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# Content of chloroplast pigments and anthocyanins in the leaves of *Ocimum basilicum* L. depending on nitrogen doses

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ABSTRACT

The effect of fertilization with different nitrogen doses (0, 1.35 and 1.80 g N per plant) and different harvest times (beginning of blooming and in full bloom) on the content of chlorophyll a and b, carotenoids and anthocyanins in the leaves of sweet basil 'Wala' and 'Dark Opal' were investigated. It has been found that the level of nitrogen fertilization as well as the harvest time and the cultivar have a significant effect on the content of chloroplast pigments, but they exert no influence on the synthesis of anthocyanins. The highest leaf yield was obtained in the case of later harvest and with the use of 1.35 g N per plant (0.9 + 0.45).

## INTRODUCTION

In the wide assortments of herbal plants, sweet basil is becoming more and more important. The production of high yield with high biological value requires, among others, the use of suitable fertilization and the right time of harvest. The yield depends to a large degree on the content of photosynthetically active pigments. Authors of numerous papers showed a close correlation between the level of these pigments and nitrogen content in leaves determined by the dose and time of fertilization (Biczak et al. 1998, Smith 1999, Baghour et al. 2000, Swiader and Moore 2002).

Anthocyanins are coloured flavonoids occurring mainly in flowers and fruits. They may also be present in leaves. The accumulation of anthocyanins is observed under conditions of nitrogen deficiency, since carbohydrates not used in nitrogen metabolism can be used in the synthesis of these pigments (Taiz and Zeiger 1998).

The aim of the undertaken studies was to determine the effect of different nitrogen doses of fertilization and the effect of harvest time on the content of chloroplast and anthocyanin pigments in sweet basil leaves.

## MATERIAL AND METHODS

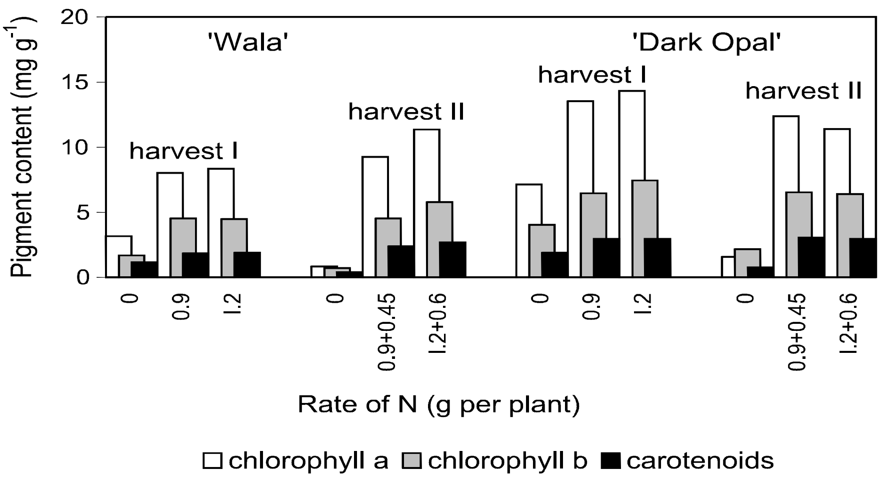
Two sweet basil cultivars – ‘Wala’ (green-leafed) and ‘Dark Opal’ (anthocyanin-tinted) - were grown in the years 2001 and 2002 in an unheated greenhouse in 5 $dm^3$ pots filled with substrate (mineral soil with raised peat in 4 : 1 proportion v/v). In the phase of 4-5 leaves and ±10-12 cm height the plants were planted into pots (1 plant per pot). The fertility of substrate (mean of two years) was (mg $dm^{-3}$ substrate): 36 ($N\text{-}NO_3$ + $N\text{-}NH_4$), 68 P, 116 K, 306 Ca, 49 Mg, 22 Na, 68 $S\text{-}SO_4$. The salinity was 0.24 g NaCl $dm^{-3}$ substrate and pH $_{H_2O}$ = 6.8. Nitrogen in the form of $NH_4NO_3$ was applied in two different doses - 0.9 and 1.2 g N prior to vegetation and after the first harvest in two other doses - 0.45 and 0.6 g N per plant, i.e. 1.35 g N (0.9 + 0.45) and 1.8 g N (1.2 + 0.6) per plant. The combination without nitrogen constituted the control. The remaining macro- and microelements were the background of the experiment. The studied plants were harvested at the beginning of blooming (harvest I) and in full bloom (harvest II), and the yield of air-dried leaves was estimated.

The leaves were preliminarily treated with hot water to remove anthocyanins, after which they were extracted by acetone to determine spectrophotometrically the content of chlorophyll a and b content as well as carotenoid content using the method of Arnon (1960). The leaf content of anthocyanins was determined using the method of Fuleki and Francis (1968) permitting their extraction from the leaves by 0.1 N HCl after which their absorbance in pH 1.0 and 4.5 at $\lambda$ = 510 nm was measured. The content of anthocyanins was calculated from the difference of measurements using the absorbance coefficient 77.5 determined by Fuleki and Francis (1968) and being the mean for four anthocyanins (cyanidin-3-galactoside, cyanidin-3-arabinoside, peonidin-3-galactoside, and peonidin-3-arabinoside). The content of chloroplast pigments was determined in 3 replications and that of anthocyanin pigments - in 6 replications.

The obtained results were statistically elaborated by the method of the analysis of variance and regression analysis.

## RESULTS

It was found that nitrogen fertilization had a significant effect on the increase of the content of all chloroplast pigments – both chlorophylls and carotenoids (Fig. 1). The regression analysis showed a positive correlation between the level of nitrogen fertilization and the content of these pigments (Fig. 3). ‘Dark Opal’ was characterized by a higher content of all chloroplast pigments in comparison with ‘Wala’. This relationship became evident especially in the initial period of sweet basil blooming (harvest I), whereas in full bloom (harvest II) this difference faded away.




| $LSD_{0.05}$ for: | chlorophyll a | chlorophyll b | carotenoids |
|---|---|---|---|
| cultivar x N fertilization | 0.95 | 1.01 | 0.24 |
| cultivar x harvest time | 0.86 | 0.95 | 0.24 |
| cultivar x N fertilization x harvest time | 1.23 | 1.09 | 0.51 |

Figure 1. The content of chloroplast pigments in sweet basil leaves

The presence of anthocyanins was found only in the leaves of 'Dark Opal' (Fig. 2). Statistical analyses showed no significant differences of these pigments in their dependence either on the nitrogen dose, or on the harvest time.

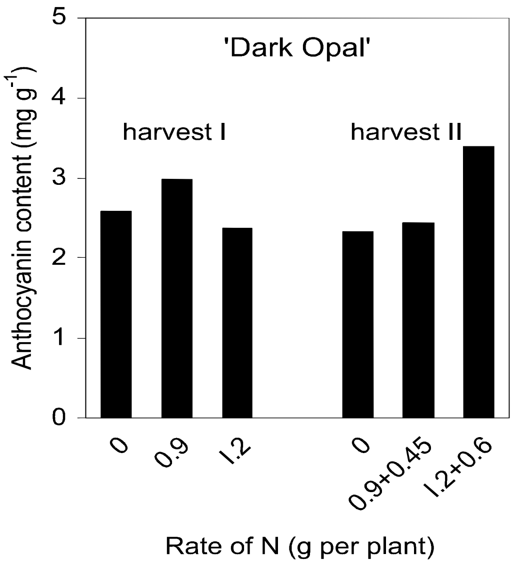



Figure 2. The content of anthocyanins in sweet basil leaves of 'Dark Opal'. There are no significant differences for harvest time and nitrogen rates

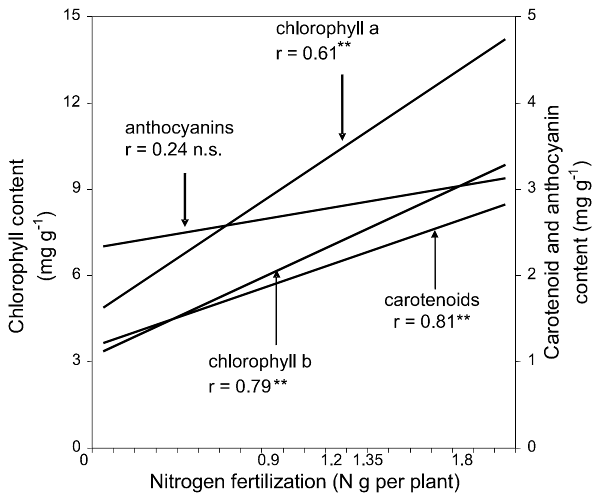



Figure 3. Correlations between the nitrogen fertilization rate and the content of pigments in sweet basil leaves

The yield of air-dried leaves (Table 1) was by about 100% higher in ‘Wala’ in comparison with ‘Dark Opal’. The leaf yield of both cultivars at the later harvest time was 2-3-fold higher than that from the earlier harvest. The most effective N dose regarding the yield was the dose of 1.35 g N per plant (0.9 + 0.45).

Table 1. Yield of air-dried leaves of sweet basil (g per plant)

| Cultivar | Harvest time and developmental stage | Rates of N applied prior to vegetation and in top-dressing fertilization (g per plant) | | | |
|---|---|---|---|---|---|
| | | 0.0 | 0.9 + 0.45* | 1.2 + 0.60* | Mean |
| ‘Wala’ | I – start of blooming | 3.38 | 5.63 | 5.42 | 4.81 |
| | II – in fool bloom | 3.02 | 15.96* | 13.51* | 10.83 |
| | Mean | 3.20 | 10.79 | 9.46 | |
| ‘Dark Opal’ | I – start of blooming | 2.29 | 2.30 | 2.50 | 2.36 |
| | II – in full bloom | 3.17 | 7.21* | 6.52* | 5.63 |
| | Mean | 2.73 | 4.75 | 4.51 | |
| $LSD_{0.05}$ for: cultivar x N fertilization | | 1.06 | | | |
| cultivar x harvest time | | 0.98 | | | |
| cultivar x N fertilization x harvest time | | 2.03 | | | |

* the nitrogen rate in top-dressing

## DISCUSSION

Most of the results obtained in the described pot experiment are in agreement with those reported by other authors for other species (Biczak et al. 1998, Smith 1999, Baghour et al. 2000, Swiader and Moore 2002). Basil yield depended on the level of photosynthetic active pigments, the content of which was positively correlated with nitrogen fertilization. However, the applied higher nitrogen doses had no significant effect on the increase of chloroplast pigments in basil plants and in the majority of cases they showed a tendency to the yield reduction. A similar dependence was observed in celery grown using different nitrogen doses (Biczak et al. 1998). Independent of the basil cultivar, the content of chlorophyll a was, on the average, twofold higher than that of chlorophyll b. Such proportions were found in many plant species, whereby there is usually 2-4 times more of chlorophyll a than chlorophyll b (Carter and Spiering 2002).

The yield of air-dried leaves was twofold higher in ‘Wala’ in comparison with ‘Dark Opal’. A similar and even greater difference in yield between green-leafed and purple-leafed form of basil was found by Nurzyńska-Wierdak (2002). Comparing the yield and the content of chloroplast pigments in the leaves of both studied cultivars, attention should also be drawn to the fact that ‘Dark Opal’ had a higher chlorophyll content, while its yield was lower by half than that of ‘Wala’. It seems that one of the reasons for this is the presence of anthocyanins in the leaves of ‘Dark Opal’. Anthocyanins occurring in leaf epidermis absorb some part of light energy including that in the range of photosynthetically active radiation (PAR). Their presence in leaves has an adaptation character and constitutes a defense against light occurring with the intensity exceeding its optimal value (Kacperska 2002). Both cultivars grew under greenhouse conditions, which limited light intensity but it is not known whether the yield of 'Dark Opal' would not be higher under field conditions.

The synthesis of anthocyanins is regarded as one of nitrogen deficiency symptoms (Taiz and Zeiger 1998). In the authors' experiment no anthocyanin accumulation was found in the leaves of sweet basil grown with the lowest nitrogen content in the substrate. Concluding from the chlorophyll level in this combination, one may suppose that photosynthesis proceeded with such a low intensity that the level of organic compounds was insufficient not only for plant growth but also for the synthesis of anthocyanins caused by the stress. However, it is interesting that nitrogen deficiency did not change the level of anthocyanins which determined the leaf colour of 'Dark Opal'.

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ZAWARTOŚĆ BARWNIKÓW CHLOROPLASTOWYCH
I ANTOCYJANOWYCH W LIŚCIACH BAZYLII WONNEJ
(*OCIMUM BASILICUM* L.) W ZALEŻNOŚCI OD DAWKI AZOTU

Streszczenie: Badano wpływ zróżnicowanego nawożenia azotowego (0; 1,35 i 1,80 g N na roślinę) oraz terminu zbioru (początek kwitnienia lub pełnia kwitnienia) na zawartość chlorofilu a i b, karotenoidów i barwników antocyjanowych w liściach dwóch odmian bazylii wonnej ‘Wala’ i ‘Dark Opal’. Stwierdzono, że zarówno poziom nawożenia azotowego, jak i termin zbioru wywierały istotny wpływ na zawartość barwników chloroplastowych, natomiast nie wpływały na zmiany poziomu barwników antocyjanowych. Najwyższy plon liści uzyskano w późniejszym terminie zbioru przy nawożeniu 1,35 g N na roślinę (0,9 + 0,45).

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