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# Plant regeneration through somatic embryogenesis of pseudostem callus culture response in In-vitro condition of palmarosa grass (*Cymbopogon martinii*) with special reference to hardening and pot culture

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**Abstract:** This study successfully achieved somatic embryogenesis and plant regeneration from callus culture of the plant *Cymbopogon martinii* (Palmarosa Grass). The ability of medicinally significant palmarosa grass to regenerate through somatic embryogenesis of pseudostem callus culture has been investigated using hardening and pot culture. *Cymbopogon martinii* pseudostem explants were employed, and the Murashige and Skoog medium (MS basal media) was added with 2,4-dichlorophenoxyacetic acid (2,4-D). In MS basal medium supplemented with 2,4-D (3 mg/1it.)+ 6-Benzyl Adenine Purine (BAP) (1 mg/1it.), plant regeneration through embryogenesis occurred. In MS baseline media supplemented with α-Naphthelene Acetic Acid (NAA) (2 mg/1it.)+BAP (0.01 mg/1it.) and NAA (1.5 mg/1it.)+BAP (0.01 mg/1it.), whole plantlets were formed. Microplant for hardening was transplanted with well-developed rootlets from *in-vitro* to *in-vivo*. Sand, soil, and cow dung cake were mixed in a 1:1:1 ratio for the potting mixture, which was then exposed to light, temperature, and humidity. The results demonstrate that MS medium enriched with 1 mg/lit. of 2,4-D allowed for the greatest induction of callus and proliferation. In the current investigation, it was discovered that 2,4-D supplementation to MS medium was successful in triggering *Cymbopogon martinii*'s callus response.

## Introduction

*Cymbopogon martinii*, a Gramineae (poaceae) perennial herbaceous grass with high essential oil concentration and therapeutic value (Patnaik et al., 1977; Abe and Futsuhara, 1984; Baruah and Bardoloi, 1989; Raina et al., 2003; Shah et al., 2011; Wifek, et al., 2016). The plant is indigenous to Asia, South Central America, and Africa's tropical and subtropical regions. The plant, also referred to as "Palmarosa grass," produces a range of essential oils. It is primarily grown in marginal west lands and on land that is covered with forests. Palmarosa grass grows best in sunny, warm, and humid situations. Additionally, it is drought-tolerant. Palmarosa oil, which resembles a rose and has a sweet aroma, is frequently used in perfumery, cosmetics, and pharmaceuticals (Wang and Nguyen, 1990; Khajuria et al., 2021). Typically, vegetative slips are used to produce palmarosa grass (Acharya, 2012). In addition to its traditional usage for rheumatism, hair loss, arthritis, lumbago, and other conditions, it has significant medical uses for cough, bronchitis, and other respiratory illnesses (Sreenath and Jagadishchandra, 1991; Malla et. al., 2015). Additionally, the C. martinii contains antifungal, antiviral, and antibacterial activities (Gupta et al., 1969, 1970, Bharati et al., 2013; Bussmann et al., 2015; Malla et al., 2015).

Looking over the literature, it seems that studies of the generation of embryogenic callus from somatic tissue in *C. martinii* were not conducted as part of the tissue cul-

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ture work done on *Cymbopogon species*. The plant species has, however, been the subject of extensive research on somatic embryogenesis (Sharifi-Rad et al., 2020; Chawla and Wengel, 1987; Dey et al., 2010; Méndez-Hernández et al., 2019). Numerous researchers have reported on the callus culture and plant regeneration through somatic embryogenesis from pseudostem explants of palmarosa grass (Mathur et al., 1988; Sreenath and Jagadishchandra, 1991; Armstrong and Philips,1988). It has a significant degree of heritability. Cross-pollination, therefore, occurs frequently in the grass crop. This crop's primary source of oil is the leaves themselves. The stream distillation technique is used to extract oil. Because of this, oil content has decreased during the past year. Therefore, tissue culture is crucial for this particular *Cymbopogon* species. In the current work, an effort was made to standardize the hardening and pot culture techniques and attempt *Cymbopogon martinii* plant regeneration using somatic embryogenesis of pseudostem callus culture.

## Materials and Methods

### Callus culture

Nodal segments of the pseudostem of mature tillering *Cymbopogon martinii* plant parts measuring about 3-5cm were cleaned with 5% (v/v) Teepol' detergent solution for 30 min and surface sterilized with 0.15% (w/v) HgCl, for 7-10 min followed by 4-6 times wash in sterile distilled water before inoculation into nutrient agar medium (modified MS-1962) supplemented with 2,4 D. NAA and kinetin are added in different concentrations. One explant (about 1 cm) was placed in a culture tube and replicated ten (10) times. Regenerated plants developed on basal medium supplemented with 2 mg./lit. NAA. 1mg./lit., BAP and 4 mg./lit. NAA, 1mg./BAP with 2-4.5 sucrose (Armstrong and Philips, 1988; Baruah and Bordoloi, 1989). For each treatment, 20–40 gm./lit. of sucrose, 0.7–0.9% agar, and a pH adjustment to 5.7–5.9 were added to the modified medium before autoclaving. Cultures were kept alive for four weeks during a 16-hour photoperiod in 25mm x 15mm culture tubes or bottles under cool fluorescent lighting. 26.22°C and 80% humidity were maintained in the culture room. For plant regrowth, calli were subcultured every 30 days. For the production of somatic embryoids, 2 month old calli were introduced to MS basal medium supplemented with different combinations and concentrations of auxin and cytokinin. After 4-5 weeks, somatic embryoid production increased dramatically (Gupta and Jain, 1978; Hussain, 1982; Dhawan, 1993).

### Hardening and pot culture

The micro plant size for hardening was transplanted with fully formed rootlets from *in-vitro* to *in-vivo*. Plants with a height of 3–4 cm were chosen for transplantation. Sand, loamy soil, and soilrite (1:1:1) or sand, loamy soil, and cow dung cake were used to make the potting mixture (1:1:1). The specified circumstances were: temperature ranged from 25 to 32°C, relative humidity ranged from 82 to 85%, the light was 100% and shade for the first month, then 75% and shade for the following month. Spraying water and using plant protectants as a fungicide was also required.

## Results and Discussion

On MS media enriched with 2,4-D at dosages of 0.1, 0.5, 1, 2, and 3 mg/ lit. either alone or in combination with 0.1, 0.25, and 1 mg/lit. kinetin, callus initiation was achieved from the pseudostem explant. Callus induction was seen on a medium without kinetin and 2,4-D (2 mg/lit.). Interestingly, neither at lower nor higher doses of 2,4-D did callus initiation occurs (Tables 1, 2, and 3).

On MS medium enriched with 1 mg/lit. of 2,4-D, the callus and proliferation were inducted to their highest levels. A stock was kept on this media by repeated subculturing after every 3–4 weeks. The callus was whitish yellow in colour and developed into a nodular mass (Fig. la). To investigate induction and plantlet regeneration from callus culture, hormone combinations comprising 2,4-D (1-3 mg/lit.) with kinetin or BAP (0.5-1 mg/lit.) and alternatively IAA with kinetin and BAP were utilised. Similar techniques were successfully established by Gorge and Sherrington (1984) for plant tissue culture propagation. 2,4-D alone was shown to be unsuccessful in causing somatic embryoids to form. Still, combined with BAP or kinetoin caused somatic embryos to form to variable degrees (Fig. 2). The callus surface initially had a few green patches visible (Fig. 1b), and then a shoot started to grow. Before being placed in a medium for regeneration, no roots formed. In MS basal medium enriched with NAA (2 mg/1.4 mg/ 1it.) and BAP (0.5 and 1 mg/1it.), Somatic embryoids (Fig. 1c) quickly grew into green plantlets with distinct shoots and roots (Gamborg, 1975a, 1975b).

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**Table 1. Growth regulator effects on induction of callus of *Cymbopogon martinii* in different basal mediums. All concentrations are expressed in mg/lit.**

| Growth regulators [mg/lit] | Basal medium | Callus induction |
|---|---|---|
| 2,4-D[0.1] | MS | - |
| 2,4-D[0.5] | Do | - |
| 2.4-D[1] | Do | + |
| 2.4-D[2] | Do | +++ |
| 2,4-D[3] | Do | ++ |
| NAA[1] | Do | - |
| NAA[2] | Do | - |
| Kinetin[0.1] | Do | - |
| Kinetin[0.5] | Do | - |
| 2,4-D{2} + Kinetin [0.1] | Do | + |
| 2,4-D[2] + Kinetin (0.25) | Do | + |
| 2,4-D[2]+ BAP [1] + NAA[1] | Do | + |
| Symbols growth nil: + slow growth: ++ moderate growth: +++ Fast growth | | |

**Table 2. Effects of 2,4-D and BAP on callus for regulation of somatic embryos of *Cymbopogon martinii*. All concentrations are expressed in mg/lit.**

| Growth regulators | Basal medium | Regulation of somatic embryos |
|---|---|---|
| a)2,4-D[1] + Kin [0.5] | MS | - |
| b) 2,4-D[1] + Kin [1] | Do | - |
| c) 2,4-D[2] +Kin [0.5] | Do | + |
| d) 2,4-D[2] + Kin [1] | Do | + |
| e) 2,4-D[3] + Kin [0.5] | Do | + |
| f) 2.4-D[3] + Kin [1] | Do | ++ |
| g) 2,4-D[1]+ BAP[0.5] | Do | - |
| h)2,4-D[1]+ BAP[1] | Do | - |
| i) 2,4-D[2]+ BAP [0.5] | Do | + |
| j)2,4-D[2]+ BAP [1] | Do | + |
| k) 2,4-D[3] + BAP [0.5] | Do | ++ |
| 1) 2,4-D[3] + BAP [1] | Do | +++ |
| m) IAA[1]+BAP [0.5] | Do | - |
| n) IAA [1] + Kin[0.5] | Do | - |
| Symbols growth nil: + slow growth: ++ moderate growth: +++ Fast growth | | |

**Table 3. Effects of growth regulators on somatic embryos of complete plantlet *Cymbopogon martinii*. All concentrations are expressed in mg/lit.**

| Growth regulators | Basal medium | Formation of complete plants |
|---|---|---|
| NAA[2]+ BAP [0.5] | MS | ++ |
| NAA[4]+ BAP (0.5] | Do | + |
| NAA[2]+BAP[1] | Do | +++ |
| NAA[4]+ BAP[1] | Do | ++ |
| Symbols: + slow growth; ++ moderate growth; +++ Fast growth | | |

The frequency of embryo germination was based on MS medium containing NAA (2 mg/l) and BAP (1 mg/lit.) shown in Fig. 1d. Similar findings were reported by Ghosh, (1995) and Jagadischandra (1975). The media supplemented with kinetin showed no remarkable effect compared to those supplemented with BAP. The embryoids were consistently obtained from the callus culture followed by regeneration of embryos of plantlet (Fig. le) on such medium tested so far followed by the study (Ber, 1953; Kole et al., 1980).

According to the study, *C. martinii*'s hormonal needs for callus induction, embryo development (Gupta, 1969), maintenance, and plant regeneration were broadly consistent with those of other grass and grain crops. While NAA and IAA were found to be ineffective for callus induction with the addition of low-level kinetin, MS medium supplemented with 2,4-D showed very slow callus induction and development (Baruah and Bordoli, 1989; Patnaik et al., 1997). This is consistent with previous research on other *Cymbopogon* species (Bansil, 1971; Armstrong and Philips, 1988).

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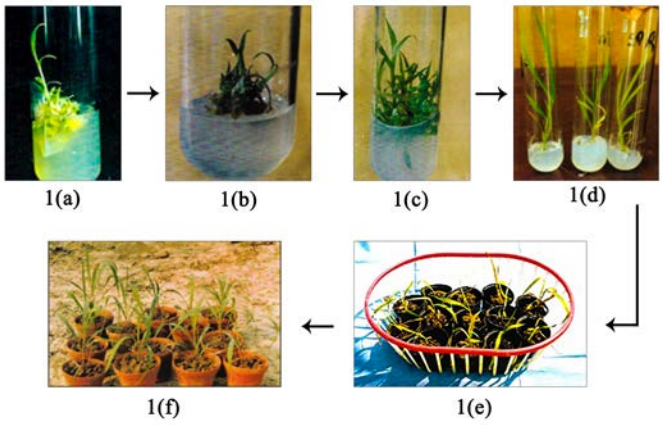


**Figure 1. Plant regeneration from pseudostem callus cultures of *Cymbopogon martinii*. Callus regenerated from the surface part of pseudostem *of Cymbopogon* sp. (a) Callus culture showing embryo-like structure maintained on MS medium with 3 mg/1it. 2,4-D and 1 mg/lit.**

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