Chilling stress applied to broccoli transplants of different age affects yield of the plants cultivated in summer

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Abstract

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Broccoli is native to moderate climatic zone, and summer cultivation of this species in Central Europe conditions leads to a decrease of heads quality. The aim of present investigations was application of dark-chilling at 2°C for 1 and 2 weeks to transplants of various ages to enhance its tolerance against adverse conditions in the field and to modify the yield potential. Broccoli cv. Monaco F_1 can be recommended for summer production in Central Europe, because of high yield potential and lack of buttoning in high temperature conditions. Dark-chilling of 4-week-old transplants for two weeks significantly increased the yield, but also the percentage of stems with hollows as compared to control. Significant advancing of harvest together with prolongation of the harvest's period can be achieved by the use of 6 and 8-week-old transplants chilled for 2 weeks. Dark-chilling of 10-week-old transplants resulted in forming of smallest heads with the highest percentage of hollow stems.

Keywords: *Brassica oleracea* var. *botrytis*; dark-chilling; transplant age; cross-tolerance; stress memory

Broccoli is a moderate climate species originating from the Mediterranean region. Harvest maturity date, head yield and quality are all affected by climatic variations during the production cycle, particularly low and high temperature episodes. Its cultivation requires relatively low temperatures, about 14°C, combined with daily mean irradiation of 450 μmol/m^2·s (Iglesias-Acosta et al. 2010). In Poland the best conditions for broccoli growing are usually during early spring and autumn. Due to the fact that the vegetable markets of economically developed countries require a constant supply of fresh broccoli heads this vegetable is cultivated on an increasing scale also in summer, despite the fact that heat stress together with water deficiency in summer generates a great risk of loss of yield and its quality (Wurr et al. 1997). Heather et al. (1992) showed that the most sensitive stage of broccoli development is a phase of head diameter 5 to 10 mm, and heat stress that occurs at this stage resulted in reduction in head weight. According to Björkman and Pearson (1998) high temperature causes un-

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evenly-sized flower buds on broccoli inflorescences and therefore this deformity limits production of broccoli to areas where summer temperatures rarely exceed 30°C. In the aspect of broccoli production during the whole vegetation season many authors demonstrated the possibility of its cultivation in different climatic conditions (Kunicki et al. 1996; Rekowska, Słodkowski 2002; Sermenli et al. 2011).

Broccoli is commonly grown from transplants. Nowadays, high quality vegetable transplants are the market product and low-temperature storage in darkness is commercially used to preserve its quality standards (Sato et al. 2004). Grabowska et al. (2007, 2013) proved that broccoli transplants can be subjected to dark-chilling in order to modify the time of transplanting, yield level and inflorescence quality. Dark-chilling applied in a manner described by the above cited authors (2°C for 1–2 weeks) can be conducted for coordinating the supply of transplants to the market according to variable demands. Broccoli transplants of different age vary in response to dark chilling, because low temperature is a common factor determining the transition from the vegetative to generative stage of development. Grabowska et al. (2013) showed that dark-chilling for 2 weeks of 4-week-old transplants increased the percent of plants in a generative phase as compared to non-chilled control, whereas Kałużewicz et al. (2002) observed the initiation of a generative phase for older plants, depending on the time of cultivation. Kalisz et al. (2013) showed that chilling of transplants positively influenced the weight of broccoli heads in spring production, and the heaviest heads were obtained as a result of transplants exposure to 6°C.

The scientific problem of high novelty is the investigation of the existence and character 'cross tolerance' or 'global tolerance' in plants ontogeny against different stress factors. Plants use the common physiological pathways and biochemical components as a response against different stress factors. This phenomenon allows plants to adapt or acclimate to a wide range of stressors after exposure to one specific stress (Pastori, Foyer 2002). 'Global tolerance' lies in activation and inhibition of some groups of gens and physiological mechanisms which lead to an increase of resistance to stress caused by reactive oxygen species during plant ontogeny (Ball et al. 2004). The acquirement of resistance as a result of chilling seems to be a good research model of interaction of biotic and abiotic stresses during crop species development, affecting yield and biological value. This phenomenon was described only for early developmental stages of warm climate vegetables i.e. cucumber (Mangrich et al. 2006), tomato (Martinez-Andujar et al. 2011), eggplant (Sękara et al. 2012). It is still poorly described for vegetables of Brassicaceae family and there are no investigations in this area with such economically important species as broccoli is.

The aim of the present investigation was to assess how dark chilling stress, applied at juvenile stage of broccoli development, can impact yield quantity and chosen head quality parameters in summer field cultivation, characterized by heat stress conditions. It was determined which combination of chilling duration and age of transplants is the most efficient in increasing the tolerance to stress factors, which is a common denominator of success in crop production.

MATERIAL AND METHODS

Experiment design. The experiment was carried out in the years 2008–2010. The object of investigations was broccoli (*Brassica oleracea* L. var. *italica* Plenck) medium late cv. Monaco F_1. The examined factors were the age of transplants (4, 6, 8, and 10-week-old) and the dark-chilling lasting for 1 or 2 weeks; non-chilled plants of the above mentioned ages were considered as control. The seeds were sown to multipots PV 96 (VEFI, Drammen, Norway), transplants were grown in a greenhouse. Dates of seed sowing were calculated to obtain transplants of different age (4, 6, 8, and 10-week-old), which were an initial material. On June 17, 2008, June 27, 2009 and June 18, 2010 one part of the initial material was placed in a cold room and treated with dark-chilling (2°C) and the other part (control) was planted into the field at the Experimental Station of the University of Agriculture in Krakow, south Poland (50°04'N, 19°51'E). Transplants chilled for one week were transferred to the field on June 25, 2008, July 2, 2009 and June 26, 2010; and transplants chilled for two weeks on July 2, 2008, July 9, 2009 and July 3, 2010. The soil was classified as a typical brown type, grey brown subtype of stabilised fluvial alluvium, silt loam laying on medium-heavy soil, underlain by very fine sandy soil. The climate of the experimental station is humid continental climate according to the Köppen's classification. Broccoli was grown in spac-

Table 1. Mean monthly air temperature, sum of rainfall, and air relative humidity during the vegetation seasons 2008–2010

Month	Temperature (°C)			Sum of rainfall (mm)			Air relative humidity (%)		
	2008	2009	2010	2008	2009	2010	2008	2009	2010
June	18.7	15.5	17.7	23	128	96	60.3	61.4	63.3
July	18.9	19.5	20.9	134	83	111	79.4	84.9	79.1
August	18.6	18.9	18.8	47	53	97	82.3	52.5	89.2
September	12.8	15.1	12.5	65	35	92	93.7	80.7	94.3
October	9.9	7.6	5.6	53	82	12	95.7	96.5	92.1

ing of 67.5 × 40 cm, 30 plants per experimental plot, in the random block design, in four replications. The agronomic treatments were applied in accordance with the requirements of the species. The doses of fertilizers were calculated on the basis of soil analyses to achieve the content of N – 140 mg, P – 60 mg, K – 200 mg, Mg – 70 mg and Ca – 1,500 mg/dm^3 of soil.

Weather condition measurements. During the vegetation seasons, the air temperature, air relative humidity and sum of rainfalls were measured at 1-h interval by automatic sensors HOBO Pro RH/Temp. and HOBO Weather Station (Onset Comp. Corp., Bourne, USA), localized at the experimental plots. Data were elaborated as means for months and presented in Table 1.

Mean monthly temperature was slightly differentiated in the experimental years. June was by 3.2°C and 2.2°C colder in 2009 as compared with 2008 and 2010, respectively. In July and August, differences in temperature between experimental years did not exceed 2°C. September 2009 and October 2008 were characterized by highest mean temperatures in comparison with the same months remaining experimental years. In 2008, the highest sum of rainfall was noted in July, but September and October were characterized by the highest air relative humidity. In 2009, the highest sum of rainfall occurred in June, but the lowest air relative humidity was noted in August. June to September 2010 were characterized by uniform sum of rainfalls, and relative humidity between 63.3–94.3%.

Phenological phases course and yield characteristics. The course of phenological phases was assessed for each experimental object. The number of days from transplanting to head setting (when more than 50% of plants had a flower bud of 1 cm diameter) as well as to the beginning and the end of harvest was assessed. Heads were harvested (July 29–October 1, 2008; August 7–September 24, 2009; August 12–September 20, 2010) together with a floral stem of 10–15 cm length, depending on the head diameter. The ratio between the diameter of the head and the floral stem was not less than 2:1. Heads were divided into two quality classes according to UNECE Standard FFV-48 (2010).

The 1st class included compact heads with flower buds of a max. size 0.002 m and intensive green colour, without visible quality defects and empty spaces within the inflorescence stem. The 2nd class heads were less compact, with flower buds discoloration and empty spaces within the inflorescence stem. The 1st and 2nd class heads were treated as a marketable yield, the remaining heads, which did not meet the above mentioned requirements, were treated as an unmarketable yield. The total yield was defined as a sum of marketable and unmarketable yields. The mean weight of 1st and 2nd class heads was assessed. The percentage of stems with hollows in the marketable yield as well as the diameter and length of hollows on the basis of the inflorescence stem were measured on 30 plants per replication. The percentage of heads with hollow stems in the total yield was assessed.

Statistical analyses. The results were evaluated statistically with the ANOVA variance with the use of Statistica Ver. 10 package (StatSoft Inc., Tulsa, USA), and the Tukey's test was used to determine homogeneous groups at $P < 0.05$.

RESULTS

With the increase of the age of transplants smaller number of days passed to attain successive phenological phases (Table 2). Chilling of 4-week-old transplants for two weeks advanced the phase of head setting of 5 and 6 days as compared with the

Table 2. Effect of the age of broccoli transplants and chilling duration on the course of phenological phases in 2008–2010

Chilling duration (weeks)	Age of transplants (weeks)											
	4			6			8			10		
	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010
Number of days from transplanting to head setting and date of observation												
Control	51	51	52	48	48	49	41	33	47	30	26	41
	07.08.	17.08.	09.08.	04.08.	14.08.	06.08.	28.07.	30.07	04.08.	17.07.	23.07.	29.07.
1	54	51	52	52	44	52	34	26	44	34	19	38
	18.08.	21.08.	17.08.	16.08.	14.08.	17.08.	29.07.	28.07.	09.08.	28.07.	21.07.	03.08.
2	51	46	46	47	46	37	33	29	37	33	19	37
	22.08.	24.08.	18.08.	18.08.	24.08.	09.08.	04.08.	07.08.	09.08.	04.08.	28.07.	09.08.
Number of days from transplanting to beginning of harvest and date of observation												
Control	58	63	63	58	63	61	51	41	61	42	41	55
	14.08.	29.08.	20.08.	14.08.	29.08.	18.08.	07.08.	07.08.	18.08.	29.07.	07.08.	12.08.
1	69	70	68	63	65	60	44	34	58	44	34	47
	02.09.	09.09.	25.08.	27.08.	04.09.	25.08.	08.08.	04.08.	23.08.	07.08.	04.08.	12.08.
2	69	63	53	63	58	53	50	33	53	50	27	53
	09.09.	10.09.	03.09.	03.09.	05.09.	25.08.	21.08.	11.08.	25.08.	13.08.	05.08.	25.08.
Number of days from transplanting to the end of harvest and date of observation												
Control	79	77	70	79	77	71	72	69	70	67	69	71
	04.09.	12.09.	27.08.	04.09.	14.09.	27.08.	28.08.	04.09.	27.08.	22.08.	04.09.	28.08.
1	89	80	76	88	79	76	76	62	73	69	62	73
	20.09.	19.09.	10.09.	21.09.	12.09.	10.09.	09.09.	01.09.	07.09.	01.09.	01.09.	07.09.
2	91	77	79	90	72	73	78	69	73	78	55	73
	01.10.	24.09.	20.09.	30.09.	19.09.	14.09.	18.09.	16.09.	14.09.	10.09.	02.09.	14.09.
Length of harvest period (days)												
Control	21	14	7	21	14	10	21	28	9	25	28	16
1	20	10	8	25	14	16	32	28	15	25	28	26
2	22	14	26	27	14	20	28	36	20	28	28	20

control in 2009 and 2010, respectively, but this effect was not observed in further developmental phases. Chilling of 6- and 10-week-old transplants gave unrepeatable effect on the phenological phases course in successive experimental years. Chilling of 8-week-old transplants for one and two weeks resulted in advancement of head setting and beginning of harvest, but harvest period was longer in comparison to control plants. Generally, chilling of transplants, especially those older than 4 weeks, resulted in shortening of the period from transplanting to the phase of head initiation. In the case of transplants chilling for 1 week, advancing ranged from 3 to 7 days (excluding 4-week-old transplants in each year of the study, 6-week-old ones in 2008 and 10-week-old ones in 2010). However, in the case of chilling for 2 weeks, it was 1 to 12 days (with the exception of 4- and 10-week-old transplants in 2008).

Transplant chilling significantly changed the duration from transplanting to the first harvest but the effect was unrepeatable in experimental years, so a significant influence of meteorological conditions on the course of phenological phases can be

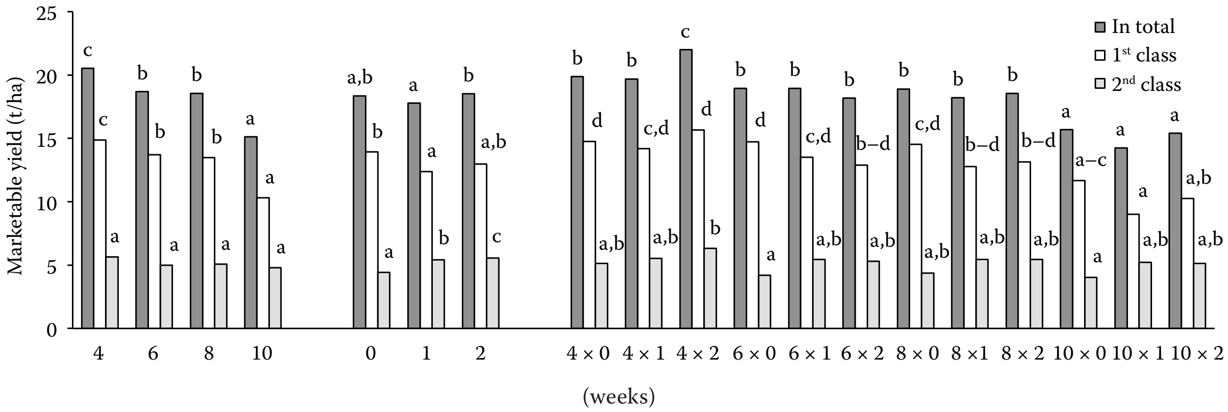

Fig. 1. Marketable yield of broccoli divided into first and second class (t/ha) as affected by the age of transplants (weeks) and chilling duration (weeks)

bars of the same colour marked with the same letter do not differ significantly at $P < 0.05$

concluded. The greatest delay of the first harvest of chilled plants was observed for 4-week-old transplants chilled for one week (11, 7 and 5 days in successive years), as compared with the control. In the case of 6-week-old transplants chilled for one week, the delay was noted only in 2008 and 2009 and it was equal 5 and 2 days, respectively. Control 8-week-old transplants as well as 10-week-old transplants in 2009 and 2010 reached the harvest maturity later than chilled ones.

Regardless of the chilling duration, the greatest yield was noted in the case of plants grown from 4-week-old transplants, and the smallest from 10-week-old transplants (Fig. 1). Independently of the transplant age, chilling generally had no significant effect on the marketable yield. The analysis of the interaction of experimental factors let show that chilling of 4-week-old transplants for two weeks resulted in greatest heads' yield, and the use of both chilled and control 10-week-old transplants resulted in lowest yield among all treatments.

The use of 10-week-old transplants in broccoli cultivation resulted in the lowest yield of 1st class heads. The most positive effect on the yield quantity had transplanting of 4-week-old plants. Regardless of the age of transplants, chilling for one week significantly decreased the yield of 1st class heads as compared with control. The analysis of the interaction showed significant differences between 1st class heads' yield from 10-week-old transplants chilled for 1 week (lowest yield) to 4-, 6-, and 8-week-old transplants, both control and chilled (greatest yield). The age of transplants had not significant effect in the yield of 2nd class heads but, independently of the transplant age, chilling increased the yield of such quality heads. Taking

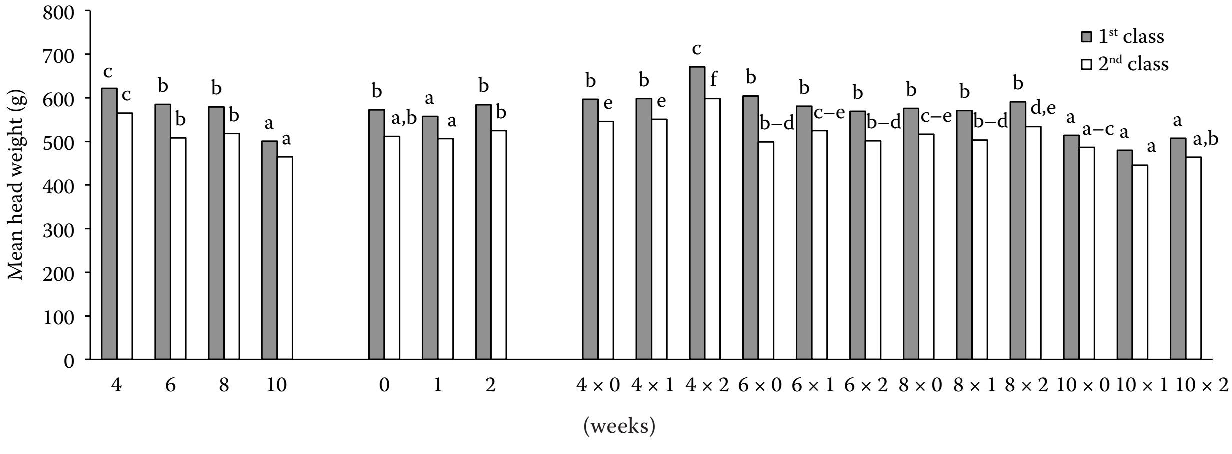

Fig. 2. Mean broccoli head weight (g) of 1st and 2nd class as affected by the age of transplants (weeks) and chilling duration (weeks)

bars of the same colour marked with the same letter do not differ significantly at $P < 0.05$

Table 3. Percentage of hollow stem depending on the age of transplants and chilling duration

Chilling duration (weeks)	Age of transplants (weeks)				Mean for chilling
	4	6	8	10	
0	12.86	35.81	36.94	45.95	32.89
1	39.73	44.18	43.61	55.84	45.84
2	20.28	37.50	48.33	63.34	42.36
Mean for age	24.29	39.16	42.96	55.04	

into consideration the interaction of experimental factors, the lowest yield of 2nd class heads was harvested from control 6- and 10-week-old transplants as compared to 4-week-old transplants chilled for two weeks.

The age of transplants had a significant effect on the 1st and 2nd class heads' weight (Fig. 2). The greatest 1st and 2nd class heads were harvested from 4-week-old transplants, the smallest – from 10-week-old ones. Taking into consideration the chilling duration, the non-chilled transplants and those chilled for two weeks formed the greatest 1st class heads. Analysis of interaction showed significant differences in 1st and 2nd class heads weight between 10-week-old transplants, both control and chilled and that received for all younger ones regardless of chilling treatments. For 2nd class, clear significant differences were noticed only between heads from 4-week-old transplants and 10-week-old ones (chilled or not).

The highest percentage of heads with hollow stem (Table 3) was observed in the case of plants gained from the oldest transplants (55.0%) and the lowest – from the youngest ones (24.3%). Chilling increased the percentage of stems with hollows regardless of transplants age. Taking into consideration the interaction of experimental factors, the highest percentage of hollow stem was found in plants obtained from 10-week-old transplants chilled for 2 weeks (63.3%).

Stem hollow length and diameter depended significantly on the age of transplants (Fig. 3). Heads of shortest caves in the stem with smallest diameter were formed by 4-week-old transplants. Longer period of transplants production resulted in a significant increase of hollow stem length but its diameter was significantly higher only for 10-week-old transplants as compared to 4- and 6-week-old ones. Regardless of the age of transplants, chilling for one week significantly increased the length of hollows in the stem in comparison to control and chilling for two weeks treatment. Taking into consideration the chilling duration, it had no effect on hollow stem diameter. Analysis of interaction showed that 4-week-old transplants, both control and chilled, were characterized by significantly shorter stem hollows as compared to 6-week-old transplants chilled for one week and 10-week-old, both control and chilled. 4-week-old transplants control or chilled for one week formed heads with

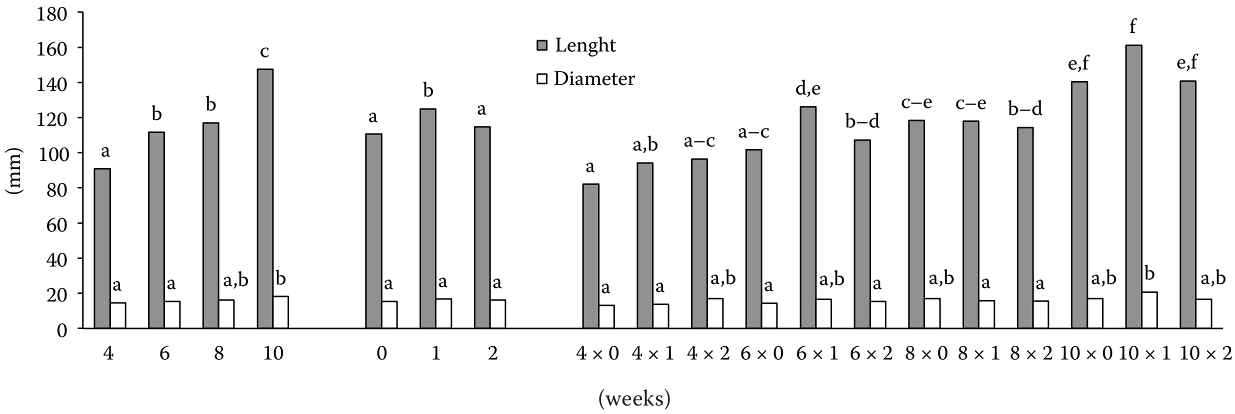

Fig. 3. Hollow stem in length and diameter (mm) in broccoli heads as affected by the age of transplants (weeks) and chilling duration (weeks)

bars of the same colour marked with the same letter do not differ significantly at $P < 0.05$

significantly lower diameter of stem hollows compared to 10-week-old transplants, chilled for one week.

DISCUSSION

Depending on the period of the vegetation season, the recommended age of the broccoli transplants for cultivation in the temperate climatic zone ranges from 4 to 7 weeks (Vavrina 1998). Planting of younger as well as older transplants can be a reason of premature heads forming, so called buttoning, which significantly decreases the marketable yield. Fritz et al. (2009) stated that buttoning can be also induced by improper hardening conditions, cold weather for more than 10 days at 4–10°C, diseases, insects or micronutrient deficiencies. It was not observed in the present experiment, so broccoli transplants of different age, chilled or not, did not negatively react to high temperatures during summer production and did not formed premature, small, unmarketable heads.

Low temperature storage in darkness is used as a holding method for numerous plants species (Heins et al. 1992; Kaczperski, Armitage 1992; Sato et al. 2004). In the present experiment, applying of dark-chilling stress to broccoli transplants of different age did not gave clear and stable effect on the course of phenological phases. Grabowska et al. (2013) observed the broccoli inflorescence's initiation between 4 and 6 week of the plant ontogeny. 4-week-old transplants chilled for two weeks, were more advanced in the generative development in comparison to non-chilled control. In the conditions of the present experiment, chilling of 4-week-old transplants for two weeks caused the advancing of head setting by 5–6 days as compared to non-chilled control; this acceleration was also noted at the beginning of harvest but only in 2010. Only in the case of 8-week-old transplants the advance of head setting and maturing was noted in all years of investigations, so this treatment can be taken into consideration as the potential way of early yield increase and elongation of harvest period. It can be useful for producers who supply the market in small batch of goods for longer period. The use of younger (4- to 6-week-old) transplants had no repeatable effect on the term of heads' maturation and harvest period. Chilling did not seem to be an effective factor to influence 4- to 6-week-old transplants' development during summer production in field conditions of Central Europe.

Marketable yield decreased with the increase of transplant age. The greatest yield of 1st and 2nd class heads was assessed at 4-week-old transplants, which is in accordance with the results of Babik (2000), Grabowska et al. (2007), Kaymak et al. (2009), and Todorova (2011). Our experiment showed that high yield can be achieved also from 6 and 8-week-old transplants, but additional effect is advancing of harvest maturity and elongation of harvest duration. Jones et al. (1991) observed the yield increase by the use of 49-day-old transplants of cauliflower but in the case of broccoli, transplant age did not influence early and total yield. Preliminary experiment of Wlazło and Kunicki (2003), conducted in similar conditions, showed higher yielding potential of 8-week-old transplants as compared to 4-, and 6-week-old ones, regardless of the term of production. Babik (2000) showed lowest yielding of 10-week-old transplants, which is in accordance with the present results. Low quality of heads formed from 10-week-old transplants was precisely described on the basis of its morphology by Grabowska et al. (2013).

Lamont (1992) stated that transplant age did not significantly influence the marketable quality of broccoli heads at harvest, because the old transplants resumed active growth after planting and at harvest could not be visually distinguished from plants grown from normal-aged transplants. Kalisz et al. (2013) stated that significantly higher weight of broccoli heads was observed at plants chilled in 6°C (1 and 2 weeks) than in control in a stage of 3.5- and 4.5-week-old transplants. In present investigations 4-week-old transplants chilled by two weeks, formed heads of the greatest weight. The use of 10-week-old transplants, both chilled and non-chilled control, resulted in a significant decrease of head's weight.

The presence of hollow stem was observed for all plants used in the experiment. However boron deficiency is widely accepted as the main cause of hollow stem in broccoli, Boersma at al. (2009) hypothesized that also the high growth rate of plants is involved in the development of hollow stem. In the next study, Boersma at al. (2013) compared the development of hollow stem in plants grown in the field at standard commercial density and at high density. All plants grown at commercial density developed hollow stem just after inflorescence initiation, together with the stem rapid enlargement and starch content decrease. This may explain that the longer and larger hollows in the head stems in this research observed in plants were obtained from the oldest transplants which showed the shortest period from planting to harvest. Chilling increased the percentage of hollows in all investigated treatments but this factor seemed to have a less significant effect as compared to transplant age.

The reaction of broccoli transplants to dark-chilling is still not comprehensively elaborated, so the effect application of this method to other cultivars or growing periods needs future investigations. On the basis of effects of the present experiment unstable in years, we can only suggest the need of precise validation of our results in subsequent experiments on other genotypes and environmental conditions.

CONCLUSIONS

Broccoli cv. Monaco F_1 can be recommended for summer production in Poland, because of high yield potential and lack of buttoning in high temperature conditions. Present research showed that dark chilling for 1 and 2 weeks at 2°C can be adapted for broccoli transplants of different age for control of their yield potential. Dark-chilling of 4-week-old transplants for two weeks significantly increased the marketable yield, but also increased the percentage of stems with hollows in comparison with non-chilled control. A significant advance of harvest together with prolongation of the harvest's period can be achieved by the use of 8-week-old transplants chilled for one or two weeks. We expected 'cross-tolerance' of broccoli for heat stress during summer production as an effect of chilling in a stage of transplants but present results did not show clearly that the existence of this phenomenon depended on a stage of plant development. The novelty of the present article is showing that transplants quality can be controlled by dark-chilling in different ways, depending on transplant age. Dark-chilling can be applied for effective transplant storage and transportation.

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