

REVIEW ARTICLE

GENETIC MODIFICATION AND TRANSFORMATION STUDIES IN AROMATIC GRASSES: ADVANCES IN *CYMBOPOGON* AND *CHRYSOPOGON*

Manju M. George*

Plant Biotechnology Lab, Department of Botany, Union Christian College (Autonomous), Aluva,
Ernakulam District, Kerala – 683102, India

Email: manju@uccollege.edu.in

Received-20.05.2026, Revised-10.06.2026, Accepted-29.06.2026

Abstract: Aromatic grasses of the genera *Cymbopogon* and *Chrysopogon* (vetiver) represent economically important crops valued for their essential oils, phytoremediation capabilities, and diverse industrial applications. This manuscript provides a comprehensive review of genetic modification and transformation studies in these aromatic grasses, synthesizing recent advances in tissue culture, regeneration protocols, and genetic engineering approaches. The review examines in vitro regeneration systems, Agrobacterium-mediated transformation protocols, molecular characterization of essential oil biosynthesis pathways, and emerging biotechnological applications. Key findings reveal that while efficient regeneration systems have been established for multiple *Cymbopogon* species and vetiver, successful stable genetic transformation remains limited, with only a few reports of transgenic vetiver plants. The manuscript highlights critical bottlenecks in transformation efficiency, discusses the molecular basis of terpenoid biosynthesis, and identifies future directions for metabolic engineering and crop improvement in these valuable aromatic grasses.

Keywords: Aromatic grasses, *Chrysopogon zizanioides*, Genetic transformation, Essential oil biosynthesis

INTRODUCTION

Aromatic grasses belonging to the genera *Cymbopogon* and *Chrysopogon* represent a diverse group of economically important plants cultivated worldwide for their essential oils, medicinal properties, and environmental applications. The genus *Cymbopogon*, comprising approximately 140 species, includes commercially significant species such as lemongrass (*C. citratus*, *C. flexuosus*), citronella (*C. winterianus*, *C. nardus*), palmarosa (*C. martinii*), and other aromatic grasses valued for their monoterpene-rich essential oils (Bhat *et al.*, 2023; Meena *et al.*, 2016). Vetiver (*Chrysopogon zizanioides*, syn. *Vetiveria zizanioides*), while taxonomically distinct, shares similar agronomic characteristics and has gained prominence for its sesquiterpene-rich essential oil and exceptional phytoremediation capabilities (Nakbanpote *et al.*, 2024; Obaleye *et al.*, 2023; Pandey and Praveen, 2020).

The economic importance of these aromatic grasses extends beyond essential oil production to include applications in perfumery, pharmaceuticals, food flavoring, cosmetics, and environmental biotechnology (Chahal *et al.*, 2015). Global demand for natural essential oils has driven research into crop improvement strategies, including conventional breeding, mutation breeding, and biotechnological approaches (Smitha and Manivel, 2021; Bhardwaj *et al.*, 2020). However, the genetic improvement of

these grasses faces significant challenges, including complex polyploid genomes, apomictic reproduction in some species, long breeding cycles, and limited understanding of the molecular mechanisms governing essential oil biosynthesis (Bellido *et al.*, 2024; Vimala *et al.*, 2022).

Biotechnological interventions, particularly genetic transformation and metabolic engineering, offer promising avenues for targeted improvement of these aromatic grasses (Giri and Praveena, 2015; Shabir, 2021). Recent advances in tissue culture, regeneration protocols, transcriptome sequencing, and gene discovery have laid the foundation for molecular breeding and genetic engineering approaches (Bhat *et al.*, 2023; Meena *et al.*, 2016; Devi *et al.*, 2016). This manuscript synthesizes current knowledge on genetic modification and transformation studies in *Cymbopogon* and vetiver, examining the state of the art in regeneration systems, transformation protocols, molecular characterization, and future prospects for biotechnological enhancement of these valuable aromatic grasses.

BOTANICAL AND ECONOMIC SIGNIFICANCE

Cymbopogon Species: Diversity and Applications

The genus *Cymbopogon* encompasses a wide range of aromatic grasses distributed across tropical and subtropical regions, with major centers of diversity in

*Corresponding Author

Asia and Africa (Adhikari *et al.*, 2017; Kumar *et al.*, 2009). Key commercial species include East Indian lemongrass (*C. flexuosus*), West Indian lemongrass (*C. citratus*), Java citronella (*C. winterianus*), Ceylon citronella (*C. nardus*), palmarosa (*C. martinii* var. *motia*), and gingergrass (*C. martinii* var. *sofia*) (Adeyemo and Osibote, 2023; Ng *et al.*, 2016; Padalia *et al.*, 2011). These species are distinguished by their essential oil composition, with lemongrass characterized by high citral content (70–85%), citronella by citronellal and geraniol, and palmarosa by geraniol and geranyl acetate (Gupta and Ganjewala, 2015; Kumar *et al.*, 2008; Mieso and Befu, 2020).

The essential oils of *Cymbopogon* species possess diverse biological activities, including antimicrobial, antifungal, antioxidant, insecticidal, and therapeutic properties (Ali *et al.*, 2017). Lemongrass oil, rich in citral (geranial and neral), is widely used in the fragrance industry, traditional medicine, and as a natural food preservative (Hartatie *et al.*, 2020; Bergan, 2022). Recent studies have also explored the anticancer potential of lemongrass extracts, with evidence of cytotoxic effects against colon cancer cells and enhancement of chemotherapy efficacy. The phytochemical diversity and biological activities of *Cymbopogon* essential oils have positioned these grasses as valuable crops for sustainable agriculture and natural product industries (Yadav *et al.*, 2023).

Vetiver: Multifunctional Grass for Environmental and Industrial Applications

Vetiver (*Chrysopogon zizanioides*) is a perennial grass native to India, characterized by its deep, extensive root system that can penetrate up to 3–4 meters into the soil (Nakbanpote *et al.*, 2024; Lavania and Lavania, 2009). This unique morphological feature, combined with its tolerance to extreme environmental conditions, has made vetiver a preferred species for soil and water conservation, slope stabilization, and phytoremediation of contaminated sites (Pandey and Praveen, 2020; Balangcod *et al.*, 2015). The vetiver root system produces a complex essential oil rich in sesquiterpenes, particularly vetiverol, khusimol, and α - and β -vetivone, which are highly valued in the perfume industry for their woody, earthy fragrance and fixative properties (Chahal *et al.*, 2015; Pripdeevech *et al.*, 2006).

Beyond its traditional use in perfumery, vetiver has emerged as a powerful tool for environmental biotechnology. Extensive research has demonstrated vetiver's capacity to remediate soils and waters contaminated with heavy metals (lead, cadmium, chromium, nickel, arsenic), organic pollutants (petroleum hydrocarbons, pesticides, antibiotics), and industrial effluents (Sengupta, 2014; Nakbanpote *et al.*, 2024; Nugroho *et al.*, 2021; Datta *et al.*, 2013). The grass exhibits remarkable tolerance to toxic elements through various detoxification mechanisms, including metal sequestration in roots, enzymatic

degradation of organic contaminants, and symbiotic associations with pollutant-degrading microorganisms (Melato *et al.*, 2016; Das *et al.*, 2017). Recent studies have also explored vetiver's potential as a lignocellulosic feedstock for bioethanol production and biogas generation, adding value to phytoremediation applications (Sun *et al.*, 2014; Geiger *et al.*, 2019; Neve *et al.*, 2022).

In Vitro Regeneration Systems

The development of efficient in vitro regeneration systems is a prerequisite for genetic transformation and biotechnological improvement of aromatic grasses. Successful regeneration protocols have been established for multiple *Cymbopogon* species and vetiver, employing various explant types and culture conditions (Giri and Praveena, 2015; Ozias-Akins and Vasil, 1988). (George and Subramanian, 1999a, 1999b, 2000)

Tissue Culture in *Cymbopogon* Species

Regeneration via Organogenesis

Organogenesis, the formation of shoots directly from explant tissues or through callus intermediates, has been successfully employed for several *Cymbopogon* species. For *C. jwarancusa*, Mehandru *et al.* developed an effective regeneration protocol using meristematic bases of spikelet explants (Mehandru *et al.*, 2014). Callogenesis was induced on Murashige and Skoog (MS) medium supplemented with 22.62 μ M 2,4-dichlorophenoxyacetic acid (2,4-D), while culture multiplication was optimized with 18.1 μ M 2,4-D, 4% sucrose, and 0.5% glucose. The addition of 4.65 μ M kinetin enhanced regeneration, yielding approximately 40–45 plantlets per explant with 98% survival rate under greenhouse conditions (Mehandru *et al.*, 2014).

For *C. martinii* (palmarosa), Baruah and Bordoloi reported high-frequency plant regeneration through both organogenesis and embryogenesis from long-term callus cultures derived from nodal segments (Baruah and Bordoloi, 1989). Organogenesis was achieved on MS basal medium with 2 mg/L kinetin, 1 mg/L 6-benzylaminopurine (BAP), 0.2 mg/L biotin, 0.2 mg/L calcium pantothenate, and 0.1 mg/L naphthaleneacetic acid (NAA). This protocol demonstrated the feasibility of maintaining regenerative capacity in long-term callus cultures, an important consideration for genetic transformation experiments (Baruah and Bordoloi, 1989).

Somatic Embryogenesis

Somatic embryogenesis represents an alternative regeneration pathway with potential advantages for genetic transformation, including the possibility of obtaining clonal plants from single cells. For *C. citratus* (lemongrass), Quiala *et al.* developed two efficient propagation protocols: one based on organogenesis via temporary immersion systems (TIS) for shoot production, and another utilizing somatic embryogenesis (Quiala *et al.*, 2016). The somatic embryogenesis protocol involved semisolid

culture for callus formation and multiplication, followed by liquid culture in rotatory shakers and conventional bioreactors for maintaining embryogenic cultures. These methods offered fast and efficient technology for mass propagation, leveraging liquid culture and automation (Quiala *et al.*, 2016).

A rapid regeneration protocol for *C. citratus* was also reported by Lin, achieving a 90% induction rate for callus production using lamina and terminal bud explants on MS medium with 1.0-2.0 mg/L BAP and 0.2 mg/L NAA (Lin, 2008). The bud differentiation rate reached 85.6-86.7%, with a multiplication coefficient of 6. Root induction was achieved at 100% efficiency on half-strength MS medium with 0.1-0.3 mg/L NAA, and transplanted seedlings showed 100% survival after 20 days (Lin, 2008).

Regeneration Protocols for Vetiver

Callus Induction and Organogenesis

Vetiver regeneration has been achieved through multiple pathways, including organogenesis and somatic embryogenesis. Leupin *et al.* established a protocol for compact callus induction and plant regeneration from a non-flowering Java vetiver using crown and leaf slices as explants (Leupin *et al.*, 2000). Callus induction occurred on modified MS medium with 2.26 μ M 2,4-D, 2.22 μ M BAP, and 75 g/L sucrose. The optimized procedure regenerated plantlets on approximately 60% of crown or leaf slices, yielding up to 100 plantlets per slice (Leupin *et al.*, 2000).

Monisha *et al.* developed a high-frequency regeneration protocol for *Chrysopogon zizanioides* via both organogenesis and somatic embryogenesis (Monisha *et al.*, 2021). For organogenesis, node explants cultured on MS medium with 2.0 mg/L BAP achieved 72.2% shoot regeneration. White friable compact (WFC) callus derived from leaf or root explants on MS medium with 1.0 mg/L kinetin and 1.0 mg/L BAP showed 75.49% shoot regeneration efficiency. Somatic embryogenesis from leaf explants on MS medium with 0.5 mg/L BAP and 1.0 mg/L 2,4-D achieved 52.50% frequency, with optimal rooting at 76.97% (Monisha *et al.*, 2021).

Shoot Multiplication and Root Induction

Cedo *et al.* investigated enhanced plantlet regeneration and in vitro root production in vetiver using shoot base explants (Cedo *et al.*, 2013). Extensive shoot proliferation was maintained with 5 mg/L BAP, while root production was greatly enhanced by 0.25 mg/L NAA or 20 mg/L indole-3-butyric acid (IBA). Notably, roots induced by NAA were short and thick, whereas IBA induced long, thin, fibrous roots. Supplementation with 0.5 mg/L NAA and 0.5 mg/L kinetin significantly enhanced root proliferation (Cedo *et al.*, 2013).

Widoretno *et al.* achieved clonal propagation of vetiver through tissue culture using crown explants (Widoretno *et al.*, 2017). Shoot formation was

initiated on MS medium with 2 mg/L BAP, while shoot multiplication with 3 mg/L BAP yielded an average of 126 shoots per explant. High BAP concentrations (3-5 mg/L) induced more but shorter shoots, while low BAP (1-2 mg/L) resulted in fewer but longer shoots. Plantlet regeneration involved root induction on MS medium with 1 mg/L NAA, and in vitro plants were successfully acclimatized to soil under greenhouse conditions (Widoretno *et al.*, 2017).

Somatic Embryogenesis in Vetiver

Ma *et al.* established an effective cyclic induction and regeneration system for vetiver through somatic embryogenesis (Guohua *et al.* (2000), 2000). Using young leaf sheath segments and subsequently basal segments of test-tube seedlings as explants, they identified auxin as a crucial factor for inducing dedifferentiation and somatic embryo formation. Shoot formation originated from the germination of somatic embryos, induced by NAA or low concentrations of 2,4-D. This research laid a foundation for further biotechnological studies of vetiver grass (Guohua *et al.* (2000), 2000).

Genetic Transformation Approaches

Despite significant progress in regeneration protocols, genetic transformation of aromatic grasses remains challenging, with limited reports of stable transgenic plants. The recalcitrance of grasses to transformation, coupled with species-specific regeneration requirements, has hindered the application of genetic engineering to *Cymbopogon* and vetiver (Giri and Praveena, 2015; Bellido *et al.*, 2024).

Agrobacterium-Mediated Transformation Transformation of Vetiver

The most comprehensive report of genetic transformation in aromatic grasses comes from Yang *et al.*, who established an efficient *Agrobacterium*-mediated transformation system for vetiver (Yang *et al.*, 2008). The protocol utilized embryonic calli as target tissue, which were infected with *Agrobacterium tumefaciens* strain EHA105 harboring the plant expression vector p1301UN-otsA. The transformation procedure involved co-cultivation on callus induction medium (CIM) in the dark at 25°C for 4 days, followed by selection on shoot induction medium (SIM) with 50 mg/L hygromycin B and 500 mg/L cefotaxime (Yang *et al.*, 2008).

Key achievements of this study included an embryonic calli induction frequency of up to 96.7%, with maintained regeneration ability over two years (92.0% at 18 months, 81.6% at 24 months). The transformation efficiency, measured as the percentage of infected calli showing hygromycin B resistance, was 18%. Transient integration and expression of the transgene were confirmed by GUS (β -glucuronidase) assay. This work demonstrated the feasibility of genetic transformation in vetiver and

established a foundation for future metabolic engineering efforts (Yang *et al.*, 2008).

Transformation Attempts and Challenges

Sengupta attempted to develop hairy root culture of vetiver using *Agrobacterium rhizogenes* ATCC 15834 for enhanced phytoremediation applications (Sengupta, 2014). The protocol involved wounding and infecting the root-shoot junction of young vetiver plantlets with overnight-grown *A. rhizogenes*. However, no successful hairy root formation was observed in three independent trials, highlighting the recalcitrance of vetiver to *A. rhizogenes*-mediated transformation (Sengupta, 2014).

For *Cymbopogon* species, no published reports of successful stable genetic transformation were identified in the current literature survey. This gap represents a significant bottleneck in the biotechnological improvement of these economically important aromatic grasses. The absence of transformation protocols for *Cymbopogon* may be attributed to several factors, including the lack of optimized regeneration systems compatible with transformation procedures, genotype-specific recalcitrance, and insufficient research investment in these crops compared to major cereals (Giri and Praveena, 2015; Bellido *et al.*, 2024).

CHALLENGES AND CONSTRAINTS IN GRASS TRANSFORMATION

General Constraints in Grass Biotechnology

Genetic transformation of grasses faces multiple technical challenges that have been extensively reviewed in the context of forage and turf grasses (Liang and Wang, 2006; Wang and Ge, 2006; Giri and Praveena, 2015). Key constraints include: (1) recalcitrance to tissue culture and regeneration, with many grass species exhibiting low regeneration frequencies or loss of regenerative capacity during prolonged culture; (2) genotype dependency, where transformation efficiency varies significantly among cultivars and accessions; (3) somaclonal variation, which can lead to off-type plants with altered phenotypes; (4) low transformation efficiency, particularly for *Agrobacterium*-mediated methods in monocots; and (5) gene silencing, which can reduce or eliminate transgene expression in subsequent generations (Wang and Ge, 2006; Giri and Praveena, 2015).

Apomixis as a Constraint

For certain aromatic grasses, particularly some *Cymbopogon* species and related apomictic grasses, the mode of reproduction presents additional challenges for genetic improvement. Bellido *et al.* reviewed genetic transformation of apomictic grasses, highlighting both progress and constraints (Bellido *et al.*, 2024). Apomixis, the asexual reproduction through seeds, can complicate breeding efforts but also offers potential advantages for fixing hybrid vigor and maintaining desirable traits.

However, the molecular complexity of apomixis and its interaction with transformation procedures remain poorly understood (Bellido *et al.*, 2024).

Recent advances in transformation of apomictic grasses have been reported for species such as *Cenchrus ciliaris* (buffelgrass), where Shashi *et al.* developed an *Agrobacterium*-mediated genetic engineering system (Shashi *et al.*, 2024). Such developments in related grass species provide valuable insights that may be applicable to aromatic grasses, particularly for species with apomictic reproduction (Shashi *et al.*, 2024).

Lessons from Related Grass Species

Transformation protocols developed for other grass species offer valuable templates for aromatic grass improvement. Wang and Ge reported rapid and efficient production of transgenic bermudagrass and creeping bentgrass bypassing the callus formation phase, achieving transformation through direct shoot regeneration (Wang and Ge, 2005). This approach minimized somaclonal variation and reduced the time required for transgenic plant production (Wang and Ge, 2005).

For forage grasses, Liu *et al.* demonstrated successful *Agrobacterium*-mediated transformation of centipedegrass (*Eremochloa ophiuroides*) (Liu *et al.*, 2012), while Faleiro *et al.* generated transgenic Napier grass (*Cenchrus purpureus*) through biolistic gene transfer using minimal expression cassettes (Faleiro *et al.*, 2021). These studies demonstrate that both *Agrobacterium*-mediated and biolistic transformation can be effective for grass species, depending on the specific characteristics of the target species and the optimization of culture conditions (Liu *et al.*, 2012; Faleiro *et al.*, 2021).

MOLECULAR CHARACTERIZATION AND METABOLIC ENGINEERING

Transcriptome Analysis and Gene Discovery

Transcriptome Sequencing in *Cymbopogon*

Recent advances in next-generation sequencing technologies have enabled comprehensive transcriptome analysis of aromatic grasses, providing unprecedented insights into the molecular basis of essential oil biosynthesis. Bhat *et al.* developed and analyzed de novo transcriptome assemblies of multiple genotypes of *Cymbopogon* species, revealing candidate genes involved in the biosynthesis of aromatic monoterpenes (Bhat *et al.*, 2023). This comparative transcriptomics approach identified genotype-specific expression patterns and highlighted key regulatory points in the terpenoid biosynthesis pathway (Bhat *et al.*, 2023).

Meena *et al.* performed de novo sequencing and analysis of lemongrass transcriptome, providing the first comprehensive insights into essential oil biosynthesis in aromatic grasses (Meena *et al.*, 2016). The study identified genes encoding enzymes of the methylerythritol phosphate (MEP) pathway,

mevalonate (MVA) pathway, and terpene synthases responsible for monoterpene production. This foundational work established a genomic resource for future molecular breeding and metabolic engineering efforts in lemongrass (Meena *et al.*, 2016).

For *C. winterianus* (Java citronella), Devi conducted transcriptome sequencing and characterized metabolic pathway genes (Devi, 2015). Subsequently, Devi *et al.* performed genome-wide transcriptome profiling that revealed differential gene expression in secondary metabolite pathways (Devi *et al.*, 2016). These studies identified key genes involved in citronellal and geraniol biosynthesis, including geraniol synthase, citronellol dehydrogenase, and various cytochrome P450 enzymes involved in monoterpene modification (Devi, 2015; Devi *et al.*, 2016).

Functional Characterization of Biosynthetic Genes

The functional characterization of genes involved in essential oil biosynthesis represents a critical step toward metabolic engineering of aromatic grasses. While direct genetic transformation of *Cymbopogon* species has not been reported, heterologous expression studies in model systems have validated the function of key biosynthetic enzymes. The identification of terpene synthases, prenyltransferases, and modifying enzymes from transcriptome data provides a foundation for future transgenic approaches aimed at enhancing essential oil yield or modifying oil composition (Bhat *et al.*, 2023; Meena *et al.*, 2016; Devi *et al.*, 2016).

Essential Oil Biosynthesis Pathways

Terpenoid Biosynthesis in Aromatic Grasses

Essential oils in *Cymbopogon* species are predominantly composed of monoterpenes (C10) and sesquiterpenes (C15), derived from the universal five-carbon building blocks isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). In plants, these precursors are synthesized via two distinct pathways: the cytosolic mevalonate (MVA) pathway and the plastidial methylerythritol phosphate (MEP) pathway (Meena *et al.*, 2016; Devi *et al.*, 2016). While the MEP pathway primarily supplies precursors for monoterpene biosynthesis in plastids, the MVA pathway provides precursors for sesquiterpene biosynthesis in the cytosol (Meena *et al.*, 2016).

The committed step in monoterpene biosynthesis is catalyzed by geranyl diphosphate synthase (GPPS), which condenses IPP and DMAPP to form geranyl diphosphate (GPP). GPP serves as the substrate for various monoterpene synthases that generate the diverse array of monoterpenes found in *Cymbopogon* essential oils. In lemongrass, citral (a mixture of geranial and neral) is the predominant component, while citronella oil is characterized by citronellal, citronellol, and geraniol (Gupta and

Ganjewala, 2015; Kumar *et al.*, 2008; Padalia *et al.*, 2011).

Metabolic Engineering Strategies

Although direct metabolic engineering of aromatic grasses has not been achieved, lessons from related species provide valuable insights. Lange *et al.* demonstrated improved peppermint essential oil yield and composition through metabolic engineering, overexpressing key biosynthetic genes to enhance monoterpene production (Lange *et al.*, 2011). Similar strategies could potentially be applied to *Cymbopogon* species once efficient transformation protocols are established (Lange *et al.*, 2011).

The potential for metabolic engineering in aromatic grasses extends beyond simple overexpression of biosynthetic genes. Strategies may include: (1) upregulation of rate-limiting enzymes in the MEP or MVA pathways to increase precursor availability; (2) overexpression of specific terpene synthases to enhance production of desired monoterpenes; (3) downregulation of competing pathways to redirect carbon flux toward essential oil biosynthesis; (4) modification of regulatory genes to enhance trichome density or oil gland development; and (5) introduction of novel biosynthetic capabilities to produce non-native compounds with enhanced commercial value (Shabir, 2021; Das *et al.*, 2024; Lange *et al.*, 2011).

Conventional Genetic Improvement

While genetic transformation remains limited, conventional breeding and mutation breeding have been successfully employed for improvement of aromatic grasses.

Breeding Approaches in *Cymbopogon*

Smitha and Manivel reported genetic improvement of palmarosa (*C. martinii* var. *motia*) for herbage yield and essential oil quality through a polycross system of breeding (Smitha and Manivel, 2021). This approach exploited natural genetic variation and recombination to develop improved cultivars with enhanced agronomic performance and oil quality (Smitha and Manivel, 2021).

Phenotypic recurrent selection has been applied to citronella grass (*C. nardus*) grown in Egypt, demonstrating the effectiveness of conventional breeding for improving herb growth yield (Ibrahim and Khalid, 2013). Such approaches, while time-consuming, have proven valuable for incremental improvement of aromatic grass cultivars (Ibrahim and Khalid, 2013).

Mutation Breeding

Mutation breeding using physical and chemical mutagens has been employed to generate novel variation in aromatic grasses. Srivastava *et al.* induced mutants in the M2 generation of palmarosa and selected for enhanced essential oil yield and quality (Srivastava *et al.*, 2000). This approach successfully generated variants with improved commercial characteristics (Srivastava *et al.*, 2000).

Choudhary and Kaul reported a radiation-induced methyl-eugenol deficient mutant of *C. flexuosus*, demonstrating the potential for mutation breeding to alter essential oil composition (Choudhary and Kaul, 1979). More recently, Munda *et al.* investigated induced variations by gamma radiation and ethyl methanesulfonate (EMS) on agronomic traits, essential oil yield, and quality in Java citronella (Munda *et al.*, 2022). These studies revealed significant variation in essential oil content and composition among mutant lines, providing valuable germplasm for breeding programs (Munda *et al.*, 2022).

Ploidy Manipulation

Ploidy manipulation represents another approach for genetic improvement of aromatic grasses. Vimala *et al.* achieved lodging tolerance in *C. khasianus* through ploidy intervention, demonstrating that polyploid induction can improve agronomic characteristics (Vimala *et al.*, 2022). For vetiver, Maikami *et al.* investigated in vitro tetraploid induction to improve salt tolerance, although the relationship between ploidy level and stress tolerance requires further investigation (Maikami *et al.*, 2017).

Somaclonal Variation

Somaclonal variation, the genetic and epigenetic variation observed in plants regenerated from tissue culture, has been explored as a source of novel variation. Resmi investigated induction of somaclones in vetiver, identifying variants with altered morphological and biochemical characteristics (Resmi, 2013). While somaclonal variation can be a valuable source of genetic diversity, it also represents a challenge for maintaining genetic fidelity in micropropagation and transformation experiments (Anil *et al.*, 2018; Resmi, 2013).

Emerging Technologies and Future Directions

CRISPR/Cas9 and Genome Editing

The advent of CRISPR/Cas9 and related genome editing technologies has revolutionized plant biotechnology, offering unprecedented precision for targeted genetic modification. Recent reviews have highlighted the potential of CRISPR/Cas9-mediated genome editing in medicinal and aromatic plants (Shabir, 2021; Das *et al.*, 2024; Hefferon *et al.*, 2025). These technologies enable precise modification of endogenous genes without introducing foreign DNA, potentially addressing regulatory concerns associated with transgenic crops (Shabir, 2021).

For aromatic grasses, genome editing could be applied to: (1) knockout negative regulators of essential oil biosynthesis to enhance production; (2) modify terpene synthase genes to alter product specificity; (3) eliminate undesirable compounds from essential oil profiles; (4) enhance stress tolerance through targeted modification of stress-responsive genes; and (5) improve agronomic traits such as lodging resistance, disease resistance, and

biomass yield (Shabir, 2021; Das *et al.*, 2024; Hefferon *et al.*, 2025).

However, the application of CRISPR/Cas9 to *Cymbopogon* and vetiver requires the prior establishment of efficient transformation and regeneration protocols, as well as genomic resources to identify target genes and design guide RNAs. The recent completion of the *Cymbopogon citratus* genome sequence represents an important milestone that will facilitate genome editing efforts (Chakravartty *et al.*, 2023).

Cisgenesis and Intragenesis

Cisgenesis and intragenesis represent alternative genetic engineering approaches that utilize genes from the same species or sexually compatible species, potentially offering greater public acceptance than transgenic approaches (Sarmah *et al.*, 2021). For aromatic grasses, cisgenic approaches could involve overexpression of endogenous terpene synthases or regulatory genes to enhance essential oil production without introducing foreign genetic material (Sarmah *et al.*, 2021).

Synthetic Biology and Metabolic Engineering

Advances in synthetic biology offer new possibilities for engineering complex metabolic pathways in aromatic grasses. The modular nature of terpenoid biosynthesis, with well-characterized enzymes and regulatory elements, makes these pathways amenable to synthetic biology approaches. Future efforts may involve: (1) construction of synthetic operons containing multiple biosynthetic genes under coordinated regulation; (2) introduction of novel biosynthetic modules to produce non-native terpenoids; (3) engineering of subcellular compartmentalization to optimize metabolic flux; and (4) development of inducible expression systems for controlled production of essential oils (Shabir, 2021; Das *et al.*, 2024).

Genomic Resources and Molecular Markers

The development of comprehensive genomic resources is essential for advancing molecular breeding and biotechnology in aromatic grasses. Recent studies have employed various molecular marker systems for genetic characterization and diversity analysis in *Cymbopogon* species, including RAPD, ISSR, SSR, and AFLP markers (Adhikari *et al.*, 2017; Adeyemo and Osibote, 2023; Ganjewala, 2008; Kumar *et al.*, 2009; Adhikari *et al.*, 2021). These markers facilitate germplasm characterization, cultivar identification, and marker-assisted selection in breeding programs (Adhikari *et al.*, 2017; Adeyemo and Osibote, 2023; Adhikari *et al.*, 2021).

The completion of the *Cymbopogon citratus* genome sequence by Chakravartty *et al.* represents a significant milestone, providing a reference for comparative genomics, gene discovery, and genome editing (Chakravartty *et al.*, 2023). Similar genomic resources for other *Cymbopogon* species and vetiver would greatly accelerate molecular breeding efforts (Chakravartty *et al.*, 2023).

Integration with Phytoremediation and Bioeconomy

For vetiver, future biotechnological efforts should consider the integration of essential oil production with phytoremediation and bioeconomy applications. Genetic engineering could potentially enhance both phytoremediation capacity and essential oil yield, creating dual-purpose crops that provide environmental services while generating valuable products (Nakbanpote *et al.*, 2024; Pandey and Praveen, 2020). Strategies may include: (1) overexpression of metal transporters or detoxification enzymes to enhance heavy metal accumulation; (2) introduction of genes for degradation of organic pollutants; (3) enhancement of root biomass and architecture for improved soil stabilization; and (4) optimization of essential oil biosynthesis to maintain oil quality in contaminated environments (Nakbanpote *et al.*, 2024; Pandey and Praveen, 2020).

The potential for vetiver as a lignocellulosic feedstock for biofuel production adds another dimension to its bioeconomy value (Geiger *et al.*, 2019; Neve *et al.*, 2022). Genetic modification to improve biomass yield, reduce lignin content, or enhance saccharification efficiency could increase the economic viability of vetiver-based phytoremediation systems (Geiger *et al.*, 2019; Neve *et al.*, 2022).

CONCLUSION

Genetic modification and transformation studies in aromatic grasses, particularly *Cymbopogon* and vetiver, remain at an early stage of development compared to major crop species. While efficient *in vitro* regeneration systems have been established for multiple species through organogenesis and somatic embryogenesis, successful stable genetic transformation has been reported only for vetiver, with an 18% transformation efficiency using *Agrobacterium*-mediated methods (Yang *et al.*, 2008). No published reports of stable transformation in *Cymbopogon* species were identified, representing a significant gap in the biotechnological toolkit for these economically important aromatic grasses.

Recent advances in transcriptome sequencing and molecular characterization have provided valuable insights into the genetic basis of essential oil biosynthesis, identifying candidate genes for metabolic engineering (Bhat *et al.*, 2023; Meena *et al.*, 2016; Devi, 2015; Devi *et al.*, 2016). The completion of the *Cymbopogon citratus* genome sequence marks an important milestone that will facilitate future molecular breeding and genome editing efforts (Chakravartty *et al.*, 2023). However, the translation of this molecular knowledge into practical crop improvement through genetic engineering requires the development of robust,

reproducible transformation protocols for *Cymbopogon* species.

Conventional breeding and mutation breeding have demonstrated success in improving aromatic grasses, generating variants with enhanced essential oil yield, altered oil composition, and improved agronomic characteristics (Smitha and Manivel, 2021; Srivastava *et al.*, 2000; Munda *et al.*, 2022; Ibrahim and Khalid, 2013). These approaches will continue to play important roles in germplasm improvement, particularly in regions where genetically modified crops face regulatory or market acceptance challenges.

The future of aromatic grass biotechnology lies in the integration of multiple approaches: conventional breeding for near-term improvements, development of efficient transformation protocols to enable genetic engineering, application of genome editing technologies for precise modification of endogenous genes, and synthetic biology approaches for engineering complex metabolic pathways. For vetiver, the integration of essential oil production with phytoremediation and bioeconomy applications offers unique opportunities for creating multifunctional crops that provide both environmental services and valuable products (Nakbanpote *et al.*, 2024; Pandey and Praveen, 2020; Geiger *et al.*, 2019).

Key priorities for future research include: (1) development of efficient, reproducible transformation protocols for major *Cymbopogon* species; (2) functional characterization of key biosynthetic genes and regulatory elements controlling essential oil production; (3) application of genome editing technologies to modify endogenous genes without introducing foreign DNA; (4) investigation of epigenetic regulation of essential oil biosynthesis; (5) development of tissue-specific or inducible expression systems for controlled production of essential oils; and (6) integration of molecular breeding with conventional breeding to accelerate cultivar development.

The successful application of genetic modification technologies to aromatic grasses will require sustained research investment, interdisciplinary collaboration among plant biotechnologists, biochemists, breeders, and industry stakeholders, and careful attention to regulatory frameworks and public acceptance. With continued advances in genomic resources, transformation technologies, and our understanding of essential oil biosynthesis, the prospect of genetically improved aromatic grasses with enhanced productivity, quality, and environmental performance is increasingly within reach.

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