Prospective Effects of Induced Mutation by Gamma Radiation in Essential Oil Production of Lemongrass (*Cymbopogon citratus)*

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Abstract: This review discusses the prospective effects of induced mutation in lemongrass, *Cymbopogon citratus* by gamma radiation towards its essential oil production by comparing available literatures on radiation studies in *Cymbopogon* genus. Previous studies on *Cymbopogon* mutation breeding program have shown that most of the analyses were limited to physical characteristic observation and concise chemical analysis in their essential oil yield. The issue that comes into view is the on-going things that happen in their essential oil biosynthesis correlated with its biological properties and chemical composition changes. Finding the exact cause of where and how the radiation had triggered the differences in essential oil production between mutant and its control variety need to be studied. This concern possibly could be answered by analysing the changes between both varieties on their interactions within chemical, biological and genetic perspectives. Thus, strong understanding could be build for better analysis in lemongrass mutation breeding.

Keywords: Induced mutation; gamma radiation; west indian lemongrass; *cymbopogon citratus*; essential oil; biological properties; chemical properties; plant breeding

1. Introduction

Lemongrass is an aromatic, perennial, tufted grass with numerous erect culms arising from a short, ring shape, sparingly branch rhizome (Oyen, 1999). Their genus, *Cymbopogon* (Poacea) is known to include about 140 species. Among this, more than 52 have been reported to occur in Africa, 45 in India, six each in Australia and South America, four in Europe, two in North America and the remaining are scattered in South Asia (Jagadish Chandra, 1975a). Lemongrass that is cultivated in the tropics and subtropics designate two different species that are East Indian lemongrass, *Cymbopogon flexuosus* and West Indian lemongrass, *C. citratus*. East Indian lemongrass also known as Cochin Malabar grass is a native to India, Cambodia, Sri Lanka, Burma and Thailand while West Indian lemongrass is a native to Southern India, Ceylon (Weiss, 1997, Simon, 1984); and Malaysia (Tiwari, 2010).

C. citratus is only known from cultivation and it has been cultivated for several centuries in South and South-East Asia as industrial and garden crop. After the First World War, large scale cultivation was taken up in South and Central America, later in Madagascar and nearby islands and also in Africa. Lemongrass is now found cultivated and often grown naturally throughout the tropics and warm subtropics, e.g. in southern parts of the Russian Federation and northern Australia. In Malaysia, it is planted in nearly every home garden (Oyen, 1999).

Lemongrass has a lemony flavour and can be dried and powdered, or used fresh. It is generally used in teas, soups, and curries besides as remarkably ingredients for cooking poultry, fish and seafood. The original use of *C. citratus* leaves was probably as a food and beverage flavouring, but it is currently grown both for its essential oil and as a condiment (Oyen, 1999). Both species of lemongrass (East Indian and West Indian lemongrass) produce up to 75-85% citral in their essential oils (Formacek and Kubeczka, 1982). Citral, a monoterpene that gives characteristic of lemon aroma in lemongrass is also an important raw material used in the pharmaceutical, perfumery and cosmetics industries, especially for synthesis of Vitamin A and ionones; which are usually synthesized from synthetic citral that derived from conifer turpentine (Dawson, 1994).

2. Chemical and Biological Properties of Lemongrass Essential Oil

Both East Indian lemongrass and West Indian lemongrass essential oils are yellow or amber-coloured, literally viscous liquid with a very strong, fresh-grassy, lemon-like, herbaceous or tea-like odour with an earthy undertone reminiscent of Ceylon citronella oil. West Indian oil differs from East Indian oil in that it is less soluble in 70% alcohol and has slightly lower citral content (Oyen, 1999). The quality of lemongrass oil is generally determined by the content of citral, the aldehyde responsible for the lemon odour. Citral is the name given to a natural mixture of two isomeric acyclic monoterpenes aldehydes: geranial (trans-citral, citral A) and neral (cis-citral, citral B). Normally, one isomer does not occur without the other. In addition to citral, the essential oil of *Cymbopogon* sp. consists of small quantities of geraniol, geranylacetate and monoterpene olefins, such as limonene (in *C. flexuosus*) and myrcene (in *C. citratus*)

(Formacek and Kubeczka, 1982; Weiss, 1997). The geranial content is 40-62% and the neral content is 25-38%. The solubility of freshly distilled oil of *C. citratus* declines rapidly in storage, due to polymerization of myrcene (Oyen, 1999).

Sacchetti *et al*. (2005) have obtained an interesting result from *C. citratus* essential oil, which has been the best performing extract in terms of both antimicrobial activity and ability to neutralize free radicals and prevent unsaturated fatty acid oxidation. *C. citratus* essential oil had remarkably shown strong antioxidant activity in reducing the concentration of DPPH free radical, with an efficacy slightly lower than of reference oil *Thymus vulgaris* (75.6±0.53% inhibition). This is due to the fact that citral isomers are the most abundant compounds in *C. citratus* essential oil which seems to be amenable with citral radical-scavenging activity reported by Choi *et al*. (2000). Citral has been reported to have antifungal activity against plant and human pathogens (Yousef *et al*., 1978; Rodov *et al*., 1995), inhibits seed germination (Dudai *et al.,* 1994) and also has bactericidal (Asthana *et al*., 1992; Kim *et al.*, 1995) and insecticidal properties (Rice and Coasts, 1994).

Previous studies undertaken with the ethanol extract of *C. citratus* had found that it possess antimutagenic properties towards chemical-induced mutation in *Salmonella typhimurium* strains TA98 and TA100. The extract was not found to be mutagenic in the Salmonella mutation test with or without metabolic activation (Vinitketkumnuen *et al*., 1994). It is thus possible that lemongrass contains some components that may be cancer chemo-preventive. In fact, the extract has shown to inhibit rat colon carcinogenesis in animal models (Puatanachokchai *et al*., 2002). Quite recently, Dudai *et al*. (2005) have found out that the concentrations of 44.5 mM, comparable to the concentration of citral in a cup of tea prepared from 1 g of lemon grass, induced apoptosis in several hematopoietic cancer cell lines. Other than that, Cheel *et al*. (2005) have obtained C-glycosylflavones orientin and isoorientin as well as chlorogenic acid from *C. citratus*. The content and free radical scavenging/antioxidant activity of the extracts and main compounds of *C. citratus* as well as the absence of cytotoxicity at doses up to 1 mM support the reputed beneficial properties of the tea and soft drinks prepared from lemongrass.

3. Market Demand of Lemongrass Essential Oils

The countless health promoting findings of lemongrass essential oil will definitely create a great demand for citral. The worldwide demand for citral has increased significantly in recent years due to the increasing need for high-quality products in perfumery, pharmaceutical, animal nutrition and food production. Although there are renewable raw materials of citral from lemongrass essential oil; competition from fine chemistry companies is quite challenging. Thus, several fine chemistry companies that have been investing in citral and its derivatives intend to expand the production scale to meet the market demand. For example; in 2004, BASF company inaugurated a world-scale plant in Ludwigshafen, Germany, with an annual capacity of 40,000 metric tons, replacing an existing plant with a capacity of 10,000 metric tons/year (Trasarti *et al*., 2007). The company is using a new plant which has more ecologically and economically favorable process that starts with cheaply available isobutene and formaldehyde to synthesize citral (Chauvel *et al*., 1994).

The actual information of production and trade of lemongrass oil from the producing countries is not available and only can be estimated. Singh *et al*. (2000) has calculated the current production of oil which is approximately at 1300 tonnes/annum, with India contributing the tune of 350 tonnes. India is a major producer of lemongrass oil with about 80% of the production is exported to West Europe, United States and Japan. Currently, other major lemongrass-oil-producing countries are Brazil, China, Guatemala, Haiti, Nepal, Russia and Sri Lanka (Anonymous, 2003-2007a and b).

Although East Indian Lemongrass is preferred due to its high citral content, West Indian Lemongrass oil is likely to remain another important source of citral for use in low-cost perfumery, since its use as a masking fragrance in deodorants, detergents and pesticides is still growing. However, the essential oil from *Litsea cubeba* (generally known as May Chang in China) is a serious competitor since citral is also one of its main component (47.1%) (Ma *et al.,* 1988). China is the major producer of May Chang oil, with an annual production of about 2000 tonnes and more than 50% of their production is exported to worldwide. May Chang oils fetch a price of USD 4/kg (1994), thus competing strongly with lemongrass oils (Nor Azah and Susiarti, 1999) which currently fetch a price of around USD 18/kg (2007). Although this tough competition has given a fluctuation result in demand for lemongrass oil, the global demand for the essential oil is still robust (Tiwari, 2010).

Another important competitor for natural source of citral and commercially available will be Lemon Myrtle *(Backhousia citiodora)* which contain about 95% of citral (Southwell *et al*., 2000). Lemon Myrtle come from the Myrtaceae family, is a native to subtropical rainforests of Queensland, Australia. It is probably the most commercialised native spice of Australia with thousands of trees is currently under cultivation. The production level has now already reaches approximately 5.5 ton/ha and the dried and milled leaf is available from the growers for AUD 22-25/kg (Robins, 2004). This world highest natural source of citral will be expected to competing strongly with current competitor of lemongrass, i.e. May Chang.

Even though the competition from natural and synthetic fragrances keeps coming for lemongrass oil, the world trade in essential oil is expected to continue to expand tremendously. This is due to the growing number and preferences of consumers together with the wider spectrum of natural essential oils produced. The agro-industrial usage will be the momentum factor to the present rate of inter-disciplinary research on essential oil plants that targeting towards increasing the production and quality of the essential oils (Sangwan *et al*., 2001). Thus, effort towards in finding the way to enhance the natural essential oil production of lemongrass is greatly important demand.

4. Plant Breeding Program of Lemongrass

Producing new strong of citral bearing plants is probably the main strategy to compete the synthetic citral producers. Moreover, natural essential oil of lemongrass have many advantages compared to synthetic citral. As for example; the American consumers are getting more aware of the side affects of the synthetics, and are turning towards products using essential oils in the hope of getting safer and milder products (Verlett, 1995). There are several breeding programmes for *Cymbopogon* spp. conducted by established research institution. Their aim is to produce new cultivars which gave higher yield of essential oil that primarily use for their main components. Those institutions located in South and South-East Asia that involved in germplasm collection and conservation of aromatic grasses are such as the Central Institute of Medicinal and Aromatic Plants, Lucknow, India; the National Board of Genetic Resources, New Delhi, India; the Research Institute for Spice and Medicinal Crops (RISMC), Bogor, Indonesia; the Thailand Institute of Scientific and Technological Research (TISTR), Bangkok, Thailand; and the University of the Philippines, Los Baňos, Philippines. Of all, India is the leading country in developing plant breeding programs for *Cymbopogon* spp. Their systematic collection of *Cymbopogon* spp. germplasm has began since 1951 at the Lemongrass Breeding Station, Odakkali, Kerala, India. It is now known as Aromatic and Medicinal Plants Research Station (AMPRS). Their collection now has over 450 accessions and has become world largest germplasm collection of lemongrass (Oyen, 1999).

Cymbopogons are highly heterozygous plants due to cross pollination. Thus intense genetic variations are widespread in the species (Sreenath and Jagadishchandra, 1991). Taxonomically, the species of *Cymbopogon* have been divided into three series namely 'Shoenanthi', 'Rusae' and 'Citrati' (Stapf, 1906). The leaves of the species in 'Schoenathi' series are thin, in 'Rusae' subcordinate and in 'citrati' lanceolate. The identification and classification of *Cymbopogon* species have become difficult because of the occurrence of numerous transitional forms which are supposed to have arisen by hybridisation (Bor, 1960) and the existence of many varieties and races (Jagadish Chandra, 1975b). There are considerable numbers of cultivars (e.g. Pragati, Krishna, Praman) which have been released in India but most of them are not from *C. citratus* (Sangwan *et al*., 2001). Some species, especially *C. citratus*, are not known to flower. Others like *C. nardus* and *C. winterianus* flower infrequently and the polyploidy forms of *C. flexuosus* and *C. coloratus* do flower, but do not produce seed. There is no known conventional breeding program for *C. citratus*. It flowers very rarely or not at all which is the main obstacle to do crossing (Khanuja *et al*., 2005; Oyen, 1999). *C. citratus* is propagated vegetatively, while *C. flexuosus* is commercially propagated by seed as well as vegetative by ramets (Kulkarni, 1994).

5. Application of Mutation Induction in Crop Improvements

The successful use of plant breeding for improving crops requires the existence of genetic variation of useful traits.

Unfortunately, the desired variation is often lacking (Brunner, 1995). When genetic variability by using traditional breeding methods is obstructed by plants characteristic limitation, induced mutations are one of the possible approaches for broadening the genetic variation in lemongrass to get out of the bottleneck conditions. (Ahloowalia and Maluszynski, 2001). FAO/IAEA have established a database of mutant varieties to help geneticists, molecular biologists and plant breeders to assess the value of mutation techniques in germplasm enhancement and stimulate the use of induced variation. The numbers of mutant varieties officially released and recorded in Mutant Varieties Database (MVD) have reached 2,252 varieties before the end of year 2000. Almost half of the varieties (1,019) have been released during the last 15 years. From the total 2,252 mutant varieties, 1,585 were developed 'directly' after mutagenic treatment and selection in the subsequent generations. From this portion, a great majority (1,411) were obtained with the use of radiation as the mutagen. Gamma rays radiation was found to be the most favoured techniques in development of mutant varieties compared to *x*-rays, gamma chronic, fast neutrons, thermal neutrons and others (Maluszyski *et al*., 2000).

Ionizing radiation is typically classified into low light emission transfer, LET radiation, such as γ-rays and *x*-rays, and high LET radiation, such as α-particles and heavy ions particles. Low-LET radiation gives relatively low energy per unit length of the particle's path and transfers energy evenly to the irradiated area. High-LET radiation gives relatively high energy per unit length of the particle's path and transfer energy to very limited regions. This difference is thought to cause different type of damage to chromosomal DNA (Sachs *et al.,* 2000). The use of ionizing radiations such as *x*-rays, gamma rays and neutrons, and chemical mutagens for inducing variation, is well established. This radiation can be used to induce mutations and thereby generate genetic variations from which desired mutants may be selected. Mutation induction has become a proven way of creating variations within a crop variety. It offers the possibility of inducing desired attributes that either cannot be expressed in nature or have been lost during evolution (Brunner, 1995).

There are many consequences that could be observed once mutation induction is introduced to the plants in which it may possibly lead to either desired or undesired effects. Application of mutation inductions has been used in seed propagated plants such as *Arabidopsis thaliana*, maize, barley, pea and tobacco to isolate and identify genes, which regulate plant development, particularly the onset of flowering, formation of floral parts, seed and fruit formation and also fruit ripening (Ahloowalia and Maluszynski, 2001). While in vegetative propagated plants, mutation induction has resulted drastic enhancement on the frequency of somatic mutations from which useful traits may be selected. Phenomenon of chimera's formation (i.e. single organisms composed of two genetically different types of tissue that may originate from the same zygote) is common after initiating irradiation on multicellular cell whether in seeds, buds, or tissue culture of vegetative propagated plants. Thus, it would be ideal to avoid chimeras by induced mutation on single cells (Brunner, 1995).

To give overall perspective, after mutation induction is successfully done, several prospective effect factors could be taken as considerations. Highlighting lemongrass, *C. citratus* as plant of interest in this review, the factors are changes in chemical composition, biological properties of essential oil, together with genetic differences and physiological characteristic of the plants (Figure 1). These four factors are the main aspects that are suggested to be analysed once mutation has occurred.

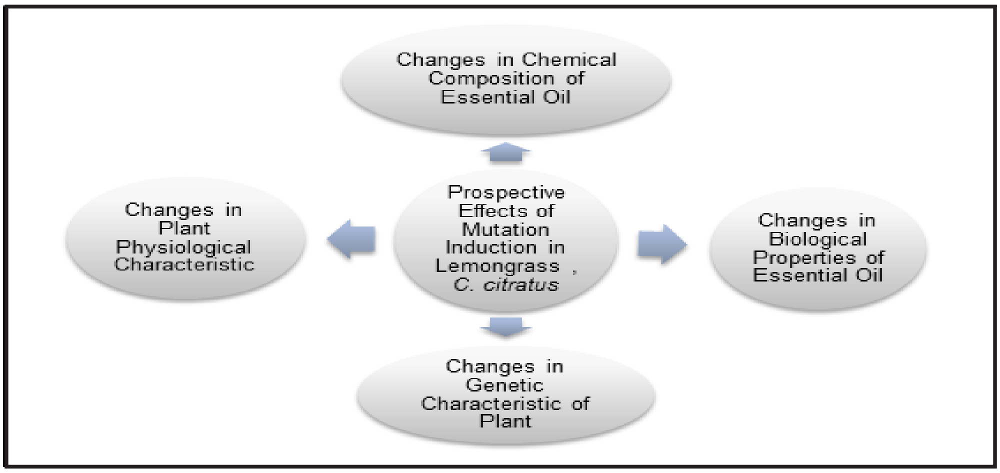

Figure 1: Prospective effects of mutation induction in lemongrass (*C. citrates)*.

6. Studies on Mutation Induction Effect in Lemongrass

Study on mutation breeding program of the genus *Cymbopogon* has started about 30 years ago. *X*-rays radiation experiment on *C. flexuosus* (Nees ex Steud) Wats had resulted in the isolation of a methyl-eugenol deficient mutant. The results had shown that once the methyl eugenol is absent from the oil, it resembles the oil of citronella (Java type) and could be good substitute for the same oil (Choudhary and Kaul, 1979). Other studies had shown that gamma irradiation on seed of Palmarosa (*C. Martini* Stapf.) has resulted an increase in yield and quality of the essential oil. This study have proved that induced mutations could changes the composition of the essential oil and would favourably alter the quantity of terpenes and minor constituents (Srivastava and Tyagi, 1986). FAO/IAEA Mutant Varieties Database (MVD) has reported that only six mutant varieties cultivars of *Cymbopogon* genus (i.e. *C. winterianus*) officially been released for past two decades (Bhanumati, Bibhuti, Niranjan, Phullara, Sourar and Subir) (Malusznski *et al*., 2000). These varieties of *C. winterianus* have been developed to adapt at North Indian and South Indian conditions. Mutation breeding techniques using *x*-rays radiation to this citronella have resulted in genetic divergence including changes in oil composition, but have not resulted in improved cultivars (Oyen, 1999, Maluszynski *et al*., 2000).

Other study of radiation on *C. citratus* had shown that short duration exposure of mild supplemental ultraviolet-B (sUV-B) irradiation on its field grown had resulted an increased in essential oil yield to 117.56%

compared to control. This does suggested that treatment of sUV-B exposure could stimulate the production of oil cells in changing the quality and percentage of essential oil contents of lemongrass. However, recommendation of this essential oil as high medicinal value products could not be confirmed as evaluation with some important parameters such as antimicrobial and antifungal activities has not been done (Rima *et al*., 2009). Previously, there are not much studies of mutation induction in *C. citratus* have been done, especially with gamma radiation technique. Quite recently, dosimetry study of gamma radiation irradiated on *C. citratus* vegetative stalk conducted by Herman *et al*. (2006) had successfully developed new mutant lines from cultivar of Riau. They had identified the lethal dose, LD_{50} of gamma rays that had triggered the differences of physical characteristic at early growth phase in M_1 generation. They also found a longer above ground stem (not stalk) with obvious appearance of nodes and internodes in one of the new mutant lines. This unique long stem can be exploited to facilitate mechanically harvesting of lemongrass. Conversely, further analysis on genetic differences and changes in essential oil biosynthesis together with chemical composition and biological properties of this new mutant variety has not yet been extensively studied.

The identification and analysis of mutants are based on the use of molecular techniques of DNA fingerprinting and mapping on PCR based markers, such as RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism) and STMS (Sequence-Tagged Microsatellite Sites) (Ahloowalia and Maluszynski, 2001). Due to that, Khanuja *et al*. (2005) have successfully established species relationship in *Cymbopogon* genus by using RAPD markers and essential oil constituents. However, it has been said that molecular markers do have certain problems like reproducibility across laboratory due to their dominant nature (Neale and Harry, 1994). On the contrary, recent studies of RAPD and ISSR (Inter-Simple Sequence Repeat) fingerprinting were found to be a powerful tool for molecular analysis of different geographically scattered populations of *C. winterianus* across West Bengal. This could be related to the number of polymorphism detected with each marker technique rather than a function of which technique is employed (Battacharya *et al*., 2010). Thus, it is still relevantly applicable to use these markers into other species of lemongrass.

It is important to analyse both chemical composition and biological properties of essential oil from mutants that have been produced. Chemical composition differences in essential oil could lead to differences in antioxidant activities. This has been shown by Choi *et al*. (2000) which have found the radical-scavenging effects of α-terpinene, not-katone, citronellal, citral, γ-terpinene, terpinolene, and geraniol were much higher than the stable antioxidant of Trolox ($p < 0.05$). Citral has shown appreciable antimicrobial activity against Gram-positive and Gram-negative bacteria as well as fungi. Media composition and inoculums size had no observable effect on activity but alkaline pH increased citral activity. Besides that, citral has been showed posses appreciable antimicrobial activity against Gram-positive and Gram-negative bacteria as well as fungi. Addition to that, increasing the concentration of citral will produced rapid growth reduction in bacteria cultures (Onawunmi, 1989). Thus, the changes of citral percentage in essential oil of lemongrass mutant are vital to its antimicrobial properties.

Comparing previous studies of *Cymbopogon* mutation induction breeding program have showed that most of the analyses were limited to physical characteristic observation and some were together with concise chemical change analysis in their essential oil yield. The main issue here is the correlation between the essential oil biosynthesis with its biological properties and chemical composition changes. Determination of exact cause of where and how the radiation had triggered the differences in essential oil yield between mutant and its control variety has not been clearly explained. This issue possibly could be answered by analysing the changes between both varieties on their interactions within chemical, biological and genetic perspectives. Regulation of essential oil production in lemongrass should also be taken into consideration in understanding the prospective changes in mutant lines produced.

7. Regulation of Essential Oil Production in Lemongrass

There are several factors involved in regulating the essential oil production in aromatic plants. Sangwan *et al*. (2001) have divided the regulator factors into three major categories: 1) the biosynthesis pathway of essential oils, 2) plant physiology and 3) impact of abiotic stress. Whilst considering the induced mutation in plants, especially by radiation such as gamma rays, the effects that come from genetic differences have to be identified. Chromosome abnormality may trigger major or minor changes to the plant physiology, physical characteristic, and also towards the biosynthesis of essential oil. However, initially, the biosynthesis pathway of essential oil has to be truly understood.

The definition described by Oyen and Nguyen (1999) has defined essential oil is a mixture of fragrant and volatile compounds that named after the aromatic plant material of a single type and is identified from which it has been derived, either by a physical process or whose odour it has. The components of plant essential oils fall into two completely different chemical classes, terpenoids and phenylpropanoids. Although terpenes correspond to the major components, occurring much more frequently and abundantly, whenever phenylpropanoids are present they provide essential and significant flavour and odour to the oil (Sangwan *et al*., 2001). From the biogenetical perspective, terpenoids and phenylpropanoids originate from different primary metabolic precursors and are generated through wholly different biosynthetic pathway. In volatile oil plants, the biosynthesis of terpenoids has been relatively much more widely investigated and regularly reviewed (Chappel, 1995).

Phenylpropanoids are not ordinary constituents of plant essential oils, even if the essential oils of certain species have abundant of significant proportions of such compounds. This is due to their occurrence that has leaded their nature and properties to significantly alter the sensory value of the oil. The main phenylpropanoids, which have been identified in the oil of certain grass species and chemotypes are eugenol, methyl eugenols, myristicin, methyl cinnamate, elemicin, chavicol, methyl chavicol, dillapiole, anethole, estragole, apiole etc. (Sangwan *et al*., 2001). The phenylpropene skeletal compounds are derived from phenylalanine, synthesized *via* the shikimate pathway. The shikimate pathway, which operates only in microorganisms and plants, represents the biosynthetic route to the aromatic amino acids (Herrmann, 1995). Citral in the lemongrass essential oil which is also called as terpene or monoterpene, biogenetically arise from two simple five-carbon moieties. Isoprenyl-diphosphate (IPP) and dimethylallyldiphosphate (DMAPP) serve as universal precursors for the biosynthesis of terpenes. IPP and DMAPP lead to geranylpyrophosphate (GPP), which is an immediate precursor of monoterpenes. In plants, there are two isoprenoid biosynthetic pathways existing to provide precursor IPP and DMAPP. One is mevalonate pathway (MVA) and the other is non-mevalonate 2-*C*-methyl-D-erythritol 4-phosphate /1-deoxy-D-xylulose 5-phosphate pathway (MEP/DXP pathway) (Rohmer, 1996; 1999; Rohdich *et al*., 2005).

Monoterpene biosynthesis in MEP pathway can be divided into four phases (Mahmoud and Croteu, 2002; Dudareva *et al*., 2004), namely, (1) construction of the basic C5 units IPP and DMAPP; (2) condensation of IPP and DMAPP by prenyltransferase to form geranyl diphosphate (GPP; C10); (3) conversion of GPP to the parent structure of the various monoterpene subfamilies, catalyzed by terpene synthases; and (4) transformation of the parent structure of the various derivatives. This can be clearly seen in Figure 2. Whether or not the concentration of certain isoprenoid precursors is limiting for the production of monoterpenes probably depends on the species, the tissue, and the physiological state of the plant (Ahaoni *et al*., 2005). In Muñoz-Bertomeu *et al*. (2006) study, a cDNA coding for the Arabidopsis (*Arabidopsis thaliana*) DXS has been constitutively expressed in spike lavender which resulting an increased of its essential oil production (101.5-359.0%). Their results demonstrated that the MEP pathway contributes to essential oil production in spike lavender. Besides that, they also confirmed that the DXS enzyme plays a crucial role in monoterpene precursor biosynthesis and thus, in essential oil production in spike lavender.

Another factor that regulates essential oil production is developmental stage/phase of the plant *per se*; as well as its concerned part/organ, tissue and the cells that contribute in oil accumulation. In *C. flexuosus*, there is a close co-ordination between leaf ontogeny with oil accumulation and biogenesis. Early growth of this plant has been reported to give high essential oil production (Singh, 1989).

In case of *C. winterianus*, maximum oil content (1.18%) in its leaves has been reported towards the end of blooming, while flowers and inflorescences produce more oil than leaves (Akhila *et al.,* 1987; Sangwan *et al*., 1982). Other than that, enzyme level of geraniol dehydrogenase has been shown to well correlate with citral: geraniol ratio, not only with a differences species but also with developmental stages (Sangwan *et al*., 1993a; 1993b; 1993c; 1990; Singh, 1990).

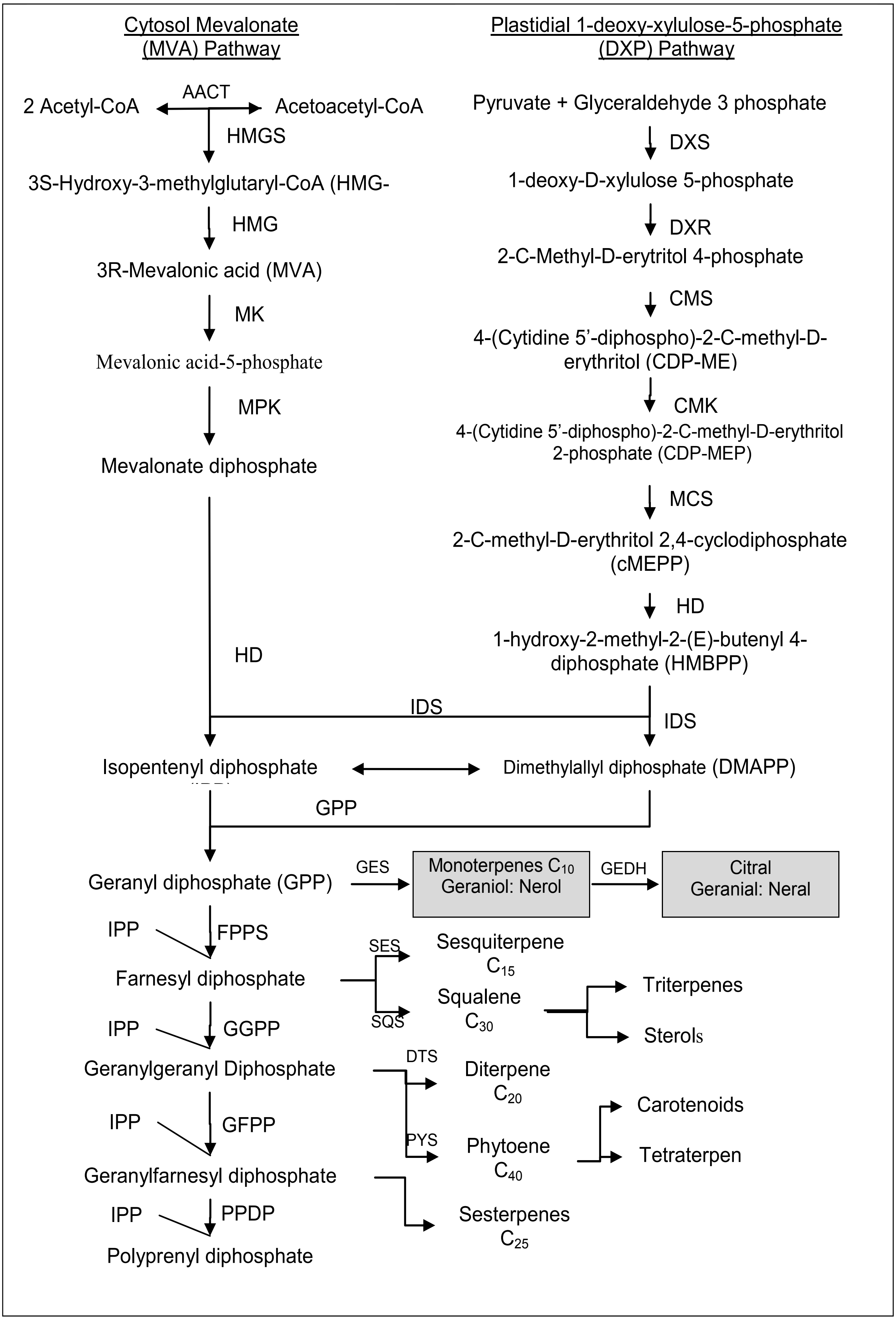

Figure 2. Terpenoid biosynthetic pathway

AACT acetyl-coenzyme (CoA) thiolase
CMK 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol-4-phospate
CMS 2-C-methyl-D-erythritol-4-phospate cytidyl transferase
DTS diterpene synthase
DXR 1-deoxy-D-xylulose-5-phosphate reductoisomerase
DXS 1-deoxy-D-xylulose-5-phosphate synthase
FPPS farnesyl diphosphate synthase
GES geraniol synthase
GEDH geranial dehydrogenase
GGPPS geranylgeranyl diphosphate synthase
GFPPS geranylfarnesyl diphosphate synthase
GPPS geranyl diphosphate synthase
HDS 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase
HMGR 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase
HMGS HMG-CoA synthase
IDS isopentenyl diphosphate isomerase
MCS 2-C-methyl-D-erithritol 2,4-cyclodiphosphate synthase
MDD mevalonate diphosphate decarboxylase
MK mevalonate kinase
MPK mevalonate-5-phosphate kinase
MTS monoterpene synthase
PPDPS polyprenyl diphosphate synthase
PSY phytoene synthase
SES sesquiterpene synthase
SQS squalene synthase

Hence, the effect of mutation induction towards this regulation has to be considered. Monoterpene biosynthesis in plants, is commonly associated with the presence of specialized secretory structures, such as oil and resin cells, glandular trichomes, oil and resin ducts, or glandular epidermis, that compartmentalize these often toxic components from metabolically active cells (Fahn, 1979; Croteau, 1986). Unlike many other aromatic plants, the surface of lemongrass leaves does not contain glandular trichomes (Croteau, 1986; Gershenzon *et al.,* 1989; Werker, 1993; Werker et al., 1993; Serrato-Valenti *et al*., 1997). In previous reports, based on staining methods for lipids, oil drops were observed in axadial epidermal and mesophyll cells, as well as in bulliform and phloem cells in lemongrass leaves (Tsai, 1978; Ming *et al*., 1996). Adding more, histochemical analysis done by Lewinsohn et al., 1998 have suggested that citral accumulation takes place in individual oil cells within the leaf tissues of lemongrass. Thus, it is important to know either mutation induction had triggered differences in essential oil storing capability within the oil cells as essential oil production has increased or decreased.

8. Conclusion

It can be concluded that the prospective effects of mutation induction should not be limited/measured on desired character only, while lots of other potential aspect could be met. Although this review has listed four main factors (chemical compositions, biological properties, genetic differences and plant physiology) that need to be studied once mutation induction is introduced in lemongrass, there are still lots of potential factors that could be explored in answering the phenomenon of mutation. By doing deep and overall study, perhaps the wise decision on the usefulness of mutant lines produced could be made.

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