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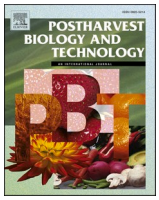

Far-red light during cultivation improves postharvest chilling tolerance in basil

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ABSTRACT

Basil (*Ocimum basilicum* L.) is a temperature sensitive plant and suffers from chilling injury (CI), especially during the postharvest storage. We investigated the effect of additional far-red light (FR) during cultivation at two temperatures on postharvest chilling tolerance. Basil was cultivated under red-white Light Emitting Diodes (LED) at 25 °C. During the last 3 weeks before harvest, plants were maintained at a high temperature (25 °C) or exposed to a low temperature (15 °C). Furthermore, plants were exposed to additional FR (180 μmol m^{-2} s^{-1}) for different durations (0, 1 or 3 weeks). After harvest, leaves were stored at 4 and 12 °C in darkness. Overall visual quality and maximum quantum yield of PS II (Fv/Fm) as indicators of chilling injury were monitored every third day for 15 d. Abscisic acid (ABA) and jasmonic acid (JA), carbohydrates, and antioxidants were measured at harvest and after 9 d of storage at 4 °C. Additional FR improved the chilling tolerance at both cultivation temperatures. Cultivation temperature had no effect on postharvest chilling tolerance. Hormone levels in basil leaves at harvest were not affected by FR. This indicates that ABA and JA are not involved in development of FR-induced chilling tolerance in basil. FR had no effect on the levels of antioxidants at harvest whereas the levels of soluble sugars and starch increased under additional FR. The positive effect of adding FR during cultivation on chilling tolerance in basil may be due to the increase in soluble sugars and starch.

1. Introduction

Basil (*Ocimum basilicum* L.) is of tropical origin and sensitive to temperatures below 10–12 °C during growth, transport or storage (Lange and Cameron, 1994), which results in chilling injury (CI). CI symptoms in basil are the development of dark necrotic spots on the leaf surfaces. Thus, basil cannot be transported or stored together with many other herbs and leafy greens which often occurs at about 2–7 °C. Low temperature induces the transition of the cell membrane lipid bilayer from a liquid to a solid gel phase, which leads to membrane malfunctioning and loss of membrane semi-permeability which finally is results in cell death (Raison and Orr, 1986). During CI the chloroplasts are the major organelle which is affected. CI results in a decrease in maximum quantum yield of photosystem II (PSII) also known as F_v/F_m (Hogewoning and Harbinson, 2007). CI does not occur homogenous in the leaves, therefore chlorophyll fluorescence imaging has been shown to be a good measurement for assessing the degree of chilling damage to the leaves (Hogewoning and Harbinson, 2007). Apart from the transition from liquid to gel phase of cell membranes, CI has been reported to cause oxidative stress with an increase in reactive oxygen species (ROS) (Mittler, 2002). However, in basil ROS have not been consistently shown to increase during cold storage (Larsen et al., 2022). Several methods have been attempted to improve postharvest quality in basil. Ethylene has been associated with development of CI (Hassan and Mahfouz, 2010). Postharvest application of 1-MCP blocks the ethylene sensitivity and was able to lessen CI (Berry et al., 2010; Hassan and Mahfouz, 2010). Hot air treatments, where application of one stress factor should make more resistant to another stress (i.e. low temperature) were successfully applied to improve chilling tolerance (Aharoni et al., 2010). Pre-harvest light treatments with high light intensity have also been

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investigated. Although the high light considerably increased the antioxidant content, it did not improve chilling tolerance in basil (Larsen et al., 2022). Light spectrum plays a role in the cold acclimation of many plants of temperate origin, particularly the ratio between red (R, 600–700 nm) and far-red (FR,700–800 nm) has been found to be important (Franklin and Whitelam, 2007; Wang et al., 2016). In nature increased amount of FR light (i.e. resulting in a R:FR ratio<1) is a signal for plant to cold acclimate along with the induction of the C-REPEAT BINDING FACTOR (CBF) pathway (Linkosalo and Lechowicz, 2006; Franklin and Whitelam, 2007). R and FR light are sensed by the plant photoreceptors from the phytochrome family (phyA to phyE). The biologically inactive form (Pr) of phytochromes absorbs red light and converts to the physiologically active (Pfr) form which absorbs FR through chromophore isomerization when red light is absorbed. The light activated phytochromes then translocate into the nucleus where they can interact with the Phytochrome Interacting Factors (PIFs) where they control a wide range of processes (Franklin and Whitelam, 2004). The phytochrome mediated responses are induced by the transition of Pr to Pfr. The degree and rate at which the response occurs are defined by the Pr:Pfr ratio which is mainly determined by the R:FR ratio (Duek and Fankhauser, 2005). This can also be described as the phytochrome photostationary state (PSS). The definition of PSS is the ratio of the active Pfr to the total amount (Pfr+Pr) of phytochrome at equilibrium (Sager et al., 1988; Both et al., 2017).

FR light improved the chilling tolerance of tomato leaves through induction of *CBF* gene expression and the accumulation of abscisic acid (ABA) and jasmonic acid (JA) (Wang et al., 2016). CI in tomato fruit was also improved when cultivated with additional FR (Affandi et al., 2020). The objective of this study was to improve chilling tolerance of basil through additional FR and lowered temperature during cultivation.

2. Materials and methods

2.1. Experimental set-up

Basil (*Ocimum basilicum* L.) cv. Emily (Enza Zaden, the Netherlands) were grown in a climate chamber, in a vertical farming set-up previously described by Larsen et al. (2020). The individual growth compartments had a size of 0.8 × 1.3 × 1 m (w x l x h) and were separated from each other by with reflective white plastic. The seeds were sown as individual seeds in polystyrene trays with 240 stone wool plugs (Grodan Rockwool B.V., The Netherlands). Plants were cultivated under 150 ± 5 μmol m^{-2} s^{-1} red-white LED light (GreenPower LED production module 120 cm, DeepRedWhite, Philips Eindhoven, the Netherlands). The red-white LED light consisted of 9% blue (B) (400–500 nm) 19% green (G) (500–600 nm) and 70% red (R) (600–700 nm) and 1% far-red (FR) (700–800 nm) light as in the study of Larsen et al. (2020). The light spectrum was measured with a spectroradiometer (SS-110; Apogee Instruments, Logan, UT, United States). Day length was 18 h. The temperature during the day and night was 25 ± 1 °C the relative humidity was set at 75 ± 10%, logged with Keytag dataloggers (KTL-508, Keytag, the Netherlands). The CO_2 level was at ambient concentrations. The plants with the most similar morphology were transplanted after 10 d to 7.5 × 7.5 × 6.5 cm stone wool blocks (Grodan Rockwool B.V., The Netherlands), after which the planting density was 123 plants m^{-2}. Throughout the growth plants were well-watered with an ebb and flood system based on plant needs and growth stage with a nutrient solution consisting of NO_3^- 8.5 mM, SO_4^{2-} 3.9 mM, HPO_4^{2-} 1.5 mM, NH_4^+ 1.5 mM, K^+ 5.5 mM, Ca^{2+} 4.0 mM, Mg^{2+} 1.5 mM, Cl^- 0.2 mM, Fe^{3+}/Fe^{2+} 30 μM, Mn^{2+} 5 μM, Zn^{2+} 5 μM, $H_2BO_3^-$ 35 μM, Cu^+/Cu^{2+} 1 μM, MoO_4^{2-} 1 μM nutrition of pH 5.7 with EC 1.7 dS m^{-1} before transplanting as described by Larsen et al. (2020). After transplanting the nutrient solution had an EC of 2.3 dS m^{-1} and the concentration of nutrients were adjusted accordingly.

2.2. Treatments

During the last 3 weeks before harvest, basil plants were cultivated at either a high (25 °C) or low temperature (15 °C). Plants cultivated at high temperature received before harvest either no, 1 or 3 weeks additional FR. Plants cultivated at low temperature received before harvest either no or 3 weeks additional FR. The light intensity of FR was 180 μmol m^{-2} s^{-1} (GreenPower Production module, 120 cm, Far Red, Philips Eindhoven, the Netherlands) (Table 1). The number of degree-days was kept the same between the high and low temperature cultivated plants. The low temperature plants were grown for a total of 5 weeks after transplant of which 2 weeks were at 25 °C and thereafter for 3 weeks at 15 °C; the high temp plants were grown for 3 weeks after transplant at 25 °C. The degree-days were calculated with 10 °C as base temperature. It was estimated that below 10 °C no growth activity would occur. For the low temperature treatments, the shift in temperature from 25 °C to 15 °C was instant.

Morphological data and growth parameters for the far-red at high temperature have been reported previously by Larsen et al. (2020). These data include plant height, leaf area, fresh and dry mass at the day of harvest.

2.3. Postharvest sampling

For postharvest storage, leaves were stored in plastic boxes, 16×11×6 cm. In each box leaves from 2 plants were stored. The leaves from each plant included three leaf pairs from excluding the oldest and youngest (underdeveloped) pair. The boxes were mounted with 2 pieces of wet filter paper in the bottom, plastic on top of the paper to avoid direct contact with the leaves and a plastic separator between the leaves from the two plants. In the clear lids 9 holes with a 1 mm syringe needle were made (Witkowska and Woltering, 2010; Min et al., 2021). Due to the wet filter paper a high humidity was maintained in the boxes; the holes in the lid prevented any built up of ethylene and CO_2 and depletion of O_2. The boxes were randomized and stored in darkness at 4 or 12 °C. To monitor the temperature and relative humidity in the boxes keytag dataloggers (KTL-508, Keytag, the Netherlands) were used. The relative humidity and temperature were within 90–100% RH and ± 1 °C of the temperature (4 or 12 °C) in the boxes.

The sampling and measurements were carried out on day 0 (at harvest) and 3, 6, 9, 12 and 15 d after harvest. During each sampling day, leaves from four plants per block per light treatment were sampled (i.e., two storage boxes each containing leaves from two plants). The chilling injury was determined by an overall visual quality (OVQ) score and measurement of maximum quantum yield of dark-adapted leaves of PSII (F_v/F_m). Following the measurements, the leaves from 4 °C were frozen in liquid nitrogen and ground with an IKA-A 11 basic analytical mill (im-

Table 1
Overview of treatments. Treatments consisted of different temperature during the last 3 weeks before harvest in combinations with different durations of far-red light during the last weeks before harvest. Furthermore, the table provides photosynthetic photon flux density (PPFD) from 400 to 700 nm, photon flux density of FR (700–800 nm) Photon flux density (PFD) from 400 to 800 nm, R:FR ratio and PSS value. R:FR was calculated as red (600–700 nm): far-red (700–800 nm). Phytochrome photostationary state (PSS) were calculated according to Sager et al. (1988).

Temperature during the last 3 weeks (°C)	Duration of FR (week)	PPFD (μmol m^{-2} s^{-1})	Far-red (μmol m^{-2} s^{-1})	PFD (μmol m^{-2} s^{-1})	R: FR	PSS
25	-	150	2	152	61.7	0.88
25	1	150	180	330	0.6	0.62
25	3	150	180	330	0.6	0.62
15	-	150	2	152	61.7	0.88
15	3	150	180	330	0.6	0.62

lab, Belgium). Before further analysis, samples were stored at − 80 °C. Metabolites were analyzed on d 0, d 6 and 9.

2.4. Overall visual quality

Overall visual quality (OVQ) was evaluated according to Larsen et al. (2022). Based on visual symptoms a score was given from 1 to 8, with 1 indicating the worst quality and 8 the best. Symptoms which reduced the score included dark spots and discoloration, fungal appearance, degree of crispness, degree of wilting, leaf shininess and presence of characteristic curved leaf shape (Table S1). The decrease in overall visual quality was calculated as a percentage of the initial score. Data was expressed as percentage of the initial score.

2.5. PSII efficiency F_v/F_m

Fv/Fm was measured as described previously by Larsen et al. (2022). Per stored box one leaf from the upper leaf-pair and one leaf from the middle leaf pair of each plant were measured. Leaves were dark adapted at 20 °C for 20 min and chlorophyll fluorescence was measured using a PSI closed Fluorcam 800-C chlorophyll fluorescence imaging system (PSI, Czech Republic). Fluorcam software Version 7 was used to operate the fluorcam and analyze the obtained images, following the method of (Hogewoning and Harbinson, 2007).

2.6. Carbohydrates

Carbohydrates were measured according to Larsen et al. (2022). Carbohydrates were extracted from 0.300 ± 0.030 g frozen ground basil leaves with 5 mL of 85% ethanol for 20 min at 80 °C in a shaking water bath. For further analysis of carbohydrates 1 mL of the supernatant was used and the pellet was stored at − 20 °C for starch analysis. One milliliter of the supernatant was dried in a vacuum centrifuge (Savant SpeedVac SPD2010, Thermo Fisher Scientific) at 50 °C and 5.1 mbar for 120 min

Samples were re-suspended in 2 mL of 0.01 N hydrochloric acid, sonicated for 10 min (Branson 2800). Amino acids other amino compounds were removed by trapping on a SPE column (UCT CLEAN-UP BCX columns, 100 mg/1 mL), eluted with 0.01 N hydrochloric acid.

Glucose, fructose and sucrose were quantified using High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD; Dionex ICS5000, Thermo Fisher Scientific), with a CarboPac1 column (250 ×2 mm) eluted with 100 mM NaOH at a flow rate of 0.25 mL min^{-1} at 25 °C. Data was expressed on the base of dry weight (DW) as g Kg^{-1}.

For starch determination the pellet was washed with 80% ethanol three times and dried for 20 min in a vacuum centrifuge at 50 °C and 5.1 mbar. The dried pellet was resuspended in 2 mL 1 g L^{-1} thermostable alpha-amylase (SERVA Electrophoresis GmbH) in MilliQ water and incubated for 30 min at 90 °C. After which 1 mL of 0.5 g L^{-1} amyloglucosidase (Sigma 10115) in 50 mM citrate buffer (pH 4.6) was added and samples were incubated at 60 °C for 15 min. Glucose was quantified using HPEAC-PAD as described above. Data was expressed on the base of dry weight (DW) as g Kg^{-1}.

As described by Larsen et al. (2022), a conversion factor for each sample was made to convert to dry weight from fresh weight; 0.100 ± 0.020 g of fresh material for each sample were weighed into a reaction tube and vacuum dried for 120 min in a vacuum centrifuge (Savant SpeedVac SPD2010, Thermo Fisher Scientific) at 50 °C and 5.1 mbar. Data was expressed on the base of dry weight as g Kg^{-1}.

2.7. Total ascorbic acid

Total ascorbic acid was measured as described previously by Larsen et al. (2022). Ascorbic acid was extracted from 0.200 ± 0.020 g frozen ground basil leaves with 1 mL of 3.3% meta-phosphoric acid (MPA) in an ultrasonic bath (BRANSON 2800) at 0 °C for 10 min in darkness. Samples were centrifuged at 21.100 RCF (Sorvall Legend Micro 21 R, Thermo Fisher Scientific) at 4 °C for 10 min. The supernatant was filtered through a 0.45 μm cellulose filter into an HPLC vial for analysis of ascorbic acid (AsA). For total AsA analysis 100 μL of the extract was mixed with 50 μL of 5 mM dithiothreitol in 400 mM Tris base and after 15 min 50 μL 8.5% o-phosphoric acid was added. AsA was quantified using a HPLC consisting of a GS50 pump (Dionex), a 340 S UV–VIS detector (Dionex) and a MIDAS autosampler (Spark Holland) equipped with a ProntoSIL 120–3 C18 AQ, 250×3mm column (Knauer). The column was eluted with 400 μL L^{-1} H_3PO_4 + 2.5 mL L^{-1} MeOH + 0.1 mM EDTA in H_2O followed by a wash step with 30% acetonitrile in H_2O at a flow rate of 0.35 mL min^{-1}. AsA was detected at 243 nm. The TAsA amount was calculated as the sum of the AsA directly measured and the AsA measured following conversion of dehydroascorbic acid (DHA) to AsA. Data was expressed on the base of dry weight as g Kg^{-1}.

2.8. Rosmarinic acid and chicoric acid

The most abundant antioxidants in basil rosmarinic and chicoric acid were measured as described previously by Larsen et al. (2022). Rosmarinic and chicoric acids were extracted at room temperature from 0.400 ± 0.020 g frozen ground leaves with 2.5 mL of acetonitrile with 2.5% formic acid. Samples were extracted for 15 min in an ultrasonic bath (Branson 2800) and centrifuged at 10000 RCF for 15 min at 4 °C (Universal 320 R, Hettich). The supernatant was filtered through a 0.45 μm cellulose syringe filter into a HPLC vial. Rosmarinic acid and Chicoric acid were analyzed according to the method of Kwee and Niemeyer (2011), with modifications on a HPLC (Waters) with a UV dual wavelength detector and autosampler, and a Vydac 201TP54 column (C18, 5 μm, 300 Å, 4.6 mm × 250 mm). The compounds were eluted with 2.5% formic acid in H_2O (mobile phase A) and acetonitrile (mobile phase B) with a linear gradient of: 85% A, 0.0 min; 75% A, 6.0 min; 0% A, 8.5 min; [0% A, 9.0 min; 85% A, 11.5 min; 85% A, 14.0 min]. Phenolic acids were detected at 330 nm. Data was expressed on the base of dry weight as g Kg^{-1}.

2.9. Hormones

Hormone content (jasmonic acid and abscisic acid) was measured according to Gühl et al. (2021). Briefly, 0.010 g of ground frozen basil leaves were extracted with 1 mL of 100% methanol (MeOH) with internal standards 1 mL of 100% methanol (MeOH) containing stable isotope-labeled internal standards (IS, Supplemental Table S2). Internal standards were used at an end concentration of concentration of 100 nM per compound per sample. All solvents were evaporated in a speed vacuum system (SPD121P, ThermoSavant, Hastings, UK) at RT and the residue stored at − 20 °C until further analysis. Samples were resuspended in 100 μL of acetonitrile/water (0.1% formic acid) (20:80, v/v), and filtered through a 0.45 mm Minisart SRP4 filter (Sartorius, Goettingen, Germany). Analyses of plant hormones was performed by comparing retention times and mass transitions with those of unlabeled standards (Supplemental Table S2) using a Waters XevoTQs mass spectrometer equipped with an electrospray ionization source coupled to an Acquity UPLC system (Waters, Milford, USA) as previously described by Schiessl et al. (2019) and Gühl et al. (2021). Chromatographic separations were conducted on an Acquity UPLC BEH C18 column (100 mm, 2.1 mm, 1.7 mm; Waters, USA) by applying an acetonitrile/water (0.1% formic acid) gradient. The column was operated at 40 °C with a flow rate of 0.25 mL min^{-1}. The acetonitrile/water (0.1% formic acid) gradient started from 20% (v/v) acetonitrile, increasing to 70% (v/v) acetonitrile in 17 min. The sample injection volume was 3 μL. Cone and desolvation gas flows were set to 150 and 1000 L h^{-1}, respectively. The capillary voltage was set at 3.5 kV, the source temperature at 150 °C, and the desolvation temperature at 550 °C (Waters, Milford, USA). Argon was used for fragmentation by

collision-induced dissociation. Multiple reaction monitoring (MRM) was used for quantification (Gühl et al., 2021). To determine sample concentrations, a 10-point calibration curve was constructed for each compound ranging from 1 μM to 190 pM and each dilution also contained a known amount of an appropriate deuterium-labeled internal standard.

2.10. Statistical set up and analysis

The experiment was carried out in a complete randomized block design. Each light treatment was repeated in separate compartments (each compartment was considered a block). Due to treatments with different temperature the high and low temperature treatments were sown with 2 weeks between them and treatments were done sequentially. First the high temperature plants were harvested after which the temperature was lowered to initiate treatment of the low temperature treated plants. The border plants were not used in the analysis. At harvest, in each block, 4 replicate plants of each treatment were sampled for chemical analyses. The remaining plants were prepared for postharvest storage. The leaves from 2 plants, were packed together in one plastic box (as described above) and the boxes were stored at 4 and 12 °C. At each postharvest sampling point, 2 boxes (leaves from 4 plants) per block were taken from the storage for visual observation and fluorescence measurements (4 replicate plants). For chemical analyses the stored leaves at 4 °C were measured. For the chemical analysis in each block the leaves from 4 plants were analyzed as a pooled sample. Each mean is based 2 blocks x 4 replicate plants.

All data was analyzed with Genstat (VSN International, 19th Edition). For all parameters the assumptions of homogeneity and normality of the residuals were tested with Bartlett's test and Shapiro-Wilk test respectively. If data did not follow these assumptions, it was transformed with the natural logarithm. Subsequently data was analyzed by a two-way ANOVA for each time point and individual storage temperature. The posthoc test Fishers protected LSD was conducted with a probability level of $\alpha = 10\%$ because the experiment had two blocks.

3. Results

3.1. Changes in hormones and metabolites

Hormone content (abscisic acid and jasmonic acid) and metabolite content (soluble sugars and starch, antioxidants: rosmarinic acid, chicoric acid and total ascorbic acid), and volatile organic compounds (VOCs) were measured at harvest and at d 6 and 9 of storage at 4 °C. The two time points (d 6 and d 9 of storage) were averaged as the values between d 6 and d 9 did not differ (data not shown).

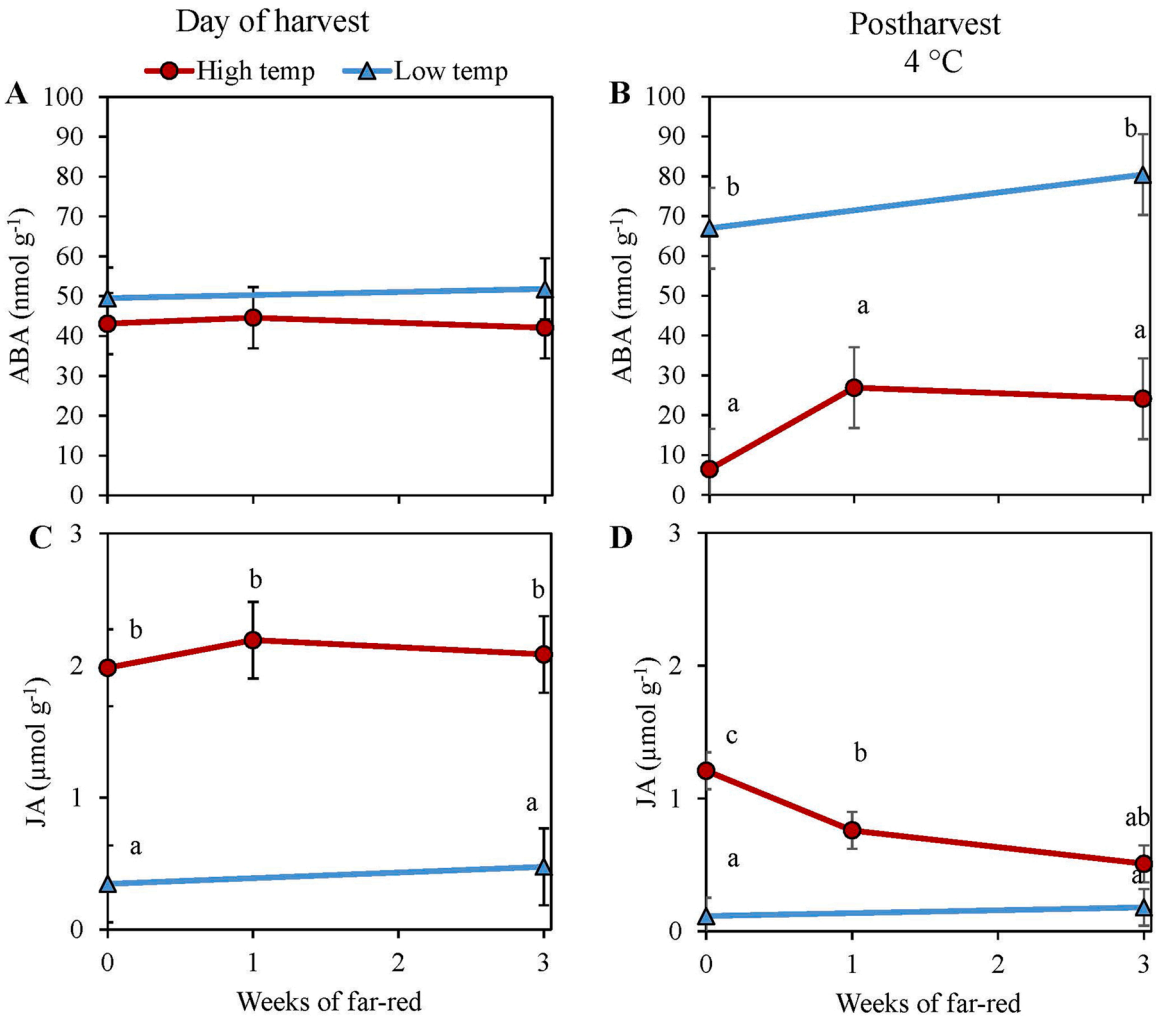

Fig. 1. The content of abscisic acid (ABA) (A,B) and jasmonic acid (JA) (C,D) in basil cv. Emily at harvest and during postharvest storage. Plants were grown under different far-red and temperature treatments i.e. at a high temperature (25 °C) and no, 1 or 3 weeks additional FR (circle, red) or at a low temperature (15 °C) and no or 3 weeks FR (triangle, blue). During postharvest storage leaves were stored at 4 °C for 6 and 9 d and the average values are shown. Each data point is a mean of 2 blocks (n = 2) and 4 replicate plants from each block. The error bars are errors of means. If letters are present, they indicate significant differences among all treatments at harvest or during postharvest storage ($\alpha = 10\%$).

The ABA content at harvest was not affected by the cultivation temperature nor by the addition of FR (Fig. 1A). ABA content in low temperature cultivated plants showed a slight increase during subsequent cold storage; ABA content in high temperature cultivated plants showed a slight decrease during subsequent cold storage. This effect was independent of the FR application (Fig. 1B).

The JA content at harvest was up to five-fold higher in high temperature cultivated plants than in low temperature cultivated plants (Fig. 1C). The JA content at harvest was not affected by FR application. After 9 d of cold storage, the JA content had decreased up to four-fold compared to the content at harvest. During postharvest storage the JA content decreased with 58% in high temperature cultivated plants that had received FR whereas no decrease was observed in the low temperature cultivated plants (Fig. 1D).

Soluble sugars and starch content at harvest increased in response to FR and to the low temperature. At both high and low temperature, the addition of FR resulted in a doubling of the soluble sugar content (Fig. 2A) compared to the no FR treatment. At low temperature FR increased the starch content three-fold, whereas FR had no effect on the starch content in high temperature cultivated plants (Fig. 2C). During postharvest storage at 4 ºC the content of soluble sugars (Fig. 2B) increased two- and five-fold, respectively, for the high and low temperature compared to the levels at harvest (Fig. 2A,C). The opposite occurred for the content of starch (Fig. 2D) which decreased with 45 % and 60 %, respectively, for the high and low temperature compared to the levels at harvest (Fig. 2A,C).

The increase in carbohydrate status at harvest in plants from low temperature cultivation and additional FR was reflected in considerable higher carbohydrate (in particular soluble sugar) levels during the postharvest storage.

Cultivation at low temperature increased the levels of rosmarinic acid at harvest (Fig. 3A). There was no effect on chicoric and total ascorbic acid (Fig. 3C,E). At both cultivation temperatures, there was no clear effect of FR on the levels of these antioxidants at harvest. A trend was observed towards lower levels of rosmarinic and chicoric acids at the longer FR applications. But the trend was only statistically significant for the rosmarinic acid level in low temperature cultivated plants. After 6–9 d (data were averaged) of storage at 4 °C, rosmarinic and chicoric acids levels were generally higher than the levels at harvest, irrespective of the cultivation temperature and FR treatments (Fig. 3B, D). The opposite was true for the total ascorbic acid levels that decreased during postharvest storage (Fig. 3F). There was little effect of FR application on the postharvest levels of these antioxidants. In general, the trends observed at harvest were still existing after storage. The levels

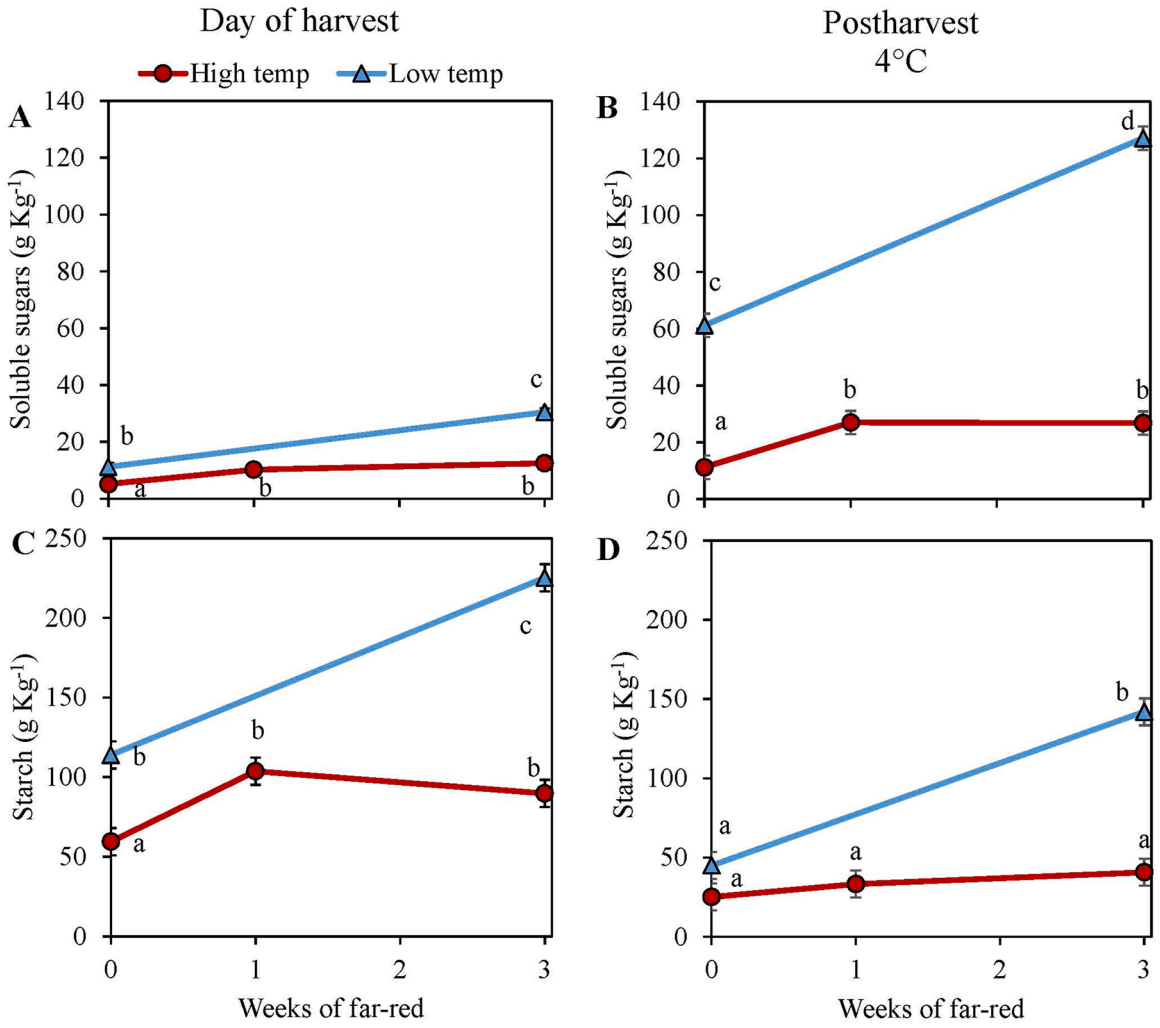

Fig. 2. Soluble sugars (sum of glucose, fructose and sucrose) and starch in basil cv. Emily at harvest (A,C) and in postharvest storage (B, D). Plants were grown under different far-red and temperature treatments i.e. at a high temperature (25 ºC) and no, 1 or 3 weeks additional FR (circle, red) or at a low temperature (15 ºC) and no or 3 weeks FR (triangle, blue). During postharvest storage leaves were stored at 4 °C for 6 and 9 d and the average values are shown. A,B. Soluble sugars, C,D. Starch. The data are given per gram dry weight in the leaves. Each data point is a mean of 2 blocks (n = 2) and 4 replicate plants from each block. The error bars are errors of means. If letters are present, they indicate significant differences among all treatments at harvest or during postharvest storage (α = 10%).

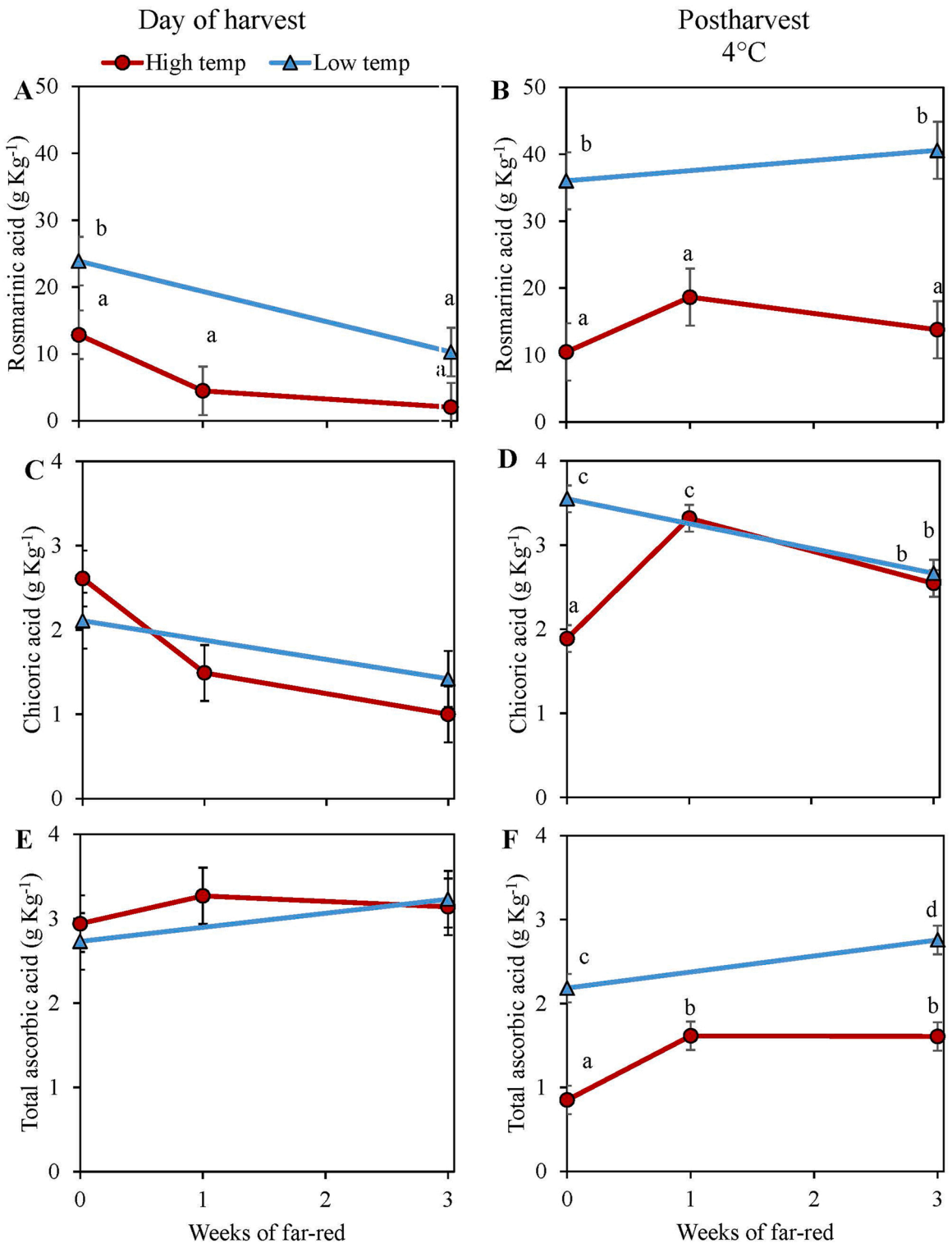

Fig. 3. Antioxidants in basil cv. Emily at harvest (A,C,E) and in postharvest storage (B,D,F). Plants were grown under different far-red and temperature treatments i. e. at a high temperature (25 °C) and no, 1 or 3 weeks additional FR (circle, red) or at a low temperature (15 °C) and no or 3 weeks FR (triangle, blue). During postharvest storage leaves were stored at 4 °C for 6 and 9 d and the average values are shown. A,B. Rosmarinic acid, C,D. Chicoric acid, E,F Total ascorbic acid. The data are given per gram dry weight in the leaves. Each data point is a mean of 2 blocks (n = 2) and 4 replicate plants from each block. The error bars are errors of means. If letters are present, they indicate significant differences among all treatments at harvest or during postharvest storage (α = 10%).

of the main volatile organic compounds (VOCs) in the cv. Emily at harvest were not affected by cultivation temperature nor by the application of FR (Fig. S1). During postharvest storage at 4 °C, the content of volatiles did not show a significant change compared to the at harvest content (Fig. S1).

3.2. Changes in chilling injury parameters during postharvest storage in response to FR and temperature

During postharvest storage at 4 and 12 °C overall visual quality (OVQ) and maximum quantum yield of PSII of dark-adapted leaves (F_v/

F_m) as a marker for chilling injury were measured. Low temperature during cultivation resulted in a lower overall visual quality (OVQ) at harvest due to yellowing of the youngest leaves (Fig. 4). OVQ of leaves decreased faster during storage at 4 ºC than at 12 °C as the leaves showed clear signs of chilling injury at 4 ºC (i.e. dark necrotic spots). Both at 4 and 12 °C storage, the decrease in OVQ over time was not affected by cultivation temperature.

Additional FR during cultivation delayed the decrease in OVQ, especially in the leaves stored at 4 °C. This indicates that additional FR induced chilling tolerance both in plants cultivated at high and at low temperature. In leaves stored at 12 °C the longer duration of FR treatment in high temperature cultivated plants delayed the decrease in OVQ compared to the other treatments. The F_v/F_m values of leaves at harvest of the low temperature cultivated plants were lower than the high temperature leaves indicating that the plants were stressed during cultivation at 15 °C (Fig. 5). F_v/F_m values showed a decrease during storage at 4 °C and stayed at the original level during storage at 12 °C. This indicates that chilling injury was apparent at 4 °C. Additional FR, apart from increasing the value at harvest (in low temperature cultivated plants) also delayed the decrease during storage at 4 °C with up to 2 days in leaves from low and high temperature cultivated plants. Overall, the patterns observed in F_v/F_m were very similar to the patterns observed in QVQ.

(25 ºC) and no, 1 or 3 weeks additional FR (closed symbols or at a low temperature (15 ºC) and no or 3 weeks FR (open symbols). Each data point is a mean of 2 blocks (n = 2) and 4 replicate plants from each block. The error bars are errors of means. If letters are present, they indicate significant differences (α = 10%) for the different time points at each storage temperature.

4. Discussion

4.1. Addition of FR during cultivation improves postharvest chilling tolerance in basil

Addition of FR (i.e. a R:FR ratio < 1) has been shown to increase cold tolerance in a number of plant species (i.e. *Arabidopsis*, tomato, barley) (Franklin and Whitelam, 2007; Ádám et al., 2016; Wang et al., 2016). We investigated if addition of FR during cultivation would improve the postharvest chilling tolerance in basil. We found that additional FR indeed improved the chilling tolerance as based on measurements of overall visual quality and F_v/F_m (Fig. 5 A,C, 6 A,C). This was true for basil leaves both from plants cultivated at a low (15 °C) and a high (25 °C) temperature. Overall visual quality has previously been shown

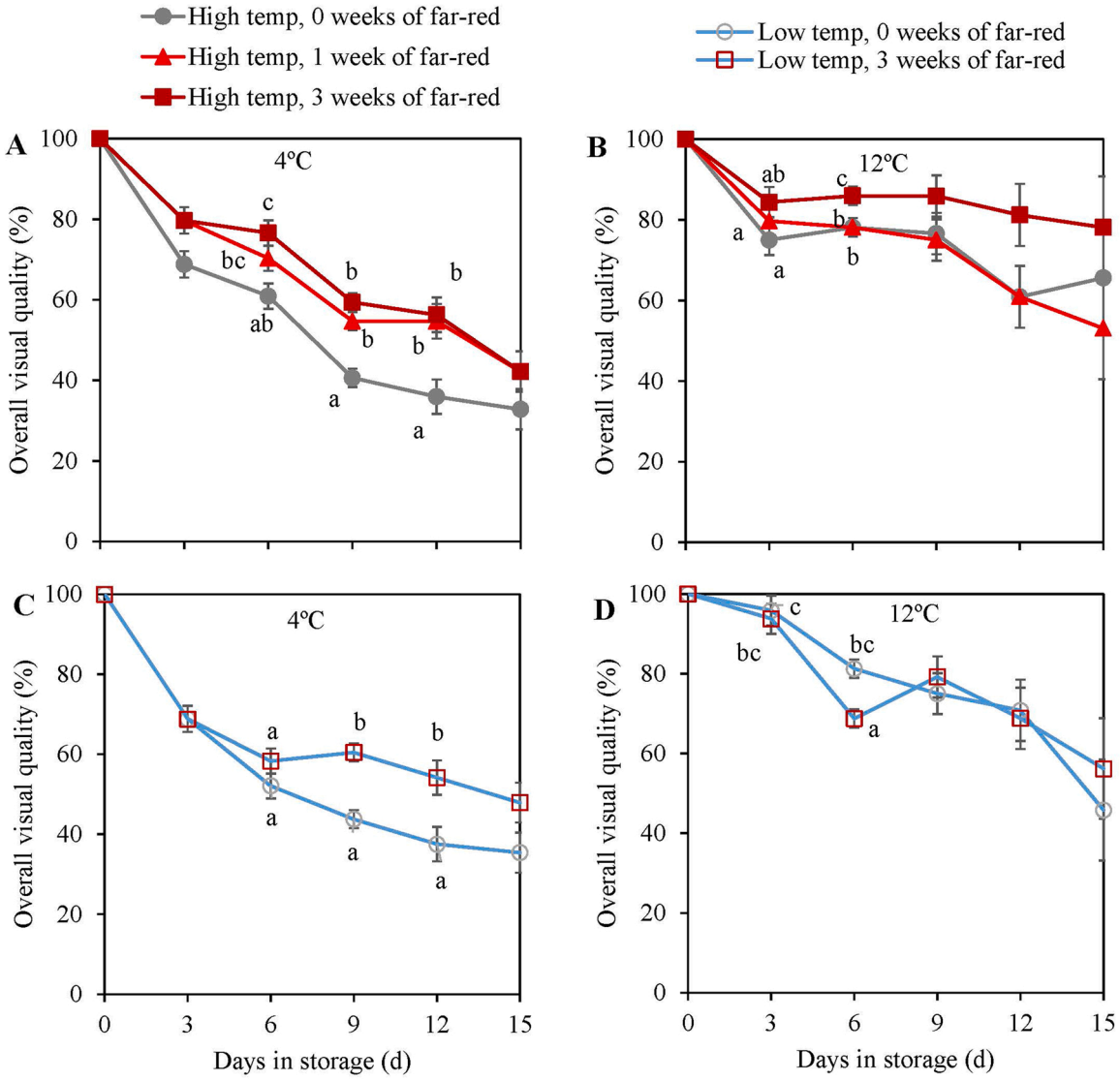

Fig. 4. Changes in overall visual quality during postharvest storage at 4 (panels A,C) and 12 °C (panels B,D) in basil cv. Emily. Plants were previously grown under different far-red and temperature treatments at a high temperature.

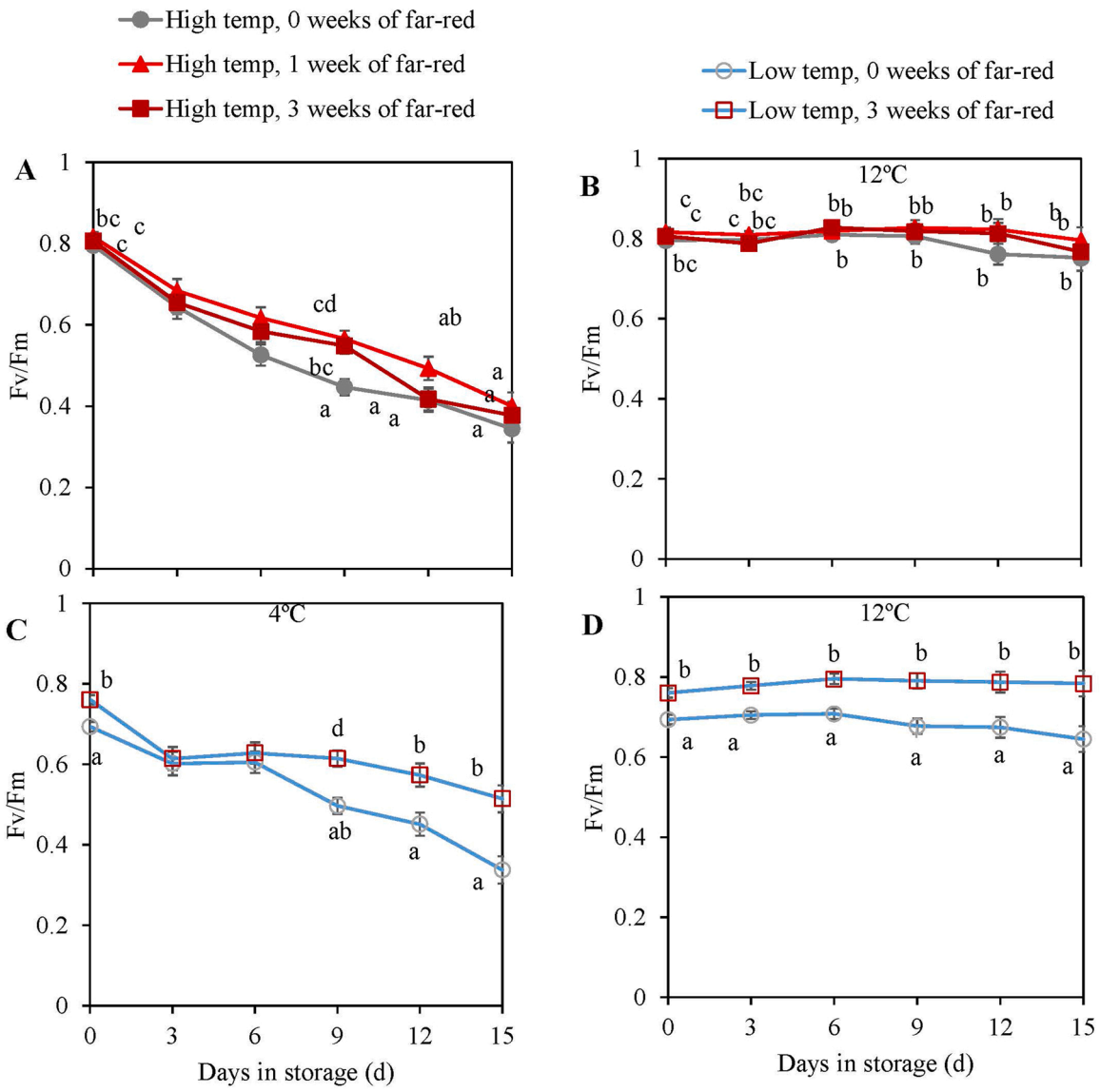

Fig. 5. Changes in maximum quantum yield of PSII of dark adapted leaves (F_v/F_m) (i.e. a marker for chilling injury) during postharvest storage at 4 (panels A,C) and 12 °C (panels B,D) in basil cv. Emily. Plants were grown under with different far-red and temperature treatments at a high temperature (25 °C) no, 1 or 3 weeks additional FR (closed symbols) or at a low temperature (15 °C) and no or 3 weeks FR (open symbols). Each data point is a mean of 2 blocks (n = 2) and 4 replicate plants from each block. The error bars are errors of means. If letters are present, they indicate significant differences (α = 10%) for the different time points at each storage temperature.

to correlate well with measurements of chilling injury (i.e. measured with chlorophyll fluorescence) (Larsen et al., 2022). Similar to our observations, Affandi et al. (2020) found that addition of FR light during cultivation improved the postharvest chilling tolerance in tomato fruit. However, Affandi et al. (2020) used a much lower intensity of FR (of 30–50 μmol m^{-2} s^{-1}). In tomato fruit, application of FR can improve the physical and biochemical properties of the fruit i.e. through an increase of biosynthesis of the cuticle wax and ascorbic acid content eventually leading to less weight loss in chilled fruit (Cozmuta et al., 2016).

In tomato leaves additional FR and chilling tolerance have been related to an increase in both ABA and JA content, which that play a role in the induction of the CBF pathway (Wang et al., 2016). In contrast to the findings of Wang et al. (2016) we did not find an increase in ABA and JA content at harvest in response to additional FR (Fig. 1A,C). During postharvest storage at 4 °C JA content in basil leaves decreased. The strongest decrease was found in the FR treated plants at high cultivation temperature (Fig. 1). Thus, FR did not positively stimulate the hormone content in the present study. An increase in ABA and JA have been found to be beneficial for the plant to overcome abiotic stress such as cold stress (Hu et al., 2017; Prerostova et al., 2021). Application of exogenous ABA in basil (Satpute et al., 2019) and JA in Arabidopsis (Hu et al., 2013) and JA in blood oranges (Habibi et al., 2019) increased chilling tolerance functioning upstream of the CBF pathway. We hypothesized that low temperature cultivation to increase ABA and JA content at harvest, however, neither ABA nor JA increased in response to low temperature cultivation. Similar to our study Liu et al. (2020) did not find an increase in JA in response to low temperature and cold acclimation of *Rhododendron*. In general, the optimal sampling time for studies involving hormones may be difficult to pinpoint as the peak of hormones may be transient. Based on our findings of ABA and JA (i.e. which should activate the CBF pathway) we cannot confirm that the CBF pathway is present in basil.

The improved chilling tolerance in basil cultivated under additional FR may have been due to the observed increase in soluble sugars and starch. Similar to our findings increases in soluble sugars were found in a number of species such as cucumber (leaves, stem and roots) (Xiong

et al., 2011), watermelon (leaves and stem) (Ranwala et al., 2002) and tomato (leaves) (Courbier et al., 2020) when additional FR was applied during cultivation. Phytochromes are highly involved in the allocation of carbon to leaves and production of biomass. *Arabidopsis* mutants had a lower biomass compared to the wild type when they are missing phytochromes (Yang et al., 2016). When plants are subjected to low temperature sugar and starch often accumulate in the leaves as an acclimation response (Ristic and Ashworth, 1993). This was also the case in our experiment where the low cultivation temperature also increased the soluble sugars and starch in the leaves. A high content of starch may help the plant mitigate abiotic stress such as cold stress as starch can serve as an energy reserve. The low temperature cultivation may result in limited photosynthesis resulting in limited carbon availability (Thalmann and Santelia, 2017). Under storage (in darkness) conditions starch functions as a carbon reserve that can provide energy for respiration. Yet, a delicate balance between carbohydrates and reactive oxygen species may exist. Larsen et al. (2022) showed that high light intensities increased the content of soluble sugars and starch, but it was not sufficient to improve chilling tolerance in basil as also a high light induced increase in ROS may have been in play. Sugar, in addition, can act as an osmoprotectant to protect thylakoid membranes in the chloroplast (Santarius, 1973), which in turn could improve chilling tolerance. This is in line with our results, which suggest that an increase in sugar and starch may aid in basil chilling tolerance.

4.2. Additional FR had no effect on antioxidants or VOCs

Antioxidants may improve chilling tolerance as they can scavenge reactive oxygen species (ROS) (Das and Roychoudhury, 2014). Additional FR has been found to increase the content of rosmarinic acid in basil (Schwend et al., 2016) and ascorbic acid in lettuce (Chen et al., 2016) and *Phaseolus vulgaris* (Bartoli et al., 2009). In contrast, Li et al. (2021) found that additional FR had a negative effect on the total ascorbic acid in lettuce. We found that addition of FR had no effect on antioxidants; in combination with low temperature cultivation rosmarinic acid decreased whereas no effect on chicoric or total ascorbic acid was observed at harvest (Fig. 3). During storage at 4 °C the content of phenolic antioxidants such as rosmarinic acid is expected to decrease due to their scavenging of ROS (Fratianni et al., 2017). However, we found that the content of rosmarinic and chicoric acid significantly increased during cold storage. Thus, the increase in ROS during cold storage may not have surpassed the scavenging potential of rosmarinic and chicoric acid.

Ascorbic acid decreased during cold storage which may either be related to its role in scavenging oxidants or due to the fact that the product was stored in darkness. Ascorbic acid biosynthesis is increased with light intensity (Ntagkas et al., 2018). When *Arabidopsis* plants were stored in darkness the ascorbic acid decreased (Yoshimura et al., 2014). Volatile organic compounds are important for the quality of basil as they make up the characteristic aroma and flavor profile of basil (Carvalho et al., 2016). FR increased the volatiles in petunia (Colquhoun et al., 2013), whereas it had no effect on sensory properties in tomato fruit (Dzakovich et al., 2017). Carvalho et al. (2016) found an increase in sesquiterpenoids but not monoterpenoids. In accordance, we did not find an effect of additional FR or the low temperature treatments on the main volatile organic compounds (the monoterpenoids eugenol, eucalyptol and linalool) in basil at harvest (Fig. S1A,C).

4.3. Low temperature during cultivation did not improve chilling tolerance

In the present experiment basil was cultivated at both a high and a low temperature (i.e. 15 and 25 °C). It was hypothesized that the cultivation at 15 °C (i.e. without FR) would improve chilling tolerance. Low temperature cultivation may have a positive effect on accumulation of carbohydrates and antioxidants. In an acclimation response to low temperature plants accumulate soluble sugars (Yuanyuan et al., 2010). When plants were cultivated at 15 °C we indeed found an increase soluble sugars and starch (Fig. 2). Furthermore, we found rosmarinic acid to be higher at low temperature cultivation. Low temperatures were found to have a positive effect on rosmarinic acid content in spearmint (Fletcher et al., 2005) and in coleus (*Plectranthus scutellarioides*) (Dörr et al., 2019). However, in Moldovian dragonhead (*Dracocephalum moldavica*) temperature did not have an effect on rosmarinic acid content (Khaleghnezhad et al., 2019). Different from our findings, ascorbic acid has also been found to increase in spinach cultivated at low temperature (Proietti et al., 2009) whereas we did not find a difference in total ascorbic acid between high and low temperature cultivated basil.

Plants may better tolerate low temperature if they have prior been exposed to short duration of low temperature resulting (as a priming effect) (Baier et al., 2019) or during prolonged exposure (acclimation) (Yuanyuan et al., 2010). In both cases we would expect leaves from plants cultivated at low temperature to have a better postharvest chilling tolerance. However, when OVQ and F_v/F_m was measured for the plants cultivated at 15 °C we observed that the youngest leaves were light green to yellow at harvest and had a lower value of F_v/F_m indicating that the plants were stressed by the low temperature. The temperature was probably too low and/or duration too long resulting in stress rather than a priming or acclimation effect. Thus, the increase in soluble sugars, starch and rosmarinic acid in low temperature cultivated plants was probably not sufficient to overcome the stress induced by low temperature. Therefore, cultivation at low temperature (15 °C) as applied in this research, does not aid in basil chilling tolerance. Applying a more moderate decline of the temperature or applying low temperature for a shorter duration may be an interesting avenue for further research.

5. Conclusion

Cultivating basil at low temperature did not improve postharvest chilling tolerance. Addition of FR either applied at low or high cultivation temperature improved postharvest chilling tolerance. The improved chilling tolerance was associated with an increase in soluble sugars and starch at harvest. There was no effect of FR on the content of antioxidants (rosmarinic acid, chicoric acid and total ascorbic acid) or hormones (abscisic acid and jasmonic acid) at harvest. This suggested that antioxidants, JA and ABA do not play a role in regulating FR-induced CI, but that carbohydrates do play a role in basil.

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CRediT authorship contribution statement

Dorthe H. Larsen designed and carried out the experiment, analyzed the data and wrote the manuscript. Ernst J. Woltering and Leo F.M. Marcelis supervised, designed and reviewed the experiment and manuscript. Diederick van Kempen acquired data and reviewed the manuscript. Wouter Kohlen acquired data on hormones and reviewed the manuscript. Celine C.S. Nicole reviewed the experimental design and manuscript.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ernst Woltering reports financial support was provided by Signify NV. Celine Nicole reports a relationship with Signify NV that includes: employment.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.postharvbio.2022.112232.

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