The effect of growth conditions on flavonols and anthocyanins accumulation in green and red lettuce

Vplyv podmienok pestovania na akumuláciu flavonolov a antokyánov v zelenom a červenom šaláte

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Abstract

The aim of the study was to investigate the effect of different growth conditions on anthocyanins and flavonols accumulation in leaves of green and red loose leaf lettuce (*Lactuca sativa* var. *crispa*). Lettuce plants were grown in three types of conditions, in greenhouse (I. variant), behind clear glass in field (II. variant) and in open field conditions (III. variant). Estimation of anthocyanins and flavonols content was done by non-destructive measurements with optical fluorescence sensor Multiplex® 3 (Force-A, France). It was estimated that green lettuce varieties had a greater flavonols content compared to red lettuce varieties in all experimental variants. The highest level of flavonols was detected in leaves of green variety Zoltán (1.218 RU) and in red lettuce had the highest amount of flavonols in variety Carmesi (1.095 RU). At the same time red lettuce varieties were characterized by higher anthocyanins content. Parameter anthocyanin index is correlated with visible red coloration of leaves. The highest content of anthocyanins was detected in variety Oakly (0.867 RU). Under the open field conditions was found statistically significant higher ($P < 0.05$) flavonols and anthocyanins level in both green and red lettuce leaves compared to greenhouse conditions. It may be connected with intensification of flavonoids biosynthesis and accumulation which normally stimulated by sun irradiation, especially UV-B radiation.

Keywords: anthocyanins, flavonols, growth conditions, lettuce, UV radiation

Abstrakt

Cieľom práce bolo zistiť vplyv rozličných podmienok pestovania na akumuláciu antokyánov a flavonolov v listoch zelených a červených šalátov (*Lactuca sativa* var. *crispa*) netvoriacich hlávku, ale iba ružicu skučeravených listov. Rastliny šalátu boli pestované v troch rôznych podmienkach, a to v skleníku (I. variant), za čírym sklom v poľných podmienkach (II. variant) a vo vonkajšom prostredí (III. variant). Hodnotenie obsahu antokyánov a flavonolov bolo realizované prostredníctvom nedeštrukčných meraní s využitím optického fluorescenčného snímača Multiplex ® 3 (Force-A, Francúzsko). Zelené odrody šalátu mali v porovnaní s červenými odrodami šalátu vyšší flavonolový obsah vo všetkých experimentálnych variantoch. Najvyššia hladina flavonolov bola zaznamenaná v listoch zelenej odrody Zoltán (1,218 relatívnych jednotiek) a v červenom šaláte bolo pozorované najvyššie množstvo flavonolov v odrode Carmesi (1,095 relatívnych jednotiek). Červené odrody šalátu sa vyznačovali vyšším obsahom antokyánov. Parameter antokyánový index koreloval s viditeľným červeným sfarbením listov. Najvyšší obsah antokyánov bol zistený v odrode Oakly (0,867 relatívnych jednotiek). Štatisticky významne vyššie hladiny flavonolov a antokyánov (P < 0,05) v listoch zeleného i červeného šalátu boli zaznamenané pri pestovaní v poľných podmienkach v porovnaní s podmienkami v skleníku. Toto zistenie môže súvisieť s intenzifikáciou biosyntézy a akumulácie flavonoidov, ktoré sú bežne stimulované slnečným ožiarením, obzvlášť UV-B žiarením.

Kľúčové slová: antokyány, flavonoly, podmienky pestovania, šalát, UV žiarenie

Introduction

Due to the fact that numerous crops are originally cultivated in greenhouses or shade constructions, where ultraviolet radiation (UV radiation) is either missing or very low, from an agricultural aspect is very important that after a certain period of growth crops are removed to the field conditions where UV irradiances are much higher. This process can lead to an important transplant “shock” and the possibility for UV-induced inhibition of growth and photosynthesis while plants can adequately acclimate to the new solar radiation regime (Bogenrieder and Klein, 1977). However, it was found that exposure to UV-B during the process of acclimation improves leaf photosynthetic carbon uptake and enhances the concentration of key secondary compounds, such as flavonoids and other derivatives of the shikimate pathway probably involved in UV photoprotection (Wargent et al., 2015).

Therefore, UV-B exposure improved the acclimation and seemed to “harden” plants for growth under field conditions. Exposure to UV can thereby supply a useful effect during seedling development that could eventually have significant consequences for crop vigor and agricultural production (Wargent and Jordan, 2013). UV-B radiation has been sufficiently proved to induce specific changes in gene expression (Hectors et al., 2007), elevated accumulation of UV-screening pigments (Agati and Tattini, 2010) and changed phytochemical content (Schreiner et al., 2012). A lot of these responses have been associated with enhanced UV-B tolerance and can be caused by below ambient, chronic UV-doses which do not involve important damage. Abovementioned responses can be described as eustress. However, while productivity may not be immediately influenced by UV radiation under eustress conditions, regulatory changes in photosynthate allocation and morphology may be responsible for slight reductions in biomass accumulation (Ballaré et al., 2011). Response of plants to days or weeks of exposure to UV-B radiation demonstrated by their ability to produce and increase protective UV absorbing compounds and change their optical properties (mainly epidermal transmittance) has long been known (Caldwell et al., 1983).

Nevertheless, the response of plants to ultraviolet radiation depends on the species and the growth conditions as well as species and varieties within species may differ broadly in their response to UV-B (Krizek, 2004). Flavonoids and hydroxycinnamic acids have been proposed for compounds to provide UV protection (Landry et al., 1995), but the role of anthocyanins is less explained. Hada et al. (2003) showed that anthocyanins do not contribute to UV protection, whereas Gitz et al. (1998) attribute to anthocyanins contribution to UV protection. Flavonoids and related compounds absorb efficiently in the UV and incline to accumulate in the vacuoles of epidermal cells (Schnitzler et al. 1996). The level of flavonoids and phenolic acids in lettuce is susceptible to environmental conditions. Increasing evidence indicates that both genotype and cultivation conditions can affect the antioxidant composition, due to their role in adaptive reactions of selected agricultural crops (Liu et al., 2007). Changed content and chemical composition of epicuticular waxes (Kakani et al., 2003) and increased production of UV-B absorbing pigments, mainly flavonoids are generally described as key elements in acclimation to exposure of UV-B radiation (Bassman, 2004).

In the present work are interpreted data from measurements of flavonols and anthocyanins content in green and red lettuce of 12 varieties grown in different growth conditions with using non-destructive method worked on principle chlorophyll fluorescence recording.

Materials and methods

Plant material and growth conditions

For experiment was chosen 12 lettuce varieties from Czech company Semo, a.s. (Dubagold, Dubared, Roden, Rosaura, Zlatava, Zoltán) and from Bejo Zaden B.V., Netherlands (Aleppo, Biondonna, Carmesi, Kiribati, Oakly, Spectation) belonging to common group of morphotypes - leaf lettuce (*Lactuca sativa* var. *crispa*) not forming head and resistant to bolting. Morphotype, origin and color of varieties is described in Table 1. Seeds of lettuce were sown in plastic seedling pot and germinated under standard laboratory conditions. After germination were lettuce transplanted in growth chamber (air-conditioned box Snijders Scientific) and grown under photoperiod 14/10 hours (day/night), temperature 21/18 °C, humidity 60% and light intensity 250 $\mu mol*m^{-2}*s^{-1}$. When the lettuce plants developed second true leaf, each plant was thinned to 0.5 l pot and transplanted to greenhouse. After 35 days, part of lettuce was removed to prepared open field conditions. The measurements were realized on 96 lettuce plants, growing in three different types of conditions. The plants of first variant were grown in greenhouse (3 plants from each variety), the plants of second variant were grown behind clear glass in field (2 plants from each variety) and the plants of third variant were exposed to direct sunlight in open field conditions (3 plants from each variety). Throughout the whole experiment lettuces were regularly and well irrigated and in the early growing season was applied Previcur for fungal disease control.

Table 1. Morphotype, origin and color of lettuce varieties

Tabuľka 1. Morfotyp, pôvod a farba odrôd šalátu

Variety	Origin	Morphotype	Color
Aleppo	Netherlands	lollo	blond
Biondonna	Netherlands	lollo	green
Carmesi	Netherlands	lollo	red
Dubagold	Czech Republic	oakleaf	yellow-green
Dubared	Czech Republic	oakleaf	red
Kiribati	Netherlands	oakleaf	green
Oakly	Netherlands	oakleaf	red
Roden	Czech Republic	lollo	dark red
Rosaura	Czech Republic	lollo	reddish
Spectation	Netherlands	lollo	red
Zlatava	Czech Republic	lollo	yellow-green
Zoltán	Czech Republic	lollo	light green

Assessment of flavonols and anthocyanins content

Estimation of flavonols and anthocyanins content was realized by optical fluorescence sensor Multiplex® 3 (Force-A, France). The Multiplex is hand-held battery-operated multi-parametric sensor working on principle of light-emitting-diode excitation and filtered-photodiode detection that is proposed to work in the field under daylight. The apparatus has six UV-light sources at 375 nm and three red-blue-green LED-matrices emitting lights at 470 nm (blue), 516 nm (green) and 635 nm (red-orange). There are three, synchronised, photodiode detectors for fluorescence recording: yellow, red and far-red (Ghozlen et al., 2010). Different combinations of the red (RF) and far-red fluorescence (FRF) signals at the various excitation bands could be used as indices of different compounds, such as flavonols and anthocyanins. Considering the fluorescence signals FRF_R, excited with red light (R), and FRF_G and FRF_UV, excited with green (G) and ultraviolet (UV) radiation, respectively, were defined FLAV and ANTH indices. The leaf flavonols content, estimated by the FLAV index, is deduced from flavonoids UV absorbing properties, localized mainly in epidermis. FLAV index was calculated as decimal logarithm of the red (FRF_R) to UV excitation ratio (FRF_UV) of far-red chlorophyll fluorescence:

FLAV index = log (FRF_R/FRF_UV),

where FRF_R is far red fluorescence under red excitation and FRF_UV is far red fluorescence under UV excitation.

The decimal logarithm of the ratio of far-red fluorescence excited at red FRF_R and green FRF_G wavelengths is anthocyanin index because it is proportional to skin anthocyanin content:

ANTH index = log (FRF_R/FRF_G),

where FRF_R is far red fluorescence under red excitation and FRF_G is far red fluorescence under green excitation.

Levels of observed parameters are expressed in relative units (RU), because the ratio of the optical densities gives a dimensionless number.

Statistical analysis

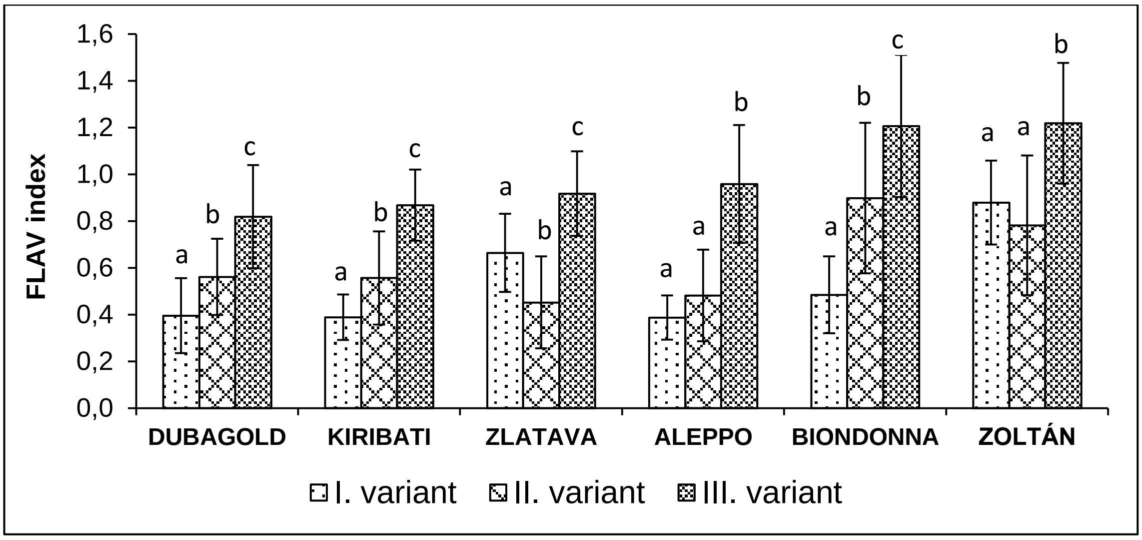
The quantitative data were evaluated by the Microsoft Office version 2010. For the significance of the results the analysis of variance (ANOVA - Scheffe´s test) was used in programe IBM SPSS 20.0 (Chicago, USA). All results were expressed as mean ± standard deviations from replications n = 54 per variety and experiment.

Results and discussion

In presented research, prescreening analysis of red and green lettuce plants under different growth conditions was done by non-invasive method based on multi-wavelength induced chlorophyll fluorescence records. In previous research was found appropriate to use Multiplex measurements for flavonoids prescreening in the different type of medicinal plants (Sytar et al., 2015).

As was expected, the highest flavonols and anthocyanins content was found in lettuce grown in open field conditions (III. variant) considering the direct UV protective role of flavonoids. The biosynthesis and accumulation of flavonoids, hydroxycinnamic acids and other related phenylpropanoid compounds are some of the most usually announced responses of plants to UV-B radiation. Sun irradiation, light intensity and quality also have a significant effect on phenolic metabolism, as they influence flavonoid biosynthesis (Dussi et al., 1995). A meta-analysis of 62 studies has demonstrated that the elevated production of UV-B-absorbing compounds is one of the most frequently observed responses of field-grown plants to UV-B-supplementation conditions (Searles et al., 2001). Wargent et al. (2015) showed that lettuce leaves grown under typical greenhouse conditions practically lack any epidermal UV shielding and that at least 6-8 days is needed to completely acclimate to UV-B conditions in a growth chamber imitating field conditions. It was also reported that lettuce developed in a polycarbonate greenhouse were characterized by a lower content of flavonoids compared to lettuce grown in the open field (Romani et al., 2002). Tsormpatsidis et al. (2010) deal with the effect of UV radiation on phenol concentration in red and green lettuce types and found that anthocyanin, flavonoid and phenolic content of lettuce plants developed constantly under a UV transparent film were 7.3-fold, 2.0-fold and 2.3-fold higher, respectively, than lettuce plants developed under a UV blocking film. Oh et al. (2011) also determined significantly higher phenolic concentration in both green and red lettuce cultivars grown in open field than those grown in high tunnels.

The highest content of parameter of FLAV index expressing relative quantity of flavonols was detected in variety Zoltán (1.218 RU) and second highest flavonol content (1.207 RU) was in variety Biondonna (Figure 1), wherein was also observed the highest percentage difference (149.26%) in flavonols content between third and first variant. Dubagold was estimated as variety with the lowest FLAV index. Nicolle et al. (2004) registered small amounts of quercetin 3-*O*-glucuronide in green lettuce cultivars and Hohl et al. (2001) noticed that small amounts of flavonols in lettuce sample could be considering to the genotype characteristics or to the ability of lettuce leaves to subject light-dependent flavonoid biosynthesis. In addition to, based on previous comparisons of polyphenol contents in lettuce between methods of cultivation, higher flavonols content was found in lettuce plants grown in open-air than in greenhouse conditions (Romani et al., 2002).

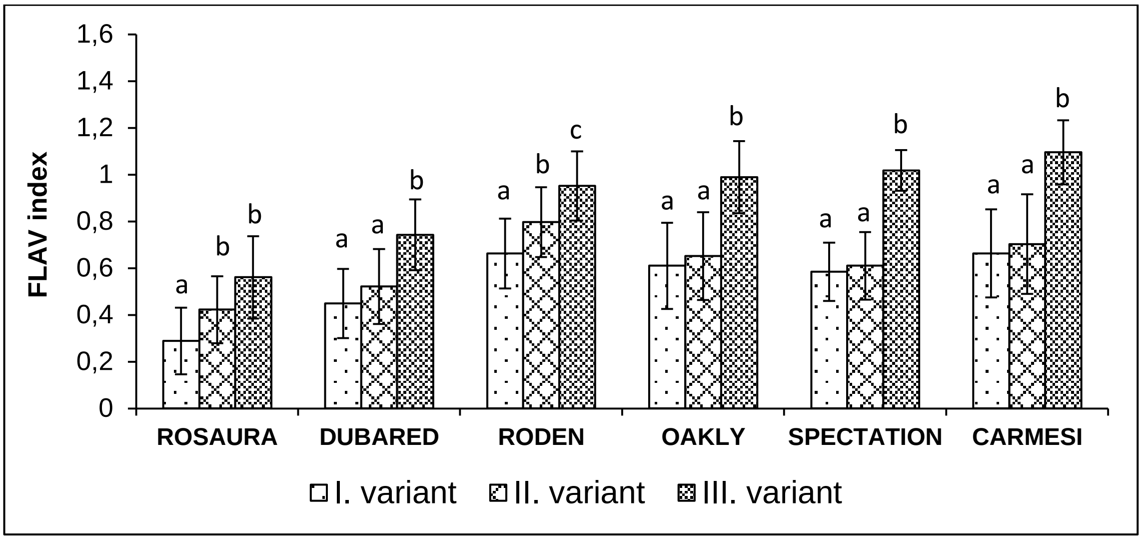


* different letters in column are significant at the P ≤ 0.05

Figure 1. Flavonols content estimated by the FLAV index in the leaves of green lettuce varieties

Obrázok 1. Obsah flavonolov hodnotený FLAV indexom v listoch zelených odrôd šalátu

In red lettuce varieties (Figure 2) were found similar values of flavonols to green varieties, mainly in case of third variant of growth conditions. Llorach et al. (2008) observed higher quantities of flavonols in red lettuce varieties compared to green lettuce varieties. DuPont et al. (2000) also recorded large amounts of flavonols in red lettuces types. The highest FLAV index was found in variety Carmesi (1.095 RU) and markedly lower flavonols content was observed in leaves of variety Rosaura. Values of flavonols in red lettuce leaves grown in greenhouse and behind clear glass conditions were not statistically different (P > 0.05) except of varieties Rosaura and Roden which got statistical difference (P < 0.05).

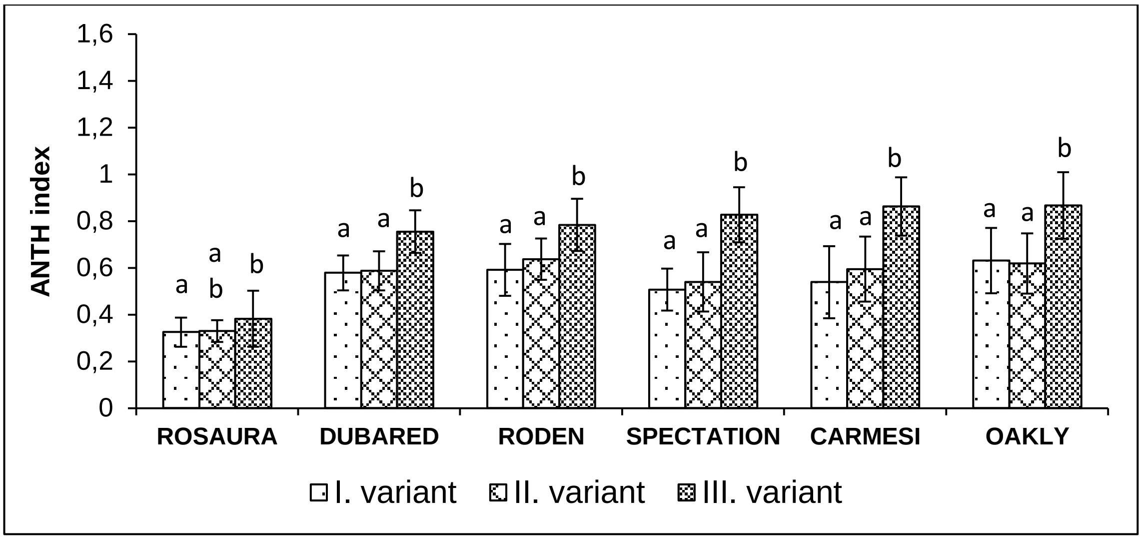


* different letters in column are significant at the P ≤ 0.05

Figure 2. Flavonols content estimated by the FLAV index in the leaves of red lettuce varieties

Obrázok 2. Obsah flavonolov hodnotený FLAV indexom v listoch červených odrôd šalátu

Parameter of ANTH index expressing relative anthocyanins content correlated with red coloration of leaves (Figure 3). Generally, red-leafed vegetables are characterized by a higher content in various aglycones and caffeic acid derivatives compared to green types of vegetable (Llorach et al., 2008). It was reported a higher phenolic concentration in red leaf lettuce plants than in green lettuce types by other researchers too (DuPont et al., 2000; Zhao et al., 2007). The fact that colored varieties of vegetables, for example red onion, red cabbage and red pepper are particularly abundant in phenolic compounds was also shown by Nicolle et al. (2004) and Stratil et al. (2006). Level of anthocyanins ranged from 0.325 to 0.867 RU, as follows: Rosaura > Dubared > Roden > Spectation > Carmesi > Oakly.

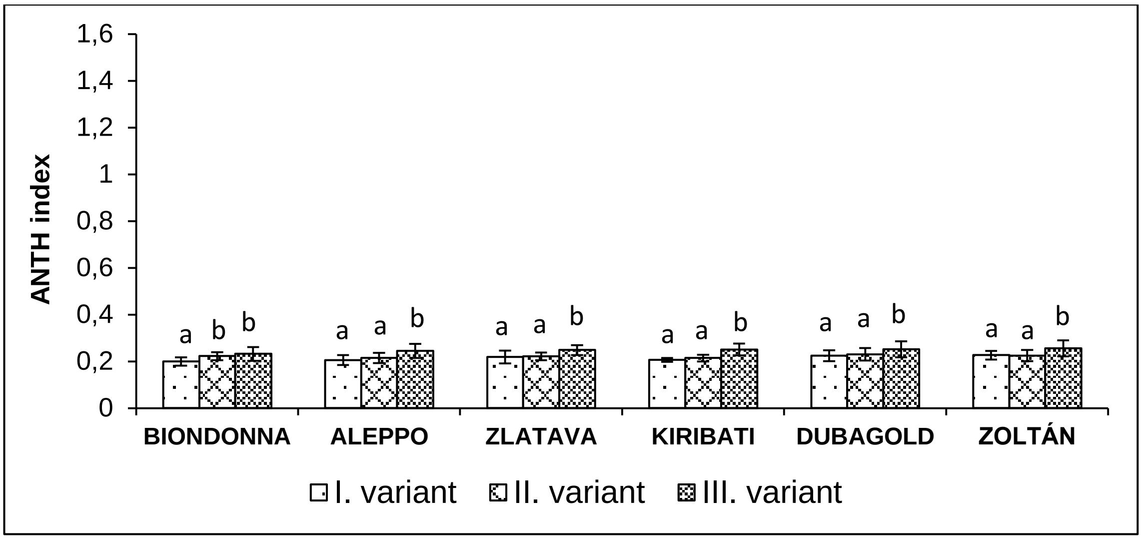


* different letters in column are significant at the P ≤ 0.05

Figure 3. Anthocyanins content estimated by the ANTH index in the leaves of red lettuce varieties

Obrázok 3. Obsah antokyánov hodnotený ANTH indexom v listoch červených odrôd šalátu

Anthocyanins content in green lettuce varieties was very low and ranged from 0.232 to 0.256 (Figure 4). Comparison of ANTH index between third and first variants of growth conditions has shown statistically significantly higher ($P < 0.05$) values of anthocyanins in open field conditions in all green lettuce varieties. In previous experiments were not detected anthocyanins in green types of lettuce plants grown in UV blocking film. Increase of anthocyanins content in lettuce leaves could be achieved by transferring lettuce plants from a UV blocking to a UV transparent film 6 days before final harvest (Llorach et al., 2008; Tsormpatsidis et al. 2010).



* different letters in column are significant at the P ≤ 0.05

Figure 4. Anthocyanins content estimated by the ANTH index in the leaves of green lettuce varieties

Obrázok 4. Obsah antokyánov hodnotený ANTH indexom v listoch zelených odrôd šalátu

Anyway, the content of phenolic compounds in various types of vegetable could be influenced by different agronomic or environmental conditions, as well as the tissue type and genetic background of plants (DuPont et al., 2000; Tomás-Barberán et al., 2000; Nicolle et al., 2004).

Conclusions

Presented data from the content analysis of flavonols and anthocynanins contents in red and green lettuce varieties under different growth conditions, provide valuable information regarding nutritional capacities of analysed lettuce plants. Higher level of flavonols and anthocyanins has been recorded in lettuce cultivars grown in open field conditions compared to lettuce grown in greenhouse or behind clear glass in field. The higher contents of flavonols and anthocyanins in all lettuce varieties were significantly different ($P < 0.05$) in open field conditions compared to the greenhouse conditions. Green lettuce varieties had higher level of flavonols and red lettuce varieties were characterized by high anthocyanins content. It was confirmed that cultivation of lettuce plants under open field conditions with normal sun irradiation, especially UV-B radiation is more efficient for accumulation of flavonoid compounds.

Acknowledgements

This work was supported by EC under the project no. 26220220180: "Construction of the "AgroBioTech" Research Centre" and projects VEGA 1/0923/16 and APVV-15-0721.

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