JJBS

Volume 8, Number 1, March .2015
ISSN 1995-6673
Pages 1 - 10

Jordan Journal of Biological Sciences **Review**

Influencing the Contents of Secondary Metabolites in Spice and Medicinal Plants by Deliberately Applying Drought Stress during their Cultivation[1]

Ahmad Al-Gabbiesh, Maik Kleinwächter and Dirk Selmar*

Institute for Plant Biology, Technische Universität Braunschweig, Mendelssohnstraße 4, D-38106 Braunschweig, Germany

Received: September 9, 2014 Revised: September 30, 2014 Accepted: October 25, 2014

Abstract

Medicinal plants grown under semi-arid conditions generally reveal significantly higher concentrations of relevant natural products than identical plants of the same species, which are cultivated in moderate climates. So far, there is very limited information on this well-known phenomenon. In the present study, corresponding data are compiled and relevant aspects are discussed. It turns out that metabolic reactions triggered by drought stress are responsible for the higher natural product accumulation in plants grown in semi-arid regions. The related plant physiological and biochemical background can be summarized as follows: In plants suffering drought stress, the water shortage caused by limited water supply triggers stomata closure. As a result, the uptake of CO_2 is markedly decreased and the consumption of reduction equivalents ($NADPH+H^+$) consumed in the course of CO_2-fixation via Calvin cycle declines considerably, generating a massive oversupply of $NADPH+H^+$. Consequently, all metabolic processes are pushed towards the synthesis of highly reduced compounds, such as isoprenoids, phenols or alkaloids. Based on these considerations, impulses for novel practical approaches for enhancing the product quality by deliberately applying drought stress during the cultivation of medicinal plants are outlined. However, as drought stress concomitantly also leads to massive reductions in biomass production, special emphasis must be put on the interference of these stress related effects.

Keywords: Drought stress, Secondary metabolites, Over reduced state, Surplus of energy, Medicinal plants.

1. 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. Obviously, under semi-arid conditions the concentrations of aroma relevant natural products are enhanced. Analogous quality differences are observed with regard to medicinal plants, i.e., the content of the corresponding relevant secondary plant products in general is less in plants grown in a moderate Atlantic climate than in those cultivated in semi-arid regions. In the past, this phenomenon was frequently explained by the unsophisticated statement that plants grown in Southern Europe "*are exposed to much more sunlight, resulting in enhanced rates of biosynthesis*". Indeed – on the first sight – such an assertion seems to be consistent; however, we have to bear in mind that the sunlight, generally, is not at all a limiting factor for plant growth. In consequence, even in Central Europe, most plants, growing in open areas without any shading, absorb much more light energy than what they require and what they utilize for photosynthetic CO_2-fixation (Wilhelm and Selmar, 2011). Nonetheless, we have to take into account that - at least in the subtropics - high irradiation often co-occurs with water deficiencies. In consequence, under semi-arid conditions, plants are frequently exposed to drought stress, which affects the entire metabolism. Accordingly, the synthesis and accumulation of secondary metabolites should also be affected. Unfortunately, in the past, these coherences were neglected and had never been given due adequate consideration (Selmar, 2008). Recently, Selmar and Kleinwächter (2013a) have compiled the relevant literature in order to get a comprehensive picture of this

---

[1] This article summarizes a lecture, on this topic, held by Dirk Selmar at the International Humboldt conference "Building International Network for Enhancement of Research in Jordan" in Amman at Princess Sumaya University for Technology (PSUT) in April 2014; it is also based on a synopsis of three relevant review articles: Selmar and Kleinwächter (2013a), Selmar and Kleinwächter (2013b), Kleinwächter and Selmar (2014).

* Corresponding Author: d.selmar@tu-bs.de

issue; and, for the first time, the metabolic background for the stress related enhancement of natural product synthesis was outlined (Selmar and Kleinwächter, 2013b; see Figure 1). The drought stress related water shortage leads to stomata closure and, thus, the uptake of CO_2 is markedly decreased. In consequence, the consumption of reduction equivalents ($NADPH+H^+$) for CO_2-fixation via Calvin cycle declines considerably, generating a massive oversupply of $NADPH+H^+$. According to the law of mass action, any enhancement of reductive power shifts all reactions consuming $NADPH+ H^+$ towards the reduction side of the equilibrium. We have to take into account that these processes might be overlaid by complex regulatory mechanisms. However, even without any change in the enzyme activity, the rate of synthesis of the highly reduced secondary plant products, such as isoprenoids, phenols or alkaloids, should rise under this highly reduced metabolic status.

The main objective and intention of this contribution is to illustrate how these insights could be used for elaborating new practical approaches. By intentionally applying drought stress during the cultivation of spice and medicinal plants, the product quality of the related commodities could be enhanced. However, as drought stress concurrently leads to massive reductions in biomass production, special emphasis must be put on the interference of these two stress related effects.

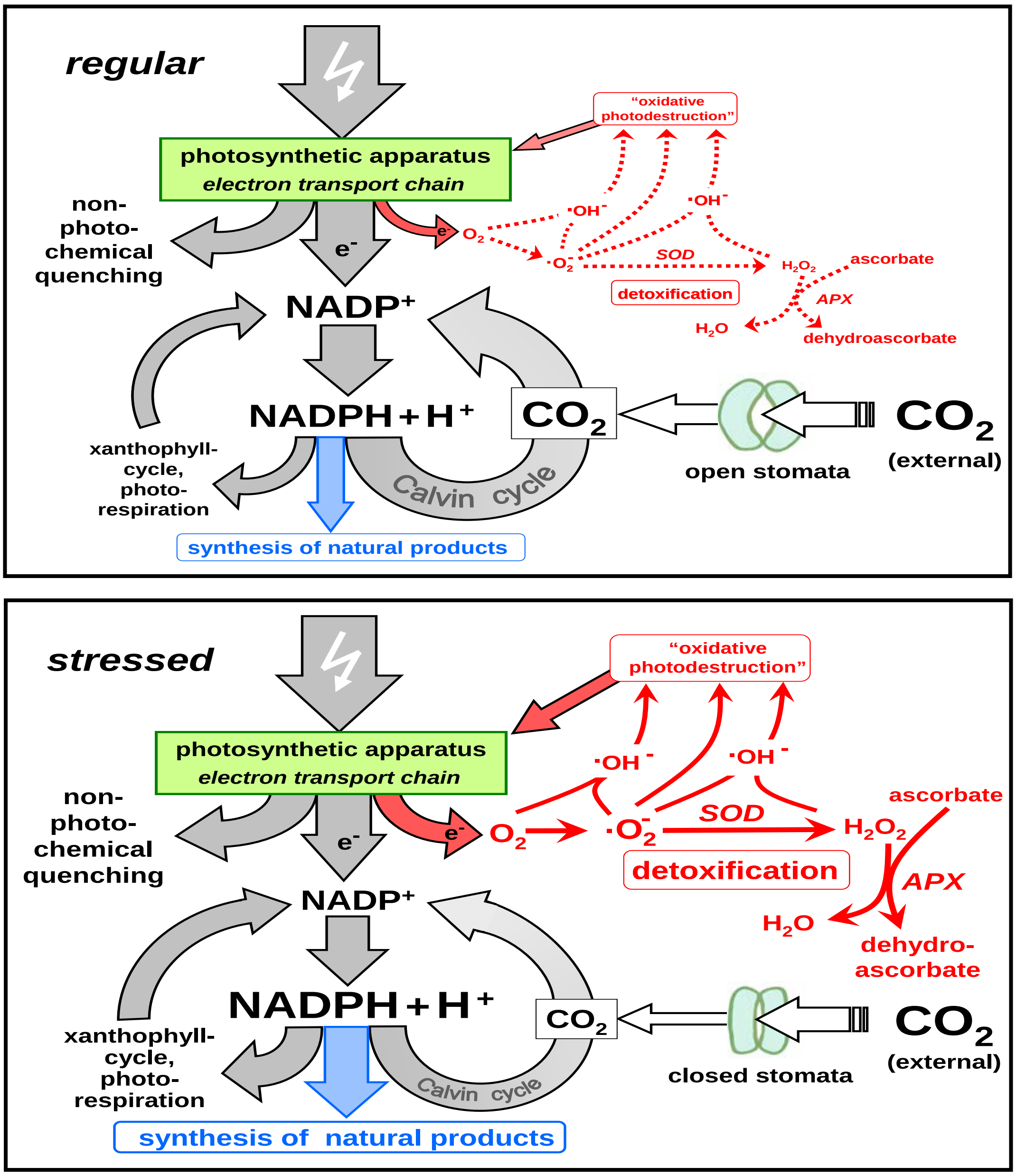

Figure 1. Model scheme energy dissipation and the drought stress related increase of natural product biosynthesis according to Selmar and Kleinwächter (2013b).

2. Synthesis and Accumulation of Natural Products is Enhanced in Drought Stressed Plants

It is well known that the synthesis and accumulation of secondary plant products strongly depends on growth conditions, e.g., on the temperature, the light regime, the nutrient supply, etc. (Gershenzon, 1984; Falk *et al.*, 2007). Consequently, much more severe environmental influences, such as the various stress situations, which are known to drastically influence general metabolism (Bohnert *et al.*, 1995), should also impact on secondary metabolism, i.e., on the biosynthesis and accumulation of natural products. Astonishingly, there is a tremendous lot of information dealing with the impact of biological stress (e.g., pathogen or herbivore attack) on the synthesis of secondary plant products (Harborne, 1988; Hartmann, 2007; Wink, 2010). Yet, corresponding information on how abiotic stress changes the secondary metabolism is rare. This especially accounts for the knowledge on the related physiological background (Ramakrishna and Ravishankar, 2011; Selmar and Kleinwächter, 2013b). A putative reason for these discrepancies might be due to the fact that host-pathogen and host-herbivore interactions are in general quite assessable, since only one distinct factor is involved, whereas the situation related to abiotic stress is frequently much more complex, since various interferences between numerous factors co-occur, e.g., the increase in light intensity mostly correlates with elevated temperatures, or lower water availability, inducing drought stress, often entails higher salt concentrations in the soil. As a consequence, the results of numerous studies on the impact of a certain abiotic stress on secondary metabolism lack the distinction of influences to other putative stress factors. Notwithstanding, a thorough reviewing of the relevant literature allows to evaluate and to filter out the putative impact of one certain factor on the accumulation of natural products, and thereby enabling decisive deductions. This treatise focuses on the effects of drought stress.

Up till now, numerous studies have shown that plants exposed to drought stress indeed reveal higher concentrations of secondary metabolites than those cultivated under well watered conditions (Table 1). Actually, the increase of this drought stress related concentration seems to be a common feature of all different classes of natural products and corresponding enhancements are reported to occur in the case of simple as well as complex phenols, and also for the various classes of terpenes (Table 1). Similarly, nitrogen containing substances, such as alkaloids, cyanogenic glucosides and glucosinolates, are influenced by drought stress (Table 1). As a general statement, it could be concluded that drought stress frequently enhances the concentration of secondary plant products. In this context, however, we have to consider that drought generally reduces the growth of stressed plants. Thus, due to the reduction in biomass – even without any increase in the overall amount of natural products – their concentration on dry or fresh weight basis could, simply, be enhanced. Accordingly, corresponding explanations are frequently adduced in the literature. As in most of the studies published, solid data on the overall biomass of the plants are lacking, only limited information is available on whether or not the observed concentration increase is also correlated with an enhancement of the total amount of related natural products. Certainly, one reason for this lack of information seems to be due the fact that mostly only certain plant parts or organs, e.g., roots, leaves or seeds, had been the focus of study and investigation, whereas the overall content of natural products on whole plant basis was not of interest. Notwithstanding, at least in some studies, the total contents of secondary plants products are given, or could be calculated from the data presented.

Table 1. Drought stress related concentration increase of natural products - Compilation of literature

	Simple Phenols		
Helianthus annuus	chlorogenic acid	massive increase (tenfold)	del Moral, 1972
Prunus persica	total phenols	higher contents	Kubota *et al*., 1988
Thymus capitatus	phenolics	higher contents	Delitala *et al*., 1986
Echinacea purpurea	total phenols	strong increase (67 %)	Gray *et al*., 2003
Crataegus spp.	chlorogenic acid	massive increase (2 - 6fold)	Kirakosyan *et al*., 2004
Hypericum brasiliense	total phenols	strong increase (over 80 %)	de Abreu *et al*., 2005
Trachyspermum ammi	total phenols	strong increase (100 %)	Azhar *et al*., 2011
Labisia pumila	total phenols	significant increase (50 %)	Jaafar *et al*., 2012
	Complex Phenols		
Pisum sativum	flavonoids	strong increase (45 %)	Nogués *et al*., 1998
Pisum sativum	anthocyanins	strong increase (over 80 %)	Nogués *et al*., 1998
Crataegus spp.	catechins / epicatechins	massive increase (2 - 12fold)	Kirakosyan *et al*., 2004
Hypericum brasiliense	rutine / quercetin	massive increase (fourfold)	de Abreu *et al*., 2005
Hypericum brasiliense	xanthones	strong increase (over 80 %)	de Abreu *et al*., 2005
Camellia sinensis	epicathecins	higher contents	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	anthocyane / 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 & citral	strong increase	Singh-Sangwan *et al*.,1994
Pinus halepensis	α-pinen, carene	strong increase	Llusià and Peňuelas, 1998
Cistus monospeliensis	caryophyllene	enormous increase	Llusià and Peňuelas, 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	massive increase (2 - 4fold)	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
Ocimum basilicum	essential oils	significant increase	Forouzandeh *et al*., 2012
Di- and Triterpenes			
Solanum tuberosum	steroid alkaloids	strong increase	Bejarano *et al*., 2000
Hypericum brasiliense	betulinic acid	strong increase	de Abreu *et al*., 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	quinolizidin alkaloids	strong increase	Christiansen *et al*., 1997
Solanum tuberosum	steroid alkaloids	strong increase	Bejarano *et al*., 2000
Glycine max	trigonelline	higher contents	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
Triglochin maritima	cyanogenic glucosides	strong increase	Majak *et al*., 1980
Brassica napus	glucosinolates	massive increase	Jensen *et al*., 1996
Coffea arabica	γ-aminobutyric acid	massive increase (tenfold)	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
Tropaeolum majus	glucotropaeolin	significant increase	Bloem *et al*., 2014

In stressed peas (*Pisum sativum*), the massive stress related increase in the concentration of phenolic compounds reported by Nogués *et al*. (1998) resulted in a 25% higher overall amount of anthocyanins (product of biomass and anthocyanin concentration), despite the fact that the total biomass of the pea plants grown under drought stress is just about one third of those cultivated under standard conditions. In the same manner, in *Hypericum brasiliense*, not only the concentration but also the total content of the phenolic compounds is drastically enhanced under drought stress in comparison to the control plants (de Abreu and Mazzafera, 2005). Although the stressed *H. brasiliense* plants had been quite smaller, the product of biomass and concentration of the related phenolics yields in a 10% higher total amount of these compounds. Similarly, in *Labisia pumila*, not only the concentration but also the overall production of total phenolics and flavonoids per plant is enhanced in plants suffering from drought stress (Jaafar *et al*., 2012).

Also, when evaluating the literature on terpenoids, it becomes obvious that, in some studies, an increase of the overall content of these natural products is reported, i.e., the total amount per plant. In sage (*Salvia officinalis*), drought stress induces a massive increase of monoterpenes, which over-compensates the reduction in biomass (Nowak *et al*., 2010). As a result, the total amount of monoterpenes accumulated in sage is significantly higher in plants suffering from moderate drought stress as compared to well watered controls. In contrast, in catmint and lemon balm plants the slight drought stress related increases in the concentrations of monoterpenes do not compensate the stress related detriment of growth. Consequently, the overall content of terpenoids is lower in the drought stressed plants of *Melissa officinalis* and *Nepeta cataria* than in the corresponding controls (Manukyan, 2011). Obviously, depending on the plant species and the stress conditions applied, the drought stress related concentration enhancement of essential oils does not always result in a corresponding increase in the total amount of natural products, but is compensated or even overcompensated by the related retards in growth. Nonetheless, as a cardinal assertion, it could be stated that in nearly all plants analyzed, the concentrations of secondary plant products are significantly enhanced under drought stress conditions. Only in few cases, apart from the stress induced concentration increase, an enhancement of the total content of corresponding natural compounds is reported. This could be either due to the lack of data on the biomass of the corresponding plants, or to the fact that the stress related decrease in biomass generally over-compensates the increase in the concentration of relevant natural products.

3. Metabolic Background: Higher Reduction Capacity in Drought Stressed Plants

Based on our recurring experience in daily life, we all have internalized that energy saving represents one of the most crucial issues in our subsistence, and corresponding statements have become fundamental. Indeed, on the first sight, it seems appropriate to transfer these considerations into plant biology, and even in reputable textbooks and in scientific publications; corresponding claims and statements can frequently be found. Despite the fact that we are aware of the fact that light energy, in general, is not the limiting factor of photosynthesis in plants (Wilhelm and Selmar, 2011), it is generally stated: "*In order to save energy, plants have evolved a certain mechanism…*" or "*Due to cost-benefit equations, the energy costs for a certain metabolic process must be minimized*". However, when considering this more closely, it becomes obvious that in contrast to heterotrophic organisms, quite other cardinal coherences are crucial for autotrophic plants. In general, plants absorb much more energy than that being required for photosynthetic CO_2-fixation. This is easily demonstrated by the massive enhancement of photosynthesis, when CO_2 concentration is elevated (for details see Wilhelm and Selmar, 2011). Under ambient environmental conditions, the surplus of energy is dissipated effectively by various mechanisms, i.e., non-photochemical quenching, photorespiration, or xanthophyll cycle (Figure 1). However, under drought stress the situation changes markedly. Due to water shortage, stomata are closed, and thus the CO_2-influx is diminished. As a result, far less reduction equivalents are consumed (re-oxidized) within the Calvin cycle. Thus, although the energy dissipating mechanisms are enhanced, they are overstretched. Therefore, electrons are transferred to molecular oxygen and superoxide radicals are generated (Figure 1), which are generally detoxified by superoxide dismutase (SOD) and ascorbate peroxidase (APX). Nonetheless, the chloroplastic reduction status increases and the ratio of $NADPH+H^+$ to $NADP^+$ is enhanced. In consequence, all reactions consuming $NADPH+H^+$, such as the biosynthesis of highly reduced secondary plant products, will be favoured without the need for any change in enzyme activity (Figure 1). As a result of the stress related increase in the biosynthesis rate of highly reduced natural products, large amounts of $NADPH + H^+$ are consumed and the over-reduced state is mitigated. Accordingly, this process could be evaluated as a further and supplementary mechanism for energy dissipation. The extent of this $NADPH + H^+$ re-oxidation is impressively demonstrated by the strong isoprene emission of various drought stressed plants. Several authors postulated that isoprene emission could significantly contribute to the dissipation of the excess of photosynthetic energy (Fall, 1999; Sharkey and Yeh, 2001). A calculation by Magel *et al.* (2006) revealed that under standard conditions, the energy consumption for isoprene biosynthesis accounts for less than 1%. However, at elevated temperatures, when isoprene emission massively increases, the amount of energy dissipated by isoprene biosynthesis could rise up to 25% of the energy supply for net photosynthesis. These considerations demonstrate that the biosynthesis of natural products, indeed, may represent a relevant system to dissipate a surplus of energy. Accordingly, secondary metabolites, apart from their ecological significance, could also be crucial as a part of the supplemental energy dissipation machinery (Grace and Logan, 2000; Wilhelm and Selmar, 2011).

4. Interferences and Interactions with other Factors

In principle, there are two quite different explanations for why the concentrations of natural products are generally enhanced in plants suffering drought stress. This effect could either be due to a stress related change in the benchmark, i.e., the dry or fresh weight used as reference value, or to a real increase in biosynthesis. In the first case, the drought stress related decline in biomass production is associated with a more or less unchanged rate of biosynthesis of natural products. Accordingly, when calculated on a dry or fresh weight base, in the case of the stressed plants, the corresponding concentration is increased. In the second case, stress does result in an authentic increase in the total content of secondary plant products caused by an enhanced biosynthesis. This increase is putatively due to the stress related over-reduction, boosting the biosynthesis of highly reduced compounds. Unfortunately, these simplified causal coherences become much more intransparent, since both issues are frequently overlaid and interfere with numerous factors and side effects.

It is well established that secondary plant products reveal a high significance for the plants by accomplishing various ecological functions within the complex interactions of plants with their environment, e.g., to repel herbivores, to protect against pathogens, or to attract pollinators (Harborne, 1988; Hartmann, 2007; Wink, 2010). Furthermore, with respect to abiotic stress, various putative functions for secondary plant products are described, e.g., protection against UV-light or too high light intensities, action as compatible solutes, radical scavenging, or reduction of transpiration (Edreva1 *et al*., 2008; Wink, 2010). Due to the tremendous progress in molecular biology, a lot of information is available on how the synthesis of the secondary metabolites is induced, modulated and regulated by various biotic and abiotic factors, respectively (e.g. Hahlbrock *et al*., 2003; Saunders and O´Neill, 2004; Ferry *et al*., 2004). Consequently, the actual synthesis and accumulation of a certain natural product is influenced and determined by numerous factors. Moreover, a particular stress situation, in general, influences several factors, e.g., a high irradiation is frequently accompanied by elevated temperatures, a high irradiation often parallels the UV-radiation, and elevated temperatures co-occur with higher evaporation rates. As drought also impacts on the entire ecosystem, it might be associated with a higher herbivore pressure, but a lesser number of pathogens. Consequently, we have to be aware that complex interferences of numerous factors occur, influencing the metabolism of plants exposed to drought stress, and thus, the synthesis and accumulation of natural products. Therefore, we have to consider that complex interferences of numerous factors occur, additionally influencing the metabolism of plants exposed to drought stress, and thus, the synthesis and accumulation of natural products.

A comprehensive and solid scientific investigation of drought stress requires an explicit differentiation between those effects, which are due to the osmotic potential, i.e., the reduced water availability within the cell, and those which are induced by the shift in redox potential caused by the decrease in CO_2 influx, when stomata are closed. Upon first sight, with respect to the decrease in water availability, the actual water potential seems to be an appropriate parameter. However, in response to drought, many plants produce and accumulate osmotic active substances. These substances, denoted as compatible solutes, significantly reduce the water potential of the cell without changing the actual water content. Accordingly, the water content seems to be a better option to describe the impact of drought stress on the water household. Just recently, in addition to the classical gravimetrical methods, a new methodology based on terahertz technology has been presented to determine the actual water content (Breitenstein *et al*., 2011).

The most appropriate marker for the stress related shift in the redox potential would be the enhanced ratio of $NADPH + H^+$ to $NADP^+$. Unfortunately, the *in situ* ratio of the reduction equivalents could not be quantified without great efforts and inappropriate expenditures. The next choice for the estimation of the over-reduced status in the chloroplastic electron transport chain would be the quantification of oxygen radicals generated. However, due to the extremely high reactivity of these oxygen species, such approach is even more problematic. Alternatively, the enzymes responsible for detoxification of toxic reactive oxygen species (ROS) generated, the superoxide dismutase (SOD) and the ascorbate peroxidase (APX), are frequently estimated as characteristic stress related enzymes. Unfortunately, these enzymes occur in various isoforms and are also part of various signal transduction chains. Accordingly, they do not reliably indicate a specific stress situation. A further alternative is the evaluation of the glutathione system, which is also part of the antioxidative defence against ROS. Yet, the interrelation between the glutathione concentrations and the redox status does not seem to be consistent (Tausz *et al*., 2004). To follow another approach, the occurrence of characteristic stress-metabolites, which are synthesized and accumulated more or less specifically in response to a particular stress situation, is analysed. In this context, proline is in the centre of focus. This amino acid is accumulated as compatible solute in plants suffering from drought stress (Rhodes *et al*., 1999). Unfortunately, the drought stress induced proline accumulation does not occur in all plant species. Alternatively, γ-amino butyric acid (GABA) is quantified, a stress metabolite produced by decarboxylation of glutamic acid (Kinnersley and Turano, 2000). GABA is accumulated in response to drought stress, but, unfortunately, it is also produced, to a high extent, under other various stress conditions (Satya Narayan and Nair, 1990; Bown and Shelp, 1997). Thus, further markers are required; the most promising complementation to the classical markers mentioned so far, is the determination of the abundance of dehydrins. These small protective proteins were first discovered in maturing seeds in the course of late embryogenesis. Moreover, it is well known that dehydrins are also frequently synthesized in plant cells suffering from drought stress (Close, 1997; Allagulova *et al*., 2003; Bouché and Fromm, 2004). These small hydrophilic proteins are thought to reveal various protective functions in desiccating cells (Hara, 2010). Meanwhile, the expression of dehydrins is well established to monitor the impact of drought stress on the metabolism. However, the complexity of such stress reactions was in general vividly demonstrated by Kramer *et al*. (2010), who demonstrated that the expression of dehydrins and the accumulation of the stress metabolite GABA follow different time patterns in coffee seeds whilst drying. Clearly, various metabolic responses occur in parallel. So, we have to consider (as outlined above) the fact that in leaves exposed to drought stress, apart from the impact of the decrease in water availability, the overreduction due to stomata closure entails numerous metabolic responses. Therefore, the elucidation of the entire metabolic syndrome, induced in drought stressed medicinal plants, requires a combination of several markers.

5. Practical Applications

Indeed, the stress related impacts on metabolism are multilayered. However, the corresponding effects with respect to the secondary metabolism are even more multifarious, since many mechanisms of elicitation and induction are overlaid, and numerous effects and processes are counteracting. This especially accounts for drought stress. In addition to the direct influence on secondary metabolism outlined above, we have, first of all, to consider the consequences and aftereffects of drought stress on general metabolism, which causes severe repercussions, such as losses in biomass, retards in development, or changes in growth behaviour. Moreover, marked changes in the general metabolic status regularly shift the ratio between generative and vegetative characteristics of tissues and organs (Houter and Nederhoff, 2007). This means that the drought stress might also change the *source-sink* properties of the entire plant. This – in addition to the inducing factors already mentioned – also impacts on the overall performance of the biosynthesis, translocation and accumulation of secondary plant products. Consequently, apart from the enhanced biosynthesis due to the stress related over-reduction, drought stress also impacts on the allocation and accumulation of natural products. In consequence, the overall effects of drought stress on secondary metabolism, and, thus, on the quality of the medicinal plants are multi-layered and very complex. Indeed, as outlined above, a deliberate application of drought stress during the cultivation of medicinal plants should principally result in an increase in the biosynthesis of secondary metabolites. However, due to the other co-occurring impacts and the various effects mentioned above, this enhancement is frequently compensated or even over-compensated. Thus, a general recommendation for the deliberate application of drought stress to increase the quality of medicinal plants cannot be made. Notwithstanding, in many cases, such approach undoubtedly could be successful. However, we always have to sound out the advantages and the drawbacks of a corresponding approach. To facilitate corresponding assessments, Selmar and Kleinwächter (2013a; 2014) have proposed to answer some simple questions:

- What kind and which level of stress enhance the accumulation of the desired compounds without causing too high losses in biomass?
- What is required, a high concentration or a large bulk? (total amount of natural products versus high concentrations in the drug)
- Are the substances synthesized and accumulated in source or in sink tissues?
- Are the substances synthesized and accumulated in generative or vegetative organs?
- Could the accumulation also be increased by phytohormone treatments (e.g., methyl jasmonate, salicylic acid)?
- Should the stress be applied only within a certain phase of cultivation or during a special developmental phase in order to obtain maximal quality?

In forthcoming approaches, we have to elaborate how these objectives could be transferred into appropriate agricultural applications. Indeed, most simple approaches should be based on altering the irrigation regime (Radovich *et al*., 2005); but this seems to be restricted to semi-arid regions, where supplemental watering is required. In moderate climates, the water supplied by rainfall cannot be diminished. However, the moisture content of the soil could be influenced by the choice of the cultivation area or by some simple measures, e.g., fields with slopes will retain markedly less water than flat plains consisting of soils with the same water holding capacity. Even in plains, the drainage effect could be realized artificially by certain cultivation measures. The creation of a furrow and ridge system, which is frequently used for surface irrigation in arid regions is also appropriate for generating drought stress situations by establishing significant gradients of soil moisture contents. Without any irrigation, the soil water content in the furrows will nearly be the same as in untreated fields, but the moisture content in the ridges strongly decreases due to an enhanced drainage effect. Moreover, elevated drainage could be achieved by increasing the proportion of sand in the soil. However, such an approach would irreversibly change the character of the soil and should only be applied in exceptional cases.

Alternative strategies to influence the product quality are based on the application of phytohormones or growth regulators to induce and modulate the stress related signal transduction chain for secondary metabolite synthesis. In this context, jasmonic acid is of special interest. Due to its physico-chemical properties, in general, the active growth regulator is not applied; what is actually applied is its volatile methyl ester. After uptake into the cells, it is hydrolysed, and the active jasmonic acid is generated. Corresponding approaches had been successfully employed in numerous tissue and cell culture systems to enhance the concentration of secondary metabolites (Namdeo, 2007). Meanwhile, methyl jasmonate was also used for effective elicitation of natural products in intact plants, e.g., to increase the synthesis of indole alkaloids in seedlings of *Catharanthus roseus* and *Chinona ledgerina*, respectively (Aerts *et al*., 1994). The application of methyl jasmonate also enhanced the contents of phenols and monoterpenes in *Ocimum basilicum* (Kim *et al*., 2006) and the concentration of glucosinolates in *Brassica rapa* (Loiavmäki *et al*., 2004). These promising approaches indicate that the usage of methyl jasmonate seems to be an encouraging alternative for quality improvement of medicinal and spice plants instead of a direct drought treatment. Analogously, salicylic acid, another endogenous signal substance, was also used for impacting on the synthesis and accumulation of secondary metabolites. The application of this signal transducer, which is involved in systemic resistance of plants (Durrant and Dong, 2004), strongly increased the content of phenolics in *Echinacea purpurea* plants (Kuzel *et al*., 2009) and the accumulation of glucosinolates in oilseed rape (*Brassica napus*) (Kiddle *et al*., 1994). However, apart from their effects on secondary metabolism, salicylic acid and methyl jasmonate also impact on primary metabolism and developmental processes, e.g., by inducing senescence and thereby causing retardation of growth. Thus, in the same manner as outlined for the deliberate application of drought stress, when the application of growth regulators and phytohormones is considered to increase the product quality, a thorough balancing of pros and cons is required. This however, necessitates a sound and comprehensive knowledge of the related metabolic background.

6. Conclusion

The metabolic background for the stress related enhancement of natural product synthesis is outlined: Drought stress leads to stomata closure, and thereby generating a highly over-reduced metabolic status. According to the law of mass action, the enhancement of reductive power (NADPH+ H^+) causes an increase in the synthesis of all highly reduced secondary plant products, such as isoprenoids, phenols or alkaloids. By intentionally applying drought stress during the cultivation of spice and medicinal plants, the concentration of natural products, and thus the product quality of the related commodities, could be enhanced. However, due to numerous interactions, the related increase could be compensated by other metabolic responses, such as growth reduction, decrease in biomass production, or the onset of senescence. Thus, for each particular case, a thorough balancing of pros and cons is necessary, which, of course, requires much more research in order to comprehensively elucidate the entire issue.

References

Aerts RJ, Gisi D, de Carolis E, de Luca V and Baumann TW. 1994. Methyl jasmonate vapor increases the developmentally controlled synthesis of alkaloids in *Catharanthus* and *Cinchona* seedlings. *Plant J,* **5**: 635-643.

Allagulova CR, Gimalov FR, Shakirova FM and Vakhitov VA. 2003. The plant dehydrins: structure and putative functions. *Biochem,* **68**: 945–951.

Azhar N, Hussain B, Ashraf YM and Abbasi KY. 2011. Water stress mediated changes in growth, physiology and secondary metabolites of desi ajwain (*Trachyspermum ammi*). *Pak J Bot,* **43**: 15-19.

Baher ZF, Mirza M, Ghorbanli M and Rezaii MB. 2002. The influence of water stress on plant height, herbal and essential oil yield and composition in *Satureja hortensis* L. *Flavour Frag J,* **17**: 275–277.

Ballhorn DJ, Kautz S, Jensen M, Schmitt S, Heil M and Hegeman AD. 2011. Genetic and environmental interactions determine plant defences against herbivores. *J Ecol,* **99**: 313–326.

Bejarano L, Mignolet E, Devaux A, Espinola N, Carrasco E and Larondelle Y. 2000. Glycoalkaloids in potato tubers: the effect of variety and drought stress on the α-solanine and α-chaconine contents of potatoes. *J Sci Food Agric,* **80**: 2096–2100.

Bettaieb I, Zakhama N, Aidi Wannes W, Kchouk ME and Marzouk B. 2009. Water deficit effects on *Salvia officinalis* fatty acids and essential oils composition. *Sci Hortic,* **120**: 271-275.

Bloem E, Haneklaus S, Kleinwächter M, Paulsen J, Schnug E and Selmar D. 2014. Stress-induced changes of bioactive compounds in *Tropaeolum majus* L. *Ind Crops Products,* **60:** 349–359.

Bohnert HJ, Nelson DE and Jensen RG. 1995. Adaptations to environmental stresses. *Plant Cell*, **7**: 1099-1111.

Bouché N and Fromm H. 2004. GABA in plants: just a metabolite. *Trends Plant Sci,* **9**: 100–115.

Bown AW and Shelp BJ. 1997. The metabolism and functions of γ-aminobutyric acid. *Plant Physiol,* **115**: 1–5.

Breitenstein B, Scheller M, Shakfa MK, Kinder T, Müller-Wirts T, Koch M and Selmar D. 2011. Introducing terahertz technology into plant biology: A novel method to monitor changes in leaf water status. *J Appl Bot Food Qual,* **82**: 158-161.

Briske DD and Camp BJ. 1982. Water stress increases alkaloid concentrations in threadleaf groundsel (*Senecio longilobus*). *Weed Sci,* **30**: 106-108.

Bytof G, Knopp S-E, Schieberle P, Teutsch I and Selmar D. 2005. Influence of processing on the generation of γ-aminobutyric acid in green coffee beans. *Eur Food Res Technol,* **220**: 245–250.

Çakir R and Çebi U. 2010. The effect of irrigation scheduling and water stress on the maturity and chemical composition of Virginia tobacco leaf. *Field Crop Res,* **119**: 269-276.

Charles DJ, Joly RJ and Simon JE. 1990. Effects of osmotic stress on the essential oil content and composition of peppermint. *Phytochemistry,* **29**: 2837-2840.

Chen Y, Guo Q, Liu L, Liao L and Zaibiao Z. 2011. Influence of fertilization and drought stress on the growth and production of secondary metabolites in *Prunella vulgaris* L. *J Med Plants Res,* **5**: 1749-1755.

Cho Y, Njitiv N, Chen X, Lightfood DA and Wood AJ. 2003. Trigonelline concentration in field-grown soybean in response to irrigation. *Biol Plantarum,* **46**: 405-410.

Christiansen JL, Jørnsgard B, Buskov S and Olsen CE. 1997. Effect of drought stress on content and composition of seed alkaloids in narrow-leafed lupin, *Lupinus angustifolius* L. *Eur J Agron,* **7**: 307-314.

Close TJ. 1997. Dehydrins: a commonality in the response of plants to dehydration and low temperature. *Physiol Plantarum,* **100**: 291–296.

de Abreu IN and Mazzafera P. 2005. Effect of water and temperature stress on the content of active constituents of *Hypericum brasiliense* Choisy. *Plant Physiol Bioch,* **43**: 241–248.

de Bruijn GH. 1973. The cyanogenic character of cassava (*Manihot esculenta*). In: Nestel B and MacIntyre R (Eds), **Chronic Cassava Toxicity**. International Development Research Centre, Ottawa, pp. 43-48.

Delitala I-F, Gessa C and Solinas V. 1986. Water stress and flexibility of phenolic metabolism in *Thymus capitatus. Fitoterapia,* **57**: 401–408.

del Moral R. 1972. On the variability of chlorogenic acid concentration. *Oecologia,* **9**: 289–300.

Durrant WE and Dong X. 2004. Systemic aquired resistance. *Annu Rev Phytopathol,* **42**: 185-209.

Edreva1 A, Velikova V, Tsonev T, Dagnon S, Gürel A, Aktaş L and Gesheva E. 2008. Stress-protective role of secondary metabolites: Diversity of functions and mechanisms. *Gen Appl Plant Physiol,* **34**: 67-78.

Falk KL, Tokuhisa JG and Gershenzon J. 2007. The effect of sulfur nutrition on plant glucosinolate content: Physiology and molecular mechanisms. *Plant Biol,* **9**: 573-581.

Fall R. 1999. Biogenic emissions of volatile organic compounds from higher plants. In: Hewitt CN (Ed), **Reactive hydrocarbons in the atmosphere**. Academic Press, pp. 41-95.

Ferry N, Edwards MG, Gatehouse JA and Gatehouse AMR. 2004. Plant–insect interactions: Molecular approaches to insect resistance. *Curr Opin Biotech,* **15**: 155-161.

Forouzandeh M, Fanoudi M, Arazmjou E and Tabiei H. 2012. Effect of drought stress and types of fertilizers on the quantity and quality of medicinal plant Basil (*Ocimum basilicum* L.). *Ind J Innov Develop,* **1**: 734-737.

Gershenzon J. 1984. Changes in the levels of plant secondary metabolites under water and nutrient stress. *Recent Adv Phytochemistry*, **18**: 273-320.

Grace SC and Logan BA. 2000. Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. *Philos T Roy Soc B*, **355**: 1499-1510.

Gray DE, Pallardy SG, Garrett HE and Rottinghaus G. 2003. Acute drought stress and plant age effects on alkamide and phenolic acid content in purple coneflower roots. *Planta Med*, **69**: 50-55.

Hahlbrock K, Bednarek P, Ciolkowski I, Hamberger B, Heise A, Liedgens H, Logemann E, Nürnberger T, Schmelzer E and Somssich IE. 2003. Non-self recognition, transcriptional reprogramming, and secondary metabolite accumulation during plant/pathogen interactions. *P Natl A Sci USA*, **100**: 14569-14576.

Hara M. 2010. The multifunctionality of dehydrins - An overview. *Plant Signaling Behav*, **5**: 1-6.

Harborne JB. 1988. **Introduction to Ecological Biochemistry**, third ed. Academic Press, London.

Hartmann T. 2007. From waste products to ecochemicals: Fifty years research of plant secondary metabolism. *Phytochem.*, **68**: 2831–2846.

Hernández I, Alegre L and Munné-Bosch S. 2006. Enhanced oxidation of flavan-3-ols and proanthocyanidin accumulation in water-stressed tea plants. *Phytochem.*, **67**: 1120–1126.

Houter B and Nederhoff E. 2007. Controlling plant growth - Part 3. Plant balance: ‘vegetative’ or ‘generative’. *Grower*, **62**: 42-44.

Jaafar HZ, Ibrahim MH and Mohamad Fakri NF. 2012. Impact of soil field water capacity on secondary metabolites, phenylalanine ammonia-lyase (PAL), maliondialdehyde (MDA) and photosynthetic responses of Malaysian kacip fatimah (*Labisia pumila* Benth). *Molecules*, **17**: 7305-7322.

Jaleel CA, Manivannan P, Sankar B, Kishorekumar A, Gopi R, Somasundaram R and Panneerselvam R. 2007. Induction of drought stress tolerance by ketoconazole in *Catharanthus roseus* is mediated by enhanced antioxidant potentials and secondary metabolite accumulation. *Colloid Surface B*, **60**: 201-206.

Jensen CR, Mogensen VO, Mortensen G, Fieldsend JK, Milford GFJ, Andersen MN and Thage JH. 1996. Seed glucosinolate, oil and protein contents of field-grown rape (*Brassica napus* L.) affected by soil drying and evaporative demand. *Field Crop Res*, **47**: 93-105.

Kiddle GA, Doughty KJ and Wallsgrove RM. 1994. Salicylic acid-induced accumulation of glucosinolates in oilseed rape (*Brassica napus* L.) leaves. *J Exp Bot*, **45**: 1343-1346.

Kim HJ, Chen F, Wang X and Rajapakse NC. 2006. Effect of methyl jasmonate on secondary metabolites of sweet basil (*Ocimum basilicum* L.). *J Agric Food Chem*, **54**: 2327-2332.

Kinnersley AM and Turano FJ. 2000. Gamma aminobutyric acid (GABA) and plant responses to stress. *Cr Rev Plant Sci*, **19**: 479–509.

Kirakosyan A, Kaufman P, Warber S, Zick S, Aaronson K, Bolling S and Chul Chang S. 2004. Applied environmental stresses to enhance the levels of polyphenolics in leaves of hawthorn plants. *Physiol Plantarum*, **121**: 182-186.

Kirk H, Vrieling K, van der Meijden E and Klinkhamer PGL. 2010. Species by environment interactions affect pyrrolizidine alkaloid expression in *Senecio jacobaea*, *Senecio aquaticus*, and their hybrids. *J Chem Ecol*, **36**: 378-387.

Kleinwächter M and Selmar D. 2013. Influencing the product quality by applying drought stress during the cultivation of medicinal plants, In: Wani, M.R. (Ed.), **Physiological Mechanism and Adaptation Strategies in Plants Under Changing Environment**. Springer Verlag, pp. 57-73.

Kleinwächter M and Selmar D. 2014. Drought stress to enhance the quality of medicinal plants. A review. *Agron Sustainable Dev, - in press*

Kramer D, Breitenstein B, Kleinwächter M and Selmar D. 2010. Stress metabolism in green coffee beans (*Coffea arabica* L.): Expression of dehydrins and accumulation of GABA during drying. *Plant Cell Physiol*, **51**: 546–553.

Kubota N, Mimura H and Shimamura K. 1988. The effects of drought and flooding on the phenolic compounds in peach fruits. *Scientific Reports of the Faculty of Agriculture, Okayama University*, **171**: 17-21.

Kuzel S, Vydra J, Triska J, Vrchotova N, Hruby Mand Cigler P. 2009. Elicitation of pharmacologically active substances in an intact medical plant. *J Agric Food Chem, 57*: 7907-7911.

Liu H, Wang X, Wang D, Zou Z and Lianga Z. 2011. Effect of drought stress on growth and accumulation of active constituents in *Salvia miltiorrhiza* Bunge. *Ind Crop Prod*, **33** :84-88.

Llusià J and Peñuelas J. 1998. Changes in terpene content and emission in potted Mediterranean woody plants under severe drought. *Can J Bot*, **76**: 1366-1373.

Loivamäki M, Holopainen JK and Nerg AM . 2004. Chemical changes induced by methyl jasmonate in oilseed rape grown in the laboratory and in the field. *J Agric Food Chem*, **52**: 7607-7613.

Magel E, Mayrhofer S, Müller A, Zimmer I, Hampp R and Schnitzler JP. 2006. Photosynthesis and substrate supply for isoprene biosynthesis in poplar leaves. *Atmos Environ*, **40**: 138-151.

Majak W, McDiarmid R E, Hall JW and van Ryswyk AL. 1980. Seasonal variation in the cyanide potential of arrowgrass (*Triglochin maritima*). *Can J Plant Sci*, **60**: 1235-1241.

Manukyan A. 2011. Effect of growing factors on productivity and quality of lemon catmint, lemon balm and sage under soilless greenhouse production: I. Drought stress. *Medicinal Aromatic Plant Sci Biotech*, **5**: 119-125.

Namdeo AG. 2007. Plant cell elicitation for production of secondary metabolites: A review. *Pharmacogn Rev*, **1**: 69-79.

Nogués S, Allen DJ, Morison JIL and Baker NR. 1998. Ultraviolet-B radiation effects on water relations, leaf development, and photosynthesis in droughted pea plants. *Plant Physiol*, **117**: 173–181.

Nowak M, Manderscheid R, Weigel H-J, Kleinwächter M and Selmar D. 2010. Drought stress increases the accumulation of monoterpenes in sage (*Salvia officinalis*), an effect that is compensated by elevated carbon dioxide concentration. *J Appl Bot Food Qual*, **83**: 133-136.

Petropoulos SA, Daferera D, Polissiou MG and Passam HC. 2008. The effect of water deficit stress on the growth, yield and composition of essential oils of parsley. *Sci. Hortic*, **115**: 393-397.

Radovich TJK, Kleinhenz MD and Streeter, JG. 2005. Irrigation timing relative to head development influences yield components, sugar levels, and glucosinolate concentrations in cabbage. *J Amer Soc Hort Sci*, **130**: 943-949.

Ramakrishna A and Ravishankar GA. 2011. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal Behav*, **6**: 1720-1731.

Rhodes D, Verslues PE and Sharp RE. 1999. Role of amino acids in abiotic stress resistance. In: Singh BK (Ed.), **Plant Amino Acids: Biochemistry and Biotechnology.** Marcel Dekker Inc, New York, Basel, Hong Kong, pp. 319-356.

Satya Narayan V and Nair PM. 1990. Metabolism, enzymology and possible roles of 4-aminobutyrate in higher plants. *Phytochem.,* **29**: 367–375.

Saunders J and O´Neill NR. 2004. The characterization of defense responses to fungal infection in alfalfa. *Biocontrol,* **49**: 715-728.

Schreiner M, Beyene B, Krumbein A and Stützel H. 2009. Ontogenetic changes of 2-propenyl and 3-indolylmethyl glucosinolates in *Brassica carinata* leaves as affected by water supply. *J Agric Food Chem,* **57**: 7259-7263.

Selmar D. 2008. Potential of salt and drought stress to increase pharmaceutical significant secondary compounds in plants. *Landbauforsch Völk,* **58**: 139–144.

Selmar D and Kleinwächter M. 2013a. Influencing the product quality by deliberately applying drought stress during the cultivation of medicinal plants. *Ind Crop Prod,* **42**: 558-566.

Selmar, D and Kleinwächter, M. 2013b. Stress enhances the synthesis of secondary plant products: The impact of the stress-related over-reduction on the accumulation of natural products. *Plant Cell Physiol,* **54**: 817-826.

Sharkey TD and Yeh S. 2001. Isoprene emission from plants. *Annu Rev Plant Phys,* **52**: 407-436.

Singh-Sangwan N, Abad Farooqi AH and Sangwan RS. 1994. Effect of drought stress on growth and essential oil metabolism in lemongrasses. *New Phytol,* **128**: 173-179.

Smirnoff N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytol,* **125**: 27-58.

Szabó B, Tyihák E, Szabó LG and Botz L. 2003. Mycotoxin and drought stress induced change of alkaloid content of *Papaver somniferum* plantlets. *Acta Bot Hung,* **45**: 409-417.

Tausz M, Šircelj H and Grill D. 2004. The glutathione system a stress marker in plant ecophysiology: is a stress-response concept valid? *J Exp Bot,* **55**: 1955-1962.

Turtola S, Manninen A-M, Rikala R and Kainulainen P. 2003. Drought stress alters the concentration of wood terpenoids in Scots pine and Norway spruce seedlings. *J Chem Ecol,* **29**: 1981-1995.

Wang DH, Du F, Liu HY and Liang ZS. 2010. Drought stress increases iridoid glycosides biosynthesis in the roots of *Scrophularia ningpoensis* seedlings. *J Med Plants Res,* **4**: 2691-2699.

Wilhelm C and Selmar D. 2011. Energy dissipation is an essential mechanism to sustain the viability of plants: the physiological limits of improved photosynthesis. *J Plant Physiol,* **168**: 79-87.

Wink M. 2010. Introduction: Biochemistry, Physiology and Ecological Functions of Secondary Metabolites. In: Wink M (Ed.), **Biochemistry of Plant Secondary Metabolism**. Wiley-Blackwell, pp. 1-19.

Xia L, Yang W and Xiufeng Y. 2007. Effects of water stress on berberine, jatrorrhizine and palmatine contents in amur corktree seedlings. *Acta Ecol Sin,* **27**: 58-64.

Zhu Z, Liang Z, Han R and Wang X. 2009. Impact of fertilization an drought response in the medicinal herb *Bupleurum chinense* DC.: Growth and saikosaponin production. *Ind Crop Prod* **29**: 629–63.