HIGH FREQUENCY REGENERATION OF VETIVERIA ZIZANIOIDES (L.) VIA MESOCOTYL CULTURE

MANJU M. GEORGE & R.B. SUBRAMANIAN
Department of Biosciences, Sardar Patel University,
Vallabh Vidyanagar-388 120, Gujarat, India

Abstract

Callus induction and high frequency regeneration was achieved from mesocotyl parts of young seedlings of 'Khus' (Vetiver) when cultured on MS medium supplemented with 2,4-D and Kinetin. Calli were maintained in MS medium with 100 mg\textsuperscript{1} CH and PVP each alongwith 2-4,D and Kinetin. Shoot formation occurred from 40-50 days old pale yellow, nodular callus when subcultured on MS basal medium or MS medium augmented with BA. Rooting of shoots occurred in MS medium supplemented with NAA. Plantlets were then transferred to soil for acclimatization.

Key Words : Vetiver, grasses, essential oil, in vitro propagation.

Vetiveria zizanoides, commonly known as ‘khus’ is a member of Poaceae indigenous to India. This plant is cultivated mainly in the tropical and sub-tropical regions for the essential oil produced in its roots. Vetiver is mainly propagated through vegetative slips as the seed setting is very poor and their viability is too short. This precludes the possibility of genetic variation that leads to genetic erosion in this plant.

The oil, mainly sesquiterpenes is used in perfume, cosmetic and pharmaceutical industries. In perfume industry, this oil is used as a basic element in perfume blends and to fix the odour of more volatile materials (Sethi & Gupte 1960).

The fragrant roots are used for making mats, baskets, fans, sachets and ornaments. The living grass is also used as a soil binder to prevent soil erosion (National Research Council 1993). Medicinally, the oil is used as a diaphoretic and a preservative against cholera (Kammathy 1968).

Plant regeneration from totipotent cultured cells is a prerequisite for genetic manipulation through somatic hybridization or genetic engineering. Somatic embryogenesis is reported to be the most common method of plant regeneration in members of poaceae (Vasil 1988).

On the basis of a detailed characterization of embryogenic tissue cultures, Vasil & Vasil (1981) emphasized the need to use explants from immature organs such as embryos and seedlings that contain undifferentiated cells. Mucciarelli et al. (1993) reported regeneration of Vetiver using basal nodal explants from mature plants. The present paper describes...
the callus induction and high frequency regeneration of Vetiver from the mesocotyl explants of young seedlings as an essential step for genetic manipulation in this plant through tissue culture techniques.

**Material and Methods**

Plants of *Vetiveria zizanoides* var-ODV III were collected from Aromatic and Medicinal Plants Research Station (AMPRS), Odakkali, Kerala and cultivated in the Sardar Patel University Botanical garden. Seeds were collected in the months of October - November. For callus initiation, young leaves and basal part of the stem from mature plants and mesocotyl parts from young seedlings were used as explants. For mesocotyl explants, seeds were soaked in distilled water for 24 hr and the viable seeds were selected on the basis of sedimentation in water, out of these 40-50% seeds germinated (data not shown) when transferred to Petri dishes on water-soaked whatman's filter paper. Seedlings of four to five days were sterilized with 0.1% HgCl₂ for 1 min and thoroughly washed with sterile distilled water five times. For all experiments, Murashige & Skoog's (1962) medium supplemented with 3% sucrose (Hi Media, India) was used. The pH of the medium was adjusted to 5.8 followed by the addition of 0.8% agar (Hi Media, India) before autoclaving at 15 psi for 15-20 min. For callus induction, growth hormones such as 2,4-dichlorophenoxyacetic acid (2,4-D), naphthalene acetic acid (NAA), kinetin (KN) and benzyl adenine (BA) at concentrations ranging from 0.1 to 4mg l⁻¹ were used either alone or in combinations. Two seedlings were used per culture as explants for callus induction along with in vivo young leaves, and mature leaf blades basal part of the stem. Cultures were maintained under 14 hr light provided with cool white fluorescent lamps (3000 lux) at a temperature of 25 ± 2°C. The standard error for the number of shoots obtained in various combinations of shoot induction media was calculated from 12 replicates.

**Observations and Discussion**

Callus initiation was attempted from various explants such as young leaves, basal part of the stem and mesocotyl parts on MS medium supplemented with 2,4-D at concentrations ranging from 0.1 to 4mg l⁻¹ either alone or in combination with 0.1 to 4mg l⁻¹ Kinetin. The young leaves did not respond and even failed to survive. They exuded a brown leachate, probably phenolics in nature, into the medium. The basal part of the stem exhibited some callus formation. However, the exudation of brown leachate retarded the callus growth. Similar observations were made in another aromatic grass *Cymbopogon* by Mathur et al. (1988). Callus formation occurred at the cut ends of the mesocotyl explants after three to four days of culture (Fig. 1A). After 40 to 50 days, nodular, pale yellow callus was obtained on medium augmented with 1mg l⁻¹ 2,4-D along with 1mg l⁻¹ Kinetin (Fig. 1B). These calli were maintained in MS medium supplemented with 100 mg l⁻¹ polyvinyl pyrrolidone (PVP) and casein hydrolysate(CH) each. Such callus cultures maintained their morphogenetic potential for more than seven subcultures (subcultured every 35-40 days). Vasil (1987) reported that in grasses the morphogenetic potential of the callus is either lost or rhizogenesis from callus occurs within two subcultures. In the present study it was observed that in the absence of either PVP or CH the calli lost their morphogenetic potential after few subcultures.
as reported for other grasses. This clearly indicates that, the presence of both PVP and CH is essential for maintaining the morphogenetic potential of the callus of this plant species. MS medium supplemented with 2,4-D or NAA alone gave rise to brownish or creamy callus having no morphogenetic potential. The source of explants was found to be important in inducing morphogenic callus. Sreenath & Jagadishchandra (1991) also observed in Cymbopogon that mature embryos, seedlings, mesocotyl and young inflorescence of the mature plants gave rise to morphogenic calli while calli from the rhizome, root and vegetative culms were non-morphogenic.

Fig.1A-D - Various stages of regeneration in Vetiver from mesocotyl explants. A. Callus initiation in cut end portion from mesocotyl explants. B. Nodular pale yellow callus in MS medium with 1mg/l 2,4-D and 1mg/l KN. C. Multiple shoot induction in MS with 1mg/l BA. D. Rooting of the shoots in MS with 0.05mg/l NAA.
Supplementation of cytokinins such as Kinetin or BA alone (0.1 to 2mg⁻¹) led to the elongation of mesocotyl explants without any callus formation. The morphogenic callus appeared to be pale yellow, nodular in nature. Morphogenic callus formation has been reported in other gramineae species also (Wernicke & Brettel 1980).

In our experiments, microtillering started when the 40 to 50-d-old callus obtained from mesocotyl explants was transferred to MS basal medium, devoid of any growth hormones or MS medium fortified with 0.5 to 2mg⁻¹ BA (Fig. 1C). Addition of lower concentrations of NAA to the medium containing BA suppressed the number of shoots. Similar observations were made by Baruah & Bardoloi (1991) in another aromatic grass *Cymbopogon*.

MS medium augmented with 1mg⁻¹ BA was found to be highly suitable for the induction of more number of shoots. About 30 shoots formed per culture. However in MS basal medium, the frequency of shoots formed was less (Table 1). When kinetin was used alone, shoot buds differentiated but failed to elongate further.

<table>
<thead>
<tr>
<th>MEDIUM USED</th>
<th>AVERAGE NUMBER OF SHOOTS (SE)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS only</td>
<td>12.75 ± 0.82</td>
</tr>
<tr>
<td>MS+0.5 mg⁻¹ BA</td>
<td>13.2 ± 1.03</td>
</tr>
<tr>
<td>MS+1 mg⁻¹ BA</td>
<td>29.75 ± 0.69</td>
</tr>
<tr>
<td>MS+2 mg⁻¹ BA</td>
<td>22.67 ± 0.93</td>
</tr>
<tr>
<td>MS+1 mg⁻¹ BA+0.2 mg⁻¹ NAA</td>
<td>19.67 ± 0.55</td>
</tr>
<tr>
<td>MS+2 mg⁻¹ BA+0.2 mg⁻¹ NAA</td>
<td>14.75 ± 0.64</td>
</tr>
</tbody>
</table>

*Mean ± Standard error (SE) calculated from 12 replicates

It may be concluded that MS medium with 1mg⁻¹ BA was found to be optimal for regeneration in Vetiver. Removal of 2,4-D from the medium was necessary for regeneration in Vetiver as has been reported for other grass species (Sethi & Gupte 1960).

Healthy shoots were transferred to MS medium with different concentrations of IAA, IBA or NAA (0.02 to 1mg⁻¹) for rooting. Among these, MS medium with 0.05 mg⁻¹ NAA was found to be suitable for rhizogenesis, and root formation occurred within seven days of culture (Fig. 1D). Higher concentrations of NAA produced callus at the base of the shoots. In the present study rooting could not be induced with IAA or IBA which is in contrast to earlier reports on aromatic grasses such as *Cymbopogon* (Sreenath & Jagadishchandra 1991) and Vetiver (Mucciarelli et al. 1993). The plantlets were transferred to sterile cocopeat under high humid condition for 15 days and later transferred to soil.

This study revealed that the hormonal requirement for callus induction, its maintenance and regeneration of plantlets in Vetiver largely conformed to that of other grasses and cereal crops reported earlier (Mathur et al. 1988, Ammirato et al. 1984). Supplementation of synthetic auxin 2,4-D in the medium was sufficient to induce callus formation as has been reported in *Cymbopogon* (Sreenath & Jagadishchandra 1980). Vasil (1987) reported that
Addition of lower levels of Kinetin (< 0.5 mg\(^{-1}\)) in 2,4-D (> 1 mg\(^{-1}\)) containing medium was beneficial for callus growth and development in grass species. In the present study, 1 mg\(^{-1}\) each of 2,4-D and Kinetin was required for the induction and maintenance of morphogenic calli. The frequency of shoot formation obtained in the present investigation is much higher than those reported by Muciarelli et al. (1993). Since Vetiver roots start synthesizing essential oils only after 18-20 months of growth, field trials on the in vitro raised plants are yet to be conducted. Regeneration of Vetiver through tissue culture will prove to be beneficial for producing improved variants such as disease resistant and higher oil yielding varieties.

**Literature Cited**


Baruah A & Bardoloi D N 1991 Growth and regeneration of palmorosa (Cymbopogon martini (Roxb) Wats.), callus tissue under varied nutritional status; *Indian J. Exptl. Biol.* 29 582-583


Mathur A K, Ahuja P S, Pandey D, Kokreja A K, & Mandel S 1988 Screening and evaluation of somaclonal variations for quantitative and qualitative traits in an aromatic grass, Cymbopogon winterianus jowitt; *Plant Breeding* 321-334


Murashige T & Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures; *Physiologia Pl.* 15 473-497

Sethi K L & Gupte R 1960 Breeding for high essential oil content in Khus (*Vetiveria zizanioides*) roots; *Indian Perfumer* 24 72-78

Sreenath H L & Jagatshchandra K S 1980 In *Plant Tissue Culture, Genetic Manipulation and Somatic hybridization of Plant cells*, p 328-334 eds P S Rao, M R Heble & M S Chadha (BARC Publication)

Sreenath H L & Jagadishchandra K S 1991 Cymbopogon Spreng (Aromatic grasses): In Vitro Culture Regeneration and Production of Essential oils; *Biotechnology in Agriculture and Forestry, Medicinal and Aromatic Plants* 15 211-236

Vasil I K 1987 Developing cell and tissue culture systems for the improvement of cereal and grass crops; *J. Pl. Physiol.* 128 153-218

Vasil I K 1988 Progress in the Regeneration and Genetic Manipulation of Cereal Crops; *Biotechnology* 6 387-402

Vasil V & Vasil I K 1981 Somatic embryogenesis and Plant Regeneration from tissue cultures of *Pennisetum americanum* and *P. purpureum* hybrid; *Am. J. Bot.* 68 862-872

Wernicke W & Brettel R 1980 Somatic embryogenesis from Sorghum bicolor leaves; *Nature* 287 138-139