize the photosynthetic assimilation rate (Hikosaka et al., 2006).

Carbon and Nitrogen-Use Efficiency

The results of this study showed that the $\Delta^{13}C$ response to stress treatments paralleled the trends seen in the g_s response. The ratio of the C_i to the ambient CO_2 fraction (C_i/C_a) is the main driver of the $\Delta^{13}C$ variation in C_3 terrestrial plants. This is influenced mainly by stomatal conductance (Farquhar et al., 1982). It is attributable to the shared path of the transpired H_2O and the inbound CO_2 fluxes, which stop in both directions when the stomata are closed in response to a water deficit. Several studies of plants in pots and in fields have demonstrated a strong relationship between water availability and the leaf carbon isotopic composition (Swap et al., 2004; Hartman and Danin, 2010; Cabrera-Bosquet et al., 2011). Given the environmental stability under which this experiment was conducted, the observed $\Delta^{13}C$ variations were attributed solely to soil moisture availability. Under the optimal water supply, an increase in the air temperature did not affect the leaf $\Delta^{13}C$. However, $\Delta^{13}C$ was lowest under heat + drought treatment compared to drought alone, suggesting a synergistic effect of the two stressors ("– *synergistic*" according to Piggott et al., 2015) which is supported by the significant H × D interaction effect on $\Delta^{13}C$ ($P < 0.001$). An increased water use-efficiency (i.e., lower $\Delta^{13}C$) under heat + drought reflects a scenario that goes beyond the effects of single stresses, whereby a higher canopy temperature results from drought-induced stomatal closure in combination with heat treatment.

Several studies conducted in natural ecosystems along a rainfall gradient (in addition to a temperature gradient) showed higher $\delta^{15}N$ values in C_3 plants. In the current experiment, the $\delta^{15}N$ values were influenced more by heat than drought. It is unclear, therefore, whether the variation in $\delta^{15}N$ enrichment was the result of heat or drought or a combination of both. In contrast, in the natural stands, the $\delta^{15}N$ values were higher in high nitrogen soils, and

the nitrogen availability was higher in the warm and dry areas (Craine et al., 2009). Given the uniform soil characteristics and the short-term nature of this experiment, it is unlikely that heat and moisture deficits could influence the ^{15}N vs. ^{14}N fractions in the soil and, subsequently, the variations in $\delta^{15}N$ observed in the leaves (Hartman and Danin, 2010). Therefore, it is proposed that the observed $\delta^{15}N$ variation in the current experiment resulted from plant internal fractionation during the physiological processes that occurred during the nitrogen uptake, assimilation, allocation, and remobilization (Evans, 2001). In plant roots, the assimilation of nitrogen occurs for NH+4NH4+ through the glutamine synthetase–glutamate synthase (GS–GOGAT) pathway. However, the assimilation of NO−3NO3- occurs in the roots and the leaves by nitrate reductase (NR) and the nitrite reductase (NiR) pathway, producing NH+4NH4+, which is subsequently assimilated through the GS-GOGAT reactions (Evans, 2001). Given that the $\delta^{15}N$ of the leaves but not the roots was measured, the site of the nitrogen fractionation in *B*. *napus* remains inconclusive. Thus, a thorough understanding of *B. napus* metabolic pathways for the nitrogen uptake (NO−3NO3- and NH+4NH4+)-, assimilation-, allocation-, and remobilization-inducing $\delta^{15}N$ variations would provide more information about plant nitrogen use, in turn a time-integrated measure of crop nitrogen-use efficiency.

Seed Yield

Heat stress during flowering was reported to reduce seed yield markedly by altering the gametogenesis (from meiosis to maturity), embryo sac differentiation, fertilization, and post-fertilization structures, such as the growth of the endosperm and the embryo (Wahid et al., 2007; Barnabás et al., 2008; Rieu et al., 2017), particularly in cool environment crops like *B. napus*. A higher sensitivity to heat for the female reproductive structures (ovary and embryo sac) than for the male structures was reported (Peet et al., 1998). In contrast, other studies have found pollen to be the most sensitive to heat (Saini and Aspinall, 1982). Drought also alters the reproduction and seed set in crops; however, the magnitude of this effect is generally less than that of heat. Drought stress lessens the available nutrients and photo-assimilation reserves that are essential for the development of reproductive structures (e.g., pollen tube elongation; see Barnabás et al., 2008).

As was hypothesized, the results showed a prevalent effect of heat, over that of drought, on yield as the seed weight of the plants exposed to heat reduced by 84% (vs. 31% for drought) and the silique number decreased by 43%. Angadi et al. (2000) observed that heat stress during the flowering stage, as opposed to the seed filling stage, had a pronounced effect on the *B. napus* yields. A threshold temperature close to 30°C during flowering has been reported as critical for yields for many herbaceous crops. The temperature threshold depends also on the plant species and the duration of exposure. For example, threshold temperatures range is 26°C for wheat (Stone and Nicolas, 1994) and 45°C for cotton (Ur Rahman et al., 2004), but for *Brassica* species, it is ~29.5°C (Morrison and Stewart, 2002). The results showed a considerable effect of heat at 29°C on seed yield, suggesting that the temperature threshold is much lower for canola (Gan et al., 2004; Aksouh-Harradj et al., 2006).

Oil Yield and Composition

Oil is the most profitable product from canola seed processing, and its content and composition are affected by environmental factors (Jensen et al., 1996; Si et al., 2003). Seed oil stems mostly from photosynthetic carbon assimilation of leaves and green silique walls, later carbohydrates converted into triacylglycerol through a metabolic pathway occurring in the plastid, cytosol, and endoplasmic reticula (Baud and Lepiniec, 2010; and references therein). The effects of drought and heat on the oil content in oilseed crops have varied remarkably and have most likely been the result of a high G × E interaction (Pritchard et al., 2000; Sinaki, 2009; Zhang et al., 2014). Champolivier and Merrien (1996) reported a 6–12% decrease in oil content in the *B. napus* when the plants were subjected to a water deficit during flowering and silique development, but Aslam et al. (2009) reported a mere 3.2% reduction. In a different field study, Zarei et al. (2010) found no differences in canola oil content (an average of 37.27%) with or without irrigation. Similar to the observations made under drought conditions, Zhang et al. (2014) reported a significant effect of heat on oil content, which decreased by 52.5%. The results of this study were similar to those of Zhang et al. (2014). Heat, through its effect on the enzymatic panel involved in the lipid biosynthesis pathways, has been reported to decrease oil content (Iyer et al., 2008; Baud and Lepiniec, 2010).

In addition, the silique walls, along with the leaves during the post-flowering stages, are significant sources of photosynthates (Aschan and Pfanz, 2003; Bennett et al., 2011; Hua et al., 2012). Thus, abiotic stressors during flowering would affect silique development and subsequently reduce the available photo-assimilates for triacylglycerol biosynthesis and oil accumulation in the seeds. In addition, oxygen availability in silique was also cited as a limiting factor in seed development (Porterfield et al., 2000). Vigeolas et al. (2003) reported that a low oxygen content in *B*. *napus* seeds resulted in reductions in the adenosine triphosphate (ATP) level and the triacylglycerol content. Similarly, Rolletschek et al. (2007) observed a negative correlation between the ambient temperatures and the oxygen levels in sunflower seeds, a relationship that affects oil composition.

As was hypothesized, heat noticeably altered the oil profile, but drought had a marginal effect. Previous studies on heat have reported changes in the oil composition, particularly the fatty acids, and protein content in oil seed crops (Dornbos and Mullen, 1992; Flagella et al., 2002; Wang and Frei, 2011). This effect has been attributed to the enzymes involved in biosynthesis and the conversion of fatty acids in various cellular compartments (Flagella et al., 2002; Di Caterina et al., 2007; Hernández et al., 2009). For example, Martínez-Rivas et al. (2003) found that in sunflowers, the activity of oleate desaturase, an enzyme involved in the desaturation of fatty acids in oilseeds, was altered by heat. Moreover, oil composition has been shown to be influenced by the action of abiotic stressors on the transport of fatty acids through various organelles, particularly from plastids to cytosol, where oleic acid (18:1) is converted into linoleic acids (18:2) and linolenic acids (18:3) (Browse and Somerville, 1991). In general, the fraction of polyunsaturated fatty acids decreased; however, the fraction of saturated fatty acids and, concurrently, oleic acid (mono-unsaturated) increased in response to stressors (Pritchard et al., 2000; Wang and Frei, 2011). The results of the current study were similar to those previously seen for heat conditions, which increased the proportion of saturated fatty acids, but not for drought, which increased the relative content of unsaturated fatty acids (Aslam et al., 2009).

Along with triacylglycerol, seed proteins represent a major form of energy reserves in the *Brassica* species, and their respective contents are negatively correlated (Grami et al., 1977; Jensen et al., 1996). Thus, stressors decreasing the oil content in seeds would concurrently increase the protein fraction (Henry and MacDonald, 1978; Rossato et al., 2001; Rathke et al., 2006). Overall, the results of this study are in agreement with these previous findings although the drought treatment increased the seed proteins without affecting the oil content. For the most part, heat exceeded the effect of drought in augmenting the seed protein content (e.g., heat shocks proteins as chaperones; Kotak et al., 2007) but not the osmoprotectants (polyamine, glycine betaine, and proline; Singh et al., 2015). Under the heat treatment, the well-watered plants might maintain the optimal nitrogen uptake and accumulation in the vegetative parts, subsequently nitrogen remobilized from the senescent tissue to the seeds. Given that the seed number was considerably reduced by heat, the nitrogen supply could have been superior to the demand, thus boosting the seed protein content. Lohaus and Moellers (2000) demonstrated that the external nitrate supply is determinant of the total amino acid content of the phloem sap of leaves and is positively correlated with the seed protein content in two *B*. *napus* cultivars. The partitioning of the oil and protein content was under G × E control, and the molecular basis of this trade-off is still unclear (Si et al., 2003; Chao et al., 2017).

Conclusions

Overall, the results of this study showed a divergence between the effects of drought, heat, and heat + drought on canola seed yield and oil quality. Drought affected the carbon assimilation rate mainly through the limitation of CO_2 diffusion through the stomata and the seed yield components. The effects of the heat conditions were clearly manifested in the alteration of the reproductive organs and process, leading to a substantial reduction in the seed yield and the number of siliques. To a lesser extent, heat impaired the internal CO_2 diffusion and the RuBisCO carboxylation and regeneration. This was most likely the result of thermal damage to the enzymes involved in photosynthetic assimilation. Similarly, heat had a prevailing effect over drought on seed composition, which is greatly influenced by the conversion and transport of photo-assimilates to the seeds, in turn higher levels of saturated fatty oils. Such higher levels of saturated fat under warmer climates could affect industrially relevant traits: the taste, freshness, and shelf life of canola oil. The adverse effects of moderate to severe drought can be

mitigated by irrigation and/or using genotypes with greater water-use efficiency. However, heat requires the breeding of heat tolerant canola as a major tool to manage the harmful effects of such environments. Such breeding efforts could target the carboxylation capacity and diffusion of CO_2 along the mesophyll pathway as well as the tolerance of the reproductive organs for elevated temperatures.

Greenhouse study #3: ***Sulfate feeding into cysteine indirectly triggers ABA induced stomata closure to impart drought tolerance in Brassica napus L.***

Results

Two spring canola cultivars (*Brassica napus* L.), drought-resistant Polish cultivar "Czyzowska" (NAM1, referred herein as DT) and drought-sensitive Indian cultivar "BN-1" (NAM5, referred herein as DS), previously screened for drought tolerance were selected from a diversity panel. Drought condition here refers to as 40% of water holding capacity. We investigated morphological and agronomic traits, analyzed gas exchange parameters and metabolic responses under drought and well-watered conditions.

Agronomic traits

Fully mature plants of DT cultivar were taller and had higher normalized difference vegetation index (NDVI) values than DS cultivar under well- watered condition. They flowered approximately 7 days later (46 DAS) compared to DS cultivar (39 DAS). Plants under drought condition were shorter and flowered earlier (43 DAS and 37 DAS respectively for DT and DS cultivars). Similarly, seed yield and seeds per pod were significantly lower in DS cultivar compared to DT cultivar. Drought treatment further reduced the yield for both cultivars. Number of branches (Figure S1) and number of pods (data not shown) however were higher in DS cultivar both of which were reduced under drought conditions.

Diurnal changes in leaf temperature and transpiration water loss

Leaf temperature was recorded from 08:00h to 18:00h at 2 hours interval (Figure S2). Lowest leaf temperature was recorded at 08:00h and highest at 10:00h. For both DT and DS, leaf temperature was significantly higher under drought compared to those under well-watered conditions at each time points recorded. Water loss was measured by weighing every two hours for two nights and one full day. Based on daylight time and greenhouse conditions night time was considered to be from 21:00pm to 05:00am. Fully watered pots of DT and DS were initially weighed and the pot was covered to minimize soil water evaporation and allow for leaf transpiration. Amount of water loss every two hour reduced as night progressed until 03:00am, started to increase throughout the morning, remained high during the afternoon and finally the decreased as the evening progressed. A subtle difference between the two cultivars were recorded during night but the difference became significant during the day at 15:00pm and 17:00pm (Figure 1a). Average water loss per hour was significantly different between day and night and between DT and DS during the day (Figure 1b).

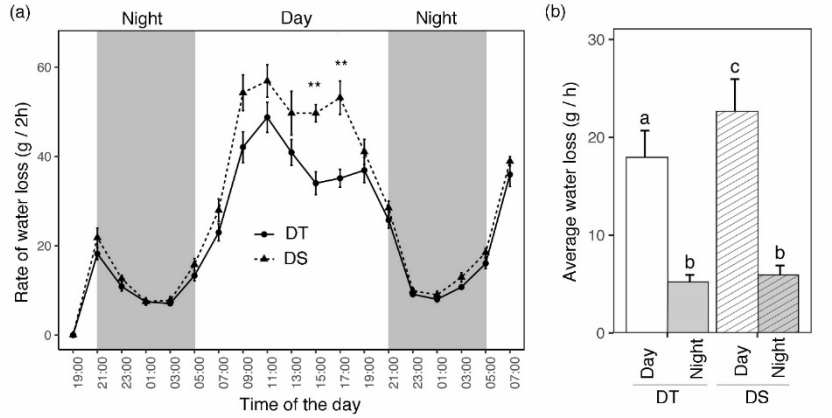


Figure 1. Gravimetrically measured day and night water loss of drought-tolerant (DT) and drought-sensitive (DS) canola lines grown in pots. **(a)** The total amount of water lost per pot was measured every two hours. The grey

background represents nighttime (21:00 pm to 5:00 am) and the observations were recorded for two full nights and one full day. Data points represent mean ± SE (n=5 plants). Statistically significance between DT and DS are indicated by asterisks ($p < 0.05$ * and $p < 0.01$ **). **(b)** Day and night average water loss per hour. The statistically significant differences between the lines during day and night are labeled with different letters at $p < 0.05$ (Tukey's HSD).

Leaf stomatal conductance

Leaf stomatal conductance (g_s) was recorded at night, pre-dawn and in the morning for both DT and DS cultivars under well-watered and drought conditions. In each of the three time points and under both conditions g_s was higher in DS cultivars (Figure 2). Among the time points recorded, there was no significant increase from night to pre-dawn however there was a drastic increase during early morning (Figure 2). The early morning, stomatal conductance under well-watered condition for DS (0.19 mol m^{-2} s^{-1}) and DT (0.12 mol m^{-2} s^{-1}) cultivar were higher than those under drought treatments (0.042 and 0.025 mol m^{-2} s^{-1} respectively for DS and DT).

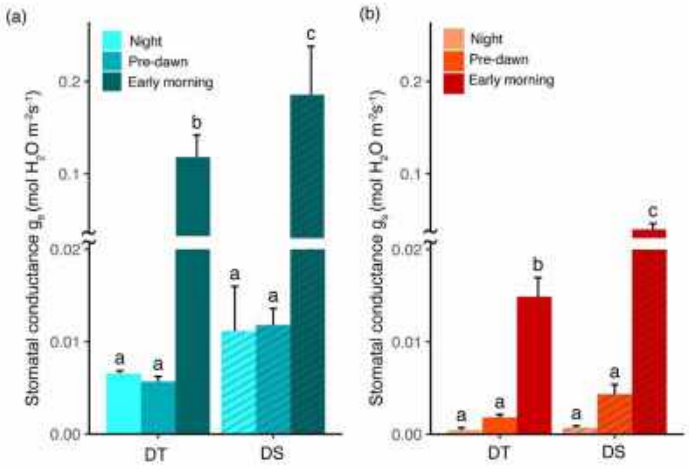


Figure 2. Stomatal conductance (g_s) during the night, pre-dawn, and early morning for the drought-tolerant (DT) and drought-sensitive (DS) canola lines under well-watered **(a)** and drought conditions **(b)**. The statistically significant differences between the lines at different time points are labeled with different letters at $p < 0.05$ (Tukey's HSD).

Relationship between ABA, sucrose, cysteine and leaf CO_2 diffusion

Under well-watered condition, Leaf ABA content for both cultivars (DT and DS) was similar (323.8 and 273.1 ng g^{-1} respectively). Under drought, the values increased significantly to 1322.7 and 869.7 ng g^{-1} for DT and DS respectively (Figure S3a). Sucrose content under well-watered conditions was higher in DS (53.4 ug mg^{-1}) compared to DT (20.3 ug mg^{-1}) and increased in both cultivars (by 3 folds and 4.5 folds respectively) under drought (Figure S3b). Similarly, cysteine content was low in both cultivars under well-watered condition (0.72 umol g^{-1} on average) and then increased by 83% and 174% in DS and DT respectively under drought (Figure S3c). To analyze the physiological impact of the drought treatments, we looked at leaf mesophyll conductance measurements in both cultivars. Mesophyll conductance decreased under drought stress and was not significantly different between the DS and DT cultivar (Figure S4).

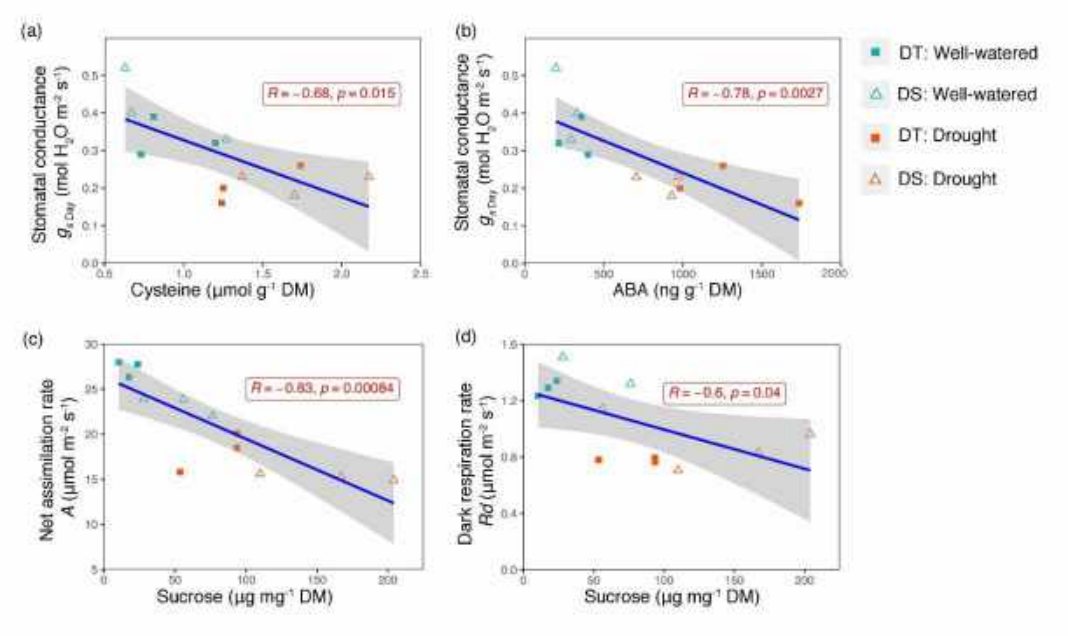


Figure 3. Scatter plot showing linear association between cysteine and stomatal conductance **(a)**, ABA and stomatal conductance **(b)**, sucrose and net assimilation **(c)** and Sucrose and dark respiration **(d)**. Pearson correlation coefficient (*R*) and p-value for each linear relation is illustrated. The gray shading represents the 95% confidence interval around the line of best fit.

Stomatal conductance was negatively correlated to leaf Cysteine and ABA content (R = -0.68 and R = -0.78 respectively) (Figure 3). In addition, although ABA content was positively correlated to cysteine (R= 0.49, data not shown), the correlation was not significant (p = 0.1). Further Sucrose showed significant negative correlation with Net assimilation rate (R = -0.83) and Dark respiration rate (R = -0.6) (Figure 3).

Leaf epicuticular waxes load and seed oil and protein content

Scanning Electron Microscopy (SEM) images were taken for both abaxial and adaxial surfaces of leaves from plants under well-watered and drought conditions. Wax morphology was different between treatments, and to a lesser extent between the two genotypes (Figure 4a and 4b). Adaxial surface appeared to have more wax platelets while the abaxial surface contained more wax tubules. Under drought, the DT cultivar developed more wax load (platelets and β-diketone tubules).

Lipid unsaturation of the epicuticular waxes, measured by C=C content, decreased by 22.8% and 37.7%, for DT and DS respectively, when plants were exposed to drought stress. Under this treatment, no significant difference was observed between the two cultivars (data not shown). CH_2/CH_3 ratio had the same variation pattern as C=C with a higher value in DS cultivar under the same treatment (Fig 4c). Drought treatment significantly reduced CH_2/CH_3 ratio in DS cultivar (Figure 4c). C=O functional group increased by 30% under drought stress for DS cultivar however there was no significant difference between two cultivars (data not shown). We also looked at the stomata pore length/breath (l/b) ratio in both adaxial and abaxial surfaces for both cultivars under well-watered and drought conditions. On both surfaces, the ratio increased significantly under drought treatment in DT cultivar but not in DS cultivar (Figure 4d, 4e).

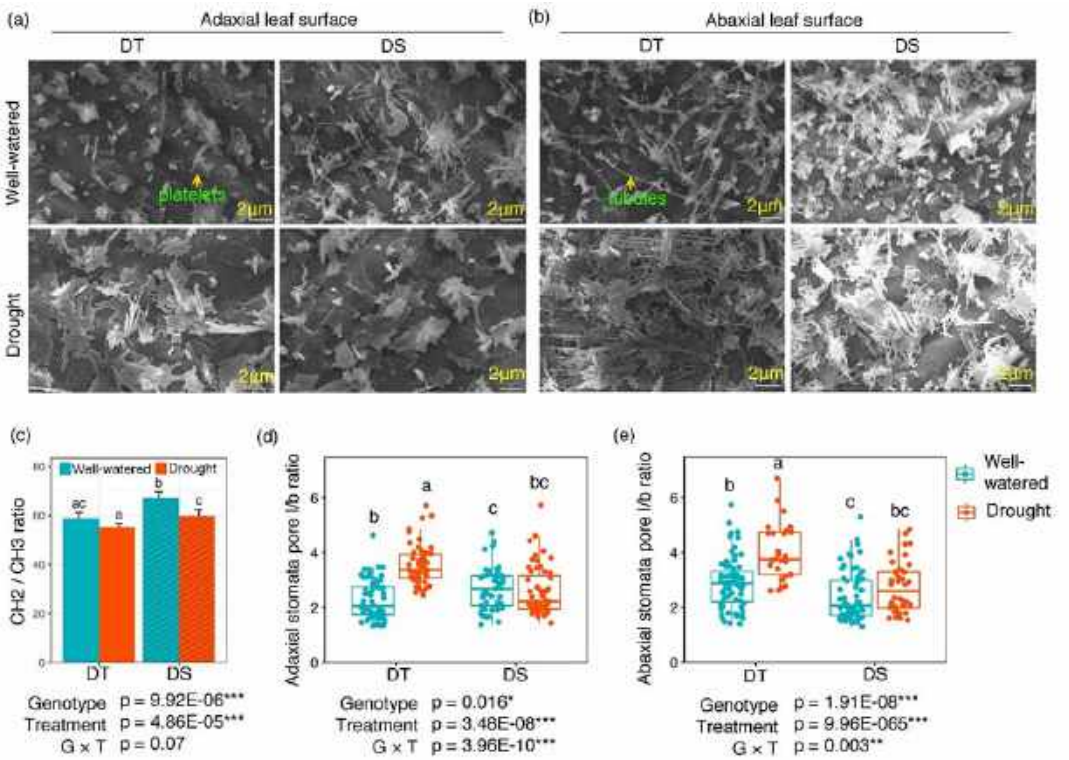


Figure 4. SEM morphology of adaxial **(a)** and abaxial **(b)** leaf surfaces from well-watered and droughted canola lines. Leaf surface wax elements were detected at 5000× magnification (scale bars = 2μm). The CH2/CH3 ratio **(c)**, Stomatal pore opening represented as (l/b) ratio of pore length and width in adaxial **(d)** and abaxial **(e)** leaf surfaces for the drought-tolerant (DT) and drought-sensitive (DS) canola lines under well water and drought conditions. Data was analyzed using two-way ANOVA with post hoc Tukey tests (letters indicate significant differences between groups at $p < 0.05$). Error bars depict standard deviation. Asterisks represent statistically significant difference between treatment and genotype (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

Oil content (% seed DM) was similar between DT and DS cultivars under WW conditions (35.3% and 34.5%, respectively) (Table S1). Under drought treatment, oil content decreased significantly to 23.8% and 23.7% for DT and DS respectively. Similarly, seed protein content in seeds was not significantly different between the DT and DS under WW treatment (Table S1). Drought conditions significantly increased protein content in both cultivars (Table S1).

Metabolic analysis

Principal component analysis (PCA) of sucrose and organic acids was performed to provide a preliminary understanding of the metabolic differences between DT and DS cultivar under well-watered and drought conditions. The PCA showed cultivar as a main factor for variance (37.5%) along PC1.

We further looked at how well-watered, drought and drought recovery affected amino acids in both cultivars. We found that out of the 17 essential and non-essential amino acids analyzed, 12 of them had genotype and/or treatment × genotype effect. These 12 aminoacids were grouped into 5 families based on their biosynthetic pathway from the intermediates of the carbon metabolism pathway (Figure 5). When we ranked these aminoacids, multi-trait genotype ideotype distance index (MGIDI) based on well-watered and drought conditions selected Histidine, Aspartic acid and Proline. Similarly, Proline, Histidine and Aspartic acid were selected when the drought and recovery selection criteria was applied (Figure 6).

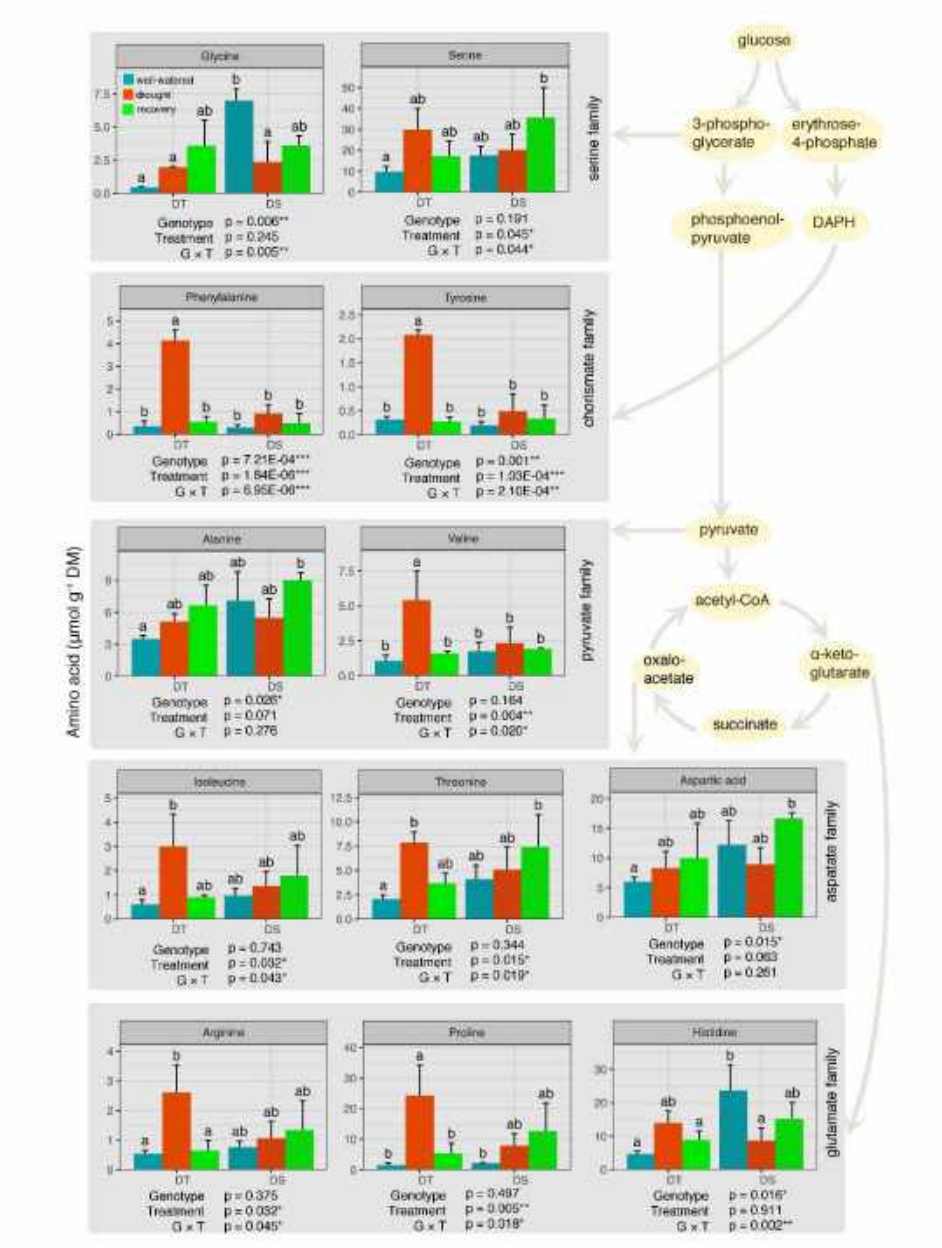


Figure 5. Leaf amino acid composition for the drought-tolerant (DT) and drought-sensitive (DS) canola lines under well-watered, drought and water recovery conditions. Data was analyzed using two-way ANOVA with post hoc Tukey tests (letters indicate significant differences between groups at $p < 0.05$). Error bars depict standard deviation. Amino acids are grouped based on different intermediates of the carbon metabolism pathway. Some intermediate components of the pathway are omitted for convenience.

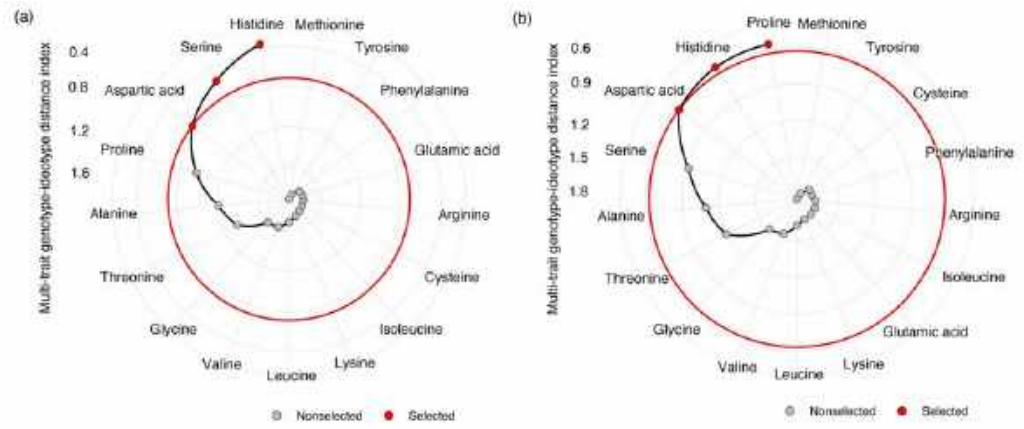


Figure 6. Amino acid ranking in ascending order for a multi-trait genotype ideotype distance index (MGIDI) from well-watered to droughted condition **(a)** and from droughted to recovery **(b)**. The selected amino acids are shown in red circles.

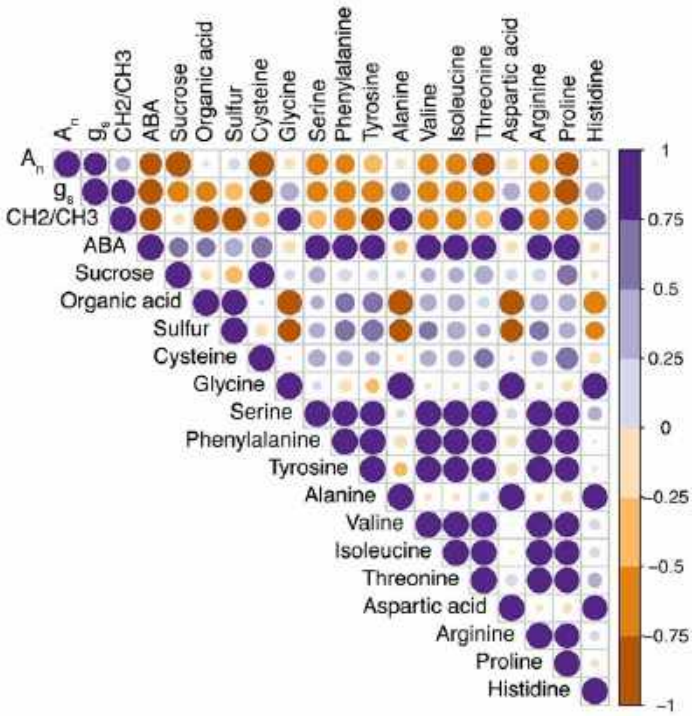


Figure 7. Correlation matrix of the physiological traits, wax load and metabolites for drought-tolerant (DT) and drought-sensitive (DS) canola lines under well-watered and drought conditions. The increasing intensity of color and size of the bubble indicates positive (purple) and negative (brown) Pearson's correlation.

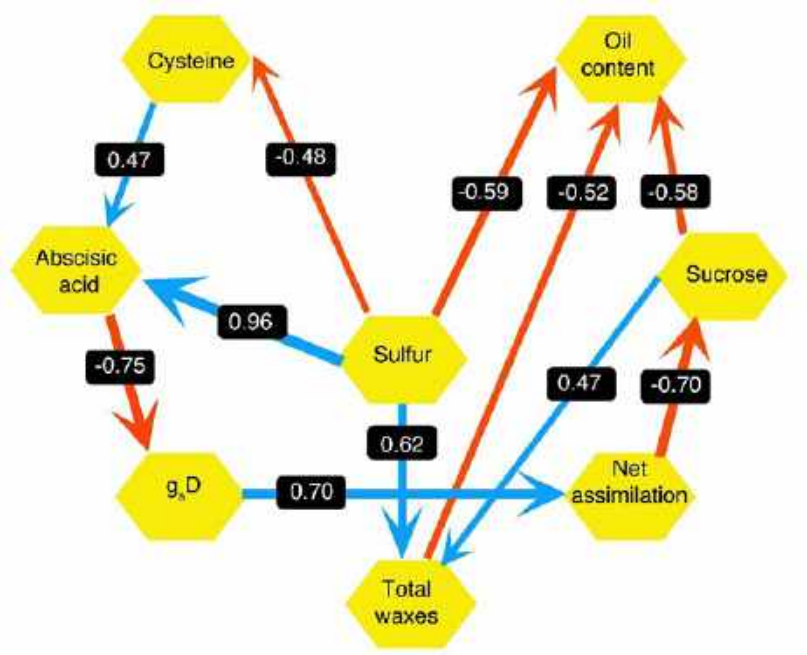


Figure 8. Pathway model hypothesized from agronomic, physiological and metabolite traits for drought-tolerant (DT) and drought-sensitive (DS) canola lines under well-watered and drought conditions. Values on the arrow are path coefficients. Blue arrows indicate positive effects and those in red indicate negative effects.

A Correlation matrix was plotted between physiological and metabolic variables that showed significant Treatment Genotype differences (Figure 7). Finally we performed confirmatory factor analysis using semPlot. Our analysis shows that oil content is strongly and directly affected by leaf sulfur and sucrose content (Figure 8) and highlights a negative relationship between oil content and leaf waxes. The diagram also showed a strong link between ABA versus S and cysteine leaf content (Figure 8).

Discussions

Nocturnal stomatal conductance was considered as a consequence of a non-complete control of stomata closing which leads to water leaking during nighttime (Resco De Dios et al. 2019). From a water balance perspective, this can be perceived as a loss which might compromise plant water status and growth. However, other explanations reporting a positive role of night transpiration have emerged, like the anticipation hypothesis stating that a higher predawn stomatal conductance is concomitant with a rapid opening of stomata and higher carbon assimilation at early morning (Resco De Dios et al. 2016). Under optimal conditions of our work (absence of water deficit), night g_s was lower in the drought tolerant cultivar with no gain in yield (oil content in % of DM). However, under drought conditions, night g_s decreased but was not significantly different between the two cultivars. Then, though night g_s was genotype-specific and responsive to drought in canola, no evidence of a relationship with yield could be demonstrated under our experimental conditions. However early morning and day stomatal conductance were both higher for the drought susceptible cultivar under optimal and stressful water status of plants, showing that anticipation hypothesis could not be confirmed under our experimental setup. On the other hand, water loss also occurs through the leaf cuticle which is a continuous lipid barrier, but not completely impermeable to water evaporation (Heredia-Guerrero et al. 2014). Indeed, epicuticular waxes load and composition affect the whole water transpiration rate and are responsive to environmental cues (Domínguez et al., 2011, Lu et al. 2012). Total waxes load of leaves that we measured did not discriminate between the two cultivars under optimal watering conditions. Genetic factors influence waxes quantity and composition as well (Laila et al. 2017), but this seems to be triggered by the genotype-environment interaction in our case, rather the genotype alone. Actually, in *Brassica sp*, high wax load and low wax load genotypes could be distinguished within the same species (Laila et al. 2017, Jin

et al. 2020). Our results showed that drought-susceptible cultivar, unlike drought-tolerant, was responsive to drought stress by increasing total wax load, suggesting a Genotype × Treatment driven response. Epiculticle composition parameters measured by FTIR were all influenced by exposure to the drought treatment, but only CH_2/CH_3 was affected by genotype. In *Arabidopsis*, the analysis of the transcriptome showed that metabolism of leaf epicuticular waxes was upregulated by exposure to soil water deficit (Bernard et Joubès, 2013). The observed variations of water loss through stomata and cuticle showed that early morning and day stomatal conductance were indictors of drought tolerance among the two cultivars rather than EW load and structure and night stomatal conductance, which were predominately affected by the environment (drought treatment).

Leaf ABA increased by exposure to water deficit as expected and was higher in the DT cultivar which is consistent with the variation of day stomatal conductance, significantly lower in the DT cultivar and the strong negative correlation between g_s and ABA. This is in agreement with the ABA-mediated stomatal closure that has been observed in many species under drought and salinity stress (Christmann et al. 2007; Cutler et al. 2010). ABA is actually considered a good target to improve tolerance to drought since biosynthesis pathways, signaling and regulation are now much better understood and action on guard cells and stomata are well documented among staple crops (Wan et al. 2009, Vishwakarma et al. 2017). Still, the 'signalosome' of leaf ABA in response to drought stress is complex and needs further investigation (Raghavendra et al, 2010). ABA-mediated closure of stomata (through cytosol alkalization or Ca^{2+} release in the cytosol) might be directly triggered by soil water deficit detected in roots or mediated by other signaling molecules through various biosynthesis cascades like that of hydrogen disulfide (H_2S) (Finkelstein, 2013; Thakur and Anand, 2021). Our results also showed a positive relationship between leaf cysteine, a sulfur amino acid, and ABA content. Previous studies highlighted the importance of sulfur in mitigating the effect stress in plants and showed that Cysteine and other S derivatives are involved in signaling and in enhancing antioxidative response to abiotic stressors (Cao et al. 2014, Ma et al. 2016). Also, there is a clear evidence of interactions between S and other metabolites involved in stress tolerance like phytohormones, hydrogen peroxide (H_2O_2) and polyamines. In *Arabidopsis thaliana*, sulfate can induce stomata closure by activating NADPH oxidase to produce reactive oxygen (ROS) under a water stress and ROS acted as an additional messenger of ABA signaling (Batool et al. 2018). *Arabidopsis* mutants lacking chloroplast sulfate transporter3;1 function (sultr3;1) showed lower ABA levels in seeds and seedlings (Cao et al. 2014). In addition, studies reported that H_2S, a signaling gas molecule produced by degradation of L-cysteine by L-cysteine desulfhydrase, was associated with ABA-dependent closure of stomata under biotic (pathogens) and abiotic stress (oxidative, heavy metals) (Zhang et al. 2010, Sun et al.2013, Pantaleno et al. 2020. The strong link between ABA, S and cysteine demonstrated by the path analysis diagram of our work is consistent with these observations. In addition, metabolites containing Sulphur were associated with tolerance to low temperature by an enhanced antioxidant capacity in the extremophile *Colobanthus quitensis* (Clemente-Moreno et al. 2019). In response to a high light, Arabidopsis transgenic plants expressing cysteine proteases in chloroplasts showed a better acclimation of photosynthesis by limiting the decrease of RubisCo large subunit abundance (Alomrani et al. 2021). Then, it is not surprising that S is involved in signaling for drought tolerance in plants even though the current understanding of sulfate action on stomata remains quite limited (Batool et al. 2018).

Under drought, Lee et al. (2019) reported an accumulation of sugars, and particularly sucrose, due in part to high expression of ABA-dependent sucrose signaling genes in *Brassica napus*. In rice, Mathan et al. (2020) showed that an ABA-responsive transcription factor, OsbZIP72, directly binds to the promoters of two sugar transporters (OsSWEET13 and OsSWEET15) and activates their expression. This increase of sucrose content was also observed in our study for both cultivars with a higher content in the drought-sensitive cultivar. However, we couldn't observe a significant correlation between ABA and sucrose as shown in Fig. S2.

Seed oil content (% DM) decreased while protein content increased in both cultivars under drought treatment but did not discriminate between the two cultivars. Our study on a canola elite cultivar (Elferjani and Soolanayakanahally, 2018) showed a similar increase of protein seed content while oil content was not significantly different between control and water-stressed plants. Other studies reported a decrease of oil content by soil water deficit (Moaveni et al. 2010, Tesfamariam et al. 2010, Hatzig et al. 2018). This differential response might be

explained by a cultivar-specific response of oil and protein content to drought as reported in the study of Hatzig et al. (2018) on a group of 8 canola cultivars. Similarly, Guo et al. (2017) showed a significant genotype x environment effect on seed oil content of nine semi-winter rapeseed lines and their 72 F1 hybrids. Accumulation of triacylglycerols, the main components of canola oil, might be influenced by environment at every step of their biosynthesis. For example, oil content is dependent on silique wall photosynthates which are transported to seed coat and transformed into fatty acids (Baud and Lepiniec, 2010). Then, drought which might affect silique development and growth would consequently decrease photosynthates produced and ultimately oil content (Ghobadi et al. 2006, Naderi and Imam, 2010). However, along with photosassimilates availability, oil content also depends on the seed intrinsic capacity for oil accumulation, particularly controlled by embryo and genotype-environment interactions (Weselake et al. 2009).

Conlcusions

Our study shows a noticeable response of leaf cysteine and Sulphur to drought exposure in both *Brassica napus* cultivars, which is in agreement with previous studies demonstrating the involvement of Sulphur and its derivatives in mitigating the effect of abiotic stressors on plants. The correlations between cysteine, ABA and stomatal conductance suggest a role of cysteine and sulfur in modulating the stomata movement under water deficit conditions. Our study also demonstrated the responsiveness of the load and composition of epicuticular waxes to drought in *Brassica napus* and the importance of water losses through stomata during dark period and early morning. Attributes related to CO_2 diffusion and photosynthetic capacity were significantly different between the two cultivars and could indicate a higher drought tolerance for the DT cultivar. Most of metabolites measured in this study were responsive to stress but did not show difference in response magnitude between the two cultivars. Using a larger panel of drought tolerant and sensitive cultivars might better differentiate the two groups and identify metabolites associated with drought tolerance.

Supplementary information

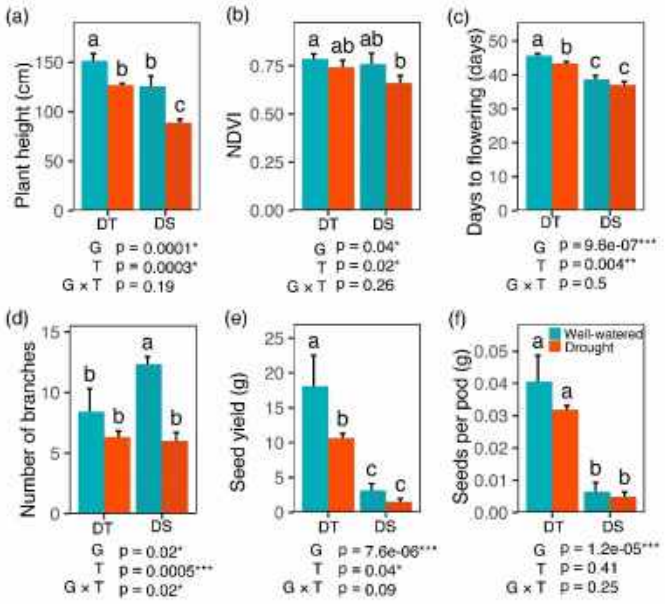


Figure S1. Various agronomic traits for drought-tolerant (DT) and drought-sensitive (DS) canola lines under well-watered and drought conditions; plant height **(a)**, normalized difference vegetation index (NDVI) **(b),** days to flowering **(c)**, number of branches **(d)**, seed yield **(e)** and seed yield per pod **(f)**. Data was analyzed using two-way ANOVA with post hoc Tukey tests (letters indicate significant differences between groups at $p < 0.05$. Error bars

depict standard deviation. Each value represents the mean ± SD and the asterisks represent statistically significant difference between treatment (T) and Genotype (G) (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

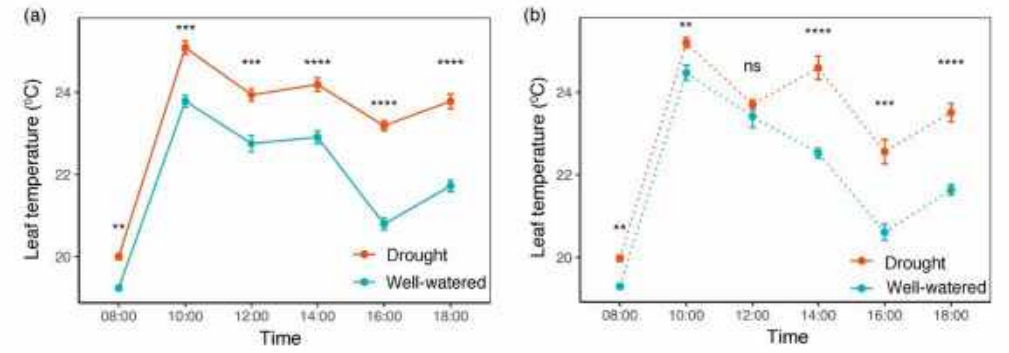


Figure S2. Leaf temperature obtained from thermal images between 8:00 to 18:00 hrs for **(a)** drought-tolerant (DT) and **(b)** drought-sensitive (DS) canola lines under well-watered and drought conditions. Each value represents the mean ± SE and the asterisks represent statistically significant difference between treatment (ns= not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, ****= $p < 0.0001$).

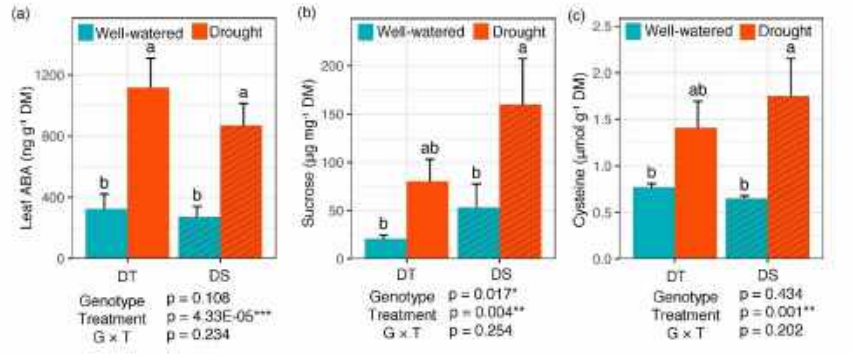
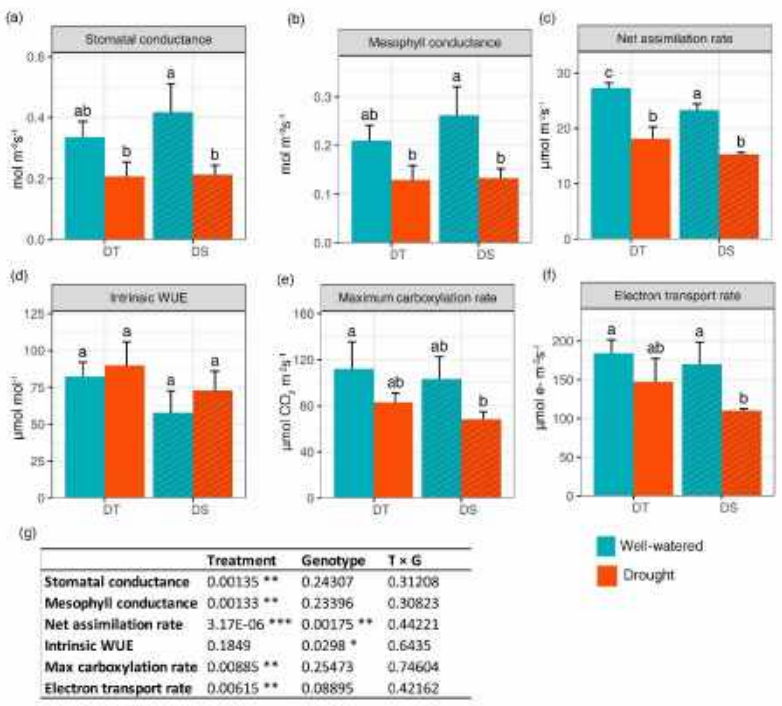


Figure S3. Leaf ABA (a), sucrose (b) and cysteine (c) values for the drought tolerant (DT) and drought sensitive (DS) cultivars under well-watered and drought conditions. Data was analyzed using two-way ANOVA with post hoc Tukey tests (letters indicate significant differences between groups at $p < 0.05$. Error bars depict standard deviation. asterisks represent statistically significant difference between treatment (T) and Genotype (G) (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).



(g)

	Treatment	Genotype	T × G
Stomatal conductance	0.00135 **	0.24307	0.31208
Mesophyll conductance	0.00133 **	0.23396	0.30823
Net assimilation rate	3.17E-06 ***	0.00175 **	0.44221
Intrinsic WUE	0.1849	0.0298 *	0.6435
Max carboxylation rate	0.00885 **	0.25473	0.74604
Electron transport rate	0.00615 **	0.08895	0.42162

Figure S4. Various physiological traits for the drought tolerant (DT) and drought sensitive (DS) cultivars under well-watered and drought conditions. Data was analyzed using two-way ANOVA with post hoc Tukey tests (letters indicate significant differences between groups at $p < 0.05$. Error bars depict standard deviation. Numbers in **(g)** are p values and asterisks represent statistically significant difference between treatment (T) and Genotype (G) (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

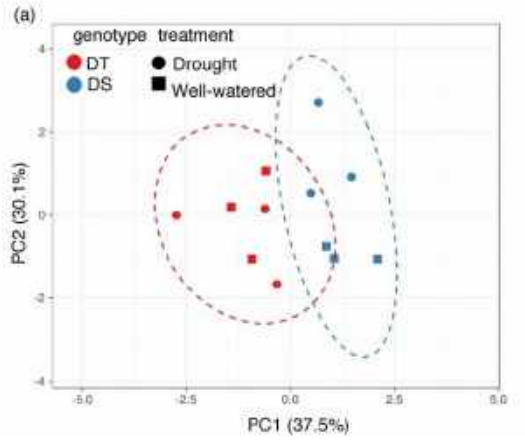


Figure S5. Principle component analysis of drought-tolerant (DT) and drought-sensitive (DS) canola lines under well-watered and drought conditions using organic acids and sucrose values. X and Y axis show principal component 1 and principal component 2 explains 37.5% and 30.1% of the total variance, respectively. A dotted eclipse represents a 95% confidence level.

Table S1. Seed oil and seed protein content in DT and DS cultivars under well-watered and drought treatment.

	DT		DS	
	WW	D	WW	D
seed oil (%)	35.3 ± 0.30 [a]	23.8 ± 1.7 [b]	34.5 ± 0.85 [a]	23.7 ± 2.55 [b]
seed protein (%)	32.2 ± 0.57 [a]	38.5 ± 1.02 [b]	33.2 ± 0.64 [a]	38.4 ± 0.16 [b]

Greenhouse study #4: ***Characterization of ABA accumulation in NAM FLs under the combinational drought and heat stresses.***

ABA is an important signal for plant stress tolerance, including drought stress (Cutler et al. 2010). Plant drought responses are categorized into two; ABA-dependent and independent regulations (Fujita et al. 2011). ABA accumulates when plants are dehydrated, and its level decreases when plants are re-watered (Nambara et al. 2005). In the early stage of dehydration, ABA closes the stomata to reduce the transpiration rate and induces gene expression for cell protection. However, in the latter stages of stress, accumulated ABA has negative impacts on both growth and yield including inhibition of photosynthesis. In this regard, the ideal pattern of ABA accumulation during drought stresses is the early increases in response to drought and the decrease for the extended period of stress.

Field-grown plants adapt to changing environments and often experience the combination of multiple stresses. In this project, we studied the ABA accumulation of NAM FLs under drought, heat and both drought and heat for 2 weeks. This experiment aims to investigate the difference in ABA accumulation of NAM FLs under various stresses. In total, 1,600 samples (50 NAM lines x 4 treatments (control, drought, heat, both drought & heat) x 4 replicates x 2 independent experiments) were analyzed for ABA levels. In addition, these samples were also analyzed for other physiological analyses. Thus, in addition to breeding data with yield performance, ABA data would complement these data to understand the genetic and physiological variations of NAM FLs.

Results

The average ABA levels of 4 replicate with two independent experiments (Exp 1 and Exp 2) are shown in Supplemental Table 1. Many lines show consistent results between Exp 1 and Exp2, but some lines have variations results between 2 experiments. So, ABA levels in individual treatment are shown by the scattered plots in which ABA levels in Exp 1 are on the X-axis, while those in Exp 2 are on the Y-axis (Fig. 1~3). ABA levels in well-watered (WW) control plants are variable, ranging from 80~500 ng/gDW. NAM FLs are compared among drought stressed (D), heated (H) and drought plus heat (D+H) based on ABA levels.

Drought stress (D)

As depicted in Fig. 1, Some NAM FLs accumulated ABA at extremely high levels in D plants, such as NAM-36, NAM-0, NAM-85, NAM-56, NAM-23, NAM-33, NAM-42, NAM-75. ABA inhibits photosynthesis during a long period of stress. Therefore these lines are expected to be susceptible to D and decrease the growth and yield. On the other hand, some lines show low ABA levels in D plants, such as NAM-4, NAM29 and NAM-88 after a long period of D. At present, we cannot evaluate these lines by ABA levels alone. The presented ABA levels are D plants after a long period of D treatment. Some of these lines may have low ABA levels throughout D, which is D susceptible. Otherwise, some other lines may accumulate ABA at the early D, then decrease due to acclimation, which is D tolerant. ABA measurements in earlier stages of these lines will validate if ABA dependent D responses act to yield positively or not. On the other hand, NAM-88 is unique, and its ABA levels are lower in D plants than WW for both Exp 1 and Exp2. Most of NAM lines accumulated ABA > 2-fold in D plants when compared with well-watered (WW) control (36 lines for Exp1, 43 lines for Exp 2 out of 51 lines) (Supplemental Table 1).

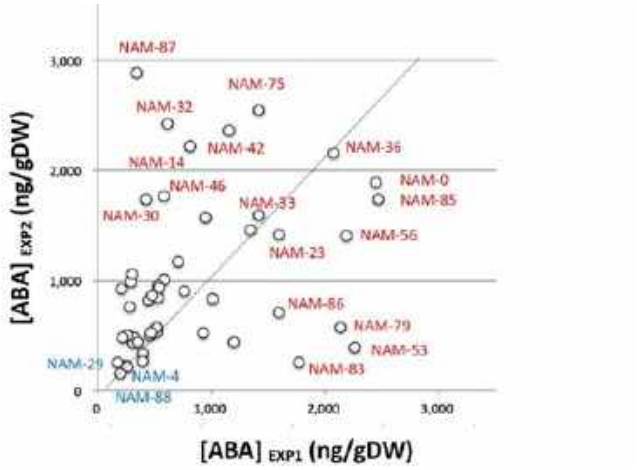


Figure 1. [ABA] levels in drought NAM FLs. Two independent ABA measurements (Exp1 and Exp2) were performed. Each ABA measurement data is the average of 4 replicates. Dashed line indicates the line that show no variations between Exp1 and Exp2.

Heat stress (H)

H alone has less impacts on ABA levels. All NAM FLs show low ABA levels for both Exp 1 and Exp 2 (Fig. 2).

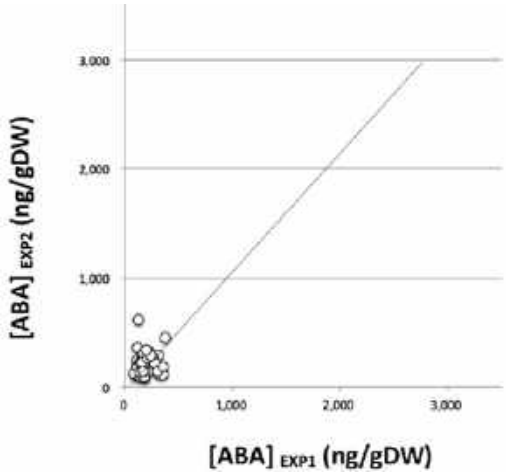


Figure 2. [ABA] levels in heat stressed NAM FLs.Two independent ABA measurements (Exp1 and Exp2) were performed. Each ABA measurement data is the average of 4 replicates. Dashed line indicates the line that show no variations between Exp1 and Exp2.

Drought and heat (D+H)

Although the limited impact of H alone on ABA accumulation, the combination of D and H have pronounced effects on ABA accumulation (Fig. 3). The effect of D+H is more complex when compared with D alone. Depending on NAM FLs, H either enhances or suppresses the ABA accumulation under D. NAM-0 accumulated ABA in D+H plants at extremely high levels (Fig. 3). Most of NAM lines accumulated ABA > 1,000 ng/gDW for both Exp 1 and Exp 2. These suggest that D+H treatment negatively impacts these lines. Only 4 lines, NAM-29, NAM-4, NAM-34, NAM-45, show low ABA levels (<1,000 ng/gDW) for Exp 1 and Exp 2. The ABA levels in the early stages of stress will explain how ABA dependent stress pathways contribute to these lines.

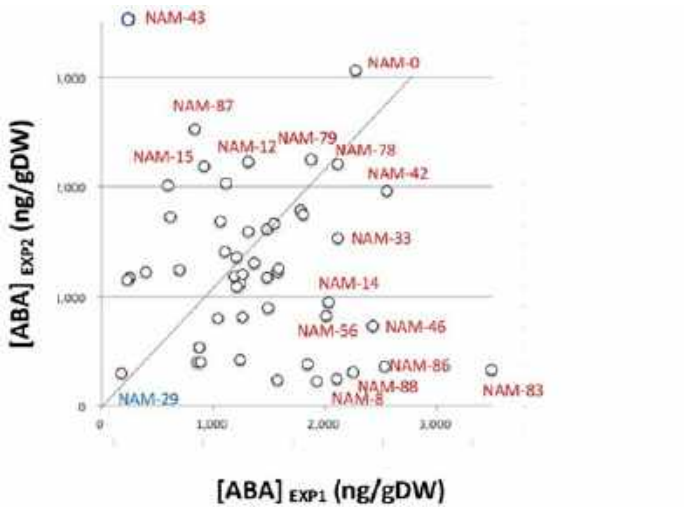


Figure 3. [ABA] levels in drought & heat stressed NAM FLs.Two independent ABA measurements (Exp1 and Exp2) were performed. Each ABA measurement data is the average of 4 replicates. Dashed line indicates the line that show no variations between Exp1 and Exp2.

This is the first demonstration of ABA levels of NAM FLs under various stresses. Four sets (WW, D, H and D+H) of data for 2-week stress treatment. Long-term ABA accumulation has a negative impact on growth and yield, so NAM lines with high ABA accumulation after long-term stress are likely to be stress susceptible. Other lines with low ABA accumulation after long-term stress requires further analysis on early stages of stress.

In addition, we found strong correlations of Fv/Fm (i.e., photosytem II effciency) and NDVI with yield across drought, heat and drought+heat stress treatments (Fig. 4, 5). So, these non-destructive estimates are good predictors for estimating canola yields.

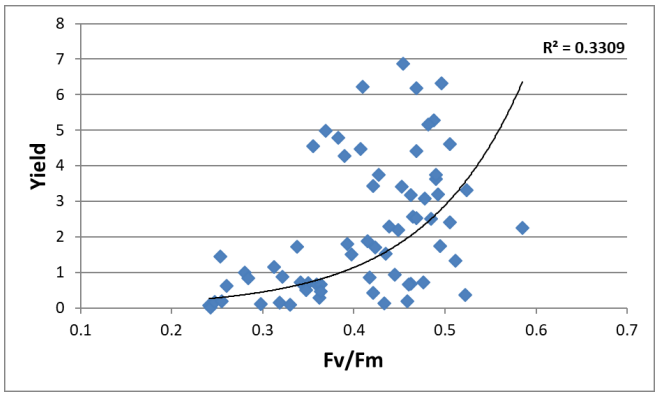


Figure 4. Correlation between Fv/Fm and seed yield in 22 NAM FLs subjected to control, drought, heat and drough+heat treaments 7 days after stress.

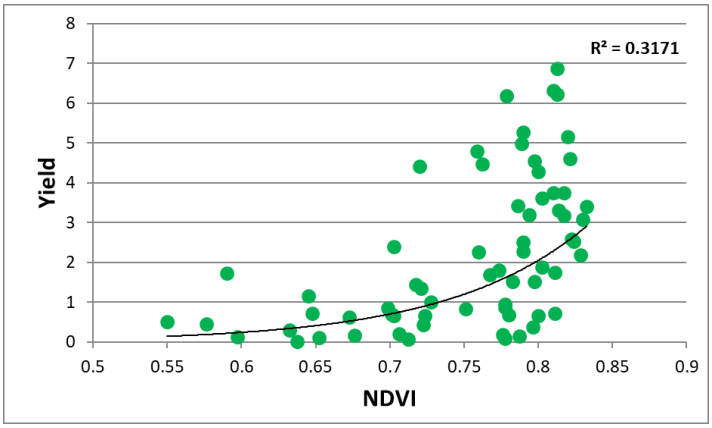


Figure 5. Correlation between NDVI and seed yield in 22 NAM FLs subjected to control, drought, heat and drough+heat treaments 7 days after stress.

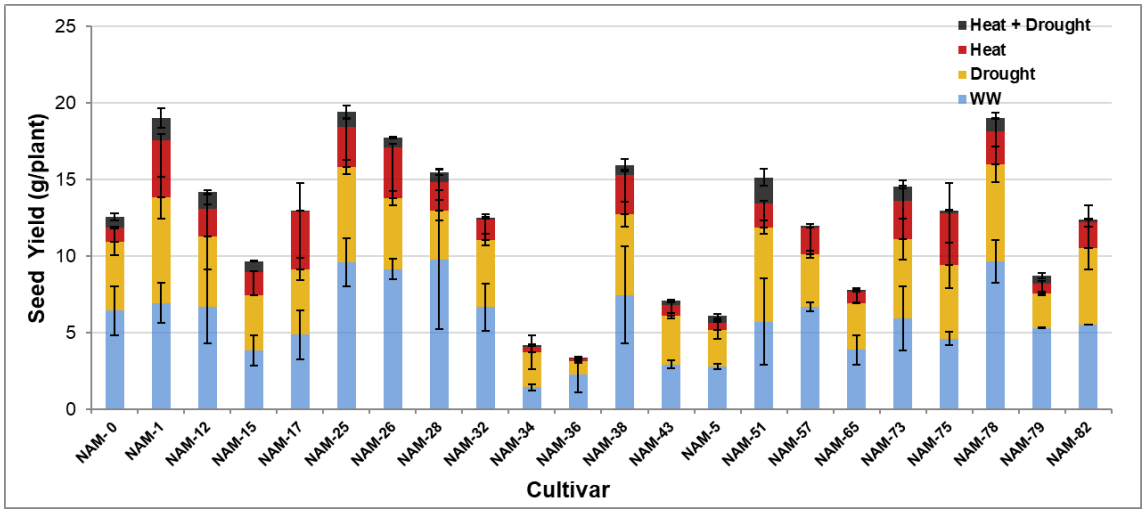


Figure 6. Seed yield in 22 NAM FLs subjected to control, drought, heat and drough+heat treaments 7 days after stress.

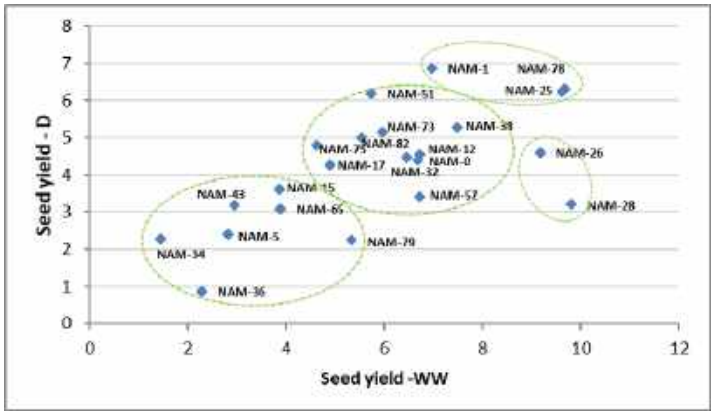


Figure 7. Seed yield of control and drought stressed 22 NAM FLs collected at crop maturity.

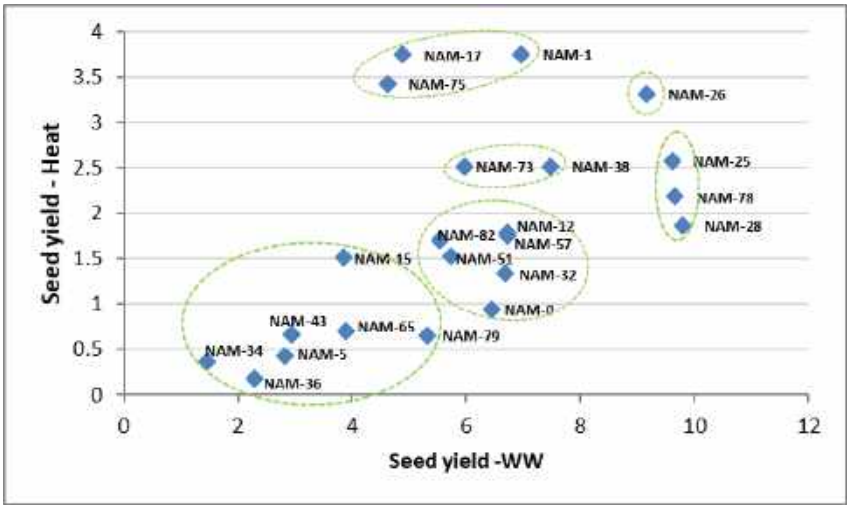


Figure 8. Seed yield of control and heat stressed 22 NAM FLs collected at crop maturity.

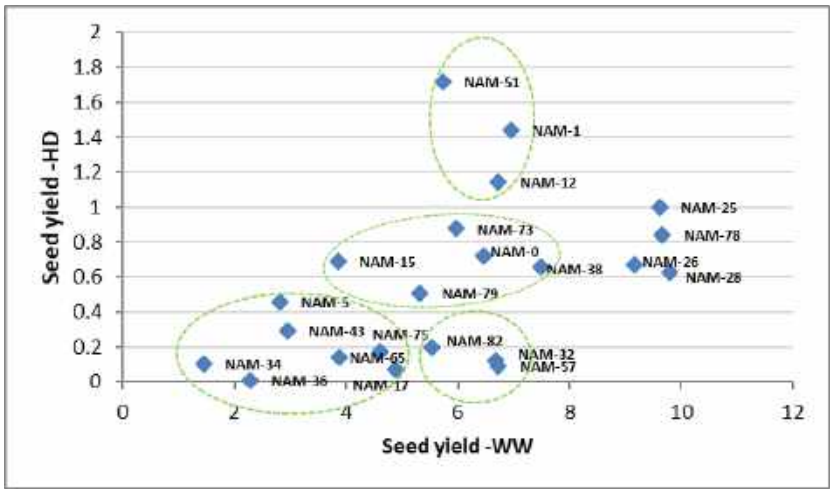


Figure 9. Seed yield of control and drought+heat stressed 22 NAM FLs collected at crop maturity.

Overall, based on our four treaments (control, drought, heat and drought+heat), heat had a significant negative effect on canola plant yields (Fig. 6, 7, 8, 9).

10. Conclusions and Recommendations: *Highlight significant conclusions based on the findings of this project, with emphasis on the project objectives specified above. Provide recommendations for the application and adoption of the project findings. (**Maximum of 500 words**)*

- Heat significantly reduced canola yields by preventing seed set. Heat treatment had a more deleterious effect than drought on the seed oil composition, leading to enhanced levels of saturated fatty oils and, consequently, desaturation efficiency, a measure of oil frying ability.
- In some ways, wax deposition on canola leaf surfaces protects against drought damage.
- As part of the cysteine pathway, sulfur contributes to stress resilience in canola, which is further linked to ABA, which leads to stomatal closure. Due to the redirected sulfur, oil content is adversely affected.

- Based on the ABA profiling in this project, we were able to identify canola NAM FLs that produce less ABA and are able to cope with drought and drought plus heat.
- Carbon and oxygen stable isotopes (proxies for water-use efficiency and transpiration efficiency, respectively) could be used as physiological markers for selecting high yielding canola lines since they integrate growing season signals.

11. Is there a need to conduct follow up research? *Detail any further research, development and/or communication needs arising from this project*.

Yield losses of approximately 35% in 2021 are the prime example of the devastation that heat and drought stress can elicit on canola production. Heat and drought stresses directly affect the water uptake and retention capacity of plants, halting photosynthesis and reducing growth and yield. Higher than normal temperatures often observed during heat waves also lead to reduced pollination and flower abortion. Similarly, drought stress disrupts hormonal balance leading to seed abortion in pods (Liu et al. 2003). The early morning flowering trait is proposed to be effective in escaping heat-induced seed sterility at flowering by shifting flower opening time to cooler early morning times. At higher temperatures, flowers that open at later hours tend to be sterile with a higher proportion; hence, shifting that flower opening time to the cooler early morning, even by one-hour advancement, is effective in mitigating heat-induced sterility at flowering (Pokharel et al. 2020).

Recently, several studies have shown that a balanced and timed reactive oxygen species ROS accumulation is critical for pollen tube growth and pollen viability (Santiago et al. 2019). If the ROS synthesis surpasses the cell quenching potential, the excess accumulation of ROS results in oxidative damage and cell death. However, the ROS-scavenging antioxidant enzymes (superoxide dismutase, peroxidase, glutathione reductase, ascorbate peroxidase) and antioxidant metabolites (Asc, Glut) have a role in alleviating heat stress damage from ROS. During pollen development, specific stages that are affected by heat stress could be targeted to improve the thermotolerance of canola flowers.

Improvement in environmental stress tolerance is considered a primary driving force of yield stability in canola. Studies based on model simulation showed a 2 °C increase in temperature is likely to reduce crop yields by more than 20%. Identifying the environmental factors contributing to the gap between average and potential yields in canola across Western Canada will provide prospects for narrowing the yield gap.

This study highlights how plants can integrate different signals to mitigate stress survival (climate, physiology, hormones, metabolomes). There are a number of intriguing questions related to this new and emerging field of system biology integration during abiotic stress in crops that need to be addressed through further research.

- Liu F et al., (2003) https://doi.org/10.1071/FP02185
- Pokharel et al., (2020) https://doi.org/10.1111/jac.12408
- Santiago et al., (2019) https://doi.org/10.1111/pce.13576

12. Patents/ IP generated/ commercialized products: *List any products developed from this research.*

Not Applicable to this proposal. The canola NAM consotirum is handling it separately.

13. List technology transfer activities: *Include presentations to conferences, producer groups or articles published in science journals or other magazines*.

Peer-reviwed article:

- Elferjani, R., and Soolanayakanahally, R.Y. (2018). Canola responses to drought, heat, and combined stress: shared and specific effects on carbon assimilation, seed yield, and oil composition., Frontiers in Plant Science doi: 10.3389/fpls.2018.01224.
- Dhanyalakshmi, K.H., Soolanayakanahally, R.Y., Rahman, T., Tanino, K.K., and Karaba, N. N. (2019). Leaf cuticular wax, a trait for multiple stress resistance in crop. IntechOpen 2019, DOI: http://dx.doi.org/10.5772/intechopen.84565.

Oral presentation:

- Soolanayakanahally, R.Y. (2021). Digital Phenotyping for Crop Breeding and Precision Agriculture. The Digital Think Tank a research and policy arm of the Information and Communications Technology Council (ICTC) May 26, 2021. Invited Seminar.
- Elferjani, R., Soolanayakanahally, R.Y., and Gjetvaj, B. (2017). Canola yields under hot and dry climates. , 2017 CSPB-CSHS Joint Meeting, Vancouver, BC, Canada, July 4-7, 2017.

Poster presentation:

- Elferjani, R., Soolanayakanahally, R.Y., and Gjetvaj, B. (2019). Contrasting responses of canola leaf traits under drought., 3rd Agriculture and Climate Change, Budapest, Hungary, March 24-26, 2019.

Print, Radio coverage:

- Canola Watch (14 August, 2021) - Harvesting thin, low-yielding canola: common questions. https://www.canolacouncil.org/canola-watch/fundamentals/harvesting-thin-low-yielding-canola-common-questions/
- Alberta Farmer (17 June, 2019) - Impact of smoke on crops is more than a little hazy https://www.albertafarmexpress.ca/2019/06/17/impact-of-smoke-on-crops-is-more-than-a-little-hazy/
- Country Guide (7 November, 2018) - Canola growth stalled under a shroud of smoke https://www.country-guide.ca/2018/11/07/canola-growth-stalled-under-a-shroud-of-smoke/92674/?module=carousel&pgtype=homepage&i=2

Field days:

- CSIDC 2015 Field Day, Rapeseed and canola genetic diversity. July 9, 2015, Outlook, Canada.

14. List any industry contributions or support received.

None.

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- At field days, oral prsentations and poster presentations the Ministry of Agriculture, SK, SaskCanola logos were dsipalyed. Ina ddditional proper acknowldegment for providing the fudning for this project was highlsihted.
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16. Appendices: *Include any additional materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications, literature cited (Use a consistent reference style throughout).*

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