DOI: 10.2429/proc.2019.13(1)007 2019;13(1)

Adriana SZUTT[1*] and Agnieszka DOŁHAŃCZUK-ŚRÓDKA[1]

CHLOROPHYLL CONTENT IN SENESCENT *Pelargonium graveolens* LEAVES

ZAWARTOŚĆ CHLOROFILU W STARZEJĄCYCH SIĘ LIŚCIACH *Pelargonium graveolens*

Abstract: *Pelargonium graveolens* belongs to Geraniaceae family. It is an aromatic and hairy herbaceous shrub that can reach up to 1.2 m height and a spread of 1 m. Its leaves are deeply incised, soft to the touch and strongly scented, while flowers are small and usually pink. *P. graveolens* is native to South Africa, but it is now widely cultivated in many countries, e.g. Reunion Island, Egypt, Russia, China, and Morocco, mainly for the production of essential oil. It is used in many different industries, e.g. perfumery, cosmetic, food and beverages industry as well as in veterinary drugs and medicine. Literature data shows that chloroplasts content in leaves reflects a whole plant condition, as they are able to produce chemical energy from the solar energy. Most important of all pigments are chlorophylls that can be found in almost every green part of a plant. However, during plant senescence, photosynthetic pigments are degraded. This research was carried out to determine a chlorophyll a, chlorophyll b and also total chlorophylls content in *Pelargonium graveolens* at different age: one year old, two years old and three years old plants. To prepare samples, fresh leaves from each cultivation have been harvested, than homogenized in a chilled mortar with organic solvent 80% (v/v) acetone and then centrifuged. The absorbance of supernatants was immediately measured at 647 and 663 nm in a spectrophotometer. The outcome shows that in a first stage (first year) of plant growth, when it absorbs nutrients and synthesizes proteins to achieve efficient photosynthesis and undergo rapid expansion, chlorophyll a and b content is the lowest from all studied plants. The best quality pelargoniums are these at the age of two in which there is the biggest amount of green photosynthetic pigments.

Keywords: chlorophyll a, chlorophyll b, *Pelargonium graveolens*, senescent leaves

Introduction

Pelargonium graveolens

Pelargonium graveolens belongs to *Pelargonium* genus and is a member of Geraniaceae family. It is an aromatic and hairy herbaceous shrub that can reach up to 1.2 m height and a spread of 1 m. *P. graveolens* has deeply incised, soft to the touch, curved and strongly scented leaves, small and usually pink flowers [1, 2]. This plant is native to South Africa, but it is now widely cultivated in many countries, e.g. Reunion Island, Egypt, Russia, China and Morocco, mainly for the production of essential oil [3, 4] which is used in perfumery, cosmetics, food and beverages industry as well as in medicine [1, 5, 6]. Beside this, it is also useful for regulating bloodstream, stimulating adrenal glands, fighting against cellulite or treating various skin problems [1]. It has also repellent properties [5].

[1] Institute of Biotechnology, University of Opole, ul. kard. B. Kominka 6a, 45-035 Opole, Poland, phone +48 77 401 60 50
[*] Corresponding author: adriana.szutt@uni.opole.pl

Contribution will be presented during ECOpole'19 Conference, Polanica-Zdroj, 9-12.10.2019

Photosynthesis and photosynthetic pigments

The main source of life on earth is solar energy which can be entrapped by different photosynthetic pigments such as carotenoids or phycobilins, but the most important are chlorophylls. Photosynthesis is a process in which the solar energy is converted into the energy of chemical bonds. It is a crucial element of a plant metabolic system that enable a plant growth and development [7-9]. In conditions of environmental stresses such as low light intensity, not only a photosynthetic rate but also a crop productivity are low [9].

Chlorophyll is a green pigment found in most plants, algae and cyanobacteria. Its name is derived from the Greek *chloros* meaning "green" and *phyllon* "leaf" [8, 10]. Chlorophyll can be found in almost every green part of a plant and its chemical structure is made up of carbon and nitrogen atoms together with a magnesium ion in central position. As the numbers of naturally occurring chlorophylls may not yet be fully known, till now, chlorophylls have been divided into five classes: a, b, c, d, and f. Additionally there is also a chlorophyll e which is rare and was reported in algae *Vaucheria hamata* and *Tribonema bombycinum* [8]. Chlorophyll a ($C_{55}H_{72}MgN_4O_5$), previously called chlorophyll α, is the most universal type and can be found in i.e. plants, algae, cyanobacteria. In solution it has a greenish-yellow colour and absorbs mainly red spectrum from the sun light. Chlorophyll b ($C_{55}H_{70}MgN_4O_6$), previously called chlorophyll β, occurs as an accessory pigment in higher plants and also green algae. This pigment absorbs blue spectrum from the whole solar spectrum. Other chlorophylls occur in: chlorophyll c (various algae), chlorophyll d (various algae) and chlorophyll f (cyanobacteria) [8, 11]. Carotenoids serve as accessory light-harvesting pigments and are involved in plants protection against oxidative stresses a nonenzymatic compounds. Another elements participating in the protection system as antioxidative enzymes are SOD (superoxide dismutase), POD (peroxidase), APX (ascorbate peroxidase) and CAT (catalase). Carotenoids under the heat stress can protect chlorophylls biosynthesis. [12, 13].

Chloroplast is one of plants plastids where a biosynthesis of pigments and a process of photosynthesis takes place [7]. Chlorophyll biosynthesis is highly complicated because of the complex combination of enzymes and many resulting compounds [8]. Chloroplasts produce energy and glucose in association with sunlight, water and CO_2 [8], that is why chloroplasts content in leaves reflects a whole plant condition. Changes in the structure of these plastids impact a development and plant growth which is due to alterations in metabolism and photosynthesis process. There is a variety of factors that can affect plastids formation, most important are environmental conditions e.g. light intensity, temperature and mineral substances [7].

Plant senescence

Leaves senescence as a naturally occurring process is a type of programmed cell death, a self-destructive cellular process, that is one of leaves development stages. Three major stages in plant growth can be distinguished: initially, by absorbing nutrients and synthesizing proteins to achieve efficient photosynthesis, a plant undergoes rapid expansion. Next it enters the stage of maturation and finally approaches the state of senescence. This degradation process results in photosynthesis, crop yield and also plant biomass production decrease, during this stage macromolecules such as proteins and nucleic acids deteriorates [14].

Plant, especially leaf senescence is a complex molecular and physiological process that involves a chlorophyll degradation, increase in ethylene production reduction, increase in reactive oxygen species (ROS) levels as well as cell membrane damage and may be a result of numerous factors including phytohormones, environmental changes that work as environmental stresses, e.g. heat stress, drought stress or darkness [13]. Plant senescence can be also a result of different naturally occurring mechanisms such as germination, flowering or fruit ripening, which are considered to be a part of the aging processes [8]. During this process, plants integrate variety of internal and external signals as well as information about growing age through complex regulatory pathways. A transition from anabolism to catabolism resulting in nutrient redistribution to newly developing organs occurs [15]. Heat stress (heat shock) is defined as the rise of temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant and it is considered a transient elevation of temperature, usually 10 to 15 °C above ambient. Effects of heat stress can lead to photosynthetic pigments content (changes in chlorophyll a and b content), photosynthesis inhibition and are seen as foliar bleaching or leaf yellowing, but the response to high temperature varies within the species and its stage of growth [12, 16]. During drought stress conditions plants close their stoma to avoid further water loss, also internal CO_2 concentration decreases [16].

The aim of this study was to determine a chlorophyll a and chlorophyll b as well as total chlorophylls content in *Pelargonium graveolens* plants of different age. The outcome was a base for deciding in what year the plants are the strongest.

Materials and methods

There were three cultivations of *Pelargonium graveolens* set up. All of them were growing in the same environmental conditions. To prepare samples, fresh leaves were harvested from one year, two years and three years old plants. For each year, there were 4 samples made, each one has the same weight of 0.1 g. Fresh material was homogenized in a chilled mortar with 5 cm^3 of 80 % (v/v) acetone and then centrifuged at 10 000 g for 10 min at 4 °C. The absorbance of supernatants transferred into cuvettes was immediately measured at 647 and 663 nm in a spectrophotometer RAYLEIGH UV-2601. The contents of chlorophyll a and chlorophyll b as well as total chlorophylls content $Chl_{(a+b)}$ were calculated according to Lichtenthaler equations [17] and are given in mg/g f.m.

Results and discussion

As it was expected, a chlorophyll quantity in plants has changed over time. The data shows that in a first year of *Pelargonium graveolens* plants growth, when they absorb nutrients and synthesize proteins to achieve efficient photosynthesis and undergo rapid expansion, chlorophyll a and b content is lower than in all other studied plants. Chlorophyll a content in first year of plants growth equalled on average 393.00 mg, while a secondary chlorophyll b equalled 203.50 mg of pigment per gram of fresh leaves weight. In three years old plants the lowest level of both chlorophylls content was determined which indicates that they probably reached the stage of senescence and the process of chloroplasts degradation has already started. Two years old plants has respectively 494.80 mg and 294.70 mg/g f.m. of primary chlorophyll a and chlorophyll b. This experiment indicate that the best quality pelargoniums are at this age in which there is the biggest amount of green photosynthetic pigments, as variation in leaf chlorophyll content provides information about the physiological condition of a leaf or whole plant. Primary chlorophyll a is in a bigger quantity that chl b in every studied sample which is also observed in [18]. Determination of a chlorophyll a and chlorophyll b content in *Pelargonium graveolens* plants of different age is shown in Figure 1. According to [19], Chl a and Chl b occur together in the higher plants in the ratio of 2:1. The typical Chl a/b for shade plants is about 1.6-2.2. It is also mentioned that the Chl a:b ratio plays an important role to higher plants to adapt to new light regions to make optimal use of ambient light intensities and quantities. In this study the closest to 2:1 ratio was determined in one year old plants which suggests that the youngest ones are the most capable of adapting to new environmental conditions (Table 1).

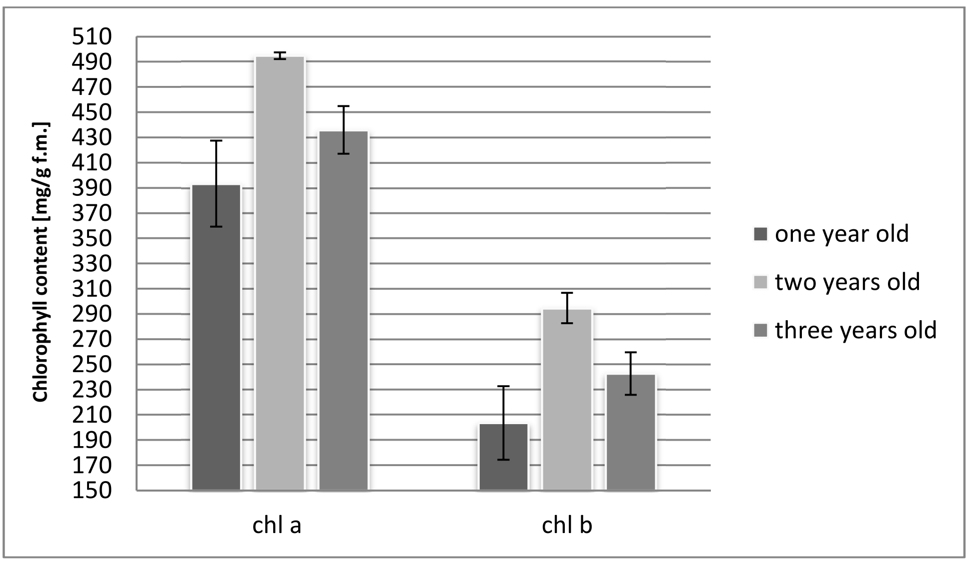

Fig. 1. A chlorophyll a and chlorophyll b content in one year old, two years old and three years old *Pelargonium graveolens* plants. chl a - chlorophyll a; chl b - chlorophyll b

Chlorophyll a/chlorophyll b ratio in each cultivation has been determined.

Table 1

Chlorophyll a/ chlorophyll b ratio in one year old, two years old and three years old *Pelargonium graveolens* plants

	One year old plant	**Two years old plant**	**Three years old plant**
Chl a/chl b	1.93	1.68	1.80

Figure 2 shows the total chlorophyll content in *Pelargonium graveolens* leaves in each of three years of plants cultivation.

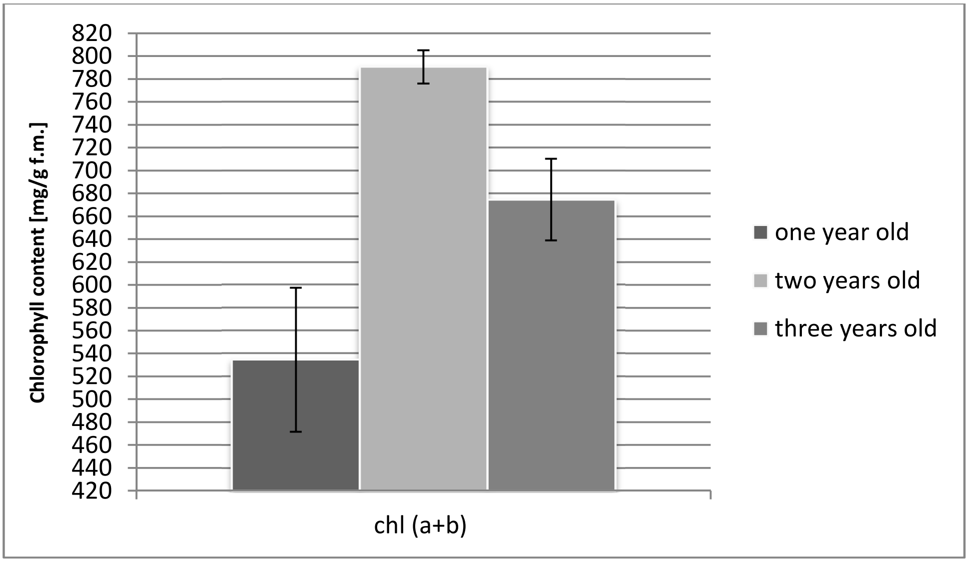

Fig. 2. Total chlorophyll content chl $_{(a+b)}$ in one year old, two years old and three years old *Pelargonium graveolens* plants. Chl $_{(a+b)}$ - total chlorophylls content

The data confirms that the highest level of primary chlorophyll a along with secondary chlorophyll b is in two years old plants, while the lowest total content is observed in the first year of plants growth.

Conclusions

This study was carried out to determine a chlorophyll a and chlorophyll b as well as total chlorophylls content in *Pelargonium graveolens* plants of different age. The data indicates that the best quality plants are these at the age of two in which there is the biggest amount of green photosynthetic pigments that enables plants to carry out photosynthetic mechanisms and process of metabolism. The lowest green pigments content was found in one year old pelargoniums, which can suggest that plants at this stage absorb nutrients and concentrate on rapid growth. However, it turned out that these one year old plants are most capable of adapting to new environmental conditions as they had chl a/chl b ratio closest to 2:1 ratio from all other ones. Chlorophylls content in three years old *P. graveolens* plants shows that after reaching the stage of maturity, the process of senescence occurs and the chlorophylls content starts to decrease.

References

[1] Džamić AM, Soković MD, Ristić MS, Grujić SM, Mileski KS, Marin PD. Chemical composition, antifungal and antioxidant activity of Pelargonium graveolens essential oil. J Appl Pharm Sci. 2014;4:1-5. DOI: 10.7324/JAPS.2014.40301.

[2] Sharopov FS, Zhang H, Setzer WN. Composition of geranium (Pelargonium graveolens) essential oil from Tajikistan, Am J Essent Oil. Nat Prod. 2014;2:13-16. https://pdfs.semanticscholar.org/

4b9d/ac0150fa0bd0815f06e6105392f769c060a3.pdf?_ga=2.177354915.769993666.1562760263-1371641520.1560859486.

[3] Rana VS, Juyal JP, Blazquez MA, Chemical constituents of essential oil of Pelargonium graveolens leaves. Int J Aromather. 2002;12:216-218. DOI: 10.1016/S0962-4562(03)00003-1.

[4] Juliani HR, Koroch A, Simon JE, Hitimana N, Daka A, Ranarivelo L, et al. Quality of geranium oils (pelargonium species): Case studies in Southern and Eastern Africa. J Essent Oil Res. 2016;18:116-121. https://www.researchgate.net/publication/285660973_Quality_of_geranium_oils_Pelargonium_species_Case_studies_in_Southern_and_Eastern_Africa.

[5] Hsouna AB, Hamdi N, Phytochemical composition and antimicrobial activities of the essential oils and organic extracts from pelargonium graveolens growing in Tunisia. Lipids Health Dis. 2012;11. DOI: 10.1186/1476-511X-11-167.

[6] Verma RS, Verma RK, Yadav AK, Chauhan A, Changes in the essential oil composition of rose-scented geranium (Pelargonium graveolens L'Herit. ex Ait.) due to date of transplanting under hill conditions of Uttarakhand. Indian J Nat Prod Resour. 2010;1:367-370. https://pdfs.semanticscholar.org/bf49/980624f754b5701d43c98462154966892ed5.pdf.

[7] Zaika V, Bondarenko T. The content of chlorophyll a and chlorophyll b in leaves of undergrowth species in hornbeam-oak forest stands of the forest-steppe zone in Wetern Ukraine. Leśne Prace Bad. Forest Research Papers. 2018;79(1):23-28. DOI:10.2478/frp-2018-0003.

[8] Pareek S, Sagar NA, Sharma S, Kumar V, Agarwal T, González-Aguilar GA, et al. Chlorophylls: Chemistry and Biological Functions. Fruit and Vegetable Phytochemicals: Chem Hum Health, Volume I, Second Edition. Edited by Elhadi M. Yahia. John Wiley&Sons Ltd; 2018. ISBN 9781119157946.

[9] Biswal AK, Pattanayak GK, Pandey SS, Leelavathi S, Reddy VS, Govindjee, et al. Light intensity-dependent modulation of chlorophyll b biosynthesis and photosynthesis by overexpression of chlorophyllide a oxygenase in tobacco. Plant Physiol. 2012;159:433-449. DOI: 10.1104/pp.112.195859.

[10] İnanç AL. Chlorophyll: Structural properties, health benefits and its occurrence in virgin olive oils. Akademik Gıda. 2011;9:26-32. https://www.researchgate.net/publication/267786661_Chlorophyll_Structural_Properties_Health_Benefits_and_Its_Occurrence_in_Virgin_Olive_Oils.

[11] Folly P, Engels N. Chlorophyll b to chlorophyll a conversion precedes chlorophyll degradation in Hordeum vulgare L. J Biological Chem. 1999;31:21811-21816. DOI: 10.1074/jbc.274.31.21811.

[12] Dhir R, Harkess RL. Physiological responses of ivy geranium „beach" and „butterfly" to heat stress. J Amer Soc Hort Sci. 2013;138:344-349. DOI: 10.21273/JASHS.138.5.344.

[13] Hatami M, Ghorbanpour M. Defense enzyme activities and biochemical variations of Pelargonium zonale in response to nanosilver application and dark storage. Turkish J Biol. 2014;38:130-139. DOI: 10.3906/biy-1304-64.

[14] Li L, Zhac J, Zhao Y, Lu X, Zhou Z, Zhao C, et al. Comprehensive investigation of tobacco leaves during natural early senescence via multi-platform metabolomics analyses. Sci Rep. 2016;6. DOI: 10.1038/srep37979.

[15] Woo HR. Plant senescence: how plants know when and how to die. J Exp Bot. 2018;69:715-718. DOI: 10.1093/jxb/ery011.

[16] Rahbarian R, Khavari-Nejad R, Ganjeali A, Bagheri A, Najafi F. Drought stress effects on photosynthesis, chlorophyll fluorescence and water relations in tolerant and susceptible chickpea (Cicer arietinym L.) genotypes. Acta Biol Cracov. 2011;53:47-56. DOI: 10.2478/v10182-011-0007-2.

[17] Lichtenthaler HK. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol. 1987;148:350-382. DOI: 10.1016/0076-6879(87)48036-1.

[18] Kamble PN, Giri SP, Mane RS, Tiwana A. Estimation of chlorophyll content in young and adult leaves of some selected plants. Univers J Environ Res Techn. 2015;6:306-310. http://www.environmentaljournal.org/5-6/ujert-5-6-5.pdf.

[19] Gogoi M, Basumatary M. Estimation of the chlorophyll concentration in seven Citrus species of Kokrajhar district, BTAD, Assam, India. Trop Plant Res. 2018;5:83-87. DOI: 10.22271/tpr.2018.v5.i1.012.

ZAWARTOŚĆ CHLOROFILU W STARZEJĄCYCH SIĘ LIŚCIACH *Pelargonium graveolens*

Instytut Biotechnologii, Uniwersytet Opolski, Opole

Abstrakt: *Pelargonium graveolens* należy do rodziny Geraniaceae. Roślina ta jest aromatycznym, owłosionym krzewem, sięgającym 1,2 metra wysokości i osiągającym 1 m szerokości. Jej liście są mocno powcinane, miękkie w dotyku i wydzielają intensywny zapach, natomiast kwiaty są małe, zwykle różowe, zebrane w baldachy. Ojczyzną *P. graveolens* jest Afryka Południowa, ale uprawia się ją obecnie w wielu krajach świata, np. wyspie Reunion, Egipcie, Rosji, Chinach czy Maroko, głównie z przeznaczeniem na produkcję olejku eterycznego. Wykorzystywany jest on w wielu gałęziach przemysłu, m.in. perfumiarstwie, kosmetyce, przemyśle spożywczym czy medycynie. Dane literaturowe pokazują, że o kondycji całej rośliny świadczy zawartość chloroplastów w jej liściach, ponieważ te produkują niezbędną do wzrostu i rozwoju energię chemiczną z dostarczanej przez słońce energii cieplnej. To właśnie w chloroplastach znajdują się chlorofile, które są najważniejszymi pigmentami fotosyntetyzującymi. Jednakże podczas starzenia rośliny pigmenty te ulegają degradacji. Obecne badanie zostało przeprowadzone w celu ustalenia zawartości chlorofilu a i chlorofilu b w jedno-, dwu- i trzyletnich roślinach z gatunku *Pelargonium graveolens*. Zebrane zostały świeże liście z każdej ww. upraw, następnie zhomogenizowane w schłodzonym moździerzu w obecności 80% acetonu, a później odwirowane. Absorbancja uzyskanych supernatantów została niezwłocznie zmierzona w spektrofotometrze przy dł. fal 647 i 663 nm. Uzyskane dane pokazały, że w pierwszym stadium (pierwszym roku) wzrostu rośliny, kiedy pobiera ona składniki odżywcze i syntetyzuje białka, aby osiągnąć wydajną fotosyntezę i kiedy przechodzi gwałtowny rozwój, zawartość chlorofilu a i chlorofilu b była najmniejsza w porównaniu z pozostałymi roślinami. Najlepszą jakością wykazały się pelargonie dwuletnie, w których ilość chlorofilu była największa.

Słowa kluczowe: chlorofil a, chlorofil b, *Pelargonium graveolens*, starzenie liści