International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 18-11-2020; Accepted: 27-11-2020: Published: 13-12-2020
www.botanyjournals.com
Volume 5; Issue 6; 2020; Page No. 797-800

In vitro propagation of aromatic grasses: Explant sources

Manju M George
Department of Botany, Union Christian College, Aluva, Ernakulam, Kerala, India
Correspondence Author: Manju M George

Abstract
Aromatic grasses of the genera *Cymbopogon* and *Vetiveria* are economically important sources of essential oils used in perfumery, pharmaceuticals, and cosmetics. Micropropagation and callus-based regeneration systems offer efficient alternatives to conventional vegetative propagation for these species. This short communication reviews the types of explants employed in tissue culture of five key aromatic grasses: *Cymbopogon flexuosus, C. citratus, C. winterianus, C. martinii,* and *Vetiveria zizanioides*. Evidence from the literature indicates that mesocotyl segments, immature inflorescences, nodal segments, leaf bases, crown and tiller tissues, pseudostem sections, and rhizome explants have been successfully utilized for callus induction and plant regeneration. Explant selection significantly influences regeneration efficiency, with juvenile meristematic tissues generally exhibiting superior morphogenic potential. Mesocotyl explants from young seedlings and immature inflorescences are particularly effective for somatic embryogenesis, while nodal segments support both organogenesis and embryogenesis pathways. Understanding explant-specific responses is critical for optimizing micropropagation protocols and germplasm conservation strategies in aromatic grasses.

Keywords: Aromatic grasses, *Cymbopogon, Vetiveria*, explant, tissue culture, micropropagation, somatic embryogenesis

Introduction
Aromatic grasses belonging to the genera *Cymbopogon* and *Vetiveria* (family Poaceae) are cultivated worldwide for their essential oils, which have applications in perfumery, flavoring, traditional medicine, and industrial chemistry. Key species include lemongrass (*Cymbopogon flexuosus* and *C. citratus*), Java citronella (*C. winterianus*), palmarosa (*C. martinii*), and vetiver (*Vetiveria zizanioides*). Conventional propagation of these grasses relies on vegetative methods such as root slips and tillers, which are labor-intensive, slow, and susceptible to pest and disease transmission.

Tissue culture techniques, including micropropagation and callus-based regeneration, offer significant advantages for rapid clonal multiplication, germplasm conservation, and genetic improvement of aromatic grasses. The success of *in vitro* regeneration systems depends critically on the choice of explant, which determines the morphogenic pathway (organogenesis or somatic embryogenesis), regeneration frequency, and genetic stability of regenerants. This short communication synthesizes current knowledge on explant types used in tissue culture of the five major aromatic grass species, with emphasis on their regeneration responses and practical implications for propagation protocols.

Explant types and their regeneration responses

1. Mesocotyl Segments
Mesocotyl explants, derived from 3–4 day-old seedlings, have proven highly effective for callus induction and somatic embryogenesis in several *Cymbopogon species* and *vetiver*. These juvenile tissues possess high meristematic activity and low phenolic content, making them ideal for initiating embryogenic cultures. Mesocotyl-derived callus cultures have been successfully established in *C. flexuosus* and *V. zizanioides*, with regeneration frequencies often exceeding those of other explant types. The compact, nodular callus produced from mesocotyl explants typically exhibits sustained embryogenic potential over multiple subcultures when maintained on appropriate media. (George and Subramanian, 1999a, 199b, 2000) [8, 10]

2. Immature Inflorescences
Immature inflorescences represent another highly responsive explant source, particularly for *C. citratus* and *C. winterianus* (Bobade *et al*., 2010), (Mathur *et al*., 1988) [2, 14], (Sreenath & Jagadishchandra, 1991) [18]. The meristematic tissues within developing spikelets and floral primordia are naturally predisposed to morphogenesis. Inflorescence explants have been used to induce both embryogenic and organogenic callus, with regeneration occurring through somatic embryogenesis or direct shoot formation. In *C. citratus*, immature inflorescences cultured on MS medium supplemented with BA and 2,4-D produced high-frequency callus capable of sustained plant regeneration (Bobade *et al*., 2010) [2]. Similarly, *C. jwarancusa* regenerated efficiently from the meristematic base of spikelets (Mehandru *et al*., 2014) [13]. The use of inflorescence explants is particularly valuable for non-flowering or poorly flowering genotypes when alternative explant sources are available.

3. Nodal Segments
Nodal explants, containing axillary buds and associated meristematic tissues, have been widely employed for both direct organogenesis and callus-mediated regeneration in aromatic grasses (Nayak, 1996), (Nayak *et al*., 1996), (Patnaik *et al*., 1997) [15, 16]. In C. flexuosus, nodal segments cultured on MS medium with 2,4-D, NAA, and kinetin produced embryogenic callus that retained regeneration potential for over two years (Nayak *et al*., 1996) [15]. Nodal callus of C. martinii was successfully used to establish cell suspension cultures capable of somatic embryogenesis and plantlet regeneration (Patnaik *et al*., 1997) [16]. The advantage of nodal explants lies in their accessibility from

mature plants and their relatively consistent regeneration response across genotypes. However, contamination rates may be higher compared to seed-derived explants due to the presence of endophytic microorganisms.

4. Leaf-Based Explants

Leaf tissues, including young leaves, leaf bases, and leaf slices, have been tested as explants in several aromatic grass species with variable success (Leupin *et al*., 2000), (Das, 1999), (El-Bakry *et al*., 2012) [3, 5, 11]. In *V. zizanioides*, leaf slices from crown regions regenerated plantlets on approximately 60% of explants, with up to 100 plantlets per slice (Leupin *et al*., 2000) [11]. Young leaf explants of *C. schoenanthus* produced both embryogenic and organogenic callus capable of plant regeneration (El-Bakry *et al*., 2012) [5]. However, mature leaf tissues often exhibit poor callus response due to high phenolic exudation, which inhibits cell division and causes browning of culture media. The use of antioxidants such as polyvinylpyrrolidone (PVP) or activated charcoal can mitigate phenolic toxicity and improve callus induction from leaf explants.

5. Crown, Tiller, and Pseudostem Explants

Crown tissues, which contain multiple meristematic centers, have been successfully used for callus induction and regeneration in *vetiver* (Leupin *et al*., 2000), (Fauziah *et al*., 2017) [6, 11]. Crown slices cultured on modified MS medium with 2,4-D and BAP produced compact callus with high regeneration frequency (Leupin *et al*., 2000) [11]. Tiller explants, representing young vegetative shoots, have also been employed for callus initiation in *V. zizanioides*, though their response may vary with tissue age and physiological state (Fauziah *et al*., 2017) [6]. Pseudostem explants from *C. martinii* have been used to establish somatic embryogenesis systems, with successful plant regeneration and subsequent hardening. These explant types are particularly useful for vegetatively propagated species where seed availability is limited.

6. Rhizome Explants

Rhizome tissues, which serve as natural perennating organs in aromatic grasses, have been explored as explants for tissue culture. In *C. winterianus*, rhizome explants cultured on MS medium supplemented with 2,4-D and kinetin produced embryogenic callus capable of plant regeneration (Dey *et al*., 2010) [4]. Rhizome-derived cultures offer the advantage of maintaining clonal fidelity and accessing mature plant material year-round. However, rhizome explants may require more intensive surface sterilization protocols and exhibit slower initial growth compared to seed-derived explants.

7. Other Explant Types

Additional explant sources reported in the literature include basal stem portions, culm segments, axillary buds, and adventitious buds (Ma *et al*., 2006), (Sreenath *et al*., 1980) [17]. While these tissues can produce callus under appropriate conditions, they often exhibit lower regeneration frequencies or higher rates of phenolic exudation compared to the primary explant types discussed above. Seed-derived explants (including whole seeds and seedling segments) have been used for callus induction in *C. nardus*, with 2,4-D proving most effective for callus initiation (Sreenath *et al*., 1980) [17].

Factors influencing explant performance

The regeneration response of explants in aromatic grasses is influenced by several interacting factors beyond explant type. Tissue age and physiological state are critical determinants of morphogenic competence, with juvenile, actively dividing tissues generally exhibiting superior regeneration capacity (Fauziah *et al*., 2017) [6]. The genotype or cultivar also significantly affects explant response, as demonstrated by variation in callus induction and regeneration frequencies among different accessions of the same species (Das, 1999), (Mathur *et al*., 1988) [3, 14].

Media composition, particularly the type and concentration of plant growth regulators, interacts strongly with explant type to determine regeneration pathway and efficiency. Auxins such as 2,4-D are typically required for callus induction from most explant types, often in combination with cytokinins such as BAP or kinetin (Leupin *et al*., 2000), (Bobade *et al*., 2010), (Baruah *et al*., 1989), (Dey *et al*., 2010), (Gahukar *et al*., 2014), (Nayak *et al*., 1996) [1, 2, 4, 7, 11, 15]. The optimal auxin-to-cytokinin ratio varies with explant type and species. For example, mesocotyl explants of *C. flexuosus* responded best to MS medium with 1 mg/L 2,4-D and 0.1 mg/L kinetin, while immature inflorescences of *C. citratus* required 1 mg/L BA and 1 mg/L 2,4-D for optimal callus induction.

Regeneration from callus cultures typically requires transfer to media with altered growth regulator composition, often with reduced auxin and increased cytokinin levels to promote shoot formation, (Ma *et al*., 2006), (Baruah *et al*., 1989) [1], (Sompornpailin *et al*., 2016), (Nayak *et al*., 1996) [15]. Somatic embryogenesis pathways may be favored by media containing 2,4-D alone or with minimal cytokinin (Ma *et al*., 2006), while organogenesis is typically promoted by cytokinin-dominant media. The inclusion of additives such as casein hydrolysate, PVP, or riboflavin can enhance regeneration frequency and maintain morphogenic potential over extended subculture periods. (George and Subramanian, 1999) [8]

Comparative analysis of explant types

A synthesis of the literature reveals distinct patterns in explant performance across aromatic grass species. Mesocotyl segments and immature inflorescences consistently emerge as the most reliable explant sources for establishing high-frequency regeneration systems, particularly for somatic embryogenesis (Bobade *et al*., 2010), (Mathur *et al*., 1988) [2, 14], (Sreenath & Jagadishchandra, 1991), (Nayak *et al*., 1996) [15, 18]. These explants combine high meristematic activity with low phenolic content and relatively uniform developmental stage, facilitating reproducible culture initiation.

Nodal explants offer a practical alternative when seed material is unavailable or when direct organogenesis is preferred over callus-mediated regeneration (Nayak, 1996), (Nayak *et al*., 1996), (Patnaik *et al*., 1997) [15, 16]. The regeneration potential of nodal callus can be maintained for extended periods (over two years in some cases), making this explant type valuable for long-term germplasm conservation and somaclonal variation studies (Nayak *et al*., 1996) [15].

Leaf-based explants and crown tissues provide additional options for species or genotypes where other explant sources are limiting (Leupin *et al*., 2000), (El-Bakry *et al*., 2012) [5, 11]. However, these explants often require more

careful optimization of culture conditions, including the use of antioxidants to manage phenolic exudation. Rhizome explants represent a specialized category useful for maintaining clonal fidelity in vegetatively propagated cultivars (Dey *et al*., 2010) [4].

The choice of explant should be guided by the specific objectives of the tissue culture program. For rapid clonal multiplication and commercial micropropagation, mesocotyl or nodal explants are generally preferred due to their high regeneration frequencies and genetic stability. For somaclonal variation studies or genetic transformation, embryogenic callus derived from mesocotyl or inflorescence explants may be more suitable due to their sustained proliferative capacity and amenability to selection procedures. (See Table 1.)

Table 1: Explants used for *in vitro* propagation of aromatic grasses and their regeneration responses

Reference	Species	Explant Used	Regeneration Response / Major Findings
George & Subramanian (1999a) [8]	*Vetiveria zizanioides*	Mesocotyl	High-frequency embryogenic callus induction and complete plant regeneration.
George & Subramanian (1999b) [8]	*Cymbopogon flexuosus*	Mesocotyl	Efficient callus induction and high-frequency regeneration through somatic embryogenesis.
George & Subramanian (2000) [10]	*Cymbopogon citratus*	Immature inflorescence	High-frequency callus induction and regeneration through somatic embryogenesis.
Baruah *et al*. (1989) [1]	*Cymbopogon martinii*	Young shoot/meristematic tissue	High-frequency plant regeneration through both somatic embryogenesis and organogenesis.
Bobade *et al*. (2010) [2]	*Cymbopogon winterianus*	Immature inflorescence	Efficient shoot regeneration via embryogenic callus.
Das (1999) [3]	*Cymbopogon polyneuros*	Leaf explants	Callus induction and regeneration influenced by genotype and culture conditions.
Dey *et al*. (2010) [4]	*Cymbopogon winterianus*	Rhizome	Embryogenic callus formation followed by successful plant regeneration.
El-Bakry *et al*. (2012) [5]	*Cymbopogon schoenanthus*	Young leaves	Embryogenic and organogenic callus formation with successful regeneration.
Fauziah *et al*. (2017) [6]	*Vetiveria zizanioides*	Crown and tiller tissues	Callus induction affected by explant type and kinetin concentration.
Gahukar *et al*. (2014) [7]	*Cymbopogon winterianus*	Callus (derived from vegetative explants)	Efficient *in vitro* regeneration through callus cultures.
Leupin *et al*. (2000) [11]	*Vetiveria zizanioides*	Leaf slices, crown slices	Compact embryogenic callus; approximately 60% regeneration with up to 100 plantlets per leaf slice.
Ma *et al*. (2006)	*Vetiveria zizanioides*	Basal stem and culm segments	Somatic embryogenesis and regeneration influenced by explant type and culture conditions.
Mathur *et al*. (1988) [14]	*Cymbopogon winterianus*	Immature inflorescence	High-frequency embryogenic callus and regeneration; useful for somaclonal variation studies.
Nayak (1996) [15]	*Cymbopogon* (Jamrosa)	Nodal segments	Direct organogenesis and callus-mediated regeneration from nodal explants.
Nayak *et al*. (1996) [15]	*Cymbopogon flexuosus*	Nodal segments	Embryogenic callus with regeneration potential retained for more than two years.
Patnaik *et al*. (1997) [16]	*Cymbopogon martinii*	Nodal callus/cell suspension	Somatic embryogenesis and complete plant regeneration from suspension cultures.
Sreenath *et al*. (1980) [17]	*Cymbopogon nardus*	Whole seeds/seedling tissues	Efficient callus induction using 2,4-D with subsequent shoot regeneration.
Sreenath & Jagadishchandra (1991) [18]	*Cymbopogon* spp.	Immature inflorescences and meristematic tissues	Comprehensive review indicating immature inflorescences as one of the most responsive explants for regeneration.
Mehandru *et al*. (2014) [13]	*Cymbopogon jwarancusa*	Meristematic base of spikelet	Direct regeneration from immature spikelet meristem.

Conclusion

The successful micropropagation and callus-based regeneration of aromatic grasses depends critically on appropriate explant selection. Mesocotyl segments from young seedlings and immature inflorescences represent the most effective explant sources for establishing high-frequency somatic embryogenesis systems in *Cymbopogon* and *Vetiveria* species. Nodal segments provide a reliable alternative for both organogenesis and embryogenesis, with the added advantage of accessibility from mature plants. Leaf-based explants, crown tissues, pseudostem sections, and rhizome explants offer additional options for specific applications, though they may require more intensive optimization of culture conditions. The integration of explant selection with optimized media formulations, culture conditions, and acclimatization procedures will continue to advance the efficiency and reliability of tissue culture systems for aromatic grass propagation and improvement.

References

1. Baruah A, Bordoloi AK, Deka PC. High frequency plant regeneration of *Cymbopogon martinii* (Roxb.) Wats by somatic embryogenesis and organogenesis. Plant Cell Reports,1989:8:483-486.
2. Bobade PB, Kale SD, Ingle SR. *In vitro* shoot regeneration studies in Java citronella. Asian Journal of Biological Sciences,2010:5(2):134-138.
3. Das S. Effects of different physiochemical factors on regeneration of *Cymbopogon polyneuros* Stapf via callus culture and subsequent chromosomal stability. Israel Journal of Plant Sciences,1999:47(3):181-188.
4. Dey A, Mukherjee S, Kundu M. Somatic embryogenesis from rhizome explants of *Cymbopogon winterianus*. Biologia Plantarum,2010:54(2):325-328.
5. El-Bakry AA, Abo El-Nil AM, Saker MM. Regeneration from embryogenic callus and suspension cultures of the wild medicinal plant *Cymbopogon*

schoenanthus. African Journal of Biotechnology,2012:11(32):8115-8127.
6. Fauziah F, Ratnasari E, Indriyani S. The effect of explant types and kinetin concentration on *in vitro* callus induction in *Vetiveria zizanioides* (L.) Nash. Journal of Experimental Life Science,2017:7(2):82-87.
7. Gahukar SJ, Kale SD, Ingle SR. An efficient protocol for *in vitro* regeneration in Java citronella (*Cymbopogon winterianus*) through callus. Asian Journal of Biological Sciences,2014:9(2):267-272.
8. George MM, Subramanian RB. High frequency regeneration of *Vetiveria zizanioides* (khus) via mesocotyl culture. Phytomorphology,1999:49(3):309-313.
9. George MM, Subramanian RB. *In vitro* plant regeneration in Lemongrass (*Cymbopogon flexuosus*) via mesocotyl culture. Journal of Plant Biology,1999:26(3):243-246.
10. George MM, Subramanian RB. Regeneration of West Indian Lemongrass *Cymbopogon citratus* DC (Stapf) from immature inflorescence cultures. Journal of Plant Biology,2000:27(2):189-192.
11. Leupin M, Kammacher M, Morel P. Compact callus induction and plant regeneration of a non-flowering vetiver from Java. Plant Cell, Tissue and Organ Culture,2000:63:169-177.
12. Ma Y, Xu X, Chen Y. Effecting factors of somatic embryogenesis and plant regeneration in vetiver. Journal of Tropical and Subtropical Botany,2006:14:321-326.
13. Mehandru P, Shekhawat A, Shekhawat I. *In vitro* plant regeneration of *Cymbopogon jwarancusa* (Jones) Schult from meristematic base of spikelet. National Academy Science Letters,2014:37(2):165-169.
14. Mathur K, Ahuja SK, Pandey A, Mandal A, Mathur AK. Screening and evaluation of somaclonal variations for quantitative and qualitative traits in an aromatic grass, *Cymbopogon winterianus* Jowitt. Plant Breeding,1988:100:145-153.
15. Nayak PK, Sahoo P, Patnaik S. Rapid propagation of lemongrass (*Cymbopogon flexuosus* (Nees) Wats.) through somatic embryogenesis *in vitro*. Plant Cell Reports,1996:15:367-370.
16. Patnaik S, Sahoo P, Chand PK, Patnaik SC. Somatic embryogenesis and plantlet regeneration from cell suspension cultures of palmarosa grass (*Cymbopogon martinii*). Plant Cell Reports,1997:16:430-434.
17. Sreenath HL, Jagadishchandra KS, Sulladmath K. Callus induction and growth in two varieties of *Cymbopogon nardus* (L.) Rendle (Ceylon citronella). Current Science,1980:49(18):712-713.
18. Sreenath HL, Jagadishchandra KS. *Cymbopogon* Spreng. (aromatic grasses): *In vitro* culture, regeneration, and the production of essential oils. In: Bajaj YPS, Ed. Biotechnology in Agriculture and Forestry. Berlin: Springer-Verlag, 1991, 211-236.