Stress Enhances the Synthesis of Secondary Plant Products: The Impact of Stress-Related Over-Reduction on the Accumulation of Natural Products

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This review is dedicated to Eric E. Conn on the occasion of his 90th birthday, an outstanding plant biochemist who has contributed exceptionally to our understanding of plant secondary metabolism.

Spice and medicinal plants grown under water deficiency conditions reveal much higher concentrations of relevant natural products compared with identical plants of the same species cultivated with an ample water supply. For the first time, experimental data related to this well-known phenomenon have been collected and a putative mechanistic concept considering general plant physiological and biochemical aspects is presented. Water shortage induces drought stress-related metabolic responses and, due to stomatal closure, the uptake of CO_2 decreases significantly. As a result, the consumption of reduction equivalents ($NADPH + H^+$) for CO_2 fixation via the Calvin cycle declines considerably, generating a large oxidative stress and an over-supply of reduction equivalents. As a consequence, metabolic processes are shifted towards biosynthetic activities that consume reduction equivalents. Accordingly, the synthesis of reduced compounds, such as isoprenoids, phenols or alkaloids, is enhanced.

Keywords: Drought stress • Over-reduction • Secondary plant products • Surplus of energy.

Abbreviations: APX, ascorbate peroxidase; Chl, chlorophyll; GABA, γ-aminobutyric acid; SOD, superoxide dismutase.

Introduction

It is well known that plants grown under Mediterranean or semi-arid climate conditions have a much more pronounced taste and are more aromatic than those obtained from similar plants of the same species cultivated in areas with a moderate climate, such as Central Europe (Selmar 2008). Analogous quality differences have also been substantiated in medicinal plants, i.e. the concentration of the corresponding secondary plant products was less in plants grown in a moderate Atlantic climate compared with the concentrations found in plants cultivated in semi-arid regions. In general, this phenomenon is frequently explained by the fact that plants grown in Southern Europe 'are exposed to much more sunlight, which results in enhanced rates of biosynthesis'. Albeit that—at first glance—such an assertion seems to be rational, sunlight does not usually represent a limiting factor for plant growth. Even in Central Europe, when plants are grown in open areas without any shade, the light energy absorbed by the leaves is far higher than the plants require for photosynthetic CO_2 fixation (de Bianchi et al. 2010, Wilhelm and Selmar 2010). However, at least in the subtropics, high irradiation frequently co-occurs with water deficiency. Accordingly, in regions of semi-arid climate, plants often suffer drought stress. As stress-related metabolic changes impact on all plant processes extensively, the synthesis and accumulation of secondary metabolites should also be affected (Selmar 2008). Unfortunately, these coherences have not been considered adequately so far (Selmar and Kleinwächter, 2012). In order to obtain a clearer picture of this issue, we have compiled a comprehensive survey of the relevant literature on the impact of drought stress on natural product metabolism. Subsequently, the metabolic background behind the stress-related enhancement of natural product synthesis has been outlined in this review.

Synthesis and accumulation of secondary plant products in drought-stressed plants

It is well established that the accumulation of natural products strongly depends on the growing conditions, such as the temperature, the light regime and the nutrient supply (Gleadow and Woodrow 2002, Falk et al. 2007, Ballhorn et al. 2011; for review see Gershenzon 1984). In addition, more severe environmental influences, such as various stress conditions, will also impact on the metabolic pathways responsible for the accumulation of secondary plant products (Bohnert et al. 1995). Unfortunately, to date, only limited information on this complex issue has been made available. In most of the few existing

studies dealing with the influence of stress on the accumulation of secondary plant products, only one certain stress factor has been comprehensively investigated, i.e. by comparatively quantifying the content of equivalent natural products in stressed and unstressed plants, without being aware that various interactions between numerous factors may have occurred. In this manner, any increase in light intensity may be correlated with elevated leaf temperatures, and a decreased water potential adjusted to induce drought stress frequently also leads to higher salt concentrations in the soil. Moreover, elevated temperatures also affect transpiration and therefore plant water usage. Because of these considerations, many studies are not fully conclusive. Nonetheless, after thoroughly reviewing the literature, certain deductions about the effects of single factors on the accumulation of secondary plant products can be made. This treatise focuses only on drought stress.

A wide range of experiments have shown that plants exposed to drought stress did indeed accumulate higher concentrations of secondary metabolites. Such enhancement is reported to occur in nearly all classes of natural products, such as simple or complex phenols, numerous terpenes, as well as in nitrogen-containing substances, such as alkaloids, cyanogenic glucosides or glucosinolates (**Table 1**). There is therefore no doubt that drought stress frequently enhances the concentration of secondary plant products. However, this stress-related increase in natural product concentrations does not mean that the rate of biosynthesis of natural products in the plants has increased, since the drought stress applied also reduces growth and biomass production in most plants. Consequently, a simple and, at first sight, obvious and rational explanation for this effect is that: 'Plants suffering drought stress, in principle, synthesize and accumulate the same amounts of natural products as under well watered conditions but, due to the reduction in overall plant biomass, product concentration on a fresh or dry weight basis is enhanced!' In order to obtain a clear picture of the impact of drought stress on natural product biosynthesis, the total amount of secondary plant products has to be calculated. For such an assessment, however, in addition to the determination of the secondary plant product concentration (mol g^{-1} FW or DW) in stressed and well-watered plants, there also needs to be an estimation of the entire biomass of the plants under both stressed and unstressed conditions. Unfortunately, in most of the studies published so far, few or no data on the total biomass of the plants analyzed have been published. This lack of information is generally due to the fact that in the related studies only certain plant parts (e.g. roots, leaves or seeds) had been analyzed, whereas the total content of natural products on a whole-plant basis was not the central focus of the studies. However, in a few studies, the total content of secondary plant products has been given or could be calculated from the data presented.

Using the whole-plant criterion, the data presented by de Abreu and Mazzafera (2005) showed that in *Hypericum brasiliense*, the concentration and total amount of phenolic compounds were drastically enhanced in plants grown under drought stress in comparison with the control plants. Despite the fact that the stressed plants were smaller, the product of biomass and natural product concentration yields a 10% increase in the total content of phenolic compounds found in the stressed plants. Furthermore, a similar conclusion can be drawn when evaluating the results of Nogués et al. (1998), who reported a significant increase in the concentration of phenolic compounds in stressed pea (*Pisum sativum*). The authors found that although the total biomass of the pea plants grown under drought stress was only one-third of that of those cultivated under standard conditions, the overall anthocyanin content (product of biomass and anthocyanin concentration, i.e. the anthocyanins g^{-1} FW) was around 25% higher in the stressed plants compared with the unstressed plants. In contrast, the total amount of flavanoids was nearly the same, whether the plants were grown under drought stress or under non-stress conditions. In red sage (*Salvia miltiorrhiza*), the overall content, i.e. the total amount per plant, of furoquinones even decreased slightly under drought stress conditions, although there was a significant increase in their concentration (Liu et al. 2011).

With respect to terpenoids, there have been many reports showing that—apart from the drought stress-related increase in their concentration (terpenes g^{-1} biomass) putatively due to the reduction in dry weight—there was also an effective increase in the terpenoid content on the whole-plant basis. In this manner, the large, drought stress-related increase in monoterpene concentration in sage (*Salvia officinalis*) was much greater than the corresponding loss in biomass (Nowak et al. 2010). Accordingly, in sage suffering moderate drought stress, the total content of monoterpenes per plant was significantly higher than in the well-watered controls. In contrast, Manukyan (2011), who detected only a slight drought stress-related increase in monoterpene concentration (terpenes g^{-1} biomass) in catmint and lemon balm plants, calculated and reported a stress-related decrease in the total content of terpenoids per plant in *Melissa officinalis*, *Nepeta cataria* and *Salvia officinalis*. In parsley leaves (*Petroselinum crispum*), the drought stress-related increase in the concentration of essential oils was more or less completely compensated by the related loss in biomass, resulting in almost the same overall essential oil contents in both drought-stressed and well-watered plants. With respect to nitrogen-containing secondary plants products, corresponding data on a whole-plant basis are rare: Ballhorn et al. (2011) demonstrated that in *Phaseolus lunatus* not only the concentration of cyanogenic glucosides (μmol HCN equivalents g^{-1} FW), but also their total amount per plant, is enhanced under drought stress conditions.

In summary, for most plants examined, the concentrations of secondary plant products (on a gram biomass basis) were significantly enhanced under moderate drought stress conditions. However, this stress-induced increase was often counteracted by a corresponding decrease in biomass. Due to the lack of data, in most cases, the impact of drought stress on the total content of secondary metabolites could not be estimated. Therefore, a stress-related enhancement in the total amount

Table 1 Drought stress-related increase in the contents concentrations of various classes of natural products

Simple phenols

Helianthus annuus	Chlorogenic acid	Massive increase (10-fold)	del Moral (1972)
Prunus persica	Total phenols	Higher content in stressed plants	Kubota et al. (1988)
Thymus capitatus	Phenolics	Higher content in stressed plants	Delitala et al. (1986)
Echinacea purpurea	Total phenols	Strong increase (67%)	Gray et al. (2003)
Crataegus spp.	Chlorogenic acid	Massive increase (2- to 6-fold)	Kirakosyan et al. (2004)
Hypericum brasiliense	Total phenols	Strong increase (>80%)	de Abreu and Mazzafera (2005)
Labisia pumila	Total phenols	Significant increase (50%)	Jaafar et al. (2012)

Complex phenols

Pisum sativum	Flavanoids	Strong increase (45%)	Nogués et al. (1998)
Pisum sativum	Anthocyanins	Strong increase (>L80%)	Nogués et al. (1998)
Crataegus spp.	Xatechins/epicatechins	Massive increase (2- to 12-fold)	Kirakosyan et al. (2004)
Hypericum brasiliense	Rutin/quercetin	Massive increase (4-fold)	de Abreu and Mazzafera (2005)
Hypericum brasiliense	Xanthones	Strong increase (>80%)	de Abreu and Mazzafera (2005)
Camellia sinensis	Epicatechins	Higher content in stressed plants	Hernández et al. (2006)
Salvia miltiorrhiza	Furoquinones	Significant increase	Liu et al. (2011)
Prunella vulgaris	Rosmarinic acid	Slight increase	Chen et al. (2011)
Labisia pumila	Anthocyanin/flavonoids	Significant increase	Jaafar et al. (2012)

Monoterpenes/essential oils

Mentha x piperita ssp.	Essential oils	Significant increase	Charles et al. (1990)
Cymbopogon pendulus	Geraniol and citral	Strong increase	Singh-Sangwan et al. (1994)
Pinus halepensis	α-Pinene and carene	Strong increase	Llusià and Peñuelas (1998)
Cistus monspeliensis	Caryophyllene	Enormous increase	Llusià and Peñuelas (1998)
Satureja hortensis	Essential oils	Increase	Baher et al. (2002)
Picea abies	Monoterpenes	Strong increase	Turtola et al. (2003)
Pinus silvestris	Monoterpenes	Strong increase	Turtola et al. (2003)
Petroselinum crispum	Essential oils	Strong increase (double)	Petropoulos et al. (2008)
Salvia officinalis	Essential oils	Nassive increase (2- to 4-fold)	Beattaieb et al. (2009)
Salvia officinalis	Monoterpenes	Strong increase	Nowak et al. (2010)
Scrophularia ningpoen.	Iridoid glycosides	Increase	Wang et al. (2010)
Nepeta cataria	Essential oils	Significant increase	Manukyan (2011)

Di- and triterpenes

Solanum tuberosum	Steroid alkaloids	Strong increase	Bejarano et al. (2000)
Hypericum brasiliense	Betulinic acid	Strong increase	de Abreu and Mazzafera (2005)
Bupleurum chinense	Saikosaponin	Significant increase	Zhu et al. (2009)
Prunella vulgaris	Triterpenes	Slight increase	Chen et al. (2011)

Alkaloids

Senecio longilobus	Pyrrolizidine alkaloids	Strong increase	Briske and Camp (1982)
Lupinus angustifolius	Quinolizidine alkaloids	Strong increase	Christiansen et al. (1997)
Solanum tuberosum	Steroid alkaloids	Strong increase	Bejarano et al. (2000)
Glycine max	Trigonelline	Higher content in stressed plants	Cho et al. (2003)
Papaver somniferum	Morphine alkaloids	Strong increase	Szabó et al. (2003)
Catharanthus roseus	Indole alkaloids	Strong increase (with Ca^{2+})	Jaleel et al. (2007)
Phellodend. amurense	Benzylisoquinolines	Strong increase	Xia et al. (2007)
Senecio jacobaea	Pyrrolizidine alkaloids	Massive increase	Kirk et al. (2010)
Nicotiana tabacum	*Nicotiana* alkaloids	Strong increase	Çakir and Çebi (2010)

Various classes

Manihot esculenta	Cyanogenic glucosides	Strong increase	de Bruijn (1973)

(continued)

Table 1 Continued

Triglochin maritima	Cyanogenic glucosides	Strong increase	Majak et al. (1980)
Brassica napus	Glucosinolates	Massive increase	Jensen et al. (1996)
Manihot esculenta	Cyanogenic glucosides	Higher content in stressed plants	Bokanga et al. (1994)
Eucalyptus cladocalyx	Cyanogenic glucosides	Massive increase	Gleadow and Woodrow (2002)
Coffea arabica	γ-Aminobutyric acid	Massive increase (10-fold)	Bytof et al. (2005)
Brassica oleracea	glucosinolates	Significant increase	Radovich et al. (2005)
Brassica carinata	Glucosinolates	Significant increase	Schreiner et al. (2009)
Phaseolus lunatus	Cyanogenic glucosides	Higher content in stressed plants	Ballhorn et al. (2011)

of natural products per plant has been detected in relatively few published studies. In contrast, stress may also result in the same total content in both stressed and well-watered plants, and in other studies drought stress elicited a decline in the total content of secondary plant products, even though their concentration was significantly increased by drought stress.

Efficient energy dissipation is essential for plant viability

Due to our recurring experience in daily life, we all have internalized that energy saving represents one of the most important requirements for our subsistence, and corresponding statements have become fundamental and general denominations. Accordingly, these considerations are frequently transferred imprudently into plant biology. Although most biologists recognized that—apart from plants in shade—the light energy absorbed by leaves generally is not the limiting factor for photosynthesis, common teleological statements such as 'In order to save energy, plants have evolved a certain mechanism' or 'Due to cost–benefit equations, the energy supply for a certain metabolic process must be minimized' are found in the literature (for a review, see Wilhelm and Selmar 2010). Accordingly, many plant biologists have been confronted with such appraisals, and frequently the fundamental limitations of these deductions are neglected. In contrast to heterotrophic organisms, many other factors are crucial to autotrophic plants, which generally do not suffer from energy shortage. However, the principle of 'energy saving' is frequently transferred unreflectingly to plants and their metabolic requirements. That plants usually obtain excess energy can easily be deduced from the well known observation that the photosynthetic rate of CO_2 fixation, e.g. as determined for the estimation of light saturation curves, increases considerably when the ambient CO_2 concentration is significantly elevated. Such enhancement of photosynthetic activity could result in CO_2 fixation rates of more than twice that of those observed under the ambient CO_2 concentration. The additional CO_2 is apparently fixed and reduced without any problems; accordingly, large amounts of additional NADPH + H^+ and ATP are required. This vividly documents that far more reduction and energy equivalents are generated and available under ambient conditions than actually are being consumed.

However, the corresponding massive surplus of reduction capacity would immediately result in a complete reduction of the $NADP^+$ pool to NADPH + H^+. As a consequence, no further electrons from the photosynthetic electron transport chain could be transferred to the pool of reduction equivalents and they would, instead, be transferred to oxygen, generating superoxide radicals. This process, known as the Mehler reaction (Hideg et al. 1995, Chen et al. 2004), is also denoted as the water–water cycle (Hormann 1993). The superoxide radicals produced are very reactive and would enter numerous further reactions and thereby generate various types of reactive oxygen species, which would finally destroy the entire photosynthetic apparatus (Jakob and Heber 1996), a process called 'oxidative photodestruction' (**Fig. 1**). Thus, the major energy-related issue that plants face concerns dissipation of surplus energy without damaging their photosynthetic machinery (Wilhelm and Selmar 2010).

Although plants generally gain far more energy than they require for photosynthetic CO_2 fixation, generation of oxygen radicals, as outlined above, is normally suppressed through the prevention of their over-reduction by various protective mechanisms (for reviews, see de Bianchi et al. 2010, Wilhelm and Selmar 2010). A very large proportion of energy is dissipated by so-called non-photochemical quenching, a mechanism which converts the absorbed light energy already within PSII into heat (for a review, see Nilkens et al. 2010). The extent of this energy dissipation is regulated by a specific mechanism: light-induced acidification within the thylakoids induces a protonation-related conformational change of the so-called PsbS proteins as well as activation of a de-epoxidase, which converts violaxanthin into zeaxanthin. As a consequence, zeaxanthin comes into direct contact with the Chls of the light-harvesting proteins, and excitation energy is effectively transferred from Chl to xanthophyll (Li et al. 2004, Szabó et al. 2005). Additional mechanisms which prevent over-reduction of the photosynthetic transport chain are based on the effective re-oxidation of NADPH + H^+ via photorespiration (Smirnoff 1993, Wingler et al. 2000) or the xanthophyll cycle (Lin et al. 2002, Latowski et al. 2004). Therefore, under regular conditions, effective energy dissipation mechanisms prevent the formation of oxygen radicals and thereby prevent any oxidative photodestruction. Accordingly, there is no requirement for any removal of oxygen radicals by detoxification via superoxide dismutase
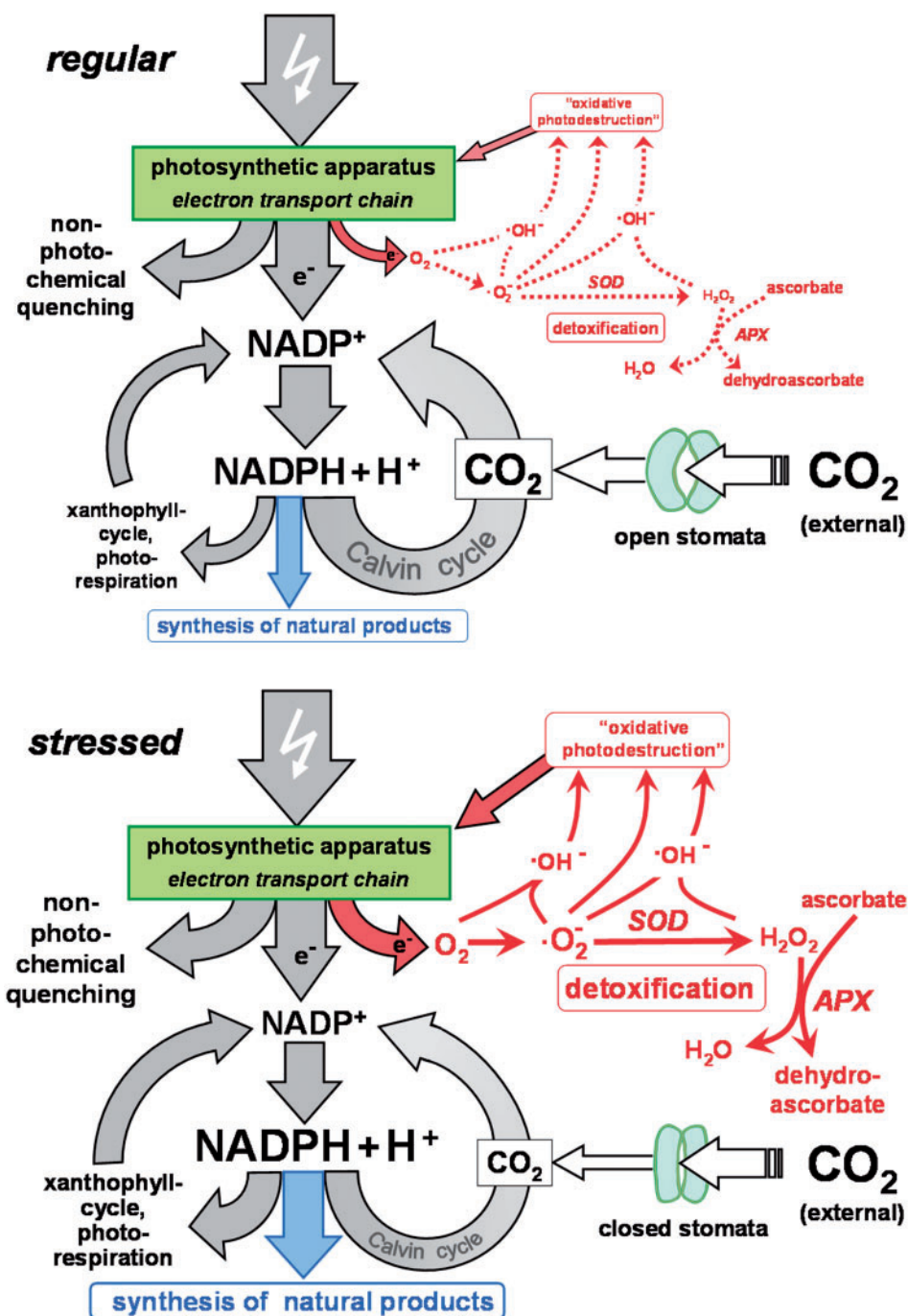
Fig. 1 Energy dissipation in plants according to Selmar and Kleinwächter (2012). As the light energy absorbed by the photosynthetic apparatus is much higher than the energy required for CO_2 fixation, large amounts of energy have to be dissipated via non-photochemical quenching and effective re-oxidation of $NADPH + H^+$, e.g. via photorespiration and the xanthophyll cycle. Due to the action of this protective machinery, the surplus reduction capacity does not result in a significant increase in the amount of radicals produced by the Mehler reaction, i.e. the transfer of electrons to molecular oxygen, leading to the production of superoxide radicals. In contrast to normal conditions in well-watered plants, energy fluxes in stressed plants are markedly shifted. Due to the elevated diffusion resistance caused by stomatal closure, the concentration of CO_2 is much lower within the stressed leaves. As a result, much less $NADPH + H^+$ can be consumed within the Calvin cycle for the fixation and reduction of CO_2. Accordingly, a much greater share of the energy has to be dissipated. Although the corresponding processes (non-photochemical quenching, photorespiration and the xanthophyll cycle) are enhanced by feedback mechanisms, numerous electrons are transferred to molecular oxygen. The superoxide radicals generated subsequently produce a wide range of further reactive oxygen species (ROS). Due to the stress-related induction of superoxide dismutase (SOD) and ascorbate peroxidase (APX), superoxide radicals are detoxified and thus production of large amounts of ROS is prevented. The strong increase in the reduction potential (ratio of $NADPH + H^+$ to $NADP^+$) enhances, according to the law of mass action, the synthesis of highly reduced natural products.

(SOD) and ascorbate peroxidase (APX), which is characteristic of stress conditions. Under normal, non-stress conditions, however, due to the co-occurrence of the various dissipation mechanisms outlined above, a certain balance between $NADPH + H^+$ and $NADP^+$ will be achieved that accounts for and determines the velocity and extent of all additional reactions, in particular the biosynthesis of highly reduced natural products (**Fig. 1**).

Putative biochemical mechanism of stress-related enhancement of secondary metabolite synthesis

In contrast to coping effectively with the surplus of energy under regular conditions, the metabolic situation for the plants is far more severe when they are suffering from drought stress. Water shortage induces partial stomatal closure (Chaves 1991), increasing diffusion resistance for all gases. Accordingly, under these conditions, the concentration of CO_2 within the leaves decreases to a low level, and far less $NADPH + H^+$ and ATP are consumed for CO_2 fixation within the Calvin cycle (**Fig. 1**). This condition results in a strong decline in the $NADP^+$ concentration and thereby the amount of adequate electron acceptors for the photosynthetic electron transport chain drastically decreases. Although energy dissipation via non-photochemical quenching and the re-oxidation of $NADPH + H^+$ will be enhanced by the corresponding feedback mechanisms, the resulting over-reduction leads to a large increase in the amount of oxygen radicals being produced. As a consequence, under drought stress conditions, an efficient detoxification machinery is induced (**Fig. 1**); gene expression of typical stress response enzymes, such as SOD and APX, is strongly up-regulated (Mittler and Zilinskas 1994, Acar et al. 2001, Gratao et al. 2005). SOD catalyzes dismutation of superoxide radicals to O_2 and H_2O_2. The latter is subsequently reduced to water by APX (Smirnoff 1993, Shalata et al. 2001) (**Fig. 1**). This means that drought stress-related over-reduction, at least up to a certain level, does not lead to massive oxidative photodestruction. The ability of the energy dissipation to deflect the large surplus of energy and its significance have been clearly demonstrated by various experimental approaches where these protective mechanisms were repressed. As outlined above, non-photochemical quenching requires the xanthophyll cycle to operate. Accordingly, the carotenoids, apart from their putative function of enhancing the amount of light energy absorbed under shady conditions, are also needed for efficient energy dissipation (for a review, see Cunningham and Gantt 1998). Without these accessory pigments, the illuminated leaves would suffer from photobleaching due to the energy surplus not being dissipated. By using virus-induced gene silencing (VIGS), phytoene desaturase, a key enzyme in carotene biosynthesis (Cunningham and Gantt 1998), is commonly used as a marker for successful gene silencing (Dalmay et al. 2000, Turnage et al. 2002). Successfully infected leaves or leaf areas do not synthesize any carotenes and show characteristic photobleaching symptoms, resulting from the lack of adequate energy dissipation because the xanthophyll cycle, and thus non-photochemical quenching, is not functional.

Apart from stress-induced production of superoxide radicals, an increase in the ratio of $NADPH + H^+$ to $NADP^+$ has strong metabolic consequences. According to the law of mass action, any enhancement of reductive power shifts all reactions involving $NADPH + H^+$ consumption towards the reduction side of the equilibrium. Yet, it has to be taken into account that these processes might be overlaid by complex regulatory mechanisms. However, even without any change in enzyme activity, the rate of synthesis of the highly reduced secondary plant products should rise under a highly reduced metabolic status. This also accounts for the synthesis of highly reduced precursors, e.g. aromatic amino acids. It should be noted that the oxidation number of the carbon atoms in sugars and carbohydrates is mostly 0, whereas the average oxidation numbers of the carbon atoms in monoterpenes and in alkaloids are generally far lower (e.g. -1.8 for menthol, -1.6 for limonene and -1.4 for lupinine), indicating the high degree of reduction of these natural products.

To date, there have been only limited data available to confirm these insights. Nowak et al. (2010) performed various drought stress experiments where additionally the concentration of CO_2 was also altered. The underlying approach is quite intriguing: as outlined above, because of stomatal closure, in drought-stressed plants the internal CO_2 concentration decreases markedly, causing extensive over-reduction. However, when in similar trials the CO_2 concentration is concomitantly markedly elevated (i.e. to 700 p.p.m.)—despite the increased diffusion resistance—the internal CO_2 concentration is significantly enhanced. As a result, the reduction capacity, i.e. the ratio of $NADPH + H^+$ to $NADP^+$, should decline. According to the law of mass action, this, in turn, should directly cause a decrease in the rate of biosynthesis of highly reduced secondary metabolites. These series of corresponding experiments were performed with *S. officinalis*. As the content of monoterpenes strongly depends on leaf age, the concentration of these secondary metabolites in the plants of different trials were quantified separately for young, middle-aged and old leaves (Nowak et al. 2010). The concentration of monoterpenes in leaves of all ages increased in sage plants grown under stress conditions. Moreover, the total content of monoterpenes in the whole plant increased despite an overall reduction in biomass production (**Fig. 2**). These findings strongly support the theory that terpene synthesis increases under stress conditions due to the enhanced reduction pressure. As predicted, the elevation of the CO_2 concentration resulting in a decline of the over-reduced status leads to significant decreases in monoterpene concentrations. These results, for the first time, illustrate a direct relationship between the biosynthesis of natural products and the reduction pressure, and thus suggest that an enhanced synthesis of secondary plant products may contribute to prevent damage caused by radicals evolved due to the drought-related over-reduced states.
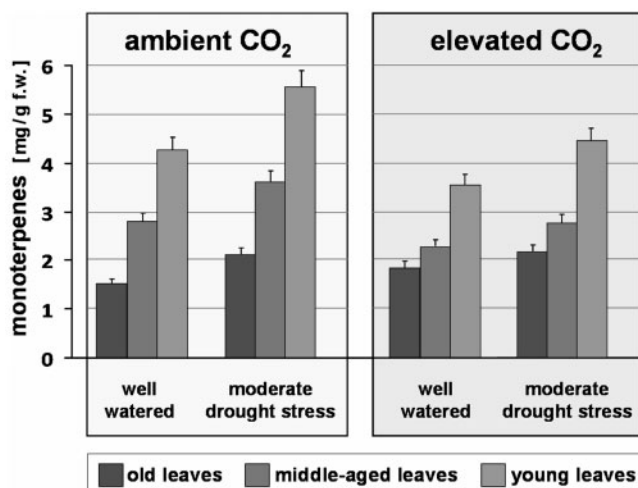
Fig. 2 Monoterpene concentrations in leaves of differentially stressed sage plants (leaves of various ages). The monoterpene concentrations were quantified after 8 weeks of cultivation under the particular conditions mentioned. The overall values correspond to the sum of the concentration for cineole, camphor and the mixture of both the stereoisomers of α-/β-thujone (data from Nowak et al. 2010). Elevated CO_2 concentration was adjusted to 700 p.p.m. Further comprehensive quantification revealed that, on average, about 59.9 mg of monoterpenes were accumulated in each control plant, while the drought-stressed individuals contained about 73.9 mg. This surplus resulted despite the fact that the biomass of the well-watered plants was significantly greater than that of the stressed plants (24.5 g vs. 21.8 g).

Interference with other factors

Secondary plant products possess remarkable relevance for interaction of plants with their environment (Harborne 1988). For example, many repel herbivores, protect against pathogens or attract pollinators (Hartmann 2007 Wink 2010). Some of these substances are also related to abiotic stress, for example protection against UV light or high light intensities or reduction of transpiration, or have relevance as compatible solutes or radical scavengers (Edreva1 et al. 2008, Wink 2010). The syntheses of secondary metabolites are considered to be frequently induced and modulated by numerous environmental factors, e.g. the synthesis of phytoalexins is elicited by pathogen attack (Hahlbrock et al. 2003, Saunders and O'Neill 2004), and relevant defense compounds against herbivores are synthesized as a result of complex induction mechanisms (Ferry et al. 2004). Thus, the actual synthesis and accumulation of a particular natural product is influenced and determined by numerous elements. In this context, it is important to consider that a particular stress situation usually influences several factors. For example, high irradiation is frequently accompanied by elevated temperatures; high irradiation by high UV radiation, and elevated temperatures by a higher evaporation, and drought stress may be associated with a greater herbivore pressure but a reduced number of pathogens. Patterns of complex interferences involving numerous factors should be considered

due to these contemporary occurrences. The actual influence of a certain component remains to be elucidated by more comprehensive studies using suitable and reliable markers or indicators for certain stresses.

With respect to drought stress, the most appropriate markers would be either the ratio of $NADPH + H^+$ to $NADP^+$ or the amount of oxygen radicals generated. Unfortunately, the actual *in situ* concentration of either of these components cannot be quantified without major effort and expenditure. Alternatively, the levels of certain so-called stress metabolites that are synthesized and accumulated more or less specifically in a particular stress situation have to be analyzed instead. In this context, proline is especially useful as it is accumulated as a compatible solute in plants suffering drought stress (for review see Rhodes et al. 1999). However, because drought stress-related proline accumulation does not occur in many plant species, the most cited and commonly used stress marker is γ-aminobutyric acid (GABA; for a review see Kinnersley and Turano 2000). Unfortunately, GABA is not only produced in great quantities in response to drought stress, but it is also accumulated under various other stress conditions (Satya Narayan and Nair 1990, Bown and Shelp 1997). Therefore, other markers are required. In this context, the abundance of dehydrins is notable. Dehydrins, first discovered in seeds during late embryogenesis in the course of maturation drying, are also frequently synthesized in plant cells suffering drought stress (for reviews, see Close 1997, Allagulova et al. 2003, Bouché and Fromm 2004). These small hydrophilic proteins are thought to be involved in various protective functions in desiccating cells (Hara 2010). However, the expression of dehydrins and the accumulation of the stress metabolite GABA may follow different time patterns in the same stressed organism; this has been demonstrated for coffee seeds during drying (Kramer et al. 2010). As in leaves of plants exposed to drought stress, apart from over-reduction due to stomatal closure (see above), the water availability is also reduced, and various metabolic responses should occur concomitantly or subsequently. As a consequence, the elucidation of all the metabolic changes induced in drought-stressed plants requires a combination of several markers, e.g. the accumulation of GABA and the expression of dehydrins.

Conclusion

Concentrations of natural products are generally enhanced in plants suffering drought stress. This increase could either be due to a stress-related decline in biomass production associated with an unchanged biosynthesis rate of natural products or to an authentic enhancement of the total secondary metabolite content. The latter option is obviously related to drought stress-induced over-reduction, which favors biosynthesis of highly reduced compounds. However, this simplified model becomes much more complex as these reactions are frequently overlaid by and interact with numerous other factors that also impact on the biosynthesis of secondary plant products. Yet, these considerations suggest that an enhanced synthesis of

secondary plant products may also contribute to prevent damage caused by radicals evolved due to the drought-related over-reduced states.

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