NTNU – Trondheim
Norwegian University of
Science and Technology

Stress responses induced by temperature and light conditions in Brassica napus

Ane Negard

MSc in Biology
Submission date: July 2015
Supervisor: Richard Strimbeck, IBI
Co-supervisor: Ishita Ahuja, IBI
Atle Bones, IBI

Norwegian University of Science and Technology
Department of Biology

Responses of transgenic oilseed rape (*Brassica napus)* MINELESS plants to light and temperature stress factors

Acknowledgments

I would like to thank my supervisor prof. Richard Strimbeck for help planning the work for the thesis, and for helpful comments.

I would also thank my co-supervisor Dr. Ishita Ahuja for help and guidance regarding *B. napus* seedlings, and for comments on the thesis. Thanks to prof. Atle Bones for help during the first planning of the thesis.

I would like to thank Kjersti Andresen for help with pigment extraction and analysis, Dr. Jens Rohloff for help with analysis of plant metabolites and thanks to Rolf-Arve Skille for technical help when nothing worked.

Abstract

Brassica napus, also known as oilseed rape or canola, belongs to the family *Brassicaceae*. An important characteristic of Brassicaceae is the production of specific secondary metabolites, glucosinolates. Intact glucosinolates are biologically inactive but when the plants are damaged by insect herbivory or tissue disruption, glucosinolates are hydrolysed by the enzyme myrosinase to other glucosinolate hydrolysis products that are toxic to many insects. B. napus *MINELESS* plants have been genetically engineered to further study the role of myrosin cells and the glucosinolate-myrosinase system against biotic and abiotic stress factors. This study examined the effect of light and temperature stress factors on wild-type and transgenic *MINELESS B. napus* plants by measuring chlorophyll fluorescence, pigment composition and plant metabolites. We undertook these analyses by combination of light and temperature stress factors. In addition, we examined how the interaction between light and temperature shock treatments affect *B. napus* wild-type and transgenic *MINLESS* plants. The study showed that *MINELESS* plants differed with wild-type for Fv/Fm after cold shock, and for plant metabolites after temperature and light stresses. Pigments were unaffected by the lack of myrosin cells in the *MINELESS* plants. The interaction between temperature and light gave unique responses, different from single stressor responses.

Contents

1. Introduction

1.1 Brassica napus

B. napus, also known as oilseed rape or canola, belongs to the family *Brassicaceae*. The well-known cultivated species of *Brassicaceae* include cabbage, cauliflower, broccoli, Brussels sprouts, turnip, kale, swede and various types of mustard, in addition to the canola (Ahuja et al., 2011). The model plant Arabidopsis thaliana also belongs to *Brassicaceae*.

The production of oilseed *Brassica* for the last three decades has been increasing, and Canada, China and India are among the main producers (Table 1). The edible oils have high nutritional value and the oilseed meal functions as an important source of protein for livestock (Font et al., 2003). The two major oilseed crops are *B. napus* and *B. rapa* (Raymer, 2002). There is also an increase in the interest of oil seeds in the production of oil for biofuels.

Table 1: Oilseed rape production in million tonnes in 2011 (faostat.org).

Country	**Rapeseed (million ton)**
Canada	14.16
China, mainland	13.43
India	8.18
France	5.37
Germany	3.87
Australia	2.36
Hungary	0.53
Bulgaria	0.52
Denmark	0.51
Lithuania	0.48

1.2 Glucosinolate – myrosinase system

An important characteristic of the Brassicaceae family is the production of specific secondary metabolites, glucosinolates (anionic thioglucosides) (Fahey et al., 2001). The glucosinolates constitute a large group of non-volatile, sulphur- and nitrogen- containing compounds (Kliebenstein et al., 2001).

Glucosinolates can be grouped based on whether they are derived from aliphatic amino acids like methionine, phenylalanine or tyrosine, or tryptophan (Halkier and Du, 1997). The different glucosinolates derivatives come from modifications like hydroxylation, methylation, oxidation and desaturation of the side chains (Halkier and Du, 1997).

Intact glucosinolates are biologically inactive (Mawson et al., 1993), but when the plants are damaged by insect herbivory or tissue disruption, glucosinolates are hydrolysed by the enzyme myrosinase to other compounds such as isothiocyanates, nitriles, thiocyanates, epithionitriles and oxazolidines, depending on the nature of the glucosinolates, reaction conditions, and the presence of protein cofactors (Figure 1) (Bones and Rossiter, 1996, Rask et al., 2000). Isothiocyanates are the most common hydrolysis products, and are unstable compounds that decompose spontaneously into indole-3-carbinol, indole-acetonitrile, thiocyanates ions and 3,3´- diindolylmethane (Fahey et al., 2001).

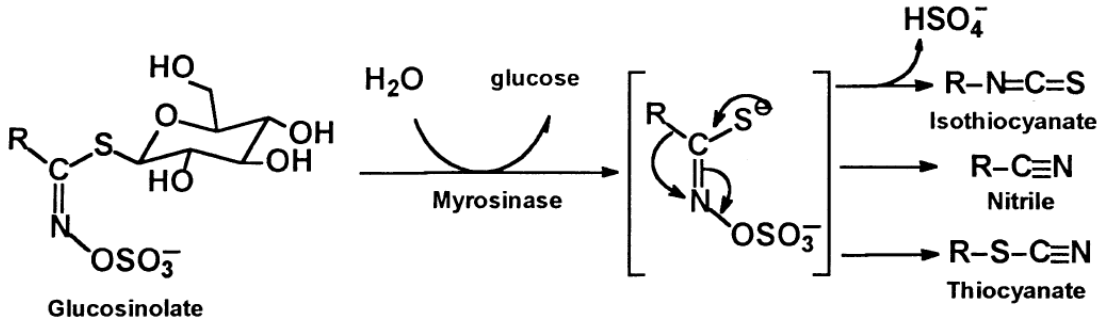

Figure 1. Hydrolysis of glucosinolates by myrosinase, from (Fahey et al., 2001).

The enzyme myrosinase is present in specialized cells known as myrosin cells (Bones and Iversen, 1985, Kissen et al., 2009), which are dispersed throughout plant tissue.

The *B. napus MINELESS* plants have been genetically engineered to further study the role of myrosin cells and the glucosinolate-myrosinase system against biotic and abiotic stress factors (Borgen et al., 2010, Ahuja et al., 2011). *MINELESS* plants lack myrosin cell, also known as the "toxic mines". This has been done in *B. napus* by inducing cell death of myrosin cells using the seed myrosin cell-specific promoter, *Myr1.Bn1* to express ribonuclease, a barnase (Borgen et al., 2010). The wild-type and MINELESS plants differed for growth parameters such as plant height, leaf traits, matter accumulation, and yield parameter. The growth and developmental patter of *MINELESS* plans was relatively slow compared with the wild-type (Ahuja et al., 2011).

Some of the degradation products after hydrolysis of glucosinolate are active in defence against pathogens and herbivores. In addition to an increase in secondary metabolites because of biotic stress caused by herbivores, the stress caused by abiotic factors such as temperature

(Velasco et al., 2007), drought (Radovich et al., 2005), light (Engelen-Eigles et al., 2006), and salinity (Qasim et al., 2003) might also modify the composition of glucosinolate breakdown products. Seasonal changes in temperature and light give rise to changes in glucosinolate content in different *Brassica* species and the amount of the different glucosinolates vary through the growth season for the crop plants (Rosa et al., 1996). In the spring season with increasing light intensity and longer photoperiods plants accumulate higher concentrations of glucosinolates than during the autumn and winter seasons (Rosa et al., 1996). Glucosinolates and the products created during hydrolysis may induce a higher heat tolerance in the plant as part a of stress acclimation process (Ludwig-Muller et al., 2000).

1.3 Plant metabolites

In addition to glucosinolate and associated compounds, abiotic stresses like temperature and excess light, may cause shifts in metabolic pathways (Taiz and Zeiger, 2010). Temperature and light stress can causes different distinct changes in the metabolic pathways and the combination of the two is distinct from the single stress response.

Compounds that are important for abiotic stress responses in plants can be involved in acclimation processes, like antioxidants and molecules involved in signal transduction pathways, or they can be a by-product of stress in the cells (Taiz and Zeiger, 2010). The signalling molecules can be plant hormones, synthesized as a response to stress, and the by-products might be different oxidized compounds such as phenolic compounds (Shulaev et al., 2008). Organic compounds of low molecular mass, such as sugars and sugar alcohols are an important adaptation to abiotic stresses. These compounds are accumulated and function as osmolytes, enhancing stress tolerance during abiotic stress (Wahid et al., 2007).

Several metabolites are linked to defence mechanisms to biotic stresses. These metabolites are generally derived from secondary metabolism, such as phenylpropanoid, alkaloid or fatty acid/ polyketide pathways (Kaplan et al., 2004). Secondary metabolites are synthesised from intermediates of primary metabolism. For example, aromatic acids like tryptophan and tyrosine serve as precursors for indole glucosinolates (Figure 2), while branched amino acids, like valine, are precursors for glucosides (Kaplan et al., 2004).

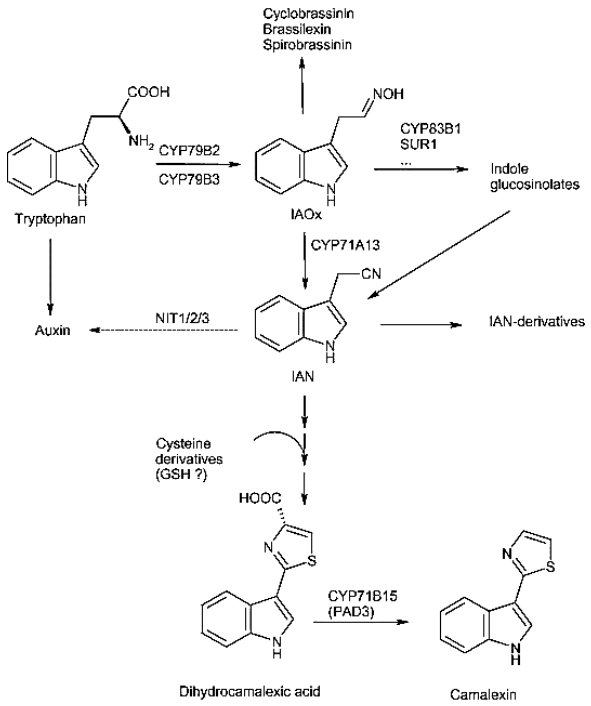

Figure 2. Biosynthesis of camalexin (phytoalexin that increase during pathogen attacks) and related indolic compound in *Arabidopsis thaliana.* Tryptophan is converted to indole-3 (IAOx). In the biosynthesis of camalexin, IAOx is dehydrated to indole-3-acetonitrile (IAN). When IAN is present in excess, it is hydrolysed by nitrilases yielding the auxin IAA. From (Rauhut and Glawischnig, 2009).

Secondary metabolites can also be produced in response to abiotic stress. For example during high irradiance phenolics, like sinapoyl malate, is transferred to the upper epidermis of seedlings to prevent harmful effects from excess light, or they can as caffeoylquinic acid work as powerful antioxidants, reducing harmful oxygen radicals (ROS) (Dean et al., 2014, Mondolot et al., 2006).

1.4 Pigments

Under stressful conditions the two photosystems (PSI and PS II) can be damaged, and protection of the two photosystems is therefore important for higher plants. Plants in the Brassicaceae are rich in carotenoid pigments, and the carotenoids can function both as accessory pigments in the light harvesting complex (LHC), and as a part of the photoprotection of the plant. The carotenoids can be divided into xanthophylls and carotenes, and can often be seen as red, orange and yellow in fruit and flowers (Taiz and Zeiger, 2010). The xanthophylls convert excess excitation energy from the chlorophyll to heat through non-photochemical quenching (NPQ) (Bjorkman et al., 2011). In LHC, xanthophyll is present in form of violaxanthin, lutein and neoxanthin. Under high light conditions, which have the potential to cause damage to the photosynthetic membrane, violaxanthin is converted into antheraxanthin and zeaxanthin through a two-step de-epoxidation (Figure 3) (Ashraf and Harris, 2013, Dall'Osto et al., 2006, Taiz and Zeiger, 2010). With increase in leaf temperature

the rate of de-epoxidation of violaxanthin to zeaxanthin increases, while with lower leaf temperature the de-epoxidation rate decreases (Dall'Osto et al., 2006).

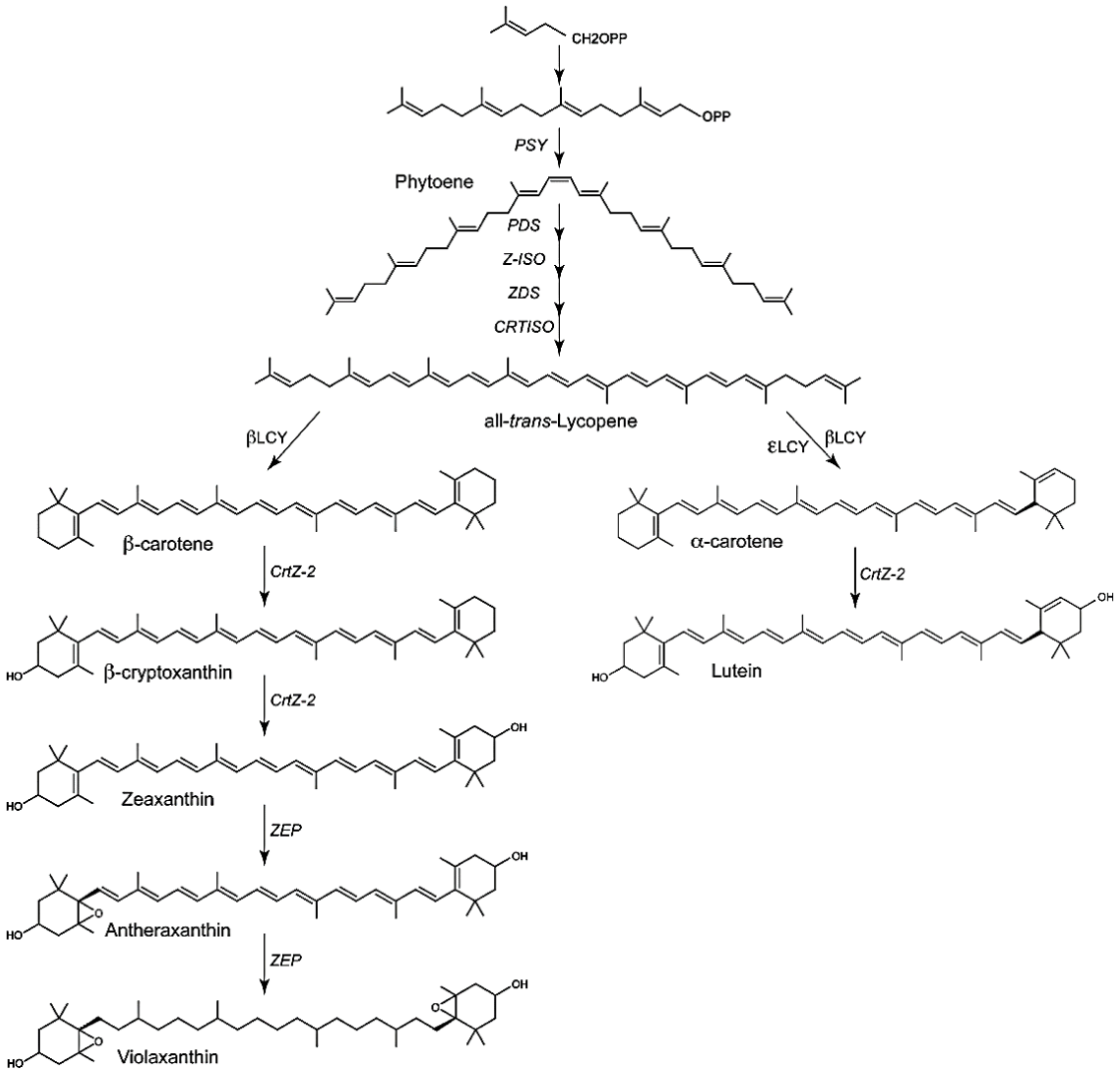

Figure 3. Carotenoid biosynthetic pathway, with carotenoids synthesised from geranylgeranyl pyrophosphate. The xanthophyll cycle consist of the de-epoxidation of violaxanthin to antheraxanthin and finally zeaxanthin under high light condition, and epoxidation back to violaxanthin under low light condition. From (Maurer et al., 2014).

Violaxanthin is also a precursor to neoxanthin, but while violaxanthin concentration varies among species and environment, neoxanthin has a more consistent concentration throughout different species and growth environments (Bungard et al., 1999) .

Another carotenoid, lutein, is the most abundant xanthophyll in higher plants, and it might also have a role in energy dissipation during the first few minutes of increased irradiance (Matsubara et al., 2001, Bungard et al., 1999). It has been shown that neoxanthin can be replaced by lutein-5, 6-epoxide (Lx) in *Cuscuta reflexa*, a holoparasitic angiosperm,

and it is assumed that lutein and Lx are interconvertible through the enzymes in the xanthophyll cycle.

The relationship between chlorophyll a (chl a) and chlorophyll b (chl b) is not constant and can be altered to adapt to varying light conditions, and chl a/b ratios has been used to define sun and shade tolerance in plants (Grimm et al., 2006, Maina and Wang, 2015). This is possible with a conversion of chl a into chl b, and the other way with chl b converted to chl a, termed the "chlorophyll cycle", or with degradation of chl b and synthesis of new chl a (Grimm et al., 2006, Ito et al., 1996). A higher chl a/b ratio can often be found in light acclimated leaves; while the ratio is lower in shade acclimated leaves (Maina and Wang, 2015).

In addition to the effect on the xanthophyll, abiotic stress may also affect chl a and chl b. Chlorophylls are the main component of the photosynthetic apparatus, and the photosynthetic apparatus can be reorganized in response to environmental conditions. During high temperature stress, the accumulation of chlorophylls in plants has been reported to drop. This might be caused by reduced biosynthesis of chlorophyll because of impaired enzymes, increased degradation or a combination of both (Ashraf and Harris, 2013).

1.5 Chlorophyll fluorescence

When light strikes the plant, the energy in the light is absorbed by the pigments found in the antenna complex. The energy is then transferred from the antenna systems to the reaction centres by transferring the excitation energy from one pigment molecule to another. The energy transfer is normally very efficient, and around 80-90 % of the photons absorbed have their energy transferred to the reaction centre (Krause and Weis, 1991, Taiz and Zeiger, 2010). The absorbed light energy absorbed in PS2 can be used to transfer electrons from P680, the reaction centre chlorophyll, to Q_A, the primary quinone acceptor. The remaining energy that is not used for photochemistry in PS2 can be lost either as chlorophyll fluorescence or as heat (Baker, 2008). In a dark adapted leaf, Q_A is oxidized maximally and the PS2 reaction centre can perform photochemical reduction of the quinone. In a dark adapted leaf, a weak measuring light gives F_0, which works as a measurement for the minimal level of fluorescence in a dark adapted leaf when the Q_A is oxidized. Exposing the dark adapted leaf to a saturation pulse gives Fm, which is the maximal level of fluorescence for the leaf (Baker, 2008). These two parameters are used to find Fv/Fm, termed as the maximum quantum efficiency of PS2 (Figure 4).

$$Fv/Fm = (Fm - F_0)/Fm \quad (1)$$

Fv/Fm is the potential efficiency of PS2 if all of the reaction centres are open. A plant growing under stressful conditions will often have a lower Fv/Fm than one in optimal conditions. A non-stressed plant will have Fv/Fm values at about 0.8-0.83 (Baker, 2008, Krause and Weis, 1991). This decrease might, among others, come from lower Fm because of an increase in nonphotochemical quenching (Baker, 2008).

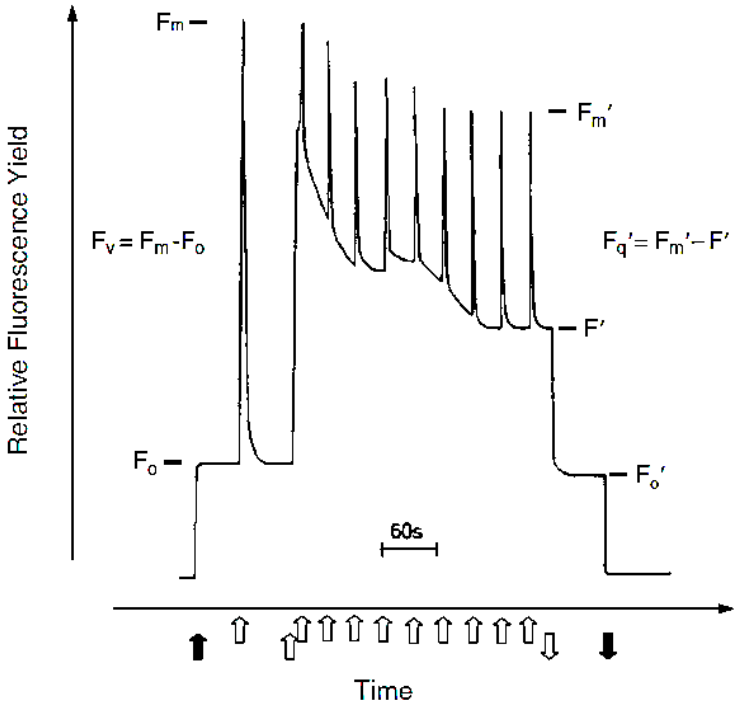

Figure 4. Fluorescence induction kinetics including application of the saturation pulse method. F_0 is measured with weak measuring light, and first dark arrow indicates measuring light on (up). The second dark arrow indicates measuring light off (down). First open arrow indicate application of a short pulse of saturating light; open arrows indication activation (up) and deactivation (down) of actinic light. Fm from in a dark adapted leaf is measured during the first saturation pulse. From (Cosgrove and Borowitzka, 2010)

In this study, the Fv/Fm measurements, pigment analyses and metabolite profiling were performed to compare how seedlings of *B. napus* wild-type and transgenic *MINELESS* plants tolerate and respond to different levels of light and temperature stresses. Different light intensities and temperatures were applied in combination to get an understanding of how the interaction between multiple stresses can give different reactions in the plants rather than considering light and temperature stress factors completely separately.

By using *B. napus MINELESS* plants that possess high levels of glucosinolates, and very low amounts of myrosinase and glucosinolate-hydrolysis products, we examined how plants with a modified glucosinolate-myrosinase defence system respond to environmental stress factors such as light and temperature. Since in a natural environment, these stress

factors affect growth of plants in combination, we applied these stress factors in combination. We aimed to see how these stress factors affect photosynthesis and other metabolites in B. napus wild-type and genetically modified MINELESS plants. We targeted to see if the plant metabolites get affected in seedlings in response to abiotic stress, or if these are produced to increase the plants defence against biotic factors, like pathogens and herbivores, in a situation where a plant might be vulnerable to attacks because of the strains caused by abiotic stress.

2. Material and methods

2.1 Plant material

2.1.1 Sowing and germination

Seeds of *B. napus* cv. Westar (designated as the wild-type) and *MINELESS* plants were sown in pots with four seeds per pot with autoclaved soil.

To avoid evaporation from the pots they were covered with a plastic sheet until germination. Germination occurred 4 days after sowing, and sheets were removed to allow for growth.

The young seedlings were watered when necessary.

Germination of the seeds occurred in growth chamber with a light intensity of 80 $\mu molm^{-2}s^{-1}$ and a temperature of 21 ºC during the photoperiod, and 0 $\mu molm^{-2}s^{-1}$ and a temperature of 18 ºC during the night. Humidity was kept at 70 % RH regardless of light and temperature conditions. The seedlings were grown under 16 h daylight and 8 h of darkness.

All wild-type seeds germinated, while nearly 80% of the *MINELESS* showed germination. Except differences in germination rate, no phenotypic differences were observed between wild-type and *MINELESS* plants (Figure 5).

Figure 5. Eleven days old B. napus seedling grown in 80 $\mu molm^{-2}s^{-1}$ at 21ºC. Wild-type (left) and *MINELESS* (right).

2.1.2 Treatment of plants with light and temperature stresses

Shock treatments were given to eleven day old plants (DAG; days after germination) when the first true leaf appeared. Six pots with four seedlings per pot of each of the wild-type and *MINELESS* plants respectively were used for each treatment. The treatments that were given are shown in Table 2. A PAR of 80 $\mu molm^{-2}s^{-1}$ at 21 ºC is the control treatment for both wild-type and *MINELESS* plants, respectively.

Table 2. Treatment of *B. napus* wild-type and *MINELESS* plant seedlings with different light intensities and temperatures, where 80 µmolm^{-2}s^{-1} at 21ºC represents control (non-stressed.

Light intensity	**Temperatures**	**Stress combinations**	
80 µmolm^{-2}s^{-1}	5 ºC	Medium light, low temperature	ML-LT
	21 ºC	Medium light, medium temperature	ML-MT
	30 ºC	Medium light, high temperature	ML-HT
200 µmolm^{-2}s^{-1}	5 ºC	High light, low temperature	HL- LT
	21 ºC	High light, medium temperature	HL- MT
	30 ºC	High light, high temperature	HL-HT

Because of space limitations, the shock treatments were preformed over two days. The shock treatment that required 30 ºC was done on day 1, while the shock treatments that required 5 ºC and 21 ºC were done on day 2. On both days, the controls (non-stressed plants) were harvested.

The plant material was harvested at different intervals after start of shock treatments as described in Table 3. For measuring chlorophyll fluorescence, the cotyledons and the single true leaf from one seedling per pot were removed. The rest of above-ground tissue was harvested and snap frozen in liquid nitrogen in tubes. The tubes were stored in -80 ºC. The tissue was homogenised with a spatula and was used for pigments and analysis of plant metabolites.

Table 3. Plan for shock treatment, with start of photoperiod as 0 hour.

Hours after start of photoperiod	
3	Start of shock-treatment
9	Harvesting of two cotyledons and one true leaf for chlorophyll fluorescence measurements
11 – 12	Harvesting of above-ground plant tissue for pigments and analysis of plant metabolites
16	End of photoperiod

2.2 Chlorophyll fluorescence

The harvested cotyledons and true leaves were placed on moist tissue paper in a 12 well cell culture plate and dark adapted for at least 15 min before chlorophyll fluorescence measurements. F_0 and Fm measurements were done in a dark room using PAM 210 Walz

fluorometer (Walz, Effeltrich, Germany). At least three measurements were taken per leaf and per cotyledon.

2.3 Extraction of pigments

Before we proceeded for pigment analysis, samples were weighed to optimize the amount of tissue, which would be used for pigment analysis. The appropriate amount of tissue after test analysis came out to be 60 mg, which was used for analysis in this experiment. The plant material was transferred to Teflon-lined screw-capped tube and 1.6 mL ice cold methanol was added and samples were vortexed. The samples were placed in a freezer and pigments were extracted overnight. The extracts were vortexed and filtered through Millipore 0.45µm filter over in new tubes and N_2 was added to replace the air in the top of the tubes before they were sealed.

2.4 Pigment analysis

The separation of pigments were performed with Hewlett-Packard HPLC 1100 Series system (Hewlett-Packard, Waldbronn, Germany) with a quaternary pump system and diode array detector, equipped with a Waters Symmetry C_8 column (150x4.6 nm, 3.5 µm particle size), using the HPLC method of Zapata et al (2000) (Rodriguez et al., 2006, Zapata et al., 2000). Column temperature was kept at 25 ºC. The mobile phase consisted of eluent A and B. Eluent A was a mixture of methanol:acetonitrile:aqueous pyridine solution (50:25:25, 0.25 M pyridine). Eluent B was a mixture of acetonitrile:acetone (80:20).

Chlorophyll and carotenoids were detected by absorbance at 440 nm and identified by a diode array detector (λ-350 750 nm, 1.2 nm spectral resolution) (Rodriguez et al., 2006).

2.4 Analysis of plant metabolites

500 mg of homogenised plant tissue was transferred to Eppendorf-tubes. 1mL of precooled solvent mixture was added. The solvent contained CCl_3:MeOH:H_2O in ratio of 1:2.5:1, and 70 µg/mL ribitol. The tubes were treated in ultrasonic bath for 60 min at 60 ºC. The room tempered samples were then placed in centrifuge precooled to 4 ºC, and centrifuged for 10 min at highest rpm.

800 µL of the aliquots were transferred to Eppendorf-tubes, and tubes were sealed with lids with holes punched in the centre. The samples were dried in SpeedVac without heating.

Dried residue was dissolved in 80 µL of 20 mg/ml methoxyamine hydrochloride in pyridine. After 90 min of incubation at 30 ºC, 80 µL N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) was added and the samples were incubated at 37 ºC for 30 min. The samples were transferred to 1.5 autosampler vials.

The separation of samples were performed on Agilent 6890/5975 GC-MS (Agilent Technologies, Palo Alto, CA) with a HP-5MS capillary column (30nm x 0.25 mm i.d., film thickness 0.25 µm) (Agilent Technologies, Palo Alto, CA) Sample volumes of 5 µL were injected with a split ratio of 15:1. Injection and interface temperature were set to 230 ºC and 250 ºC, respectively. The GC temperature program was held isothermically at 70 ºC for 5 min, ramped from 70 ºC to 310 ºC at 5 ºC/min, and finally held at 310 ºC for 7 min (run time: 60 min). The MS source was adjusted to 230 ºC and a mass range of m/z 70-700 was recorded (EI mode).

Further data alignment and processing was varied out using the MetAlign software (Rikilt, Wageningen, NL) (Lommen, 2009). Compound identification was achieved using MS libraries, such as NIST/ EPA/ NIH MassSpectralLibrary NIST05 (National Institute of Standards and Technology, Gaithersburgh, MD), the Golm Metabolome Database containing MS spectra of derivatized metabolites (Hummel et al., 2013), in combination with an in-house retention index library of trimethylsilylated (TMS) metabolites. The Automated Mass Spectral Deconvolution and Identification System (AMDIS; National Institute of Standards and Technology, Boulder, CO) software was used to interpret GC/MS data.

2.5 Statistical analysis

Factorial ANOVA with light, temperature, genotype, and all interactions, was used as to explore the differences between the treatments. ANOVA for metabolites was performed using MiniTab (MiniTab17), and for pigments and chlorophyll fluorescence R was used (R studio, version 2.14.1).

Tukey honestly significant difference (HSD) test for multiple comparisons with light, temperature and genotype as factors was used to look for the significant differences between treatment groups. Tukey HSD was performed using R with foreign and agricolae packages (R studio, version 2.14.1).

Figures, standard deviations and standard errors were obtained using Sigmaplot (Sigmaplot 13.0).

Heat maps for metabolites were constructed using MeV, by dividing data points from GC-MS by the median of the samples for each metabolite, and transforming the data to a

logarithmic scale with base 2. Calculations and log transformation were performed in Microsoft Excel.

3. Results

3.1 Pigments

Neoxanthin, violaxanthin, lutein, two unidentified carotenoids (designated as carotenoid 1 and carotenoid 2), chlorophyll a and b and β- carotene were detected in traceable amounts (Table S1, appendix). Carotenoid 1 and carotenoid 2 were given numbers 1 and 2 on the basis of their retention time, where carotenoid 1 represents the one with shortest retention time.

Both the carotenoids and β-carotene were either found in low concentration or were absent in the extracted samples with the method used in this analysis. The method was also unable to detect zeaxanthin in any of the samples.

The combination of different light intensities (ML and HL) and temperature treatments (LT, MT and HT) had a significant effect ($P < 0.05$) on all pigments, except lutein.

The interactions between light and temperature were significant for both of the xanthophylls (violaxanthin and neoxanthin) and β-carotene. The only pigments to show a variation between the wild-type and *MINELESS* plants effect were chl b and carotenoid 1. Chl b showed a significant change to the combination of temperatures and wild-type/ *MINELESS* plants. Carotenoid 1 was affected by wild-type/*MINELESS* without interactions from temperature and light.

There was no difference between wild-type and *MINELESS* plants on the amount of violaxanthin detected in any of samples for any of the light and temperature combinations (Table S2). At 21ºC, the light intensities (ML and HL) had no effect on the amount of violaxanthin (Figure 6A). However at both LT and HT, the seedlings grown under ML had a higher amount of violaxanthin than the seedlings at HL. At both ML and HL there was an increase in violaxanthin from LT to HT, but where the major part of the increase happens is different. At 80 $\mu molm^{-2}s^{-1}$ the increase happens from 21 ºC to 30 ºC. At 200 $\mu molm^{-2}s^{-1}$ the increase is from 5 ºC to 21 ºC. The trend observed for violaxanthin was also observed for neoxanthin (Figure 6B)

Chl a had no difference in the amount of chlorophyll (ng chlorophyll per mg plant tissue) between wild-type and *MINELESS* (Table S1, appendix).

Chl a was detected at around the same amounts for both light intensities (80 $\mu molm^{-2}s^{-1}$ and 200 $\mu molm^{-2}s^{-1}$) at 5 ºC and 21 ºC (Figure 6C). At 30 ºC, seedlings that were treated with 80 $\mu molm^{-2}s^{-1}$ had a higher amount of chl a than seedlings treated with 200 $\mu molm^{-2}s^{-1}$. The

same relationship between the level of chlorophyll, light and temperature was also detected for chl b (Figure S1, appendix).

There were similar amounts of beta - carotene in seedlings treated with both light intensities (80 $\mu molm^{-2}s^{-1}$ and 200 $\mu molm^{-2}s^{-1}$) at 5 ºC and 21 ºC, and there were no significant differences between the two genotypes (wild-type and *MINELESS*) (Figure 6D). At 30 ºC the plants grown under low light intensities (80$\mu molm^{-2}s^{-1}$) showed a strong increase in the detected amount of beta-carotene, under high light conditions (200 $\mu molm^{-2}s^{-1}$) the amount of beta-carotene decreased.

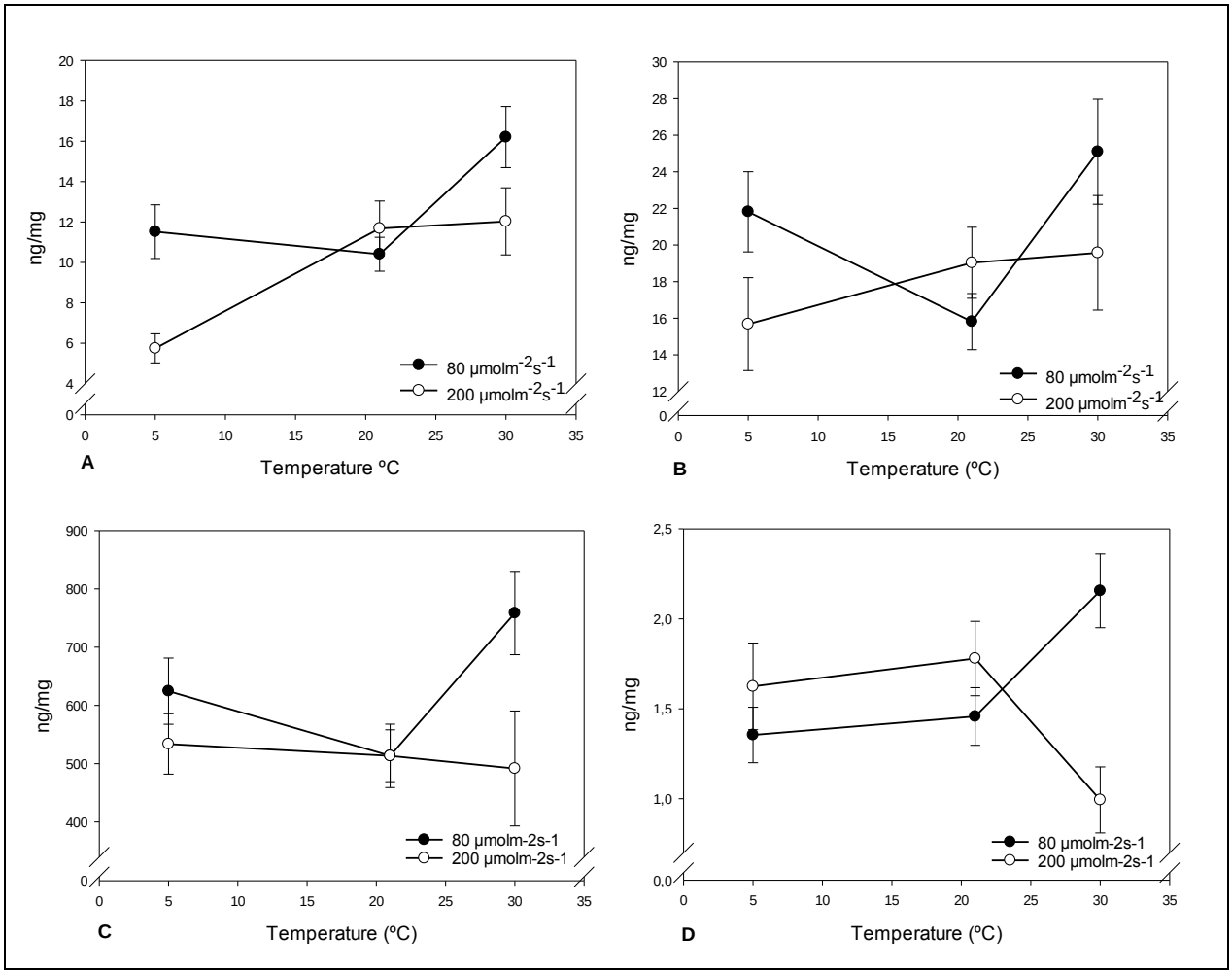

Figure 6: A) Violaxanthin (ng/mg), B) Neoxanthin (ng/mg) C) Chl a (ng/mg) and D) beta-carotene (ng/mg), in 11-12 days old *B. napus* seedlings grown in all six combinations of the following of light intensities; 80 $\mu molm^{-2}s^{-1}$ and 200 $\mu molm^{-2}s^{-1}$, and temperatures; 5 ºC, 21 ºC, and 30 ºC. Black circles represent 80 $\mu molm^{-2}s^{-1}$ and open circles 200 $\mu molm^{-2}s^{-1}$. Error bars represent standard error. n=12.

The ratio between chl a and chl b, was calculated to look into how the relationship between these two changed. The calculated ANOVAs are shown in Table 4.

The ratio between chl a and b seems to be sensitive to the interaction between light intensity and genotype, and temperature and genotype.

The ratio between chl a and b showed a consistent level with about the same levels for 5ºC and 21 ºC except for wild-type 80 µmolm^{-2}s^{-1} at 21ºC (Figure 7). At 30 ºC the ratio gets smaller, and most so for wild-type 200 µmolm^{-2}s^{-1}.

Table 4. ANOVA table for the ratios between chl a and b, and viola- and neoxanthin. All P values < 0.05 are marked with bold font. *P < 0.05 = '*', P < 0.01='**', P < 0.001= '***'.* n= 6.

			Temperature	Genotype	Light* Temperature	Light* Genotype	Temp* Genotype	Light* Temperature* Genotype
	df	1	2	1	2	1	2	2
Chl a/ Chl b	MS	4.17990	2.80582	0.06676	1.28346	2.06968	1.47994	0.77849
	F	9.77	6.56	0.16	3.00	4.85	3.46	1.82
	P	**** 0.003**	**** 0.002**	0.694	0.056	*** 0.031**	*** 0.037**	0.17

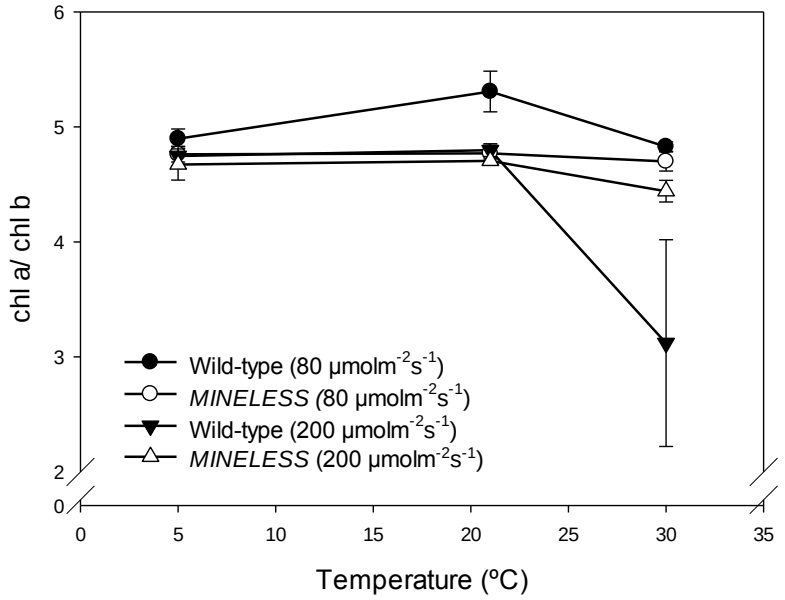

Figure 7: A) Chl a/ b in 11-12 day old true leaves on *B. napus* seedlings of both *MINELESS* and wild-type. Black symbols represent wild-type and open symbols *MINELESS*. Circles represent 80 µmolm^{-2}s^{-1} and triangles 200 µmolm^{-2}s^{-1}. Error bars represents standard error. n=6 for chla/b.

3.2 Chlorophyll fluorescence

There was a difference in the effect on Fv/Fm from light and temperature between true leaves and cotyledons, and the effects of stress factors on these were therefore considered separately (Table S1 and S2, appendix). After treatment with different light intensities and temperature stress (cold and heat shock), significant changes were observed in Fv/Fm between the combinations of light intensities (80 µmolm^{-2}s^{-1} and 200 µmolm^{-2}s^{-1}) and temperature stress

(5 ºC, 21 ºC, and 30 ºC) for the cotyledons (Figure S2 and Table S1, appendix). For true leaves, changes were observed between combinations of light intensities (80 µmolm^{-2}s^{-1} and 200 µmolm^{-2}s^{-1}) and wild-type and *MINELESS* groups (Figure 8 and Table S1, appendix)

In true leaves, there were a significant decrease in Fv/Fm from non-stressed wild-type (21 ºC and 80 µmolm^{-2}s^{-1}) to both *MINELESS* groups (80 µmolm^{-2}s^{-1} and 200 µmolm^{-2}s^{-1}) and high light stressed (200 µmolm^{-2}s^{-1}) wild-type at 5 ºC and 21 ºC. Within each light treatment group (wild-type; 80 µmolm^{-2}s^{-1} and 200 µmolm^{-2}s^{-1}, and *MINELESS*; 80 µmolm^{-2}s^{-1} and 200 µmolm^{-2}s^{-1}) there was little effect of temperature on Fv/Fm.

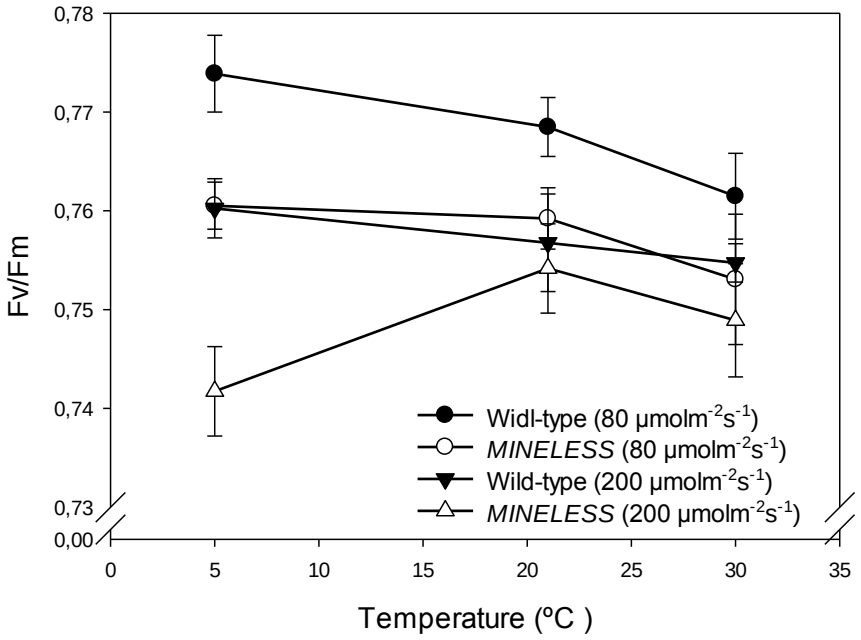

Figure 8: Fv/Fm in 11-12 day old true leaves on *B. napus* seedlings of both *MINELESS* and wild-type. Black symbols represent wild-type and open symbols *MINELESS*. Circles represent 80 µmolm^{-2}s^{-1} and triangles 200 µmolm^{-2}s^{-1}. Error bars represents standard error. n= 12.

3.3 Analysis of plant metabolites

Detected metabolites with P values < 0.05 for one or more of the stress factors are listed in Table S2 (appendix). Some of the metabolites are listed with full name, others only by chemical structure (for example sugar or amino acid).

Indole-3-acetonitril (IAN) (Figure 9A) had similar values for wild-type and *MINELESS* at 5 ºC, while at 20 ºC and 31 ºC *MINELESS* had higher values.

Wild-type had higher levels of ascorbic acid than *MINELESS* at 5 ºC, but from 5 ºC to 21 ºC *MINELESS* seedlings had an increase in ascorbic acid, and at 21 ºC MINELESS levels of ABA were higher than in wild-type. At 30 ºC there was no difference between wild-type and *MINELESS* (Figure 9B).

Some of the metabolites the showed significant P values for light intensity and not wild-type/ *MINELESS* are shown in figure 10.

Indole (Figure 10A) had similar levels in both 80 µmolm^{-2}s^{-1} and 200 µmolm^{-2}s^{-1} treated plants at 5 ºC. At 21 ºC the indole levels in the high light treated plants (200 µmolm^{-2}s^{-1}) were higher than in non-stressed plants (80 µmolm^{-2}s^{-1} and 21 ºC). At 30 ºC this changed, and it was the non-stressed plants that had the highest indole levels, while the high light plants (200 µmolm^{-2}s^{-1}) had lower indole levels. The indole levels for light stressed plants (200 µmolm^{-2}s^{-1}) at 30 ºC were also lower than for light stressed plant at 5 ºC and 21 ºC.

Sinapoyl malate (Figure 10B) had similar levels in both 80 µmolm^{-2}s^{-1} and 200 µmolm^{-2}s^{-1} treated plants at 5 ºC. From 5 ºC to 21 ºC there were a decrease in the plants treated with 80 µmolm^{-2}s^{-1}, and at 21 ºC and 30 ºC the 80 µmolm^{-2}s^{-1} treated plants had lower sinapoyl malate levels than those treated with 200 µmolm^{-2}s^{-1}.

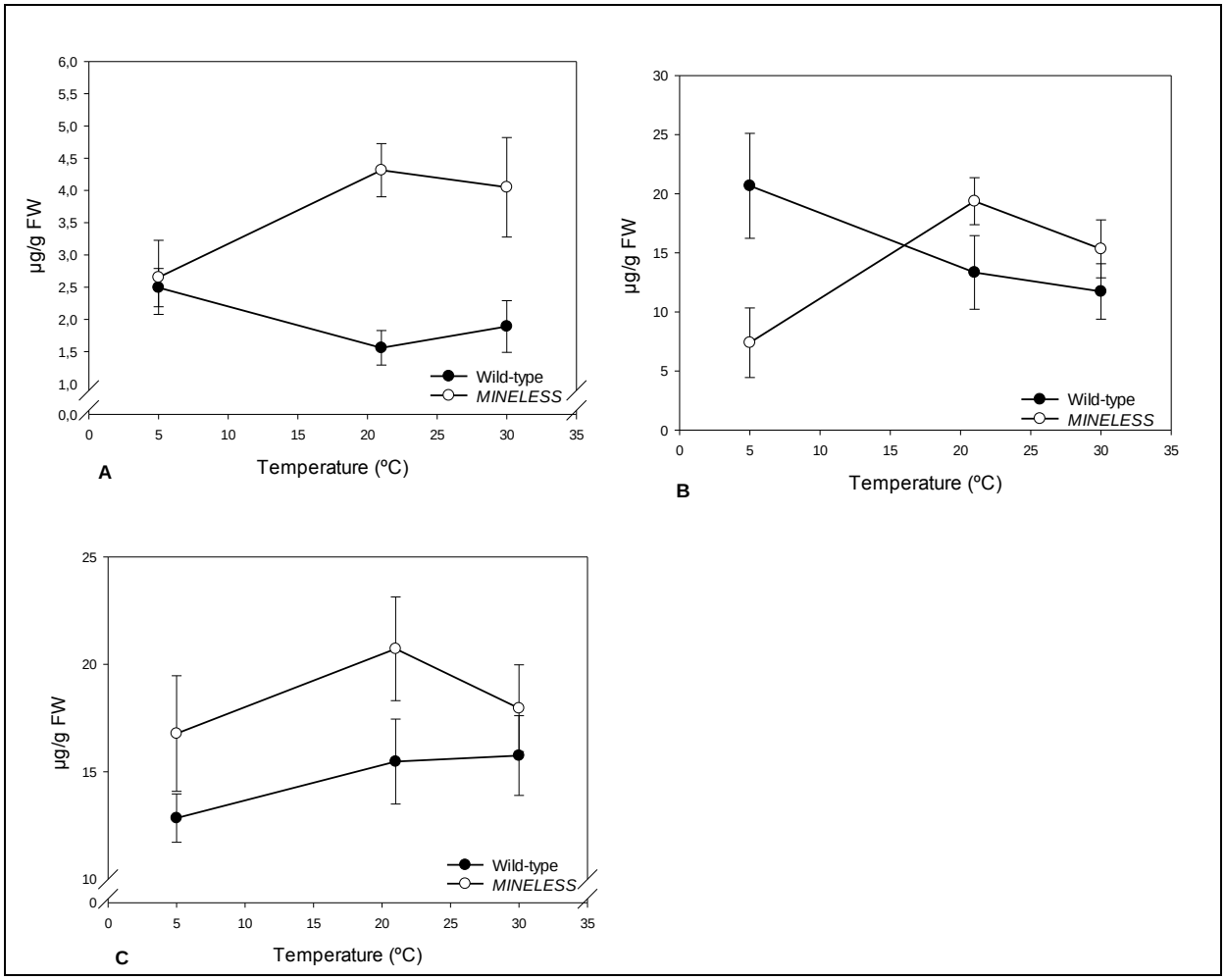

Figure 9. A) Indole-3-acetonitrile (µg/g FW) and B) ascorbic acid (µg/g FW), C) (E)-sinapic acid (µg/g FW) in 11-12 days old *B. napus* seedlings grown in all six combinations of the following of light intensities; 80 µmolm^{-2}s^{-1} and 200 µmolm^{-2}s^{-1}, and temperatures; 5 ºC, 21 ºC, and 30 ºC. Black circles represent wild-type and open circles *MINELESS*. Error bars represent standard error. n=12. FW=fresh weight.

(Z)-3-caffeoylquinic, E)-5-caffeoylquinic acid, sinapoyl glucoside (1) (all three, secondary metabolites) and tryptophan (Figure 10; C, D, E and F, respectively) all showed the same responses to light treatments (80 $\mu molm^{-2}s^{-1}$ and 200 $\mu molm^{-2}s^{-1}$) and temperatures (5 ºC, 21 ºC and 30 ºC). At 5 ºC, 80 $\mu molm^{-2}s^{-1}$ and 200 $\mu molm^{-2}s^{-1}$ have the same amount of the respective metabolite. From 5 ºC to 21 ºC the levels decrease in 80 $\mu molm^{-2}s^{-1}$ treated plant while the levels in the 200 $\mu molm^{-2}s^{-1}$ treated plants stay the same. From 21 ºC to 30 ºC the levels in the high light intensity treated plants (200 $\mu molm^{-2}s^{-1}$) decreases to the same levels as the 80 $\mu molm^{-2}s^{-1}$ treated plants.

Detected sugars with $P < 0.05$ are shown in heat maps in figure 11. Heat map for all the detected metabolites is shown in figure S3, appendix. Comparing the two heat maps (left, wild-type and right, *MINELESS*) there are few differences in the increase and decrease of the sugar levels between the two, and the trends are similar. There are however some higher levels in the *MINELESS* plants (Figure 11)

Cold shock (5 ºC) resulted in higher levels of sugar than heat shock in both non-stress (80 $\mu molm^{-2}s^{-1}$) and high light stress (200 $\mu molm^{-2}s^{-1}$) treatments, for both wild-type and *MINELESS* seedlings (Figure 11 and Figure S3, appendix). Heat shock under non-stress light conditions (80 $\mu molm^{-2}s^{-1}$, 30 ºC) gave several sugars (fructose (1), glucose (1) and raffinose) the lowest observed levels for both wild-type and *MINELESS*.

In the high light treatments (200 $\mu molm^{-2}s^{-1}$) there were in general higher sugar levels in both wild-type and *MINELESS* plants, and especially so for the disaccharides. In the cold shock treatment (5 ºC), combined with high light (200 $\mu molm^{-2}s^{-1}$) several of the disaccharides had high changes in the amount of detected sugar, and this was observed for both wild-type and *MINELESS*. At 21 ºC, the high light treatment (200 $\mu molm^{-2}s^{-1}$) had less of an effect, but at 30 ºC high light gave either an increase or no change in the disaccharides in wild-type, while they had a slight decrease in the *MINELESS* plants.

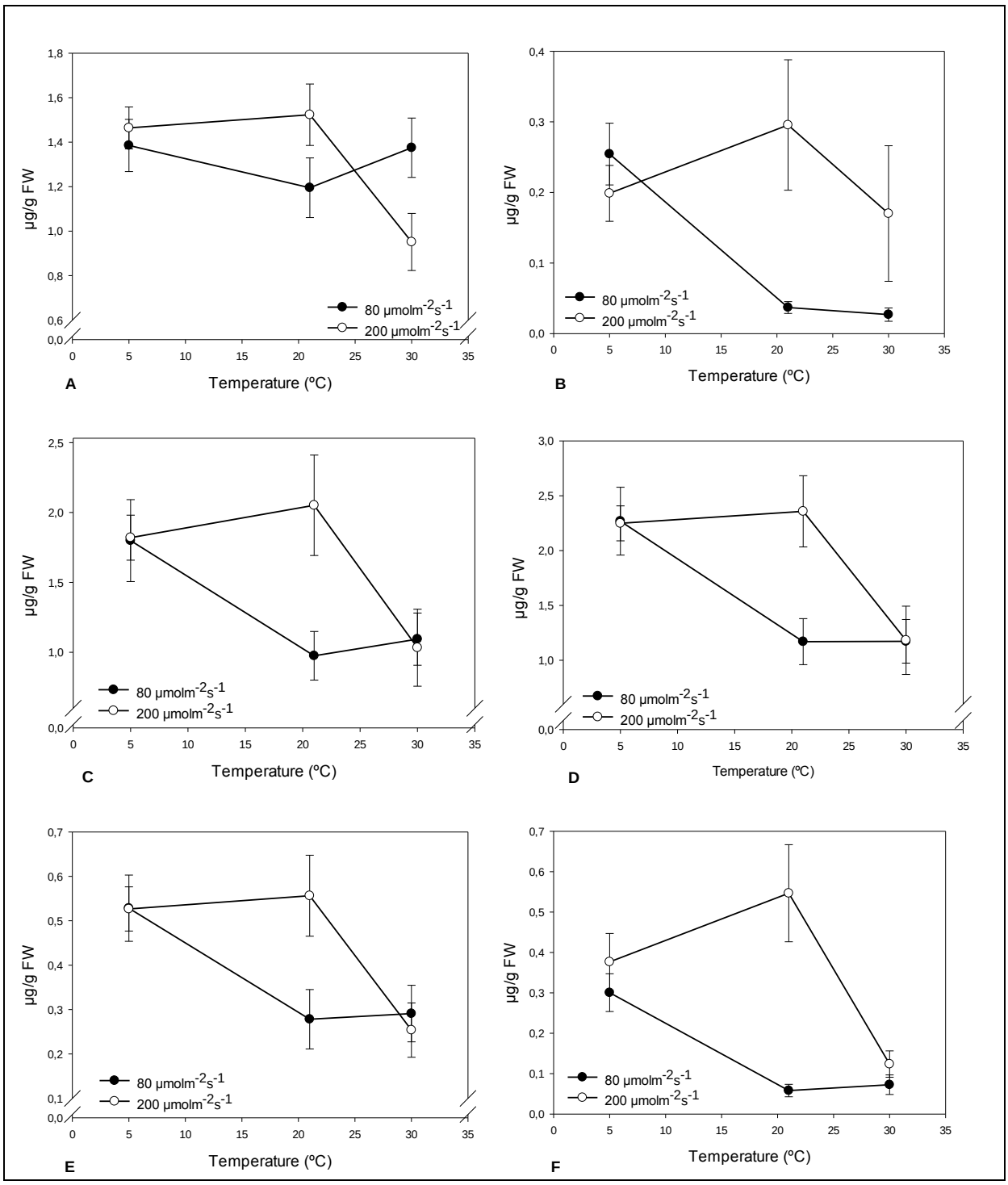

Figure 10. A) Indole (µg/g FW (fresh weight)), B) sinapoyl malate (µg/g FW), C) (Z)-3-caffeoylquinic acid (µg/g FW), D) (E)-5-caffeoylquinic acid (µg/g FW), E) sinapoyl glucoside(1) (µg/g FW), F) tryptophan (µg/g FW) in 11-12 days old *B. napus* seedlings grown in all six combinations of the following of light intensities; 80 µmolm^{-2}s^{-1} and 200 µmolm^{-2}s^{-1}, and temperatures; 5 ºC, 21 ºC, and 30 ºC. Black circles represent 80 µmolm^{-2}s^{-1} and open circles 200 µmolm^{-2}s^{-1}. Error bars represent standard error. n=12.

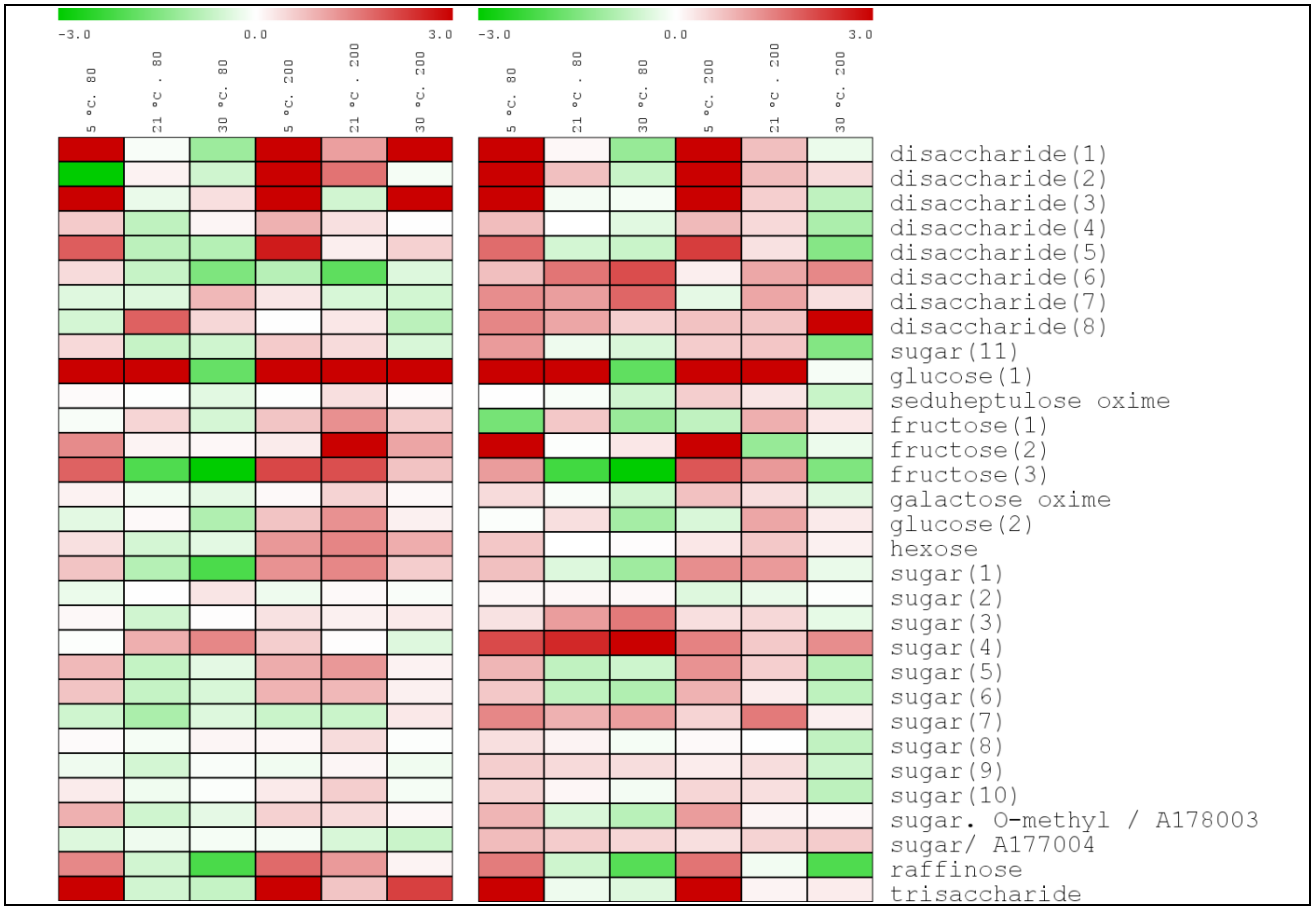

Figure 11: Sugars from GC-MS of 11-12 days old wild-type and transgenic *MINELESS B. napus* seedlings grown in all six combinations of the following of light intensities; 80 µmolm^{-2}s^{-1} and 200 µmolm^{-2}s^{-1}, and temperatures; 5 ºC, 21 ºC, and 30. Left panel shows treatments done with wild-type plants, the right *MINELESS*. First three columns in both panels represent the non-stressed light condition (80 µmolm^{-2}s^{-1}) at the three temperatures, and the last three the high light treatments (80 µmolm^{-2}s^{-1}) with the three temperatures.

PCA calculated from all plant metabolites with significant P values ($P < 0.05$) is shown in figure 12. There are no indication of a clear groupings between the six different treatment groups (HT-HL, MT-HL, LT-HL, HT-ML, MT-ML and LT-ML). There is some indication for a difference between temperature treatments, with LT treatment gathered mostly to the right and lower on the graph (blue data points), while the HT treatments are gathered higher and to the left (red data points).

There is also some indication for a difference in HL and ML treatments, with more data points representing HL treatments (filled circles and triangles) to right, while ML data points are located more to the left (open circles and triangles).

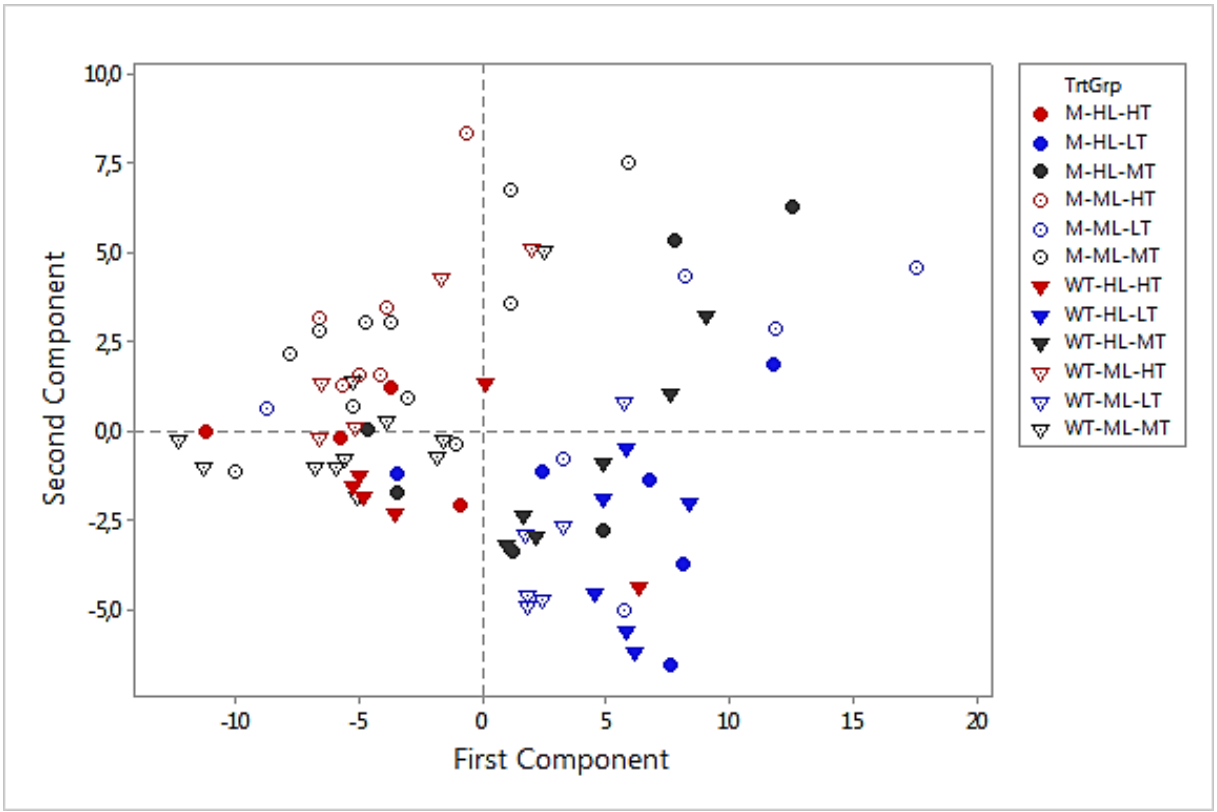

Figure 12. Principal component analysis. Blue point represent LT treatments, red HT, and black MT. Filled circles and triangles represent HL treatments and open circles and triangles ML treatments. Wild type is indicated with triangles and MINELESS with circles.

4. Discussion

In this study, we examined the effect of light and temperature stresses on wild-type and transgenic *MINELESS B. napus* plants by measuring Fv/Fm, pigment composition and metabolic profiles. With two different light intensities (non-stressed and high) and three different temperatures (non-stressed, low and high) the interactions between temperature shocks and high light stress were examined. The study showed that there was a difference in how the *MINELESS* plants reacted to temperature and light stress when compared to the wild-type for some factors. When it comes to pigment composition, there is little difference between wild-type and *MINELESS B. napus*, and the metabolite profile is mainly affected by light and temperature, but on some metabolites there were observed a difference between the wild-type and *MINELESS* plants.

Stressful environments do have an effect on *B. napus* seedlings. Using the potential efficiency of PS2 (Fv/Fm measurements) as an indicator for how stressed the seedlings are, LT and heat HT shock at ML had little impact. The observed decrease in Fv/Fm when seedlings were exposed to multiple stressors through combinations LT and HT shock and HL show that the combination of multiple types of stressors has a different effect than the effect of a single stresses.

Fv/Fm differed between wild-type and *MINELESS* plants at HL, and there seems to be some indication for a difference between the stress levels in the wild-type plants when compared with the *MINELSS*.

Since Fv/Fm measurements differ not only because of the environmental conditions, but also because of age of the leaves, the results from the young true leaves might be skewed (Baker, 2008). The results from the true leaves indicate that the 1-2 days old leaves are more sensitive to high irradiance than temperature shocks. The lower Fv/Fm in *MINELESS* leaves indicates that the glucosinolate-myrosinase system has an effect on the potential efficiency of PS2 at cold shock.

The changes in Fv/Fm between the light and wild-type/*MINELESS* groups are similar with increasing temperature, and there is therefore no indication for interaction effects between stresses on Fv/Fm for *B. napus* seedlings in this experiment.

With only chl b being affected by the lack of myrosin cells, and when affected only in combination with change in irradiance, there is no indication of glucosinolate-myrosinase system affecting pigment composition in *B. napus*. Both cold and heat shock combined with

high irradiance, do, as expected, have an effect on the pigment composition. In the amount of pigment in the seedlings, there were some interaction effects between temperature and light.

The changed levels of violaxanthin in response to increased irradiance after MT and HT shocks can be explained by the role of the pigment in the xanthophyll cycle and NPQ. High levels of violaxanthin at HL after HT shock is a part of the plants photoprotection, and differences in xanthophyll levels at different temperatures and light conditions was expected, and can be explained with the de-epoxidation of violaxanthin with excess light and the slower de-epoxidation at lower leaf temperatures. The higher levels of violaxanthin and neoxanthin at ML after LT shock when compared at HL after LT shows that there is an interaction between light and temperature in the use of the xanthophyll cycle in NPQ.

Also, the two chlorophylls and beta-carotene show a reaction to interactions between light and temperature, indicating that both light and temperature has to be considered together when predicting the seedlings reaction to stresses. Both chl a and b had about the same amount of pigment in LT and MT temperatures under both HL and ML light levels. At HT however increased irradiance gave a higher amount of chlorophyll while LT gave lower. It been shown that higher temperatures can give decreased levels of chlorophyll a and chlorophyll b, but this does not seem to be the case in *B. napus* seedlings after temperature shock treatments during high light conditions.

The metabolite profile of the plants was affected by the glucosinolate-myrosinase system, but temperature and irradiance had an effect on more of the compounds than whether or not the plants were transgenic *MINELESS*.

Among the metabolites that the glucosinolate-myrosinase system had an effect on was ascorbic acid, where the *MINELESS* had higher concentrations in non-stressed (21 ºC) plants, while during LT shock the MINELESS seedlings had less ascorbic acid. Ascorbic acid is the most abundant antioxidant found in plants (Chen and Gallie, 2004), and the antioxidant defence system can be used by plants to cope with cold stress (Li et al., 2013). It has also been shown that myrosinases are strongly activated by ascorbic acid (Shikita et al., 1999). This experiment shows that ascorbic acid levels are lower in *B. napus* with a defect glucosinolate-myrosinase system after cold shock.

With the low levels of myrosinase present in *MINELESS* plants, there is less hydrolysis of glucosinolates, and low production of glucosinolate-myrosinase hydrolysis products. The increase in indole-3-actonitril (IAN) levels found in MINELESS plants treated with temperatures of 21 ºC and 30 ºC is therefore not a product of glucosinolates hydrolysis.

Another pathway where IAN is found is in the biosynthesis of camalexin, a secondary metabolite known to increase with pathogen attacks and which is also has tryptophan as a precursor (Rauhut and Glawischnig, 2009). The glucosinolate-myrosinase system had no effect on the amount of tryptophan detected, but temperature and irradiance did. Irradiance had however no effect on the amount of IAN. The increase of tryptophan alone can therefore not explain the increase in IAN found at 30 ºC in *MINELESS*.

Phenolic compounds like sinapoyl malate and caffeoylquinic acid had, as expected, an increase when the light intensity increased (HL) at non-stress temperature (MT). During cold and heat shock, however high light stress gave no increase in the concentration of in sinapoyl glucoside and caffeoylquinic acid, and a very small increase in sinapoyl malate, when compared to the non-stressed, ML treated, plants. Chapple et. al. showed that a *A. thaliana* mutants that lack sinapic acid and sinapoyl malate are UV-B sensitive (Chapple et al., 1992, Page et al., 2012), and this could be attributed either to the screening effect of the phenolic compounds or to the antioxidant scavenging properties of the compounds . After heat shock there is little or no difference between high light and no-stress treatments, and the reasons for this might be the effect of temperature on the enzymes in the metabolic pathways. Grace et. al. (Grace et al., 1998) showed in their experiment that *Mahonia repens* leaves exposed to light, sampled in the field, had higher caffeoylquinic acid levels than leaves sampled from the shade. They also found that leaves sampled during winter had higher caffeoylquinic acid levels than leaves sampled during summer (Grace et al., 1998). These findings are in accordance with the findings in this experiment, where the plants are shock treated. Lower temperature (LT) gave higher levels than heat shock (HT), and high light stress (HL) resulted in much caffeoylquinic acid higher levels in non-stressed temperature conditions (ML).

The metabolic profiles of sugars change after challenging to different stresses and the different interactions. This can be explained by the effect of sugars as osmolytes and it was expected that there would be changes in the sugar levels. The increase in sugar after cold shock is expected and numerous studies has shown that there are both qualitative and quantitative changes in free sugars in plants exposed to low temperatures (Guy et al., 1992). Temperature and light had a greater effect on the sugar composition than a working glucosinolate-myrosinase system. The *MINELESS* plant had more sugars with increased levels, but in general there was little difference between the wild-type and transgenic MINELESS plants.

The performed PCA did not show any clear grouping between the six treatments used in this experiment. There is however some indication for a distinction between the LT and HT treatment groups, indicating that the effect of LT on the plant metabolites can be separated from HT.

5. Conclusions

The glucosinolate-myrosinase system does have an effect on *B. napus* seedlings when it comes to how the plant reacts to cold and heat shock, and high light stresses. The effect can be measured through Fv/Fm, but it is clearer with studies of plant metabolite profiles.

There is an interaction between high light stress and cold and heat shock. The interactions between these stresses effects pigment composition and metabolite concentrations.

6. References

AHUJA, I., BORGEN, B. H., HANSEN, M., HONNE, B. I., MULLER, C., ROHLOFF, J., ROSSITER, J. T. & BONES, A. M. 2011. Oilseed rape seeds with ablated defence cells of the glucosinolate-myrosinase system. Production and characteristics of double haploid MINELESS plants of Brassica napus L. *Journal of Experimental Botany,* 62**,** 4975-4993.

ASHRAF, M. & HARRIS, P. J. C. 2013. Photosynthesis under stressful environments: An overview. *Photosynthetica,* 51**,** 163-190.

BAKER, N. R. 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annu. Rev. Plant Biol.,* 59**,** 89-113.

BJORKMAN, M., KLINGEN, I., BIRCH, A. N. E., BONES, A. M., BRUCE, T. J. A., JOHANSEN, T. J., MEADOW, R., MOLMANN, J., SELJASEN, R., SMART, L. E. & STEWART, D. 2011. Phytochemicals of Brassicaceae in plant protection and human health - Influences of climate, environment and agronomic practice. *Phytochemistry,* 72**,** 538-556.

BONES, A. & IVERSEN, T. H. 1985. MYROSIN CELLS AND MYROSINASE. *Israel Journal of Botany,* 34**,** 351-376.

BONES, A. M. & ROSSITER, J. T. 1996. The myrosinase-glucosinolate system, its organisation and biochemistry. *Physiologia Plantarum,* 97**,** 194-208.

BORGEN, B. H., THANGSTAD, O. P., AHUJA, I., ROSSITER, J. T. & BONES, A. M. 2010. Removing the mustard oil bomb from seeds: transgenic ablation of myrosin cells in oilseed rape (Brassica napus) produces MINELESS seeds. *Journal of Experimental Botany,* 61**,** 1683-1697.

BUNGARD, R. A., RUBAN, A. V., HIBBERD, J. M., PRESS, M. C., HORTON, P. & SCHOLES, J. D. 1999. Unusual carotenoid composition and a new type of xanthophyll cycle in plants. *Proceedings of the National Academy of Sciences,* 96**,** 1135-1139.

CHAPPLE, C., VOGT, T., ELLIS, B. E. & SOMERVILLE, C. R. 1992. An Arabidopsis mutant defective in the general phenylpropanoid pathway. *The Plant Cell Online,* 4**,** 1413-1424.

CHEN, Z. & GALLIE, D. R. 2004. The ascorbic acid redox state controls guard cell signaling and stomatal movement. *The Plant Cell Online,* 16**,** 1143-1162.

COSGROVE, J. & BOROWITZKA, M. A. 2010. Chlorophyll fluorescence terminology: an introduction. *Chlorophyll a Fluorescence in Aquatic Sciences: Methods and Applications.* Springer.

DALL'OSTO, L., LICO, C., ALRIC, J., GIULIANO, G., HAVAUX, M. & BASSI, R. 2006. Lutein is needed for efficient chlorophyll triplet quenching in the major LHCII antenna complex of higher plants and effective photoprotection in vivo under strong light. *Bmc Plant Biology,* 6.

DEAN, J. C., KUSAKA, R., WALSH, P. S., ALLAIS, F. & ZWIER, T. S. 2014. Plant Sunscreens in the UV-B: Ultraviolet Spectroscopy of Jet-Cooled Sinapoyl Malate, Sinapic Acid, and Sinapate Ester Derivatives. *Journal of the American Chemical Society,* 136**,** 14780-14795.

ENGELEN-EIGLES, G., HOLDEN, G., COHEN, J. D. & GARDNER, G. 2006. The effect of temperature, photoperiod, and light quality on gluconasturtiin concentration in watercress (Nasturtium officinale R. Br.). *Journal of Agricultural and Food Chemistry,* 54**,** 328-334.

FAHEY, J. W., ZALCMANN, A. T. & TALALAY, P. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry,* 56**,** 5-51.

FONT, R., DEL RIO, M., FERNANDEZ, J. M. & DE HARO, A. 2003. Acid detergent fiber analysis in oilseed Brassicas by near-infrared spectroscopy. *Journal of Agricultural and Food Chemistry,* 51**,** 2917-2922.

GRACE, S. C., LOGAN, B. A. & ADAMS, W. W. 1998. Seasonal differences in foliar content of chlorogenic acid, a phenylpropanoid antioxidant, in Mahonia repens. *Plant Cell and Environment,* 21**,** 513-521.

GRIMM, B., PORRA, R. J., RÜDIGER, W. & SCHEER, H. 2006. Chlorophylls and Bacteriochlorophylls.

GUY, C. L., HUBER, J. L. A. & HUBER, S. C. 1992. SUCROSE PHOSPHATE SYNTHASE AND SUCROSE ACCUMULATION AT LOW-TEMPERATURE. *Plant Physiology,* 100**,** 502-508.

HALKIER, B. A. & DU, L. C. 1997. The biosynthesis of glucosinolates. *Trends in Plant Science,* 2**,** 425-431.

HUMMEL, J., STREHMEL, N., BÖLLING, C., SCHMIDT, S., WALTHER, D. & KOPKA, J. 2013. Mass Spectral Search and Analysis Using the Golm Metabolome Database. *The Handbook of Plant Metabolomics.* Wiley-VCH Verlag GmbH & Co. KGaA.

ITO, H., OHTSUKA, T. & TANAKA, A. 1996. Conversion of chlorophyll b to chlorophyll a via 7-hydroxymethyl chlorophyll. *Journal of Biological Chemistry,* 271**,** 1475-1479.

KAPLAN, F., KOPKA, J., HASKELL, D. W., ZHAO, W., SCHILLER, K. C., GATZKE, N., SUNG, D. Y. & GUY, C. L. 2004. Exploring the temperature-stress metabolome of Arabidopsis. *Plant Physiology,* 136**,** 4159-4168.

KISSEN, R., ROSSITER, J. T. & BONES, A. M. 2009. The 'mustard oil bomb': not so easy to assemble?! Localization, expression and distribution of the components of the myrosinase enzyme system. *Phytochemistry Reviews,* 8**,** 69-86.

KLIEBENSTEIN, D. J., KROYMANN, J., BROWN, P., FIGUTH, A., PEDERSEN, D., GERSHENZON, J. & MITCHELL-OLDS, T. 2001. Genetic control of natural variation in Arabidopsis glucosinolate accumulation. *Plant Physiology,* 126**,** 811-825.

KRAUSE, G. H. & WEIS, E. 1991. CHLOROPHYLL FLUORESCENCE AND PHOTOSYNTHESIS - THE BASICS. *Annual Review of Plant Physiology and Plant Molecular Biology,* 42**,** 313-349.

LI, Z.-G., YUAN, L.-X., WANG, Q.-L., DING, Z.-L. & DONG, C.-Y. 2013. Combined action of antioxidant defense system and osmolytes in chilling shock-induced chilling tolerance in Jatropha curcas seedlings. *Acta Physiologiae Plantarum,* 35**,** 2127-2136.

LOMMEN, A. 2009. MetAlign: interface-driven, versatile metabolomics tool for hyphenated full-scan mass spectrometry data preprocessing. *Analytical chemistry,* 81**,** 3079-3086.

LUDWIG-MULLER, J., KRISHNA, P. & FORREITER, C. 2000. A glucosinolate mutant of Arabidopsis is thermosensitive and defective in cytosolic Hsp90 expression after heat stress. *Plant Physiology,* 123**,** 949-958.

MAINA, J. N. & WANG, Q. 2015. Seasonal Response of Chlorophyll a/b Ratio to Stress in a Typical Desert Species: Haloxylon ammodendron. *Arid Land Research and Management,* 29**,** 321-334.

MATSUBARA, S., GILMORE, A. M. & OSMOND, C. B. 2001. Diurnal and acclimatory responses of violaxanthin and lutein epoxide in the Australian mistletoe Amyema miquelii. *Functional Plant Biology,* 28**,** 793-800.

MAURER, M. M., MEIN, J. R., CHAUDHURI, S. K. & CONSTANT, H. L. 2014. An improved UHPLC-UV method for separation and quantification of carotenoids in vegetable crops. *Food chemistry,* 165**,** 475-482.

MAWSON, R., HEANEY, R. K., ZDUNCZYK, Z. & KOZLOWSKA, H. 1993. RAPESEED MEAL-GLUCOSINOLATES AND THEIR ANTINUTRITIONAL EFFECTS .2. FLAVOR AND PALATABILITY. *Nahrung-Food,* 37**,** 336-344.

MONDOLOT, L., LA FISCA, P., BUATOIS, B., TALANSIER, E., DE KOCHKO, A. & CAMPA, C. 2006. Evolution in caffeoylquinic acid content and histolocalization during Coffea canephora leaf development. *Annals of Botany,* 98**,** 33-40.

PAGE, M., SULTANA, N., PASZKIEWICZ, K., FLORANCE, H. & SMIRNOFF, N. 2012. The influence of ascorbate on anthocyanin accumulation during high light acclimation in Arabidopsis thaliana: further evidence for redox control of anthocyanin synthesis. *Plant, cell & environment,* 35**,** 388-404.

QASIM, M., ASHRAF, M., ASHRAF, M. Y., REHMAN, S. U. & RHA, E. S. 2003. Salt-induced changes in two canola cultivars differing in salt tolerance. *Biologia Plantarum,* 46**,** 629-632.

RADOVICH, T. J. K., KLEINHENZ, M. D. & STREETER, J. G. 2005. Irrigation timing relative to head development influences yield components, sugar levels, and glucosinolate concentrations in cabbage. *Journal of the American Society for Horticultural Science,* 130**,** 943-949.

RASK, L., ANDREASSON, E., EKBOM, B., ERIKSSON, S., PONTOPPIDAN, B. & MEIJER, J. 2000. Myrosinase: gene family evolution and herbivore defense in Brassicaceae. *Plant Molecular Biology,* 42**,** 93-113.

RAUHUT, T. & GLAWISCHNIG, E. 2009. Evolution of camalexin and structurally related indolic compounds. *Phytochemistry,* 70**,** 1638-1644.

RAYMER, P. L. 2002. Canola: an emerging oilseed crop. *Trends in new crops and new uses,* 1**,** 122-126.
RODRIGUEZ, F., CHAUTON, M., JOHNSEN, G., ANDRESEN, K., OLSEN, L. & ZAPATA, M. 2006. Photoacclimation in phytoplankton: implications for biomass estimates, pigment functionality and chemotaxonomy. *Marine Biology,* 148**,** 963-971.
ROSA, E. A. S., HEANEY, R. K., PORTAS, C. A. M. & FENWICK, G. R. 1996. Changes in glucosinolate concentrations in Brassica crops (B-oleracea and B-napus) throughout growing seasons. *Journal of the Science of Food and Agriculture,* 71**,** 237-244.
SHIKITA, M., FAHEY, J., GOLDEN, T., HOLTZCLAW, W. & TALALAY, P. 1999. An unusual case of 'uncompetitive activation'by ascorbic acid: purification and kinetic properties of a myrosinase from Raphanus sativus seedlings. *Biochem. J,* 341**,** 725-732.
SHULAEV, V., CORTES, D., MILLER, G. & MITTLER, R. 2008. Metabolomics for plant stress response. *Physiologia Plantarum,* 132**,** 199-208.
TAIZ, L. & ZEIGER, E. 2010. *Plant physiology,* Sunderland, Mass., Sinauer Associates.
VELASCO, P., CARTEA, M. E., GONZALEZ, C., VILAR, M. & ORDAS, A. 2007. Factors affecting the glucosinolate content of kale (Brassica oleracea acephala group). *Journal of Agricultural and Food Chemistry,* 55**,** 955-962.
WAHID, A., GELANI, S., ASHRAF, M. & FOOLAD, M. R. 2007. Heat tolerance in plants: An overview. *Environmental and Experimental Botany,* 61**,** 199-223.
ZAPATA, M., RODRIGUEZ, F. & GARRIDO, J. L. 2000. Separation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using a reversed phase C8 column and pyridine-containing mobile phases. *Marine Ecology Progress Series,* 195**,** 29-45.

7. Appendix

7.1 Pigments and chlorophyll fluorescence

Table S1. ANOVA table for Fv/Fm and all detected pigments from HPLC. All P values < 0.05 are marked with bold font. *P < 0.05 = '*', P < 0.01='**', P< 0.001= '***'.*

	Light	Temperature	Genotype	Light* Temperature	Light* Genotype	Temperature * Genotype	Light* Temperature * Genotype
df	1	2	1	2	1	2	2
Fv/Fm - cotyledon	*****0**	*****0**	0.103	0.116	0.124	0.381	0.523
Fv/Fm - true leaf	*****0**	0.167	*****0**	0.232	0.830	0.162	0.540
Neo-xanthine	0.187	0.083	0.862	*** 0.048**	0.205	0.86	0.564
Viola-xanthine	****0.006**	***** 0**	0.402	****0.008**	0.056	0.59	0.485
Lutein	0.153	0.177	0.46	0.074	0.112	0.839	0.676
Chl b	0.149	*** *0.006**	0.195	0.192	*** 0.024**	0.976	0.884
Chl a	*** 0.031**	0.163	0.208	0.087	0.173	0.752	0.599
β,β-carotene	0.311	0.735	0.527	****0.001**	0.527	0.477	0.819
Caro-tenoid 1	0.288	*** *0.012**	0.875	0.112	0.123	0.936	0.93
Caro-tenoid 2	0.305	***0.011**	*****0**	0.126	0.381	0.309	0.815

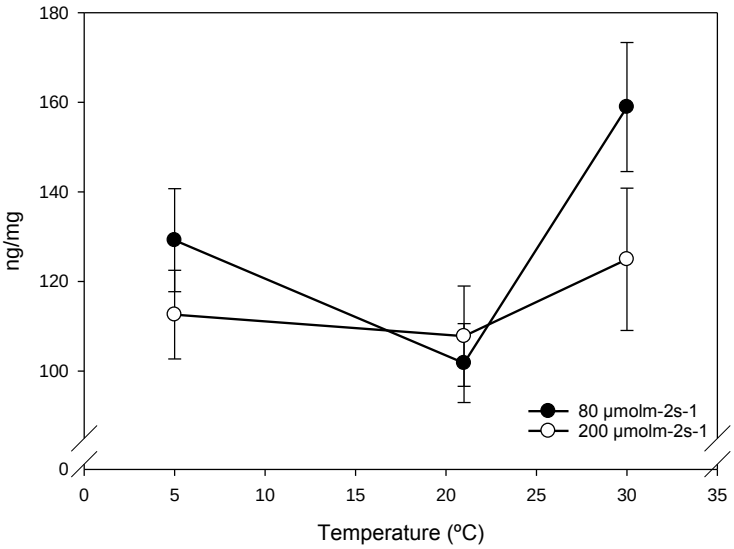

Figure S1: Chl b (ng/mg) in in 11-12 days old *B. napus* seedlings grown in all six combinations of the following of light intensities; 80 µmolm^{-2}s^{-1} and 200 µmolm^{-2}s^{-1}, and temperatures; 5 ºC, 21 ºC, and 30 ºC. Black circles represent 80 µmolm^{-2}s^{-1} and open circles 200 µmolm^{-2}s^{-1}. Error bars represents standard error. n=12.

Table S2: Tukey HSD test for temperature (5ºC, 21ºC and 30ºC), light intensity (80 µmolm^{-2}s^{-1} and 200 µmolm^{-2}s^{-1}) and wild-type/ MINELESS combinations, based on significant P values for true leaves and cotyledons (Table S1). Temperature and light combination resulting in significant changes from control (Wild-type, 80 µmolm^{-2}s^{-1} for true leaves, and 21ºC, 80 µmolm^{-2}s^{-1} for cotyledons) is highlighted with bold font. n=12 for true leaves and n= 24 for cotyledons.

True leaf			
Groups	Means	Light (µmolm^{-2}s^{-1})	Wild-type/ *MINELESS*
a	0.7681	80	Wild-type
b	**0.7581**	**80**	***MINELESS***
b	**0.7576**	**200**	**Wild-type**
c	**0.7482**	**200**	***MINELESS***
Cotyledon			
Groups	Means	Light (µmolm^{-2}s^{-1})	Temper-ature (ºC)
b	**0.783**	**80**	**5**
c	**0.7646**	**200**	**5**
a	0.7925	80	21
b	**0.7812**	**200**	**21**
a	0.7911	80	30
b	**0.7791**	**200**	**30**

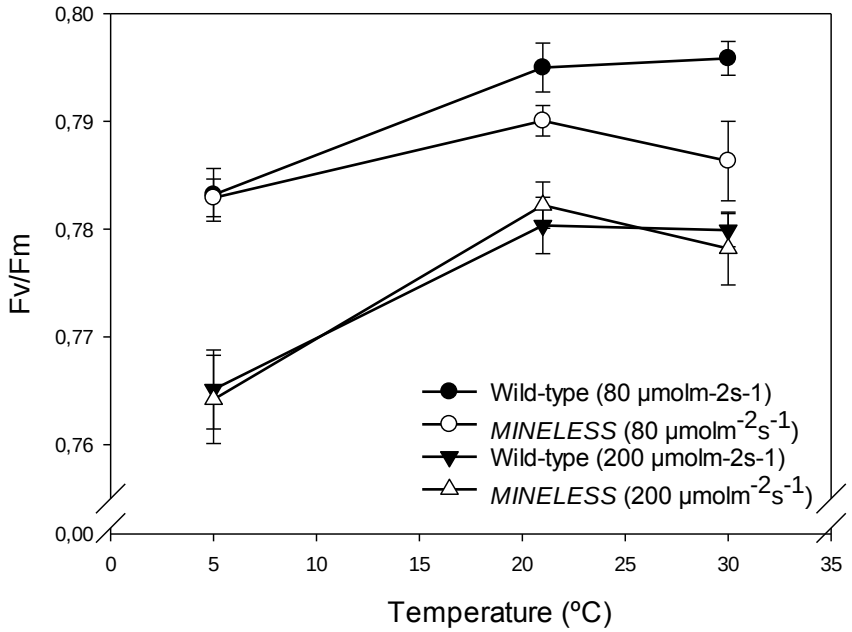

Figure S2. Fv/Fm (average) calculated from measurements obtained from two different growth periods (trial 1 and 2, with 6 independent biological replicas from each trial), in 11-12 day old cotyledons of *B. napus* seedlings of wild-type and *MINELESS* respectively. Black symbols represent wild-type and open symbols *MINELESS*. Circles represent 80 µmolm^{-2}s^{-1}, and triangles 200 µmolm^{-2}s^{-1}. n= 24. Error bars represent standard error.

7.2 Metabolites

Table S3. ANOVA table for detected metabolites with significant P values. All P values < 0.05 are marked with bold font. *P < 0.05 = '*', P < 0.01='**', P< 0.001= '***'.*

Compound	Genotype	Light	Temperature	Genotype: Light	Genotype: Temperature	Light: Temperature
Amino acid						
alanine	****0.009**	****0.007**	*****0**	0.758	***0.028**	0.57
amino acid(1)	*****0**	0.055	****0.001**	0.747	0.09	0.313
amino acid(2)	0.212	0.919	0.083	0.322	0.801	***0.027**
amino acid(3)	0.09	0.513	***0.02**	0.781	0.835	***0.017**
amino acid(4)	0.472	*****0**	*****0**	0.592	0.667	0.109
aspartic acid(5)	0.177	***0.033**	*****0**	0.465	0.884	0.226
glutamic acid	0.144	***0.013**	*****0**	0.866	0.846	***0.047**
glycine(1)	0.458	****0.005**	***0.018**	0.631	0.853	****0.008**
glycine(2)	0.555	****0.007**	0.826	0.197	0.563	0.491
pyroglutamic acid	0.805	0.906	0.331	0.874	0.983	***0.027**
serine(1)	0.446	0.79	***0.037**	0.141	0.746	0.246
serine(2)	0.404	****0.003**	*****0**	0.28	0.988	0.204
threonine	0.959	**0.001**	0.068	0.736	0.978	0.314
tryptophan	0.433	*****0**	*****0**	0.486	0.817	*****0**
tyrosine	0.563	*****0**	*****0**	0.229	0.903	***0.038**
valine (1)	0.873	0.907	**0.001**	0.892	0.762	0.602
valine (2)	0.144	*****0**	*****0**	0.312	0.313	****0.004**
Carboxylic acid						
2-methyl malic acid	*****0**	0.31	***0.027**	0.668	0.356	0.293
2-oxo-glutaric acid	0.109	0.292	*****0**	0.103	0.969	0.472
acid(1)	***0.019**	0.735	0.08	0.497	0.07	0.486
acid(2)	0.95	***0.04**	*****0**	0.846	0.904	***0.031**
acid(3)	****0.001**	*****0**	0.06	0.99	0.976	***0.039**
acid (4)	*****0**	***0.018**	0.427	0.184	0.913	0.709
acid/ A178005	0.978	***0.046**	0.306	0.739	0.462	0.091
ascorbic acid	0.602	0.769	0.527	0.836	****0.006**	0.675
citric acid	0.331	0.392	0.606	0.419	0.376	****0.003**
dehydroascorbic acid deriv.	0.835	0.297	0.471	0.665	***0.016**	0.673
fumaric acid	0.386	****0.001**	0.96	0.597	0.578	0.123
glycolic acid	0.643	***0.019**	0.54	0.146	0.795	0.644
isocitric acid	0.399	0.333	***0.022**	0.315	0.095	*****0**
maleic acid	***0.014**	****0.001**	0.122	**0.002**	0.116	0.125
malonic acid	0.066	*****0**	****0.004**	0.047	0.342	***0.017**
pyruvic acid	0.634	***0.022**	0.709	0.434	0.772	0.057
quinic acid	0.795	***0.011**	0.621	0.348	0.433	0.464
succinic acid	0.726	***0.014**	0.648	0.755	0.243	0.332
sugar acid(1)	0.855	****0.006**	****0.008**	0.44	0.681	0.297
sugar acid(2)	*****0**	0.489	0.798	0.449	0.821	0.381
sugar acid(3)	0.001	0.414	0.873	0.457	0.439	0.063

Alcohol						
1,2-ethanediol	0.97	0.584	****0.003**	0.4	0.727	0.628
galactinol	0.507	***0.032**	*****0**	0.2	0.93	***0.033**
glycerol	0.992	0.845	****0.007**	0.477	0.553	0.319
myo-inositol	0.313	0.403	***0.016**	0.332	0.115	0.061
pentitol	***0.028**	***0.013**	0.481	0.573	0.089	0.738
Amine						
amine	0.384	0.36	*****0**	0.399	0.303	0.06
aromatic	***0.014**	0.259	0.449	0.874	0.859	0.792
ethanolamine	0.2	0.319	****0.003**	0.15	0.503	0.179
amd						
uric acid	0.725	0.888	0.133	0.06	0.979	****0.024**
Aromatic						
(E)-5-caffeoylquinic acid	0.853	0.319	*****0**	0.153	0.483	***0.015**
(Z)-3-caffeoylquinic acid	0.461	0.295	****0.007**	0.31	0.311	***0.018**
4-hydroxybenzoic acid	0.718	***0.039**	0.926	0.382	0.45	***0.014**
aromatic(1)	***0.048**	0.105	0.188	0.491	0.304	0.092
aromatic(2)	0.076	0.444	****0.006**	0.166	0.28	0.058
benzylglucopyranoside	0.108	0.605	****0.007**	**0.021**	0.315	0.21
chlorogenic acid	0.889	0.145	****0.012**	0.096	0.677	0.063
F6P	0.101	0.266	*****0**	0.105	0.781	0.277
feruloylquinic acid(1)	0.594	0.354	****0.002**	0.405	0.471	0.057
feruloylquinic acid(2)	0.315	0.347	*****0**	0.135	0.591	0.052
feruloylquinic acid(2)	0.3	0.395	*****0**	0.065	0.543	0.097
sinapoyl malate	0.717	0.424	***0.044**	0.136	0.323	****0.009**
sinapoyl malate	0.982	0.487	***0.036**	0.095	0.379	***0.012**
sinapoylglucoside(1)	0.499	0.304	****0.004**	0.343	0.394	***0.038**
spermidine	0.141	0.349	***0.044**	0.332	0.932	0.405
Disaccharide						
disaccharide(1)	0.481	0.7	*****0**	0.541	0.789	0.848
disaccharide(2)	0.315	0.616	***0.033**	0.474	0.275	0.897
disaccharide(3)	0.105	0.222	*****0**	0.423	0.384	0.597
disaccharide(4)	0.732	0.154	*****0**	0.255	0.114	0.08
disaccharide(5)	0.086	****0.001**	*****0**	0.233	0.291	***0.025**
disaccharide(6)	****0.001**	0.216	0.424	0.464	0.164	0.994
disaccharide(7)	***0.016**	0.066	0.646	0.216	0.816	0.326
disaccharide(8)	***0.032**	0.54	0.357	0.08	***0.049**	***0.044**
sugar(12)	0.286	0.542	*****0**	0.164	0.297	****0.007**
Fatty acid						
(E)-sinapic acid	***0.042**	0.4	0.449	0.711	0.759	0.595
C7:0	0.826	0.367	***0.015**	***0.022**	0.896	0.174
Glucero sugar						
glycero sugar	***0.024**	****0.003**	0.277	0.35	0.825	0.991
glycero-guloheptose	0.135	0.447	***0.019**	0.186	0.309	0.406
Heptose						
seduheptulose oxime	0.874	***0.027**	****0.009**	0.648	0.147	0.711
Hexose						

glucose(1)	**0.005	***0	***0	0.265	0.978	**0.004
fructose(1)	0.15	*0.029	**0.008	0.457	0.611	0.905
fructose(2)	0.255	0.877	0.1	0.223	*0.019	0.351
fructose(3)	**0.008	***0	***0	0.141	0.919	**0.008
galactose oxime	0.426	**0.007	**0.001	0.975	*0.026	0.246
glucose(2)	0.355	***0	***0	0.068	0.635	0.104
sugar(1)	0.8	***0	**0.002	0.368	0.923	0.196
sugar(2)	0.682	*0.035	0.193	0.188	0.654	0.781
sugar(3)	*0.033	0.23	0.689	0.034	0.453	0.338
sugar(4)	***0	**0.002	0.371	0.035	0.642	0.208
sugar(5)	0.395	**0.001	***0	0.363	0.254	*0.014
sugar(6)	0.116	**0.002	***0	0.254	0.58	0.107
sugar(7)	***0	0.658	0.826	0.355	0.231	0.058
sugar(8)	0.4	0.501	0.079	*0.031	0.209	0.162
sugar10)	**0.006	0.332	0.476	0.052	0.334	0.141
sugar(11)	0.872	0.471	**0.003	0.226	0.28	*0.047
sugar, O-methyl / A178003	0.527	*0.01	***0	0.329	0.198	0.115
sugar/ A177004	***0	0.383	0.914	0.838	0.997	0.991
hexose	0.205	*0.013	0.577	*0.025	0.988	0.363
Indole						
indole	0.874	0.71	0.104	0.26	0.265	*0.022
indole-3-acetonitrile	***0	0.209	0.713	0.072	*0.023	0.469
Phosphate						
F6P	0.309	*0.016	***0	0.101	0.078	*0.01
G6P(1)	0.13	*0.014	***0	0.216	*0.043	*0.011
G6P(2)	0.158	0.231	***0	0.059	0.622	*0.043
G6P(3)	0.379	0.191	***0	*0.035	0.416	*0.042
Glycerol phospho glycerol	*0.01	**0.005	0.532	0.053	0.614	*0.017
MI1P	***0	0.051	*0.04	0.853	0.365	0.086
phosphate	0.841	**0.006	***0	0.16	0.192	**0.006
phosphoric acid	0.025	0.162	0.851	0.686	0.295	0.077
sugar(9)	0.909	0.341	***0	0.92	0.525	0.504
sugar phosphate	0.78	***0	***0	*0.021	*0.03	*0.022
Purine						
adenine	0.16	*0.036	*0.032	0.476	0.474	0.199
adenosine	0.907	0.445	*0.034	0.285	0.703	0.302
Siloxane						
polysiloxane	0.053	*0.028	0.49	0.581	0.892	0.325
Trisaccharide						
Raffinose	0.07	**0.003	***0	*0.013	0.344	0.489
Trisaccharide	0.322	*0.044	***0	0.2	0.902	0.258
NA						
A148003	0.392	***0	***0	0.624	0.972	*0.04
A195012	0.181	0.182	**0.002	0.955	0.94	**0.004
A199008	0.228	0.27	**0.002	0.475	0.791	*0.01
A238003	0.182	0.78	**0.015	0.198	0.831	0.494
A246001	0.519	0.927	0.52	0.23	0.389	**0.004

A259001	0.344	0.873	***0.046**	0.665	0.537	0.067
A279006	****0.009**	****0.007**	****0.001**	0.214	0.871	****0.001**
A286005	0.088	0.514	*****0**	***0.031**	0.289	****0.002**

Table S4: (µg/g FW) in 11-12 days old *B. napus* seedlings grown in all six combinations of the following of light intensities; 80 µ molm^{-2}s^{-1} and 200 µ molm^{-2}s^{-1}, and temperatures; 5 °C, 21 °C, and 30 °C

Temperature (ºC):	**5**				**21**				**30**			
Light (µmolm-2s-1):	**80**		**200**		**80**		**200**		**80**		**200**	
Wild-type/ *MINELESS*:	**Wild-type**	***MINELESS***	**Wild-type**	***MINELESS***	**Wild-type**	***MINELESS***	**Wild-type**	***MINELESS***	**Wild-type**	***MINELESS***	**Wild-type**	***MINELESS***
Disaccharides												
disaccharide(1)	454,40	280,77	326,27	386,54	**9,89**	11,14	22,84	17,61	4,70	4,49	8,90	90,21
disaccharide(2)	0,03	35,73	21,36	30,40	**2,00**	3,02	5,65	3,08	1,22	1,15	2,41	1,66
disaccharide(3)	177,83	145,57	241,09	161,00	**1,49**	1,61	1,21	2,61	2,29	1,63	1,05	44,52
disaccharide(4)	1,12	1,26	1,39	1,29	**0,44**	0,74	0,95	1,01	0,80	0,58	0,37	0,74
sugar(12)	0,97	1,60	1,09	1,08	**0,44**	0,62	0,95	1,12	0,48	0,52	0,26	0,52
disaccharide(5)	1,06	0,93	1,80	1,37	**0,16**	0,20	0,32	0,36	0,15	0,18	0,10	0,41
disaccharide(6)	1,72	2,15	0,71	1,46	**0,80**	3,93	0,34	2,61	0,44	5,43	3,36	0,97
disaccharide(7)	0,41	1,34	0,65	0,43	**0,40**	1,18	0,39	1,10	0,94	1,85	0,69	0,37
disaccharide(8)	0,81	3,08	1,14	1,90	**4,17**	2,39	1,40	1,86	1,60	1,70	10,44	0,66
Heptose												
seduheptulose oxime	4,58	4,41	4,33	6,54	**4,31**	4,16	5,71	5,42	3,46	2,95	2,80	4,52
Hexose												
sugar/ A177004	14,67	33,25	17,68	25,06	**16,99**	29,30	14,05	27,58	17,79	26,89	29,20	12,56
sugar, O-methyl / A178003	7,79	7,46	5,94	9,15	**2,77**	3,01	5,50	4,49	3,32	2,32	4,34	4,37
fructose(1)	1,32	0,46	2,26	0,84	**1,98**	2,20	3,48	2,68	1,00	0,61	1,71	2,12
fructose(2)	3,35	70,59	1,53	34,36	**1,43**	1,27	31,50	0,55	1,38	1,58	1,12	2,70
fructose(3)	179,58	112,13	224,82	198,71	**11,94**	10,59	209,49	114,86	0,28	0,28	17,56	81,85
hexose	10,88	13,40	19,65	10,33	**5,99**	8,45	22,94	13,22	6,72	8,66	9,56	16,57
glucose(1)	494,27	177,06	362,42	354,93	**42,60**	32,16	625,30	288,77	0,02	0,02	0,06	285,70
sugar(1)	2,31	2,39	3,50	3,62	**0,78**	1,09	3,81	3,26	0,33	0,66	1,21	2,16
glucose(2)	40,45	48,96	82,32	37,18	**53,32**	66,03	124,97	104,54	26,60	24,45	60,30	57,35
sugar(2)	2,78	3,51	2,84	2,49	**3,23**	3,48	3,43	2,74	4,02	3,46	3,18	3,11
sugar(3)	0,03	0,04	0,04	0,04	**0,02**	0,07	0,04	0,04	0,03	0,09	0,03	0,04
sugar(4)	0,03	0,15	0,05	0,09	**0,07**	0,20	0,03	0,05	0,09	0,29	0,09	0,03
galactose oxime	6,57	7,89	6,21	9,80	**5,26**	5,59	8,43	7,72	4,78	4,07	4,53	6,22
sugar(5)	1,16	1,19	1,29	1,60	**0,41**	0,39	1,52	0,97	0,53	0,44	0,37	0,73
sugar(6)	0,90	0,88	1,04	1,05	**0,35**	0,33	1,00	0,65	0,41	0,30	0,33	0,63
sugar(7)	0,12	0,49	0,12	0,26	**0,09**	0,35	0,12	0,54	0,14	0,40	0,21	0,22
sugar(8)	1,11	1,38	1,15	1,12	**0,98**	1,18	1,42	1,06	1,15	0,98	0,64	1,04
sugar(10)	0,54	0,92	0,55	0,73	**0,43**	0,84	0,67	0,82	0,59	0,82	0,41	0,55
sugar(11)	1,59	1,93	1,61	1,89	**1,20**	1,45	2,02	1,76	1,31	1,23	0,79	1,25
Trisaccharides												
raffinose	0,46	0,50	0,58	0,54	**0,12**	0,12	0,39	0,15	0,04	0,04	0,04	0,19
trisaccharide	0,99	1,10	1,64	1,25	**0,04**	0,06	0,10	0,07	0,04	0,05	0,07	0,30

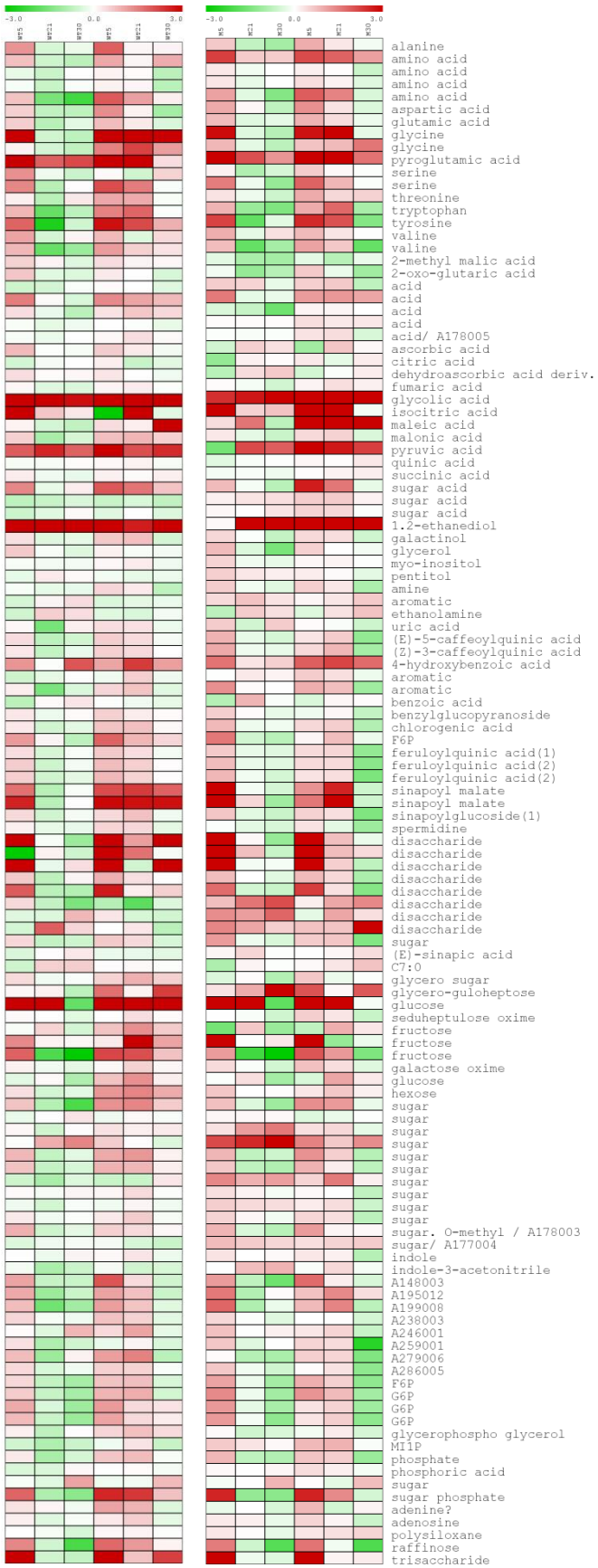

Figure S3. Metabolites from GC-MS of 11-12 days old wild-type and transgenic *MINELESS B. napus* seedlings grown in all six combinations of the following of light intensities; 80 µmolm^{-2}s^{-1} and 200 µmolm^{-2}s^{-1}, and temperatures; 5 ºC, 21 ºC, and 30. Left panel shows treatments done with wild-type plants, the right *MINELESS*. First three rows in both panels represent the non-stressed light condition at the three temperatures, and the three last the high stress treatments with the three temperatures