

REVIEW

INFLUENCE OF ULTRAVIOLET RADIATION ON PLANT SECONDARY METABOLITE PRODUCTION

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Received: 22 January 2013 **Accepted:** 13 June 2013

Summary: Classification of major secondary metabolite groups is described. A short account is also given to ultraviolet (UV) climatology and UV response in plants. Investigations regarding secondary metabolite production in plants, *in vitro* cultivated plant cell and tissue cultures under UV radiation, particularly UV-B (280-315 nm) and UV-C (200-280 nm) are reviewed. The capacity of plants, callus and tissue cultures to accumulate secondary metabolite compounds after exposure to UV is discussed. The cell and tissue cultures possess high potential for production of valuable secondary metabolites under controlled conditions, and it seems perspective to enlarge the investigations in this direction by using low doses of UV as elicitors of such compounds.

Citation: Katerova Z., D. Todorova, K. Tasheva, I. Sergiev. Influence of ultraviolet radiation on plant secondary metabolite production. *Genetics and Plant Physiology*, 2012, 2(3–4), 113–144.

Keywords: Calli; plant secondary metabolites; UV-B; UV-C.

Abbreviations: PAR – photosynthetically active radiation; ROS – reactive oxygen species; UV – ultraviolet radiation.

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1. UV radiation – classification and impact on plants

Ultraviolet (UV) wavelength (400 – 200 nm) is a small part of the solar radiation reaching the Earth's surface but with significant biological impact on the living organisms, including plants. According to the International Commission on Illumination this wavelength region is divided into UV-A (315 – 400nm), UV-B (280 – 315nm) and UV-C (200 – 280nm). The negative effect of UV radiation increases towards the shorter wavelengths. Therefore, due to its highest energy, UV-C quickly provokes high levels of injuries and it is most detrimental for the living organisms (Stapleton, 1992; Hollósy, 2002; Häder et al., 2007). Both UV-C and UV-B possess enough energy to damage different chemical bonds causing photochemical reactions, which is the main reason for the negative biological effects (Kovács and Keresztes, 2002). The composition of the UV radiation is modified due to its absorption by the atmosphere. Usually the short-wave UV-C radiation is fully absorbed with exception of the high mountain locations (Häder et al., 2007), while UV-B radiation is only absorbed by the stratospheric ozone and small part of it reaches the Earth's surface. During the last decades the surface solar UV-B radiation was found to increase which corresponds with depletion of the stratospheric ozone caused by the increased release of anthropogenic pollutants such as chlorofluorocarbons and other gaseous emissions. Along with the atmospheric ozone amount, the spectral irradiance of the environmental UV depends also on the angle at which the solar radiation reaches the Earth's atmosphere, i.e. the “solar zenith angle” (including time of day, season and

latitude), altitude, clouds, surface reflection, aerosols and even air pollution (Diffey, 1991; Paul, 2001; McKenzie et al., 2007). Since there is not selective absorber for the long-wave UV-A radiation, it is affected mainly by the light scattering, its intensity is much higher than UV-B but it is not so biologically relevant (Stapleton, 1992; Vass et al. 2005). Additionally, it was found that UV-A irradiation partially protected PSII reaction center from damages caused by UV-B by activating xanthophyll cycle by preserving the level of β -carotene in cluster bean chloroplasts during the steady phase of leaf development (Joshi et al., 2007). The report supported previous observations showing that environmentally relevant UV-A doses possess ameliorating effect on UV-B triggered damage (Newsham et al., 1998; Bischof et al., 2003). In fact, the presence of realistic doses of both UV-A and PAR are highly important in order to obtain environmentally adequate results since they both moderate the negative UV-B effects (Caldwell et al., 1994; Flint et al. 2003, Kakani et al., 2003; Dolzhenko et al., 2010).

High doses of UV-B and UV-C radiation affect negatively growth, development, photosynthesis, and other important processes in plants, leading to overproduction of reactive oxygen species (ROS) and development of oxidative stress, acting negatively on macromolecules, may decrease cell viability and cause cell death (Alexieva et al., 2001; Jansen, 2002; Frohnmeyer and Staiger, 2003; Zacchini and de Agazio, 2004; Procházková and Wilhelmová, 2007; Takeuchi et al., 2007; Danon and Gallois, 1998; Toncheva-Panova et al., 2010; Schreiner et al., 2012). However, low ROS concentrations were found to play a key role in the signaling

processes during plant acclimation (Dat et al., 2000). Along with the nucleic acids (Kucera et al., 2003; Takeuchi et al., 2007), proteins and lipids, the main target sites of UV radiation are known to be amino acids, membranes, quinones, pigments, photosynthetic machinery, mainly because of the UV absorbing aromatic chemical groups (Jansen et al., 1998; Hollósy, 2002; Jansen, 2002; Vass et al., 2005; Edreva, 2005). UV-induced effects depend also on the plant sensitivity (Lavola et al., 2003; Zu et al., 2011). Low UV-B or UV-C doses may trigger acclimation responses in plants, including activation of enzymatic and non-enzymatic defense systems (Loyall et al., 2000; Jansen, 2002; Lavola et al., 2003; Katerova and Todorova, 2009; Katerova et al., 2009; Katerova and Todorova, 2011; Rai et al., 2011), but high UV doses could activate repair mechanisms in order to cope with the stress (Frohnmeyer and Staiger, 2003). It was documented that the defense or tolerance to UV-B can be related to the induction of different signal transduction pathways, secondary metabolite production, and DNA repair mechanisms (A-H Mackerness, 2000; Brown et al., 2005; Ishibashi et al., 2006). Application of low UV-C doses (0.5–9.0 kJm⁻²) has been considered to be of commercial prospect by causing hormetic (beneficial) effects to prevent pathogen diseases and delay senescence during fruit storage (Shama and Alderson, 2005).

Although the role of some secondary metabolites such as anthocyanins is still under question (Sarma and Sharma, 1999; Hada et al., 2003), most authors claim that the production of these compounds (mainly flavonoids and UV-B absorbing metabolites) in plants subjected to low UV-B doses is a major part of the complex

plant defense system (Solovchenko and Schmitz-Eiberger, 2003; Kucera et al., 2003; Schmitz-Hoerner and Weissenböck, 2003; Frohnmeyer and Staiger, 2003; Jansen et al., 2008). Bashandy et al. (2009) also assume that the accumulation of non-pigmented flavonoids in leaves of the double *ntra ntrb* (lacking NADPH-dependent thioredoxin reductases) *Arabidopsis* mutant might lead to the observed UV-C tolerance. The authors strongly support the proposed theory by the fact that UV-C tolerance was lost after crossing the double mutant with the *tt4* (mutation in the gene encoding the first enzyme of the flavonoid biosynthesis) showing that production of flavonoids in the *ntra ntrb* mutant could protect plants against UV-C. In addition, the mRNA level of *Chs* gene (chalcone synthase gene, involved in flavonoid production) was also induced in UV-C treated plants. The authors suggest that NADPH-dependent thioredoxin reductases could be a new negative regulator of flavonoid biosynthesis.

In opposite to the high UV-fluency rate, relatively low UV-B or UV-C doses led to an increased production of secondary metabolites (Kreft et al., 2002; Antognoni et al., 2007; Nadeau et al., 2012; Schreiner et al., 2012). As the pathways for secondary metabolite production are interrelated, the fact that some of these compounds increase and other decrease is not unexpected, and the biosynthesis prevails mostly to compounds possessing higher ROS scavenging activity or UV-shielding properties (Jansen et al., 2008). In the current review, we focus on secondary metabolites, which have been reported to alter predominantly after UV-B and/or UV-C treatment.

2. Secondary metabolites – an introduction

The primary metabolites are vital for every living cell. On the other hand, the secondary metabolites are present only incidentally and are not essential for plant life (Edreva et al., 2008). They are organic compounds derived through methylation, hydroxylation or glycosylation from primary metabolites (carbohydrates, proteins, amino acids, lipids) (Korkina, 2007). Secondary metabolites could be classified into several categories according to various features like their chemical structure, solubility in different solvents, or the pathway of their biosynthesis. Another important classification is related to the presence or absence of nitrogen in their chemical structure (Gershenzon 2002). Thus, secondary metabolites form two major groups: 1) nitrogen containing – alkaloids, non-protein amino acids, amines, cyanogenic glycosides, and glucosinolates; and 2) without nitrogen – terpenes (mono-, sesqui-, di-, tri-, tetraterpenes, steroids, saponins), phenolics (phenolic acids and phenylpropanoids), polyketides and polyacetylenes.

More than 12000 alkaloids are synthesized in plants (Gershenzon, 2002; Zhang and Bjorn, 2009), and derived from amino acids such as ornithine, lysine, phenylalanine, tyrosine, tryptophan, histidine, and aspartic acid. Five major alkaloid subgroups are identified and representative alkaloids are shown in Fig. 1.

Beside the well known 20 essential amino acids involved into protein structures more than 500 other uncommon non-proteinogenic amino acids are

reported (Bell, 1980; Gershenzon, 2002). They are comparable to common amino acids and occur in a free form or as ingredients of low molecular weight compounds. The non-protein amino acids could be divided into several subgroups – neutral aliphatic amino acids; acidic amino acids; basic amino acids; heterocyclic amino acids; aromatic amino acids; imino acids; sulphur-containing amino acids; and selenium-containing amino acids (Bell, 1980).

Plant amines often derive from amino acids by decarboxylation (Smith, 1980). Several subcategories of plant amines are identified in relation to number of amino groups in their structure – simple aliphatic monoamines; aliphatic diamines; aliphatic polyamines, amines containing various heterocyclic groups. Several plant amines serve as precursors in biosynthesis of polyamine alkaloids.

Cyanogenic glycosides are natural compounds containing cyanide group in their structure and can release HCN by hydrolysis (Conn, 1980; Gershenzon, 2002). They also derive from common amino acids and could be classified on the basis of the glycosylated group.

Glucosinolates are sulfur- and nitrogen-containing compounds, distributed predominantly in dicotyledonous plant families and like other nitrogen-containing secondary metabolites are synthesized from common amino acids (Underhill, 1980; Gershenzon 2002). Glucosinolates are precursors of mustard oils and similarly to cyanogenic glycosides, they can also release toxic volatiles like isothiocyanate.

Up to date, approximately 29000 terpenoids/terpenes are discovered. Terpenes derive from their precursor

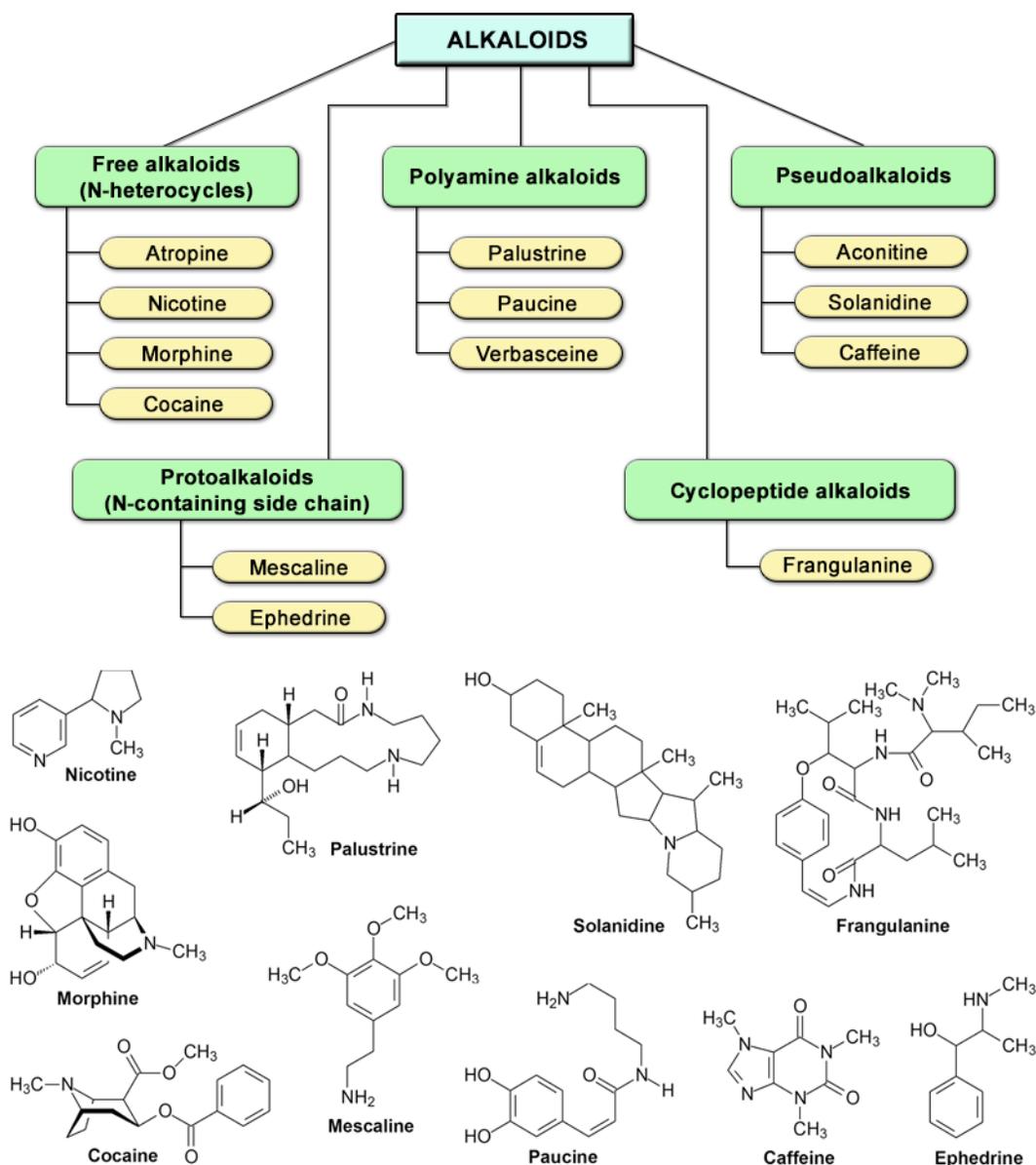


Figure 1. Major alkaloid subgroups and chemical structures of some representatives.

isopentenyl diphosphate and are classified by the number of isoprene units. There are two major pathways for biosynthesis of isopentenyl diphosphate: 1) acetate/mevalonate pathway in cytosol and endoplasmatic reticulum and 2) glyceraldehyde phosphate/pyruvate pathway in plastids (Croteau et al., 2000; Gershenzon, 2002). Additionally, the acetate/mevalonate pathway is implicated

for generation of the ubiquinone prenyl group in mitochondria (Croteau et al., 2000). The key biosynthetic enzymes are prenyltransferases and monoterpene synthase/cyclases. Some major representatives of the terpene group are presented in Fig. 2. Steroids and saponins are secondary metabolites closely related to terpenoids (Grunwald, 1980, Croteau, et al. 2000).

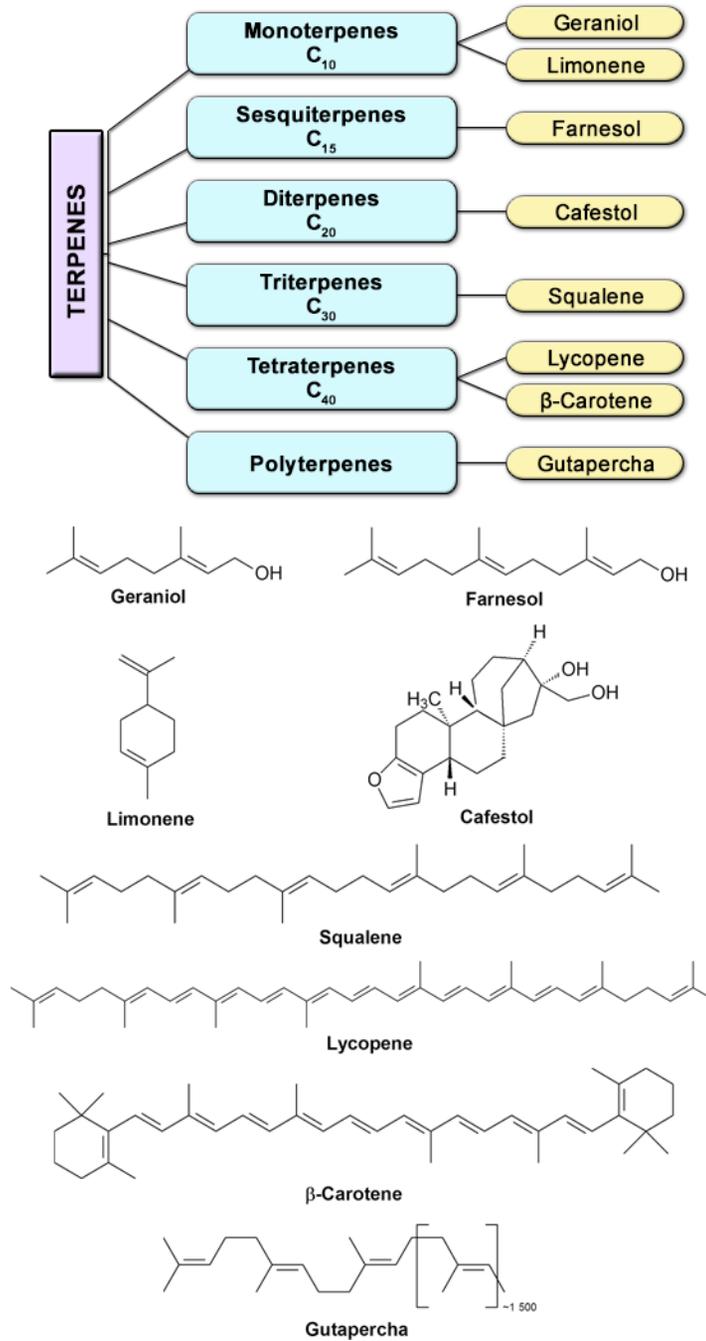


Figure 2. Terpenes classification based on isoprene unit numbers.

More than 11000 plant phenolics are synthesized mainly by two major pathways: shikimic acid pathway and malonic acid pathway (Gershenzon, 2002). The plant phenols could be classified into two major categories – benzoic acid

derivatives (C₆-C₁ skeleton), so called phenolic acids and phenylpropanoids (C₆-C₃ skeleton) (Fig. 3).

Phenolic acids are common substances widespread in plant species. Several well-known plant acids belong to this category:

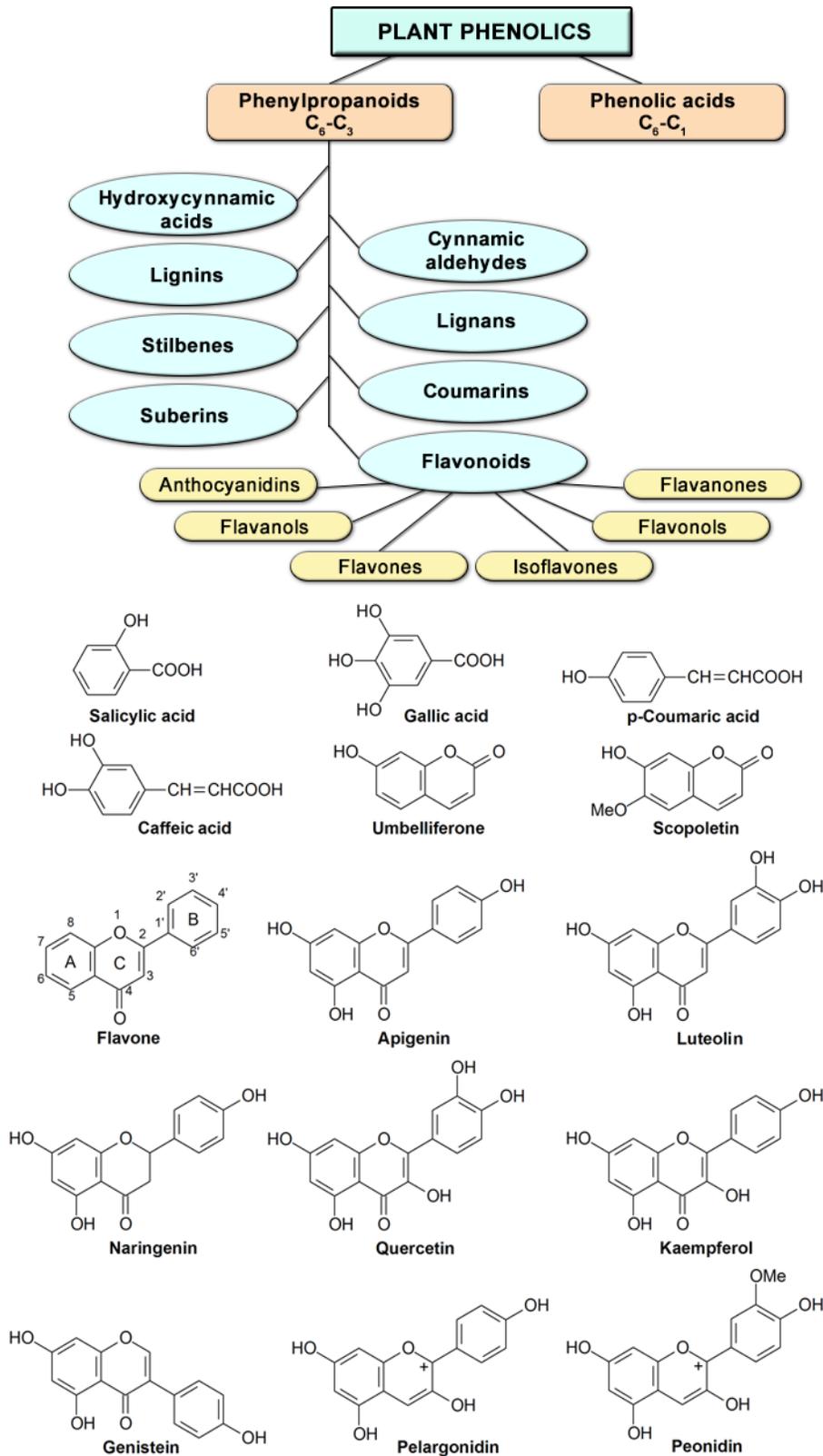


Figure 3. Classification of plant phenolics and some important representatives.

salicylic acid, vanillic acid, gallic acid, etc. (Harborne, 1980).

Phenylpropanoids are classified into several subgroups: flavonoids, hydroxycinnamic acids, cinnamic aldehydes, coumarins, lignins, lignans, stilbenes and suberins.

Hydroxycinnamic acids (C_6-C_3) are derivatives of cinnamic acid (Harborne, 1980). They are formed from *trans*-cinnamic acid by a series of hydroxylations and *O*-methylations to yield compounds like *p*-coumaric, caffeic, ferulic and sinapic acid. Hydroxycinnamic acids rarely occur in free forms and usually exist as conjugates, mostly as esters of glucose or various organic acids or amides, and less often as glycosides.

Lignins (C_6-C_3)_n are complex phenolic heteropolymers based on phenylpropanoid units resulting from the oxidative polymerization of hydroxycinnamoyl alcohol derivatives (Ibrahim, 2001b; Gershenzon, 2002; Vogt, 2010).

Lignans (C_6-C_3)₂ constitute a different group of phenylpropanoids which are biochemically related to lignins. Lignans are synthesized from phenylalanine via dimerization of substituted cinnamic alcohols to yield monolignol-derived dimers and some oligomers (Harborne, 1980; Ibrahim, 2001b).

Coumarins (C_6-C_3) are structurally considered as the lactone derivatives of 2-hydroxy-(*cis*)-cinnamic acids which result in an apyrone nucleus. More than 500 naturally occurring coumarins are known and they are mostly spread only in few plant families like Umbelliferae and Rutaceae (Harborne, 1980, Ibrahim 2001b). Beside the typical coumarins, some other classes as furanocoumarins,

4-phenylcoumarins (neoflavanoids), and isocoumarins are recognized.

Stilbenes ($C_6-C_2-C_6$) consist of a *trans* (or *cis*) ethene bond substituted with a phenyl group on both carbon atoms of the double bond. The most abundant natural stilbenes are resveratrol and lunularic acid (Harborne, 1980).

Suberins are lipid-derived biopolymers consisting of long-chain (C_{16} to C_{22}) dicarboxylic acids, long-chain (C_{20} to C_{26}) components like acids and alcohols and substantial amount of phenolic compounds (Thompson, 1980).

Among the phenylpropanoids in plants flavonoids are the most plentiful group including more than 9000 representatives. They are synthesized in plants via the flavonoid branch of the phenylpropanoid and acetate-malonate pathway. Flavonoids comprise 15 carbon atoms - two aromatic rings (A and B) connected with a 3-carbon bridge (C ring). The basic flavonoid skeleton can tolerate a large number of substitutions, for example hydroxyl groups, methyl groups, sugars (e.g. glucose, galactose, rhamnose), etc. Introducing a second hydroxyl group at *o*-position in the B ring of flavonoids is responsible for the enhancement of the antioxidant capacity of the resulting compounds (Edreva et al., 2006, 2008). Sugars and hydroxyl groups increase the water solubility of flavonoids, while methyl and isopentyl groups make flavonoids lipophilic. Flavonoids are divided into several subclasses: flavones, flavanones, flavonols, flavanols, anthocyanidins, isoflavonoids (Ibrahim 2001a; Buer et al., 2010).

Flavones are a class of flavonoids based on the basic flavone structure

with substituents mainly on 4', 5, and 7 carbon atoms and lack of –OH group in position 3. Apigenin and luteolin and their respective glycosides are commonly found in many herbaceous plant species (Harborne, 1980).

Flavanones are presented in high concentrations in citrus fruits. Flavanones have no double bond between C² and C³ of the flavone structure. The most common flavanones are hesperetin, naringenin, and their glycosides hesperidin and naringin.

The molecule of flavonols has a double-bonded oxygen atom attached to position 4 and double bond between C² and C³ of the flavone structure, and –OH group at C³. Flavonols are present in a wide variety of fruits and vegetables mainly as *O*-glycosides. The most abundant flavonols are quercetin (main glycosides – rutin and quercitrin), myricetin (glycoside myricitrin), and kaempferol. More than 200 different sugar conjugates of kaempferol are discovered.

Flavanols (catechins and epicatechins) are flavonoids without double bonds in the C ring, and have –OH group at C³. They occur in a number of plant species, but predominantly in cocoa, green tea and some woody species as birch, pine and apple (Harborne, 1980; Kostina et al. 2001; Lavola et al. 2003; Solovchenko and Schmitz-Eiberger, 2003). Another two classes of flavanols are flavan-4-ol and flavan-3,4-diol.

Anthocyanidins have a positive charge in the C ring and two double bonds in the C ring. Anthocyanins are anthocyanidin glycosides, and most common in plants are the glycosides of cyanidin, delphinidin, malvidin,

pelargonidin, peonidin, and petunidin.

Isoflavonoids (isoflavones) are similar to flavones, but the B ring is attached to C³ of the C ring. The major representatives are the isoflavones genistein and daidzein and their respective glycosides daidzin and genistin. They are found almost exclusively in leguminous plants with highest concentrations in soybean.

Tannins are secondary metabolites which can be divided in two categories – hydrolyzable (gallotannins) and condensed (Harborne, 1980, Gershenzon 2002). The hydrolyzable tannins usually contain glucose and phenolic acids (mainly gallic acid). Condensed tannins (or polyflavonoid tannins, catechol-type tannins, pyrocatecollic type tannins, non-hydrolyzable tannins or flavolans) are polymers formed by the condensation of flavans and they do not contain sugar residues. Tannins have molecular weights ranging from 500 to over 3000 (gallic acid esters) and up to 20000 (proanthocyanidins).

Quinones are aromatic dicarbonyl compounds. The two carbonyl groups usually are in *p*-position and form colored pigments. Most of the naturally occurring quinones contain a long isoprenoid side chain, and are divided in two major structural groups – naphthoquinones and benzoquinones. Usually naturally occurring quinone pigments contain phenolic or methoxyl constituents and have important role in vital physiological processes in plants (Harborne, 1980).

Polyketides are secondary metabolites from plants that are usually synthesized in a similar to fatty acids biosynthetic process through decarboxylative condensation of

malonyl-CoA. The polyketide chains produced by a polyketide synthase are often further modified into bioactive metabolites (Thompson, 1980). Polyacetylenes are natural products containing carbon-carbon triple bond functionality. There are discovered approximately 2000 polyacetylenes, and near 1200 of them are found in plants of Asteraceae (Compositae) family. Polyacetylenes derive from fatty acids and polyketide precursors (Minto and Blacklock, 2008).

Secondary metabolites play an important role in many plant physiological and developmental processes such as root nodule formation; determination of pollen germination and pollen functionality; leaf and petal pigmentation; gravity responses; regulation of auxin binding and transport; inhibition of certain enzymatic activities; influence on cellular protein phosphorylation. They also contribute to stress responses as signaling molecules, potent scavengers of ROS, and to the protection against pathogens and UV irradiation (Winkel-Shirley, 2001; Ibrahim, 2001a; Gershenzon, 2002; Velikova et al., 2004; Edreva, 2005; Velikova et al., 2007; Korkina, 2007; Edreva et al., 2007; Edreva et al., 2008; Buer et al., 2010; Samanta et al., 2011). Furthermore, plant secondary metabolites possess biological activities which are important for human life and health. A number of articles have documented the benefits of plant secondary metabolites and their use in traditional and modern medicine, food industry, perfumery and cosmetics (Havsteen, 2002; Korkina, 2007; Dinkova-Kostova, 2008; Jansen et al., 2008; Zhang and Bjorn, 2009; Caputi and Aprea, 2011; Wijesinghe and Jeon, 2011). Most of the known functions

of alkaloids are related to protection against insects, but they also contribute to animal metabolism as important neurotransmitters. Many alkaloids are used in medicine as antiarrhythmics, anticholinergics, antitumors, vasodilators, antihypertensives, anesthetics, analgesics, as well as muscle relaxants, inhibitors of acetylcholinesterase, antipyretics, and antiprotozoal agents. Terpenes and terpenoids are important components of plant essential oils that are also extensively used as natural flavor additives in food, as fragrances in perfumery, and in traditional and alternative medicine such as aromatherapy. Plant-derived phenylpropanoids (especially flavonoids) and their derivatives are among the most common biologically active components in food, wines, beer, spices, aromas, fragrances, and essential oils. Taking in account their defensive roles, these compounds are of great medicinal interest, especially as free radical scavengers, antioxidants, UV screens, anticancer, antiviral, anti-inflammatory, wound healing, antibacterial, and metal chelating agents.

3. UV radiation and secondary metabolites

3.1. Impact of UV-C on the synthesis of secondary metabolites

3.1.1. In vitro cultured plant cells, tissues and calli

UV-C light exposure of sterile cultures of *Scenedesmus quadricauda* (*Chlorophyceae*) over 1h did not influence total soluble phenols and flavonoids (Kováčik et al., 2010).

Phenolic acids were altered differently by UV-C - vanillic acid increased; gallic, caffeic, chlorogenic and *p*-coumaric acids were decreased, while protocatechuic and salicylic acids did not change significantly. Selected flavonols (quercetin and kaempferol) were not detected after UV-C treatment. The authors concluded that the exposure time to UV light was not sufficient to stimulate more considerable changes of the phenolic metabolites in *Scenedesmus quadricauda*. Exposure of *Chlamydomonas nivalis* (so-called snow alga) cells to UV-C light resulted in a three-fold increase in free proline occurred within two days after exposure to UV-C, accompanied with a 12–24% increase in phenolics after 7 days of exposure (Duval et al., 2000). The authors report that UV-C light exposure can stimulate phenolic-antioxidant production in aplanospores of *C. nivalis* which supports the idea that there is a considerable biotechnological and pharmaceutical potential incorporated within the genome of this UV-tolerant snow alga. Moreover, UV-induced secondary metabolite production, similar to that in *C. nivalis*, may provide a valuable source of pharmacological products targeted for anticancer, anticoagulant, antimicrobial, or anti-inflammatory treatments (Duval et al., 2000). Additionally, by using blue autofluorescence method Lesniewska et al. (2004) also found that UV-C light forced *Vitis vinifera* cells to produce phytoalexins - secondary metabolites with antimicrobial properties.

Ghorpade et al. (2011) used tissue culture techniques and examined the effect of UV-C on the synthesis of four

derivatives of boswellic acid - active metabolite produced in *Boswellia serrata* Roxb. (endangered medicinal plant). The authors found that 5 min of UV-C irradiation was effective for production and accumulation of acetyl-11-keto- β -boswellic acid (10-fold) and β -boswellic acid (7-fold) in the callus culture. The synthesis of stilbenes was extensively investigated in grape calli systems (Liu et al., 2010). The authors reported induced by UV-C *in vitro* production of resveratrols and their glucoside (piceids) in four grape genotypes and three tissue types of each genotype. UV-C irradiated calli accumulated stilbenes which have been already reported to have a number of health-beneficial properties, such as antioxidant capacity, cardioprotective effects, and anti-mutagenic, estrogenic and anti-cancer activity (Hung et al., 2000; Sgambato et al., 2001). Further by methylation of resveratrol via *O*-methyltransferases can be generated its methyl ether pterostilbene (trans-3,5-dimethoxy-4'-hydroxystilbene) which is known to possess fungicidal, antioxidant, anti-cancer, and anti-infective properties. Xu et al. (2012) isolated *VpROMT* (*Vitis pseudoreticulata* resveratrol *O*-methyltransferase) gene from the Chinese wild plant *V. pseudoreticulata*, which had 98.9% and 98.3% identity to the resveratrol *O*-methyltransferase gene of *V. vinifera* at the nucleotide and amino acid levels and found that gene expression level was rapidly induced by UV-C irradiation in suspension culture cells of *Vitis romanetii*. Ku et al. (2005) also found that synthesis of resveratrol and piceatannol (stilbenoids) were promoted by UV-C radiation in callus cultures of peanut.

3.1.2. Plant organs

Total polyphenols and phenol-carboxylic acids in potato and buckwheat were examined by Orsák et al. (2001) who found that the content of secondary metabolites studied was enhanced by UV-C irradiation. Schmidlin et al. (2008) reported that a whole range of stilbene derivatives (including *trans*-resveratrol, *trans*- and *cis*-piceid, *trans-ε* and *trans-δ* viniferins, and *trans*-pterostilbene), are induced in leaves of grapevine (*Vitis vinifera*, Cabernet Sauvignon variety) by UV-C (6 min, 90 mW cm⁻²). Balouchi et al. (2009) investigated the changes in photosynthetic pigments and other physiological and biochemical traits of durum wheat leaves exposed to UV-C radiation. Their results showed that carotenoids, anthocyanins, flavonoids and proline content increased significantly by UV-C as compared to the control. Other authors (Boveris et al., 2001) found that the pigment apigeninidin (3-deoxyanthocyanidin) accumulated in the epidermal areas of soybean cotyledons irradiated for 60 min with UV-C light. Interestingly, the authors stated that this pigment was not verified in soybean species principally and only UV-C (not UV-B) led to its induced accumulation. The *in vitro* test showed that apigeninidin had the ability to quench some semiquinone radicals as ascorbyl and lipid radicals in a dose-dependent manner and possessed antioxidant capacity. Twenty four hour-UV-C irradiation was also effective in the reddening of yellow saffron thistle florets to yield carthamin which is applied as a colour additive for processed foods, in cosmetic and medicinal industry (Saito, 2001).

Nadeau et al. (2012) studied the effect of hormetic UV-C dose on glucosinolates - secondary metabolites derived from amino acids which are the precursors of bioactive compounds with anti-cancer properties such as sulforaphane and indole-3-carbinol. The authors showed that UV-C tended to enhance 4-methoxyglucobrassicin, 4-hydroxyglucobrassicin and glucoraphanin in broccoli florets. So they suggested that hormetic dose of UV-C had biochemical significance to enlarge potential health effect of broccoli in cancer prevention by increasing bioactive compounds.

3.2. Impact of UV-B on the synthesis of secondary metabolites

3.2.1. *In vitro* cultured plant cells, tissues and calli

Accumulation and tissue localization of phenolic compounds in response to UV-B radiation (up to 40 d, 0.74 W m⁻²) was studied in two strains of *Camellia sinensis* L. (tea plant) callus cultures, which varied in biosynthetic capacity (Zagoskina et al., 2003). UV-B treatment affected negatively culture growth and size of the callus-forming cells. However, UV-B radiation induced a considerable increase in soluble phenolics and flavans but the rise in polymeric forms as lignin was negligible. Phenolic deposition in cell walls and intercellular space as well as the deposition of lignin-resembling substance on the callus cultures surface also rose. Further, the strain possessing a higher rate of phenolic compounds accumulation revealed greater tolerance to the UV-B radiation, demonstrating the key role of these metabolites in cell

protection to UV-B light.

UV-B irradiation induced a rise of nitric oxide (NO) production, activities of nitric oxide synthase and phenylalanine ammonia lyase (leading to flavonoid synthesis), as well as flavonoid level in *Ginkgo biloba* callus (Hao et al., 2009). The authors reported that both inhibitors of nitric oxide synthase and nitric oxide reduced phenylalanine ammonia lyase activity and the production of flavonoids. It was noted that both phenylalanine ammonia lyase activation and flavonoids synthesis in UV-B treated *G. biloba* callus were induced mainly by NO signaling molecule. Further, Loyall et al. (2000) demonstrated a contribution of the non-enzymatic and enzymatic antioxidants glutathione and glutathione S-transferase in the initial events of UV-dependent signaling to the gene encoding the key enzyme chalcone synthase (*Chs*) in parsley (*Petroselinum crispum*) cell cultures. Thus, using several short pulses of UV-B radiations, the authors proved that the oxidative cell status played a role of a central regulating element.

The effect of elicitation by methyl jasmonate and/or UV-B radiation on the production of four C-glycosyl flavonoids (isoorientin, orientin, isovitexin, vitexin) was examined in callus cultures from leaf explants of *Passiflora quadrangularis* (Antognoni et al., 2007). Flavonoids shield UV-B radiation, play a defense role against pathogen attacks, function as attractants to pollinators, and because of their high antioxidant activity, they are considered to possess health-promoting assets for humans and to provide protection against cardiovascular disease, cancer, and age-related disorders. Among all UV-B doses tested (12.6, 25.3 and

37.9 kJ m⁻²) the optimal was found to be 25.3 kJ m⁻². It was reported that exposure of *P. quadrangularis* calli to the optimal dose increased the production of all studied flavonoids, which was 6 to 40-fold higher than elicitation with methyl jasmonate. In addition, UV-B treatment led to a higher antioxidant activity compared to non-treated calli. Further, UV-B exposure of callus cultures for 7 days caused production of isoorientin similar to the quantities found in fresh leaves from glasshouse-grown plants.

The induction of monoterpenoid indole alkaloids camptothecin and 10-hydroxycamptothecin by phytohormones, heavy metals, hydrogen peroxide and UV-B radiation were evaluated in the Chinese medicinal tree *Camptotheca acuminata* cell culture (Pi et al., 2010). UV-B and salicylic acid showed the most prominent effects as elicitors of the studied alkaloids, which are valuable due to their considerable anti-tumor actions. UV-B irradiation of *Catharanthus roseus* multiple shoot cultures and cell suspension cultures was shown to induce a considerable rise in the production of terpenoid indole alkaloids, along with precursors of the dimeric alkaloids vinblastine and vincristine (composed of both vindoline and catharanthine), known to be effective in the treatment of leukemia and lymphoma (Binder et al., 2009). Other authors exposed stationary phase cell suspension cultures of *C. roseus* to low UV-B dose and succeeded to enhance substantially the amounts of catharanthine and vindoline without affecting cell growth and viability (Ramani and Chelliah, 2008). The concentrations of these secondary metabolites were found to be

highest 48-72h after UV-B treatment. In general, cell cultures of *C. roseus* produce terpenoid indole alkaloids, but fail in vindoline production, noted to be an important component of the anti-tumor dimeric alkaloids. Further, Ramani and Chelliah (2007) showed that cell surface receptor(s), calcium, medium alkalization, ROS, Ca²⁺-dependent protein kinase and mitogen-activated protein kinase have an important role in UV-B signaling, in transcriptional activation of triptophan decarboxylase (*Tdc*) and strictosidine synthase (*Str*) genes, which encode enzymes participating in biosynthesis of terpenoid indole alkaloids, and subsequent accumulation of catharanthine.

3.2.2. Plant organs

Ouwerkerk et al. (1999) reported that UV-B specifically induced a *Tdc-gusA* construct in tobacco. In addition, UV-B induced expression of *Tdc* gene and accumulation of terpenoid indole alkaloids, but the percentage induction of catharanthine and vindoline was not markedly enhanced (14 and 11%, respectively) in *C. roseus* leaves. Binder et al. (2009) revealed that up to 168h after UV-B exposure an augmented lochnericine and reduced hörhammericine amounts were found in hairy roots of *C. roseus*. When UV-B exposure time was increased up to 20 min a rise in lochnericine, serpentine, and ajmalicine and decline in hörhammericine was noted. Peebles et al. (2009) examined the role of the endogenous production of jasmonic acid via octadecanoid pathway in the production of terpenoid indole alkaloids in *C. roseus* hairy roots using octadecanoid pathway inhibitors. The

authors assumed that the octadecanoid pathway did not actively control the generation of terpenoid indole alkaloids under normal or UV-B stress conditions in *C. roseus*. Leaf concentration of another monoterpene indole alkaloid brachycerine, possessing antioxidant and antimutagenic activities was also noted to increase significantly in UV-B-treated cuttings of *Psychotria brachyceras* Müll. Arg. (do Nascimento et al., 2013). The authors supposed that brachycerine probably participated in acute UV-B responses and at least partially its accumulation might be regulated at transcriptional level. The expression of the majority genes involved in peppermint (*Mentha x piperita* L.) terpenoid biosynthesis were also modulated by exposure to UV-B (7.1 kJm⁻² day⁻¹ UV_{BE}) radiation of plants grown in field and in a growth chamber, but it did not correlate with the amount of most essential oil compounds (Dolzhenko et al., 2010). The authors documented enhanced phenolic compounds like flavonoids eriocitrin, hesperidin and kaempferol 7-*O*-rutinoside in UV-B treated plants. The interaction between terpenoid and flavonoid production in response to UV-B was proven by the higher essential oil amount in the growth chamber plants associated with lower total phenolic contents; and the decreased terpenoid concentrations in field grown peppermint related with increased content of phenolic compounds. Expectedly, it was concluded that field grown plants were better adapted to increasing UV-B irradiation than peppermints in the growth chamber due to enhanced flavonoid concentration (Dolzhenko et al., 2010). Similarly, Johnson et al., (1999) found that the

broad-leaf variety of sweet basilicum (*Ocimum basilicum*) containing phenylpropanoids in its essential oils, after UV-B exposure showed a strong increase of the phenylpropanoids (eugenol and methyl-eugenol) and the terpenoids (1,8-cineole, linalool, *trans*- β -ocimene, α - and β -pinene, sabinene, β -myrcene, limonene, α -terpinolene, borneol, α -terpineol, α -*trans*-bergamotene, γ -cadinene, germacrene D). UV-B induction of these secondary metabolites was found to be strongest in the five-leaf than in two-leaf plants. Further, UV-B-induced accumulation of the monoterpene *trans*-ocimene was also observed in leaves (predominantly in mature than in developing leaves) of linalool-rich commercial variety of sweet basilicum lacking phenylpropanoids in its essential oil (Ioannidis et al., 2002). In addition, it was reported that UV-B was necessary for the normal development of oil glands, in particular for the filling of glandular trichomes of sweet basil. Other authors revealed that short term high intensity (3d, 1.13Wm^{-2}) and long term low intensity (15d, 0.43Wm^{-2}) UV-B irradiation induced nearly 1.5-fold higher glycyrrhizin (an oleanane-type triterpenoid saponin, natural sweetener possessing anti-tumor and anti-viral activities) production in roots of only 3 month-old *Glycyrrhiza uralensis* than in control plants (Afreen et al., 2005). Using a similar model system, Afreen et al. (2006) compared the concentration of melatonin (N-acetyl-5-methoxytryptamine, an indole amine) in different tissues (seed, root, leaf and stem) of *G. uralensis* and noted the highest amount in root tissues, which increased with plant development. Exposure of

plants to different spectral quality of light (red, blue, white) and UV-B showed the highest melatonin concentration after exposure to high intensity UV-B radiation for 3 days, which decreased after longer exposure period. The authors assumed that melatonin protected *G. uralensis* plant against UV-B-triggered oxidative damage. In addition, Solhaug et al. (2003) showed that induction of melanin and parietin (anthraquinone, possessing antifungal activity) synthesis in *Lobaria pulmonaria* and *Xanthoria parietina* lichens required presence of UV-B.

Secondary metabolites, including flavonoids and terpenoids, are important for UV-B induced lessening of plant tissue quality required for microbial and herbivory pathogens (Bassman, 2004; Roberts and Paul, 2006; Izaguirre et al., 2007). Izaguirre et al. (2007) reported that both UV-B and simulated herbivory induced the accumulation of several leaf phenolic compounds (chlorogenic acid and dicaffeoylspermidine isomers) in *Nicotiana attenuata* and *N. longiflora* plants. The flavonoid rutin was specifically increased by UV-B irradiation. In another study (Kreft et al., 2002), rutin and tannin concentrations were reported to be reduced in the following order: under ambient > UV-B enhanced (simulating 17% O₃ depletion) > UV-B depleted (using Mylar foil) conditions in field grown *Fagopyrum esculentum* Moench (buckwheat). The highest amounts of these compounds were determined in flowers, followed by leaves and stems.

Tattini et al. (2004) identified and quantified the polyphenol spectrum of *Ligustrum vulgare* leaves grown outdoors and exposed to increasing sunlight (receiving PAR, UV-A and UV-

B). A prominent rise in the accumulation of singlet oxygen (1O_2)-scavengers as flavonoids (quercetin 3-O-rutinoside and luteolin 7-O-glucoside occurring in both adaxial epidermis and palisade tissue) and hydroxycinnamates (echinacoside, occurring mainly in abaxial tissues) was reported in response to solar radiation. The authors assume that a coordinated control system exists between flavonoids and hydroxycinnamate pathways and flavonoids may serve antioxidant functions in *L. vulgare* exposed to excess light. Different physiological parameters were measured in Scots pine (*Pinus sylvestris*) subjected to different UV-B levels for one growing season (Lavola et al. 2003). The authors suggested that pine plants was adequately protected against supplemental irradiation. It was noted that UV-B affected mainly secondary metabolites. Under moderate nutrient availability, the accumulation of flavonols in *P. sylvestris* needles was highest at the ambient ($4.3 \text{ kJ m}^{-2} \text{ day}^{-1}$) or near to ambient UV-radiation doses. At elevated nutrient level the UV-B doses higher than ambient (up to $13.1 \text{ kJ m}^{-2} \text{ day}^{-1}$) specifically enhanced the accumulation of diacylated flavonols (dicoumaroyl-trifolin, dicoumaroyl-isorhamnetin, dicoumaroyl-astragalol and dicoumaroyl-isoquercetin) in a dose-dependant manner. Non-acylated flavonols were increased to a lesser extent by UV-B treatment but condensed tannins were not enhanced. The major effects of UV-B radiation were on the pathway division converting dihydroflavonols to flavonols. The authors assume that the production of secondary metabolites through flavonoid pathway is multi-step regulated by UV-B exposure and nutrient

availability in pine seedlings. A similar investigation was done with silver birch (*Betula pendula*) showing that UV-B exposure did not increase phenolic acids or condensed tannins, but significantly enhanced flavonoids (quercitrin, hyperin, kaempferol-3-rhamnoside and myricetin-3-galactoside), which are important UV-B shields (de la Rosa et al., 2001). The concentration of some flavonoids was also found to depend on UV-B dose. In another study with silver birch it was shown that the amount of condensed tannins and anthocyanins in leaves was not altered by UV-B light (Tegelberg et al., 2004). The authors showed that UV-B treatment increased the concentration of quercetins, kaempferols, and chlorogenic acids but along all measured plant growth parameters only leaf area was negatively affected. In another study, Kostina et al. (2001) demonstrated a rise in (+)-catechin, quercetin, cinnamic acid derivatives, apigenin and pentagalloylglucose in leaves of birch seedlings exposed to enhanced UV-B radiation. The significant negative correlations between apigenin, and mainly quercetin amounts and levels of lipid peroxidation revealed the antioxidant role of these secondary metabolites (Kostina et al. 2001). Wulff et al. (1999) also documented UV-B-dependent accumulation of quercetin 3-glycoside in European silver birch (*Betula pendula* Roth.) seedlings exposed to high UV-B radiation levels (14.4 or $22.5 \text{ kJ m}^{-2} \text{ d}^{-1}$ UV-B_{BE}) accompanied with a transient increase of *Chs* mRNA assuming induction of flavonoid biosynthesis. The reported weaker induction of *Chs* mRNA levels in the higher UV-B dose exposure led to the suggestion that a substantial DNA

damage took place, which could partially inhibit the transcription of *Chs* as well. The concentrations of flavonol conjugates and betacyanins in the halophyte *Mesembryanthemum crystallinum* was also reported to increase after exposure to very low wavelength UV-B (like 280 or 295 nm) and the amount of feruloylglucose (the precursor of flavonol conjugates and acylated betacyanins) was much higher in leaves than in leaf tips (Ibdah et al., 2002). The authors reported that accumulation of flavonols and betacyanins could be illustrated by a weakly sigmoid dose function along with an exponential reduction of the response function of the plant with increasing wavelength.

Other authors observed that three closely related species of the sub-Arctic dwarf shrubs (*Vaccinium myrtillus* L., *Vaccinium vitis-idaea* L., and *Vaccinium uliginosum* L.) grown outdoors showed different strategies in UV-B response concerning the content and distribution of UV-absorbing phenolic compounds in leaves (Semerdjieva et al., 2003). Methanol-extractable UV-B absorbing compounds were highest in *V. myrtillus*. They increased with UV-B irradiation, and were distributed all over the leaf but concentrated in cells containing chlorophyll. The majority of phenolic compounds in *V. vitis-idaea* were cell-wall bound, concentrated in the walls of epidermis and their pool was enhanced with UV-B dose. The authors assumed that the difference in strategies for UV screening found in those two plants could be connected with leaf longevity. The response of *V. uliginosum* to UV-B was found to be flexible. This plasticity was explained with the fact that the plant is

deciduous as *V. myrtillus*, but possessed leaves with structural similarity to *V. vitis-idaea*. Kumari and Agrawal (2010) also applied supplemental to the ambient UV-B radiation (1.8 and 3.6 kJ m⁻² d⁻¹) on the aromatic perennial herb *Cymbopogon citratus* (D.C.) Stapf in field conditions and reported that only the high dose inhibited biomass production. A reduction of chlorophyll content without significant alteration in photosynthesis, and increase of carotenoids and phenolic compounds was noted in UV-B treated plants. The authors demonstrated the positive outcome of the supplemental low dose of UV-B radiation on volatile oil production accompanied by dense waxy deposition on the adaxial surface of the leaves. Further, using 45 species from different genera, Holmes and Keiller (2002) demonstrated that one of the functions of leaf waxes is shielding of UV-B. In another study, a reduction of epicuticular wax, photosynthetic pigments and flavonoid content in needles of Korean pine (*Pinus koraiensis* Sieb. et Zucc) was associated with a rise in ROS ([•]OH, H₂O₂) and malondialdehyde amounts as well as catalase activity after UV-B exposure (Zu et al., 2011). The authors reported that Korean pine had considerable sensitivity to supplementary UV-B exposure and concluded that the induced antioxidant defense system was not efficient against UV-B triggered injuries. Supplemental UV-B radiation applied for 3 months to three-year-old *Taxus chinensis* var. *mairei* led to a significant augmentation of taxol (a diterpenoid) and flavonoid content in fully expanded leaves, which play an important role in the observed plant tolerance (Zu et al., 2010). The taxol production of this shrub is well known

and it is used as an anti-tumor agent for treating breast and ovarian cancer, however up to now the major source of this molecule is obtained via extraction from *T. chinensis* var. *mairai*.

Spitaler et al. (2006) evaluated the altitudinal variation of phytochemical diversity in flowering heads (mostly unaffected by seasonal variations in regard to secondary metabolite contents) from *Arnica montana* cv. ARBO, grown between 590 and 2230m (at 9 sites near Innsbruck, Austria). An increased ratio of flavonoids with vicinal free hydroxy groups in ring B (Fig. 3), flavonoids without this trait, and elevated content of caffeic acid derivatives (most notably 1-methoxyoxaloyl-3,5-dicaffeoylquinic acid) was reported. These authors demonstrated for a first time the induction of phenolics as a major factor in the ROS scavenging system in genetically homogenous populations grown along an altitudinal gradient and most probably linked with augmented UV-B light. In opposite, sesquiterpene lactones were not observed to correlate with altitude, which was explained with the fact they did not absorb UV-B radiation nor possessed radical scavenging activity (Spitaler et al., 2006). Other authors studied some physiological effects of a natural altitudinal gradient (from 1140 to 1816m) of UV-B radiation on the aquatic liverwort *Jungermannia exsertifolia* Steph. subsp. *cordifolia* (Dumort.) Váňa collected in mountain streams (Arróniz-Crespo et al., 2006). A linear relationship between altitude and methanol-extractable UV-absorbing compounds, maximal apparent electron transport rate through PSII and the maximal non-photochemical quenching was observed, while the

photoinhibition percentage dropped. Additionally, the concentrations of the two newly found caffeic acid derivatives (5''-(7'',8''-dihydroxycoumaroyl)-2-caffeoylmalic acid and 5''-(7'',8''-dihydroxy-7-O- β -glucosyl-coumaroyl)-2-caffeoylmalic acid) also increased with altitude. The authors noted that aquatic liverwort was not negatively influenced by the changing conditions along the altitudinal gradient, including the rising UV-B irradiance, assuming that the alterations found in high-altitude populations may confer tolerance to enhanced levels of UV-B (Arróniz-Crespo et al., 2006). It was reported that total hypericin content of the plants (defined as the sum of quinones protohypericin, hypericin, protopseudohypericin and pseudohypericin) also showed the tendency to increase with altitude in spontaneously growing *Hypericum* species (*H. perforatum* L., *H. triquetrifolium* Turra, *H. empetrifolium* Willd. and *H. perforatum* L.) collected during the flowering stage on the island of Crete (Xenophontos et al., 2008). The authors proposed that the reason for the increasing levels of total hypericins with increasing altitude might be a complex of enhanced UV-B radiation accompanied by higher light intensities and lower air temperature. Concentration of hyperforin, pseudohypericin and hypericin was found to alter after exposure to UV-B (single/daily/increasing daily dose) in *Hypericum perforatum* (St. John's wort) during the vegetative growth of plants (Brechtner et al., 2011). A single UV-B exposure led to significant transient production of hyperforin (a terpenoid), which was enhanced to a concentration comparable to the initial stages of flowering when the

active ingredient concentrations in control plants are highest (Brechner et al., 2011). The authors stated that the information obtained might be valuable to optimize total product harvest for continuous production in controlled environments. Hyperforin and hypericin are the main active ingredients which contribute to the antidepressant action of St. John's wort. Hypericin and pseudohypericin possess anti-viral and anti-retroviral activity. Germ et al. (2010) reported UV-B induced production of flavonoids and tannins in leaves of St. John's wort. In opposite to Xenophontos et al. (2008), Germ et al. (2010) noted that hypericin concentration in leaves decreased in plants exposed to enhanced UV-B light and assumed that plants compete for the energy needed for synthesis of flavonoids, hypericin and tannins as the synthesis of UV-B absorbing compounds is an energy-consuming process. Exposure of fresh mulberry leaves *in vitro* to UV-B light also induced the production of two 2-aryl-benzofuran secondary metabolite compounds - chalconoracin, possessing antibacterial activity, as well as accumulation of its precursor moracin N (Gu et al., 2010).

Hofmann et al. (2003) examined nine populations of *Trifolium repens* plants after the combined exposure to UV-B (12 weeks, 13.3 kJ m⁻² d⁻¹) and drought stress. The authors noted that UV-B radiation increased the concentrations of flavonol glycosides of kaempferol and quercetin which were synergistically enhanced by drought stress. The reported UV-B-induced changes were more pronounced for the *ortho*-dihydroxylated quercetin (more than double rise in response to UV-B), rather

than the monohydroxylated kaempferol glycosides. In grape (*Vitis vinifera* cv. Silvaner) leaves UV-B also augmented specifically flavonoid (kaempferol and quercetin) amounts (Kolb et al., 2001). In contrast, the highest concentrations of hydroxycinnamic acids (caffeic and coumaric acids) accompanied with low flavonoid levels were found in the leaves of *Vitis vinifera* plants grown under strong visible light (without UV). Since the UV-B-induced inhibition of the maximum photochemical yield of photosystem II was recovered, the authors suggested that the epidermal UV screening protected efficiently photosystem II. In opposite, UV-B-dependent inhibition of CO₂ assimilation rates was not lessened by the epidermal UV-B screening (Kolb et al., 2001). It was shown that these responses were stronger after 22h than after 2h adaptation time. Solovchenko and Schmitz-Eiberger (2003) exposed two apple (*Malus domestica* Borkh.) cultivars (Braeburn and Granny Smith) differing in response to high fluxes of solar radiation to UV-B radiation. The correlation between the F_v/F_m ratio and skin content of quercetin glycoside (but not anthocyanin) was reported to increase during UV-B exposure. The authors concluded that unlike anthocyanin, the apple skin flavonoids accumulated during acclimation to strong sunlight have a considerable role in the resistance of photosynthetic apparatus to UV-B radiation. Other authors irradiated white asparagus (*Asparagus officinalis* L. cv. Gijnlim) spears (apical and basal parts) with UV-B (0.54 or 1.08 kJ m⁻²) and showed that concentration of flavonol quercetin-4'-*O*-monoglucoside increased with UV-B dose, which was accompanied

with a rise in the activity of polyphenol-related enzymes (phenylalanine ammonia-lyase and peroxidase) (Eichholz et al., 2012).

Sun et al. (2010) examined the effect of different radiation times applied by a device with UV-B intensity $82.90 \mu\text{W cm}^{-2}$ on flavonoid concentration in freshly collected *Ginkgo biloba* leaves. It was reported that younger leaves and moderate irradiation time of 120 min UV-B significantly enhanced the content of quercetin, kaempferol and isorhamnetin as well as total flavonoid concentration. The authors wished to present an innovation method enriching health-related compounds for food and pharmaceutical technology. Similarly, Ning et al. (2012) studied the effect of UV-B or UV-A radiation on secondary metabolites in freshly collected flower buds of the medicinal plant *Lonicera japonica* Thunb. Four kinds of secoiridoid glycoside (secologanic acid, secoxyloganin, secologanin and (E)-aldosecologanin) and three derivatives of chlorogenic acid (3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid) were reported to rise after UV application. Iridoids or iridoid-rich plants have been noted to possess a wide range of biological activities like anti-arthritic, anti-inflammatory, antibacterial, antifungal, anticancer, anticoagulant, antioxidant, antiviral, anti-spasmodic, immunomodulatory, wound-healing and neuroprotective activities. In addition, the antioxidant power measured by DPPH assay in methanol extracts of flower buds showed that it was higher in UV-B treated flower buds than in those subjected to UV-A.

Reifenrath and Müller (2007) reported increased relative contents of quercetin flavonols at the cost of kaempferols in *Sinapsis alba* L. leaves in UV-treated (short term UV-B plus UV-A) plants. Total flavonoid amounts in young leaves of *Nasturtium officinale* L. exposed to UV were also enhanced although being much lower when compared to *S. alba*, which was associated with the shady habitat. In both species hydroxycinnamic acid contents were unaffected, however, as important partners of the Brassicaceae defence system glucosinolates and myrosinases responded in species-specific manner to UV exposure. The authors concluded that compared to old leaves, young leaves (rich in nitrogen and soluble protein) were efficiently protected from UV light due to high flavonoid and glucosinolate amounts in *S. alba*, or enhanced flavonoid levels and myrosinase activities in *N. officinale* (Reifenrath and Müller, 2007). Other authors explored UV-B elicitor mediated changes on secondary plant metabolites in *Vaccinium corymbosum* L. (highbush blueberries) after harvest by application of different doses (0.075 and 0.15 Wh m^{-2}) and adaptation times (2h and 24h) (Eichholz et al., 2011). Both UV-B treatments enhanced the relative peak area of volatile secondary metabolites (C6-aldehydes, terpenes and ketones), responsible for the valuable flavor, stress response, environmental interaction (herbivore attack), antimicrobial and anticarcinogenic assets. The rise in volatile secondary metabolites was detected only after 2h adaptation time but an opposite tendency was reported 22h later. Total phenolic compounds responsible for the antioxidative properties of blueberries

increased rapidly with UV-B intensity. Similarly, an increment of phenolic compounds (flavonols, anthocyanins, hydroxycinnamic and hydroxybenzoic acids) was shown in UV-B treated *Ribes nigrum* L. (black currant) irrespective of the adaptation time (Huyskens-Keil et al., 2007). Based on the obtained results the authors suggest that flavonols and phenolic acids have antioxidant protection activity against UV-B mediated tissue damage and concluded that anthocyanins absorbed UV radiation within a short time.

Hordeum vulgare L. seedlings and the effect of UV-B exposure on the proteome and flavonoid amount were also examined in leaf epidermis and mesophyll (Kaspar et al., 2010). The main alteration of flavonoid compounds due to UV-B irradiation was found to be in saponarin content, which accumulated in the epidermis but not in the mesophyll. The predominant UV-B-responsive proteins, which changed their expression pattern, were reported to be implicated in initial responses typical for oxidative stress, the remaining were primary metabolism proteins, participating in the supply of secondary metabolites precursors (Kaspar et al., 2010). Global metabolite profiling of UV-induced changes also showed that the phenylpropanoid-related metabolites shikimic acid, quinic acid and phenylalanine were markedly increased in lemon balm (*Melissa officinalis*) exposed for 2h to UV-B irradiation as compared with control plants (Kim et al., 2012). Comparing *Arabidopsis* mutants impaired in biosynthesis of flavonoid (*transparent testa 4*, *tt4*; *tt5*) or sinapoylmalate (*sinapoylglucose accumulator 1*, *sng1*) with the wild-type, Kusano et

al. (2011) demonstrated that the UV-B response consists of broad reprogramming in *Arabidopsis* metabolism. It was shown that the short-term responses happened only at the level of primary metabolites, and probably provoked cell to assist the next production of UV-B-absorbing secondary metabolites. The authors described the extensive metabolism reprogramming as a gradual process by a model including early metabolite alterations resulting in an increase of anthocyanin and tocopherol together with a rise of the strong antioxidant ascorbate derivatives, and later responses characterized by remarkable changes of flavonols.

4. Concluding remarks

Different authors assume that diverse mechanisms are involved in the response to UV-B and UV-C radiation, although the high fluency of both radiations could lead to similar damages (generation of ROS, DNA damage, etc.) in plant organisms (Stapleton, 1992; Fukushima and Saito, 2000; Casati and Andreo, 2001; Katerova and Prinsen, 2008; Katerova et al., 2009). As a result, UV-C exposure has been documented to be an unusable model to study UV-B induced physiological responses by cheaper facilities (Stapleton, 1992). Nevertheless, several authors have documented that short-time UV-C could induce higher production of some valuable secondary metabolites when compared to UV-B in saffron thistle (*Carthamus tinctorius* L.), pepper (*Capsicum annuum* L.), or *Artemisia annua* L., (Fukushima and Saito, 2000; Mahdavian et al., 2008; Rai et al., 2011). However, higher accumulation of the studied secondary metabolite

compounds (carthamin, artemisinin, rutin) is associated with induction of negative effects on plants (inhibition of florets elongation, growth, or reduction in chlorophyll *a* and chlorophyll *b*) expressed much more after exposure to UV-C than to UV-B radiation. UV-B has high elicitation potential for secondary metabolite production, comparable with salicylic acid (in cell culture of Chinese medicinal tree), or even higher than methyl jasmonate (in *Passiflora quadrangularis* callus cultures) (Antognoni et al., 2007; Pi et al., 2010).

Principally, Jansen et al. (2008) conclude that the changes of stress-induced metabolites are too complex and showed the necessity to study them in more details to answer the question whether human consumers can benefit from the additional nutritional and/or pharmaceutical characteristics of UV acclimated plants. Despite the fact that recently more detailed research has appeared in regard to metabolite and proteome profile of plants subjected to UV, the question for medicinal effect of UV acclimated plants still needs to be answered (Kaspar et al., 2010; Kusano et al., 2011; Kim et al., 2012). However, secondary metabolite accumulation is highly important from biotechnological and pharmacological point of view and further studies with low-dose UV exposure are important in order to obtain higher yield of these valuable compounds (Schreiner et al. 2012). The plant cell and tissue culture methods offer an integrated approach for the production of valuable plant secondary metabolites (Tasheva and Kosturkova, 2012). The capacity of cell and tissue cultures to produce secondary metabolites after UV exposure

is not extensively studied. The existing data give us ground to propose that it is essential to extend the investigations using low doses of UV as elicitors for secondary metabolite production.

Acknowledgments

Research was supported by National Science Fund of Bulgaria – Project for Junior Scientists DMU 03/55 (leader Dr. K. Tasheva).

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