Effect of chilling on total antioxidant capacity and growth processes of basil (*Ocimum basilicum* L.) cultivars

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S u m m a r y

Total antioxidant capacity (TAC, assayed by FRAP method) of three basil (*Ocimum basilicum* L.) cultivars subjected to chilling stress (10°C) and at the recovery stage was studied. To estimate the influence of temperature on the conditions of plants, the biometric parameters and leaf water content were measured.
It was established that chill does not influence significantly growth of basil plants and leaf water content, but decreases TAC of leaves of 'Sweet Green Genovese' and 'Kasia' cvs. plants. Plants of Polish cultivars 'Kasia' and 'Wala' have a bigger biomass production by individual plant than the Dutch cultivar 'Sweet Green Genovese'. C. var. 'Wala' has the biggest productivity. However, TAC of its leaves is the lowest, whereas 'Sweet Green Genovese' c.var. plants give low biomass, but of high reducing capacity.

Key words: basil, total antioxidant capacity, chilling stress, plant productivity

Chilling periods with the temperature of 0 to 15°C are very frequent in Central and Eastern Europe. The effect of chilling on plants is different from that of frost because ice nucleation does not occur in cells. However, numerous species of crops are sensitive to such conditions which may affect growth, development and, in consequence, limit the yield.

Basil belongs to such thermophilic species. Temperature alterations may influence the term of anthesis, number of anthodia, and the content of essential oil as well as its composition [1]. The optimal temperature for basil growth and development is ca. 20–25°C. When the temperature drops to 1–2°C, plants die.

The theories explaining the phenomenon of chilling stress state that chill affects membranes directly [2] and indirectly, e.g. by maintaining transpiration where-

as hydraulic conductivity of roots is impaired, which results in so-called physiological drought observed as wilting, although the soil is properly watered [3].

Chilling stress *per se* and physiological drought are also accompanied by oxidative stress, namely the overproduction of oxidizing molecules – oxygen radicals, hydrogen peroxide, singlet oxygen and other so-called reactive oxygen species (ROS [4]). Plant cells are protected from ROS by numerous low-molecular-weight and enzymatic protections which a) prevent ROS formation, b) act as the scavengers, and c) repair the molecules damaged by radical attack. The interaction of various antioxidants implies better protection from radical injury than the protective properties of individual antioxidant [5], both for the plants themselves, but also for the consumers.

The parameter named Total Antioxidant Capacity (TAC, synonyms: Total Antioxidant Power, Total Antioxidant Potential, Total Antioxidant Efficiency) determines such interaction of antioxidants in biological sample, providing information about its redox status [5]. The main advantage of this assay is the possibility to determine all antioxidants and reduced compounds in the plant extract [6]. There are several methods of measuring TAC. One of them, Ferric Reducing Antioxidant Power (FRAP), is the most accurate and relatively fast. It also can be used for comparison of the redox status of various plant samples [7, 8].

Taking into account all these data, the aim of the study was to assess total antioxidant capacity of leaves of three basil cultivars subjected to chilling stress (10°C), on physiological background estimated as biomass production and water content. It was borne in mind that the restriction of shoot elongation during chilling is a primary plant response to stress, and the rate of regrowth after a period of unfavorable conditions reflect the resistance of plants [9, 10]. The usefulness of total antioxidant capacity parameter was considered from plant physiologist's, human nutritionist's, and breeder's point of view.

MATERIALS AND METHODS

Plant material

Three cultivars of basil (*Ocimum basilicum* L.) were used: two Polish cultivars, 'Kasia' and 'Wala', bred in Research Institute of Medicinal Plants in Poznań [11], and the popular Dutch cultivar 'Sweet Green Genovese'.

Vegetation period

Plants were grown in a phytotron of Agricultural University in Kraków, in a greenhouse, in pots of ca. 5 dm^3 of volume, containing organic soil + peat (pH 5,5–6,5) + sand; ratio 3:1:1 (v/v/v). The temperature of 17/25°C (day/night), relative humidity (RH) of ca. 50% and natural illumination with the photoperiod (12/12 h) were kept. Additional lighting on cloudy days was performed to maintain the

minimal value of photosynthetic photon flux density (PPFD) at the level of 400 mmol(quantum) m^{-2} s^{-1}. Plants were watered according to their demand, and fertilized once per week.

Chilling treatment

3-month-old plants initiating anthesis were transferred into growth chambers and lasted for 3 days at 20/17°C (day/night) to allow them to adapt to the new conditions. Then plants were divided into chilled (10/10°C) and control group (20/20°C). RH, photoperiod and PPFD were kept as they were before. After 6 days of the treatment, plants were transferred back to the greenhouse (recovery stage; the conditions as before chilling).

Measurements and analyses

For dry mass quantitation, fresh mass of leaves was recorded immediately after cutting, then the tissue was dried to the stable mass (70°C, 24 h), kept in a presence of silica gel for 24 h, and then weighed again (scale Radwag, Poland, d=0.001 g). For each case the leaf from the 4th node (counting from the top of the plant) was taken. Measurements were performed in 14–21 biological (individual leaf from individual plant) replicates.

Total Antioxidant Capacity (TAC) was assayed as Ferric Reducing Antioxidant Power (FRAP) [12, 13] with minor own modifications. In each case the leaf from the 4th node (counting from the top of the plant) was taken. Leaf samples were frozen in liquid nitrogen, and then kept at –80°C until preparation. Samples were homogenized with a mortar (kept on ice) and a pestle, in 80% ethanol in water (v/v), in 1:10 ratio (fresh weight of leaf: cm^3 of ethanol). Homogenate was centrifuged in Eppendorf-type tubes, at 15 000 g and 4°C for 10 min. Supernatant was collected and kept in new series of tubes, on ice, in darkness. FRAP of each sample (50 ml) was immediately assayed spectrophotometrically (LKB Biochrom 4050, UK) at λ=593 nm, as a reduction of ferric tripyridyl-S-triazine (Fe(III)-TPTZ) complex to ferrous tripyridyl-S-triazine (Fe(II)-TPTZ). Calibration curves were performed for standard antioxidants: Trolox – water-soluble analogue of vitamin E (0.3125 – 10 mmol·dm^{-3}) and ascorbic acid – vitamin C, AsA (0.125 – 1 mmol·dm^{-3}). Analyses were performed in 7 biological (individual leaf from individual plant) replicates, and each replicate was assayed at least twice.

Statistical analysis

The statistical significance of differences was evaluated by variance analysis supported by the Duncan multiple range test (for 4 means within the cultivar) or the Student t-test (for 2 means within the experimental stage).

RESULTS

Neither growth of the main shoot nor new leaf formation was influenced directly by chilling (fig. 1a, 1b). Even the differences in the shoot length for chilled and control plants in case of 'Wala' c. var. were not significant, as both the t-Student test (data not shown) and Duncan test revealed (fig. 1a). However, the strong varietal effect was established. Plants of c. var. 'Sweet Green Genovese' had the shortest shoots (ca. 13 cm, fig. 1a.), when plants of c. var. 'Wala' formed the longest ones (30–40 cm), and c. var. 'Kasia' a little shorter (28–31 cm). Similar relations were obtained for the number of leaves formed by individual plant (fig. 1b). 'Sweet Green Genovese' c. var. had ca. 20 leaves per plant, 'Wala' 70–80, 'Kasia' 72.

During visual observations performed at both stages of the experiment, no symptoms of wilting were noticed. The assay of leaf water content also revealed no changes in response to chilling (table 1; ca. 90% of leaf fresh weight), similarly to growth processes described above.

Table 1.

Water content in leaves of basil on the 5th day of chilling and after 7-day recovery. Means of n=6 ± standard error.

	Water content [% of fresh weigh] ± standard error		
cultivar	'Kasia'	'Wala'	'Sweet Green Genovese'
control plants, chilling stage (20°C)	89.3 ± 0.45	88.5 ± 1.11	87.8 ± 0.73
chilled plants, chilling stage (10°C)	88.1 ± 0.90	89.1 ± 0.49	88.6 ± 0.37
control plants, recovery stage (25/17°C)	90.3 ± 0.43	89.5 ± 0.81	87.8 ± 0.58
chilled plants, recovery stage (25/17°C)	89.8 ± 0.83	90.3 ± 0.61	89.6 ± 0.38

In contrary to plant growth processes and leaf water status, total antioxidant capacity (TAC) of leaves was decreased by chilling, but it was dependent on a) the cultivar, b) standard antioxidant (Trolox or ascorbic acid) and c) basis of calculation (fig. 2 and 3). Moreover, the differences between control and chilled plants were significant or not upon the experimental stage (chilling or recovery).

TAC of 'Wala' c. var. leaves was not affected, whereas TAC of 'Sweet Green Genovese' revealed a strong tendency to be diminished by low temperature treatment, and the differences between means were significant for the fresh weight of individual leaf (fig. 2b and 3b). Interestingly, these differences were often bigger at the recovery stage when plants were re-warmed than at the chilling stage itself (fig. 2 and 3). Chilling also diminished TAC of c. var. 'Kasia' leaves, but this was significant in less cases than it was established for 'Sweet Green Genovese' (fig. 2 and 3). However, similarly to 'Sweet Green Genovese', TAC of 'Kasia' was influenced mainly during the recovery.

Constitutive reducing capacity (for non-chilled plants, at the chilling stage) of both standardized fresh weight and individual leaves was strongly affected by the genotype. The highest values were obtained for 'Sweet Green Genovese', and the lowest for 'Wala' c. var. (fig. 2 and 3), irrespective of the equivalent used. During ontogenesis, TAC often increased, and this influenced the varietal comparison. At the recovery stage the means for both chilled and control plants of 'Kasia' c. var. were at least at the level of 'Sweet Green Genovese' c. var. means. However, this effect was restricted to TAC expressed as AsA equivalents (fig. 3a and b).

DISCUSSION

In this work we established that 6-day chilling treatment (10°C) does not influence the growth processes significantly, but it may affect leaf antioxidant properties. The lack of the growth response suggests the resistance of plants to such conditions, as inhibition of growth of various parts of plants is often observed during chilling, because low temperature negatively affects cell elongation and division [14]. Additionally, those processes depend on water content. In this experiment, the duration of chilling (5 days) was probably too short, and/or the chill was not strong enough to trigger big differences in water status and elongation itself between control and chilled plants.

We hypothesise the protective role of phytohormone abscisic acid (ABA). Low temperature may increase ABA content in roots, where water status is gradually changed [15]. Then, ABA fluxes in the xylem pathway via shoots to leaves. In leaves, it migrates from chloroplasts to cytosol, and then to guard cells triggering stomatal closure and inhibition of water loss [16].

The biggest differences in studied parameters are caused by the genotype. Plants of Polish cultivars 'Kasia' and 'Wala' are characterized by much longer main shoots and the number of leaves formed by individual plant than the Dutch cultivar 'Sweet Green Genovese'. However, low productivity of this cultivar is compensated for high antioxidant capacity, in contrast to 'Wala' plants which form large biomass but of relatively low TAC. Additionally, TAC often increases at the recovery stage which may reflect some adaptive metabolic changes.

We hope that presented data may be interesting for producers and consumers. The basis of TAC calculation for individual leaf may be more informative to the consumer than the comparison of TAC for standardized leaf weight. From the physiologist's and breeder's point of view, we can also discuss whether antioxidant status and high biomass production are negatively correlated. This would have required the implementation of other methods of total antioxidant capacity measurement. It must be taken into consideration that use of FRAP method reducing capacity based on ferric ions reduction is only assayed [8]. However, the direct comparison of antioxidant properties of cultivars is possible and often performed for commercial use [7, 8].

Irrespectively to this controversy, the results of this experiment reveal that for those who are interested in antioxidant properties of basil more than the biomass itself, 'Sweet Green Genovese' may be recommended. However, both studied Polish cultivars: 'Kasia' and 'Wala' are well-adapted to Polish climatic conditions and give a high yield of raw material. This is confirmed by the direct breeding studies [11, 17].

CONCLUSIONS

1. Chill (10°C) does not significantly influence the growth of basil plants and leaf water content, but there are big varietal differences. Plants of Polish cultivars 'Kasia' and 'Wala' make a bigger biomass than plants of the Dutch cultivar 'Sweet Green Genovese'.
2. Chill decreases (directly and/or indirectly) the total antioxidant capacity (TAC) of 'Sweet Green Genovese' and 'Kasia' cvs. plants. TAC of c. var. 'Wala' is not affected by the treatment, but its values are the lowest.
3. C. var. 'Wala' forms the highest biomass, although TAC of its plants is low, whereas 'Sweet Green Genovese' c. var. plants are characterized by low biomass, but of high reducing capacity.

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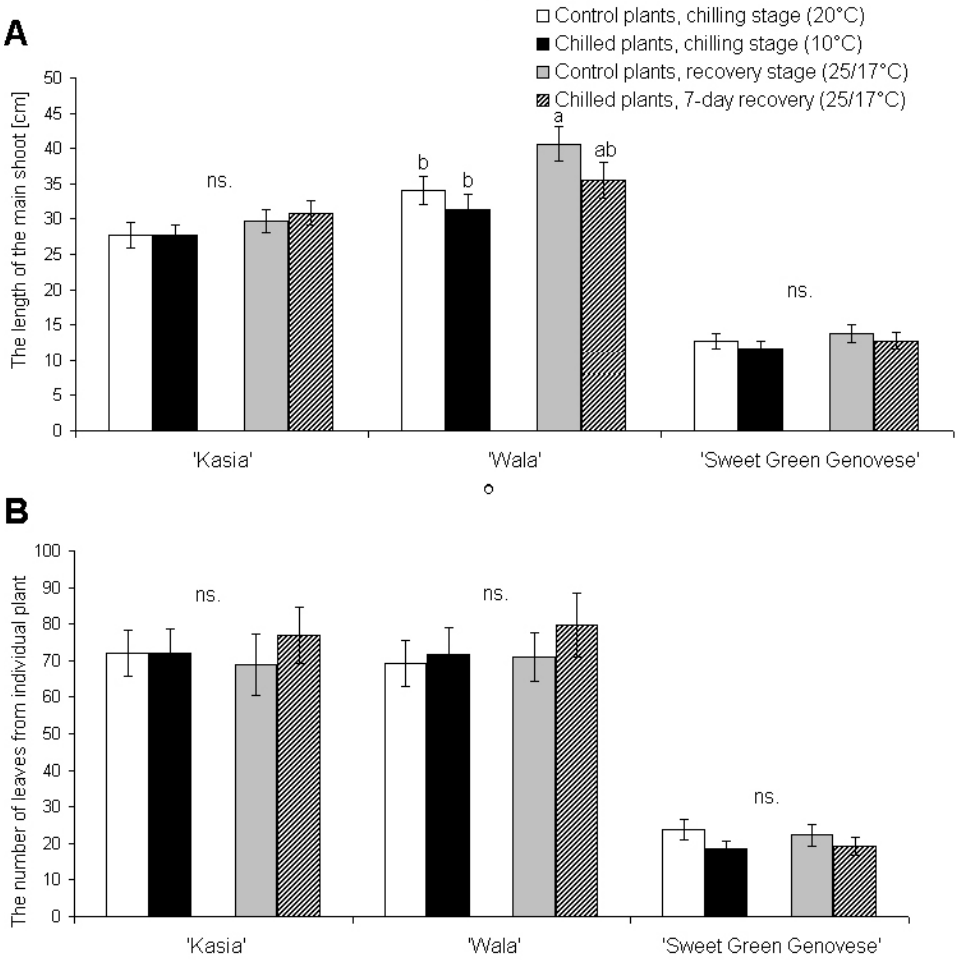

Fig. 1. The length of the main shoot of basil plants (A) and the number of leaves from individual plant (B) on the 5th day of chilling and after 7-day recovery. Means of n=14–21 ± standard error. Means within the cultivar marked with the same letter do not differ significantly ($p<0.05$) according to Duncan multiple range test.

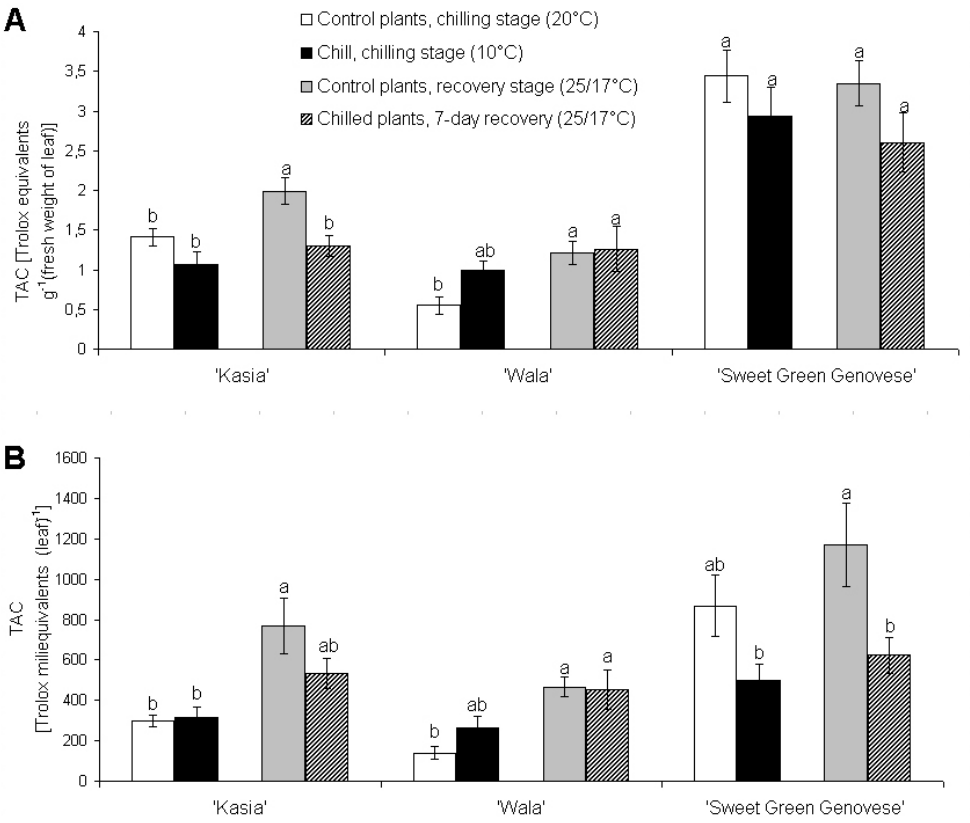

Fig. 2. Total antioxidant capacity expressed as Trolox equivalents related to 1 g of fresh weight of leaves (A) and weight of individual leaf (B) of basil, assayed on the 5th day of chilling and after 7-day recovery. Means of n=7 ± standard error. Means within the cultivar marked with the same letter do not differ significantly ($p<0.05$) according to Duncan multiple range test.

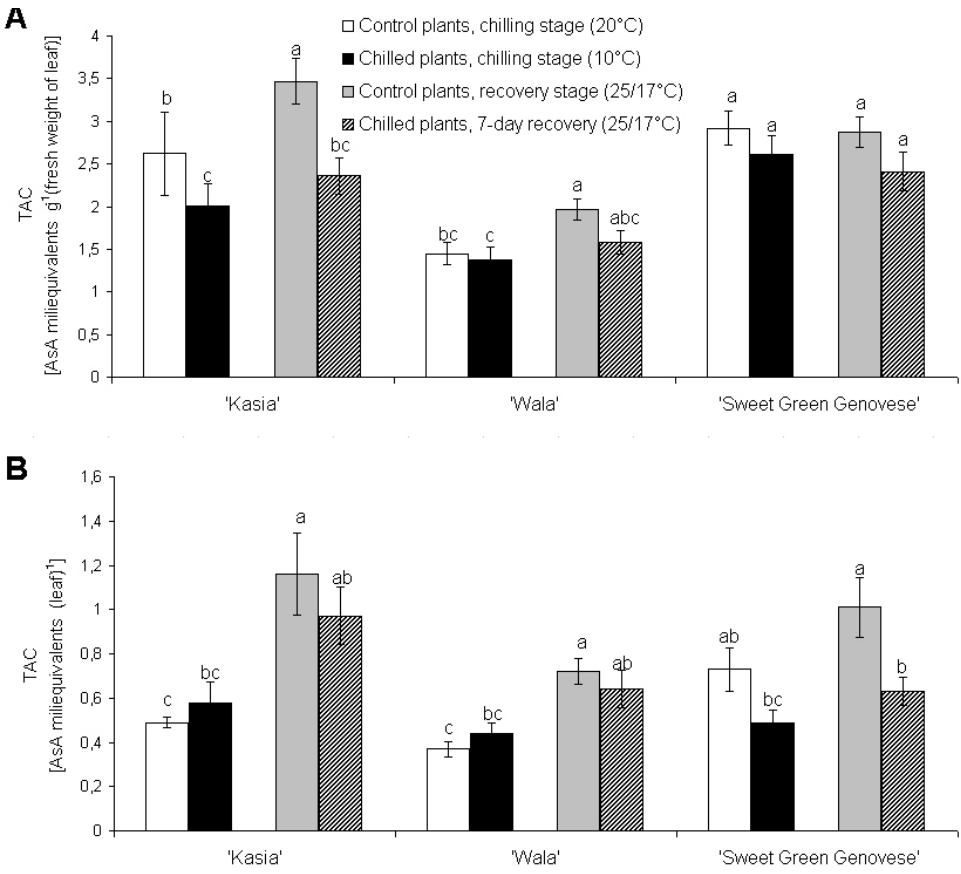

Fig. 3. Total antioxidant capacity expressed as ascorbic acid (vitamin C) equivalents related to 1 g of fresh weight of leaves (A) and fresh weight of individual leaf (B) of basil, assayed on the 5th day of chilling and after 7-day recovery. Means of n=7 ± standard error. Means within the cultivar marked with the same letter do not differ significantly ($p<0.05$) according to Duncan multiple range test.

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WPŁYW CHŁODZENIA NA CAŁKOWITĄ ZDOLNOŚĆ ANTYOKSYDACYJNĄ I PROCESY WZROSTOWE ODMIAN BAZYLII POSPOLITEJ (*OCIMUM BASILICUM* L.)

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S t r e s z c z e n i e

Badano całkowitą zdolność antyoksydacyjną (TAC, oznaczoną metodą FRAP) roślin trzech odmian bazylii właściwej (*Ocimum basilicum* L.) poddanych stresowi chłodowemu (10°C) oraz po jego ustąpieniu. W celu ustalenia stopnia wpływu temperatury na kondycję roślin analizowano parametry biometryczne oraz zawartość wody w liściach.
Stwierdzono, że chłód nie wpływa istotnie na procesy wzrostowe roślin ani na uwodnienie liści, natomiast obniża TAC liści w przypadku odmian 'Sweet Green Genovese' i 'Kasia'. Rośliny polskich odmian 'Kasia' i 'Wala' charakteryzują się większą produkcją biomasy niż holenderska odmiana 'Sweet Green Genovese'. Odmiana 'Wala' jest najbardziej produktywna, ale TAC jej liści jest najniższa, natomiast rośliny odmiany 'Sweet Green Genovese' wytwarzają najmniej biomasy, aczkolwiek o wysokiej zdolności redukcyjnej.

Słowa kluczowe: bazylia, całkowita zdolność antyoksydacyjna, stres chłodowy, produktywność roślin