Alterations in Chlorophyll $a$ Fluorescence and Pigments Concentration in the Leaves of Cauliflower and Broccoli Transplants Subjected to Chilling

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Abstract

Chlorophyll $a$ fluorescence parameters and photosynthetic pigments content in leaves of broccoli ($Brassica oleracea$ L. var. italica) cv. ‘Monaco’ F1 and cauliflower ($Brassica oleracea$ L. var. botrytis) cv. ‘Bruce’ F1 transplants were analyzed to investigate the effects of chilling temperatures (6 and 10 °C) and the exposure length (1 or 2 weeks) on the photosynthetic apparatus condition in 3-year experiment. Data were compared to control plants exposed to 14 °C for 1 and 2 weeks. The lowest values of maximum quantum efficiency of photosystem II ($F_v/F_m$) and the performance index (PI) were observed in cauliflower chilled at 6 °C for 2 weeks. This was not accompanied by any substantial reduction in chlorophylls concentration, however, significant correlations between $F_v/F_m$ or PI and chlorophylls content in cauliflower leaves were found. There was no negative effects of chilling on photosynthetic activity and chlorophyll content in broccoli leaves, the amount of carotenoids significantly increased in the broccoli chilled for 2 weeks in 6 °C. The chlorophyll $a$ fluorescence indices and accelerated carotenoids synthesis showed that broccoli had less vulnerable photosynthetic apparatus to stress-induced temperatures and more efficient protection mechanisms in comparison to cauliflower transplants.

Keywords: Brassica crops, carotenoids, chlorophylls, low temperature, $F_v/F_m$, performance index

Introduction

Abiotic stresses are major environmental factors that significantly affect plant growth and productivity, leading to substantial yield losses (Hasanuzzaman et al., 2013). An important fact is that plant species and their ecotypes developed various physiological mechanisms underlying abiotic stress responses (Sharma et al., 2012).

One of the earliest responses of plant cells under chilling conditions is the generation of large quantities of reactive oxygen species (ROS) (Bhattacharje, 2005; Suzuki and Mittler, 2006; Pospíšil, 2012). Exposure to low temperature may lead to overproduction of ROS, affects cellular components through damage to nucleic acids, protein oxidation, and lipid peroxidation (Sharma et al., 2012), ultimately results in oxidative stress, often requiring the involvement of several antioxidant enzymes or low-molecular antioxidants (Turan and Elmekici, 2011). Excessive production of ROS can disturb photosynthesis due to disarrangement of thylakoid ultrastructure (Krantsch and Wise, 2001), inhibition of de novo synthesis of D1 protein, which is needed for PSII repair (Nishiyama et al., 2011), and suppression of some chloroplast enzymes activities (Kato and Sakamata, 2014). The inherent feature of the photosystem II complex is its vulnerability to abiotic stresses, including chill-induced oxidative stress, significantly disturbing photosynthetic process (Murata, 2007; Gunarani et al., 2015).

Monitoring changes in $F_v/F_m$ can be useful for revealing different responses of various plant species to chilling temperatures (Strauss et al., 2006; Mishra et al., 2011; Gorbe and Calatayud, 2012). Measurement of chlorophyll $a$ fluorescence is a simple and non-invasive technique that has been successfully used for the evaluation of photosynthetic activity under environmental stresses (Gorbe and Calatayud, 2012; Ashrafl and Harris, 2013). The consequences of damage to the photosynthetic apparatus, caused by chill-induced oxidative stress, are reflected by changes in the chlorophyll $a$ fluorescence indices and accelerated carotenoids synthesis showed that broccoli had less vulnerable photosynthetic apparatus to stress-induced temperatures and more efficient protection mechanisms in comparison to cauliflower transplants.

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In temperate climate, cauliflower and broccoli planted in the field during early spring may experience multiple environmental stresses, for example low above-zero temperatures or ground frosts. Controlled stress, applied to plants before planting out, through lowering the temperature, activates acclimation process, and provides an improvement of plant tolerance to a subsequent stress (Hasanuzzaman et al., 2013). Chilling may play a regulatory role in cross-adaptation of the plants, in which pre-exposure to one stress confers tolerance to other types of stresses also (Thakur and Nayar, 2013). It is important because crops with enhanced tolerance to field environment may potentially exhibit higher yields (Kalise et al., 2014). The question is which combination of low temperature and chilling duration will be safe enough for cauliflower and broccoli transplants to launch the process of acclimation and not cause permanent damages to the photosynthetic apparatus. We assume that these plants, exposed to chilling treatment, predisposing them to outdoor production, may show lesser disturbances in photosynthetic activity and pigments content than thermophilic plants (Kingston-Smith et al., 1999; Liu et al., 2001; Lukasik et al., 2012; Sharma et al., 2012). We also expect the differences between broccoli and cauliflower crops in response to stress, which should be helpful to estimate their sensitivity to low temperature. Results published by Długosz-Grachowska et al. (2012) and Grabowska et al. (2014) suggest that broccoli is relatively tolerant to chilling temperatures, while the response of cauliflower plants to environmental conditions is stronger in comparison to broccoli, and the species seems to be more affected by extreme thermal factors (Diputado, 1989; Olesen and Gregsen, 2000). However, direct comparative studies of broccoli and cauliflower responses to low temperature treatment are scarce and very difficult to achieve. Within this context, our main purpose was: (1) to check the Fv/Fm of PSII and the PI parameters of the plants exposed to temperatures of 6 and 10 °C; (2) to evaluate plants’ photosynthetic apparatus respond when measured after 1 and 2 weeks of chilling; (3) to determine the changes in the pigments content of the transplant leaves previously subjected to chilling; (4) to compare sensitivity of Brassica crops at transplant stage to low temperature.

Materials and Methods

Plant material and chilling treatment

The experiments with ‘Bruce’ F1 cauliflower (Brassica oleracea L. var. botrytis) and ‘Monaco’ F1 broccoli (Brassica oleracea L. var. italica) were carried out at the University of Agriculture in Krakow, Poland, in 2011, 2012, and 2013. The seeds, obtained from Syngenta Seeds (Warsaw, Poland) were sown March 8–11 using multipots (VEFI, 96 cells, 53 cm3 single-cell volume) filled with standard peat substrate (Klasmann TS2, Klasmann-Deilmann GmbH, Geeste, Germany). The pots were then placed in the greenhouse. The temperature in the greenhouse was maintained at 24 ± 2 °C until emergence, and then the plants were grown at approximately 18/16 ± 2 °C (day/night) up to chilling treatment. Plants were watered when necessary and fertilized twice (21 and 29 days after sowing) with a soluble fertilizer Yara Kristalon Zielony (18% N, 18% P2O5, 18% K2O, 3% MgO, and 2% S) (Yara International ASA, Oslo, Norway), at a dose of 10 g dm-2 water. The 40-day-old transplants of cauliflower and broccoli were transferred to vegetative chambers with a 14-h photoperiod (Sunmaster metal halide lamps, LM 400W U/46CDX, Venture Lighting Europe Ltd., Rickmansworth, UK). The intensity of

Chlorophyll concentrations optimal for moderate temperatures become a heavy burden for a cell in lower temperatures, especially with long-acting stress stimulus (Murata et al., 2007). Some of the absorbed light quanta would be counted as excess energy that often initiates singlet oxygen overproduction, followed by photooxidative damage and cell death. Decreasing chlorophyll content in plant leaves under low temperature is often considered a typical symptom of oxidative stress (Yadegari et al., 2007). This was shown, for example, in chill-sensitive coffee seedlings (Oliveira et al., 2009), different rice genotypes (Zhang et al., 2011; Perveen et al., 2013), wheat (Habib et al., 2011), and chill-sensitive maize cultivars (Arora et al., 2001). Partelli et al. (2009) reported that coffee plants responded with a 30% reduction in chlorophyll a content and 27% reduction in chlorophyll b when the day/night temperature decreased from 25/20 to 13/8 °C.

Allostatic stresses induce an “alarm phase”, characterized with functional decline due to the stressor factor. Photosynthesis and photosynthesis-related pigments are directly affected by low temperature, which is manifested by decreases in plant photosynthetic efficiency and capacity (Ruadland et al., 2009; Ruadland and Zachowski, 2010; Theocharis et al., 2012). If intensity of the stress stimulus is not lethal and exposure to stress is not prolonged, plants may reestablish their homeostasis, repair cellular components, and reprogram the metabolic system (Wang et al., 2003; Suzuki et al., 2011).

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irradiance at canopy level was ~300 μmol m⁻² s⁻¹, and the relative humidity RH was ~75% Next, the plants were exposed to chilling of varying intensities. We investigated the effects of chilling duration (1 or 2 weeks) and chilling temperature at the constant level of 6 and 10 °C. Additional treatments of 14 °C were performed for 1 and 2 weeks, with the same irradiance intensity and relative air humidity, served as the control. The control reflects average temperature conditions acting on the broccoli and cauliflower plants after planting them to the field in Poland spring conditions.

**Chlorophyll a fluorescence**

The parameters of chlorophyll a fluorescence were quantified using a portable Handy Plant Efficiency Analyzer (Handy-PEA, Hansatech Instruments, Norfolk, UK). At the end of chilling period, 20 randomly selected plants (5 plants over 4 replications) of each variety were moved from the chambers to the laboratory. Healthy and fully expanded leaf was selected from the plants (6 leaves of 6 different plants per variety). The leaves were dark-adapted for 20 min prior to the measurements and then illuminated by using the saturation pulse method (intensity: 1500 μmol m⁻² s⁻¹). Two main parameters were measured: maximum quantum yield of PSII (F_v/F_m) and performance index (PI). The yield of variable fluorescence F_v was calculated as F_v=F_m where F_v is the initial fluorescence value, and F_m is the maximal fluorescence of a dark-adapted sample.

**Chlorophyll and carotenoids analyses**

Contents of plant pigments were evaluated in leaves which fluorescence parameters previously had been determined. Tissues were extracted directly from plant leaves without freezing. Leaf samples were poured and measured in 4 replications. A 0.4 cm-diameter discs were cut from the middle of the leaf, avoiding the midrib and major veins. Leaf samples (0.1 g) were ground with addition of 3 mg of magnesium carbonate (MgCO₃) as a pigment stabilizer and chlorophyll (Chl a and Chl b) and carotenoids (Car) were extracted in 80% (v/v) aqueous acetone (25 cm³). After 0.5 h in the dark, obtained suspension was filtered through a filter paper (POCH SA, No. 97877/4313, Gliwice, Poland), and then the absorbance readings were carried out at 663, 646, and 470 nm, respectively, using a Helios Beta spectrophotometer (Thermo Fisher Scientific Inc., Waltham, USA). The chlorophyll and carotenoids contents were calculated using the equations described by Lichtenthaler and Wellburn (1987). The ratios of chlorophyll a to b (Chl a: Chl b) are also presented.

**Statistical analyses**

The results were statistically evaluated based on the two-way analysis of variance (ANOVA) procedure in the STATISTICA program (StatSoft Inc., v. 12, USA), with a Tukey’s HSD (honest significant difference) test at 0.05, 0.01, and 0.001 levels of probability. Sources of variation were chilling temperature level (T), treatment duration (D), and interactions between them (T × D). For comparison direct response of tested B. oleracea crops to chilling temperatures, irrespectively of exposure length, two-way ANOVA involved main effects of the crop (C), chilling temperature (T), and their interactions (C × T) was additionally performed. Correlation analyses for the relationships between chlorophyll a fluorescence parameters (F_v/F_m, PI) and the pigments content were performed for the cauliflower and broccoli transplants (n = 18). Linear coefficient of correlation (r) values were calculated and assessed at different significance levels (p < 0.05; p < 0.01; p < 0.001).

**Results**

**Chlorophyll a fluorescence**

The leaves of the cauliflower plants growing at 14 °C for 1 week were characterized by high values of both F_v/F_m and PI (Fig. 1). The significantly lower values of F_v/F_m and PI were found after 1 week at both 6 and 10 °C. Further declines occurred after 2 weeks of chilling but the lowest values of both parameters were observed only under the 6 °C temperature treatment. The control temperature (14 °C) induced no significant modifications in F_v/F_m during either time period. Most measured PI values of chilled plants were significantly lower compared to the control.

The temperature variants applied during the 1- and 2-week treatments did not induce any significant changes in F_v/F_m in broccoli transplants (Fig. 2). The maximum quantum efficiency of PSII was not disturbed after 2-week long exposure to 14 °C; non-significant declines were noted in plants at temperatures of 6 and 10 °C. Interestingly, higher PI values were noted under 6 °C compared to 14 °C, although differences were statistically insignificant.

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**Fig. 1.** Effect of different temperatures (6, 10, 14 °C) and length of their application (1 or 2 weeks) on the maximum quantum efficiency of PSII (F_v/F_m) and the performance index (PI) of the leaves of cauliflower transplants (means for the years 2011-2013); means followed by the same letter are not significantly different at p < 0.05 according to Tukey’s HSD test.

**Fig. 2.** Effect of different temperatures (6, 10, 14 °C) and length of their application (1 or 2 weeks) on the maximum quantum efficiency of PSII (F_v/F_m) and the performance index (PI) of the leaves of broccoli transplants (means for the years 2011-2013); means followed by the same letter are not significantly different at p < 0.05 according to Tukey’s HSD test.
Photosynthetic pigments content

We observed significant decrease of chlorophyll $a$ and $b$ contents in cauliflower plants treated with all temperature variants for 2 weeks (Table 1).

Chlorophyll $a$ decline was not parallel with decreased temperature, and the lowest chlorophyll $a$ content was measured under control (14°C). Temperature slightly affected chlorophyll $b$ content in leaves of cauliflower transplants. With decreasing temperature, a higher Chl $a$: Chl $b$ ratio was observed. Changes in the chlorophylls, as an effect of temperature treatments, were less differentiated in broccoli (Table 2) as compared to cauliflower. Prolonged treatment did not modify chlorophyll $a$ and chlorophyll $b$ concentrations in broccoli nor the Chl $a$: Chl $b$ ratio. Generally, temperatures of 6 and 10 °C caused some increase in the content of both chlorophylls compared to 14 °C, it was not statistically significant for chlorophyll $b$. An increase in the Chl $a$: Chl $b$ ratio were noted for the plants treated with 6 and 10 °C temperatures.

Analysis of the carotenoid concentrations revealed that these pigments were affected by low temperature differently between cauliflower and broccoli transplants (Tables 1 and 2). In cauliflower leaves, the carotenoids contents were rather stable, and no interaction effects were observed. However, lower contents of these pigments in the plants treated with temperature variants for 2 weeks were noted. In broccoli, an increase was observed when plants were exposed to 6 and 10 °C, especially when applied for a 2-week period.

Chlorophyll $a$ fluorescence parameters versus pigments content

A correlation analysis was performed to find relationships between chlorophyll $a$ fluorescence parameters and chlorophyll content. The results are presented in Table 3. All of the correlations were significant in the cauliflower transplants. The $F_m/F_v$ and PI parameters were positively correlated with the chlorophyll $a$ or chlorophyll $b$ contents or with the sum of the chlorophylls. The highest correlation coefficients were found for both chlorophyll fluorescence parameters ($F_m/F_v$ and PI) and total chlorophyll content (chlorophyll $a + b$). We did not find any significant correlations between chlorophyll $a$ fluorescence and pigments for broccoli transplants.

Response of *Brassica oleracea* crops to chilling

Species and varieties may respond in different manner when exposed to extreme temperatures. In present study, additionally made statistical analysis involved chilling temperatures and *Brassica oleracea* varieties (cauliflower, broccoli), irrespective of chilling duration, showed that photosynthetic apparatus of cauliflower plants was more sensitive to low temperature action (Table 4). Maximum photochemical efficiency of PSII ($F_v/F_m$) and performance index (PI) decreased significantly in cauliflower with lowering temperature. These fluorescence parameters were not affected by chilling in broccoli. In the case of $F_m/F_v$ of broccoli transplants, differences between control (14 °C) and chilling temperatures were amounted to about 3%, while for cauliflower they were larger than 30%. No response to low temperature in carotenoids content was observed for cauliflower plants; while the lowest tested temperature (6 °C) increased significantly content of these pigments in broccoli in comparison to control. Analysis of interaction effects did not prove any significant alteration in chlorophyll $a$ content, for each tested *Brassica* crops subjected to chilling as compared to the control, although these pigments concentration tended to be higher in lower temperature. Chl $a$: Chl $b$ ratio increased due to chilling treatment in both crops, more spectacularly in cauliflower transplants.

Table 1. Carotenoids (Car), chlorophyll $a$ (Chl $a$) and $b$ (Chl $b$) content (expressed in mg 100 g FW$^{-1}$) and the ratio of chlorophylls (Chl $a$: Chl $b$) in cauliflower transplants leaves as an effect of temperature treatment (means of the years 2011-2013)

| Duration (weeks) | Temperature (°C) | Car     | Chl $a$ | Chl $b$ | Chl $a$: Chl $b$ |
|------------------|------------------|---------|---------|---------|-----------------|
|                  | 6                | 10      | 14      | 6       | 10              | 14      | 6       | 10      | 14      |
| 1                | 30.03 a           | 32.18 a | 30.88 a | 30.62 a | 104.71 ab       | 28.98 a | 98.33 a | 55.96 abc | 55.23 ab | 1.780 a |
| Temperature (T)  | ns               | ***     | ns      | ***     | ***             | ns      | ***     | ns      | ***     |***     |
| Duration (D)     | ***              | ns      | ***     | ns      | ***             | ***     | ***     | ns      | ***     |***     |
| T x D            | ns               | ns      | ns      | ns      | ns              | ns      | ns      | ns      | ns      |***     |

Note: Data were subjected to two-way ANOVA. Means within a column followed by different letters are significantly different at $p < 0.05$ according to Tukey’s HSD test; the level of significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, non-significant) for main effects (temperature level ‒ T, treatment duration ‒ D), and interactions between them (T x D) was also shown.

Table 2. Carotenoids (Car), chlorophyll $a$ (Chl $a$) and $b$ (Chl $b$) content (expressed in mg 100 g FW$^{-1}$) and the ratio of chlorophylls (Chl $a$: Chl $b$) in broccoli transplants leaves as an effect of temperature treatment (means of the years 2011-2013)

| Duration (weeks) | Temperature (°C) | Car     | Chl $a$ | Chl $b$ | Chl $a$: Chl $b$ |
|------------------|------------------|---------|---------|---------|-----------------|
|                  | 6                | 10      | 14      | 6       | 10              | 14      | 6       | 10      | 14      |
| 1                | 33.34 bc          | 31.40 abc| 30.24 ab| 34.91 c | 118.33 ab       | 32.38 bc| 27.98 a | 107.13 a | 60.66 a  | 1.766 a |
| Temperature (T)  | ***              | **      | ns      | ***     | ***             | **      | ***     | ns      | ***     |***     |
| Duration (D)     | ns               | ns      | ns      | ns      | ns              | ns      | ns      | ns      | ns      |***     |
| T x D            | ns               | ns      | ns      | ns      | ns              | ns      | ns      | ns      | ns      |***     |

Note: Data were subjected to two-way ANOVA. Means within a column followed by different letters are significantly different at $p < 0.05$ according to Tukey’s HSD test; the level of significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, non-significant) for main effects (temperature level ‒ T, treatment duration ‒ D), and interactions between them (T x D) was also shown.
Table 3. Correlation coefficients (r) at particular significance level (p) between maximum quantum efficiency of photosystem II (Fv'/Fm') or performance index (PI) in leaves of the transplants and chlorophyll pigments content

| Crop     | Parameter | Fv'/Fm | PI | r  | p   | r  | p   |
|----------|-----------|--------|----|----|-----|----|-----|
| Cauliflower | Chl a     | 0.621  | ** | 0.499 | *  |      |     |
|          | Chl b     | 0.636  | ** | 0.498 | *  |      |     |
|          | Chl a + b | 0.641  | ** | 0.510 | *  |      |     |
| Broccoli | Chl a     | 0.264  | ns | 0.414 | ns |      |     |
|          | Chl a     | 0.264  | ns | 0.103 | ns |      |     |
|          | Chl a + b | 0.275  | ns | 0.415 | ns |      |     |

Note: *, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, non-significant.

Table 4. Comparison of cauliflower and broccoli transplants response to chilling temperatures (means of the years 2011-2013)

| Crop     | Temperature (°C) | Fv/Fm | PI | Car | Chl a | Chl b | Chl a: Chl b |
|----------|------------------|-------|----|-----|-------|-------|-------------|
| Cauliflower | 6               | 0.544 a | 1.765 a |     | 30.33 a | 113.50 a | 55.88 a |
|            | 10              | 0.684 b | 5.338 b |     | 30.58 a | 118.11 a | 59.11 ab |
|            | 14              | 0.801 c | 8.468 c |     | 29.66 a | 108.55 a | 59.53 ab |
| Broccoli  | 6               | 0.781 c | 6.378 b |     | 34.13 b | 121.40 a | 63.26 b |
|            | 10              | 0.768 c | 4.508 b |     | 31.89 ab | 120.90 a | 63.25 b |
|            | 14              | 0.793 c | 4.628 b |     | 29.11 a | 109.53 a | 60.97 ab |
| (C × T)   | ***             | ns    | *** | **  | ns    | ***    | ***      |
| (C)       | ***             | ns    | *** | **  | ns    | ***    | ***      |
| (T)       | ***             | ns    | *** | **  | ns    | ***    | ***      |

Note: Data were subjected to two-way ANOVA. Means within a column followed by different letters are significantly different at p < 0.05 according to Tukey's HSD test; the level of significance (*, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, non-significant) for main effects (crop, C; temperature level, T), and interactions between them (C × T) was also shown. Carotenoids and chlorophylls contents were expressed in mg 100 g FW⁻¹.

Discussion

In this study we answered what changes have occurred in the plants of temperate-climate vegetable crops subjected to low temperature treatment. We determined the effects of chilling temperatures and its duration on photosynthetic activity of broccoli and cauliflower on the base of chlorophyll a fluorescence indices and pigments content. We found the differences in response to chilling treatment of two tested *Brassica* crops: cauliflower and broccoli, pointing to the cauliflower plants as a more sensitive to low temperature.

In present study, chlorophyll a fluorescence indices of the cauliflower showed a significant effect of low temperature (6 °C). Such temperature applied to the cauliflower plants likely caused disruption to the photosynthetic apparatus after 1 week, and much stronger after 2 weeks of the treatment, which led to reduction in maximum quantum efficiency of PSII and performance index values. Decrease in the efficiency of PSII photochemistry may be associated with the decrease activity of reaction centers and not regulation of PSII and not connected with some damages of PSII or its down-regulation (Kalisz et al., 2014). However, further research demonstrated that cauliflower plants treated with identical chilling variants were able to recover from chilling stress resulting in higher crop productivity. In present study, Fv'/Fm and PI parameters of broccoli transplants was not affected by both chilling temperature and chilling duration, which was in contrast to cauliflower. It indicates lesser inhibition of the activity of PSII reaction centers and ability of the broccoli plants to protect effectively photosystem II.

In the experiment, the lowest temperature (6 °C) was applied together with an irradiance of 300 µmol m⁻² s⁻¹. Decreases in chlorophyll a fluorescence indices (Fv'/Fm and PI) in cauliflower, but not in broccoli, indicated possible photoinhibition in the cauliflowers resulting from photooxidative damage (Powles et al., 2006). It suggested that at 6 °C, applied photosynthetically active radiation intensity was perceived as excess light and was potentially dangerous for photosynthetic components of cauliflower leaf tissues. However, when time of exposure and low temperature intensity are above lethal levels, the plants are able to recover their photosynthetic capacity (Bruce et al., 2007). Further studies on cauliflower plants, conducted in the field, confirmed this supposition (Kalisz et al., 2014). Second fluorescence parameter PI describes precise equilibrium between primary photosynthetic reactions and dark enzymatic reactions (Kaliaji and Guo, 2008). According to the present results, the lowest applied chilling temperature (6 °C) disrupted this equilibrium in cauliflower transplants but not in broccoli leaves. Because fluorescence parameters are indicators of photosynthetic performance (Hasdai et al., 2006; Strauss et al., 2006), we determined, using Fv'/Fm and PI, intensity of response to chilling between both tested *Brassica oleracea* crops (cauliflower and broccoli), pointing to the photosynthetic apparatus of broccoli transplants as less vulnerable to low temperature than that of cauliflower.

There is lack of research concerning the changes of chlorophyll and carotenoids levels in response to low temperature for temperate-climate vegetable crops, such as cauliflower and broccoli, being in juvenile stage. In present study, there was no effect of chilling duration on broccoli chlorophyll a and b concentrations, but measured values for cauliflower were significantly lower after 2 weeks of chilling in comparison to plant chilled for 1 week. There was no reduction in chlorophylls by chilling temperature, the average content of chlorophyll a was even higher in chilled broccoli.
leaves (for both low temperature levels) and in cauliflower (10 °C) than in the plants held in 14 °C. This is in contrast to results described in other reports for chill-sensitive plants (Aroca et al., 2001; Zang et al., 2010) and to results obtained by Hasdai et al. (2006) for Arabidopsis, belonging to Brassicaceae family. An explanation can be found in the work of Aghaee et al. (2011). They compared chlorophyll contents in juvenile rice of cold-sensitive and cold-tolerant genotypes stressed with low temperature (15/10 °C day/night) for 2 weeks. Although stressed plants had lower chlorophyll contents than control, much smaller decreases in cold-tolerant rice genotypes suggest that light can be efficiently used in photosynthesis by these plants even under stressful conditions. More tolerant to low temperature crops like broccoli and cauliflower may not show a significant decrease of chlorophyll content in stress-induced conditions. Protective mechanisms in tested B. oleracea crops seemed to be more effective in relation to chill-sensitive plants, moreover, it worked more sufficiently in broccoli than in cauliflower transplants. Significant correlations between the chlorophyll contents and Fv/Fm or PI in cauliflower transplants but not in broccoli were found. Lack of correlation between these parameters in the leaves of broccoli plants is likely due to different response of the crop to chilling.

Higher carotenoids concentration (statistically confirmed) in broccoli transplants treated with the low temperatures (6 and 10 °C) was observed. This proves initiation of carotenoids biosynthesis in the plants during chilling. Carotenoids play important role in protecting photosynthesis apparatus against damage via their abilities to quench singlet molecular oxygen and to trap peroxyl radicals produced thus avoiding chlorophyll photooxidation (Haldimann, 1999). These compounds also stimulate energy dissipation within light-harvesting antenna proteins by non-photochemical quenching, or enhance light-harvesting capacity of chlorophylls to compensate reduced chlorophyll content under low temperature. In present study, we did not observe reduction in chlorophylls in chilled broccoli leaves, but an increase in total carotenoids content under lower temperatures occurred. It indicated that carotenoids were involved mainly in free radical scavenging mechanisms launched in the leaves of that transplants.

Results of present study showed a diversity response of tested B. oleracea crops to chilling. We did not find any research comparisons of the effect of low temperature on cauliflower and broccoli in one study, however, cauliflower plants are regarded as sensitive to environmental conditions (Wurt et al., 1990). As was reported by Długosz-Grochowska et al. (2012) for broccoli transplants, there was no visible outer symptoms of damage caused by very low temperature (2 °C), the plants seem to be rather tolerant of low temperature, but still changes in the physiological and biochemical processes have occurred. A significant decrease of Fv/Fm and PI in cauliflower transplants treated with chilling temperatures, observed in present study, and no changes in this regard noted for broccoli plants, suggests lower disturbances in photosynthetic activity of the latter in chilling conditions. No reduction in chlorophylls concentration was observed in both Brassica crops after chilling treatment, but increased accumulation of carotenoids in broccoli leaves showed that they perform an essential photoprotective role by quenching triplet state chlorophyll molecules and scavenging reactive oxygen species formed within the chloroplast. Probably increased carotenoids content was connected with lack of negative effects of low temperature action on chlorophyll a fluorescence parameters in the case of broccoli plants.

Conclusion
We showed that the response of the photosynthetic apparatus, described by chlorophyll a fluorescence, to low temperature is more distinct in the case of cauliflower transplants than in broccoli. A temperature of 6 °C applied during 1 or 2 weeks decreased the PI and Fv/Fm of the cauliflower leaves. Chilling duration, as a long-acting stimulus, seemed to be determinant for chlorophyll concentrations, especially in cauliflower plants, than temperature level itself. We observed accelerated biosynthesis of carotenoids in broccoli transplants acting as protective compounds against ROS under low temperature. In the case of cauliflower, chlorophyll a fluorescence was a useful tool for screening the physiological response of the plants to low temperature, while biochemical analyses did not show any significant changes in the levels of photosynthetic pigments, such as chlorophylls or carotenoids. According to the results of this study, we must indicate that broccoli and cauliflower plants, as natural varieties of B. oleracea species with similar biology, showed different responses to chilling applied in the same manner, pointing to broccoli as less sensitive to low temperature at juvenile stage.

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References
Aghaee A, Moradi F, Zare-Maivan H, Zarinkamar F, Irandoust HP, Sharifi P (2011). Physiological responses of two rice (Oryza sativa L.) genotypes to chilling stress at seedling stage. African Journal of Biotechnology 10:7617-7621.
Aroca R, Irigoyen JJ, Sánchez-Díaz M (2001). Photosynthetic characteristics and protective mechanisms against oxidative stress during chilling and subsequent recovery in two maize varieties differing in chilling sensitivity. Plant Science 161:719-726.
Ashraf M, Harris PC (2013). Photosynthesis under stressful environments: An overview. Photosynthetica 51(2):163-190.
Bhattacharjee S (2005). Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transduction in plant. Current Science 89:1113-1121.
Bruce TJA, Matthes MC, Napier JA, Pickert JA (2007). Stressful “memories” of plants: evidence and possible mechanisms. Plant Science 173(6):603-608.
Diputado MT (1989). Growth and development studies with broccoli (Brassica oleracea var. italica), PhD Thesis, Massey Univ, New Zealand.
Długosz-Grochowska O, Leja M, Grabowska A, Kunicki E (2012). The effect of preliminary chilling of broccoli transplants on some antioxidative parameters. Folia Horticulturae 24(2):131-139.
Dutta S, Mohanty S, Tripathy BC (2009). Role of temperature stress on chloroplast biogenesis and protein import in pea. Plant Physiology 150(2):1050-1061.
Gorbe E, Calatayud A (2012). Applications of chlorophyll fluorescence imaging technique in horticultural research: A review. Scientia Horticulturae 138:24-35.
Kalisz A, Kunicki E, Kalisz A, Wojciechowska R, Leja M, Śękara A (2014). Chilling stress applied to broccoli transplants of different age affects yield of the plants cultivated in summer. Horticultural Science (Prague) 41(2):71-79.

Guidi I, DegnInnocenti E (2012). Chlorophyll a fluorescence in abiotic stresses. In: Venkateswarlu B, Shanker AK, Shanker CH, Maheshwari M (Eds). Crop Stress and its Management: Perspectives and Strategies. Springer Netherlands, Dordrecht, The Netherlands pp 359-399.

Gururani MA, Venkatesh J, Tran LS-P (2015). Regulation of photosynthesis during abiotic stress-induced photoinhibition. Molecular Plant 8:1304-1320.

Habibi F, Normaharnadi H, Sharifbad A, Eivazi A, Heravan M (2011). Effect of cold stress on cell membrane stability, chlorophyll a and b contain and proline accumulation in wheat (Triticum aestum L.) variety. African Journal of Agricultural Research 6:5854-5859.

Haldimann P (1997). Chilling-induced changes to carotenoid composition, photosynthesis and the maximum quantum yield of photosystem II photochemistry in two maize genotypes differing in tolerance to low temperature. Journal of Plant Physiology 151:610-619.

Haldimann P (1999). How do changes in temperature during growth affect leaf pigment composition and photosynthesis in Zas mays genotypes differing in sensitivity to low temperature? Journal of Experimental Botany 50(333):543-550.

Hasanuzzaman M, Nahar K, Fujita M (2013). Extreme temperature responses, oxidative stress and antioxidant defense in plants. In: Vahdati K, Leslie Ch (Eds). Abiotic Stress – Plant Responses and Applications in Agriculture. IntTech, Rijeka, Croatia pp 169-205.

Hasdai M, Weiss B, Levi A, Samach A, Porat R (2006). Differential responses of Arabidopsis ecotypes to cold, chilling and freezing temperatures. Annals of Applied Biology 148:113-120.

Jansen M, Gilmer F, Biskup B, Nagel KA, Rascher U, Fischbach A, Briem S, Dreissen G, Tittmann S, Braun S, De Jaeger I, Metzlaff M, Schurr U, Scharr H, Walter A (2009). Simultaneous phenotyping of leaf growth and chlorophyll fluorescence via GROWSCREEN FLUORO allows detection of stress tolerance in Arabidopsis thaliana and other rosette plants. Functional Plant Biology 36:902-914.

Kalaji HM, Guo P (2008). Chlorophyll fluorescence: a useful tool in barley plant breeding programs. In: Sanches A, Gutierrez SJ (Eds). Photochemistry Research Progress. Nova Science Publishers Inc, Haupauge, NY, USA pp 447-471.

Kalisz A, Sękara A, Cebula S, Grabowska A, Kunicki E (2014). Impact of low-temperature transplant treatment on yield and quality of cauliflower curds in late spring production. Scientia Horticulturae 176:134-142.

Kato Y, Sakamoto W (2014). Phosphorylation of photosystem II core proteins prevents undesirable cleavage of D1 and contributes to the fine-tuned repair of photosystem II. The Plant Journal 79:312-321.

Kingston-Smith AH, Harbinson J, Foyer CH (1999). Acclimation of photosynthesis, H2O2 content and antioxidants in maize (Zas mays) grown at sub-optimal temperatures. Plant, Cell & Environment 22:1071-1083.

Kranich HA, Wise RR (2001). The ultrastructure of chilling stress. Plant, Cell & Environment 23:337-350.

Lichtenthaler HK, Wellburn AR (1983). Determinations of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. Biochemical Society Transactions 603:591-592.

Liu P, Meng Q-W, Zou Q, Zhao SJ, Liu Q-Z (2001). Effects of cold-hardening on chilling-induced photoinhibition of photosynthesis and on xanthophyll cycle pigments in sweet pepper. Photosynthetica 39(3):467-472.

Lukatkin AS, Bezaiztzy A, Bobinas Ç, Dochowskis P (2012). Chilling injury in chilling-sensitive plants: A review. Žemdirbystė 99(2):111-124.

Mishra A, Mishra KB, Höermiller II, Heyer AG, Niedhal L (2011). Chlorophyll fluorescence emission as a reporter on cold tolerance in Arabidopsis thaliana accessions. Plant Signaling & Behavior 6(2):301-310.

Munata N, Takahashi S, Nishiyama Y, Allakhverdiev SI (2007). Photoinhibition of photosystem II under environmental stress. BBA-Bioenergetics 1767(6):414-421.

Nishiyama Y, Allakhverdiev SI, Munata N (2011). Protein synthesis is the primary target of reactive oxygen species in the photoinhibition of photosystem II. Physiologia Plantarum 142:35-46.

Olesen JE, Greven K (2000). A simulation model of climate effects on plant productivity and variability in cauliflower (Brassica oleracea L. botrytis). Scientia Horticulturae 83:83-107.

Oliveira JG, Da Costa Aguiar Alves PL, Vitoria AP (2009). Alterations in chlorophyll a fluorescence, pigment concentrations and lipid peroxidation to chilling temperature in coffee seedlings. Environmental and Experimental Botany 67:71-76.

Partelli FL, Vicia HD, Viana AP, Batista-Santos P, Rodrigues AP, Leitão AE, Ramalho JC (2009). Low temperature impact on photosynthetic parameters of coffee genotypes. Pesquisa Agropecuária Brasileira 44(11):1404-1415.

Perveen S, Shirwani KL, Jan M, Malook I, Rehman S, Khan MA, Jamil M (2013). Low temperature stress induced changes in biochemical parameters, protein banding pattern and expression of Zat12 and Myb genes in rice seedling. Journal of Stress Physiology & Biochemistry 9(4):193-206.

Podstil P (2012). Molecular mechanisms of production and scavenging of reactive oxygen species by photosystem II. Biochimica et Biophysica Acta 1817:218-231.

Powles SB, Berry JA, Bjorkman O (2006). Interaction between light and chilling temperature on the inhibition of photosynthesis in chilling-sensitive plants. Plant, Cell & Environment 6(2):117-123.

Ruelland E, Zachowski A (2010). How plants sense temperature. Environmental and Experimental Botany 69:225-232.

Ruelland E, Vautier M-N, Zachowski A, Hurry V (2009). Cold signaling and cold acclimation in plants. Advances in Botanical Research 49:35-150.

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany, ID 217037.

Strasser RJ, Tsimilli-Michael M, Srivastava A (2004). Analysis of the fluorescence transient. In: Papagnojciou C, Govindjke (Eds). Chlorophyll Fluorescence: a Signature of Photosynthesis. Advances in
Turan O, Ekmekci Y (2011). Activities of photosystem II and antioxidant enzymes in chickpea (Cicer arietinum L.) cultivars exposed to chilling temperatures. Acta Physiologiae Plantarum 33:67-78.

Van Heerden PDR, Viljoen MM, De Villiers MF, Krüger GHJ (2004). Limitation of photosynthetic carbon metabolism by dark chilling in temperate and tropical soybean genotypes. Plant Physiology and Biochemistry 42:117-124.

Wang Y-J, Zhang Z-G, He X-J, Zhou H-L, Wen Y-X, Dai J-X, Zhang J-S, Chen S-Y (2003). A rice transcription factor OsbHLH1 is involved in cold stress response. Theoretical and Applied Genetics 107:1402-1409.

Wurr DCE, Fellows JR, Hiron RWP (1990). The effect of field environmental conditions on the growth and development of four cauliflower cultivars. Journal of Horticultural Science 65(5):565-572.

Yadegari LZ, Heidari R, Carapetian J (2007). The influence of cold acclimation on proline, malondialdehyde (MDA), total protein and pigments contents in soybean (Glycine max) seedling. Journal of Biological Sciences 7:1431-1436.

Zhang YH, Chen LJ, He JL, Qian LS, Wu LQ, Wang RF (2010). Characteristics of chlorophyll fluorescence and antioxidative system in super-hybrid rice and its parental cultivars under chilling stress. Biologia Plantarum 54(1):164-168.