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Approaches for the genetic improvement of Lavender: A short review

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Abstract
There is a recent increase in popularity of lavenders and their essential oils due to its potential in therapeutic properties and agri-business. Farming society and different agri-entrepreneurs around the globe are preferring cultivation of lavender for commercial farming over the traditional one. Lavender oil has big domestic and international market scope. Its significance in the perfumery industry, aromatherapy and ecotourism has motivated people to opt for the cultivation of lavenders. Improvement of the yield and quality of these natural plant products in lavender through conventional breeding is still a challenge due to clonal nature of most of the species and perennial nature. To overcome these constraints genomics and post-genomics are believed to offer new opportunities for the improvement of yield and quality of oil in lavender. Also the progress made in the development of EST databases and functional markers significantly helped genetic improvement programme in lavender. This review will present an overview of different genomic and molecular approaches for enhancing the genetic potential and vigour of lavenders.

Keywords: Lavender, Lavandula, genomics, EST, essential oils, IPP, functional markers

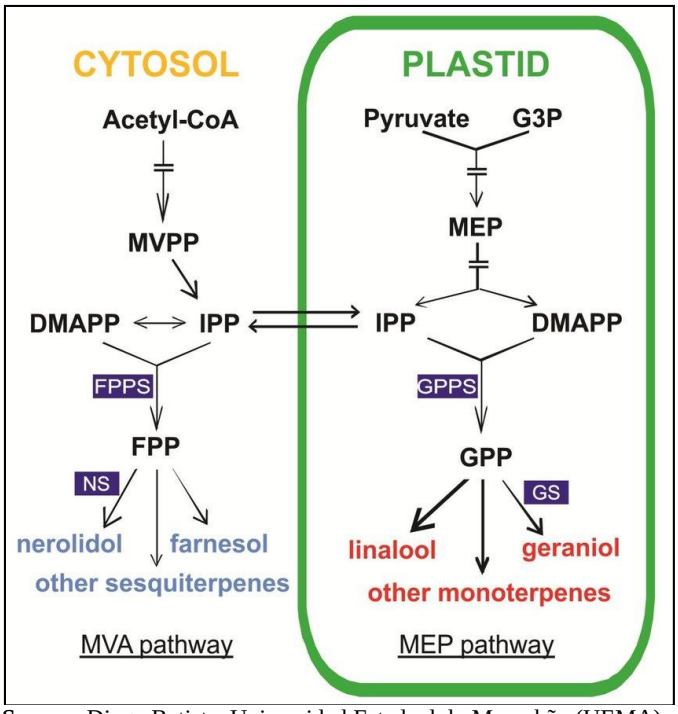
Introduction
Lavender (Lavandula) belongs to the family (Lamiaceae) is currently world's high value aromatic plant species. It has a small genus of 28 species. Out of these, only three species L. *angustifolia* (*True lavender*), L. *latifolia* (Spike lavender) and. *Hybrida* (Lavendin) is used for oil extraction.(True lavender) is the highest quality oil and used is perfumery and aroma-therapy and thus possesses both domestic and international market. A lot of pharma as well as other multinational companies are engaged in value addition of the EO for product development and marketing. Due to climate change and keeping in view the plight of farmers involved in traditional farming, lot of farmers and agri-entrepreneurs are showing their keen interest in commercial farming of Lavender. It is the dynamics of rural transformation in emerging economies. Lavender (Lavandula) plant secondary metabolites, which are important for human healthcare. Improvement of the yield and quality of these natural plant products through conventional breeding is still a challenge (Gupta *et al.* 2008) [20]. In the recent past some approaches like mutation breeding and other conventional approaches were used to increase the yield and quality. However, these methods were not that powerful due to long generation time, failure to generate mapping population and perennial nature (Gupta *et al.* 2008) [20]. To overcome these constraints genomics and post-genomics are believed to offer new opportunities for the improvement of yield and quality of oil in lavender.

For applying the genomics and molecular approaches to improve the Lavender species, it is important to generate the knowledge of its metabolic pathway and to exploit the genetic control for the biosynthesis of different constituents of Essential oils. These studies uncovered a number of pathways for secondary metabolites provide the basic carbon skeleton (central precursors) for a class of secondary metabolites, while other routes are also used by plants for biosynthesis of metabolites (Herrmann 1995; Walker 1999; Mahmoud and Croteau 2001; Liu *et al*. 2005) [18, 35, 27, 25]. In metabolic pathway of lavender, there underlies the amazing diversity among plant products of medicinal value. The major connecting pathways between the primary metabolites and secondary metabolites. A single pathway produces more that 100 products, secondary metabolic pathways are known as diversity oriented rather than target-oriented (Fischbach and Clardy 2007) [12]. The Knowledge of biosynthetic pathway in *Lavandula* will help in deciphering the mechanisms involved to produce a particular kind of secondary metabolites from common precursors.

Biosynthesis of Monoterpenes and Sesquiterpenes
Lavender oil constituents has certain physiological roles and medicinal properties. In turn, the

biosynthesis of essential oil constituents is determined by the genetic makeup of the plant. It should therefore be possible to improve medicinal properties of lavenders by enhancing the production of biologically active essential oil constituents through controlling the expression of related genes. This requires a clear understanding of the biochemical pathways that generate these phytochemicals, and a thorough knowledge of the nature and expression pattern of structural and regulatory genes driving the pathways. Lavender essential oils are primarily made up of monoterpenes (C10), although trace levels of sesquiterpenoids (C15) can also be present. Like other terpenoids, these low molecular weight biochemical are derived from condensation of the universal terpene Precursors isopentenyl diphosphate (IPP, C5) and dimethylallyl diphosphate (DMAPP, C5). The condensation reactions initially form geranyl diphosphate (GPP) and farnesyl diphosphate (FPP), which can be modified by terpene synthases to produce over 1000 monoterpenes and approximately 5000 sesquiterpenes, respectively. Monoterpenes are derived from GPP by the action of monoterpene synthases, and sesquiterpenes synthases transform FPP to various sesquiterpenes (Schilmiller *et al.* 2009) [34]. An overview of the enzymatic reactions resulting in synthesis of major lavender monoterpenoids is shown in "Fig. 1. Monoterpenes directly derived from GPP may be further modified through the actions of cytochrome P450 hydroxylases, reductases, dehydrogenases, and transferases to produce additional terpenes, which often have unique physical and chemical properties as well as biological activities (Aharoni *et al.*2003) [3]. In plants, IPP and DMAPP are derived from two distinct biochemical pathways: the classical acetate-mevalonate (MVA) pathway and the more recently monoterpenoids mainly being derived from the plastidial MEP pathway (McCaskill *et al.* 1995) [28]. However, it has been demonstrated that a considerable amount of cross-talk can occur between the MVA and MEP pathways. For example, Laule *et al.* demonstrated that Arabidopsis plants, in which the MVA pathway was blocked, continued to produce sterols, presumably through the plastidial MEP pathway.

IPP precursors destined for both mono- and sesquiterpenes assembly were derived from the MEP pathway (Dudareva *et al.* 2005) [9]. The two biosynthetic pathways can operate in concert, as studies of sesquiterpenes production in chamomile, for example, have shown that isoprenoid precursors were derived from both the plastidial and cytosolic terpenoid biosynthetic pathways (Adam *et al.* 1998) [1]. In a series of elegant experiments using tobacco cell cultures, radiolabeled precursor metabolites, and protein biochemistry techniques, Hemmerlin *et al.* 2003 [17] described the considerable cross-talk between the MVA and MEP metabolic pathways *in vivo*. The conversion of IPP/DMAPP to GPP and FPP, and the subsequent transformation of the later precursors to various terpene backbones – catalyzed by terpene synthases – have been extensively studied. Terpene synthases were first identified in tobacco (Nicotiana tabacum) (Facchini *et al*. 1992) [11] and spearmint (Mentha spicata), and subsequently in numerous other plants including Arabidopsis thaliana, Citrus spp., Abies grandis, and Zea mays. To date, only three terpene synthase genes (including limonene, linalool, and bergamotene synthases) have been cloned and biochemically characterized in lavender. However, efforts are currently underway to develop genomics resources in lavender to facilitate the discovery of additional structural and regulatory genes that control the production of essential oil constituents in these plants The discovery and cloning of terpene biosynthetic genes has recently prompted extensive genetic engineering efforts aimed at improving production of mono- and sesquiterpenes in higher plants. These studies have yielded promising results. For instance, over-expression of a key MEP pathway enzyme, 1-deoxy- D-xylulose 5-phosphate synthase (DXS), in Arabidopsis resulted in increased monoterpene production, as well as increases in quantities of chlorophyll and carotene terpenoids (Estevez *et al.* 2001) [10]. Deoxyxylulose phosphate reductoisomerase (DXR), the enzyme that catalyzes the second step of the MEP pathway resulting in increased production of lycopene (C40) (Kim *et al.* 2001) [19]. Furthermore, yields of the p-menthane monoterpenes have been shown to increase 40–60% via ectopic expression of DXR in peppermint (Mentha × piperita) (Mahmoud *et al.* 2002) [26]. These results underscore the potential of genetic engineering to improve the quality of essential oil in plants.



Source: Diego Batista, Universidad Estadual do Maranhão (UEMA)

Fig 1: Summarized biosynthetic pathway of monoterpenes and sesquiterpenes. The methyl erythritol phosphate (MEP) pathway and the mevalonic acid (MVA) pathway occurs in the plastid and cytosol, respectively. The following enzymes and metabolites are shown: glyceraldehyde 3-phosphate (G3P), 2-C-methyl-D-erythritol 4-phosphate (MEP), isopentenyl pyrophosphate (IPP), dimethylallyl pyrophosphate (DMAPP), geranyl pyrophosphate synthase (GPPS), geranyl pyrophosphate (GPP), mevalonate-5-pyrophosphate (MVPP), farnesyl pyrophosphate synthase (FPPS), farnesyl pyrophosphate (FPP), geraniol synthase (GS) and nerolidol synthase (NS). Enzymes highlighted in blue boxes were targeted for expression analysis

Genomic information/cDNA sequences in Lavender (*Lavandula)*

The metabolic pathways of secondary metabolites still have many unknowns, and we still have a poor understanding of several complex biosynthetic pathways. This could be due to limited genome/cDNA sequence information on medicinal plants, although EST sequence information has been generated in some. Three lavender terpene synthases were first identified and characterized by Landmann in 2007 [22]. Since then, the first expressed sequence tag (EST) library of L. angustifolia has recently been reported (Lane *et al.* 2010)

[21]. This EST librarycontains 9453 unigenes, with many coding terpenoid biosynthesis-related enzymes, including terpene synthases, prenyl transfereases, and representatives from both the MVA and MEP terpenoid biosynthesis pathways (Lane *et al.* 2010) [21]. These sequences help in micro array studies and gene expression, for example *LaLIMS* (limonene synthase), a gene directly responsible for the biosynthesis of limonene and co-products (terpenes) was downregulated as its corresponding terpenes decreased in full bloom inflorescences. In contrast, the expression of *LaLINS* (linalool synthase), the gene responsible for production of linalool, was at the highest level in full bloom inflorescences in Lavender with linalool and linalyl acetate, the main compounds.

Cloning genes

During the last several decades, efforts have been made to purify the key enzymes of different metabolic pathways involved in synthesis of secondary metabolites. A large number of corresponding genes encoding these enzymes have been cloned using cDNA cloning approach. By this approach, knowledge of peptide sequence determined from purified enzymes was used to isolate the corresponding genes with the help of molecular techniques. For example, cDNA Cloning of four essential oil related genes from L. *angustifolia* and L. *intermedia* plants (Sarker *et al.* 2013) [33] borneol dehydrogenase LiBDH, from the secretory cells of floral glandular trichomes of *L. x intermedia* plants was carried out. The 780 bp open reading frame (ORF) of the LiBDH cDNA encoded a 259 amino acid short chain alcohol dehydrogenase enzyme with a predicted molecular mass of ca. 27.5 kDa and transcripts were detected both in leaf and flower tissues. However, they were concentrated in floral glandular trichomes of *mature* L. *x intermedia* flowers indicating that like other *Lavandula* monoterpene synthases the expression of this gene is regulated in a tissue-specific manner. This converted borneol to camphor as the only product with *Km* and *kcat* values of 53 µM and 4.0x 10-4 s-1, enzyme specifically respectively. Likewise, cDNA cloning of other genes like: caryophyllene synthase (LiCPS), linalool acetyltransferase (LiLAT), terpene synthase like protien-I (LaTPS-I) has been also carried out. We believe that the cloning of lavender genes has far reaching implications for improving the quality of *Lavandula* EOs through metabolic engineering.

Metabolic engineering involving several structural genes of a pathway

Biosynthesis of a secondary metabolite generally involves either a pathway with a series of coordinated reactions or a complex network of pathways controlled by many genes. Therefore, multiple genes operating at multiple control points in a targeted pathway may have to be manipulated to achieve desired metabolic alterations. One such example is metabolic engineering of MEP pathway: the main source of precursors (IPP and DMAPP) for monoterpenes (Wildung and Croteau, 2005). The MEP pathway (Rohmer, 2003) starts by the transketolase-type condensation of two carbons from pyruvate with glyceraldehyde-3-P to form 1-deoxy-D-xylulose-5-P (DXP), catalyzed by DXP synthase (DXS). The subsequent synthesis of MEP, catalyzed by DXP reductoisomerase (DXR), is the previous step for the formation of the C5 units, following reactions in which the last enzyme of the MEP pathway (hydroxymethyl butyl diphosphate reductase) synthesizes IPP and DMAPP simultaneously. Targeting the first step in the MEP pathway by overexpressing the DXS cDNA from Arabidopsis in spike lavender lead to the increase of monoterpene precursors on the final production of essential oil in lavender.

Molecular Marker Technology

DNA-based molecular markers can also help in improvement of medicinal plant species. These markers can be used for assessing genetic diversity, authenticating plant material used for drugs and for marker-assisted breeding (Joshi *et al*. 2004; Canter *et al*. 2005) [10]. Unfortunately in case of lavender not many genetic and random markers like SNPs, RFLPs, RAPD and SCARs etc have not been developed due to lack of genomic resources and lack of reference genome. In future we can look forward to developing these DNA based markers by using approaches like Whole Genome Sequencing, De-Novo DNA and RNA sequencing while could further revolutionize the lavender improvement programmes.

Functional Markers

The available nucleotide sequences of genes which have been cloned can also be used to design primers for functional markers (FMs), to be used as perfect markers for detection of marker–trait associations (Gupta and Rustgi 2004) [15]. These markers can be used in marker assisted selection programmes at a very early stage like seedling stage. EST databases developed can used to associate a relationship between marker data and traits and can be also useful in marker development. For example, caryophyllene synthase (LiCPS), linalool acetyltransferase (LiLAT), terpene synthase like protien-I (LaTPS-I) cDNA clones for candidate genes involved in the synthesis of various enzymes have been used as a probe for developing SNP, SSR and RFLP markers for marker–trait associations in lavender. It has also been shown that co-linearity in gene order and content across related genera provides an opportunity to transfer perfect markers among medicinal plant species.

Conclusion

A recent increase in the popularity of alternative medicine has renewed interest in lavenders and their essential oils as potential natural remedies. The rapidly developing knowledge of metabolic pathways involved in terpenoids production lead to the improvement of lavenders both in quality and yield through breeding. genomics and modern biotechnology. The genomics and post genomics advancements have helped to understand the complex genetics and biochemistry of metabolic pathways in lavender. But due to less genomic resources and non availability of DNA based markers developing molecular maps and mapping population has always been a challenge in this value oil crop. Linkage based association mapping and functional markers can serve as an alternative to this problem. “Cisgenics” which is transferring wild strains into local cultivar can be used as a novel approach for the genetic improvement in lavenders.

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