

## **ANALYSIS OF THE COLOUR OF FLORETS AND LEAVES IN CHRYSANTHEMUM IN THE ASPECT OF ALL-YEAR-ROUND GLASSHOUSE CULTIVATION**

Justyna Lema-Rumińska, Anita Woźny, Małgorzata Zalewska,  
Sandra Łysiak

University of Technology and Life Sciences in Bydgoszcz

**Abstract.** The colour of florets and leaves, considerably determining the decorative nature of plants, can depend much on the conditions throughout the period of cultivation. The research analysed the occurrence of anthocyanins and carotenoids in ray florets and chlorophylls in leaves in *Chrysanthemum* × *grandiflorum* /Ramat./Kitam., ‘Baton Rouge’ grown in the glasshouse over 2010–2011. The plants were exposed only to short day induced by darkening, applying no supplementary lighting of the plants. From the ray floret tissues carotenoids were extracted using concentrated acetone and anthocyanins with 1% HCl in methanol, whereas to extract chlorophylls a and b from leaf explants, concentrated acetone was used. The samples with extracted pigments were exposed to studies applying the spectrophotometer UV-VIS 1601-PC at the wavelength corresponding to the maximum of the band of a given pigment. For carotenoids the wavelength was  $\lambda = 440$  nm, for anthocyanins:  $\lambda = 530$  nm, whereas for chlorophylls:  $\lambda = 645$  and  $663$  nm. There was also defined the colour of ray florets and leaves applying the RHSCC Colour Chart [1966]. It was found that the date of plant planting, and thus their flowering, affects the concentration of pigments: anthocyanins and carotenoids in ray florets and chlorophylls in leaves and, as a result, also their colour. The highest concentration of anthocyanins was reported in the plants planted into pots on 1.12., 1.01 and on 1.07., carotenoids in the growing cycles launched on 1.11., 1.12., 1.01, 1.03, 1.07 and 1.08., while chlorophylls a and b – on 1.03.

**Key words:** *Chrysanthemum* × *grandiflorum*, anthocyanins, carotenoids, chlorophylls

### **INTRODUCTION**

The decorative nature of plants as well as their practical application are considerably affected by the colour of florets and leaves which can be less or more dependent on the

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Corresponding author: Justyna Lema-Rumińska, Department of Ornamental Plants and Vegetable Crops, University of Technology and Life Sciences in Bydgoszcz, Bernardyńska 6, 85-029 Bydgoszcz, Poland, e-mail: lem-rum@utp.edu.pl

conditions of the cultivation; with the effect of such factors including mostly light and temperature. According to Czajka et al. [2009], the selection of the family Lamiaceae perennials cultivation stand in terms of their access to light, can considerably determine the final colour effect of the entire plants. The seedlings of *Salvia splendens* growing under artificial light emitted by fluorescent lamps at 20-hour day length, contains more chlorophyll a + b, as compared with the plants grown exposed to a 16-hour day [Woźny 2011]. Kozłowska et al. [2011] point to the effect of the total day temperature on the content of chlorophyll in the leaves of chrysanthemum. The reports by Bachman and McMachon [2006] claim that in *Petunia × hybrida*, when applying chambers constructed of CuSO<sub>4</sub>-filled panels acting as spectral filters removing far-red light (-FR), the chlorophyll content increased, however in the plants grown in -DIF (18°C day/24°C night) it decreased.

The photothermal conditions over the formation of respective kinds of buds in *Chrysanthemum × grandiflorum* can, according to Zalewska et al. [2002] contribute to the final inflorescence colour. The abiotic stress, connected with a water deficit, which can quite often occur in the green areas in the city, limits the content of chlorophyll in leaves in *Impatiens walleriana*, while in *Pelargonium × hortorum* no such significant changes are recorded [Chyliński and Łukaszewska 2006]. In some plants, following the application of growth regulators, the green leaf colour is more intensive, which was found e.g. in dwarf alstroemeria treated with flurprimidol [Pobudkiewicz et al. 2000], while Pogroszewska et al. [2011] point to a positive effect of gibberellic acid applied prior to harvest, on the content of assimilating pigments in cut leaves in *Asarum europaeum*. Schreiber et al. [2011], on the other hand, note a considerable effect of aluminium in red-to-blue colour changes in *Hydrangea macrophylla* sepals. The applicable literature, however, is missing detailed studies into effect of cultivation conditions on the content of flavonoid and carotenoid pigments in the florets of ornamental plants and chlorophylls in leaves in various months of the year.

The aim of that paper was the analysis of the colour of florets and leaves in chrysanthemum in the aspect of annual cultivation in the glasshouse, carried out at 10-hour day and natural light intensity.

## MATERIAL AND METHODS

The research material was made up by *Chrysanthemum × grandiflorum* /Ramat./Kitam., 'Baton Rouge'. It is a small-flowered cultivar, with red – yellow florets, of average-strong growth, early.

Rooted plant cuttings were placed in pots 18 cm in diameter, in each 5 plant cuttings in five replications. At one date the total number of plant cuttings was 25. The medium was made up by Baltic Substrate Hawita peat substrate. Both the plant cuttings and the peat substrate were provided by Vitroflora (a Trzęsacz-based firm, in the vicinity of Bydgoszcz). Planting took place on the first day of each month for successive 12 months (tab. 1). Seven days after planting, the plants were pinched over the 5<sup>th</sup> leaf (counting from the stem base). Starting from the moment of planting, the plants were exposed to a short day, produced thanks to darkening curtains 6 pm through 8 am. There

was applied no supplementary lighting of the plants over the natural light deficit. The plots of the natural insolation and the values of air temperatures during plant cultivation in the glasshouse are given in figs 1 and 2.

During the cultivation there were noted the dates of apical bud setting, the beginning of flowering and full flowering. Pigments were extracted at the stage of full flowering plant, when at least half of all the inflorescence had been fully flowering.

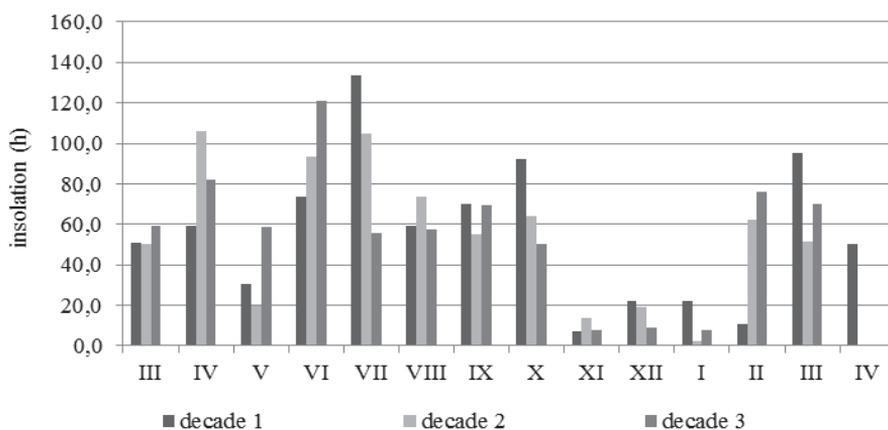


Fig. 1. Real insolation from March 2010 to April 2011

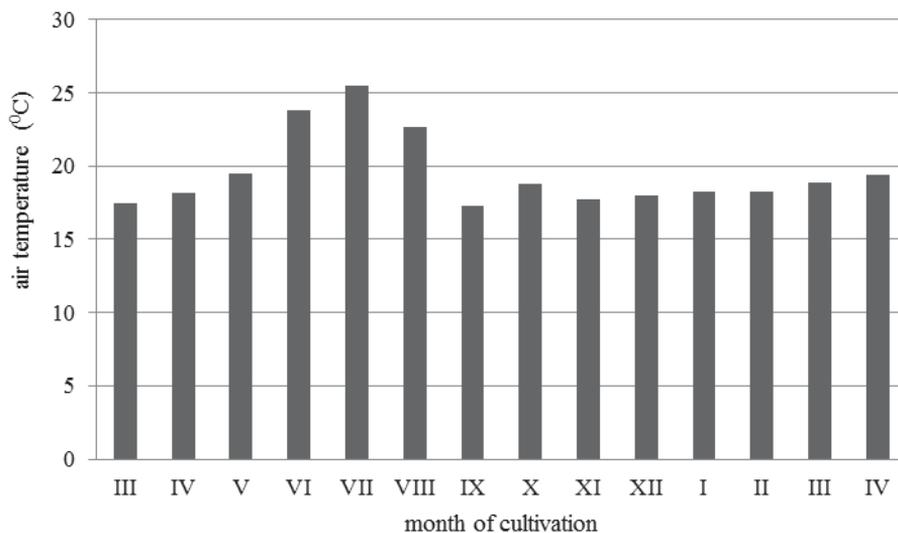


Fig. 2. The average daily air temperature in glasshouse from March 2010 to April 2011

Prior to the measurements of absorbance of pigments, there was determined the colour of ray florets (the adaxial and abaxial sides) as well as the adaxial side of the leaves using the Royal Horticultural Society Colour Chart [RHSCC 1966], under the same light conditions.

**Plant pigment extraction.** The homogenation of fresh, most characteristic fragments of ray florets, with a typical colour and about 2 cm long from the apex as well as entire leaves, was made in a porcelain mortar with some quartz sand added (a few mg). The pigments were extracted by accurate mixing of the adequate solvent with the formerly crashed plant material. Then the solution was filtered through the funnel with filter paper (quality, medium) into 10 ml volumetric flask. The absorbance was measured with the UV-VIS spectrophotometer – Shimadzu UV 1601-PC in the quartz cuvette (1 cm) at a wavelength adequate for a given pigment.

For each flowering date and the type of pigment the research was made in five replications. The results were statistically verified with the Student's t-test and the significance level of  $\alpha = 0.05$ . The concentration all of pigments was expressed per 1 g of fresh weight.

**Anthocyanins.** Anthocyanins were extracted from the fragments of ray florets of 200 mg, with 1% HCl in methanol. The absorbance was measured at the wavelength corresponding to the maximum of the anthocyanin band (for cyanidin-3-glucoside – 530 nm). The concentration of the total anthocyanins was calculated following the formula [Harborne 1967]:

$$C_A = A_{530} / h \cdot k \text{ [g} \cdot \text{dm}^{-3}\text{]}$$

$A_{530}$  – absorbance at  $\lambda = 530$  nm

$h$  – layer thickness – 1 cm

$k$  – specific extinction coefficient for cyanidin-3-glucoside = 61.7

**Carotenoids.** Carotenoids were extracted from the fragments of ray florets of the weight of 100 mg with concentrated acetone (10 ml). The absorbance was measured at the wavelength of 440 nm for carotenoids. The concentration of the total carotenoids was calculated according to the formula [Wettstein 1957]:

$$C_K = 4.695 \cdot A_{440} \text{ [mg} \cdot \text{dm}^{-3}\text{]}$$

$A_{440}$  – absorbance at  $\lambda = 440$  nm

**Chlorophylls.** Chlorophylls were extracted from the tissues of leaves (the first fully-developed leaf under the inflorescence was sampled) of the weight of 100 mg, with concentrated acetone (10 ml). Absorbance was measured at adequate wavelengths characteristic for chlorophyll a and b, respectively 645 and 663 nm. The concentration of chlorophyll a and b was calculated according to the formulae [Wettstein 1957]:

$$C_a = 12.7 \cdot A_{663} - 2.7 \cdot A_{645}$$

$$C_b = 22.9 \cdot A_{645} - 4.7 \cdot A_{663}$$

$A_{645}$  – absorbance at  $\lambda = 645$  nm

$A_{663}$  – absorbance at  $\lambda = 663$  nm

## RESULTS

The effect of the date of the start of cultivation on the photoperiodic reaction of plants and full flowering are given in Table 1. The average photoperiodic reaction in 'Baton Rouge' was 72.4 days, whereas the shortest photoperiodic reaction (60 days) was noted in the plants planted out on May 1 (date III), while the longest (87 days) on November 1 (date IX).

Table 1. Effect of the cultivation date on the photoperiodic plant reaction and full flowering date

Date	Beginning cultivation in pots	Full flowering and photoperiodic reaction (days)
I	01.03.2010	14.05.2010 (75)
II	01.04.2010	02.06.2010 (63)
III	01.05.2010	29.06.2010 (60)
IV	01.06.2010	10.08.2010 (71)
V	01.07.2010	17.09.2010 (79)
VI	01.08.2010	14.10.2010 (75)
VII	01.09.2010	04.11.2010 (65)
VIII	01.10.2010	16.12.2010 (77)
IX	01.11.2010	26.01.2011 (87)
X	01.12.2010	18.02.2011 (80)
XI	01.01.2011	11.03.2011 (70)
XII	01.02.2011	08.04.2011 (67)

Table 2. Concentration of anthocyanins and carotenoids extracted from ray florets depending on the flowering date, expressed per 1 g fresh weight

Date	Pigments	
	anthocyanins	carotenoids
concentration (mg·dm <sup>-3</sup> )		
I	77.73 b*	35.65 a
II	62.50 b	17.13 d
III	27.51 d	27.16 bc
IV	20.26 d	25.52 c
V	107.98 ab	34.10 a
VI	69.45 b	36.51 a
VII	40.73 c	29.08 b
VIII	46.48 c	29.16 b
IX	64.24 b	31.33 ab
X	128.02 a	34.37 a
XI	109.97 a	31.69 a
XII	64.40 b	30.90 b

\* – means in columns marked with the same letter do not differ significantly at  $\alpha = 0.05$

Table 3. Concentrations of chlorophylls extracted from leaves depending on the flowering date, expressed per 1 g fresh weight

Date	Chlorophyll a	Chlorophyll b
	concentration (mg·dm <sup>-3</sup> )	
I	98.41 a*	43.60 a
II	76.72 b	38.38 bc
III	63.81 c	30.94 c
IV	53.84 c	30.03 d
V	58.81 c	29.22 cd
VI	72.03 c	34.77 c
VII	84.34 b	38.65 b
VIII	77.86 c	34.46 bc
IX	55.97 c	22.15 cd
X	72.95 bc	37.48 c
XI	73.92 c	33.64 c
XII	86.38 b	36.87 b

\* – means in columns marked with the same letter do not differ significantly at  $\alpha = 0.05$

Table 4. Colour of ray florets and leaves according to the RHSCC color code depending on the flowering date

Date	Colour code (RHSCC)			
	ray florets		margins of ray florets	adaxial side of leaf
	side			
Adaxial	Abaxial			
I	46A	10A	9A	137B
II	46A	9A	9A	143A
III	46A	4A	3A	146A
IV	46A	4A	3A	146A
V	46A	9B	46A	137C
VI	46A	10A	46A	137C
VII	46A	10B	46A	137B
VIII	46A	10C	46A	137C
IX	45B	10C	45B	137D
X	46A	8C	46A	137C
XI	46A	10B	46A	146A
XII	45A	11B	45A	137A

There were noted clear differences in the content of pigments at respective plant flowering dates (tab. 2, 3). The highest concentration of anthocyanins was recorded in the plants planted into pots in December (date X), January (date XI) and July (date V), which flowered on 18.02., 11.03 and on 17.09, respectively. The minimum concentra-

tion of those pigments, on the other hand, was reported in the ray florets of the plants planted on 1.05. at date III as well as on 1.06. (date IV) which flowered on 29.06 and on 10.08. As for carotenoids, their maximum concentration was reported in the ray florets of the plants planted out into pots in the growing cycles started in December, January, March, July, August and in November (the dates: October, November, January, May, June and September), they flowered on 18.02., 11.03., 14.05., 17.09. 14.10. as well as on 26.01., respectively, and the lowest concentration occurred in the plants flowering at date II, on 2.06. A similarly low concentration of carotenoids, similarly as anthocyanins, was noted in the plants flowering at dates III and IV (29.06. and 10.08). Both for anthocyanins and carotenoids there were observed the highest values of concentrations at the flowering date coinciding with 11.03. (at date XI).

The concentration of chlorophylls a and b in leaves, on the other hand, was highest in the plants planted out into pots in March, the flowering of which was observed on 14.05 (date I). That date also corresponded to one of the highest values of carotenoid concentrations (tab. 3).

Table 4 presents the colour of ray florets and the leaves determined with the colour chart. The most characteristic inflorescence colour is determined by the adaxial side of the ray florets. Almost at all the dates it remained unchanged, marked as 46A; only at dates IX and XII, at which flowering was reported on 26.01 and on 8.04, respectively, it differed slightly (45B and 45A). Greater differences in the colour were noted, however, on the abaxial side of the ray florets (tab. 4). Similarly significant differences, which can affect the level of the pigments extracted, occurred on the margins of the ray florets, especially at dates I and II, at which flowering was reported on 14.05 and 2.06 were definitely lighter in colour (9A) than the middle parts of florets (46A), and at dates III and IV during flowering on 29.06 and 10.08 the margins of ray florets were totally yellow (according to the chart – 3A).

The colour of leaves defined with the colour chart showed various shades (A, B, C, D) of basic colour 137 reported at as many as 8 flowering dates. The other colours demonstrated greater variations (tab. 4).

## DISCUSSION

All-year-round cultivation of 'Baton Rouge' chrysanthemum identified a varied photoperiodic reaction of plants directly dependent on real insolation and the temperatures at the growing site. The greatest insolation coincided with the months June through July and at that time there were found in that cultivar the shortest photoperiodic reaction, which was also shown by earlier reports by Jerzy and Borkowska [2002]; an exception from that rule was a lack of plant flowering identified in July 2010, which can come from an excessively high temperature at the growing site, exceeding 25°C. According to Jerzy and Borkowska [2002], an excessively high temperature has a significant effect on the microscopic bud development by inhibiting it, which results in a delayed plant flowering. As for chrysanthemum, the optimal development temperature is assumed at 16–18°C, which is difficult to maintain throughout the cultivation period. However, the factor, which is more important than temperature, inducing flowering in

chrysanthemum is the photoperiod not exceeding 10–11 hours. Such a day length can be received by plant darkening. When exposed to the insolation deficit, however, to avoid the prolonging photoperiodic plant reaction, their supplementary illumination can be applied.

In all-year-round chrysanthemum cultivation we have also noted that there was also changing the level of pigments (anthocyanins and carotenoids) in ray florets and chlorophylls in leaves. The highest concentrations of anthocyanins were reported exclusively in the plants planted in the period of insolation deficit the flowering of which was recorded in February and March. Over that period the number of sunny hours in 2011 increased even seven-fold, as compared with the light deficit November through January 2010. Griesbach [1992] claims that the effect of light is especially important in the biosynthesis of anthocyanins, and in *Eustoma grandiflorum* he found that the florets in which the light intensity was decreased by 25% demonstrated as much as a 30% lower content of anthocyanins and 40% less intensive colour. Similarly in February and March we observed the highest level of carotenoid pigments, however, their high concentration was also reported in other plant flowering periods, e.g. in May, September and in October. As such it was not as specific as for anthocyanins. Czajka et al. [2009] found that in Lamiaceae the level of carotenoid pigments affects the cultivation stand and it is higher when the plants are grown at the semi-shady place than in a sunny place. However, Nguyen and Cin [2009] suggest that based on research in *Solenostemon scutellarioides* that despite the biosynthesis of anthocyanins, also the biosynthesis of carotenoids depends on a high light intensity. Over plant flowering during the insolation deficit, we identified a significantly lower degree of anthocyanins and carotenoid pigments, however, the definitely lowest level of those pigments occurred during the highest insolation and temperature, June through August. In the plants flowering over that period there was also identified the lightest colour of the margins of ray florets which have become completely yellow (according to the RHSCC Colour Chart, marked as 3A). And so the light deficit is a factor determining the content of pigments and thus the intensity of the colour of chrysanthemum inflorescence. It seems that that factor is an excessively high temperature which, at that same time, prolongs the photoperiodic reaction and the development of flower buds and thus prolongs the period required for flowering chrysanthemums. In July temperature exceeded 26°C and triggered a delay in flowering. The reports by Zhang et al. [1997] and Dela et al. [2003] suggest that the biosynthesis of anthocyanins is influenced not only by light but also by temperature. As reported by Zhang et al. [1997], the optimal temperature is 20°C. Dela et al. [2003] considers temperature to be the key factor affecting the concentration of anthocyanins in *Rosa hybrida*.

In the present research it was also found that the content of chlorophylls in the leaves in chrysanthemum changes throughout the year and depends on the cultivation date. The highest value of the concentrations for chlorophylls a and b were recorded at date I at which flowering was reported in May, while the lowest concentration of chlorophyll b occurred in August, September and January. Czajka et al. [2009] found that the effect of the cultivation stand on the content of chlorophylls in leaves depends on the cultivar and the plant species. Džugan [2006], on the other hand, reports on chlorophylls being very sensitive to high temperature and to effect of light and so they get broken down into pheophytin. The same is also confirmed by Nguyen and Cin [2009] who found that an excessively high light intensity results in a decrease in the content of chlorophylls.

## CONCLUSIONS

1. The real insolation and temperature throughout chrysanthemum growing affects the content of pigments: anthocyanins and carotenoids in ray florets and chlorophylls in leaves and, as a result, also on their colour.

2. The highest level of pigments, anthocyanins and carotenoids in ray florets in chrysanthemum is observed at the dates at which flowering was reported on 18.02 and 11.03, while chlorophylls a and b in leaves – on 14.05 (the beginning of cultivation in pots: 1.12, 1.01 and 1.03, respectively).

3. The light conditions and temperature do not have a considerable effect on the colour of the adaxial side of ray florets, however, they have a significant effect on the colour of the abaxial side and the margins of ray florets and the adaxial side of the leaves.

4. The greatest insolation and the highest air temperature resulted in lower concentration of pigments in inflorescences and leaves, which reduced the intensity of the colour and decorative value of the cultivar.

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## ANALIZA BARWY KWIATÓW I LIŚCI CHRYZANTEM W ASPEKTCIE CAŁOROCZNEJ UPRAWY W SZKLARNI

**Streszczenie.** Barwa kwiatów i liści, stanowiąca w istotny sposób o dekoracyjności roślin, w dużej mierze może być uzależniona od warunków panujących w okresie ich uprawy. W badaniach analizowano występowanie antocyjanów i karotenoidów w kwiatach języczkowatych oraz chlorofili w liściach *Chrysanthemum × grandiflorum* /Ramat./Kitam. ‘Baton Rouge’ w uprawie prowadzonej w szklarni w latach 2010–2011. Rośliny prowadzono wyłącznie przy dniu krótkim uzyskanym przez zaciemnianie, nie stosując przy tym doświetlania roślin. Z tkanek kwiatów języczkowatych wyekstrahowano karotenoidy za pomocą stężonego acetonu oraz antocyjany przy udziale 1% HCl w metanolu, zaś do ekstrakcji chlorofili a i b z eksplantatów liściowych wykorzystano stężony aceton. Próbkę z wyekstrahowanymi barwnikami poddano badaniom za pomocą spektrofotometru UV-VIS 1601-PC przy długości fali odpowiadającej maksimum pasma danego barwnika. Dla karotenoidów długość ta wynosiła  $\lambda = 440$  nm, dla antocyjanów:  $\lambda = 530$  nm, natomiast dla chlorofili  $\lambda = 645$  i  $663$  nm. Określano także barwę kwiatów języczkowatych i liści za pomocą katalogu barw RHSCC (1966). Stwierdzono, że termin posadzenia roślin, a tym samym ich kwitnienia, ma wpływ na stężenie barwników: antocyjanów i karotenoidów w kwiatach języczkowatych oraz chlorofili w liściach, a w konsekwencji także na ich zabarwienie. Największe stężenie antocyjanów uzyskano u roślin posadzonych do doniczek 1.12., 1.01. oraz 1.07., karotenoidów w cyklach uprawowych rozpoczynanych 1.11., 1.12., 1.01, 1.03, 1.07. oraz 1.08., zaś chlorofili a i b – w terminie 1.03.

**Słowa kluczowe:** *Chrysanthemum × grandiflorum*, antocyjany, karotenoidy, chlorofile