

Effects of Mineral Nutrients on Physiological and Biochemical Processes Related to Secondary Metabolites Production in Medicinal Herbs

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ABSTRACT

Although biosynthesis of plant metabolites (e.g. alkaloids, terpenoids, phenolic compounds, etc.) is primarily controlled genetically, environmental factors such as stresses and nutrient elements affect the production of metabolites in plants. Many biological activities have been ascribed to these metabolites. In addition to their therapeutic effects, they play a role as chemical defense agents against microorganisms and herbivores. Therapeutic effects of medicinal plants are associated with their chemical peculiarities. Chemical features of these plants serve as an integral determinant of their species specificity and pharmacological properties and facilitate their wide use in medical practice and other uses. The relationship between the synthesis of physiologically active substances and accumulation of elements is mediated by several levels of molecular regulation. There is general agreement that various mineral nutrients increase the growth of individual plants and, consequently, enhance the total plant biomass yield. However, the effects of certain macro- and micro-nutrients on the production of secondary metabolites are also modulated by environmental conditions and depend on plant species. Incorporation of one or more of the trace elements could increase or decrease the production of secondary metabolites depending on the plant species as well as on the concentrations of these elements. The effect of the interaction of certain nutrients on the production of secondary metabolites in herbs is discussed in the present review as well.

Keywords: nutrient fertilization, C/N balance, plant secondary metabolites, medicinal herbs

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INTRODUCTION

Plant secondary metabolites (SMs) are more than 30,000 different substances exclusively produced by plants (Baranauskiene *et al.* 2003; Scheible *et al.* 2004; Rice-Evans 2004; Zhou 2006). Therapeutic effects of medicinal plants are associated with their chemical peculiarities. As differentiated from other plants, medicinal plants synthesize and accumulate physiologically active natural substances with marked physiological activities (Havsteen 2002; Lisiewska *et al.* 2006; Maisuthisakul *et al.* 2007). Plants produce numerous SMs have historically been used as pharmaceuticals, fragrances agents, flavor compounds, dyes, antioxidants, antimicrobial agents and agrochemicals (Said *et al.* 2002; Silva *et al.* 2004; Ljubuncic *et al.* 2005, 2006; **Table 1**). Even today, these metabolites are a major source of new drugs. Because their profiles vary among species, extracts from both known and newly discovered plants are subjected to screening for use as new medications. SMs are produced *in vivo* usually at low concentrations; therefore, large-scale production systems have been developed using plant cell cultures. Despite the enormous commercial efforts made for several decades, only a few compounds have successfully been produced at low cost this way com-

pared to those produced by direct plant extraction or through chemical synthesis. These SMs include alkaloids, terpenoids (triterpene and steroid saponins), phenolic compounds, glycosides, polysaccharides and others (**Table 1**). Generally, drug plants are unique in containing compounds that are end-products of long biosynthetic pathways and are usually not needed as such in plants metabolic processes. Wild plants and cultivated species exhibit great variation in the content of SMs as well as in the biological activities as affected by different growth environments (e.g. soil type, nutrients, topography, salinity, drought, allelochemicals, etc.) and genetic differences among species (El-Darier and Youssef 2000; Khudsar *et al.* 2004; Akerstroem *et al.* 2009; Babalar *et al.* 2010). There is general agreement that the effect of farmyard manure, nitrogenous fertilizers and other macro- and micro-elements of various kinds is to increase the growth of individual plants in certain soil and so to increase the total yield of plant biomass (Demeyer and Dejaegere 1992, 1995; Babalar *et al.* 2010; Ibrahim and Jaafar 2011). The mineral nutrition can exert an effect with respect to relative ionic composition, pH of the culture medium, and total mineral fertilizer dose. These parameters can also simultaneously exert their own specific influence (Demeyer and Dejaegere 1992, 1995; Babalar *et al.* 2010;

Table 1 Secondary metabolites originating from plants, their sources, some effects and uses.

Class	Compounds	Sources	Effects and Uses	References
N-containing SMs				
Alkaloids	Nicotine cocaine theobromine	Tobacco coca plant chocolate (cocoa), <i>Datura</i>	Interfere with neurotransmission, block enzyme action	Sikuli and Demeyer 1997; Fritz <i>et al.</i> 2006
N- and S-containing SMs				
Glucosinolates	Sinigrin	Cabbage, relatives	Anticancer	Wang <i>et al.</i> 2011
Terpenoids				
Monoterpenes	Menthol linalool	Mint and relatives and other plants	Antimicrobial, anesthetic	Bassole <i>et al.</i> 2010
Sesquiterpenes	Parthenolid	Parthenium and relatives (<i>Asteraceae</i>)	Contact dermatitis, anti cancer	Cheng and Xie 2011
Diterpenes	Gossypol	Cotton	Block phosphorylation; toxic, anti cancer	Moon <i>et al.</i> 2011
Tetraterpenoids	Carotene	Many plant species	Antioxidant; orange coloring	Lewinsohn <i>et al.</i> 2005
Sterols	Phytoecdysteroids, spinasterol	Spinach	Pesticides, interfere with animal hormone action	Bakrim <i>et al.</i> 2008
Phenolics				
Phenolic acids	Caffeic, chlorogenic	Many plant species	Antioxidants, browning in fruits and wine	Predrag <i>et al.</i> 2005; Balasundram <i>et al.</i> 2007; Maisuthisakul <i>et al.</i> 2007
Coumarins	Umbelliferone	Carrots, parsnip	Cross-link DNA, block cell division	Matsmoto <i>et al.</i> 2012
Lignans	Podophyllotoxin	<i>Podophyllum peltatum</i>	Cathartic, vomiting, anti cancer	Avula <i>et al.</i> 2011
Flavonoids	Anthocyanin, catechin	Many plant species	Flower, leaf color; inhibit enzymes, anti- and pro-oxidants, estrogenic	Havsteen 2002; Hoelzl and Petersen 2003; Rice-Evans 2004; Li <i>et al.</i> 2005
Tannins	Gallotannin, condensed tannin	Oak, hemlock trees, birdsfoot trefoil, Lentisk	Bind to proteins, enzymes, block digestion, antioxidants	Landau <i>et al.</i> 2010
Quinonoids	Benzoquinones, naphthoquinones, anthraquinones	Many plant species	Insect repellants	Hazra <i>et al.</i> 2004
Xanthones	Xanthone	Mainly Gentianaceae and Guttiferae	Antioxidant and neuroprotective activities	Aberham <i>et al.</i> 2011; Sun <i>et al.</i> 2011
Anthocyanins	Anthocyanidin	<i>Vaccinium myrtillus</i> (berries) and other plants	Antioxidants, insect repellants	Okamoto <i>et al.</i> 2003; Akerstroem <i>et al.</i> 2009

Ibrahim and Jaafar 2011).

Several SMs are natural antioxidants that occur in all plant parts in various concentrations. The typical compounds that exhibit antioxidant activities include phenolics, carotenoids, vitamins and others (Chanwitheesuk *et al.* 2005). Among the various kinds of natural antioxidants, polyphenols constitute the main powerful group of compounds owing to their multiple applications in food industry, cosmetics, and pharmaceutical and medicinal products (Maisuthisakul *et al.* 2007). In addition to their role as antioxidants, these compounds, originated from plant herbs, exhibit a wide spectrum of medicinal properties, such as those concerned with anti-allergic, anti-atherogenic, anti-inflammatory, anti-microbial, anti-thrombotic, cardio-protective, vasodilatory and other effects (Said *et al.* 2002; Ljubuncic *et al.* 2005; Balasundram *et al.* 2007). Several studies have shown that the amount of polyphenolics in plants and their antioxidant activities depend on biological factors (genotype, organ and ontogeny) as well as on the edaphic and environmental conditions (temperature, salinity, nutrients, water stress and light intensity) (Lisiewska *et al.* 2006). Nutrients are important factors for plant growth and production of primary metabolites as well as SMs during the plant growth and development. This chapter will focus the effects of nutrients on the production of secondary metabolites in plant herbs.

EFFECT OF NUTRIENTS ON SECONDARY METABOLITES OF PLANT HERBS

Plants have an almost limitless ability to synthesize medicinal and aromatic substances that have been evaluated for their therapeutic potential. These include alkaloids, coumarins, saponins and flavonoids (Watson *et al.* 2001; Barnes *et al.* 2002). Flavonoids are probably the best known of these substances due to their antioxidant properties (Havsteen 2002; Rice-Evans 2004). Data indicate that cultivation suc-

cess should not rely solely on parameters of growth but also it should incorporate the assessment related to indices of therapeutic potential and contents of SMs. Increasing the amount of fertilizer caused a significant concentration-dependent increase in antioxidant activity of the cultivated plant of *Teucrium polium* compared with the wild type plant (Azaizeh *et al.* 2005). In contrast, increasing the amount of fertilizer caused a significant concentration-dependent reduction in the antioxidant activity of the powder prepared from the cultivated plants of *Eryngium creticum* when compared with that of wild plants.

Cysteine sulfoxides (e.g. alliin) are the characteristic sulfur-containing SMs in garlic, which account for taste and pharmaceutical quality. The influence of sulfur (S) and nitrogen (N) supply was tested under field conditions on the content of alliin, cysteine and glutathione, which are used as possible precursors (Bloem *et al.* 2010). Sulfur fertilization significantly increased the contents of cysteine, glutathione and alliin in the leaves and bulbs, while N fertilization had no significant influence. Cysteine content was increased by a factor of 1.3 to 1.5 in leaves and 1.0 to 2.0 in bulbs. Glutathione content increased significantly in bulbs by a factor of 0.9 to 1.6 but only at vegetative stage of growth and not at maturity. The alliin concentration in bulbs increased with S fertilization significantly at all harvesting dates as well as at maturity. High S application in combination with low N fertilization increased the alliin concentration in garlic significantly during vegetative growth until the beginning of ripening. Garlic bulbs, fertilized with a high level of S, may have a higher potential to accumulate alliin after harvest because of higher contents of alliin precursors (Bloem *et al.* 2010). Therefore, a high S fertilizer level may be beneficial to produce high-quality garlic bulbs. In contrast, N should be fertilized at a lower dose to avoid adverse effects on crop quality, such as that related to alliin content.

The effect of ion-balance on biomass production and

alkaloid yield of *Datura stramonium* L. revealed that the ionic interactions between macro-elements influenced the biomass production and alkaloid yield differently. The highest biomass yield was found with NO_3^- and K^+ -dominance, whereas hyoscyamine yield increased to the highest extent with the culture medium in which SO_4^{2-} and K^+ were dominant (Sikuli and Demeyer 1997). Although N fertilization usually increases yield, especially of *Hyoscyamus* sp., unbalanced fertilization with other nutrients tends to decrease yield, or if there is increase, it is of small amount. Balanced N, P and K nutrition is very important factor regarding the growth and development of medicinal plants; for example, balanced nutrition of N and P is required for *Datura stramonium* and that of N and K for *Hyoscyamus niger*. In several studies, it was emphasized that balanced fertilization was essential for healthy growth for most plants, and that over-fertilization with mineral nutrients (except perhaps that with N in the form of nitrate) may be harmful to plants. The effect of all mineral fertilizers is greatly influenced by the composition of the soil in which the plants grow, and possibly also by the age of the plant (Sikuli and Demeyer 1997). Biosynthesis of alkaloids, although controlled genetically, could be affected by different environmental factors, such as light, high temperature, drought stress and nutrients. Results obtained from different authors investigating the effect of various manures and fertilizers on the alkaloid content of different herbs are much less consistent than those dealing with growth and yield of the plants. Several studies have shown the influence of mineral nutrition on alkaloid biosynthesis, depending upon the plant species (Tamaru 1980; Gorinova and Atanassov 1993; Tawfik 1997; Poutaraud and Girardin 2005). In *Datura stramonium*, high Co application rates decreased the yield of all plant parts and the alkaloid content of leaves; while in roots, the alkaloid content rose with increasing Co rates (Tamaru 1980). In *Leucojum aestivum*, the highest plant galanthamine content was recorded when the soil was dressed with a good supply of N, K, Mg, B, Mo, Zn, Fe, and Cu (Gorinova and Atanassov 1993). In *Atropa belladonna*, Petrishek *et al.* (1983) indicated a significant relationship between Co supply, Co content in the plant and the synthesis and accumulation of tropane alkaloid. Application of Fe and Mn and their combination increased the total content of seed-alkaloids in *Lupinus hartwegii* (Tawfik 1997). Soil analyses were performed on different natural sites examining seed content (SEC) of Meadow saffron (*Colchicum autumnale* L.) for different alkaloids (Poutaraud and Girardin 2005). Four mineral elements (Ca, Fe, Co, and Mn) were assayed to determine mineral soil element potentially active on alkaloid synthesis. Linear relationships were observed between the soil content (SOC) of some mineral elements and SEC, especially with regard to Co and Ca (Poutaraud and Girardin 2005). Furthermore, a high correlation was observed between the SEC of Co + Ca and the SEC of alkaloids. Biosynthesis of the colchicine and colchicoside alkaloids occurs in seeds, with the Co and Ca playing an important role on the regulation of these alkaloids. Optimization of application-dose of micronutrient appears to be a good way for controlling production of *C. autumnale* with a high SEC of alkaloids (Poutaraud and Girardin 2005).

St. John's wort (*Hypericum perforatum* L.) (Family: Hypericaceae/Guttiferae) is a perennial flowering plant used in traditional folk medicines to treat various ailments (Mukherjee *et al.* 2002; Reichling *et al.* 2002). Extracts of *H. perforatum* are known to contain compounds from six major groups of natural products, viz. naphthodianthrones, acylphloroglucinols, flavonol glycosides, biflavones, proanthocyanidins and phenylpropanes (Hoelzl and Petersen 2003). Hypericins, which include five known compounds (hypericin, pseudohypericin, protohypericin, protopseudohypericin and cyclopseudohypericin), are considered the principal agents in the range of biological activities reported for *H. perforatum*. The concentration of the medicinally active compounds in St. John's wort has been shown to be influenced by the available N in the soil (Briskin *et al.* 2000,

2001), light quantity and quality, seasons and growing region. The levels of hypericins in plant tissue increased as a result of chemical elicitor treatments; total hypericin levels increased as much as 3.3 times of the control levels when treated with methyl jasmonate and salicylic acid. A loss in seed germination and a significant decrease in abundance of hypericin (21-fold) and pseudohypericins (15-fold) was observed in Ni-treated seedlings of *H. perforatum* L. (Murch *et al.* 2003). A complete inhibition of hyperforin biosynthesis was also observed in this investigation due to Ni-toxicity. The hypocotiles of *H. perforatum* (sp. *Angustifolium*) treated with various concentrations (within the mM range) of Cr(VI) for seven days produced a marked increase in the biosynthesis of protopseudohypericin, hypericin and pseudohypericin (Tirillini *et al.* 2006). Baroni Fornasiero (2003) reported the effect of fluoride on plant growth parameters of *H. perforatum*, making no observation on the SMs. The effect of certain nutrient(s) on the production of SMs (e.g. alkaloids and phenols) depends on plant species as well as on plant organ under investigation. Hence, the analysis of separate plant parts (stems, leaves, seeds, roots, etc.) for the content of SMs is essential in order to determine the effect of certain nutrients or of their combination on the metabolism of secondary compounds.

EFFECT OF NITROGEN FERTILIZATION AND C/N BALANCE ON SECONDARY METABOLITES IN PLANT HERBS

Carbon/nitrogen balance (CNB) hypothesis is used to explain the effects of nutrient availability on the concentration of plant SMs such as flavonoids, alkaloids, phenolics, etc. (Bryant *et al.* 1983, 1988; Tuomi *et al.* 1988; Reichardt *et al.* 1991). Nitrogen is one of the most important nutrients for crop production and influences both the content and quality of plant SMs (Scheible *et al.* 1997, 2004; Aires *et al.* 2006; Sifola and Barbieri 2006). The CNB hypothesis asserts that plants allocate carbon and N to the SMs only after growth requirements are met and that growth is constrained more by nutrient limitations than by photosynthesis. According to this theory, the excess carbohydrates that accumulate in nutrient-limited plants are diverted to the production of carbon-based SMs (e.g., phenolics). Increasing fertilization increases tissue nutrient concentrations and allows growth to outpace photosynthesis. Therefore, according to the CNB hypothesis, production of N-based SMs (e.g. alkaloids) should be increased as N is acquired in excess of growth requirements. Conversely, concentrations of carbon-based phenolic compounds are predicted to decrease with fertilization due to decreased rates of photosynthesis relative to growth. Although some studies on the effects of N fertilization on SMs support the CNB hypothesis, others contradict it (Hermes and Mattson 1982; Koricheva *et al.* 1998).

The effect of ammonium nitrate fertilization level and that of gender (male/female) on the production of caffeine and related alkaloids and phenolic compounds in the leaves of pot-grown Aquifoliaceae plant yaupon (*Ilex vomitoria*) was studied by Palumbo *et al.* (2006). The CNB hypothesis predicts that additional N should result in increased alkaloid concentrations accompanied with a decrease in phenolic concentrations. Yaupon responded to N-fertilization with large increases in concentrations of caffeine and total methylxanthine alkaloids, but it did not respond to decreases in concentrations of cinnamic acid derivatives, flavonoids, or total phenolics. Furthermore, neither alkaloid nor total N concentrations differed on account of gender, although an interaction between gender and fertilization influenced caffeine production (Palumbo *et al.* 2006). Even when N fertilization stimulates biomass accumulation but not photosynthetic rates, phenolic concentrations do not always decrease (Reichardt *et al.* 1991; Bezemer *et al.* 2000). Similarly, no correlations were found between relative growth rates and the relative foliar mass of phenolic glycosides among N-fertilized *Populus tremuloides* (Donaldson *et al.* 2006).

An extension of the CNB hypothesis to dioecious plants predicts that females have higher C/N ratios and therefore accumulate higher phenolic but lower alkaloid concentrations than the male of the same plant species (Palumbo *et al.* 2006). The researchers showed that caffeine and total alkaloid concentrations were 5-10 times higher in fertilized than in control plants but did not vary by gender. Nevertheless, an observed interaction between gender and fertilization suggests that females respond more to fertilization than males with regard to caffeine production. In addition, fertilized plants not only contained higher concentrations of alkaloids and total N but also allocated a larger proportion of their N content to alkaloid production than the control plants (Palumbo *et al.* 2006). Total phenolic concentrations were higher in control females than in control males as predicted by the CNB hypothesis, but did not vary by treatment nor were there significant differences among fertilized plants due to gender. Higher correlations were observed between antioxidant capacity and the content of both classes of phenolic compounds (cinnamic acid derivatives and flavonoids), indicating that in addition to their putative defensive function against herbivores, phenolic compounds protect yaupon from oxidative stress (Palumbo *et al.* 2006). Explanation of the inconsistencies between these data and predictions of the CNB hypothesis may benefit from a reappraisal of the physiological mechanisms, where resource availability affects the accumulation of SMs as well as consideration of the selective pressures to which secondary metabolism responds in addition to environmental conditions.

The extracts of *Satureja hortensis* L. contain flavonoids and caffeic acid derivatives. Rosmarinic acid (RA) is present in higher concentration and possesses a multitude of biological activities, e.g. antiviral, antibacterial, anti-inflammatory and antioxidant activities, making it a valuable plant product for the pharmaceutical and cosmetic industries (Li *et al.* 2005). The presence of RA in medicinal plants, herbs and spices has beneficial and health-promoting effects. The production of RA by plant cells can be modified by many factors. Babalar *et al.* (2010) investigated the effects of calcium carbonate (CaCO₃) and N application on RA content, plant growth and yield of *S. hortensis*. Nitrogen fertilization significantly increased the plant height, fresh and dry weights of herb as well as RA content and yield. CaCO₃ treatment did not affect either content or yield of RA, while it increased the fresh and dry weights of the herb significantly.

Ibrahim *et al.* (2012) studied the effect of four levels of N fertilization (from 0 to 270 kg N/ha) on three varieties of *Labisia pumila* for 15 weeks in terms of antioxidant activities (FRAP and DPPH), production of glutathione (GSH), oxidized glutathione (GSSG), and total contents of flavonoid, anthocyanin and ascorbic acid. Neither varietal nor interaction (N level × variety) effects were observed. The production of GSH, GSSG, anthocyanin, total flavonoid and ascorbic acid increased steadily as the N levels decreased from 270 to 0 kg N/ha. At the highest N level, *L. pumila* exhibited significantly lower antioxidant activities (DPPH and FRAP) than those when exposed to limited N supply. Significant positive correlation was obtained between antioxidant activities (DPPH and FRAP), total content of flavonoid, GSH, GSSG, anthocyanin and ascorbic acid, suggesting that an increase in the antioxidant activities in *L. pumila* under low N fertilization could be attributed to higher contents of these compounds. The authors advised to avoid excessive application of N fertilizer for *L. pumila* cultivation for its medicinal uses. Previous studies on *L. pumila* have shown that high N application might reduce the production of SMs in the herb due to correlation of reduced phenyl alanine lyase (PAL) activity with low C/N ratio, photosynthetic rates and total non structural carbohydrate (Ibrahim and Jaafar 2011). *Vaccinium myrtillus* berries (bilberries) contain antioxidants, in particular anthocyanins, which are the SMs that have proven health-promoting effects. Different levels of N fertilization were tested for 3 years, observing

no significant effect on total anthocyanidin level (Akerstroem *et al.* 2009). The results indicated that climatic factors and yearly fluctuations influenced the biosynthesis as well as degradation of anthocyanidin more strongly than the N availability. Okamoto *et al.* (2003) found higher anthocyanin concentrations in samples of grape skins taken from moderately N-fertilized plots (1.5 times higher than normal levels for the studied site) than in samples obtained from both unfertilized and heavily fertilized plots (2.0 times higher than normal levels for the studied site). Therefore, fertilization levels, genotypes as well as environmental conditions affected the production of SMs significantly.

Studies have revealed that N fertilization did not necessarily decrease the starch or total sugar concentrations, though it decreased the phenolic concentrations (Balsberg 1992); further, N additions might stimulate the increases in total content of nonstructural carbohydrates (Kaakeh *et al.* 1992). A refined understanding of the mechanisms by which N availability influences the accumulation of carbohydrate, N, and SMs can clarify the influence of N application on carbon and N allocation in plants for the production of both alkaloids and phenolics. Another study indicated that nitrate, not merely downstreamed the production of N metabolites, but also it elicited the signals for the biosynthesis of organic acids, amino acids, and those for nucleotide metabolism (Scheible *et al.* 1997, 2004). It did, however, repress the starch biosynthesis. In *Nicotiana tabacum*, nitrate induced the biosynthesis of nicotine by a similar mechanism, inhibiting the biosynthesis of phenylpropanoid and flavonoid, on the contrary (Fritz *et al.* 2006). These studies indicated that accumulations of carbohydrate, N, and SMs in plants are, in fact, coordinated according to N availability. C/N ratio alone, however, may be insufficient for predicting resource allocation to SMs because their production is influenced by nitrate-induced signals and not merely by the mass flow of carbon and N through various metabolic pathways (Stitt and Krapp 1999). The multiple functions of phenolic compounds may render it difficult to predict their production as a result of N additions as well. It may be ascribed to the interaction of N with other elements that might affect the production of phenolics and other SMs during plant growth.

According to Briskin *et al.* (2000), N supply to *H. perforatum* plants might offer another example proving a profound impact on production of phenolics and other SMs. Growing the *H. perforatum* plants in a low-N soil and sand culture might result in elevated levels of hypericins (2- to 3-fold). Soil supplemented with N induced a 3-fold decrease in total hypericin production in fresh plant material, but did not affect the mean number of dark glands per leaf. The researchers noticed that the relative ratio of hypericin and pseudohypericin was not affected by N supplementation. Only a moderate decrease in N supplementation was, however, advisable to avoid the induction of chlorosis and subsequent undesirable reduction in biomass production. However other contradictory reports are available on N supplementation to *H. perforatum*. In fact, plants cultivated in Iran and fertilized with 250 kg N/ha and 100 kg P/ha increased the number of flowering stems per plant and leaf-hypericin content (Azizi and Omidbaigi 2002). Further research, performed on plants supplemented with 150 kg N/ha and 100 kg P/ha evidenced an increase in the content of hypericin, hyperforin and flavonoidic. Thus a definitive statement in this regard is yet to come and it may also depend upon the composition of the tested soil (Dias *et al.* 2001). The combined effect of light exposure and decreased N supplementation indicated additive and independent results, facilitating the continuous enhancement both in the number of red glands and hypericins content (Briskin and Gawienowski 2001). Effect of interaction between N and other nutrients in combination with that of environmental conditions, light intensity, plant species and age are important factors in determining the metabolism of SMs in plants. Not enough data are available on these aspects to predict the optimum metabolism of a certain plant in order to pro-

duce the best combination of SMs for pharmaceutical or agricultural purposes.

CONCLUDING REMARKS

Enhancing the plant biomass and biosynthesis of SMs through mineral nutrition is a challenge that should be pursued with a multidirectional approach. Economical factors should also be considered, to evaluate if higher biomass yield, enhanced production of SMs and higher cultivation density might cover the costs of this kind of cultivation.

Genetic factors strongly affect both plant yield and the biosynthesis of bioactive SMs in different plant herbs, making the availability of elite germplasm a fundamental factor for field cultivation of superior quality herbal drugs based on plant cultivation. As a result of different climatic factors such as soil characteristics, nutrients, C/N balance, fertilization practices and geographic differences, each region may offer unique ecological conditions and, thus, might help to determine different phytochemical profiles in quantitative terms. Comparative breeding, long-term selection of lines and fertilization regimes should be evaluated not simply for their agronomic advantages (gross yield, stress resistance, etc.) but also for their phytochemical profile and content. A synergistic effort between agronomists and phytochemists must be encouraged in this regard. In fact, the complete optimization of agronomic conditions according to phytochemical production requires longtime efforts, needing years to conduct long term experimentation and financial support. Fertilization with nutrients and maintaining the favorable C/N balance in combination with proper environmental conditions, light intensity, plant species and age are important factors in determining the accumulation of SMs in plant herbs. However, factors to be considered should emerge not only from in-field agronomic results, but also from physiological, genetic, biotic, abiotic and phytochemical data that could be scaled up to the application level.

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