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Standard Review

Plant terpenoids: applications and future potentials

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The importance of terpenes in both nature and human application is difficult to overstate. Basic knowledge of terpene and isoprene biosynthesis and chemistry has accelerated the pace at which scientists have come to understand many plant biochemical and metabolic processes. The abundance and diversity of terpene compounds in nature can have ecosystem-wide influences. Although terpenes have permeated human civilization since the Egyptians, terpene synthesis pathways are only now being understood in great detail. The use of bioinformatics and molecular databases has largely contributed to analyzing exactly how and when terpenes are synthesized. Additionally, terpene synthesis is beginning to be understood in respect to the various stages of plant development. Much of this knowledge has been contributed by the plant model, *Arabidopsis thaliana*. Considering the advances in plant terpene knowledge and potential uses, it is conceivable that they may soon be used in agrobiotechnology.

Key words: Terpenes, terpene synthase, secondary metabolites, transgenic plants

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INTRODUCTION

Plants produce primary and secondary metabolites which encompass a wide array of functions (Croteau et al., 2000). Primary metabolites, which include amino acids, simple sugars, nucleic acids, and lipids, are compounds that are necessary for cellular processes. Secondary metabolites include compounds produced in response to stress, such as the case when acting as a deterrent against herbivores (Keeling, 2006). Plants can manufacture many different types of secondary metabolites, which have been subsequently exploited by humans for their beneficial role in a diverse array of applications (Balandrin et al., 1985). Often, plant secondary metabolites may be referred to as plant natural products, in which case they illicit effects on other organisms. Although this review focuses on plant terpenes, it should be realized

that other organisms are able to synthesize terpenes. For example, the endophytic fungus isolated from St. John's Wort (*Hypericum perforatum*) was recently shown to produce hypericin and emodin, two types of terpene lactones (Kusari et al., 2008). There are three broad categories of plant secondary metabolites as natural products; terpenes and terpenoids (~25,000 types), alkaloids (~12,000 types), and phenolic compounds (~8,000 types) (Croteau et al., 2000).

Terpene chemistry and biosynthesis

Ever since techniques such as low-temperature chromatography, were used to separate plant terpenes nearly a half of a century ago (Clements, 1958), great strides have been made to explore molecular details of terpenes. For instance, subjecting plant vegetation to pyrolysis techniques and gas chromatography has allowed for identifi-

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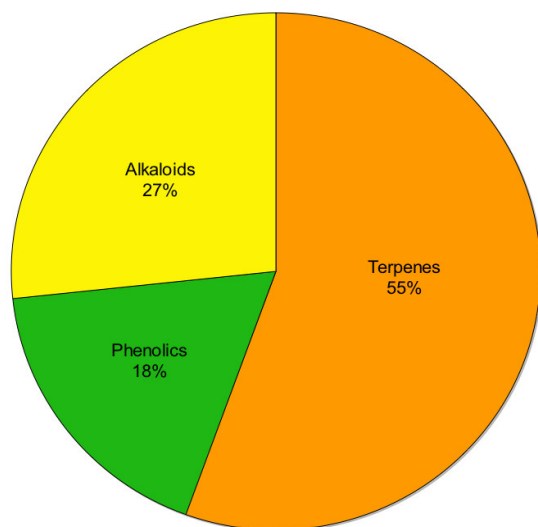


Figure 1. Pie chart representing the major groups of plant secondary metabolites according to Croteau et al. (2000). Based on their numbers and diversity, terpenes offer much potential in an array of industrial and medicinal applications.

cation of different volatile organic compounds (VOCs) (Greenberg et al., 2006). Some extraction techniques have relied on using ultra pure water, also dubbed sub-critical water. Although this method works relatively well, it has been pointed out that increasing the temperature of the water decreases the stability of the terpenes (Yang et al., 2007). Lai et al. (2005) performed a crude extraction of terpenes from the leaves of *Ginkgo biloba* by refluxing the leaves in ethanol and then dissolving the extracts in water. They subsequently isolated terpenes with either column chromatography or a liquid-liquid extraction using ethyl acetate as a solvent.

Ma et al. (2007) used two-dimensional gas chromatography time-of-flight mass spectrometry (GC x GC-TOF MS) to analyze volatile oils in the leaves and flowers of *Artemisia annua*. The authors concluded that the number of components was close to 700 and the majority was terpenes. In a comparative analysis they used the same extraction techniques but instead of GC x GC-TOF MS they used GC-MS, which resulted in a much lower sensitivity for molecular diversity. This illustrates the fact that newer isolation and identification methods are helping with terpene analysis.

It has long been known that the basic unit of most secondary plant metabolites, including terpenes, consists of isoprene, a simple hydrocarbon molecule. The term terpene usually refers to a hydrocarbon molecule while terpenoid refers to a terpene that has been modified, such as by the addition of oxygen. Isoprenoids are, therefore, the building blocks of other metabolites such as plant hormones, sterols, carotenoids, rubber, the phytol tail of chlorophyll, and turpentine.

Terpenes are the most numerous and structurally diverse plant natural products (Figure 1). For this reason, a system of nomenclature has been established. The nomenclature of terpene compounds is ostensibly complex, yet can be quickly elucidated upon closer examination. The isoprene unit, which can build upon itself in various ways, is a five-carbon molecule. The single isoprene unit, therefore, represents the most basic class of terpenes, the hemiterpenes. An isoprene unit bonded with a second isoprene is the defining characteristic of terpene, which is also a monoterpene (C_{10}). While sesquiterpenes contain three isoprene units (C_{15}), diterpenes (C_{20}) and triterpenes (C_{30}) contain two and three terpene units, respectively. Tetraterpenes consist of four terpene units and polyterpenes are those terpenes containing more than four terpene units (i.e., more than eight isoprene units).

McGarvey and Croteau (1995) reviewed terpene biosynthesis and suggested that a more detailed study of the terpene synthases were needed and that this in turn would increase the role of terpenes in, perhaps, commercial uses such as flavor enhancers. Since their publication more than a decade ago, terpene biosynthesis enzymes have been studied in detail. For instance, Greenhagen et al. (2006) used mapping strategies to determine the variance and composition of amino acids within terpene synthase active sites. This is, arguably, very useful in determining the evolutionary divergence of the terpene synthases and elucidating relationships among plants. Trapp and Croteau (2001) reviewed the genomic organization of terpene synthase genes across different species. They suggest that terpene synthase genes may impact phylogenetic organization of some plants. For example, some terpene genes are more closely related in certain plant species, in which the species themselves were previously thought to be distantly related.

In a more recent review of terpene synthase genes, Zwenger and Basu (2007) performed *in silico* analysis of publicly available microarray data using Genevestigator software (Zimmerman et al., 2004). Such software allows for assaying an organism of choice, in this case, *Arabidopsis thaliana*. In their study, more than 2,500 microarrays were simultaneously compared for expression of terpene synthase genes. Multiple biotic and abiotic factors, which may or may not induce expression, were also considered in respect to terpene synthase gene expression. Possibly even more important is the fact that expression of terpene synthases were examined across the life cycle of *Arabidopsis*, which countered some wet lab experimental data previously published. In addition to expanding the comprehension of terpene synthase genes across the life cycle of *Arabidopsis*, the authors determined that five terpene synthase genes, which appeared in many microarray analyses, were lacking in experimental studies. As discussed by the authors, further experiments may lead to better understanding the roles of

previously uncharacterized genes.

Terpenes in nature

The distribution of terpenes in nature has been studied extensively. Indeed, the distribution of terpenes within species has received attention. To better understand terpene and other volatile organic compound emissions, from loblolly pine (*Pinus taeda*), Thompson et al. (2006) analyzed tree core samples. They found the highest concentrations of terpenes in heartwood, lowest in outer sapwood, and moderate levels in the inner sapwood. In a less invasive study by Martin et al. (2003) methyl jasmonate was applied onto foliage of Norway spruce (*Picea abies*) trees which led to a two fold increase of terpenes within the needles. In another investigation, the amounts of different terpenes in Scots pine (*Pinus sylvestris*) needles varied across Finnish and Turkish regions, showing the diversity of terpene distribution can vary within a species (Semiz et al., 2007).

Although more commonly associated with coniferous species, terpenes have been detected in other plant phyla, including angiosperms. Aside from terpenes manufactured by plants in response to herbivory or stress factors, it has also been shown that flowers can emit terpenoids to attract pollinating insects (Maimone and Baran 2007). Interestingly, terpenoids have also been shown to attract beneficial mites, which feed on the herbivorous insects (Kappers et al., 2005). Terpene emissions and subsequent attracting mechanisms have been shown to play an indirect role in plant defense mechanisms in other studies as well. Kessler and Baldwin (2001) have shown that herbivorous insects can induce terpene release from a plant, and also cause the plant to release signals which attracts predatory species. These experiments provide not only powerful evidence for the role of terpenes for plant defense, but also give an exemplary model for co-evolution between plants, mites, and insects. Chen et al. (2003) have shown that many different volatiles, including terpenes, may be emitted from flowers of *Arabidopsis*. They propose that the role as insect attractants of at least some emitted terpenes seems inconclusive, but still strongly suggest they might play a role in reproduction.

Of course, other studies have extended the understanding of plant terpenes and insects. Johnson et al. (2007) examined fragrance mixtures including terpenes and found scent chemistry of the emitted fragrance played a role in beetles and wasps pollinating an orchid species (*Satyrion microrrhynchum*). After performing GC-MS to identify fragrance compounds, they manipulated antennae to determine electrophysiological responses. Molecules which elicited effects included monoterpenes and sesquiterpenes. While the beetles were generalists in pollination, the wasps were more specific. However, Urzúa et al. (2007) studied terpenoids from an Asteraceae (*Haplopappus berterii*) and suggested little or no

correlation between fragrance molecules and insect preference.

Ecological roles of terpenes extend beyond plant-insect coevolution. Cheng et al. (2007) discuss ecological impacts of terpenes. These include their roles above and below ground in attracting predatory species upon herbivory attack. Additionally, they point out terpenes may act as chemical messengers which influence the expression of genes involved in plant defense mechanisms or even influence gene expression of neighboring plants.

Terpenes have been studied with great interest, due to their roles in the earth's atmosphere. It has been estimated that the annual global emission of isoprenes is 500 teragrams (Guenther et al., 2006). Therefore, it is tempting to speculate on their interactions with solar radiation. Due to the abundance of citrus plantations in the mediterranean area, Thunis and Cuvelier (2000) helped identify the influence and composition of VOCs on ozone formation in this region and found some of the biogenic VOCs included α -pinene and *d*-limonene. In a study by VanReken et al. (2006) a biogenic emissions chamber was used to measure terpenoids released from Holm oak (*Quercus ilex*), loblolly pine and a dilute mixture of α -pinene. They suggested a large majority of emissions are chemically oxidized or otherwise transformed into different aerosol compounds. Llusia and Penñuelas (2000) have examined stomatal conductance to better understand how plants interact with abiotic atmospheric conditions such as temperature, water availability, and irradiance to alter the diffusive resistance of terpenes from plant leaves. They also describe the seasonal fluctuation of terpenes.

Since many plants contribute to the earth's atmospheric composition by releasing volatile organic compounds, which include terpenes, they should arguably be studied more extensively. Future research may therefore help pave the road to understanding the global influence of terpenes.

Society and terpenes

There have been many applications of terpenes in human societies. Pharmaceutical and food industries have exploited them for their potentials and effectiveness as medicines and flavor enhancers. Perhaps the most widely known terpene is rubber, which has been used extensively by humans. Rubber is a polyterpene, composed of repeating subunits of isoprene. The addition of sulfur to rubber by Charles Goodyear led to vulcanized rubber, which yields various degrees of pliability depending on the mixture ratio (Stiehler and Wakelin, 1947). Other important terpenes include camphor, menthol, pyrethrins (insecticides), cleaners, antiallergenic agents, and solvents. Rosin (a diterpene), limonene, carvone, nepetalactone (in catnip), hecogenin (a detergent), and digitoxigenin are also important terpenes (Croteau et al., 2000).

Agriculture has also shown an increasing interest in

terpenes. In a study by Villalba et al. (2006) sheep were suggested to have increased tolerance for terpene consumption if they consumed more grains. They also showed terpenes can influence ungulate herbivory on other plants. This may help agronomists balance diets of ruminants if they consume plants such as sagebrush (*Artemisia* sp.). Terpenes have also shown antimicrobial activities (Islam et al., 2003). This is important due to the increase in antibiotic resistant bacteria, which is occurring globally and at an alarming rate. Addition of terpenes into livestock feed may replace conventional antibiotic addition, which in turn would slow the rate of antibiotic resistance in bacteria.

The effect of some terpenes on microorganisms has been seriously studied since at least the 1980's (Andrews et al., 1980). Plant oils, which contain terpenes, have shown increasing promise *in vivo*, inhibiting multiple species of bacteria. For example, cinnamon oil has shown broad-spectrum activity against *Pseudomonas aeruginosa* (Prabuseenivasan et al., 2006). The various compositions of terpenes can be markedly different from one species to another. For example, John et al. (2007) found plant oils from *Neolitsea foliosa*, which also showed some antibacterial properties, included sesquiterpenes such as β -caryophyllene but lacked monoterpenes.

Other microbes have also shown inhibition by terpenes. Murata et al. (2008) extracted numerous compounds from stem bark of the cape ash (*Ekebergia capensis*) growing in Kenya. Ten of these were triterpenes, whose structures were determined using spectroscopic analysis such as NMR (nuclear magnetic resonance). Determining the precise molecular activities of these triterpenes may be an important step towards finding newer and more effective drugs against *Plasmodium falciparum*, the causative agent of malaria. Susceptibility to terpenes has been tested by Morales et al. (2003), in which extracts from *Artemisia copa* showed inhibitory effects against yeast (*Candida albicans*). They also showed that some plant extracts containing terpenes tested showed biotoxicity effects against brine shrimp (*Artemia salina*).

Cumene (isopropylbenzene) is a terpene that has been used in bioremediation studies. In an experiment carried out by Suttinun et al. (2004), bacteria used in bioremediation of trichloroethylene (TCE) showed an increased capability to uptake TCE in the presence of cumene. In their study, 75% of the TCE present was successfully metabolized, allowing for a more robust degradation and bioremediation. Additional terpenes included in the study were limonene, carvone, and pinene. However, cumene showed the most beneficial effects. Without the knowledge and application of cumene, such success in bioremediation studies might not have been possible.

Because terpenes have been incorporated into much antibacterial soaps, cosmetics and household products, descriptive studies have been published on absorption and penetration into skin. Due to their properties of lipid organization disruption, Cal et al., (2006) studied the ab-

sorption kinetics of four cyclic terpenes; α -pinene, β -pinene, eucalyptol, and terpinen-4-ol. Each terpene varied in accumulation and elimination time with terpinen-4-ol showing the fastest penetration. Matura et al. (2005) investigated the role some terpenes play as causative agents of contact dermatitis and fragrance allergies. Out of approximately 1500 patients tests, just over 1% had reactions to oxidized linalool.

To better understand the vast array of terpenes, genetically modified organisms have been used. For example, the biosynthesis of terpenes has been studied in transformed *E. coli* (Adam et al., 2002). As described by Adam et al. (2002), modification of organisms is important to help understand the various pathways of terpene synthesis for the purpose of producing antimicrobial and antiparasitic drugs (Goulart et al., 2004).

Transgenic plants and future research

Plant tissue culture is an *in vitro* technique that allows clonal propagation of transformed clones. A review of tissue culture methods and applications by Vanisree et al. (2004) discusses the importance of inserting genes for plant secondary metabolites, including taxol (a diterpene alkaloid), a well-known anticancer agent. They point out that *in vitro* cell culture methods provides systematic advantages such as the ability to manipulate plant environment, control of cell growth, and regulation and extraction of metabolic products.

In contrast to producing terpenes in the laboratory, others have suggested extending the methods to create transgenic crops for terpene synthesis and production. Genetic modification of *Arabidopsis* has been performed to study the production of different terpenoids by up-regulating terpene synthase genes (Aharoni et al., 2003), which has led to an increase in understanding of how terpenes might function. For example, it has been shown that genetic engineering of *Arabidopsis* plants has allowed for an increase in pest resistance (Kappers et al., 2005). Others who have shown terpenes to influence insect behavior have suggested the use of terpene expression as a possible control mechanism for aphid infestations (Harmel et al., 2007). A study by Lweinsohn et al. (2001) determined that genetically modified tomatoes could be produced, which had enhanced levels of linalool and thus enhanced flavor and aroma. Degenhardt et al. (2003) discuss how monoterpenes and sesquiterpenes are the two most common terpenes emitted from plants post-herbivory. They suggest that finding the proper mixture and timing of terpene release from crop plants is key to creating an adequate transgenic plant. Additionally, the properties of terpene emission should be tightly regulated by an herbivore-responsive promoter.

Genetic transformation of tobacco (*Nicotiana tabacum*) was carried out by Lückert et al. (2004) (Table 1). After inserting monoterpene synthase genes the plants showed

Table 1. Partial representation of organisms which have been genetically transformed with at least one terpene synthase gene

Species	Organism	Citation
<i>Escherichia coli</i>	bacteria	Adam et al., 2002
<i>Candida albicans</i>	yeast	Jackson et al., 2006
<i>Arabidopsis thaliana</i>	thale cress	Aharoni et al., 2003
<i>Lycopersicon esculentum</i>	tomato	Lweinsohn et al., 2001
<i>Nicotiana tobacum</i>	tobacco	Lücker et al., 2004
<i>Lactuca sativa</i>	lettuce	Wook et al., 2005
<i>Mentha piperita</i>	mint	Wildung et al., 2005

an increase of terpene emission from leaves. To better understand terpene biosynthetic pathways Pateraki et al. (2007) isolated multiple cDNAs from *Cistus creticus*. They used polymerase chain reaction (PCR) techniques to amplify sequences from the plant and found additional terpene synthase genes after searching expressed sequence tag (EST) libraries. Expression of genes is at least partly dependent on their promoters. Davidovich-Rikanati et al. (2007) used a ripening-specific promoter to modify the aroma and flavor of tomatoes. Unfortunately, although levels of flavor-causing monoterpenes increased, the lycopene decreased.

The medicinal value of terpenes has not been ignored. Canter et al. (2005) discuss some areas within biotechnology to improve medicinal plant cultivation. These include incorporating agronomic traits into medicinal plants, pathway engineering and exploring additional transformation systems. In a more recent examination, Tyo et al. (2007) describe methods for studying and engineering cells such as using 'omics' technologies, screening libraries, and synthetic and computational systems biology. As they have mentioned, some of these technologies are currently being used to extend comprehension of metabolic pathways in plants. In a study by Yao et al. (2008) bioinformatics helped characterize a terpene synthase pathway after comparative analysis of isolated cDNA from a cultured callus line of an endangered medicinal plant (*Camptotheca acuminata*) native to China. Similar to many other bioinformatic-based approaches, they benefited from NCBI's (National Center for Biotechnology Information) BLAST (Basic Local Alignment Search Tool), which can help understand phylogenetic relationships among nucleotide sequences (Altschul et al., 1990).

Melvin Calvin, the Nobel laureate known for his contribution to the scientific understanding of the carbon fixation pathways in plant chloroplasts, studied the tropical copiba (*Copaifera langsdorfii*) for its natural biofuel production (Calvin, 1980). Although it has not yet been examined, diesel from *C. langsdorfii* is largely composed of terpenes. The current interest in biofuels is not only in the United States but also other countries such as Brazil and the European Union. This has sparked new interest in finding renewable sources, or plants which may contribute to biofuels. Considering this global interest in

biofuels, research describing the up-regulation of terpene synthase genes in *C. langsdorfii* may prove very beneficial. This research would be very useful, for instance, in providing a more cost effective extraction and by-pass typical conversion of biomass (corn ethanol) to more contemporary biofuels (Demirbas and Balat, 2006). Therefore, future studies may include terpene production in the diesel tree or related biofuel plants.

Conclusions

Many terpenes remain to be discovered so they will undoubtedly intrigue scientists for years, as their applications are only beginning to be fully realized. Arguably, society has benefited tremendously from terpenes. In addition, understanding the function of genes in terpene production could lead to discovering novel compounds or pathways, which might reveal new important aspects for many human applications. For instance, the ability to up-regulate terpene synthesis in *C. langsdorfii* could result in an increase in the diesel-like resin harvested from this tree, might prove beneficial to the global market of biofuels. As we continue into the agrobiotechnology age, it is highly likely the applications and potentials of terpenes will be further explored.

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