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Shift in accumulation of flavonoids and phenolic acids in lettuce attributable to changes in ultraviolet radiation and temperature

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ABSTRACT

Environmental conditions influence the content and metabolic profile of phenolic compounds in vegetables. The present study focused on distinguishing between the effects of ultraviolet (UV) radiation and temperature on lettuce plants with green or red leaf color when grown in either a greenhouse or outdoors. A combination of the non-destructive, fluorescence-based method with spectrophotometric and HPLC analyses enabled us to assess the effects of environment, cultivar, and plant-leaf color on concentrations of flavonoids, anthocyanins, and phenolic compounds. The accumulation of total phenolics, flavonoids, anthocyanins, and phenolic acids (benzoic acid derivatives and cinnamic acid derivatives) increased in direct sunlight (high UV radiation, moderate temperature) conditions outdoors as compared to the greenhouse conditions (low UV radiation, high temperature). The comparison of the effects of UV radiation and temperature (indoor-outdoor effect) indicated that the level of UV radiation plays a dominant role in the accumulation of flavonoids, anthocyanins, and methoxycinnamic acid; while temperature predominantly influences the accumulation of phenolic acids (rosmarinic, p-anisic, vanillic acid. Although, the leaf color (green vs. red) was strongly related to the content of majority of studied phytochemicals, environmental conditions affected their concentrations in both color types similarly. The concentrations of compounds estimated with the non-invasive, fluorescence excitation ratio method were highly consistent with those obtained by standard analytical approaches. Our results show that this fast, non-invasive method can be effectively used for determining concentrations of flavonoids and phenolic acids in lettuce plants.

1. Introduction

The metabolic profile of fresh vegetables reveals their quality and indicates their potential effect on human health. The accumulation of secondary metabolites in plants depends on both external (environmental) and internal (biological) factors. Although, the variation observed among cultivars is generally considered to be more important than environmental effects (Kleinhenz et al., 2003), the content of phytochemicals in plants can be influenced by adjusting growing conditions (Steyn et al., 2002) such as concentration of CO_2, or light intensity, quality, and photoperiod (Park et al., 2012). The variation in concentration of phytochemical was extensively studied in the relationship with exposure of plants to light (Kopsell and Kopsell, 2008; Perez-Balibrea et al., 2008). The effects of light are determined by the light spectra. Ultraviolet radiation (280–400 nm) represents a relatively small, but important part of the solar spectrum, with specific effects on higher plants (Ballaré et al., 2011). UV-B radiation (280–315 nm) is well known to induce changes in gene expression (Hectors et al., 2007), enhancing synthesis of UV-absorbing pigments (Agati and Tattini, 2010; Csepregi et al., 2017), and other changes that modify the metabolic profile of plant tissues (García-Macías et al., 2007; Schreiner et al., 2012; Hectors et al., 2014).

The roles of flavonoids and phenolic compounds in plants are very diverse. In addition to UV-shielding screen, their roles in plant resistance to biotic stressors has been also broadly examined. Despite being not simply acting as broad spectrum defensive mechanisms, the

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role of phenolics in protection against herbivores and plant diseases was clearly documented by numerous studies (for review see Treutter, 2006). In this respect, it was documented that UV-induced accumulation of flavonoids may increase the resistance of crops to biotic agents (Raviv and Antignus, 2004). Using artificial sources of UV radiation in some growing systems may improve both quality in terms of content of bioactive compounds and resistance to pests in plants. In addition to UV-B, the small doses of short-wave UV-C (200–280 nm) were found to be efficient to stimulate accumulation of different phenolic compounds (Rivera-Pastrana et al., 2014; Tiecher et al., 2013). Although the atmosphere efficiently absorbs all UV-C and plants are not exposed to this part of the spectrum naturally, low doses that are needed by plants and availability of sources of UV-C allow supplemental application of UV-C. Such application may improve the quality of vegetables, by increasing concentrations of health promoting phytochemicals and triggering the mechanisms for adaptation to abiotic and biotic stresses (Urban et al. 2016).

In addition to biochemical processes, UV-B radiation influences the responses at anatomical, morphological and physiological level (Kakani et al., 2003; Hectors et al., 2007; Caldwell et al., 2007). A typical example of physiological processes is the phenolic metabolism which sensitively responds to any change of UV-B radiation compared to the ambient level, thus altering the content of the UV-absorbing compounds and phenolic acids (Meijkamp et al., 1999). However, changes in biochemical processes may considerably vary when different stress effects co-occur with a high solar radiation and UV-B (Rozema et al., 1997; Hideg et al. 2013), as stress factors specifically influence the synthesis of individual phytochemicals in plants (Oh et al., 2009a, 2009b).

Lettuce (*Lactuca sativa* L.) is commercially the most popular leafy vegetable, cultivated mainly in moderate climates around the world (Simko et al., 2014). The phenolic compounds of lettuce have been widely investigated due to their antioxidant properties (Altunkaya et al., 2009; Llorach et al., 2008). The content of phenolic compounds and the antioxidant capacity of selected leafy vegetables were analyzed in a broad range of environmental and cultivating conditions, as leafy vegetables are grown in open fields, but also in greenhouses or polycarbonate tunnels (Romani et al., 2002; Llorach et al., 2008). The results of analyses varied depending on the species, growth stage, and the specific phenolic compounds. It was demonstrated that lettuce grown under typical greenhouse conditions practically lack epidermal UV shielding in leaves (Wargent et al., 2015). Both lettuce and Okinawan spinach (*Gynura bicolor* DC.) cultivated in fields with the ambient solar radiation (including natural UV-B radiation) accumulated higher concentrations of flavonoid compounds than those grown in a greenhouse (Brücková et al., 2016; Schirrmacher et al., 2004).

We have previously demonstrated (Zivcak et al., 2017) that fast, fluorescence record analyzed by the fluorescence excitation ratio method (Bilger et al., 2001; Cerovic et al., 2002) can efficiently determine environmentally induced changes in the content of flavonoids and anthocyanins in lettuce leaves with different colors. These fluorescence techniques are broadly used in photosynthetic studies, particularly when investigating changes in photochemical responses due to abiotic stress factors (Kalaji et al., 2016, 2017). The fluorescence excitation ratio method uses the same fluorescence signal, but a different measuring procedure that is based on fast subsequent excitations by different light spectra (UV, blue, green, red) is used. This approach has been effectively used to determine flavonol and anthocyanin content in grapes (Agati et al., 2007; Ghozlen et al., 2010; Tuccio et al., 2011), vegetables (Pfündel et al., 2011), and medicinal plants (Sytar et al., 2015).

The aim of the present study was to test the hypotheses that (1) the accumulation of UV-absorbing and other related phenolic compounds responds specifically to altered UV radiation and temperature; (2) the differences exist in the accumulation of phenolic compounds between green-colored and red-colored lettuce cultivars when exposed to different environmental conditions; (3) the non-destructive quantifications of flavonoids and phenolics using the fluorescence excitation ratio method can produce the data consistent with the results of standard analytical methods; and (4) there is a close relationship between the content of specific phenolic acids and major groups of polyphenols.

2. Material and methods

2.1. Plant material and cultivation

Twelve cultivars of leaf-type lettuce (*Lactuca sativa* var. *crispa*) were selected for the study. This type of lettuce forms open heads with loose leaves that do not close to cover younger leaves. Six green-colored cultivars originated from Semo, a.s., Czech Republic (Dubagold, Zlatava, and Zoltan) and Bejo Zaden B.V., Netherlands (Aleppo, Biondonna, and Kiribati); six red-colored cultivars were also from Semo (Dubared, Roden, and Rosaura) and Bejo Zaden (Carmesi, Oakly, and Spectation). The experiments were performed in the spring period (April, May). Lettuce seeds were sown in plastic pots and germinated under standard laboratory conditions (ca. 21 °C, 12-hour photoperiod). After germination, the lettuce plants were transplanted into a growth chamber (air-conditioned box model MC1750 (Snijders Scientific, Tilburg, Netherlands) and grown under 14/10 h (day/night) photoperiod, 21/18 °C temperature, 60% humidity, and 250 $\mu mol.m^{-2}.s^{-1}$ light intensity. The commercial peat substrate (Klasmann, Germany) was used (pH 6.0, nutrient content: N: 220 mg/L, P_2O_5: 110 mg/L, K_2O: 220 mg/L, Mg: 80 mg/L). Approximately at the stage of second, fully expanded, true leaf the plants were transplanted to 0.5 l pots and kept at the same conditions for seven days to recover. After recovery, the plants were transferred into one of the three experimental conditions described below. Plants were watered regularly to avoid drought stress. Considering a high level of nutrients in the substrate and a short duration of the experiments, no additional nutrition was applied to plants.

2.2. Experimental design and testing environments

The experiments were held at SAU in Nitra (48°19′7″N, 18°4′55″E, 144 m asl). To distinguish the effects of UV radiation from other environmental factors such as temperature, humidity, and light intensity, the plants were grown in three different environments: 1. direct sunlight (outdoor conditions with high UV), 2. under clear glass (outdoor conditions with low UV), and 3. greenhouse (indoor conditions with low UV)

Plants grown under direct sunlight conditions (1) were placed into a vegetation cage (a walk-in cage surrounded by the thin wire mesh from the top and side to protect the experimental plants against birds and animals) and exposed to almost unrestricted sunlight and ambient temperature and humidity. Plants were watered as needed to achieve a fully hydrated state. Temperature outdoors was monitored (temperature trend is presented in Results, Fig. 2).

Plants grown under clear glass (2) were placed in similar environmental conditions as those cultivated under direct sunlight, but were grown in the glass shelter constructed from clear glass sheets (thickness of 8 mm). The clear glass sheets were positioned such as to eliminate UV light coming to plants from the south and above. The backside (oriented to the north) of this glass shelter was covered by the plastic-coated wire mesh not impeding the flow of air, so the temperature and other conditions were almost identical to fully open outdoor conditions. Temperature outdoors and under the glass sheets was occasionally compared using hand-held thermometers, showing only insignificant differences. The glass cover lowered the intensity of photosynthetically active radiation (PAR) by 10–15% at noon due to absorbance and reflectance of radiation by the glass. The overhang of the glass shelter, wire mesh from the north part as well as composition of buildings from the north west and east direction was very favorable to prevent