REVIEW

Volatile organic compounds: plant natural defense mechanisms against herbivorous arthropods and an opportunity for plant breeding of cotton

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Abstract

Plants' natural defense mechanisms against herbivorous arthropods include the emission of volatile organic compounds (VOC). Nowadays field observations about plant-insect interactions are better understood thanks to the increasingly scientific investigations over recent decades. There are now more precise data about molecules, action modes and physiological and genetic bases of these plant defense mechanisms. VOC present an important potential for crop protection and pesticide use reduction. In the present review, we focus on the latest research advances about plant protection provided by VOC, considering experimental methods of volatile analysis and the involved genes toward genetic improvement of natural defense in the future varieties, particularly for Upland cotton *Gossypium hirsutum*.

Keywords: stress; insect; terpene; secondary metabolites; *Gossypium hirsutum*.

Introduction

Plants can produce a high diversity of volatile organic compounds (VOC). The emission of these secondary metabolites can be strongly increased as a result of certain biotic or abiotic stresses (Dicke and Baldwin, 2010; Dong *et al.*, 2016; Schoonhoven *et al.*, 2005). Several VOC are emitted as a natural defense mechanism (NDM) against the attack of arthropods and pathogens (Dicke and Baldwin, 2010; Rodriguez-Saona and Frost, 2010; War *et al.*, 2012). VOC play an important role in communication between plants, as well as with animals (Dicke and Baldwin, 2010). Particularly, many evidences show that the attack of herbivore arthropods induces the emission of VOC with repellent effect on other arthropods (Krempl *et al.*, 2016; Zhou *et al.*, 2013), or with attractive effect for predators of herbivorous arthropods (carnivores, egg and larvae consumers)

(Turlings *et al.*, 1990; D'Alessandro and Turlings, 2005; Erb *et al.*, 2009). Researchers have also noted that protection against herbivore attack extends to neighboring plants from which they were initially attacked (Arimura *et al.*, 2002; Kost and Heil, 2006).

In some regions of the world, despite of attempts to combat insect pests by pesticides, they are still considered one of the main causes of economic losses in crop production (Oliveira *et al.*, 2014). Several scientific research efforts to improve pest management have been carried out. On the other hand, other relevant aspects such as global climate change, land use, biological invasions, conservation of genetic resources and food security are also considered in plant protection programs (Brévault *et al.*, 2014). Many observations confirm the use of VOC-based NDMs as a tool to combat herbivores (Alborn *et al.*, 1997; Chen, 2008; Huang *et al.*, 2015;

Köllner *et al.*, 2008; Köpke *et al.*, 2008; Robert *et al.*, 2013; War *et al.*, 2011). However, many questions remain to be solved in order to achieve a total understanding of these self-protection tools that are very peculiar to plants (Paré and Tumlinson, 1999). Regarding to its aromatic characteristics, these are determined by the profiles of each VOC, and may vary depending on each genotype (Iijima, 2014). During the last two decades several researchers have aroused their interest in the study of NDM, which has allowed several researches to be carried out at the genetic, biochemical, physiological and ecological level (Alborn *et al.*, 1997; Atiama-Nurbel *et al.*, 2015; Bernasconi *et al.*, 1998; Catola *et al.*, 2018; D'Alessandro and Turlings, 2005; Loughrin *et al.*, 1994; Schnee *et al.*, 2006; Ton *et al.*, 2007). Considering discernment of the functionality of VOC, encouraging results could be obtained to achieve a better management of pests in crops, and in effect, decrease dependence on the large quantities of insecticides used to control phytophagous insects (War *et al.*, 2012).

Upland cotton (*Gossypium hirsutum*) is a tetraploid annual crop and the most cultivated worldwide (d'Eeckenbrugge and Lacape, 2014). From planting to production, it is attacked by a large number of pests, which requires a high consumption of synthetic phytosanitary products to control herbivorous arthropod populations (Villamar-Torres *et al.*, 2016). Consequently, its production is considered to be highly dangerous for the environment and farmers (Renou *et al.*, 2012). Some evidence shows that plant breeding could provide possible answers by VOC to increase their NDM in cotton cultivation, in order to obtain new and improved varieties. This review addresses some relevant aspects of VOC, detailing certain advances generated in recent years in the different studies on these volatile molecules, due to the progress of the technologies used such as genomics and transcriptomics. The techniques used to capture of VOC, both in the field and in greenhouse experiments have advanced, which has allowed study VOC' biosynthesis, their biochemical profiles and some genes linked to their emission, in a better way. Finally, a vision regarding the future perspectives of the uses and applications of VOC as NDM in modern agriculture, particularly against the attack of herbivorous arthropods in cotton cultivation is presented.

Natural defenses: herbivores induce the synthesis of VOC

Plants' NDMs can be direct or indirect (Bruce *et al.*, 2010; Dicke and Baldwin, 2010; Loreto *et al.*, 2014; Rodriguez-Saona and Frost, 2010; War *et al.*, 2012). The former are mediated by characteristics that affect the biology of herbivores, among them the best known are appearing villi, spines, wax, trichomes and leaves with greater thickness, or in turn, the production of toxic molecules such as terpenes, alkaloids , anthocyanins, phenols and quinones (War *et al.*, 2012; 2011).
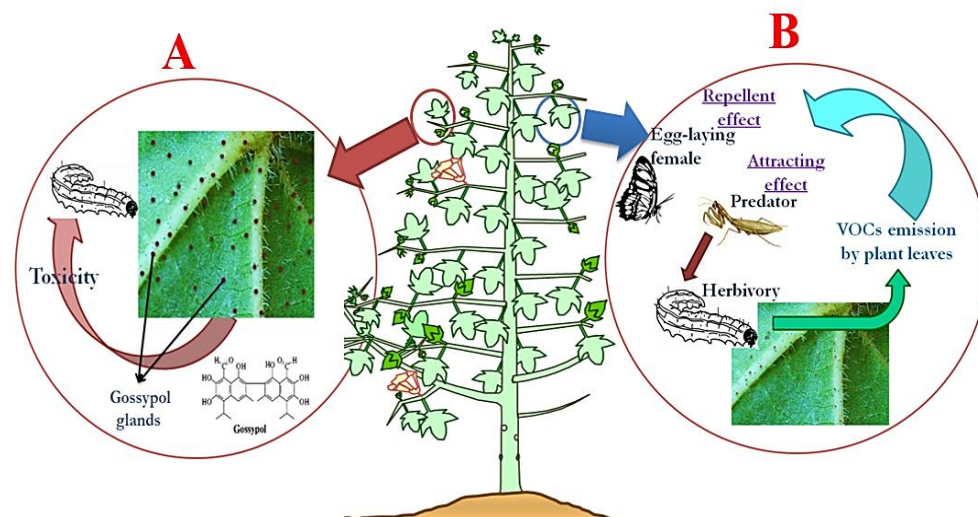

Figure 1. A) Direct mechanism of natural defense in the cotton plant: gossypol glands containing highly toxic terpenoids affecting the physiology of the herbivorous arthropods and causing mortality or retarded growth, **B)** Indirect mechanism of natural defense by means of volatile organic compounds (VOC) in the cotton plant. It modifies the behavior of certain pests and predators aiming attraction of arthropod-pest enemies and repulsion of arthropods seeking to put their eggs.

A well-known example of this system are glands emitting gossypol, a highly toxic sesquiterpenoid found in the leaves of cotton plants (*Gossypium hirsutum*), and are considered a direct NDM due to mortality or retarded growth (Câmara *et al.*, 2016; Hagenbucher *et al.*, 2017; Leghari *et al.*, 2001; Gershenzon and Dudareva, 2007; Krempl *et al.*, 2016). This system has been extensively investigated also in other crops of interest during the last decades (Figure 1A).

On the other hand, plants also possess less evident defense systems, considered as indirect NDM, which imply the emission of chemical messages that influence the behavior of certain herbivores, their predators and even other plants cohabiting with them (D'Alessandro and Turlings, 2005; Huffaker *et al.*, 2013; Villamar-Torres *et al.*, 2016) . It seems that these volatile mixtures exert on plants a function of repellency, and consequently they allow distancing female arthropods having eggs. Additionally, these signals or messages serve as an attraction for enemies of certain arthropods. As evidenced in rice, through the emission of around 30 volatiles, including MeSA (Methyl salicylate) and MeBA (Methyl benzoate), they attracted certain predators of the insect *Spodoptera frugiperda* (Bruinsma *et al.*, 2008; Kessler and Halitschke, 2007; Pinto-Zevallos *et al.*, 2018). These molecules have seductive characteristics of natural enemies and were also reported in bean, (*Phaseolus lunatus*) and Arabidopsis (Chen *et al.*, 2003; War *et al.*, 2012). Potentially, this NDM has many benefits in different aspects (Figure 1B).

Description, biosynthesis of VOC and classification of terpenes

Plant volatiles are metabolites that have low molecular weight and boiling point, approximately < 300 Da and < 260 ° C, respectively. Plants are capable of emitting VOC from some of their organs, including leaves, flowers, fruits, and roots; the latter scatter them in atmosphere and soil.

Therefore, all these characteristics allow plants to have an adequate interaction with other organisms and their environment in general (Dong *et al.*, 2016; Negre-Zakharov *et al.*, 2009). In terms of their diversity, to date, about 1700 volatile compounds from more than 90 plant families are known, belonging to both angiosperms and gymnosperms. Globally they include only 1% of all plant-specific metabolites (Dong *et al.*, 2016; Dudareva *et al.*, 2013; Knudsen *et al.*, 2006).

According to their structural chemical classification VOC are formed by alcohols, hydrocarbons, ketones, aldehydes, ethers and esters (Dong *et al.*, 2016). Based on their biosynthesis in plants, VOC have their origin from precursor primary metabolites, and are subject to the availability of carbon, nitrogen, sulfur, as well as, the energy that comes from these precursors, including but not limited to Acetyl-CoA, Pyruvate, phosphoenolpyruvate (PEP) and erithrose 4-phosphate (Ery4P). Metabolic routes including Shikimate/phenylalanine, methyl-eritritol phosphate (MEP), mevalonic acid (MVA) and lipoxygenase (LOX), are those from which the emissions of VOC such as phenylpropanoid/benzenoid, all terpenoids / terpenes, methyl jasmonate, volatile derived from green leaves, carotenoids and certain derived fatty acids are derived (Figure 2) (Dudareva *et al.*, 2013). A large amount of VOC derives from biosynthetic pathways of terpenes or terpenoids more specifically from monoterpenes, sesquiterpenes and diterpenes as the most representative VOC in plants (Table 1) (Chen et al., 2003; Dudareva et al., 2013).

Based on their biosynthesis, this could be summarized in four main phases among the vast majority of plants. The first phase consists of the synthesis of IPP (isopentenyl pyrophosphate) that comes from the isomerization in dimethylalylpyrophosphate (DMAPP). It is formed in the classic metabolic route of mevalonate (MEV) within the cytoplasm or mitochondria, although it can also to be formed, on the metabolic pathway of methyleritritol 4-phosphate (MEP) (Dudareva *et al.*, 2013; Rohmer, 2009). The second phase starts from the condensation of molecules by prenyltransferases in order to transfer geranyl diphosphate (GPP; C10), farsenyl diphosphate (FPP; C15), and finally geranylgeranyl diphosphate (GGPP; C20) , corresponding predecessors of mono, di and sesquiterpene (Liang *et al.*, 2002). The third phase is where prenyl diphosphates are subject to cyclization reactions catalyzed by several enzymes called terpene synthases (TPS), resulting in base skeletons and transforming the orga-nization of properties created by the precursors (Irmisch *et al.*, 2014; Schwab *et al.*, 2008). Finally, the fourth phase is given by skeleton remodeling through the maturation enzymes of terpenes during the reactions of hydroxylation, oxidation, acylation, double bond deflation and methylation (Dudareva *et al.*, 2013; Pare and Tumlinson, 1997; Schwab *et al.*, 2008).

As the particular case of cotton (*Gossypium spp.*), the biosynthesis of mono, sesqui and diterpene does not present great differrences compared to other plants, since the biochemical reactions basically remain the same. From the Geranyl-pyrophosphate GPP synthase that is derived from the IPP/DMAPP, some involved enzymes transform several molecules through some reactions such as cyclization, hydroxylation and dehydration (Yang *et al.*, 2013a).

On the other hand, TPS in general have been divided into three different classes and according to their phylogenetic relationship in seven sub-families. All these subfamilies have been clearly confirmed by functional studies in some plants such as *Solanum lycopersicum* (Falara *et al.*, 2011), *Populus trichocarpa* (Irmisch *et al.*, 2014), *Vitis vinifera* (Martin *et al.*, 2010), *Eucalyptus grandis* and *E. globulus* (Külheim *et al.*, 2015) and *Arabidopsis thaliana* (Aubourg *et al.*, 2002). According to the classification by their classes, class I covers TPS-c (copalyl diphosphate and ent-kaurene), TPS-e/f (ent-kaurene and in other cases diterpenes as well as mono- and sesquiterpenes) and TPS-h (Selaginella-specific). These types of genes comprise between 12-14 introns and 13-15 exons. Consecutively, TPS-d belongs to Class II (gymnosperm-specific), which comprises 9 introns and 10 exons. Finally, Classe III includes TPS-a (sesquiterpenes), TPS-b (cyclic monoterpenes and hemiterpenes)

and TPS-g (acyclic monoterpenes) and are the class with the highest presence in plants, with 6 introns and 7 exons. A new class of terpene synthesis was reported in *Selaginella moellendorffii*, and named "microbial terpene synthesis genes", due to its similarity to the microbial synthesis of terpenes or terpenoids (Chen *et al.*, 2011; Külheim *et al.*, 2015; Padovan *et al.*, 2012; Trapp and Croteau, 2001).

Collection, extraction, and analysis of VOC
Different techniques for micro-extraction and VOC analysis have been developed (Azmir *et al.*, 2013; Fothergill, 1968; Kallenbach *et al.*, 2014; Lampson *et al.*, 2014; Loreto *et al.*, 2014; Ormeño *et al.*, 2011; Spietelun *et al.*, 2010; Tholl *et al.*, 2006; Van Bramer and Goodrich, 2015; Yang *et al.*, 2013). Although all the systems try to identify the profile of plant volatile compounds, however, the appropriate choice of system to be used in each experiment will often depend on the biological problems and the plant material under study. On the other hand, an important point to consider by researchers is the cost of establishing some techniques for capturing VOC, although in many cases it is better to develop the devices using readily available materials. Although, different devices to capture VOC in several plants have been created; but in general, the principle workflow remains the same (Figure 3).
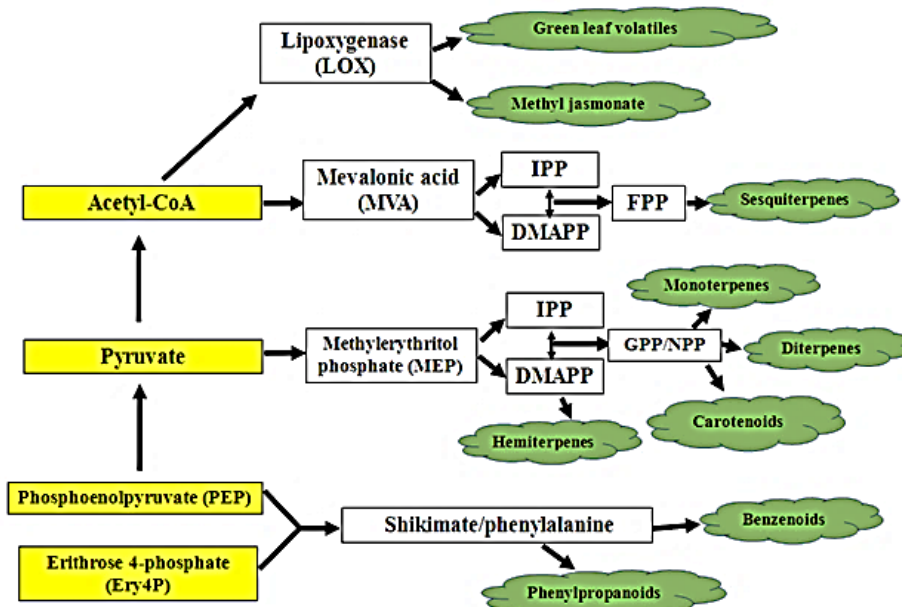

Figure 2. General view of biosynthesis processes that handle VOC emission in plants. IPP: isopentenyl pyrophosphate, FPP: farsenyl diphosphate, DMAPP: dimethylalyl-pyrophosphate, GPP: geranyl diphosphate, NPP: neryl pyrophosphate.

Table 1
Main groups of VOC terpenes produced in plants with some of their characteristics

Groups	Most common VOC terpenes	Precursors	Functional characteristics
Monoterpenes (C10)	ß-Myrcene, ß-Ocimene, Limonene, α-Pinene, ß-Pinene, Geraniol, Linalool	Metabolic pathway MEP	Pollinator attractant emit signals to herbivores
Sesquiterpenes (C15)	Farnesol, ß-Farnesene, Nerolidol, Gossypol, farnesene cadinene, ß- Cariofilene	Metabolic pathway MVA	Pollinator attractant emit signals to herbivores, antimicrobial activity
Homoterpenes (C5)	DMNT, TMTT, Isoprene	Metabolic pathway MEP	Emission of signals to herbivores

Two method types for VOC capture are mainly used static and dynamic. Of these, the first is more classic and simple; however, it is less efficient when capturing VOC, because once captured molecules are diluted inside the chamber, consequently, the headspace, accumulates humidity and heat. This occurs especially when the samples are collected under solar or artificial lighting that can directly interfere with normal physiology and the emission of plant volatiles. Its use is recommended for the study of VOC profiles in a single space of time and not in several time frequencies where VOC emissions are changing. It is mainly used to capture VOC in fruits (Iijima, 2014; Ormeño *et al.*, 2011; Tholl *et al.*, 2006).

Dynamic headspace sampling technique is presented as the most used for almost all areas of VOC collection. This method consists in allowing the flow of continuous air through a sampling container where the VOC accumulate in gas that enters and exits through attached holes in each end of the containers (one as air intake and one as exit). It is connected by means of fine hoses to a vacuum pump (Loreto *et al.*, 2014; Tholl *et al.*, 2006). While VOC are trapped in absorbents and the gas circulates through the container, it allows the collection of sufficient VOC for detection. This system prevents the accumulation of moisture due to temperature increase, unlike the static system; however, it is important to make sure that clean air is being provided when pumping air through fluids, using carbon filters for example, in order to avoid VOC impurities moving to the following analytical parts (Tholl *et al.*, 2006). This system is mainly used for synthesis elucidation studies of volatiles affected primarily by environmental events, such as flowering, attack by herbivores, and other biotic and abiotic factors (Iijima, 2014; Loreto *et al.*, 2014).

Apart of the two techniques used for collection of VOC, these mixtures are trapped by micro-extraction fibers in solid phase (SPME), due to the advantages of this system, which is capable to detect a large number of molecules, even the smallest ones. This decreases loss of VOC amounts at the time of extraction and analysis (Iijima, 2014; Azmir *et al.*, 2013; Loreto *et al.*, 2014). Latest evidence shows that an adequate choice of fiber can be highly significant when extracting VOC (Iijima, 2014; Kusano *et al.*, 2013; Risticevic and Pawliszyn, 2013).

Divinylbenzene/carboxen/polyDimethylsiloxane fibers have been reported to be suitable for metabolic studies and to provide optimal extraction coverage and sensitivity with a wide range of analyzes (Iijima, 2014; Risticevic and Pawliszyn, 2013).

With regard to the characterization of VOC, gas chromatography (GC) is employed. This technique is used to separate and analyze the profiles of analytes (the name given to each VOC), which are evaporated and treated without decomposition. Additionally, during the first phase called preparative chromatography, GC can help identify a particular compound (Azmir *et al.*, 2013; Tholl *et al.*, 2006; Yang *et al.*, 2013b). Regarding to its functionality, the chromatograph uses a flow tube through which the different VOC circulate by means of a carrier gas, according to its denomination. This phase is known as the mobile phase. Then, the analytes reach to differential speeds depending on VOC' chemical and physical properties, the latter process is called the stationary phase. After that, the samples are injected as solvent extracts, which allow them to heat up and separate and then be placed in a thermal desorption tube, this whole process occurs at a temperature of 250-300 °C. Next, the VOC are released and condensed in a cold trap and then injected into the GC column. For purely analytical purposes, the volatiles are separated into capillary columns of fused silica, with different stationary phases. Depending on column selection, relevant and detailed information are available in the market.
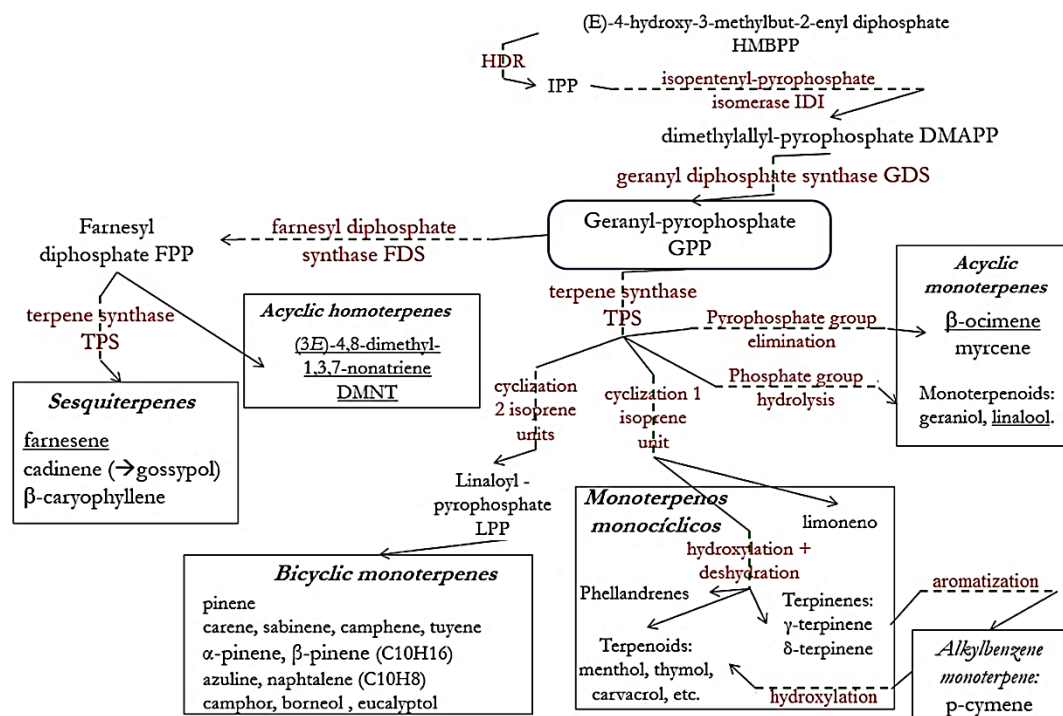
Figure 3. The biosynthesis pathway of monoterpenes, sesquiterpenes and other terpenoids emitted by cotton.

For plant-based study of the emission of VOC terpenes, the photoionization detector (PID) is used, which is considerably more sensitive to the detection of compounds with the presence of double bonds Finally, mass spectrometry (MS), a device that separates the atomic nuclei with respect to the relationship between mass and charge (m/z) is available. Globally, it allows adequate studies on the composition of chemical elements and atomic isotopes. It usually works in conjunction with the GC, as GC-MS. The process done by MS consists in heating a beam of the under study compound up to vaporize and ionize the different atoms, consequently; a detector functions as a specific pattern for each analyte. It has being widely used for VOC analysis in plants (Risticevic and Pawliszyn, 2013; Tholl *et al.*, 2006; Van Bramer and Goodrich, 2015; Vas and Vékey, 2004).

There is a great diversity of synthesis of compounds in plants that have been characterized by the techniques. In the cotton plant (*Gossypium hirsutum*), some VOC have been characterized such as Gossypol, (E) ß-Ocimene, linalool, (E)-4,8-Dimethyl-1,3,7-Nonatriene, (E, E)-a - Farnesene, (E)-b-Farnesene, (E, E)-4,8,12-Trimethyl-1,3,7,11-Tridecatetraene (Brévault *et al.*, 2014), α-Pinene, β-Pinene, (E) -b-caryophyllene, (p) -cymene, particularly linked to the biosynthesis of

monoterpenes, sesquiterpenes and terpenoids as previously mentioned (Figure 4) (Huang *et al.*, 2013). Additionally, specific VOC capture devices for this crop have been manufactured in order to facilitate the collection and subsequent analysis of VOC, the vast majority of which are used in studies of attack and induction of herbivores (Lampson *et al.*, 2014; Rose *et al.*, 1996).
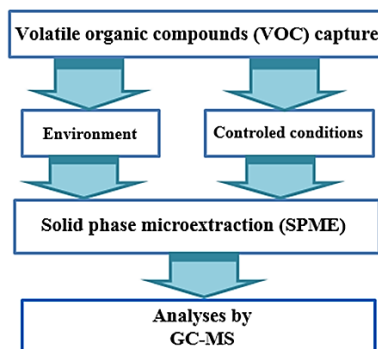

Figure 3. General workflow of VOC capture. GC-MS: Gas chromatography–mass spectrometry.

The new era of omics tools as support for genetic improvement

Thanks to the advance of fields such as functional and comparative genomics, transcriptomics and proteomics, VOC for their protective potential have been successfully studied in a better way at the molecular genetic level. Thus, the presence of genes leading some metabolic processes

and linked to some enzymes of interest has been proven in several genomes. To date, in *A. thaliana*, 40 terpene synthesis genes have been reported; of which 32 are considered functional genes while 8 are pseudogenes. According to their phylogeny, it is confirmed that they are highly related to monoterpenes, sesquiterpenes and diterpenes (Aubourg *et al.*, 2002). Another example is the grape (*Vitis vinifera*), which contains 69 putative functional genes, 20 partial and 63 pseudogenes, which were found in a study of functional annotations, genome organization and phylogeny of the gene family of terpene synthesis in 2010 (Martin *et al.*, 2010). Finally, in the balsamic poplar (*Populus trichocarpa*) 38 genes of the terpene family coded by putative proteins of an approximate length of 520 amino acids, tomato 44 terpene genes, including 29 functional ones, Eucalyptus 113 terpene genes, of which 106 highly functional have been reported (Külheim *et al.*, 2015).

On the other hand, for some varieties because of plant improvement breeding, yield have increased while having acquired resistance to certain stresses in their natural state but their production of VOC has decreased, which makes them more susceptible to any attack of herbivorous. Latest evidence in transgenic *Bt* cotton indicated that the production of sesquiterpene gossypol is diminished by "Cry" proteins of Bacillus thuringiensis (Bt) those are efficient in the control of Lepidoptera *(Hagenbucher et al.*, 2017). For some plants such as *A. thaliana* there are evidences of resistance to attack by herbivores. For example transgenic Arabidopsis showing high overexpression of a strawberry terpene synthase gene, responsible for the production of alcohol (3S)-(E)-nerolidol was reported to attract the carnivorous mite (*Phytoseiulus persimilis*) (Kappers *et al.*, 2005; War *et al.*, 2012)*.* Additionally, it was found that the synthesis of green leaf volatiles (GLV), which originate from the primary precursor Acetyl-CoA and are synthesized through the hydroxypropylide of lyoxygenase (HPL), increased after herbivory by white cabbage butterfly larvae and pathogen infection (gray mold), compared to Arabidopsis wild type (Shiojiri *et al.*, 2006). In maize (*Zea mays*), after herbivory by lepidoptera, the production of attractive volatiles for females of several species of parasitic wasps increased. These molecules are encoded by the TPS10 gene, previously reported in Arabidopsis (Kappers *et al.*, 2005), forming (E)-β-farnesene, (E)-α-

bergamotene, and other sesquiterpene hydrocarbons that are induced from farnesyl diphosphate (Dong *et al.*, 2016; Schnee *et al.*, 2006).

All these available technologies have allowed to explore slightly the genetic resources of some cultivated species (mostly maize), depending on their capacity to produce VOC (Degen *et al.*, 2004), contrasting mainly modern, semi-wild and wild varieties (Köllner *et al.*, 2008). For the semi and wild varieties, a large amount of valuable genetic information about the genes related to environmental adaptation and, particularly, resistance and tolerance to attack by herbivores (Viot, 2016) could be hidden and undisclosed. This makes it necessary to continue exploring the NDMs to learn more in details about genes that lead some metabolic routes at the time of herbivory attack like what seen for TPS10 in Arabidopsis (Kappers *et al.*, 2005). Some strategies based on the positioning of QTLs along genetic maps, nowadays are used for gene selection as this approach has outputted good results in animal genetic improvement (Viot, 2016).

Expression profiles in response to stresses have generated reliable and accurate results. RNA-seq allows us to study many genes that show differential expression pattern while overexpression or downregulation by the effect of undesirable conditions. This technology produces millions of short sequence reads for purified mRNA, based on sequencing on a high performance platform or NGS such as Ilumina, Ion Torrent, ABI, SOLID or PacBio (Shendure and Ji, 2008), routinely being applied to analysis of genomes, epigenomes and transcriptomes (Jazayeri and Villamar-Torres, 2017; Oshlack and Robinson, 2010; Griffith *et al.*, 2015; Jazayeri *et al.*, 2015).
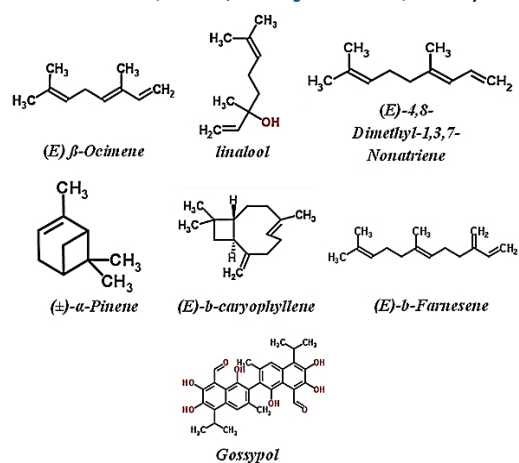

Figure 4. Chemical structure of some molecules of monoterpens, sesquiterpens and other terpenoides characterized in cotton.

A case study, cotton

In West Africa millions of small farmers depend on cotton production (*Gossypium hirsutum*) (Brévault *et al.*, 2014). Normally, cotton production in this region has been characterized by a low consumption of synthetic insecticides, however, in recent years there has been a high resistance of certain herbivores especially cotton worm to the use of pyrethroids (Brévault *et al.*, 2014; Renou *et al.*, 2012). Consequently, all these have led the researchers to initiate studies on plant – herbivore interaction and return to the exploration of NDMs to understand their function, activity, and operation. At the end, these characteristics are used for genetic improvement of new cotton varieties, but only to a field-level (classical plant breeding), which could take several years up to obtain improved varieties. However, in cotton (*Gossypium hirsutum*) there are not many studies on the genomic and molecular bases of herbivore NDM, and how these defenses are orchestrated at the level of internal molecular biological processes (Huang *et al.*, 2015). A few transcriptomic researches have reported that cotton plants show differential expression, where several genes are overexpressed or downregulated at the time of attack by arthropod insects. So that, some genes and proteins have been able to be characterized, when are subjected to attack of insects such as *Helicoverpa armigera, Aphis gossypii* and *Bemisia tabacci* (Whitefly), arthropods that cause high economic losses in cotton plantations (Dubey *et al.*, 2013; Huang *et al.*, 2015; Zhou *et al.*, 2013).

Perspectives futures

Based on an adequate use of all the tools available and detailed in this review, combined with the use of molecular genetics in laboratories, good omens could be envisaged to improve plant protection, particularly cotton cultivation. Understanding functions of some genes linked to VOC that are leading processes or metabolic pathways can provide key answers, allowing identifying future potentialities and probable solutions to solve problems due to herbivore attacks. In the Figure 6 is showed a by-plan suggested for a plant breeding program based in cotton, with the combination of plant genomics and metabolic, followed by molecular validation of genes, proteins, and enzymes. Finally, until obtaining the improved varieties with the support of MAS (Molecular Assisted Selection).
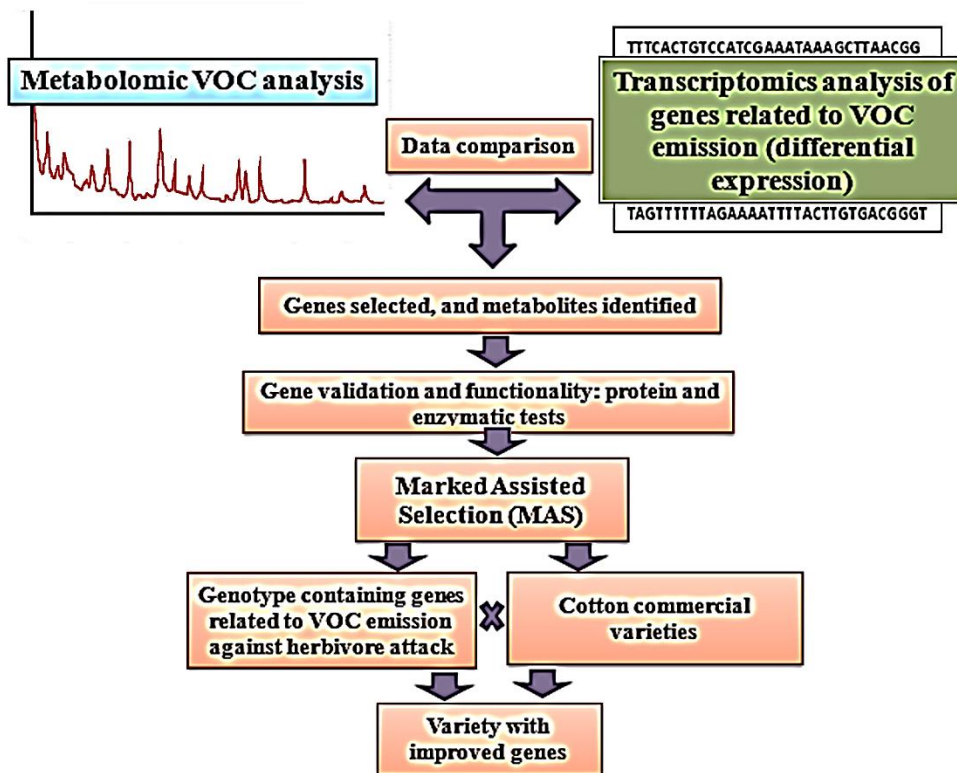

Figure 5. An overview of biotechnological tools for plant breeding based on VOC as self-defense mechanism against herbivores suggested in cotton crop.

Conclusions

All these advantages show the possibility of supporting with the use of agricultural biotechnology to create varieties in relation to NDM against predatory arthropods, mainly in the cotton and in benefit of small farmers, hence, avoiding a high consume of pesticides which representing a hazard for environment and for the health of the cotton cultivators.

Acknowledgments

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