

STRESS-PROTECTIVE ROLE OF SECONDARY METABOLITES: DIVERSITY OF FUNCTIONS AND MECHANISMS

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Summary. Secondary metabolites play a major role in the adaptation of plants to the changing environment and in overcoming stress constraints. This flows from the large complexity of chemical types and interactions underlying various functions: structure stabilizing, determined by polymerisation and condensation of phenols and quinones, or by electrostatic interactions of polyamines with negatively charged loci in cell components; photoprotective, related to absorbance of visible light and UV radiation due to the presence of conjugated double bonds; antioxidant and antiradical, governed by the availability of –OH, –NH₂, and –SH groupings, as well as aromatic nuclei and unsaturated aliphatic chains; signal transducing. In our research embracing several plant-abiotic stress stimuli systems we evidenced the multiplicity of biochemical mechanisms involved in the protective role of secondary metabolites: condensation of chlorogenoquinone with proteins yielding brown pigments limiting the spread of stress-induced tissue damage in tobacco; accumulation of polyamines and formation of phenylamides in tobacco and bean subjected to water stress and heat shock, respectively, with phenylamides performing ROS-scavenging

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ability; accumulation of anthocyanins in leaves of cotton suffering Na/K imbalance, and shift from mono- to orthodihydroxy substitution in the B-ring of anthocyanin aglycone, with this conferring a higher ROS-scavenging capacity; relation of drought tolerance in cotton to the level of ROS-scavenging polyphenol compounds. The protective effect of a gaseous secondary metabolite, isoprene, against ozone fumigation and heat shock was shown, and the ability of isoprene to scavenge singlet oxygen was demonstrated. Altogether, the data provided evidence that secondary metabolites through their diversity of functions can be involved in the non-enzymatic plant defense strategy.

Key words: antioxidant ability, photoprotection, signalling, structure stabilizing.

INTRODUCTION

The term “secondary” introduced by A. Kossel in 1891 implies that while primary metabolites are present in every living cell capable of dividing, the secondary metabolites are present only incidentally and are not of paramount significance for plant life. In the last decade secondary metabolites, low molecular compounds occurring in all living organisms while largely distributed in plants, became a subject of dramatically increasing interest relevant to their significant practical implication for medicinal, nutritive and cosmetic purposes, as well as to their indisputable importance in plant stress physiology. Plant organisms being devoid of motility and immune system, have elaborated alternative defense strategies, involving the huge variety of secondary metabolites as tools to overcome stress constraints, adapt to the changing environment and survive. The large diversity of chemical types and interactions displayed by the secondary metabolites can underlie the impressive multiplicity of protective functions ranging from toxicity and light/UV shielding to signal transduction (Yang et al., 1997; Grassmann et al., 2002; Hadacek, 2002; Osbourn et al., 2003; Vasconsuelo and Boland, 2007). Revealing the putative structure-function relationships of secondary metabolites contributes to the better understanding of their stress-protective

role in plants, and can constitute a rationale for their extensive exploitation as pharmaceuticals, food additives and cosmetic products.

The present survey is an attempt to briefly examine the diverse chemical characters and interactions of secondary metabolites as putative determinants of their defense functions in various stressful situations. The paper is based on available knowledge in this field, supplemented by authors' data.

CHEMICAL TYPES OF SECONDARY METABOLITES

In plants an enormous variety of secondary metabolites has been described, their number amounting to more than 100 000 (Hadacek, 2002). The most characteristic feature of secondary metabolites is the large diversity of chemical types, embracing representatives of all main classes of organic compounds: aliphatic, aromatic, hydroaromatic, and heterocyclic; unique carbon skeletons occur along with multiplicity of functional groups (Table 1).

CHEMICAL CHARACTERS OF SECONDARY METABOLITES AS DETERMINANTS OF THEIR INTERACTIONS AND FUNCTIONS

A broad array of protective functions is performed by secondary metabolites in both biotic and abiotic stress situations: antimicrobial, photoprotective, structure stabilizing, signalling. Diverse chemical characters can be determinants of various functions, as shown in Table 2.

Availability of electrical charges

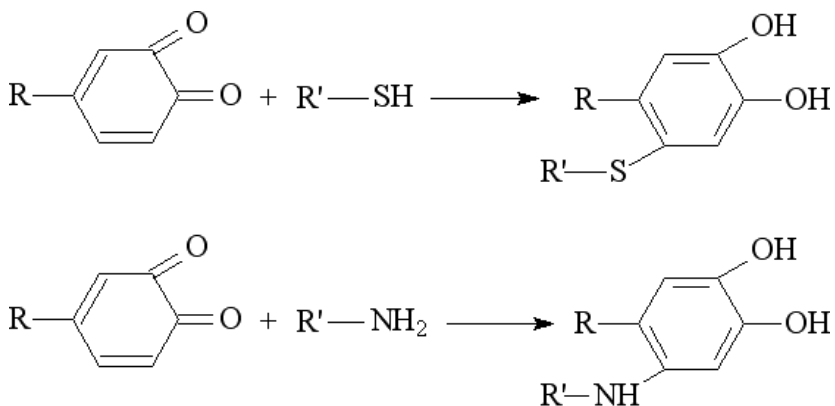
Electrostatic interactions of secondary metabolites can result in stabilizing of cell structures. Thus, positive charges in polyamines containing protonated amino- and imino groups allow electrostatic interactions with negatively charged loci in macromolecules and cellular substructures, with this exerting a stabilizing effect. Evidence is supplied that polyamine interactions with phosphoric acid residues in DNA, uronic acid residues in cell wall matrix, and negative groups on membrane surfaces contribute to the maintaining of their functional and structural integrity (reviewed by

Edreva, 1996 and Edreva et al., 2007; Berta et al., 1997).

Presence of carboxy-, hydroxy-, amino-, mono- and o-diphenol-, o-quinoid groups

Covalent bondings of the above functional groups underlie polymerization, condensation and complexation events, occurring mainly at cell wall level. Thus, polymerization of phenolic alcohols (sinapyl-, coniferyl- and p-coumaryl-) yields lignins strengthening the wall structure. Ferulic acid, an unsaturated aromatic carbonic acid, is bound to polysaccharids chains in cell wall matrix by ester bonds; complexation (cross-linking) via diferulate bridges consolidates cell wall structure (Fry, 1986).

The presence of o-diphenol groups allows formation of o-quinoid grouping; *in vitro* covalent interactions between proteins and oxidation products of caffeoylquinic acid were recently reported (Prigent et al., 2007). Quinoid grouping can interact with thiol- and amino groups in proteins as follows:



The interactions of quinoid groupings with microbial proteins can underlie toxicity of secondary metabolites against fungi, bacteria and viruses, i.e. an antimicrobial activity, given that blocking of active sites and corresponding functions in proteins may occur. Similar interactions of quinoid groupings with plant proteins can be a mechanism of the necrotic reaction against pathogens, as well as of the necrobiosis due to abiotic stresses. High molecular condensation brown products of the quinoid form of o-diphenols and proteins were evidenced to arise during both types

of necrobiosis and suggested to limit the spread of stress-induced tissue damage (Edreva, 1975). Hypersensitive response (HR) localizing invading pathogens at infection sites appears as a series of similar processes (Heath, 2000). Experimental data point to the involvement of phenolic-storing cells as keys in the expression of programmed cell death (Beckman, 2000).

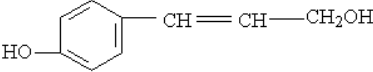
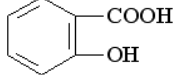
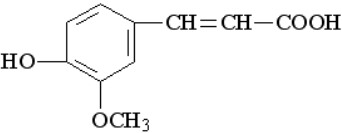
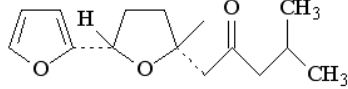
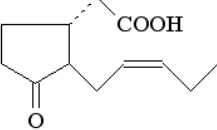
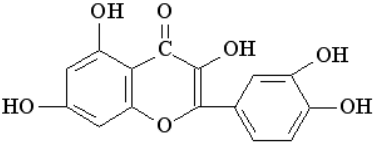
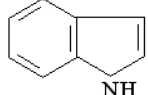
Presence of conjugated double bonds

The rationale of the photoprotective function displayed by various chemical types of secondary metabolites (flavonoids, including anthocyanins, cinnamic acid derivatives, xanthophylls) can be the presence of conjugated double bonds, i.e. delocalized π -electrons. This electronic configuration allows absorbance in the visible light- and UV- spectrum and easy electron and energy transfers (Cockell, 1997). π -electron configuration occurs in ring-closed, short side-chained and long chained-structures (Table 2). Xanthophylls containing numerous conjugated double bonds in a long chain operate in the xanthophyll cycle, performing dissipation of excess light energy as harmless heat (Demmig-Adams, 2003).

Presence of thiol-, hydroxy-, amino-, o- and p-diphenol groups and unsaturated carbon chains, including conjugated double bond-containing ones

Recently, an increasing amount of evidences substantiates the functioning of secondary metabolites as antioxidants and antiradicals, assisting the plants to cope with oxidative stress arising in hostile environments (Grace and Logan, 2000; Grassmann et al., 2002; Gould et al., 2002). The list is ever growing, involving hydroxy- and thiol-group-containing compounds, such as ascorbic acid and lipoic acid, o-dihydroxy group-containing flavonoids, such as quercetin, aliphatic and arylamines, unsaturated fatty acids, carotenoids (Edreva, 2005; Edreva et al., 2007). Chelation of transition metals (Fe) by flavonoids such as quercetin interferes with the generation of reactive oxygen species (ROS) via the Fenton reaction, thus contributing to a powerful antioxidant/antiradical performance (Leopoldini et al., 2006).

Table 1. Diverse chemical types of secondary metabolites.

Chemical types	Formulae	Representatives
Aliphatic	$\text{NH}_2\text{—CH}_2\text{—CH}_2\text{—CH}_2\text{—CH}_2\text{—NH}_2$ $\text{CH}_2=\text{CH}_2$ $\text{CH}_2=\underset{\text{CH}_3}{\text{C}}\text{—CH}=\text{CH}_2$	Polyamines Ethylene Isoprene
Aromatic	  	Phenolic alcohols Phenolic acids Unsaturated aromatic carbonic acids
Hydroaromatic	 	Terpenoids Jasmonic acid
Heterocyclic	 	Flavonoids Indole derivatives

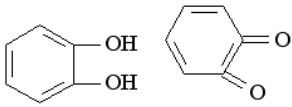

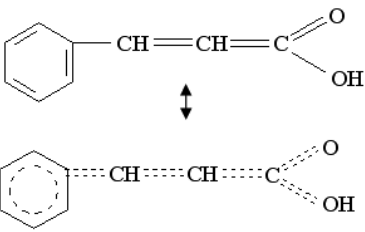
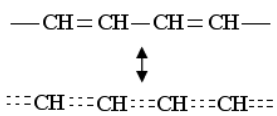
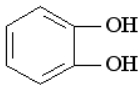
In our research with reed plants isoprene, a gaseous secondary metabolite containing two conjugated double bonds (Table 1) was shown to have an antioxidant effect in ozone- and heat-induced stress situations (Loreto and Velikova, 2001; Velikova et al., 2005). Evidence in favour of the singlet oxygen quenching ability of isoprene was obtained (Velikova et al., 2004). Moreover, for the first time singlet oxygen quenching effect of phenylamides (conjugates of unsaturated aromatic carbonic acids and polyamines) was demonstrated by *in vitro* experiments (Velikova et al., 2007). Formation of phenylamides and dramatic accumulation of polyamines in bean and tobacco following abiotic stresses were reported, thus suggesting an antioxidant role of these secondary metabolites (Edreva et al., 1995; 1998; 2007).

Ortho-dihydroxy substitution in the B-ring of anthocyanins potentiating the antioxidant capacity was proposed as a protective mechanism in a physiological disorder of cotton (leaf reddening), due to oxidative stress provoked by Na^+/K^+ imbalance (Edreva et al., 2006). Flavonoids (quercetin glycosides) and cinnamic acid derivatives endowed with high ROS scavenging capacity were suggested as a rationale of drought tolerance in cotton (Aktaş et al., unpublished).

OTHER CHARACTERS AND FUNCTIONS

Color and scent performance by secondary metabolites, such as flavonoids, terpenoids, and other volatiles can underlie attraction or repelling of insects and herbivores, while toxins can be involved in plant-plant allelopathic interactions (Hadacek, 2002). Water solubility and lack of electrical charge contributes to the compatible solutes being implicated in osmoregulation and protection of hydrophilic cellular sites, while hydrophobic molecules (carotenoids) are the best candidates to protect lipophilic surfaces such as membranes. It is becoming increasingly clear that secondary metabolites may play an important role as signal molecules. The best known examples are ethylene, salicylic acid and jasmonic acid (Table 1) (Dixon et al., 2002; Vasconsuelo and Boland, 2007). However, the chemical rationale of their involvement in the signal transduction cascades remains elusive.

Table 2. Chemical characteristics of secondary metabolites.

Chemical characteristics	Interactions	Functions
Availability of electrical charge	Electrostatic	Structure-stabilizing
Presence of $-COOH, -OH, -NH_2,$ 	Covalent bonding: polymerisation, condensation, complexation	Structure-stabilizing (cell wall-strengthening) Antimicrobial (blocking of active sites) Hypersensitive response (HR)
Availability of conjugated double bonds (delocalized π -electrons) in:  Ring-closed structures  Ring-closed Short side-chained structures  Long-chained structures	Light and UV- absorbance Energy dissipation	Photoprotective
Availability of $-NH_2, -SH, -OH,$  $-CH-CH=CH-CH=$ (unsaturated carbon chains)	H and electron transfers	Antioxidant Antiradical

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