Phytochemical diversity: The sounds of silent metabolism

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ABSTRACT

Plants produce tens of thousands of different natural products also referred to as secondary metabolites. These metabolites were once thought to be the result of aberrant metabolism, or a form of transient storage of byproducts and intermediates thereof. Although the true role of such metabolites in plants remains mostly unknown, it is evident that plants invest a great deal of resources in synthesizing, accumulating and sorting such metabolites, often produced through complex and highly regulated biosynthetic pathways operating in multiple cellular and sub-cellular compartments. There is also growing evidence indicating that many biosynthetic pathways leading to the accumulation of plant natural products are not fully active. Thus, occult enzymes exist, sometimes without any apparent endogenous substrate or function, suggesting that plants have a reservoir of metabolic capabilities that normally remain hidden or unused. It is often difficult to accurately guess what are the actual biological roles of such enzymes solely based on bioinformatics, due to promiscuity towards substrates and the relatively ease to change substrate or product specificity by introducing minor changes in sequence of the enzymes. It could be that such orphan enzyme activities are relics of a recent past that have not been fully eliminated through selection and evolution. Additionally, it could be that such occult activities possess unknown biochemical roles and coincidentally are able to accept novel substrates. We have coined the term “silent metabolism” to describe occult metabolic capacities present or induced in plants. A few examples illustrating silent metabolism in the terpenoid and phenylpropanoid pathways, as well as their repercussion in the metabolic engineering of plant secondary metabolism are discussed in this review.

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Hello darkness, my old friend
I've come to talk with you again
Because a vision softly creeping
Left its seeds while I was sleeping
And the vision that was planted in my brain
Still remains
Within the sound of silence

(P. Simon, 1964)

1. Introduction

Plants produce and accumulate a vast number of different natural products, also called secondary metabolites. Although tens of thousands of secondary metabolites have been chemically identified [1–3], the biological roles of most of these compounds remain obscure. Many natural compounds are of commercial and industrial importance, imparting colors and scents to flowers, fruits and vegetables, and are also key ingredients in medicinals and nutraceuticals. Many studies have indicated that natural products accumulated in plants have clear ecological roles. Relatively small chemical changes in a molecule, can have a major impact in its biological function [9–11], the potential to produce a large array of different compounds that can be activated when novel substrates become available.

The much greater multitude of metabolites in living organisms as compared to their unexpected low calculated number by the small number of relevant genes identified during genome sequencing projects has puzzled biologists [14]. It has been postulated that promiscuity of enzymes, including multiple product and substrate enzyme specificity as well of changes in compartmentation patterns may also contribute to plant metabolome diversity [15]. Moreover, the more knowledge we mine on specific biosynthetic pathways and the accumulation of information on the complexity of biosynthetic networks have revealed that the transcription patterns as a whole are rarely directly reflected in the proteome of an organism [16] and in turn, the proteome in many cases only vaguely reflects the metabolome [17]. The more knowledge we extract on the regulation of plant natural product biosynthesis, the more puzzling it gets. Perhaps the old-fashioned unpopular and forgotten theory of being natural products part of an “aberrant metabolism” was not so off-line. It thus seems that the combination of enzymes and substrates generated by serendipity may be a platform to generate the much needed variation to attain effectiveness in plant defenses, and this process might be favored by evolution [7]. In this scenario, it is evolutionary advantageous to keep parts of the enzymatic machinery active through generations, and this in turn is the basis of what we have defined as “silent metabolism”:

Occult biosynthetic capacities that is not easily detected but readily active when challenged. “Silent metabolism” can be either constitutive or induced by development, the environment and genetic manipulation, and might seem to the naive spectator as if active enzymes and biosynthetic pathways do not have any apparent role in the tissue studied, but are uncovered when a change in metabolism is effected.

In this review we will discuss a few of many examples of “silent metabolism” based on our own results and on other published work. They all indicate that plants have practically a network of occult biosynthetic capacities that confers them an unlimited potential to produce a large array of different compounds that can be activated when novel substrates become available.

2. Occult terpenoid pathways

Many monoterpenes are key constituents of essential oils and impart unique aromas to flowers and fruits. The metabolic engineering of the monoterpene pathway has been the focus of many studies [18]. The *Clarkia breweri* floral gene linalool synthase (LIS) encodes an enzyme that catalyzes the formation of (S)-linalool from the monoterpene precursor geranyl diphosphate. Over-expression of *Clarkia’s LIS* in tomato fruit caused the accumulation of (S)-linalool [19] as expected, but also the unexpected formation of 8-hydroxylinalool, a compound absent in control fruits (Fig. 1). The formation of 8-hydroxylinalool is thought to be mediated by an endogenous “occult” hydroxylase activity able to act on (S)-linalool. The actual biological role of this hydroxylase in tomato fruit metabolism is presently unknown, but the novel availability of substrate due to expression of a foreign gene enabled us to uncover this activity. The *Clarkia LIS* gene was also over-expressed in petunia flowers, in this case, (S)-linalool was formed but was rapidly glycosylated (Fig. 1) [20]. In turn, when the *Clarkia LIS* gene was over-expressed in carnation flowers, linalool was also detected in the transgenic flowers but it was apparently further metabolized to linalool oxides (Fig. 1) [21]. Thus, the over-expression of an identical gene in different target tissues and organisms gave rise to distinct phenotypes, according to the silent metabolism present or induced in the target plant. Therefore, in metabolic engineering experiments, one has to take into account not only the potential functions of the genes manipulated, but also the patterns of the silent metabolism in the target organism that might interact with the novel products generated.
Subsequent metabolic engineering of the terpenoid pathway in tomato fruit further demonstrated the existence of a very active grid of seemingly "occult" enzymes. The lemon basil (Ocimum basilicum L. cv. Sweet Dani) geraniol synthase (GES) gene was overexpressed in tomato fruits in attempts to modify their aroma and flavor [22]. The GES gene encodes a protein able to synthesize geraniol, a rose-scented monoterpene alcohol from geranyl diphasphate. Diversion of the plastidial terpenoid pathway of tomato to the formation of geraniol was achieved as evidenced by the accumulation of relatively high levels of geraniol in transgenic tomato fruit [22]. Nevertheless, transgenic fruit accumulated at least eleven novel additional metabolites that share a common chemical backbone and most likely are derived from geraniol. These volatiles included the monoterpenols alcohol nerol and citronellol, the monoterpenoid aldehydes geranial and neral, and citronellal, the monoterpenoid esters geranyl, neryl and citronellyl acetate, geranic and neric acid and rose oxide (Fig. 2). The conversion of geraniol to geranial and neral is catalyzed by alcohol dehydrogenase, an enzyme long known to be active in tomato fruits and characterized by a broad substrate specificity able to accept ethanol (and also geraniol) as substrates [23]. Geraniol reductase activity, able to generate citronellol from geraniol and NADPH, was readily detected in nontransgenic tomato fruit [22]. Additionally, alcohol acetyltransferase activity able to act on geraniol, nerol or citronellol and acetyl CoA to generate geranyl acetate, neryl acetate, and citronellyl acetate, respectively, was also readily detected in ripe control tomatoes [22]. The actual role of these enzymes can only be speculated, but the above evidence suggests that tomato fruits possess active but occult enzymatic activities that can readily accept novel substrates if the substrates become available. To conclude, metabolic engineering of the plastidial terpenoid pathway revealed that a "silent" array of enzymes is present or can be readily induced in tomato fruit. The metabolic flow through this occult, "silent" (but not silenced) grid can be rapidly triggered and detected when a novel substrate is present.

2.1. Silent metabolism in carotenoid biosynthesis and degradation reactions

Carotenoids are nutritionally important tetraterpenoid pigments that are also part of the photosynthetic apparatus and are needed in human nutrition as pro-vitamin A. Attempts to improve the nutritional value of rice have focused on increasing the levels of β-carotene in the endosperm yielding what we term “Golden Rice”. This was attained by the ectopic expression of PSY (phoetoene synthase and CTRLI a bacterial gene coding for carotenoid desaturase [24,25]. In the initial experiments, a construct directing the expression of lycopene β-cyclase was also employed, to further direct carotenoid biosynthesis to β-carotene but shown to be unnecessary, as transgenes overexpressing PSY and CTRL1 only displayed a similar carotenoid pattern as transgenes overexpressing the three genes [24]. The product expected to be formed by the action of the two carotenoid biosynthesis transgenes PSY and CTRL1 is lycopene, a red pigment. Still, Golden Rice accumulates β-carotene and xanthophyll derivatives. The absence of lycopene in Golden Rice shows that the pathway proceeds beyond the transgenic end point and thus that an endogenous pathway was also acting. Moreover, by using realtime PCR, it was elegantly shown that in wild-type rice endosperm the mRNA expression of the relevant carotenoid biosynthetic enzymes encoding phytoene desaturase, ζ-carotene desaturase, carotene cis-trans-isomerase, β-lycopene cyclase, and β-carotene hydroxylase were all active. Only the mRNA corresponding to PSY was virtually absent in wild type rice indicating that this was the limiting step for carotenoid accumulation [26]. Thus, an occult, silent, but potentially active biosynthetic pathway was revealed in wild-type rice. This suggests that the wild ancestor of rice contained a pigmented endosperm. This trait was probably selected against early during rice domestication and this was attained by incorporating mutants deficient in the expression of PSY in cultivated rice.
Carotenoid content not only influences fruit color, but indirectly affects the volatile composition of fruits. Pleiotropic effects on aroma and flavor caused by genes affecting carotenogenesis have been detected by utilizing defined mutants and near-isogenic lines of tomatoes that solely differ in carotenoid patterns [27,28]. Similar effects of carotenoid content and composition on the aroma apocarotenoid volatiles of watermelon, melon, bell peppers and carrots [27–29] and [Azulay, Y., Tadmor, Y., and Lewinsohn E., unpublished results] have been noted. Carotenoids give rise to apocarotenoid (norisoprene) aroma volatiles upon oxidative cleavage [30], and thus carotenoid composition dictates the composition of apocarotenoid volatiles. This relationship is probably mediated by the oxidative cleavage of carotenoids into apocarotenoid volatiles [19,27]. A group of carotenoid cleavage dioxygenase enzymes (CCD’s) catalyze such cleavages and accept a variety of carotenoids as substrates [19,27,29,31–33].

b-carotene is the predominant pigment in orange-fleshed melon (Cucumis melo) varieties, while pale-green and white cultivars have much lower b-carotene levels. In parallel, the potent odorant b-ionone, the 9, 10 (9\textsuperscript{0},10\textsuperscript{0}) cleavage product of b-carotene, is present in orange-fleshed melons and in much lower levels in pale green and white fleshed varieties. A search for a gene putatively responsible for the cleavage of b-carotene into b-ionone was carried out in annotated melon fruit EST databases yielding a sequence (CmCCD1) similar to other plant carotenoid cleavage dioxygenase genes [29]. Interestingly, the sequence originated from ‘Tam Dew’ melons, a pale green fleshed variety that lacks either b-carotene and its oxidative cleavage product b-ionone. Still, the CmCCD1 gene is up-regulated upon maturation, similarly to the corresponding CCD genes in orange-fleshed varieties that contain high levels of both b-carotene and its degradation product b-ionone [29]. To assess the biochemical functionality of CmCCD1, the clone was overexpressed in E. coli strains previously engineered to produce b-carotene. The CmCCD1 gene product efficiently cleaved b-carotene at the 9, 10 and 9\textsuperscript{0},10\textsuperscript{0} positions and b-ionone was released from the bacterial cultures. Further examination revealed that the CmCCD1 gene product cleaves many other carotenoids, but only at positions 9, 10 and 9\textsuperscript{0},10\textsuperscript{0}, generating geranylacetone from phytoene; pseudoionone from lycopene; as well as a-ionone and pseudoionone from d-carotene. These acyclic and monocyclic carotenoids are normally absent in melons, still the CCD enzyme efficiently accepted them as substrates generating novel apocarotenoid volatiles. Thus, it seems that the broad substrate specificity of the CmCCD1 allows the formation of novel volatile metabolites when the proper carotenoid substrate is available. A similar CCD enzyme with a broad substrate-specificity was also discovered in tomatoes [33]. These findings easily explain the pleiotropic effects on aroma previously observed in tomato carotenoid mutants and the general correlation between carotenoid composition and apocarotenoid aroma chemicals observed [27,28]. However, the question of why is an active CCD enzyme is needed in the fruits of a melon genotype that lacks b-carotene, the apparent substrate of this enzyme.

Carotenoids are biosynthesized and accumulated within plastids, yet the carotenoid cleavage dioxygenases are apparently cytosolic. Thus, it is likely that subcellular compartmentation limits the access of the CCD enzymes to carotenoid substrates and limits carotenoid cleavage. This might explain the vast difference (about a thousand fold) between the carotenoid levels (measured in mg/g FW) and apocarotenoid aroma levels (measured in \(\mu g/g\) FW) detected in fruits.
FW) in many fruits [19,27]. However, this leaves the question open for speculation if carotenoids are the bona fide substrate for CCD's or whether these genes actually have a different role in the cell metabolism and serendipitously accept carotenoids as substrates. One could speculate that this activity was important in the melon wild progenitors, and the gene was retained without any apparent function during domestication.

3. Silent metabolism in the biosynthesis of volatile ethers

The scent of roses is a very complex trait that is a result of the presence of dozens of aroma chemicals that have distinct biosynthetic origins; among those are the mono- and sesquiterpenes, phenylalanine derived compounds, fatty acid derivatives and phenolic methyl ether compounds. The modern hybrid tea rose cultivars emit phenolic methyl ethers, a group compounds that are only present in the Chinese ancestral species and not in the European ancestral species [34,35]. The last steps in the formation of these compounds are the sequential methylation of orcinol (3,5-dihydroxytoluene) and 3-hydroxy, 5-methoxytoluene to give rise to orcinol dimethyl ether (3,5-dimethoxytoluene). The reactions are catalyzed by O-methyltransferases (OMT's) that utilize S-adenosyl methionine as a methyl donor and an alcoholic substrate as an acceptor. Hybrid roses possess two types of orcinol methyltransferase genes with similar but not identical substrate preferences OOMT1 and OOMT2 [34,36]. Both gene products perform both of the methylations involved, but OOMT1 is much more active towards orcinol than towards 3-hydroxy, 5-methoxytoluene. Interestingly, OOMT2-like genes seem to be present in all rose types, while OOMT1 types are only present in Chinese types and their progenies [35]. Still, only those roses that possess OOMT1 emit orcinol dimethyl ether. European roses, that do not emit orcinol dimethyl ether, still express OOMT2 and possess enzymatic capability in vitro to produce orcinol methyl ether from orcinol, indicating that another biosynthetic step must be preventing orcinol dimethyl ether accumulation. Thus, if OOMT2 is not operational in European roses, why is it active in cell-free extracts? It could therefore be that the biological function of OOMT2 is not related to orcinol dimethyl ether formation, providing another example of occult metabolic capabilities driven by “silent” metabolism.

Chemical diversity with respect to volatile monoterpenes and phenylpropane contents has been documented and rationalized in sweet basil (Ocimum basilicum), using genomic and metabolic tools [12,16,37]. A sweet basil type that contains predominantly the phenylpropane derivative estragole, possess OMT activities able to methylate chavicol to estragole, while a type of basil that accumulates only methyl eugenol (a 3 methoxylated estragole derivative) possesses an activity able to methylate eugenol to methyl eugenol (Fig. 3). However, if chavicol is offered as a substrate it will be methylated to estragole in cell-free extracts, although the plant itself does not contain estragole. Moreover, some of the lines able to O-methylate chavicol in vitro, do not accumulate estragole in vivo, and some of the lines are able to O-methylate isoeugenol in vitro to generate methylisoeugenol, a compound that has not been detected in sweet basil. Interestingly, the “lemon-scented” basil line 197 contains citral (a mixture of the monoterpene aldehydes geranial and neral) but lacks volatile phenylpropanes in its essential oil (Fig. 3). Still, cell-free extracts derived from this lemon-scented line can readily O-methylate chavicol to estragole, eugenol to methyl eugenol or isoeugenol to methyl-isoeugenol (Fig. 3) although none of these compounds are present in the plant. This further indicates the presence of “silent” O-methyltransferase activities in basil lines that may readily emit orcinol dimethyl ether, still express OOMT2 and possess enzymatic capability in vitro to produce orcinol methyl ether from orcinol, indicating that another biosynthetic step must be preventing orcinol dimethyl ether accumulation. Thus, if OOMT2 is not operational in European roses, why is it active in cell-free extracts? It could therefore be that the biological function of OOMT2 is not related to orcinol dimethyl ether formation, providing another example of occult metabolic capabilities driven by “silent” metabolism.

Fig. 3. Silent metabolism in phenylpropane metabolism in basil (Ocimum basilicum). Essential oil compositions (left panels) were compared to phenylpropane O-methyltransferase activities in basil lines that may readily emit orcinol dimethyl ether, still express OOMT2 and possess enzymatic capability in vitro to produce orcinol methyl ether from orcinol, indicating that another biosynthetic step must be preventing orcinol dimethyl ether accumulation. Thus, if OOMT2 is not operational in European roses, why is it active in cell-free extracts? It could therefore be that the biological function of OOMT2 is not related to orcinol dimethyl ether formation, providing another example of occult metabolic capabilities driven by “silent” metabolism.

accept novel substrates if the ability to produce those substrates is acquired by breeding or mutation, easily yielding to novel chemotypes.

Two types of genes coding for phenylpropene specific OMT’s are found in sweet basil. Both gene product types O-methylate phenylpropene alcohols at the 4 position. CVOMT1 readily accepts chavicol to generate methylchavicol (estragole), while EOMT1 accepts eugenol to generate methyleugenol, but still retains the ability to methylate chavicol to estragole at a significant rate. A single T-to-C mutation, causing a phenylalanine residue at position 260 changed to a serine controls this substrate specificity [12]. Nevertheless, plants possessing EOMT activity still have the “silent” ability to O-methylate chavicol to estragole if chavicol is offered as a substrate. Breeders can predict that if the trait to generate the substrate chavicol was introduced by crosses or metabolic engineering the resulting progenies will contain estragole by the action of silent metabolism.

4. Silent metabolism in the phenylpropanoid pathways

The flavonoid phenylpropanoid biosynthetic pathways of plants have been studied from many angles due to the importance, ubiquity, and the visibility of the products.

4.1. Flavonoid biosynthetic pathways provide numerous examples of silent metabolism

Phenotypes that are controlled by flavonoid pigments are powerful tools for genetic investigations because they can easily be measured. The coloration of flowers, seeds, and other plant organs captures our attention, even if one is not a biochemist, geneticist, or plant biologist. It also seems that flavonoid pathways determine traits that are subject to heavy selection pressures; at least this is clearly so for domesticated crop and ornamental plants. The flavonoid pathway provides a rich source of examples to illustrate silent metabolism because it is such a large and well-studied pathway that has evolved a variety of functions that serve plants. With regard to seed crops, for most species the selection has been for lack of color. Wild wheat, rice, barley, soybean and many other crops have colored seed while most domesticated strains lack color.

4.2. Silent flavonoid metabolism in soybean seed coats

Seed coat pigmentation in soybean is determined by the accumulation of anthocyanin and proanthocyanidin molecules in the palisade cells of epidermis [38]. Nearly all commonly grown soybean cultivars are yellow-seeded but may vary with regard to pigmentation of the hilum (point of connection of the seed to the ovary wall). Conversely, wild-type soybeans are deeply pigmented and black-seeded [Fig. 4]. Over the years, through genetic crossing and inheritance studies, a bewildering array of seed coat pigmentation phenotypes have been uncovered and described [39]. There are many genes that can influence seed coat pigmentation, but three major ones, I, R, and T, have the greatest effects [40]. Among these, the I locus is the most important. This gene controls the expression and distribution of chalcone synthase in the seed coat tissues, and thus it effectively operates as a bottleneck that can restrict entry of substrate into various branches of the flavonoid pathway [41]. The I locus exerts powerful epistatic effects that can mask phenotypes conditioned by R and T, because these other genes operate in pathways that are downstream from chalcone synthase. The I locus is also tissue specific, only altering flavonoid metabolism in the seed coat. This is likely because flavonoids play essential roles in other plant tissues, such as in reproductive tissues where they are essential for fertility.

Even though most commonly grown soybeans are yellow-seeded due to a lack of chalcone synthase in the seed coat tissues, genes corresponding to steps in the downstream pathways continue to be expressed at high levels. For example, dihydro-flavonol-4-reductase (DFR) and flavonoid 3’ hydroxylase (F3’H) transcripts are highly expressed in seed coats where they could have participated in the biosynthesis of anthocyanin pigments during the late stages of seed development. Moreover, these genes are also expressed in yellow-seeded phenotypes that do not accumulate any pigments [42,43]. The isoflavonoid branch also appears to be constitutively active in seed coats, regardless of chalcone synthase levels, as evidenced by the expression of isoflavone synthase [44]. But in this case, isoflavonoid products can be detected in seed coat tissues, whereas anthocyanins clearly are not present. How does this occur? There are several possibilities, but it seems most likely that the isoflavonoids present in developing seed coats were synthesized in other plant organs and simply transported to the seed coat. In any case, it is apparent that the seed coat is programmed to make anthocyanins, isoflavonoids, and other phenylpropanoids that are dependent upon the key entry point enzyme, CHS, regardless of whether there is a sufficient amount of this enzyme to ensure flux through the pathway.
4.3. Soybean seed coat anthocyanin pigments are substrates for the seed coat peroxidase enzyme

Another example of silent metabolism in soybean seed coats that is apparently linked to anthocyanin pigmentation relates to the accumulation of peroxidase. The soybean peroxidase is a class III plant peroxidase and is the most abundant protein in mature seed coats, where it accounts for approximately 5% of the total soluble protein [45]. The occurrence of soybean peroxidase in the seed coat is controlled by a single dominant gene, Ep. Homozygous recessive epep genotypes do not accumulate soybean peroxidase due to a deletion mutation in the structural gene encoding the enzyme [46]. Despite this difference in the amount of soybean peroxidase enzyme in Ep- versus ep ep genotypes, there is no known phenotypic effect such as change in seed color, permeability, lignification, longevity, or hardness that can be associated with the Ep locus. Thus, the function of SBP in the seed coat remains enigmatic. Perhaps this is because its role is dependent on the presence of anthocyanin pigments that are absent from virtually all modern soybean cultivars.

The participation of anthocyanin pigments and other flavonoid molecules in oxidative reactions is gaining more attention, since this may constitute a significant portion of their functionality. With regards to soybean seed coats, wild-type (black) soybeans accumulate large amounts of anthocyanin pigments and soybean peroxidase in separate but adjacent cell layers of the seed coat. The anthocyanins are concentrated in the palisade cells of the epidermis of the seed coat, where soybean peroxidase accumulates within the hourglass cells of the subepidermis [45,47]. The vacuolar targeting signal of the soybean peroxidase is somewhat unusual among class III plant peroxidases, as most other enzymes of this type are secreted into the apoplast. Nonetheless, maceration of the seed or imbibition of water simultaneously releases these two soluble seed coat constituents. In fact, seed coat anthocyanin pigments are substrates for soybean peroxidase-catalyzed reactions, as shown in Fig. 5. The polymerization of seed coat anthocyanin pigments by soybean peroxidase into insoluble products may provide defense or protection for the seed. It is reminiscent of binary systems where enzymes and substrates are spatially separated in different cell types or layers, such as glucosinolates and cyanogenic glycosides [48]. Thus, it is plausible that function of SBP is related to its activity towards anthocyanin pigments, and that this has become obscured as a consequence of domestication and selection of yellow-seeded types.

5. Mechanism of action of silent metabolism

5.1. Silent metabolism and relaxed selection

It is instructive to consider the idea of silent metabolism together with the well-known genetic concepts of epistasis and relaxed selection, as well as the related topics of sub- and neo-functionalization of gene products. In cases where genes interact to produce particular phenotypes, epistatic genes may either hide or reveal the phenotypic expression of other genes. This differs from dominant and recessive interactions because the genes are not allelic. For example, epistatic interactions are commonly observed among genes that encode enzymes within a linear biochemical pathway. The catalytic efficiency, substrate specificity, or other characteristics of a particular enzyme in the pathway can alter the final products or the metabolic flux through the entire path. A mutation in a gene that results in a non-functional enzyme would effectively block the pathway at that step, possibly leading to accumulation of substrate upstream and a depletion of substrate downstream. For individuals that carry such an epistatic mutation, the enzymes that operate downstream are biochemical orphans and become part of the silent metabolism of the plant cell. Thus, epistatic genetic effects may provide a context for silent metabolism that is otherwise cryptic or puzzling.

Epistatic genetic effects that become fixed in a population may result in the degeneration of a biochemical pathway [49] or supply opportunities for enzymes that have become biochemical orphans to evolve new capabilities. Genetically, this is considered a form of relaxed selection, a reduction or removal of selective pressure that operates on a gene. Gene duplication is another genetic mechanism that results in relaxed selection and offers similar prospects. The sub-functionalization of an enzyme, so that it acquires characteristics that are somewhat different from its ancestral type, is an adaptation that is facilitated by removing any conservative selective pressure. Likewise, neo-functionalization is the acquisition of a completely new property that is distinct from its ancestral type. This occurs more rarely but potentially offers the organism novel traits that may drastically alter its fitness and success. Therefore, the silent metabolism of a plant cell represents a biochemical manifestation of the genetic notion called relaxed selection.

The impact of gene duplication on primary and secondary metabolic pathways has been estimated using the model plant Arabidopsis [50]. The relatively high levels of intraspecific variation observed in pathways of secondary metabolism contrast with the general conservation of function in primary metabolism. The flexibility of secondary metabolism is supposed to be enabled by a greater genetic redundancy of these pathways compared to primary metabolic routes [50]. It is a compelling hypothesis that plant populations maintain a diverse reservoir of secondary metabolic capability to meet the ever changing selective pressures in their environment.

6. Concluding remarks

It has become evident that the initial Darwinian view of biological systems that have been optimized by evolution is much more complex than we initially thought. The large phytochemical diversity that we find in nature is a result of the different selection pressures that plants have successfully coped with during evolution. The ubiquity of plant silent metabolism may be a great advantage in a changing and evolving world. It is likely that plants have evolved means of preserving the biochemical information to...
generate chemicals without actually generating them. Moreover, the availability of plant complete genomes and the advancement in our understanding of gene evolution, has led to the discovery that plants that do not normally accumulate a certain natural product still possess homologous genes to those present in plants that actively synthesize them [51].

It has long been known by geneticists that through hybridization it is possible to augment the generation of qualitative and quantitative variation in secondary chemistry. Qualitatively, hybrids may express all of the secondary chemicals of the parental taxa, may fail to express certain parental chemicals, or may express novel chemicals that are absent in each parent. Quantitatively, concentrations of parental chemicals may vary markedly among hybrids. This phenomenon has been termed “additive inheritance” [52], and it might be a reflection or operate in a similar or related way as the phenomenon long known as “heterosis” or “hybrid vigor”. In essence, each parent may contribute previously silent but complementary enzymes to generate complementary networks, and thus the hybrid’s phytochemistry might be very different to that of their parents. Recently, a combined metabolomic–genomic set of tools have been employed to rationalize flavonoid biosynthesis in poplar and has provided interesting insights about this phenomenon [53].

Most of the biochemical, molecular and genetic studies on secondary metabolism have been conducted using domesticated plants. All cultivated crops originated from wild relatives and many of the original traits present in the progenitors have been lost through crop domestication [54]. Cultivated plants have been subjected to directed hybridization and selected taking into account commercially important or practical traits. Thus, breeding and crop improvement has allowed for a limited set of important traits to prevail and be overrepresented in our modern crops. The number of generations in which selection pressure has been attained is very small as compared to the number of generations that wild plants have experienced selection pressures throughout natural evolution. Silent metabolism could be a relic of important and valuable traits or functions that the ancestral wild plants acquired and preserved during their evolution, but their cultivated offspring have lost. If the expression of a silent partial pathway or biochemical orphan do not have harmful effects and do not significantly affect the overall fitness of the plant, only very little selection pressure has been applied towards eliminating those traits, and therefore they will be conserved during the domestication process.

Another possibility of the widespread occurrence of silent metabolism is that many of the enzymes we detect as “silent” do have an important yet unknown metabolic function. Due to the broad substrate specificity displayed by many of these enzymes, novel substrates can be readily utilized by serendipitously recruited enzymes and novel phenotypes can be generated that are subjected to natural or human selection. Thus, if the new traits generated provide an evolutionary value, they will be retained. Increasing and retaining phytochemical diversity is key to the survival and adaptation of plants, and “silent metabolism” might be one of the key factors by which plants cost-effectively retain their flexibility to defend themselves and communicate with other organisms. In this context, silent metabolism is not at all silenced, but is a loud reservoir of metabolic pathways that can easily be activated, conferring fitness benefits to the bearing organism. Silent metabolism constitutes an evolutionary asset and thus widespread the plant kingdom, contributing to the impressive phytochemical diversity that we presently experience.

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References


