Review

Quality from the field: The impact of environmental factors as quality determinants in medicinal plants

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Abstract

Plants have been used extensively in both pharmaceutical and food industries, with consumers showing increasing interests in these products. Phytomedicines exploit a pool of biogenic resources produced by plants. However, the quality and quantity of these chemical metabolites in plants are influenced by a multitude of factors, chief among them, environmental. This review provides an overview of plant-produced chemical compounds with medicinal properties and how their production is affected by different environmental factors. An insight into how these factors can be manipulated within the plants' growing environments as a way of ensuring quality is also discussed.
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Contents

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1. Introduction

Plants constantly interact with rapidly changing and potentially damaging external environmental factors. Being organisms devoid of mobility, plants have evolved elaborate intricate alternative defence strategies, which involve an enormous variety of chemical metabolites as tools to overcome stress conditions. Secondary metabolites play a major role in the adaptation of plants to the changing environment. Plants have an almost limitless ability to synthesise these metabolites. As a result of biotic and abiotic stresses, such as temperature, light intensity, herbivory and microbial attack, plants generate these defence mechanisms, triggering many complex biochemical processes (Holopainen and Gershenzon, 2010). Changes have been reported at genetic or protein level that are brought about by stress conditions and are reflected in a profound alteration of the metabolite pool of the affected plants (Dangle and Jones, 2001; Loreto and Schnitzler, 2010; Szathmáry et al., 2001). The synthesis of secondary metabolites is, however, often tightly regulated, and is commonly either restricted to specific plant tissues or developmental stages, or induced in response to stimulation factors (Osbourn et al., 2003; Wink, 2003). As a strategy for survival and for the generation of diversity at the organism level, the ability to synthesise particular classes of secondary metabolites is also restricted to selected plant groups.

The ability of plants to carry out *in vivo* combinatorial chemistry by mixing, matching and evolving the gene products required for secondary metabolite biosynthetic pathways, creates an unlimited pool of chemical compounds, which humans have exploited to their benefit. Plant secondary metabolites therefore, offer a diverse range of benefits to humans, that includes among them, medicinal properties.

As a result of the recent expansion in the interest on secondary metabolites, the research fields of molecular biology, biochemistry, plant physiology and ecology have overlapped each other's boundaries and established the variations in molecular mechanisms and metabolism underlying the diversity and production of these compounds in plants. Here, we review literature on how different environmental factors qualitatively and quantitatively influence secondary metabolites of medicinal benefits and how these can be integrated as tools to ensure medicinal quality in phytomedicines.

2. Plant secondary metabolism

Plants are multifaceted chemical factories that produce a multitude of structurally diverse organic compounds that are not directly involved in the normal growth, development, or reproduction, but are thought to be required in the adaptation with their environment. These secondary metabolites usually contain more than one functional group and often exhibit multiple functionalities and bioactivity (Dey and Harborne, 1989). Although they are structurally diverse, secondary metabolites derive their synthesis from limited products of primary metabolism (Crozier et al., 2006). Intensive research efforts have elucidated the basic biochemistry and molecular biology of some biosynthetic pathways of secondary metabolism, with most of the findings supporting the chemical reasoning that the diversification of secondary metabolism originates from the elaboration of a few central intermediates (Facchini, 1999; Kutchan, 1995; Kutchan and Zenk, 1993; Wink, 2003). Further studies on secondary metabolism reveal that their biosynthesis is not a random process, but rather highly ordered with respect to plant development, often exhibiting controlled expression of their pathways within organs, specific cells, and/or organelles within cells (Osbourn et al., 2003). Their synthesis depends on numerous enzymes involved in different metabolic pathways and their metabolism is completely integrated into morphological and biochemical regulatory patterns of plants. Several schools of thought, substantiated with numerous scientific evidence, implicate the highly ordered interactions between plants and their biotic and abiotic environments as being a major driving force behind the emergence of specific secondary metabolites (Facchini, 1999). The genetic evolutionary phenomenon could, perhaps, be the best possible logical explanation for this attribute. Despite the complex nature and inconclusive reports on the regulatory mechanisms and biosynthesis of different secondary metabolites in plants, a significant body of research evidence indicates that their biosynthesis and accumulation remain remarkably under the control of the environment (Holopainen and Gershenzon, 2010; Szathmáry et al., 2001).

Based on this compelling evidence on the role of the environment and the associated genetic mechanisms on the regulation of the secondary metabolic profiles in plants, can the plant's environment be manipulated to tilt their metabolism in favour of the production of the secondary metabolite of interest? Logically, the answer could possibly be, yes. But the question remains; could this be a simple translation, considering the multiplicity of the factors that plants are subjected to on a day to day basis? Our extensive knowledge on the chemistry and pharmacology of some secondary metabolites has led to their use in a range of medical applications. The wide chemical diversity of secondary metabolites throughout the plant kingdom, therefore, represents an extremely rich biogenic resource for the discovery of novel and innovative drugs.

3. Environmental factors - a force behind secondary metabolism?

The central aspects in the survival of plants in a dynamic environment are the identification of the forces of evolution and diversity within communities, the plant's inherent mechanism to detect these, and the ways in which this biodiversity is maintained and/or propagated. From a general evolutionary perspective, one would then hope that such critical characteristic attributes of any organism could be intrinsically controlled within the organism's genome if survival and species perpetuation are to be ensured. Arguably, this is perhaps the only possible mechanism of ensuring sustainable survival through generations in most organisms, including plants. The most common, unavoidable interaction occurring in plant communities is the plant–environment interaction. External factors

quantitatively affect the plant's metabolic processes through their effects on plant development, growth rates and partitioning of assimilates into vital metabolites. These factors can also trigger abrupt activation of qualitative changes in secondary metabolite production (Laughlin, 1993; Lommen et al., 2008; Pérez-Estrada et al., 2000). This could possibly be one of the explanations behind plant metabolic diversity. Climatic (abiotic) factors often have an especially large influence on the biosynthetic levels and quality of secondary metabolites in plants (Coley, 1987). Since plants cannot escape from the environmental extremes of light, temperature, and drought, nor move to regions with better nutritional conditions, they have thus evolved highly complex mechanisms to integrate physiology and metabolism in order to adapt to the conditions to which they are exposed. Secondary metabolites form an integral component of these adaptive mechanisms. Plants have acquired a range of mechanisms to sense their environment and modify their growth and development as required. Constitutive defence mechanisms differentially respond to the various environmental cues but most importantly, upon exposure to a set of different environmental regimes, induced plant response takes effect (Table 1). Induced defence responses are characterised by alterations in a set of traits that lead to a reduction of the negative effect of the stress factor(s) on plant fitness.

Throughout the course of growth and development plants are exposed to various biotic and abiotic factors to which they respond with an activation of their defence system.

3.1. Light/solar radiation

Solar radiation energy is one of the most important environmental factors required for plant growth and development. The developmental plasticity of plants in any given environment is achieved, at least partly by constant monitoring of the quality (wavelength), duration and quantity of solar radiation. The survival of plants hinges much on their ability to carry out photosynthetic carbon fixation and biomass accumulation, and have thus developed extremely sensitive and accurate capacities to sense different light spectra and ultraviolet (UV) light present in the solar radiation (Kazan and Manners, 2011). To ensure optimal benefit from light incidences, plants have evolved biochemical protective mechanisms against potentially damaging elevated doses of UV radiation and extreme light intensities. In addition, corresponding mechanisms have developed in plants as a means to optimise the absorption of useful light spectra. Among these, UV-B (280–315 nm) has been perceived by plants as an environmental stressor that promotes UV acclimation and survival in sunlight (Rozema et al., 1997). UV-B radiation is potentially damaging to plants, impairing gene transcription and translation, as well as photosynthesis (Jansen et al., 1998). Upon perception, light signals are integrated into the plant's intricate signalling network system, resulting in the conversion of light inputs into outputs that shape plant growth and development. UV-B radiation impacts on the levels of a broad range of secondary metabolites, including phenolic compounds, terpenoids and alkaloids as part of outputs and/or intermediates in this complex biochemical interaction (Kazan and Manners, 2011; Rozema et al., 1997).

Phenylpropanoid derivatives are among the major classes of secondary metabolites that selectively absorb in the UV-B spectral region without decreasing penetration of photosynthetic radiation into the leaf. This has led to speculations that one of their major roles is to act as sunscreens to absorb UV-B light. Several studies using phenylpropanoid mutants and different UV-B radiation levels have yielded results that are consistent with this opinion (Laakso et al., 2000; Lavola et al., 1997; Winkel-Shirley, 2002), but a decrease in the level of these

Table 1
Influence of some environmental factors on secondary metabolite production in some medicinal plant species.

Environmental stress factor	Plant species	Secondary metabolites	Reference
Light	*Betula* spp., *Pinus taeda*, *Salix myrsinifolia*, *Arabidopsis thaliana*, *Secale cereal*, *Artemisia annua*, *Sambucus nigra*, *Cornus sanguinea*, *Prunus serotina*, *Frangula alnus*, *Corylus avellana*, *Pteridium arachnoideum*, *Solanum tuberosum*, *Diplacus* spp., *Larrea*, *Marchantia polymorpha*	Terpenoids, alkaloids, flavonoids, flavonol glycosides, hydroxycinnamic acids, tannins, artemisinin, phenolic acids, phytosterols; glycoalkaloids, luteolin, apigenin	Alonso-Amelot et al. (2007), Arnqvist et al. (2003), Burchard et al. (2000), Karolewski et al. (2010), Kliebenstein (2004), Laakso et al. (2000), Lavola et al. (1997), Lommen et al. (2008), Markham et al. (1998), Rhoades (1977), Tegelberg and Julkunen-Tiitto (2001) and Winkel-Shirley (2002)
Moisture	*Pteridium arachnoideum*, *Artemisia annua*, *Pachypodium saundersii*, *Achnatherum inebrians*	Phenolic compounds, lipophilic resins, artemisinin, tannins, isoprene, anthocyanins, alkaloids	Alonso-Amelot et al. (2007), Horner (1990), Lommen et al. (2008), Sharkey and Loreto (1993) and Zhang et al. (2011)
Temperature	*Arnica montana*, *Hypericum perforatum*, *Achnatherum inebrians*, *Quercus* spp., *Pachypodium saundersii*, *Petunia × hybrida*	Phenolic compounds, isoprene anthocyanins, alkaloids, flavonoids, tannins	Albert et al. (2009), Christie et al. (1994), Pennycooke et al. (2005), Sharkey and Loreto (1993), Singsaas and Sharkey (2000), Solecka and Kacperska (1995), Zhang et al. (2011) and Zobayed et al. (2005)
Soil nutrients	*Rhodiola sachalinensis*, *Ceratonia siliqua*, *Lithopermum erythrorhizon*, *Aradopsis thaliana*, *Betula* spp., *Eucalyptus cladocalyx*	Salidroside, phenolic compounds, gallotannins, condensed tannins, shikonin, cyanogenic glycosides	Dixon and Paiva (1995), Gleadow et al. (1998), Kim and Chang (1990), Kliebenstein (2004), Kouki and Manetas (2002), Riipi et al. (2002), Simon et al. (2010) and Yan et al. (2004)
Ozone	*Pinus taeda*, *Ginkgo biloba*, *Betula pendula*, *Petroselium crispum*	Tannins, phenolic acids, phenolic compounds,	Eckey-Kaltenbach et al. (1994), He et al. (2009), Jordan et al. (1991), Lavola et al. (1994) and Saleem et al. (2001)

metabolites in some species has also been reported (Tegelberg and Julkunen-Tiitto, 2001). These studies brought forth disparate conclusions on the range of phenolics that act as predominant UV-B protectants. Some of these include hydroxycinnamic acids, flavonoids, and complex polymeric lignin or tannin-like compounds. A prominent group of these compounds is flavonoids, but hydroxycinnamic acids and their esters have also been implicated in this role in a broad range of plant species (Burchard et al., 2000; Kliebenstein, 2004).

Structural and chemical diversity of flavonoids is related to their diverse properties and roles in plants. These compounds, in addition to affording protection against ultraviolet radiation, also serve to protect plants against pathogenic and herbivorous attack (Harborne and Williams, 2000). Hrazdina (1992), working with a *Pisum sativum* cv. *argenteum* mutant, reported that the highest accumulation of both anthocyanin and flavonol glycosides occurs in the upper epidermal layers of leaf peels, which were directly exposed to visible and UV light irradiation, confirming a UV inducible protection role for these compounds. In view of this, solar radiation seems to have an especially profound contribution to the content of flavonoids and phenolic acids in plants. Flavonoids have attracted a lot of research interest in the pharmacological and pharmaceutical spheres, and this has seen characterisation of various plant flavonoids as antifungal, antibacterial, antiviral, anti-inflammatory, antioxidant, antitumor, anti-hepatotoxic, anti-lipolytic, vasodilator, immunostimulant and antiallergic agents (Alias et al., 1995; Burda and Oleszek, 2001; Gurib-Fakim, 2006; Iinuma et al., 1994; Williams et al., 1999). Because tannins have been shown to have antibiotic, antifeedant, or biostatic effects on a variety of organisms that consume them (Haslam, 1989), their chemical properties have been exploited in the discovery of versatile medicinal agents. Tannins are thus reported to possess numerous medicinal properties such as antibacterial, antifungal, antiviral, anti-diarrhoeal, free radical scavenging, immunomodulatory, anti-inflammatory, antitumour and antidote activities (Gurib-Fakim, 2006; Okuda et al., 1992). In addition, phenolic compounds, including tannins and flavonoids are important contributors to organoleptic qualities of fresh fruits, fruit juices, and wine. Their potential contribution to human health therefore, will largely depend on the environmental factors that control their accumulation levels and quality in plants.

The relationship between plant solar radiation stress acclimation and human health is not limited to phenolic compounds as antioxidants, but comprises a broad array of other metabolites, some of which possess desirable pharmacological properties, while some are toxic to humans. Glycoalkaloids such as α-solanine and α-chaconine reportedly accumulate in potato tubers exposed to mechanical stress or light (Arnqvist et al., 2003; Marcel et al., 2008), and the compounds lead to gastro-intestinal or neurological disorders in humans. However, accumulation of these compounds is directly associated with accumulation of phytosterols (Arnqvist et al., 2003), which themselves have positive effects on human health. Phytosterols limit absorption of cholesterol from fat matrices into the intestinal tract, which in turn results in lower cholesterol levels in human consumers and decreases the incidence of cardiovascular diseases. Notwithstanding this

seemingly clear link between light-induced levels of secondary metabolites and the pharmacological benefits, a review of literature demonstrates this association as being highly complex. To ensure quality, safety and to improve on phytopharmaceuticals and human health, an understanding of the factors controlling these metabolites would thus become a fundamental prerequisite.

3.2. Soil nutrients

The effects of soil nutrient availability on plant growth, physiology, tissue chemistry, or stress tolerance are often investigated by experimentally manipulating nutrient supply. Levels of secondary metabolites in plant tissues have been reported to vary with resource availability (Coley et al., 1985). Levels of proanthocyanidins increase following nutritional stress such as limitation in available phosphate (Kouki and Manetas, 2002). Low iron levels were shown to stimulate increased biosynthesis of phenolic compounds (Dixon and Paiva, 1995). In general, source–sink relations are expected to constrain the levels of secondary metabolites accumulated by growing plants (Price et al., 1989) but their dynamics warrants further exploration.

Plant nutrient balance in the soil is thought to influence the production of secondary compounds at the level of metabolic regulation in plants (Herms and Mattson, 1992). The carbon/nitrogen balance (CNB) hypothesis, as proposed by Bryant et al. (1983), postulates that fertilisation with growth-limiting nutrients will lead to decreased concentrations of carbon-based secondary metabolites. CNB explains the concentrations of secondary metabolites in plant tissues as a function of the relative abundance of plant resources, particularly nitrogen. The theory rests on the assumptions that growth (primary metabolism) is a priority for plants over secondary metabolism, and that carbon and nitrogen are allocated to secondary metabolite production only after the requirements for growth are met. It also assumes that the rate of secondary metabolite production is determined by the concentrations of precursor molecules (Reichardt et al., 1991). When growth is limited by nitrogen deficiency in plants, CNB predicts that carbohydrates will accumulate in plant tissues, leading to an increased synthesis of carbon-based secondary compounds. The interpretation placed on such results is that the accumulation of C-based metabolites when the carbon–nitrogen ratio is high is due to a relative excess of fixed carbon being available to the plant. Thus the plant is only able to use the photosynthates for C-, H-, and O-containing metabolites, such as phenolics and terpenes (Waterman and Mole, 1994). The theory therefore, predicts that nutrient limitation leads to a decrease in the production of N-containing compounds such as alkaloids and cyanogenic glycosides. Although many studies have yielded results that are consistent with these predictions (Dixon and Paiva, 1995; Kouki and Manetas, 2002; Simon et al., 2010), CNB has also failed repeatedly in several other similar studies (Koricheva et al., 1998; Riipi et al., 2002).

In line with the opinion of the CNB theory, Jones and Hartley (1999) proposed a protein competition model (PCM) of phenolic allocation in plants. In contrast to the predictions of CNB, the PCM attempts to explain phenolic production on the

basis of the metabolic origins of pathway constituents, alternative fates of pathway precursors, and biochemical regulatory mechanisms. Protein synthesis and the phenylalanine ammonia-lyase (PAL)-catalysed committed step of phenolic and alkaloid biosynthesis, both utilise the amino acid phenylalanine as a precursor (Diallinas and Kanellis, 1994). This, therefore, presents a protein–metabolite competition for the limiting phenylalanine, leading to a process-level trade-off between rates of protein versus metabolite synthesis. When rates of protein synthesis are high, rates of phenolic synthesis should be low and vice versa (Jones and Hartley, 1999). Because of these demands, the phenylalanine pool appears to be limiting in plants (Weaver and Hermann, 1997). Allocation to phenolic biosynthesis, therefore, may be determined by the competitive dynamics between protein and phenolic demands and these are controlled by the inherent growth demands and environmental cues. To this effect, the two theories further affirm the widely held view that the rate of secondary metabolite production in plants is a result of the interaction between the extrinsic (environmental) and the plant's intrinsic (genetic) factors. Because secondary metabolism is linked to primary metabolism by the rates at which substrates are diverted from primary pathways and channelled towards the secondary biosynthetic routes, several factors affecting growth, photosynthesis and other parts of primary metabolism will also affect secondary metabolism.

In investigating the effects of soil nutrient factors on salidroside (active ingredient) production in the roots of *Rhodiola sachalinensis* (Chinese medicinal herb), Yan et al. (2004) found that rich organic matter, low pH and high levels of exchangeable nitrogen and total nitrogen in the soil were essential to high level production of salidroside. However, when soil bioavailable phosphorus content was increased to levels higher than 5 mg/L and exchangeable potassium content higher than 180 mg/L, salidroside yields were significantly reduced. Salidroside is a phenolic glycoside with some nervous stimulating effects, causes fatigue, decreases depression, and enhances work performance and resists the side effects of anoxia (Ming et al., 1988). Such investigations provide a guide to soil management practices on how to select and fertilise the soil for the cultivation of medicinal plants in order to improve their effectiveness. The shift in the soil nutrition will definitely lead to the alteration of both primary and secondary metabolism and consequently results in changes on the plant's productivity of secondary metabolites. On their investigation of the effect of a range of soil N on the correlation between growth and defence metabolites, Simon et al. (2010) found evidence that, for every N invested in cyanogenic glycosides, additional N is added to the leaf in *Eucalyptus cladocalyx*.

Given the relatively large demand that cyanogenesis makes on plant resources (up to 15% of leaf N in *E. cladocalyx*, Gleadow et al., 1998) and its effectiveness as a defence mechanism, soil nutritional status would therefore, determine cyanogenic glycoside concentrations in plants. In resource-limited environments, carbon partitioning to constitutive secondary metabolism often increases, which enhances resistance to attack by herbivores as well as stress tolerance.

Plasticity in patterns of resource allocation and partitioning also translate into variation in secondary metabolism. This trade-off in resource allocation between primary and secondary metabolism has important implications on the phytomedicinal quality of medicinal plants.

3.3. Moisture stress

Drought events of relative ranges of magnitudes and durations are commonly experienced in many environments and can drastically impact plant survival and/or stress tolerance. Growth reduction is expected under drought because water limitation reduces photosynthetic rates. Reduced water availability and high temperatures influence high phenolic production in plants (Alonso-Amelot et al., 2007; Glynn et al., 2004). Plants close their stomata and curtail photosynthesis during periods of water shortage and thus one might expect a negative relationship between water shortage and the synthesis of secondary metabolites. A more definite role of phenolic compounds in plant water-relations has been proposed for lipophilic resins accumulated in *Diplacus* and *Larrea* species (Rhoades, 1977), where an integrated antidesiccant and UV screen defence role have been assumed. Furthermore, Horner (1990) suggested a link between xylem pressure and tannin synthesis, and that the relationship can either be positive or negative, depending on the degree of water stress suffered by the plant. Phenolic and saponin levels and the corresponding bioactivity were found to vary seasonally in medicinal bulbs (Ncube et al., 2011a). In the same study, high phenolic compounds were recorded in all the species during the winter season, where moisture stress is a typical characteristic. The authors, therefore, speculated that moisture stress might have contributed to such high levels of phenolic compounds during this season.

3.4. Temperature

Temperature stress in plants is generally known to induce or enhance the active oxygen species-scavenging enzymes like superoxide dismutase, catalase, peroxidase and several antioxidants. Temperature stress may lead to a number of physiological, biochemical and molecular changes in plant metabolism such as protein denaturation or perturbation of membrane integrity. Many of these changes can alter the secondary metabolite concentrations in the plant tissues that are often used as an indicator of stress injury in the plant (Zobayed et al., 2005). High temperature (35 °C) treatment increased the leaf total peroxidase activity together with an increase in hypericin, pseudohypericin and hyperforin concentrations in the shoot tissues of St. John's Wort (Zobayed et al., 2005). Also, an exponential increase in a variety of volatile organic compounds, with a linear increase in temperature has been described in a range of plant species (Parker, 1977; Sharkey and Loreto, 1993; Sharkey and Yeh, 2001). Cold stress has been shown to stimulate an increase in phenolic production and their subsequent incorporation into the cell wall (Christie et al., 1994). The levels of PAL were shown to increase following exposure of maize seedlings and oilseed rape plants to low

temperatures, resulting in a corresponding increase in their phenolic content (Christie et al., 1994; Solecka and Kacperska, 1995). In particular, levels of anthocyanins increase following cold stress and are thought to protect plants against this effect (Pennycooke et al., 2005). Ncube et al. (2011a) attributed the high levels of total phenolic compounds obtained during the winter season in their study as being consistent with this fact and supports similar findings from previous studies (Pennycooke et al., 2005; Prasad, 1996).

3.5. Other stress factors

In addition to climatic factors, plants are subjected to a multitude of biotic stress factors such as herbivore and pathogenic attacks (Dixon and Paiva, 1995; Holopainen and Gershenzon, 2010). Herbivore damage to vegetative parts has been repeatedly shown to cause an increased release of inducible secondary compounds (Bernays and Chapman, 2000; Hagerman and Butler, 1991). Perception, transduction and propagation of stress signals are mediated by several agents including induced hormones, leading to a large number of secondary organic compounds being released as a result of the activation of different defence pathways in addition to the ubiquitous stress compounds. The compound spectrum depends on the type and magnitude of stress, and even on the attacking organism in the case of biotic stresses (Niinemets, 2010). Mechanical damage to plant foliage also causes an elevated level of defence metabolites though most types of artificial damage do not result in metabolite compound production as intense as, or with the same compositional diversity, as herbivory (Holopainen and Gershenzon, 2010). Ozone has also been demonstrated to affect secondary metabolism in plants (Eckey-Kaltenbach et al., 1994; Jordan et al., 1991). Elevated O_3 levels increased the concentrations of terpenes, but decreased the concentrations of phenolics in *Ginkgo biloba* leaves grown under greenhouse conditions (He et al., 2009). Another contributing factor to secondary metabolism in plants is salt stress. Plants adjust metabolism to acclimate to different salt levels in soil and other growth media. High levels of alkaloids were reported for *Achnatherum inebrians* plants cultivated under salt stress (Zhang et al., 2011).

4. How much of what to produce under which conditions: fine tuning the defence mechanisms

Understanding the defence signalling regulatory pathways and uncovering key mechanisms by which plants tailor their responses to a multitude of stress factors are fundamental to its manipulation towards the desired human benefits. In an attempt to advance knowledge towards this objective, plant growth regulators such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) have emerged as key players in the regulation of signalling networks involved in these responses (Pieterse and Dicke, 2007; Van Poecke and Dicke, 2004). These so called 'signal signatures' have an important role in the fine tuning of the plant's defence response.

4.1. Stress quality (degree, duration and magnitude) versus metabolite pool (quality and quantity)

In the field, plants are exposed to a multitude of environmental fluctuations during the day, between the days and during the growing season. Not only are they confronted with these abiotic factors, but concurrently face a range of pressures from herbivores and myriads of pathogenic microbes. Every environmental factor deviating from the optimum has an effect on the rate of relevant physiological processes, thereby constituting a stress to the plant. The way plants sense stress, depends on the duration and magnitude of the stress episode and its sustained effects depend on its severity, timing, duration and the physiological status of the plant (Niinemets, 2010). Stress factor intensities that reduce net photosynthetic rates are expected to lead to reduced carbon and energy allocation for the synthesis of constitutive defence metabolites and thus quantitatively affect their levels within plants. In addition, alterations in the activity of key enzymes limiting specific secondary metabolic pathways relative to the enzymes controlling carbon assimilation can also modify the secondary metabolite pool under different stress regimes (Singsaas and Sharkey, 2000). Consequently, different stress regimes differentially affect quality and quantity of the secondary metabolite pool in plants. A plant's response to the biotic and abiotic stress factors will therefore, vary depending on the type and magnitude of stimulation each plant species receives and on which part of the plant. An understanding of how the intensity and duration of each stress factor affect the composition and quantity of various metabolites in different plant species is essential in the commercial production of these compounds for pharmaceutical purposes. Regulation of these specific factors to levels and duration that favour optimum accumulation of a desired metabolite of interest provides an opportunity for people to utilise plants as chemical factories for producing pharmaceuticals.

4.2. Multiple stress effect

Under natural conditions, plants rarely experience single abiotic factors one by one, but are much more likely to be exposed to multiple stresses simultaneously. Seasonal climatic changes bring about a variety of different stress combination factors and hence the response by plants is not always predictable and very complex (Holopainen and Gershenzon, 2010). More often, these factors are investigated individually. Gouinguene and Turlings (2002) highlighted that when two or more factors co-occur, their effects are sometimes additive, while in other cases the influence of one factor has priority. In maize, a combination of high temperature and simulated lepidopteran herbivory resulted in greater metabolite accumulation than when either stress was applied alone (Gouinguene and Turlings, 2002). Niinemets (2010) reported that, fungal infection in maize reduced the emission of induced defence metabolites by lepidopteran herbivory alone by about 50%. Higher alkaloid levels were recorded for *Achnatherum inebrians* plants cultivated under salt and drought stress, with levels of ergonovine being higher than those of ergine (Zhang

et al., 2011). Concentrations of both alkaloids increased over the life-span of the plant growing period. The influence of multiple stress factors commonly experienced by plants in field environments is often interactive, implying that the combined effect of various stresses is more diverse. Research efforts aimed at understanding this diversity is essential in that the response of plants to multiple stress combinations cannot always be extrapolated from responses to individual stress factors. Knowledge of how multiple stresses affect secondary metabolite accumulation in plants will provide more information to evaluate the biological roles of these metabolites in mitigating stress and provide criteria for describing their optimum yields and quality and hence provide a means of ensuring quality in phytomedicine.

5. Towards quality regulation in phytomedicine - does the environment have a say?

The extensive exploitation of plants in various medical traditions rests on the principle of active ingredients contained in their extracts. Plants undoubtedly provide these health benefits to humans largely because of their inherent ability to provide a limitless pool of chemical compounds, with diverse ranges of medicinal properties. Since medicinal plant extracts derive their therapeutic effects from secondary metabolites, the driving forces behind the production and accumulation of these valuable compounds in plants will certainly have "*the loudest*" voice in dictating quality of phytomedicines. To what extent does the environment contribute to this effect? Perhaps the answer lies in the rich scientific literature that undoubtedly places environmental factors, among other contributors, as the chief contributing factors to the events surrounding secondary metabolism. With this in mind, the most logical conclusion that one would reach is that, yes, medicinal quality is indeed determined out in the field.

Most medicinal plants used in phytomedicines are collected from the wild and their metabolite pools continue to be shaped by the natural, external environmental factors. Their quality is determined by natural variability of these factors. But, do these shape the metabolite pool to our precise need in terms of type, quantity and optimum bioactivity? Is there room for manipulating these factors in favour of the desired types and quantities of secondary compounds? Do high concentrations of particular classes of medicinal compounds translate to better activity? Are there poisoning effects from certain classes and/or concentration levels of some of these compounds? Answers to these questions could ensure improved quality of phytomedicines.

5.1. Possible environmental solutions to quality problems

Given the need and demand for a continuous supply of medicinal plants with uniform quality attributes, increasing the number of medicinal plants through cultivation would appear to be an important strategy for meeting a growing demand (Uniyal et al., 2000). Cultivation of medicinal plants however, has not been widely adopted as a conservation strategy in most cultures. The total number of species of medicinal plants

cultivated on any scale is very few (Hamilton, 2004). China is probably the country with the greatest acreage of medicinal plants under cultivation, but, even so, only 100–250 species are cultivated (Schippmann et al., 2002). One explanation may be found in the observation that cultivated plants are sometimes considered qualitatively inferior when compared with wild gathered specimens (Schippmann et al., 2002). The reason is primarily cultural. In Botswana, for example, traditional medicinal practitioners cited that cultivated material was unacceptable, as cultivated plants did not have the power of material collected from the wild (Cunningham, 1994). The limitations of cultivation as an alternative to wild harvest have been examined in several case studies (CBD, 1992; Hamilton, 2004; Sheldon et al., 1997). In addition, the Nagoya Protocol (2011) offers provisions for the respect of cultural and traditional knowledge of communities on access to genetic resources, which further strengthen the ability of these communities to benefit from the use of their knowledge, innovations and practices. The conclusion is therefore, that, notwithstanding the level of interest in cultivation as a means for enhanced production, most medicinal plant species will continue to be harvested from the wild to some extent. Cultivation, however, particularly under controlled environments like greenhouses, allows for the control and manipulation of specific factor(s) that influence specific secondary metabolites of interest.

Various studies have explored whether UV-B acclimation responses can be exploited to manipulate for disease resistance (Wargent et al., 2006), in a commercial context. Glasshouses and polytunnels are mostly UV-B-free. The different types of glass or plastics used in the greenhouses, however, facilitate selective penetration of some, or all, UV-wavelengths into glasshouses or polytunnels (Krizek et al., 2005). Alternatively, UV-supplementation setups, based on UV-emitting fluorescent tubes, can be used to obtain higher than ambient levels of UV-B radiation. Ultraviolet supplementation has been proposed as a cultivation measure to increase levels of tocopherol and flavonoid in vegetable crops (Higashio et al., 2007; Lavola, 1998), both of which have desirable pharmacological properties. Similarly, manipulation of other stress factors such as light, mineral nutrition, water, temperature, salt stress, CO_2, etc., in similar or related cultivation setups have previously been investigated, with varying degrees of success (Glynn et al., 2004; Gouinguene and Turlings, 2002; He et al., 2009; Simon et al., 2010; Zhang et al., 2011). Furthermore, *in vitro* manipulation and maintenance of environmental factors in cell and plant cultures under controlled environments could be another potential source of ensuring uniform quality supply of phytotherapies (Aoyagi, 2011; Bruce and West, 1989; Kim and Chang, 1990; Ncube et al., 2011b). When plants grow in a relatively controlled environment, where climatic factors and disturbance could be looked at as uniform, quality of secondary compounds in a given plant species could at least be ascertained with some degree of predictability.

6. Conclusion

If secondary compounds can be induced by biotic and abiotic stresses, then a potential exists to use such stresses as tools to

increase the health-related properties of plant material. In advancing this aspect, any increase in the accumulation of pharmaceutically desirable plant metabolites should be viewed in the context of a complex, overall change in the metabolic profile in which levels of many different compounds change in parallel. Understanding the elicitation of induced metabolites and their lifetime is fundamental to the quantitative assessment of these compounds. Be that as it may, and notwithstanding the challenges and complex interactions between external factors and the plant's physiological and metabolic system, an understanding of these dynamics is arguably the ultimate potential strategy of ensuring quality in phytomedicine. Quality is, indeed, grown out in the field. It must, however, be pointed out that plant responses to environmental cues is species specific. A multidisciplinary approach to this theme, combining ecology, biochemistry, and molecular physiology, would have great potential to advance this field and unravel the extent to which plant–environment interactions contribute to phytomedicine. Timing of harvesting and/or postharvest handling of plant material also has an influence on the quality of secondary metabolites in medicinal plant extracts.

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