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EFFECT OF CHILLING STRESS BEFORE TRANSPLANTING ON MORPHOLOGICAL PARAMETERS OF BROCCOLI HEADS

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Abstract. Vegetable crops produced in the field from transplants for early harvest are exposed to various stressful conditions. Standard treatment for transplants before planting out is hardening, through lowering the temperature or less-frequent watering. This process launches several plant defensive mechanisms against stress. However, chilling must be adjusted to specific crop species or even cultivars, because their response to low temperature may be different. The aim of the present experiment was to determine the effect of transplant chilling on the morphology of broccoli inflorescences. The experiment was conducted in the years 2011–2012. Broccoli transplants were chilled for 1 week or 2 weeks at 6, 10 and 14°C. Control plants were exposed to 18°C. Bud diameter and bud stalk length of broccoli inflorescences were measured, as well as diameter and weight of mature heads. In response to longer transplant chilling period, in the following growing stages plants formed inflorescences with longer bud stalks compared to the non-chilled control. The same tendency was observed for bud diameter in inflorescences. The greatest diameter of buds was noted for plants exposed to 10°C in comparison to the control, with buds about 29% smaller in diameter. Mean diameter of mature heads was the lowest for control plants, while the widest was obtained from plants chilled at 6°C or 10°C for 2 weeks, depending on the year. Chilling of transplants positively influenced the weight of broccoli heads at harvest in most cases, and the heaviest heads were obtained as a result of transplant chilling at 6°C. Relationships between temperature and some parameters of broccoli heads were found and regression equations were created.

Key words: *Brassica oleracea* var. *italica*, chilling, bud development, head weight

INTRODUCTION

Vegetable crops cultivated in an open field are often exposed to many stress factors because of the variability of environmental conditions [Kosmala 2009, Janská et al.

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2010]. Especially in the spring, plants cultivated at the beginning of the growing season are subjected to significant decreases in ambient temperature and humidity in comparison to those in the greenhouse or hot tunnel, where transplants are produced. The method of increase of plant tolerance to stress is applying chilling in young stages of plant growth, i.e. 7–10 days prior to planting out [Jian et al. 2005, Kalisz and Cebula 2006]. Chilling stress causes many changes in the metabolism of plants which lead to biochemical, physiological and morphological modification and promote plant's defence systems against stress factors. The nature of these changes is often complex and called cross-tolerance acquisition. It allows plants to acclimate to a range of different stresses after exposure to one specific stress [Pastori and Foyer 2002]. Temperate plant species like broccoli can acclimatise to low non-freezing temperature through synthesis of cryoprotectans, increasing activity of antioxidative enzymes and launching the synthesis of non-enzymatic antioxidants [Huang and Guo 2005, Janská et al. 2010, Goraj et al. 2012]. Besides biochemical and physiological changes in the plants under low temperature conditions, modification of plant morphology also takes place [Kalisz and Cebula 2006]. Assessment of the effect of juvenile plant chilling on morphological development during field vegetation is an interesting scientific problem with high applied impact. Acclimated plants should better withstand field conditions and they should be characterized by more intensive growth. Nature of all above mentioned changes, noticeable in the plants, can be short-term, observed only in the young plants, or long-term, influencing next developmental stages and yield [Kalisz and Siwek 2006]. Chilling treatment, applied at juvenile stage of plant growth, can also induce transition to the generative phase [Michaels and Amasino 2000, Kalisz and Cebula 2006]. For such a vegetable crop like broccoli, it can affect yield through earlier initiation of flowering and further head development [Kałużewicz et al. 2012]. Launching the stress tolerance mechanisms in the plants can allow to fully exploit the yield potential of vegetable crops. Thus the aim of the present paper was to assess how chilling stress, applied at juvenile stage, could affect morphological development of broccoli in the field. It was done on the basis of some parameter of primary broccoli heads, crucial to their exterior quality.

MATERIAL AND METHODS

The experiment was conducted in the years 2011–2012. Seeds of broccoli (*Brassica oleracea* L. var. *italica* Plenck) cultivar Monaco F_1 (Syngenta) were sown on 23–25 February into multipots (VEFI A/S, 96 cells, black-coloured, cone-shaped with 53 cm^3 volume of single cell) filled with standard peat substrate and placed in the greenhouse. Temperature in the greenhouse was kept at the level of 24/21°C (day/night) until emergence, then the temperature was lowered to 18/15°C (day/night). Plants of 32-day-old (with about 4 leaves) and 25-day-old (with about 3 leaves) were transferred to cold-chambers for 1 and 2 weeks, respectively (factor I – chilling period). They were cultivated under an 14 h photoperiod (metal halide lamps Sunmaster, 400W), with an relative air humidity of ca. 75%. The temperature inside the particular chambers was constant at levels of 6, 10, 14°C (factor II – temperature level). For control plants, grown in the greenhouse, temperature was 18 ±2°C during the day and 15 ±2°C at night until planting. Transplants were fertilized twice with liquid fertilizer (Yara Kristalon Zielony, 1 dag per 1 dm^3 of water). Broccoli transplants were planted out in the field of the University of Agriculture in Krakow, southern Poland (50º04'N, 19º51'E), in the beginning of April, at 50 × 45 cm spacing, and covered with nonwoven fleece (Agryl PP, 19 $g \cdot m^{-2}$). The experiment was located on soil classified as brown type, with loess as the basement complex. The experiment was laid out in 4 replications. The plot size for harvest was equal to 6.75 m^2 and comprised 30 plants plus plants of marginal belts. Standard cultivation practices recommended for broccoli were done, including fertilization, weed control, pest and disease control. Sprinkler irrigation began when soil water potential was equal to or less than -40 kPa. The amount of fertilizers was calculated on the basis of soil analyzes to achieve the contents of nutrients in 1 dm^3 of soil of 140 mg N, 60 mg P, 200 mg K, 70 mg Mg and 1500 mg Ca. Covers were removed around 9 May. Harvests took place from the end of May to the end of June.

Table 1. Temperature and light conditions during plant growth in the field

Year	Month	10 days	Air temperature (°C)			PPFD* ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Irradiance ($W \cdot m^{-2}$)
			mean	min	max		
2011	April	I	10.2	5.6	16.2	311.2	167.4
		II	10.5	3.1	19.7	512.0	260.7
		III	15.0	8.1	23.7	627.5	339.7
	May	I	11.9	5.2	18.6	671.3	346.7
		II	14.7	6.2	22.2	755.5	379.0
		III	16.7	8.5	24.2	734.8	359.8
	June	I	18.7	13.5	24.7	575.8	288.6
		II	17.4	11.2	23.8	664.1	322.6
		III	17.7	11.1	24.3	598.2	310.7
2012	April	I	7.9	2.2	15.4	496.3	248.9
		II	11.1	5.9	18.5	506.3	259.2
		III	16.7	8.0	27.2	720.7	371.3
	May	I	17.9	9.7	26.9	682.9	328.4
		II	12.5	5.2	19.5	635.6	321.4
		III	16.2	8.4	23.4	750.4	382.2
	June	I	15.3	10.8	20.0	533.3	278.7
		II	19.0	11.6	26.1	657.3	343.1
		III	19.4	12.3	26.4	673.4	353.5

*PPFD – photosynthetic photon flux density

Meteorological conditions (air temperature, photosynthetic photon flux density, total irradiance) during plant vegetation in the field are presented in Table 1. Data were collected from the automatic HOBO Pro RH/Temp. loggers (temperature) and HOBO Weather Station (Onset Comp. Corp., USA) – light characteristics – placed 100 m from the plots. Average values for both years were very similar regarding temperature and light conditions. However, there were some differences in temperature pattern. For example, end of April and beginning of May were much cooler in 2011 than in the next year, while beginning of June was warmer. Some differences in the pattern of light parameters between 2011 and 2012 were also observed. Average values for PPFD and irradiance were higher for 2012 by 22.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 12.4 $W \cdot m^{-2}$, respectively, than in 2011.

During harvesting, the weight of each primary broccoli head was precisely assessed. Heads defined as marketable were fully developed (minimal diameter reached 6 cm), compact, with no visible quality defects, and flower buds were closed and did not exceed 2 mm in diameter. Heads were cut together with a fleshy shoot of ca. 10–15 cm length. All data concerning weight of particular heads were summed up for each experimental object, then divided into individual weight classes (up to 500 g, up to 1000 g and above 1000 g). Diameter of mature broccoli heads (20 plants per object) were measured by an electronic calliper.

The flower buds of each head were observed with a stereomicroscope SteREO Lumar V12 (Carl Zeiss AG, Germany) to determine its quality. The diameter (in length) and stalk length of successive flower buds on an oldest outward-facing whorl on the third branch were measured. According to Björkman and Pearson [1998], the size of these buds is consistent and representative of the whole curd quality. The measurements were made with an Image Tool 3.0 software, after registration of the image with a SteREO LUMAR V12 microscope (Carl Zeiss AG, Germany).

The results were evaluated statistically based on the analysis of ANOVA in the STATISTICA program (StatSoft Inc., USA) with HSD Tukey's test with $p < 0.05$. Multiple linear regression analysis (verified at $p < 0.05$), with backward elimination, was conducted with the Advanced Linear/Nonlinear Models module of STATISTICA to find the temperature variables, which most significantly influenced flower bud diameter and stalk length in inflorescence of broccoli and percentage of heads with a weight above 500 g. Air temperatures (T_{avg}, T_{max}, T_{min}) were averaged for growing season including period of chilling time.

RESULTS AND DISCUSSION

Chilling of broccoli transplants for 2 weeks extended the time from planting to head initiation in all years of the experiment (tab. 2). The same phenomenon was observed for plants chilled for 1 week, but only in 2011. However, next developmental stage (from head initiation to first harvest) was shorter for chilled plants. The duration of harvest period was shorter for control plants than chilled ones.

Broccoli flower bud size depended on the temperature and period of chilling (tab. 3, fig. 1). Flower bud diameter was lower for controls in comparison to plants chilled at 10°C for 1 and 2 weeks. Only in this case differences were significant. Taking into account means for chilling period, it should be underlined that both 1 week and 2 weeks of low temperature caused an increase of bud diameter. Although a slight increase in this parameter was noted for all chilled plants, only between controls and plants chilled at 10°C significant difference was observed and it amounted for 0.71 mm.

Table 2. Duration of broccoli developmental stages in years 2011–2012 (days)

Developmental stage	Year	Chilling						Control
		1 week			2 weeks			
		6°C	10°C	14°C	6°C	10°C	14°C	18°C
Planting → head initiation	2011	48	50	50	53	53	56	46
	2012	42	38	41	45	43	43	41
Head initiation → first harvest	2011	12	13	10	13	13	16	17
	2012	19	15	18	18	15	16	22
First harvest → last harvest	2011	11	9	11	7	11	13	7
	2012	19	19	14	13	18	14	10

Table 3. Chosen parameters of broccoli flower buds as influenced by period and temperature of chilling

Chilling period	Temperature (°C)	Bud diameter (mm)	Bud stalk length (mm)
1 week	6	2.14 ±0.27 abc	7.86 ±0.55 abc
	10	2.37 ±0.53 bc	6.75 ±1.35 ab
	14	1.78 ±0.33 a	6.85 ±0.78 ab
2 weeks	6	1.95 ±0.16 ab	8.25 ±0.79 bc
	10	2.49 ±0.22 c	8.63 ±1.26 c
	14	2.05 ±0.19 abc	6.88 ±1.13 ab
Control	18	1.72 ±0.19 a	6.45 ±0.37 a
Means for chilling period			
1 week		2.10 ±0.44 B	7.15 ±1.03 AB
2 weeks		2.16 ±0.30 B	7.92 ±1.28 B
control		1.72 ±0.19 A	6.45 ±0.37 A
Means for temperature			
6°C		2.04 ±0.23 A	8.06 ±0.68 B
10°C		2.43 ±0.39 B	7.69 ±1.59 AB
14°C		1.91 ±0.29 A	6.86 ±0.92 A
18°C (control)		1.72 ±0.19 A	6.45 ±0.37 A

Mean values within a column, followed by different capital letters for main effects and small letters for interaction effects, are significantly different at $p < 0.05$ according to Tukey's HSD test; ±SD – standard deviations

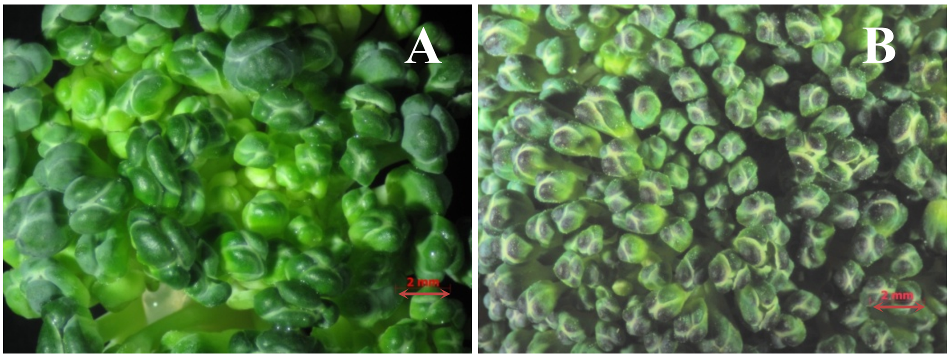

Fig. 1. Flower buds in the inflorescence developed by broccoli chilled 2 weeks at 10°C (A) and by a control plant (B)

The greatest difference in bud stalk length was measured between control and chilled plants at 6 and 10°C for 2 weeks (tab. 3, fig. 2). In those cases, bud stalks were longer by 1.80 and 2.18 mm, respectively, than controls. Analyzing means for chilling period, it was shown that 2 weeks of low temperature significantly increased bud stalk length by 1.47 mm, on average. With respect to temperature lowering the broccoli inflorescences formed longer bud stalks, but only at 6°C differences were statistically significant in comparison to 14 and 18°C.

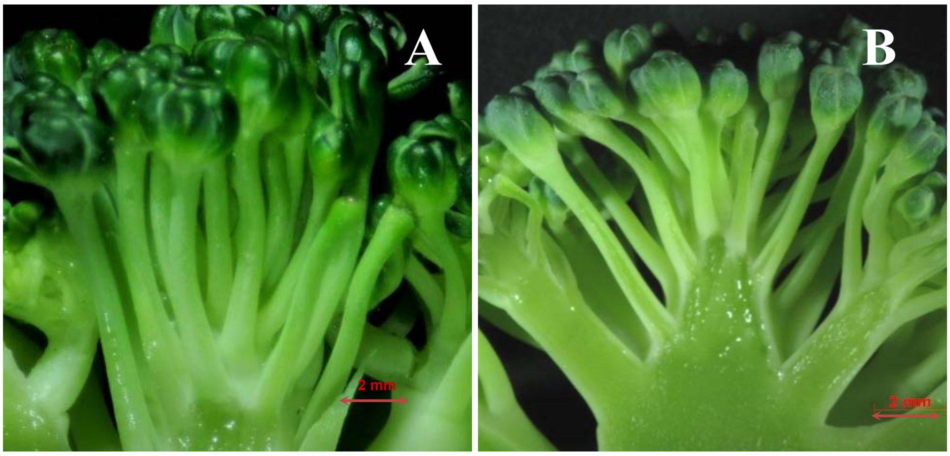

Fig. 2. Flower bud stalks in the inflorescence developed by broccoli chilled 2 weeks at 10°C (A) and by a control plant (B)

Inflorescence visual quality was characterized by a uniform shape and small size of flower buds [Björkman and Pearson 1998, Kałużewicz et al. 2010, Grabowska et al. 2013]. It was shown here that chilling applied at the transplant stage can affect further broccoli inflorescence development. Heads developed from transplants chilled at 10°C were characterized by greater flower buds than controls. This indicates that inflorescences had slightly lower quality. Also Grabowska et al. [2013] pointed out, that dark-chilling (2°C / 2 weeks) of 8- and 10-week-old broccoli transplants resulted in some deterioration of inflorescence quality.

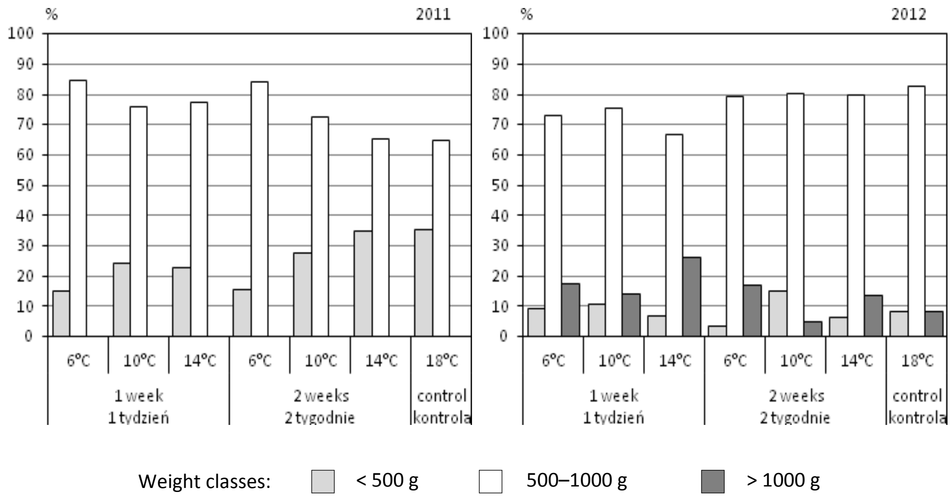

Fig. 3. Influence of transplant chilling on share of heads in particular weight classes in broccoli yield

The weight classes of broccoli heads are presented in Figure 3. There were no heads of weight above 1000 g in 2011. In this year the highest percentage of the smallest heads (below 500 g) was obtained from controls (35.4%) and from plants chilled at 14°C for 2 weeks (34.8%). A significantly higher weight of broccoli heads was the result of transplant chilling at 6°C, both for 1 and 2 weeks (84.7 and 84.3% of heads with masses above 500 g, respectively). In the next year, the smallest percentage of the heads below 500 g was observed for plants chilled at 6°C for 2 weeks (3.7%), and at 14°C for 1 and 2 weeks (6.9–6.3%, respectively). The lowest temperature of chilling (6°C) caused visible increase in head weight (1 week of chilling – 17.6%; 2 weeks – 17.1% of heads above 1000 g). It is interesting that broccoli chilled at 14°C for 1 week also had very high percentage of the heaviest heads (26.4%). Control broccoli plants gave the smallest degree (8.5%) of heads heavier than 1000 g. The only exception was in 2012, when plants chilled at 10°C for 2 weeks produced twice less heads > 1000 g than in controls. Thus, chilling of transplants positively influenced the weight of broccoli heads in most cases. Many publications describe effects of chilling on vegetable crop in a phase of transplants. However, very few focuses on the long-term effects of young plants chilling. Korkmaz and Dufault [2001a, b] found negative responses of chilling-sensitive watermelon and muskmelon to long chilling before transplanting, stunting their growth and reducing yields. The effect of chilling seems to be different in chilling-tolerant plants like species of *Brassica*. The results described in a publication on heading Chinese cabbage (*Brassica pekinensis*), where plants produced from chilled transplants finally gave heavier heads and better yield than controls [Kalisz and Siwek 2006], confirms such suggestions.

Results of regression analyses for flower bud diameter and stalk length are presented in Table 4. It was shown that the bud diameter generally depended on all tested temperature variables (T_{avg}, T_{max}, T_{min}) with a high coefficient of determinations, while bud stalk length mainly depended on T_{min}. Estimation of the bud diameter and stalk length are mistaken by an average of about 0.1 and 0.5 mm, respectively. The equation for percentage of broccoli heads with a weight > 500 g included T_{avg} (slope is negative) and T_{max} (β-coefficient is positive). The calculated error for predicting head weight was equal to 6%. The coefficient of determination for the latest model showed that approximately 70% of the data variation can be explained by T_{avg} and T_{max}.

Table 4. Relationship between temperature and flower bud (A) diameter, (B) stalk length and (C) percentage of broccoli heads with weight > 500 g

Trait	Regression equations		
A	$y = 60.298 + 23.190 * T_{avg} – 7.919 * T_{min} – 15.723 * T_{max}$		
B	$y = 16.692 – 1.127 * T_{min}$		
C	$y = –375.206 – 94.054 * T_{avg} + 87.511 * T_{max}$		
Parameters	A	B	C
R	0.960	0.858	0.848
R^2	0.921	0.737	0.720
R^2_{adj}	0.842	0.684	0.669
SE_e	0.114	0.478	6.029
p	0.037	0.014	0.001

T_{avg}, T_{max}, T_{min} – daily average, maximum and minimum temperature, respectively
R – multiple coefficient of regression
R^2 – coefficient of determination
R^2_{adj} – adjusted coefficient of determination
SE_e – standard error of estimation
p – significance level

As shown by regression analysis there are some relationships between averaged temperatures of growing season, in the present experiment modified by chilling conditions applied before planting out, and some parameters of mature broccoli heads. Flower bud diameter was affected by all tested temperatures (averaged, minimum and maximum) and bud stalk length by minimum temperature. It should be hypothesize that after-effect of chilling had inductive character. In opposite, Dufault [1996] observed, based on regression analysis, that bud size of broccoli cultivars was unaffected by growing season temperatures, however, plants were not chilled. In the present experiment daily average temperature was negatively correlated with the percentage of heads > 500 g, but surprisingly a positive relationship was found with maximum temperature. Heavier heads in cooler temperature conditions (together with chilling) were due to temperate requirements of broccoli plants and their better growth in such conditions. However, Kałużewicz et al. [2010] found no relationship between temperature from head initiation to harvests and mean weight of broccoli heads; correlation coefficients were rather high, but not significant.

In time of harvests broccoli head diameter was measured (fig. 4). In 2011, plants of control and after chilling at 14°C for 2 weeks had significantly smaller diameters of the head. Transplant chilling at 6°C and 10°C (for 1 and 2 weeks) and 14°C (1 week) resulted in significant increases in mature head diameter, especially in the case of the plants of 10°C/2 weeks combination. In the next year, clear increase in head diameter for all chilled plants was observed in comparison to control. The highest increase in size of the heads was observed for plants chilled at 6°C for 2 weeks, difference reached 22.0 mm when compared to the control.

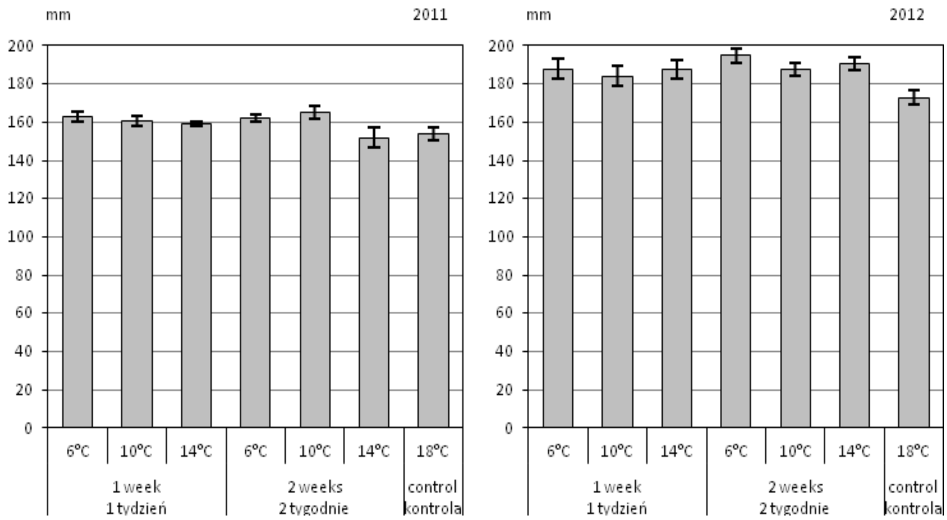

Fig. 4. Influence of transplant chilling on diameter of broccoli heads. Error bars represent standard deviations

Broccoli head diameter, measured at harvest, clearly increased as a result of transplants chilling. Greater weight of the heads of chilled plants was caused by better plant acclimatization to field conditions and more pronounced growth and development. It proves that chilling of transplants induces long-term effects, which could be noticed even during harvests.

CONCLUSIONS

1. Broccoli transplants chilling had a long-term effect on inflorescence development and its morphological quality.
2. The highest diameter of flower buds was observed for broccoli chilled at 10°C, while the lowest in control plants. Bud stalks were longer with a longer period of chilling.
3. A significantly higher weight of broccoli heads was observed for plants chilled at 6°C (1 week and 2 weeks) compared to controls.
4. Chilling applied at the juvenile stage generally caused an increase in diameter of the broccoli heads.

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WPŁYW STRESU CHŁODOWEGO PRZED SADZENIEM NA PARAMETRY MORFOLOGICZNE RÓŻ BROKUŁU

Streszczenie. Rośliny warzywne produkowane w polu na wczesny zbiór są narażone na różne stresowe warunki. Standardowym sposobem traktowania rozsady przed posadzeniem jest hartowanie polegające na obniżaniu temperatury lub ograniczeniu podlewania. Powoduje to uruchomienie mechanizmów obronnych przeciwko stresom. Chłodzenie roślin musi być dostosowane do gatunku, a nawet odmiany rośliny, gdyż ich reakcja może być różnorodna. W niniejszym doświadczeniu prowadzonym w latach 2011–2012 rozsadę brokułu chłodzono przez 1 tydzień lub 2 tygodnie w 6, 10 lub 14°C. Rośliny kontrolne znajdowały się w temperaturze 18°C. Oceniano wpływ tych warunków na średnicę pąków i długość szypułki pąków w kwiatostanie brokułu, jak również średnicę dojrzałych róż i ich masę. Wydłużenie okresu chłodzenia zwiększało długość szypułek pąków kwiatowych. Podobna tendencja została zaobserwowana dla średnicy pąków. Największą średnicę pąków stwierdzono dla roślin chłodzonych temperaturą 10°C, zwłaszcza w stosunku do kontroli, gdzie pączki były o 29% mniejsze. Średnica róży była najmniejsza dla roślin kontrolnych, podczas gdy róże o największej średnicy wykształciły rośliny chłodzone w 6°C przez 2 tygodnie oraz 10°C przez 2 tygodnie, w zależności od roku badań. Chłodzenie rozsady w większości przypadków wpłynęło pozytywnie na masę zebranych róż brokułu, a najcięższe róże były wynikiem chłodzenia roślin w temperaturze 6°C. Znaleziono związek między temperaturami a niektórymi parametrami róż brokułu, co zostało przedstawione za pomocą równań regresji.

Słowa kluczowe: *Brassica oleracea* var. *italica*, chłód, rozwój pąków, masa róży

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