

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) (Bhattacharjee, 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 Ekmekci, 2011). Excessive

production of ROS can disturb photosynthesis due to disarrangement of thylakoid ultrastructure (Kratsch 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 Sakamoto, 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; Gururani *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; Ashraf 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 parameters which was frequently reported and widely discussed (Strauss *et al.*, 2006; Dutta *et al.*, 2009; Oliveira *et al.*, 2009; Gorbe and Calatayud, 2012; Guidi and Degl'Innocenti, 2012). The F_v/F_m value is a measure of the maximum efficiency of PSII. Its optimal value, found in intact and healthy plants of most species, is about 0.830 (Guidi and Degl'Innocenti, 2012), while, for stressed plants, F_v/F_m values tend to decline. Jansen *et al.* (2009), in experiments on *Arabidopsis thaliana* plants exposed to low-temperature stress (5 °C), observed a slow decrease in the F_v/F_m values in two tested lines (polymerase-deficient PARP and C24 wild type) – until below 0.730 or 0.700 (the difference was due to genetic line). Among fluorescence parameters, the PI, also known as the “performance index” or “vitality index”, reflects the three main functional steps of photosynthetic activity by a PSII reaction center complex: light energy absorption, trapping of excitation energy, and conversion of excitation energy to electron transport (Strauss *et al.*, 2006). The PI parameter indicates the functionality of both photosystems (PSI and PSII) and provides quantitative information on the current state of plant performance under stress conditions (Strasser *et al.*, 2004). This parameter, as F_v/F_m , is usually reduced by the chilling temperature. Sun *et al.* (2009) subjected watermelon juvenile plants to chilling (4 °C) and they observed significant decrease in performance index as compared to the control. The performance index is also capable to reveal differences among plant genotypes. For example, Van Heerden *et al.* (2004) showed that dark chilling (8 °C for 1 night) did not decrease the PI parameter of chill-tolerant soybean while in chill-sensitive genotype the PI was 26% lower than in control plants.

Low temperature slows down the metabolism of the transplants, impairing the biosynthesis of main photosynthetic pigments as well as chloroplast development (Yadegari *et al.*, 2007; Dutta *et al.*, 2009). Plants subjected to low-temperature conditions may optimize their photosynthetic apparatus, since the activity of the enzymes of the main metabolic pathways is significantly down-regulated. In this case, demands of light absorption and utilization are lowered. 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.*, 2010; Perveen *et al.*, 2013), wheat (Habibi *et al.*, 2011), and chill-sensitive maize cultivars (Aroca *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.

Abiotic stresses induce an “alarm phase”, characterized with functional declines 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 (Ruelland *et al.*, 2009; Ruelland 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).

In temperate climate, cauliflower and broccoli planted in the field during early spring may experience multiply 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 Nayyar, 2013). It is important because crops with enhanced tolerance to field environment may potentially exhibit higher yields (Kalisz *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; Lukatkin *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-Grochowska *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 Grevsen, 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 F_v/F_m 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 (Klasman 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 emergences, 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⁻³ 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 U46CDX, Venture Lighting Europe Ltd., Rickmansworth, UK). The intensity of

irradiance at canopy level was $\sim 300 \mu\text{mol m}^{-2} \text{s}^{-1}$, and the relative humidity RH was $\sim 75\%$. Next, the plants were exposed to chilling of varying intensities. We investigated the effects of chilling duration (for 1 and 2 weeks) and chilling temperature at the constant level of 6 and 10 °C. Additional treatments of 14 °C maintained 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 treatment and 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 treatment). The leaves were dark-adapted for 20 min prior to the measurements and then were illuminated by using the saturation pulse method (intensity: $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$). 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 $F_m - F_0$, where F_0 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 in which fluorescence parameters previously had been determined. Tissues were extracted directly from plant leaves without freezing. Leaf samples were pool and measure was done 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 grounded with addition of 3 mg of magnesium carbonate (MgCO_3) as a pigment stabilizer and chlorophyll (Chl *a* and Chl *b*) and carotenoids (Car) were extracted in 80% (v/v) aqueous acetone (25 cm^3). After 0.5 h in the dark, obtained suspension was filtered through a filter paper (POCH SA, No. 978774513, 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 (1983). 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 ($T \times D$). 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 ($C \times T$) 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.

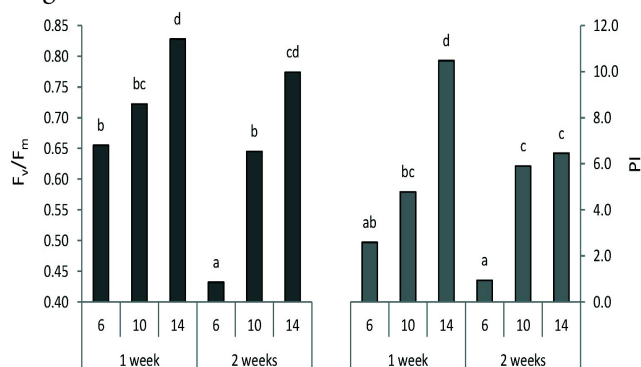


Fig. 1. Effect of different temperatures (6, 10, 14 °C) and length of their application (1 or 2 weeks) on the maximum quantum efficiency of PSII (F_v/F_m) and the performance index (PI) in the leaves of cauliflower transplants (means for the years 2011-2013); means followed by the same letter are not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

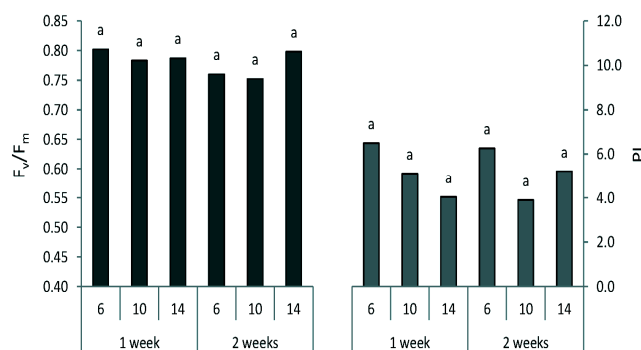


Fig. 2. Effect of different temperatures (6, 10, 14 °C) and length of their application (1 or 2 weeks) on the maximum quantum efficiency of PSII (F_v/F_m) and the performance index (PI) of the leaves of broccoli transplants (means for the years 2011-2013); means followed by the same letter are not significantly 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 increases 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 carotenoid 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_v/F_m 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_v/F_m 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 responded 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_v/F_m 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 <i>a</i>	Chl <i>b</i>	Chl <i>a</i> : Chl <i>b</i>
1	6	30.03 a	122.28 c	61.47 bc	1.989 c
	10	32.18 a	123.97 c	62.26 bc	1.991 c
	14	30.88 a	118.76 bc	63.83 c	1.861 b
2	6	30.62 a	104.71 ab	50.30 a	2.082 d
	10	28.98 a	112.25 abc	55.96 abc	2.006 c
	14	28.44 a	98.33 a	55.23 ab	1.780 a
Temperature (T)		ns	*	ns	***
Duration (D)		*	***	***	ns
T × D		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 × 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 <i>a</i>	Chl <i>b</i>	Chl <i>a</i> : Chl <i>b</i>
1	6	33.34 bc	124.46 b	65.50 a	1.900 c
	10	31.40 abc	120.41 ab	63.78 a	1.888 c
	14	30.24 ab	111.92 ab	61.28 a	1.826 b
2	6	34.91 c	118.33 ab	61.01 a	1.940 c
	10	32.38 bc	121.38 ab	62.71 a	1.936 c
	14	27.98 a	107.13 a	60.66 a	1.766 a
Temperature (T)		***	**	ns	***
Duration (D)		ns	ns	ns	ns
T × D		***	*	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 × D) was also shown.

Table 3. Correlation coefficients (*r*) at particular significance level (*p*) between maximum quantum efficiency of photosystem II (F_v/F_m) or performance index (PI) in leaves of the transplants and chlorophyll pigments content

Crop	Parameter	F_v/F_m		PI	
		<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Cauliflower	Chl <i>a</i>	0.621	**	0.499	*
	Chl <i>b</i>	0.636	**	0.498	*
	Chl <i>a + b</i>	0.641	**	0.510	*
Broccoli	Chl <i>a</i>	0.264	ns	0.414	ns
	Chl <i>b</i>	0.385	ns	0.103	ns
	Chl <i>a + b</i>	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)	F_v/F_m	PI	Car	Chl <i>a</i>	Chl <i>b</i>	Chl <i>a</i> : Chl <i>b</i>
Cauliflower	6	0.544 a	1.765 a	30.33 a	113.50 a	55.88 a	2.031 c
	10	0.684 b	5.338 b	30.58 a	118.11 a	59.11 ab	1.999 c
	14	0.801 c	8.468 c	29.66 a	108.55 a	59.53 ab	1.824 a
Broccoli	6	0.781 c	6.378 b	34.13 b	121.40 a	63.26 b	1.919 b
	10	0.768 c	4.508 b	31.89 ab	120.90 a	63.25 b	1.912 b
	14	0.793 c	4.628 b	29.11 a	109.53 a	60.97 ab	1.797 a
Crop (C)		***	ns	*	ns	**	***
Temperature (T)		***	***	**	**	ns	***
C × T		***	***	***	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 transplants 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 connected with some damages of PSII or its down-regulation (Haldimann, 1997; Guidi and Degl'Innocenti, 2012), which is associated with the decrease activity of reaction centers and conformational changes in the light harvesting complexes of PSII (Ashraf and Harris, 2013). However, further research demonstrated that cauliflower plants treated with identical chilling variants were able to recover the photosynthetic apparatus efficiency during subsequent development in the field (Kalisz *et al.*, 2014). Moreover, it was found that an application of 6 °C resulted in significant increase of cauliflower yield. These findings showed that low temperature probably caused short-term decrease of the crop photosynthesis through down-regulation of PSII and not irreversible damages of the photosystem in a transplants stage. The plants had a potential to recover from chilling resulting later in higher crop productivity. In present study, F_v/F_m 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Decreases in chlorophyll *a* fluorescence indices (F_v/F_m 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 photosystem 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 (Kalaji 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 F_v/F_m 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 carotenoid 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 chlorophylls 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 F_v/F_m 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 peroxy 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 observed 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 (Wurr *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 F_v/F_m 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 F_v/F_m 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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