Multi-scale characterization of cold response reveals immediate and long-term impacts on cell physiology up to seed composition

N. Langlade[1], Leconte Jean M.L.[1], Moroldo Marco[1], N. Blanchet[1], Bindea Gabriela[2], Carrère Sébastien[1], Catrice Olivier[1], Comar Alexis[3], Labadie Marc[3], Marandel Rémy[1], N. Pouilly[1], Tapy Camille[1], Paris Clémence[4], and Mirleau-Thébaud Virginie[4]

[1]Universite de Toulouse
[2]INSERM
[3]Hiphen
[4]Syngenta France SAS

April 04, 2024

Abstract

Early sowing can help summer crops escape drought and can mitigate the impacts of climate change on them, but it exposes them to cold stress during initial developmental stages, which has both immediate and long-term effects on development and physiology. To understand how early night-chilling stress impacts plant development and the yield, we studied the reference sunflower line XRQ under controlled, semi-controlled and field conditions. We performed high-throughput imaging of the whole plant parts and obtained physiological and transcriptomic data from leaves, hypocotyls and roots. We observed morphological reductions in early stages under field and controlled conditions, with a decrease in root development, an increase in reactive oxygen species content in leaves and changes in lipid composition in hypocotyls. A long-term increase in leaf chlorophyll suggests a stress memory mechanism that was supported by transcriptomic induction of histone coding genes. We highlighted leaf transcriptomes in cold-acclimation genes such as chaperone, heat shock and late embryogenesis abundant proteins. We identified genes in hypocotyls involved in lipid, cutin, suberin and phenylalanine ammonia lyase biosynthesis and ROS scavenging. This comprehensive study describes new phenotyping methods and candidate genes to understand phenotypic plasticity better in response to chilling and study stress memory in sunflower.

Introduction

In the near future, climate change will likely intensify extreme weather events, increase average temperatures and increase the frequency of heat waves and dry summers, thus becoming the main threat to food security worldwide (Abbass *et al.* , 2022; Seneviratne *et al.* , 2023). It will also have a profound impact on the distribution of arable land, with potential gains in Russia and northern China (Zhang and Cai, 2011) and probable losses in Africa, South America and Europe. At the global scale, aridity could degrade 40% of arable land (Prăvălie *et al.* , 2021). To address the threat of climate change in the context of a growing population, agriculture must adapt to ensure human food security, feed and non-food services (Anderson *et al.* , 2020).

Early sowing (ES) is a traditional agricultural practice used in arid regions to reduce the impact of drought. It increases yields effectively because it places the key developmental stages of crops under the most favorable growing conditions. For instance, ES increases the duration of the vegetative stage and the yield of soybean (*Glycine max*) (Taaime *et al.* , 2022). For sunflower (*Helianthus annuus*) and wheat (*Triticum aestivum*

), ES promotes flowering earlier in the year, when rainfall is more frequent, which increases the yield (Hunt *et al.* , 2019; Abdelsatar, 2020; Giannini *et al.* , 2022).

One side effect of ES is that plantlets are exposed more to chilling stress (i.e. temperatures of 1-15°C), which can decrease productivity (Li *et al.* , 2017). For sunflower, suboptimal temperatures during initial stages of development decrease yield by ca. 34.7 kg/ha/d (Debaeke *et al.* , 2017). Thus, ES seems a promising solution to mitigate impacts of drought in Mediterranean climates, but it may be more difficult to implement in temperate climates (Mangin *et al.* , 2017).

Phenotypic responses to cold stress such as a decrease in plant height, stem diameter, canopy area and root weight were observed for soybean and maize (*Zea mays*) (Hussain *et al.* , 2020; Borowska and Prusiński, 2021; Hassan *et al.* , 2021; Walne and Reddy, 2022). Morphological changes such as plant development are caused by underlying physiological processes. Low temperatures first make plant cell membranes more rigid. The decreased fluidity has a mechanical effect on calcium channels, resulting in calcium influx into the cytosol. Changes in the calcium balance then act as a signal transduction that modulates downstream pathways (Sangwan *et al.* , 2001). Membrane fluidity is an important parameter that governs other crucial processes, such as vesical trafficking and membrane permeability. Fluidity is maintained by increasing the unsaturation of lipids through the activity of desaturases (Murata and Los, 1997; Thakur and Nayyar, 2013; Manasa*et al.* , 2021)

Plant development is a direct function of photosynthetic efficiency. Low temperatures decrease the activity of enzymes in plants, which decreases photosynthesis (i.e., "photoinhibition"). For instance, low temperatures decrease the carboxylase capacity of RuBisCO and thus photosynthetic efficiency, which decreases biomass production (Galmés*et al.* , 2013).

The production of reactive oxygen species (ROS) is another negative effect of cold stress. At low concentrations, ROS such as nitric oxide and hydrogen peroxide can act as a signaling molecule that modulates genes involved in defense-signaling pathways. At high concentrations, ROS cause cellular damage if they are not associated with an effective ROS scavenging mechanism (Ritonga and Chen, 2020).

These physiological processes are under genetic control, which are triggered by low temperatures. The ICE-CBF-COR is a signaling cascade activated by low temperatures that modulates the expression of multiple genes in order to resist low temperatures. For instance, CBF can bind to the promotor of COR genes, which code for cryoprotective proteins and enhance cold tolerance (Ritonga and Chen, 2020). Proteins such as late embryogenesis abundant (LEA) proteins, anti-freezing proteins and cold shock proteins are synthetized under cold stress and protect plants from injury (Ding *et al.* , 2019). These processes have been described well for model plants such as thale cress (*Arabidopsis thaliana)* , but no information is available for sunflower. These observations were performed 3-48 h after exposure under controlled conditions, but under field conditions, chilling stress occurs, persist longer than 48 h, and influence all initial developmental stages and late traits such as oil yield (Mangin *et al.* , 2017). For instance, Allinne *et al.*(2009) observed a decreased photochemical efficiency, chlorophyll content and leaf area and increased electrolyte leakage with ES compared to normal sowing for sunflower immediately before flowering (i.e., 800 degree days).

Although cold can be a major abiotic stressor for summer crops in the context of new cropping practices, the impacts of early chilling stress throughout the life cycle is not well documented for sunflower. Here, we assessed impacts of cold stress and ES using modern phenotyping platforms to compare controlled and semi-controlled conditions to field experiments in order to distinguish environmental factors and determine their impacts throughout the life cycle. On molecular and physiological levels, we described impacts of low temperatures on root, hypocotyl and leaf transcriptomes, as well as on fatty acid composition. We aimed to describe sunflower plasticity in response to night chilling stress and provide a framework for future studies of sunflower tolerance to cold.

Materials and Methods

Twelve experiments were included in the study that differed in their experimental design and measured traits (Table 1).

Experiment code						
Experiment code	Stage of development	Growing method	No. of experiments and plants	Traits measured	Detailed data	19C

Figure 1. (A) The cold stress index, calculated as the sum of low temperatures during the vegetative growth of sunflower, c

Controlled conditions and Heliaphen phenotyping platform

To study long-term impacts of early cold stress on late traits and oil yield, plantlets were stressed for three weeks in a growth chamber as described previously (Fig. 1C), and then each was transferred outdoors to a 10 L pot filled with fertile soil (PAM2, Proveen) on the high-throughput phenotyping platform Heliaphen (Gosseau et al., 2019) until the end of its life cycle. The chlorophyll, anthocyanin and flavonoid contents and nitrogen balance of leaves 1, 3, 5 and 10 or more were measured three times per week until senescence of the leaf using a leafclip sensor (Dualex®, Force-A).

Field experiments

Two field experiments were performed to study impacts of the sowing date (i.e., early vs. normal) on sunflower development and oil yield. The first field experiment (21TE01-02) was conducted at the INRAE station in Auzeville, France, in 2021. ES and normal sowing were performed on 1 March and 27 May 2021, respectively. Once half of the plants in each plot had developed a true pair of leaves, unmanned aerial vehicle (UAV) flights were used to take images of the experiment three times per week until half of the plants had three true pairs of leaves.

Analysis was based on that of Madec *et al.* (2017). Plant height was calculated from a point cloud obtained through structure from motion of UAV RGB (i.e., red, blue and green) images. Photogrammetry was performed using Photoscan software (Agisoft LLC, St. Petersburg, Russia). We then defined two regions: the soil buffer (larger than the original plotmap), used to calculate the soil baseline, and the inner buffer, used to calculate height. Then, outlier 3D points that lay outside the 1^{st} to 99.9^{th} percentile interval for height were identified and removed. Soil height was defined as the lowest point in the point cloud (20^{th}percentile), while plant height was defined as the distance from the soil to the 99^{th} height percentile in the inner buffer, in a UTM-based reference system. To assess the variability in height, 30 cm × 30 cm cells were analyzed in the same way, and the variability was calculated as the coefficient of variation of height among the cells. The canopy was defined as the percentage of green pixels in the plot. Green pixels were detected using excess green index (ExG = 2 × G - R - B) thresholding. The green cover thus decreased as the density of yellow ligules increased.

The second field experiment was performed at Grisolles (43.815356°N, 1.290086°E), France, in 2022. ES and normal sowing were performed on 1 March and 11 April 2022, respectively. Seeds were sown every 12.5 cm, in 5 m rows separated by 60 cm. The number of plantlets with fully developed cotyledons was recorded three times per week until half of the plots developed them. Similarly, the number of plants with leaves 4 cm long was recorded three times per week until half of the plant on each plot developed them. Visual inspection of growth curves identified the exponential stage of growth for vigor. The last four measurements were used to calculate the growth rate of above-ground cover during the exponential stage, while all measurements were used to calculate the growth rate of height.

Transcriptome analysis

The first two pairs of leaves, hypocotyl and entire root system were harvested after three weeks of growth for plants in both the night chilling and control treatments (Fig. 1C). Organs from two plantlets were sampled between 10:00 and 12:00, pooled and immediately flash frozen in liquid nitrogen. Frozen samples were ground using a grinder (ZM200, Retsch, Haan, Germany) with a 0.5 mm stainless steel sieve. RNA was extracted from 100 mg of frozen powder using an RNA kit (NucleoSpin®, Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. To eliminate genomic DNA contamination, samples were then treated with DNAse (TURBO, Thermo Fisher Scientific, Waltham, MA, USA).

Concentrations and 260/230 and 260/280 ratios of the RNA were determined using a spectrophotometer (NanoDrop, Thermo Fisher Scientific), and their integrity was verified using a bioanalyzer (2100, Agilent, Santa Clara, CA, USA) and an RNA chip (6000 Nano, Agilent). For each organ per treatment, three replicates were selected for sequencing based on their concentrations and integrity (total: 18 samples). An RNA sequencing kit (Universal Plus mRNA-Seq, Tecan Genomics, Redwood City, CA, USA) was used for library preparation following the manufacturer's instructions (library type: fr-secondstrand). Paired-end 150 bp sequencing was performed by IGA Technology Services (Udine, Italy) using a sequencer (NovaSeq 6000, Illumina, San Diego, CA, USA), and FASTQ files were submitted to the Sequence Read Archive (www.ncbi.nlm.nih.gov/sra) under accession no. SRP477303.

Raw paired-end reads were processed using the *nf-core/rnaseq* v 3.0 (doi:10.5281/zenodo.4323183) pipeline and subsequently aligned to the sunflower reference genome HanXRQr2.0 (GCA_002127325.2) using*Salmon* v 1.4.0. Read counts were retrieved at the gene level. After a quality-control step based on hierarchical clustering, two samples appeared to be misplaced and were excluded from further analysis.

Read counts were converted to counts per million and then filtered by keeping only genes that had more than three counts per million in at least three libraries. Normalization factors were calculated using the*calcNormFactors* function of the *edgeR* package v 3.40.2 of R Statistical Software (v 4.0.4; R Core Team 2021), and then used to normalize the counts using the *voom* function of the *limma*package v 3.46.0. The resulting expression matrix was used to perform a scaled principal component analysis using the *prcomp* function of the *stats* package v 4.2.2.

Differentially expressed genes (DEGs) were identified using the*limma* package v 3.46.0. A linear model was fitted for each gene while setting the treatment as a fixed factor and comparing stressed and control plants. A threshold of 0.05 for Benjamini-Hochberg-corrected$p$ -values was used.

Functional analysis of the transcriptome was performed using two approaches. In the first, the three lists of DEGs were analyzed using ClueGO v 2.5.8 (Bindea *et al.* , 2009). For hypocotyls and roots, all DEGs were used, while for leaves, only the genes with |Fold Change (FC) | > 1.5 were used. A two-sided test was used to highlight enriched ontology terms. Significance was set at Bonferroni-adjusted $p < 0.05$, the 'GO fusion' option was used, the k-score was set at 0.4 and the analysis considered only gene ontology (GO) levels 3-8. Three custom ontology terms were used: (1) a GO 'biological process' term, (2) a KEGG term and (3) a ROS-specific term based on the gene lists of Willems et al. (2016) (Table S5). The three terms were developed as detailed in the supplementary materials (Supplementary Methods). The second approach used the *pathifier* package v 1.36.0 (with min_std = 0.25 and min_exp = 0.10) for the 5000 genes with the highest variance among all samples. Only the KEGG ontology term was used, and leaf samples in the control treatment were chosen as references. A matrix of pathway deregulation scores (PDSs) was then visualized by producing a heatmap using the *gplots* package v 3.1.3.

ROS wheel clusters, libraries, differential expression and ClueGO data were submitted to a public portal: https://entrepot.recherche.data.gouv.fr/dataset.xhtml?persistentId=doi:10.57745/4HNS1J

Data analysis

All data were analyzed using R with the *dplyr* (v 1.0.4*)*, *purr* (v 0.3.4) and *ggplot* (v 3.3.3) packages to manipulate data and generate graphs. We first calculated the plasticity P of each trait Y as:

$$P_{i,j} = \frac{Y_{cold,i,j}}{Y_{control,i}}$$

where i represents the experiment, j the individual plant,$Y_{cold,i,j}$ the value of the trait in the cold treatment and $Y_{control,i}$ the mean of phenotypes in the control treatment in experiment i.

We used the *emmeans* package (v 1.5.4) to calculate adjusted means of plasticity among all experiments.

For dynamic phenotypes Y at an intermediate developmental stage, the growth rate was calculated using a sigmoid model of Deng *et al.* (2012):

$$Y = \frac{1}{1 + e^{(-g \times (t - t_i))}} \times Y_{\max}$$

where g represents the intrinsic growth rate, t the number of days after sowing, t_i the constant for a given variety i and Y_{max} the maximum value of the trait.

ROS quantification

Eight leaf disks per plant were harvested and placed in 96 well microplates. Disks were stained following the protocol of Kumar *et al.* (2014). Plates were then scanned using a table scanner (11000XL, Epson). Images were processed using a custom Python script to segment disk interiors and then transformed to grayscale, and peroxide or anion oxide coloration was measured as the mode of pixel intensities.

Lipid composition

Three replicates of leaves, hypocotyls and root systems in both the cold and control treatments were used to analyze lipid composition. Samples were harvested and ground in the same way as for the transcriptome analysis

Lipids were extracted and purified according to Folch et al. (1957). Briefly, the lipids from 20mg of sample were extracted first with chloroform:methanol (1:1, v/v) and 1 volume of H2O then with 2 volume of chloroform. Polar contaminants such as proteins or nucleic acid were removed by adding 2 volume of NaCl (0.9%, w/v) solution. After phase separation, the lower organic phase, which contains lipids, was harvested and the solvent was evaporated.

Transmethylation of fatty acids was performed overnight in 1 mL of methanol:H2SO4 solution (100:2.5, v/v) containing the internal standards C17:0 (57 µg/mL) at 85°C. Aftercooling, 1 mL of hexane:2.5% NaCl (1:1, v/v) was added, and the upper hexane phase containing FAMEs was recovered. The upper hexane phase was harvested, and diluted 20 time before injection in GC-MS.

GC-MS (gas chromatograph – mass spectrometry) was performed using an Agilent 7890B gas chromatograph and coupled MS detector MSD 5977-EI (Agilent). An DB-23 capillary column (60-m, 250-mm, and 0.25-mm film thickness; Agilent) was used with helium carrier gas at 2 mL/min; injection was done in splitless mode; injector and mass spectrometry detector temperatures were set to 250°C; the oven temperature was held at 80°C for 1 min, then programmed with a 65°C/min ramp to 130°C and a 5°C/min ramp to 250°C (4-min hold). The MS instrument us a MS detector MSD 5977-EI (Agilent). Injection (1µl) was done in splitless mode; injector and mass spectrometry detector temperatures were set to 250 °C. The ion source in an electron

ionization set at 70eV, the mass range is 40-700 m/z. Quantification of fatty acid methyl esters was based on peak areas, which were derived from total ion current, and using the respective internal standards.

Lipidomic analyses were performed on the Bordeaux Metabolome Facility-MetaboHUB (ANR-11-INBS-0010).

Results

Growth response to early sowing under field conditions

To study the impact of sowing date on sunflower development\sout, we performed two field experiments with different sowing dates in 2021 and 2022. The pattern of plant and canopy growth differed between ES and normal sowing (Fig. 2). Although the growth rate of the canopy cover did not differ with ES (Fig. 2E), the latent phase was ca. 25 days longer (60 d, vs. 35 d with normal sowing). This was consistent with lower vigor (i.e., plant emergence) in the cold treatment (Fig. 2A) and the observation that the number of days required for leaves to fully develop was nearly twice as long with ES (45 d) than with normal sowing (25 d) (Figure 2D). Once emerged, plants grew significantly slower with ES (1.4 cm/d) (Fig. 2C) than with normal sowing (2.2 cm/d) (Fig. 2F).

Figure 2. Growth of sunflower for early (blue) and normal (red) sowing dates under field conditions using manual and high-

Growth response to night chilling under controlled conditions

To isolate effects of low temperatures alone, we reproduced the cold stress observed in the multi-environment experimental network of Mangin*et al* . (2017)

Figure 3. Means ± 1 standard error of sunflower plasticity in response to cold treatment (5°C night and 18°C day) for abov

After three weeks of growth under the night chilling or control treatment, several above-ground and root morphological traits were measured, and the plasticity of each trait was calculated. Night chilling decreased plant height, leaf 1 area and collar diameter significantly (Fig.3). Roots responded strongly to night chilling, with a large and significant decrease (-44%) in biomass due to a 53% decrease in root projected area, length and tip number, although the root diameter increased. This result indicated strong repression of primary root growth and induction of secondary root growth.

Physiological and metabolic responses to night chilling under controlled conditions

To identify the main physiological processes of sunflower influenced by low temperatures, as well as developmental changes, we studied physiological processes known to be influenced by low temperatures, such as lipid metabolism, production of ROS and chlorophyll content. Cold stress significantly increased leaf anthocyanin and flavonol contents but decreased chlorophyll and nitrogen contents (Fig. 4A). Leaves produced significantly more ROS (i.e., hydrogen peroxide and superoxide anion) between 8h to 10h (Fig. 4A). These results confirm the intensity and pleiotropic impact of the night chilling stress applied.

One molecular response to cold (including freezing) is to modify fatty acid saturation and length. To describe these characteristics, we measured five fatty acids in the organs: C16:0 (palmitic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid) and C18:3 (linolenic acid). The largest impact of cold stress was observed in hypocotyls, in which the cold treatment decreased the amount of saturated palmitic (-0.19%)

and stearic acids (-48%) and tripled the amount polyunsaturated linoleic and linolenic acids (+206%). Interestingly, the amounts of the two latter fatty acids decreased in leaves (-50%) but did not differ significantly in the roots.

Figure 4. Means ± 1 standard error of the plasticity of physiological parameters in response to cold treatment. (A) Plasticit

Long-term impacts of night-chilling stress

Impacts on physiology and growth until flowering

To study effects of cold stress on later plant development (i.e., vegetative growth, final yield and biomass), we transferred 3-week-old plants that had been cold stressed (or not) under controlled conditions to the Heliaphen platform and monitored their responses.

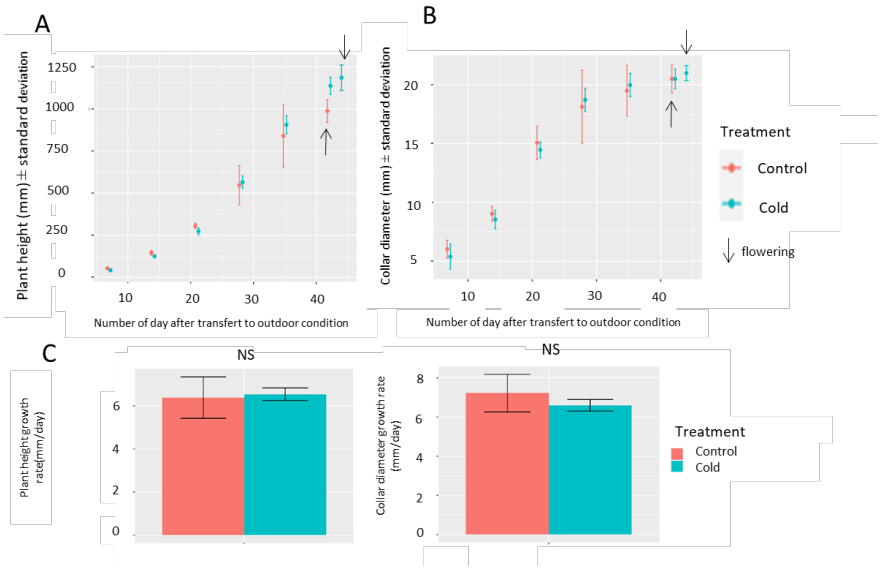

Figure 5. Means (points) ± 1 standard deviation (SD) (error bars) of (A) plant height and (B) collar diameter for sunflower in control (red) and cold (blue) treatments. Plants were grown for three weeks in a growth chamber and then transferred outside to identical conditions, in which they were phenotyped. Arrows indicate the flowering date. (C) Means ± 1 SD of the growth rate of plant height and collar diameter, which was calculated using sigmoidal functions. Corresponding experimentations were 20HP01, 21HP01, 22HP02.

After three weeks of stress and transfer to identical conditions, the growth of cold-stressed plants (i.e., height and collar diameter) did not differ in response to early stress (Fig. 5A and B), and their mean growth rates did not differ significantly (Fig. 5C). However, stressed plants flowered ca. 2 d later and continued to grow in height, which resulted in plants ca. 20 cm taller.

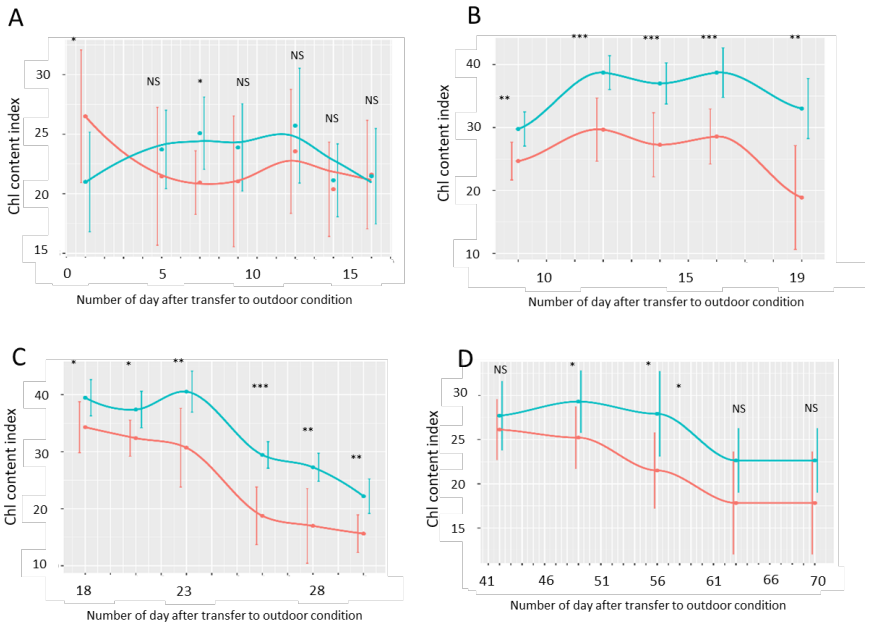

*Figure 6. Chlorophyll (Chl) content in leaf (A) 1, (B) 3, (C) 5 and (D) 10 or more for control (18°C night and day) (red) and cold treatments (5°C night and 18°C day) (blue) in a growth chamber. Asterisks indicate the significance of the Student's t-test between the cold and control treatments: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: non-significant. Corresponding experimentations was 20HP01.*

Along with measuring growth, chlorophyll was measured in leaves 1, 3, and 5 and the leaf ranked at 2/3 of the total number of leaves (i.e., leaf 10 or more). Chlorophyll content was higher in stressed plants than in control plants after being transferred outside. Chlorophyll content was higher on day 1 in leaf 1 in the control treatment than in the cold treatment, which reflected the influence of the recent cold treatment in the growth chamber (Fig. 6A). Seven days after transfer outside, the chlorophyll content became higher in the cold treatment than in the control treatment. This result was observed for all measurements for leaves 3, 5 and 10 or more, with a high level of significance for leaves 3 and 5 (Fig. 6B and C).

Impacts of early sowing on yield and yield-related traits

We studied impacts of ES date on late development and yield in the 2022 experiments (22TE01-02). We measured morphology, yield, number of seeds, thousand kernel weight, seed weight, oil content and seed lipid composition (Table 2). ES did not influence plant height but did result in later flowering (111 days) than that of normal sowing (79-86 days) (i.e., 9-16 d later). Flowering occurred earlier with ES (20 June 2022) and was more synchronized for its three replicates than those of normal sowing, which flowered from 29 June-6 July 2022. ES increased the seed yield and the oil yield (770 kg/ha) compared to that of normal sowing (430 kg/ha) (i.e., +80%) due to a slightly higher oil content. ES decreased the oleic acid content in seeds significantly and increased the contents of linoleic and palmitic acids.

Impacts of night chilling at early stages on final yield-related traits

To study impacts of early chilling stress on final traits, plants were stressed in the cold and control treatments described previously. After three weeks in growth chambers, the cold treatment was stopped, and plants were transferred outside to the high-throughput phenotyping platform Heliaphen.

Trait	Trait	Plasticity Controlled conditions (growth chamber then outdoor platform)	Plasticity Early sowing (field)
Trait Trait Plasticity Controlled conditions (growth chamber then outdoor platform) Plasticity Early sowing (field) Morphological Plant height 1.18 ± 0.03 (n=24) *** 1.15 ± 0.11 (n=4) Collar diameter 1.019 ± 0.02 (n=28) nd Total leaf area 1.08 ± 0.11 (n=23) nd No. of senescent leaves at flowering 1.07 ± 0.03 (n=40) nd Total leaf number 1.06 ± 0.02 (n=39) nd Plant weight 1.00 ± 0.05 (n=40) nd Developmental Flowering date (das) 0.94 ± 0.03 (n=38) * 1.332 ± 0 (n=6) *** Yield component Oil content 1.03 ± 0.28 (n=26) 1.16 ± 0.05 (n=6) · Oil yield 1.08 ± 0.11 (n=39) 1.80 ± 0.07 (n=6) · Number of seeds 1.05 ± 0.14 (n=40) nd Seed weight 1.03 ± 0.14 (n=40) 1.52 ± 0.06 (n=6) * Seed lipid composition Palmitic acid 1.00 ± 0.02 (n=14) 1.19 ± 0.02 (n=6) * Stearic acid 1.01 ± 0.05 (n=14) 0.84 ± 0.053 (n=6) Oleic acid 0.97 ± 0.03 (n=14) 0.66 ± 0.03 (n=6) *** Linoleic acid 1.01 ± 0.01 (n=14) 1.45 ± 0.05 (n=6) ***			

Figure 7. Number of differentially expressed genes in and between leaves, hypocotyls and roots in response to night chilling.

Leaves had the most DEGs: 5136, of which 53.3% were upregulated and 47.7% were downregulated. Hypocotyls had 653 DEGs, of which 35.4% were upregulated and 64.6% were downregulated. Roots had only 29 DEGs, of which 27.6% were upregulated and 72.4% were downregulated (Fig. 7).

Overall, the organs shared few DEGs (Fig. 8). Many DEGs in leaves and hypocotyls were potentially related to cold stress (Table S7). In leaves, this was the case for histone and heat shock proteins genes (115 and 75 genes, respectively). Interestingly, 108 of the 115 histone genes were upregulated. Other genes potentially related to cold stress included lipases, germin-like proteins, glycine-rich proteins and LEA proteins were detected. No gene related to the ICE-CBF-COR cascade was differentially expressed. In hypocotyls, many genes were related to the biosynthesis of lipids (e.g., lipases, acyltransferases, very-long-chain 3-oxoacyl-CoA synthases) or cutin, suberin and wax (e.g., casparian strip proteins, fatty acyl-CoA reductases, plant lipid transfer proteins) (41 and 29 genes, respectively). Other genes potentially involved in cold stress included proline-rich and glycine-rich cell-wall proteins and expansins. Even in this case, no gene related to the ICE-CBF-COR cascade was differentially expressed.

Figure 8. Networks of gene ontology terms enriched in differentially expressed genes in (A) leaves, (B) hypocotyls and (C) r

The enrichment analysis performed using ClueGO identified molecular pathways that were overrepresented the most in the DEG lists. In leaves, 51 individual molecular pathways were enriched (Table S8), many of which were related to nucleosome assembly (e.g., DNA conformation change, DNA packaging), ROS metabolism and carbohydrate metabolism. In hypocotyls, 18 enriched terms were detected, several of which were related to the biosynthesis of fatty acids, cutin, suberin and wax, as well as phenylpropanoid metabolism (Table S8). In roots, only two pathways were significantly enriched (Table S8).

Additional analysis performed using the *pathifier* package highlighted 116 terms whose pathway deregulation scores (PDSs) had been significantly altered among organs or between treatments (Fig. 9). Deregulation was high among organs, with roots and hypocotyls having deregulation patterns opposite to those of leaves. Fewer terms were deregulated between treatments. In leaves, they included several pathways related to energy metabolism (e.g., citrate cycle), purine and pyrimidine metabolism and signaling (Fig. 9A). In hypocotyls, some of the most deregulated pathways were related to the metabolism of fatty acids (e.g., glycerolipid metabolism, glycerophospholipid metabolism, fatty acid elongation) and to ROS metabolism, which was represented by five ROS-wheel-related ontologies (Fig. 9B, Table S8). Other deregulated pathways were related to calcium metabolism and the biosynthesis of cutin, suberin and wax. In roots, stressed and control samples differed little in pathway deregulation.

Figure 9. Heatmap obtained using unscaled pathway deregulation scores (PDSs). Each row corresponds to a pathway and e

Discussion

Cold is a major abiotic stressor to consider when adapting agriculture to the challenges of climate change, such as avoiding drought or growing short-cycle crops in northern regions. Seed germination and emergence are crucial for establishing crops, followed by the vigor and growth of above- and below-ground parts. Optimal vigor improves nutrient competition with weeds or after early herbicide treatment, enhances resistance to pathogens and increases resilience to abiotic stress. Thus, breeding for vigor is essential to benefit from ES (Houmanat*et al.* , 2016; Walne and Reddy, 2022).

Assessing vigor usually involves observing above-ground parts, but assessing the root system is equally important. For instance, a robust root system that can grow into deeper soil layers allows plants to withstand more severe drought stress later in the season (Cutforth*et al.* , 1986; Lamichhane *et al.* , 2018). Understanding the influence of chilling on early developmental stages of sunflower is crucial to identify the components of early cold tolerance, which increases oil yield in the context of ES.

These field experiments highlight the impacts of ES on early morphological traits. We observed an 80% decrease in the rate of vigor growth and a 42% decrease in the rate of height growth. However, in these ES experiments, low temperatures were correlated with the daylength and solar radiation intensity. To assess impacts of low temperatures alone, we developed an indoor experiment that reproduced the night chilling stress observed and modeled in a French field environmental network (Mangin *et al.* , 2017).

Sunflower responded similarly to night chilling (i.e., decrease in leaf area, hypocotyl diameter, root length and number of secondary roots) as maize did in other studies (Hussain *et al.,* 2020; Walne and Reddy, 2022). Low temperatures decreased sunflower development, resulting in smaller leaves and roots. These phenotypes likely result from direct effects of low temperatures on plant physiology: cold prolongs the cell cycle and slows the growth rate, which results in smaller organs (Rymen *et al.* , 2007). Low temperatures also influence the metabolism of auxin, which strongly regulates plant growth. Rahman (2013) observed that cold stress immobilized the cellular auxin transport protein and altered the auxin gradient usually associated with organ formation. This influence could explain the decrease in root length and increase in root diameter that we observed for sunflower. A decrease in the root system influences water and nutrient uptake, which is high near root tips due to increased expression of nutrient transporters and water channels. Branching is an effective way to increase the absorptive surface area of the root system (Dinneny, 2019), which ultimately decreases the yield (Hammer *et al.* , 2009). The decrease in leaf area under low temperatures is caused by lower cell division and cell elongation (Ben-Haj-Salah and Tardieu, 1995), which decreases light interception and thus the yield (Merrien, 1992).

Since early chilling stress decreases oil yield, we explored the morpho-physiological mechanisms underlying this long-term effect. We observed no increase in plant height at flowering despite a notable difference in the number of days required for flowering (+33.2% for ES). Oil yield increased (80%), as did seed weight and oil content (52.5% and 16.8%, respectively). Previous studies, such as those of Tahir *et al.* (2009), Demir (2019) and Abdelsatar (2020), also observed an increase in oil content (+7.7%), thousand kernel weight (+27.2%) and oil yield (+43.2%) with earlier planting dates for sunflower and wheat. The longer life cycle allowed plants to accumulate more photo-assimilates and remobilize them to seeds, as suggested by Evans and Wardlaw (1976) and Giannini *et al.* (2022) for wheat and rice. However, the relation between the duration of the vegetative phase and yield is complex. Some studies observed a positive correlation between the duration of vegetative growth and yield for sunflower (Gontcharov and Zaharova, 2008; Abdelsatar, 2020), while other studies observed a higher yield with a shorter vegetative phase and longer grain-filling phase for wheat (Sharma, 1992). This complexity is likely due to interactions among multiple abiotic stressors (e.g., early cold, late drought) and low temperatures combined with low solar radiation after ES.

In the present study, UAV and platform phenomics helped distinguish the impact of night chilling alone on late developmental stage and showed that it had no influence on leaf area or later growth rates, but did influence flowering time and thus growth duration, resulting in taller plants and more biomass. These results agree with those of previous studies of wheat (Tahir *et al.,* 2009) and sunflower (Alkio*et al.* , 2003; Demir, 2019). Ferreira and Abreu (2001) observed similar results for sunflower, with a 29% increase in the number of days between emergence and flowering with ES compared to normal sowing.

These morphological changes reflect underlying physiological processes related to cell division, extension, energy pathways and abiotic signaling. One well-documented physiological process related to cold resistance is an increase the unsaturation of fatty acids in cell membranes. We observed similar results, but specifically in hypocotyls rather than roots or leaves. This hypocotyl response has not been reported for other plants, but it has been observed for rapeseed (*Brassica napus)* , maize and wheat leaves and roots at varying levels of polyunsaturation (Tasseva *et al.* , 2004; Makarenko*et al.* , 2011; Nejadsadeghi *et al.* , 2015).

Low temperatures decrease cell membrane fluidity and permeability, which results in electrolyte leakage and alters internal cell homeostasis (Barrero-Sicilia *et al.* , 2017). Destabilization of the chloroplast membrane has a cascade of negative effects on chlorophyll content, photosynthetic enzyme activity and the electron-transport chain (Banerjee and Roychoudhury, 2019), resulting in the production of ROS until chlorosis symptoms appear (Wise, 1995; Suzuki and Mittler, 2006). Our results agreed with those of Fabio *et al.* (2022), given the 10.3% decrease in chlorophyll content and the 9.0% and 7.2% increase in H_2O_2 and $O_2^{\cdot-}$, respectively. Hussain *et al.* (2020) observed a 100% and 120% increase in H_2O_2 and $O_2^{\cdot-}$, respectively, in maize leaves. Zhu *et al.* (2013) observed similar results for sugarcane (*Saccharum officinarum*) leaves, as did Nejadsadeghi *et al.*(2015) for wheat leaves. As ROS concentrations increase in organs, the risk of damaging proteins, DNA and lipids increases (Apel and Hirt, 2004). This negative impact on plant physiology is managed by the ROS scavenging mechanism, which involves superoxide dismutase, ascorbate peroxidase, catalase and glutathione peroxidase (Cassia *et al.* , 2018). These results demonstrate the complex and interconnected physiological responses of sunflower to cold stress. Our results indicate that night chilling decreases the stability of cell membranes, which leak electrolytes. Disruption of chloroplast membranes is followed by a decrease in chlorophyll content and production of ROS due to the decrease in the efficiency of the electron-transport chain, which results in cell damage (Fig. 10).

To reveal the molecular mechanisms underlying the physiological responses observed, we explored the transcriptomic responses of leaves, hypocotyls and roots under chilling stress. The effects of cold stress were especially pronounced in leaves. Histone genes were the most abundant group of DEGs in leaves, which is consistent with results of Kumar and Wigge (2010) for *A. thaliana* that showed the critical role of histone H2A.Z in sensing temperature. This histone binds to the promoters of temperature-responsive genes, which can inhibit the binding of repressors. It is therefore plausible that an epigenetic mechanism of stress memory had long-term effects throughout the sunflower life cycle in our experiments.

Many other DEGs belonged to groups which are potentially linked to chilling stress, i.e. lipases, germin-like proteins, glycine-rich proteins, chaperones, heat shock proteins (HSP), and late embryogenesis abundant proteins (Winfield *et al.* , 2010; Barrero-Sicilia*et al.* , 2017).). Interestingly, we observed no DEGs related to the ICE-CBF-COR cascade, perhaps due to different sampling strategies. We sampled tissues after three weeks of exposure to chilling stress, while these genes are usually found to be differentially expressed after 3-48 h of treatment (Lee *et al.* , 2005; Song *et al.* , 2013; Londo *et al.* , 2018), which highlights the time-dependent nature of gene expression in response to chilling.

Analysis of DEGs confirmed the importance of histone and nucleosome activity, and highlighted other functions potentially related to the chilling response. This was the case for the ontologies related to carbohydrate metabolism, whose induction has been identified in peanuts (*Arachis hypogaea*) (Zhang *et al.* , 2020) and grapevine (*Vitis vinifera*) (Londo *et al.* , 2018). This was also observed for ontologies related to ROS metabolism, which have been described as a chilling stress response in species such as cotton (*Gossypium herbaceum*) (Tang *et al.* , 2021) and Chinese cottonwood (*Populus simonii*) (Song *et al.* , 2013). The increase in ROS production, particularly under high light levels, could be associated with photoinhibition (Banerjee and Roychoudhury, 2019), resulting in the degradation of chlorophyll, which may explain the decrease in chlorophyll content in leaves during the cold stress.

The transcriptomic profile of hypocotyls was characterized by two main processes. The first was lipid metabolism, including lipid unsaturation, which was represented by two genes and is consistent with the fatty acid profiles of hypocotyls. Lipid unsaturation in response to chilling stress is usually observed in leaves (Barrero-Sicilia *et al.* , 2017; Zhang *et al.* , 2020). Our study is the first to identify this process in hypocotyls. Three other lipid metabolism terms in response to chilling were alpha-linolenic acid, glycerolipid and glycerophospholipid metabolisms, all of which are related to membrane remodeling. To date, these pathways have been described only in leaves, such as those of peanuts (Zhang *et al.* , 2020).

The second main process that characterized hypocotyl transcriptomes was the biosynthesis of cutin, suberin and wax, which was represented by 29 DEGs and confirmed by enrichment and deregulation analyses. The leaf cuticle is the first line of physical defense against abiotic stresses (Barrero-Sicilia *et al.* , 2017), and

activation of cutin-related pathways under chilling stress has been described, for instance in rubber trees (*Hevea brasiliensis*) (Gong *et al.* , 2018). However, the role of cutin in plants is usually described in leaves.

In addition to these two main processes, other relevant pathways related to chilling stress were identified in hypocotyls. First, phenylpropanoid metabolism, represented by several key DEGs, is homologous to the*A. thaliana EARLI1* and phenylalanine ammonia lyase genes, respectively. The latter is induced by low temperatures in the hypocotyls of *A. thaliana* and leaves of rapeseed (Cabane *et al.* , 2012) and is related to enhanced lignin synthesis, which increases the mechanical resistance of cell walls and reduces dehydration. Overexpression of *EARLI1,* a lipid transfer protein, reduces electrolyte leakage during freezing stress, which suggests that it helps maintain membrane stability (Bubier and Schläppi, 2004).

To better understand the response of sunflower to abiotic stresses, we adapted the ROS wheel developed for *A. thaliana* (Willems *et al.* , 2016) to the sunflower genome (Badouin *et al.* , 2017). Doing so highlighted the relevance of ROS metabolism in hypocotyls, with two enriched ontologies and six ROS-wheel-related deregulated pathways, and indicated that hypocotyls and leaves had similar responses to chilling. Our transcriptomic experiment also revealed a role of the anti-oxidant vitamin B1 (thiamine) (Subki *et al.* , 2018). Chilling stress did not influence the root transcriptome greatly under hydroponic conditions, but it did deregulate the metabolism of the anti-oxidant vitamin B2 (riboflavin). Riboflavin has anti-oxidant properties in several plants, including apples (*Malus* spp.) (Zha *et al.* , 2022). Identification of pathways related to ROS metabolism and scavenging in the three organs highlighted the major role of these processes in the cell response to chilling.

We reproduced field conditions in spring in France to characterize sunflower after three weeks of exposure to moderate cold stress (night chilling). In this context, we observed no expression of well-known genes such as those involved in the ICE-CBF-COR cascade, which are usually induced shortly after exposure to cold stress. Overall, this shows that the transcriptome is highly plastic and suggests a sequential action of genes. The literature indicates that during the first few hours of exposure, the main genes are related to signal transduction and protein synthesis, indicating a potentially active response strategy. In contrast, after three weeks, we observed genes associated with long-term changes, such as those involved in lignin and lipid metabolism, which alters membrane lipid composition, which may be a passive strategy that requires less energy to resist stress by structurally modifying plants and their reactivity through epigenetic changes.

To study long-term impacts of early night chilling, we characterized sunflower using high-throughput phenotyping platforms combined with agronomic and biochemical measurements. Our field observations (e.g., 19% increase in C16:0, 44% decrease in oleic acid, 45% increase in linoleic acid) agreed with observations of Flagella *et al.* (2002) for sunflower, suggesting that the activation of desaturases required in early stages to maintain membrane fluidity remained in the seeds three months later. Similarly, the chlorophyll content increased consistently throughout the life cycle in leaves that had not developed at the time of stress. This result suggests that molecular memory may be transmitted through cell division, likely epigenetic changes, such as DNA methylation, phosphorylation or histone acetylation (Trewavas, 2016), whose stability is related to memory duration (Villagómez-Aranda*et al.* , 2022). Although our data did not identify these epigenetic markers, such as DNA methylation, in later developmental stages, future studies could explore long-term effects of morphological modifications induced in early stages or due to epigenetic modifications.

Figure 10. Immediate and long-term multiscale responses of sunflower to chilling stress applied during early developmental s

This study of the response of sunflower to night chilling under controlled and field conditions can provide valuable insights for plant biologists to help understand phenotypic plasticity and adaptation to abiotic environments. The results are also relevant for plant breeders to adapt crops to new cropping systems to mitigate impacts of climate change on agriculture. To better understand long-term impacts of early stress, future studies could focus on freezing tolerance and its similarities and differences with night chilling, and on interactions between early cold stress and later drought stress at the physiological and molecular levels, especially on epigenetically driven stress memory.

Author contributions

NL, CP and VMT designed and supervised the research. JL, NB, CT, OC and NP performed experiments under controlled conditions. JL, CT and RM performed field experiments. RM, AC and ML acquired and analyzed UAV images. JL analyzed the phenotypic data. NP and JL produced the transcriptomic data, and SC, MM and JL analyzed these data. JL, NL and MM wrote the manuscript, and VMT, OC and NB provided corrections.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

Funding was provided by Syngenta France and CIFRE (Conventions Industrielles de Formation par la Recherche) program, funded by the French National Association for Research and Technology (ANRT).

Acknowledgments

This research used the PHENOME-EMPHASIS facility Phenotoul-Heliaphen (Phenome-ANR-11-INBS-0012,*https://doi.org/10.15454/1.5483266728434124E12*) and was part of the French Laboratory of Excellence project "TULIP" (ANR-10-LABX-41; ANR-11-IDEX-0002-02). We thank the INRAE Centre for Biological Resources of Sunflower and Soybean, and the Bordeaux Metabolome Facility and MetaboHUB (ANR-11-INBS-0010 project) for lipid analysis. We thank Jean Belleville for helping to analyze field data, the research-support services of LIPME, the Experimental Unit Agroecology and Crop Phenotyping, and the Syngenta teams, including Marlène Mazas, Stéphanie Bascouert, Gaëtan Perate and Laurent Allegri.

References

Abbass K, Qasim MZ, Song H, Murshed M, Mahmood H, Younis I . 2022. A review of the global climate change impacts, adaptation, and sustainable mitigation measures. Environmental Science and Pollution Research **29** , 42539–42559.

Abdelsatar M . 2020. Effect of sowing dates on yield and yield-attributes of some sunflower hybrids. Agricultura**113–114** , 131–144.

Alkio M, Schubert A, Diepenbrock W, Grimm E . 2003. Effect of source–sink ratio on seed set and filling in sunflower (Helianthus annuus L.). Plant, Cell & Environment **26** , 1609–1619.

Allinne C, Maury P, Sarrafi A, Grieu P . 2009. Genetic control of physiological traits associated to low temperature growth in sunflower under early sowing conditions. Plant Science **177** , 349–359.

Anderson R, Bayer PE, Edwards D . 2020. Climate change and the need for agricultural adaptation. Current Opinion in Plant Biology**56** , 197–202.

Apel K, Hirt H . 2004. Reactive Oxygen Species: Metabolism, Oxidative Stress, and Signal Transduction. Annual Review of Plant Biology **55** , 373–399.

Badouin H, Gouzy J, Grassa CJ, *et al.* 2017. The sunflower genome provides insights into oil metabolism, flowering and Asterid evolution. Nature **546** , 148–152.

Banerjee A, Roychoudhury A . 2019. Cold Stress and Photosynthesis. In: Ahmad P, Abass Ahanger M, Nasser Alyemeni M, Alam P, eds. Photosynthesis, Productivity and Environmental Stress. Wiley, 27–37.

Barrero-Sicilia C, Silvestre S, Haslam RP, Michaelson LV . 2017. Lipid remodelling: Unravelling the response to cold stress in Arabidopsis and its extremophile relative Eutrema salsugineum. Plant Science **263** , 194–200.

Ben-Haj-Salah H, Tardieu F . 1995. Temperature Affects Expansion Rate of Maize Leaves without Change in Spatial Distribution of Cell Length (Analysis of the Coordination between Cell Division and Cell Expansion). Plant Physiology **109** , 861–870.

Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, Fridman W-H, Pagès F, Trajanoski Z, Galon J . 2009. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics **25** , 1091–1093.

Borowska M, Prusiński J . 2021. Effect of soybean cultivars sowing dates on seed yield and its correlation with yield parameters. Plant, Soil and Environment **67** , 360–366.

Bubier J, Schläppi M . 2004. Cold induction of EARLI1, a putative Arabidopsis lipid transfer protein, is light and calcium dependent. Plant, Cell & Environment **27** , 929–936.

Cabane M, Afif D, Hawkins S . 2012. Lignins and Abiotic Stresses. Advances in Botanical Research **61** , 219–262.

Cassia R, Nocioni M, Correa-Aragunde N, Lamattina L . 2018. Climate Change and the Impact of Greenhouse Gasses: CO2 and NO, Friends and Foes of Plant Oxidative Stress. Frontiers in Plant Science, 9:273.

Cutforth HW, Shaykewich CF, Cho CM . 1986. Effect of soil water and temperature on corn *Zea mays* root growth during emergence. Canadian Journal of Soil Science **66** , 51–58.

Debaeke P, Casadebaig P, Flenet F, Langlade N . 2017. Sunflower crop and climate change: vulnerability, adaptation, and mitigation potential from case-studies in Europe. OCL **24** , D102.

Demir I . 2019. The effects of sowing date on growth, seed yield and oil content of sunflower (helianthus annuus l.) cultivars under rainfed conditions. Fresenius Environmental Bulletin **28** , 6849–6857.

Deng J, Ran J, Wang Z, Fan Z, Wang G, Ji M, Liu J, Wang Y, Liu J, Brown JH . 2012. Models and tests of optimal density and maximal yield for crop plants. Proceedings of the National Academy of Sciences**109** , 15823–15828.

Ding Y, Shi Y, Yang S . 2019. Advances and challenges in uncovering cold tolerance regulatory mechanisms in plants. New Phytologist **222** , 1690–1704.

Dinneny JR . 2019. Developmental Responses to Water and Salinity in Root Systems. Annual Review of Cell and Developmental Biology**35** , 239–257.

Evans LT, Wardlaw F . 1976. Aspect of the comparative physiology of grain yield in cereals. Advances in Agronomy **28** , 301–359.

Fabio E, Tommasino EA, Grieu P . 2022. Physiological and biochemical contrasting responses associated with growth performances in sunflower seedlings after a cold stress. ResearchSquare doi: 10.21203/rs.3.rs-1945485/v1.

Ferreira AM, Abreu FG . 2001. Description of development, light interception and growth of sunflower at two sowing dates and two densities. Mathematics and Computers in Simulation **56** , 369–384.

Flagella Z, Rotunno T, Tarantino E, Di Caterina R, De Caro A . 2002. Changes in seed yield and oil fatty acid composition of high oleic sunflower (Helianthus annuus L.) hybrids in relation to the sowing date and the water regime. European Journal of Agronomy **17** , 221–230.

Folch J, Lees M, Sloane Stanley GH . 1957. A simple method for the isolation and purification of total lipids from animal tissues. J biol Chem **226** , 497–509.

Galmés J, Aranjuelo I, Medrano H, Flexas J . 2013. Variation in Rubisco content and activity under variable climatic factors. Photosynthesis Research **117** , 73–90.

Giannini V, Mula L, Carta M, Patteri G, Roggero PP . 2022. Interplay of irrigation strategies and sowing dates on sunflower yield in semi-arid Mediterranean areas. Agricultural Water Management**260** , 107287.

Gong X-X, Yan B-Y, Hu J, Yang C-P, Li Y-J, Liu J-P, Liao W-B . 2018. Transcriptome profiling of rubber tree (Hevea brasiliensis) discovers candidate regulators of the cold stress response. Genes & Genomics **40** , 1181–1197.

Gontcharov S, Zaharova M . 2008. Vegetation period and hybrid sunflower productivity in breeding for earliness. Breeding and Genetics. Córdoba, Spain.

Gosseau F, Blanchet N, Varès D, *et al.* 2019. Heliaphen, an Outdoor High-Throughput Phenotyping Platform for Genetic Studies and Crop Modeling. Frontiers in Plant Science **9** , 1908.

Hammer GL, Dong Z, McLean G, Doherty A, Messina C, Schussler J, Zinselmeier C, Paszkiewicz S, Cooper M . 2009. Can changes in canopy and/or root system architecture explain historical maize yield trends in the U.S. Corn Belt? Crop Science **49** , 299–312.

Hassan MA, Xiang C, Farooq M, Muhammad N, Yan Z, Hui X, Yuanyuan K, Bruno AK, Lele Z, Jincai L . 2021. Cold Stress in Wheat: Plant Acclimation Responses and Management Strategies. Frontiers in Plant Science **12** , 676884.

Houmanat K, Fechtali ME, Mazouz H, Nabloussi A . 2016. Assesment of sunflower germplasm selected under autumn planting conditions. Edirne, Turkey.

Hunt JR, Lilley JM, Trevaskis B, Flohr BM, Peake A, Fletcher A, Zwart AB, Gobbett D, Kirkegaard JA . 2019. Early sowing systems can boost Australian wheat yields despite recent climate change. Nature Climate Change **9** , 244–247.

Hussain HA, Men S, Hussain S, Zhang Q, Ashraf U, Anjum SA, Ali I, Wang L . 2020. Maize Tolerance against Drought and Chilling Stresses Varied with Root Morphology and Antioxidative Defense System. Plants**9** , 720.

Kumar SV, Wigge PA . 2010. H2A.Z-Containing Nucleosomes Mediate the Thermosensory Response in Arabidopsis. Cell **140** , 136–147.

Kumar D, Yusuf MA, Singh P, Sardar M, Sarin NB . 2014. Histochemical detection of superoxide and H2O2 accumulation in Brassica juncea seedlings. Bio-protocol **4** , e1108–e1108.

Lamichhane JR, Debaeke P, Steinberg C, You MP, Barbetti MJ, Aubertot J-N . 2018. Abiotic and biotic factors affecting crop seed germination and seedling emergence: a conceptual framework. Plant and Soil **432** , 1–28.

Lee B, Henderson DA, Zhu J-K . 2005. The Arabidopsis Cold-Responsive Transcriptome and Its Regulation by ICE1. The Plant Cell**17** , 3155–3175.

Li P-F, Ma B-L, Xiong Y-C, Zhang W-Y . 2017. Morphological and physiological responses of different wheat genotypes to chilling stress: a cue to explain yield loss. Journal of the Science of Food and Agriculture **97** , 4036–4045.

Londo JP, Kovaleski AP, Lillis JA . 2018. Divergence in the transcriptional landscape between low temperature and freeze shock in cultivated grapevine (Vitis vinifera). Horticulture Research **5** , 10.

Madec S, Baret F, de Solan B, Thomas S, Dutartre D, Jezequel S, Hemmerlé M, Colombeau G, Comar A . 2017. High-Throughput Phenotyping of Plant Height: Comparing Unmanned Aerial Vehicles and Ground LiDAR Estimates. Frontiers in Plant Science **8** .

Makarenko S, Dudareva L, Katyshev A, Konenkina T, Stolbikova A, Rudikovskaya E, Sokolova N, Chernikova V, Konstantinov Y . 2011. The effect of low temperatures on fatty acid composition of crops with different cold resistance. Biochemistry (Moscow) Supplement Series A: Membrane and Cell Biology **5** , 64–69.

Manasa L, Panigrahy M, Panigrahi K, Rout G . 2021. Overview of Cold Stress Regulation in Plants. The Botanical Review **88** , 359–387.

Mangin B, Casadebaig P, Cadic E, *et al.* 2017. Genetic control of plasticity of oil yield for combined abiotic stresses using a joint approach of crop modelling and genome-wide association. Plant, Cell & Environment **40** , 2276–2291.

Merrien A . 1992. Some aspects of sunflower crop physiology. CETIOM.

Murashige T, Skoog F . 1962. Murashige T & Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-97, 1962. Physiol Plant **3** , 473–497.

Murata N, Los DA . 1997. Membrane fluidity and temperature perception. Plant physiology **115** , 875.

Nejadsadeghi L, Maali-Amiri R, Zeinali H, Ramezanpour S, Sadeghzade B . 2015. Membrane fatty acid compositions and cold-induced responses in tetraploid and hexaploid wheats. Molecular Biology Reports**42** , 363–372.

Prăvălie R, Patriche C, Borrelli P, Panagos P, Roșca B, Dumitraşcu M, Nita I-A, Săvulescu I, Birsan M-V, Bandoc G . 2021. Arable lands under the pressure of multiple land degradation processes. A global perspective. Environmental Research **194** , 110697.

Rahman A . 2013. Auxin: a regulator of cold stress response. Physiol. Plant. **147** , 28–35.

Ritonga FN, Chen S . 2020. Physiological and Molecular Mechanism Involved in Cold Stress Tolerance in Plants. Plants **9** , 560.

Rymen B, Fiorani F, Kartal F, Vandepoele K, Inzé D, Beemster GTS . 2007. Cold Nights Impair Leaf Growth and Cell Cycle Progression in Maize through Transcriptional Changes of Cell Cycle Genes. Plant Physiology **143** , 1429–1438.

Sangwan V, Foulds I, Singh J, Dhindsa RS . 2001. Cold-activation of Brassica napus BN115 promoter is mediated by structural changes in membranes and cytoskeleton, and requires Ca2+ influx. The Plant Journal**27** , 1–12.

Seneviratne, S I, Zhang X, *et al.* 2023. Cambridge University Press. Climate Change 2021 – The Physical Science Basis: Working Group I Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge United Kingdom and New York: Cambridge University Press, 1513–1766.

Sharma RC . 1992. Duration of the vegetative and reproductive period in relation to yield performance of spring wheat. European Journal of Agronomy **1** , 133–137.

Song Y, Chen Q, Ci D, Zhang D . 2013. Transcriptome profiling reveals differential transcript abundance in response to chilling stress in Populus simonii. Plant Cell Reports **32** , 1407–1425.

Subki A, Abidin AAZ, Yusof ZNB, Subki A, Abidin AAZ, Yusof ZNB . 2018. The Role of Thiamine in Plants and Current Perspectives in Crop Improvement. B Group Vitamins - Current Uses and Perspectives. London UK: IntechOpen, 33–44.

Suzuki N, Mittler R . 2006. Reactive oxygen species and temperature stresses: A delicate balance between signaling and destruction. Physiologia Plantarum **126** , 45–51.

Taaime N, El Mejahed K, Moussafir M, Bouabid R, Oukarroum A, Choukr-Allah R, El Gharous M . 2022. Early sowing of quinoa cultivars, benefits from rainy season and enhances quinoa development, growth, and yield under arid condition in Morocco. Sustainability **14** , 4010.

Tahir M, Ali A, Nadeem MA, Hussain A, Khalid F . 2009. Effect of Different Sowing Dates on Growth and Yield of Wheat (Triticum aestivum L.) Varieties in District Jhang, Pakistan. Pak. j. life soc. sci**7** , 66–69.

Tang S, Xian Y, Wang F, Luo C, Song W, Xie S, Chen X, Cao A, Li H, Liu H . 2021. Comparative transcriptome analysis of leaves during early stages of chilling stress in two different chilling-tolerant brown-fiber cotton cultivars. (B Zhang, Ed.). PLOS ONE **16** , e0246801.

Tasseva G, Davy de Virville J, Cantrel C, Moreau F, Zachowski A . 2004. Changes in the endoplasmic reticulum lipid properties in response to low temperature in Brassica napus. Plant Physiology and Biochemistry **42** , 811–822.

Thakur P, Nayyar H . 2013. Facing the Cold Stress by Plants in the Changing Environment: Sensing, Signaling, and Defending Mechanisms. In: Tuteja N, Singh Gill S, eds. Plant Acclimation to Environmental Stress. New York, NY: Springer New York, 29–69.

Villagómez-Aranda AL, Feregrino-Pérez AA, García-Ortega LF, González-Chavira MM, Torres-Pacheco I, Guevara-González RG . 2022. Activating stress memory: eustressors as potential tools for plant breeding. Plant Cell Reports **41** , 1481–1498.

Walne CH, Reddy KR . 2022. Temperature Effects on the Shoot and Root Growth, Development, and Biomass Accumulation of Corn (Zea mays L.). Agriculture **12** , 443.

Willems P, Mhamdi A, Stael S, Storme V, Kerchev P, Noctor G, Gevaert K, Van Breusegem F . 2016. The ROS wheel: refining ROS transcriptional footprints. Plant Physiology **171** , 1720–1733.

Winfield MO, Lu C, Wilson ID, Coghill JA, Edwards KJ . 2010. Plant responses to cold: transcriptome analysis of wheat. Plant Biotechnology Journal **8** , 749–771.

Wise R . 1995. Chilling-enhanced photooxidation: The production, action and study of reactive oxygen species produced during chilling in the light. Photosynthesis Research **45** , 79–97.

Zha Z, Tang R, Wang C, Li Y, Liu S, Wang L, Wang K . 2022. Riboflavin inhibits browning of fresh-cut apples by repressing phenolic metabolism and enhancing antioxidant system. Postharvest Biology and Technology **187** , 111867.

Zhang X, Cai X . 2011. Climate change impacts on global agricultural land availability. Environmental Research Letters**6** , 014014.

Zhang H, Jiang C, Ren J, *et al.* 2020. An Advanced Lipid Metabolism System Revealed by Transcriptomic and Lipidomic Analyses Plays a Central Role in Peanut Cold Tolerance. Frontiers in Plant Science **11** .

Zhu J-J, Li Y-R, Liao J-X . 2013. Involvement of anthocyanins in the resistance to chilling-induced oxidative stress in Saccharum officinarum L. leaves. Plant Physiology and Biochemistry **73** , 427–433.

Hosted file

`Figures_Leconte_PCE.pptx` available at https://authorea.com/users/763204/articles/740538-multi-scale-characterization-of-cold-response-reveals-immediate-and-long-term-impacts-on-cell-physiology-up-to-seed-composition

Hosted file

Table_Leconte_PCE.pptx available at https://authorea.com/users/763204/articles/740538-multi-scale-characterization-of-cold-response-reveals-immediate-and-long-term-impacts-on-cell-physiology-up-to-seed-composition