vegetation, then, Limkhetar shares the first position with Kodba, due to sustainable gochar lands and selection of trees species keeping in mind the needs of forest-dependent community. Considering the number of years of protection in case of Kodba and Limkhetar it could be observed that both stand almost equal in terms of ecological (carbon sequestration, dominance and diversity indices) and economical (gochar land, forest/household and % landless) parameters, with Limkhetar slightly higher in position ecologically due to higher species diversity and due to presence of gochar land in this area and Kodba slightly higher economically. Few more factors in relation to implementation of these plantation strategies like protection given to plantation in early years resulting in increased species diversity as in case of Nani Chikhli, presence of gochar land resulting into less stress on forest also have significant impetus on success of any plantation programme.

Ecorestoration requires that the redevelopment process should go in accordance with or achieve parallel progress in environment, social and economic sectors both in short and long term and across a range of spatial scales. Such a goal could be achieved only by implementing proper planning strategies as mentioned above while carrying out the plantation activities. In this context, linking up ecological process with social process becomes significant to ensure community participation in ecorestoration leading to sustainable livelihood development of this area.

Criterion-based ranking of different villages in terms of their contribution towards restoration of the area has highlighted the significance of polyculture strategy which should go parallel with the protection of these plantation in early years and which would definitely lead to the success of any plantation programme.

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Received 25 May 2004; revised accepted 28 September 2004

Guar gum and isubgol as cost-effective alternative gelling agents for *in vitro* multiplication of an orchid, *Dendrobium chrysotoxum*

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Guar gum (isolated from the endosperms of *Cyamopsis tetragonoloba*) and isubgol (husk derived from *Plantago ovata* seeds) have been successfully used as exclusive gelling agents for *in vitro* multiplication of an orchid, *Dendrobium chrysotoxum* from leaf and protocorm explants. The explants were cultured on Mitra's medium supplemented with 2% sucrose, 1 g l^{-1} peptone and gelled with either 0.9% agar, 3% guar gum or 3% isubgol. The medium used for leaf explants contained 1 mg l^{-1} BA. Both the alternative gelling agents supported differentiation of protocorm-like bodies and their growth into shoots two and half to threefold higher than agar.

ORCHIDS constitute an outstanding royalty in the world of ornamentals because of their intricately fabricated flowers of exquisite beauty. Orchids account for 2.7% of the global cut-flower production in terms of their value[1]. Because of heterozygous nature of orchids and their extremely slow vegetative propagation, tissue-culture techniques are routinely applied for their clonal multiplication. In fact, the application of these techniques for the production of quality plants in large quantities and propagation of exquisite and rare hybrids have catapulted orchids among the top ten cut flowers in the international market[2]. As for other plants, culture media used for orchids are also gelled with agar, an expensive component. Agar has remained the most frequently used gelling agent for microbial and plant tissue

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culture media since its introduction, more than a hundred years ago by Robert Koch[3].

The properties of agar, which make it the gelling agent of choice, are stability, high clarity and resistance to metabolism during culture. However, some investigators have raised doubts about its biological inertness and non-toxic nature[4–6]. Moreover, the exclusive use of agar may result in over-exploitation of its resources[7,8]. Due to these reasons and high price of tissue-culture-grade agar, attempts were initiated in our laboratory to identify suitable alternatives. Earlier, we have reported the successful use of isubgol (derived from *Plantago ovata* seeds) and Gum Katira (exudated from *Cochlospermum religiosum* bark) as exclusive gelling agents for eliciting morphogenic responses as varied as caulogenesis, rhizogenesis, androgenesis, and embryogenesis in some model systems[8,9]. In this communication, we report the successful use of guar gum and isubgol as exclusive gelling agents in nutrient media for protocorm-like bodies (PLBs) and foliar explants of the orchid, *Dendrobium chrysotoxum*.

The medium as described by Mitra *et al.*[10], adjuvated with 1 gl^{-1} of peptone and 3% sucrose was used for all experiments. The media were gelled with 3% guar gum, 3% isubgol or 0.9% agar. For preparing agar gelled media, agar along with sucrose (HiMedia, India) was added in an amount of water less than the total volume of the medium to be made. This mixture was heated in an autoclave until the pressure reached 68.9 kPa. After switching off the autoclave and letting the pressure come to zero, molten agar along with dissolved sucrose was taken out and with other constituents of the medium, the volume was raised to the required level and pH was adjusted to 5.8 using the temperature compensation device of the pH meter, and aliquots (20 ml) were dispensed in either 25 × 150 mm culture tubes or 90 mm petri dishes. However, for all treatments (agar, isubgol or guar gum) of an experiment, the same type of culture vessel was used and autoclaved at 103.36 kPa, 121°C for 15 min. For preparing guar gum and isubgol-gelled media, guar gum or isubgol and sucrose were mixed with other constituents of the medium and the volume was raised to the required level on a magnetic stirrer before adjusting the pH to 5.8. The medium was dispensed in individual culture vessels after autoclaving.

PLBs were excised from existing one-month-old protocorm cultures maintained on agar-gelled Mitra's basal medium. Leaf explants, comprising basal half of about 1-cm-long leaves[11], were taken from the 90-day-old cultures. Leaf explants were inoculated on BA (6-benzylaminopurine, 1 $mg\,l^{-1}$)-supplemented medium, while for PLBs, hormone-free basal medium was used. All cultures were incubated at 25 ± 2°C under continuous light of 17.76 $\mu mol\ m^{-2}\ s^{-1}$ provided by cool daylight fluorescent tubes (40W, Philips, India). Individual cultures were scored for the number of PLBs and shoots differentiated after 45 and 90 days of culture respectively. All the experiments were repeated at least once, each time maintaining 48 replicates per treatment. The data were subjected to statistical analysis employing chi-square test ($P = 0.05$) to find the significance of difference.

After about a fortnight of culture, cent per cent of both the explants started differentiating PLBs on all the gelling agents. Observations recorded after 45 days of culture revealed that the number of PLBs formed from PLBs on guar gum and isubgol-gelled media were two and a half to three times more than that recorded on agar gelled medium (Table 1, Figure 1 *a–d*). The response of leaf explants was likewise higher on guar gum and isubgol-gelled media compared with agar-gelled medium. After 90 days, 70–99% of the PLBs had converted to shoots in different treatments (Table 1). Isubgol had somewhat better promotory effect on the observed response than guar gum (Table 1). The apparent better promotory effect of isubgol does not seem to be because of use of two different types of culture vessels for guar gum and isubgol treatments and their corresponding controls, as the values of these two controls though not significantly different, were numerically lower in case of agar controls for isubgol than guar gum (Table 1). The better response on guar gum and isubgol-gelled media could be due to the absence of some inhibitors which have been reported to be present in agar[4–6]. Alternatively, the promotory effect of two gelling agents used might be due to an unidentified beneficial compound(s) present in them. However, both the possibilities will remain within the realm of speculation till the exact cause(s) for the promotory effect of these gelling agents is/are identified.

During the last decade, there has been an increase in the efforts to look for suitable substitutes for agar. Consequently, a number of substances have been tested for their gelling ability. Table 2 lists some of the gelling agents which have been used with reasonable success, along with their cost per litre of the medium.

Starch, the cheapest of the gelling agents, is not expected to find universal acceptance as an alternative gelling agent because of its inferior gelling quality, lower clarity than agar and its metabolizable nature, which leads to softening of the media during the culture period[8]. Moreover, it softens

Table 1. Morphogenic response of protocorm and leaf explants of *Dendrobium chrysotoxum* cultured on media gelled with agar, guar gum or isubgol

Gelling agent	Protocorm explant		Leaf explant	
	Average no. of PLBs/ explant	Average no. of shoots/ explant	Average no. of PLBs/ explant	Average no. of shoots/ explant
Agar	5.9[c]	5.4[c]	5.8[b]	5.6[b]
Guar gum	14.8[a]	14.5[a]	10.7[a]	7.4[a]
Agar	4.9[c]	4.4[c]	4.8[c]	4.7[c]
Isubgol	15.1[a]	15[a]	12.2[a]	10.1[a]

*Values followed by the same superscript in a column are not significantly different ($P = 0.05$).

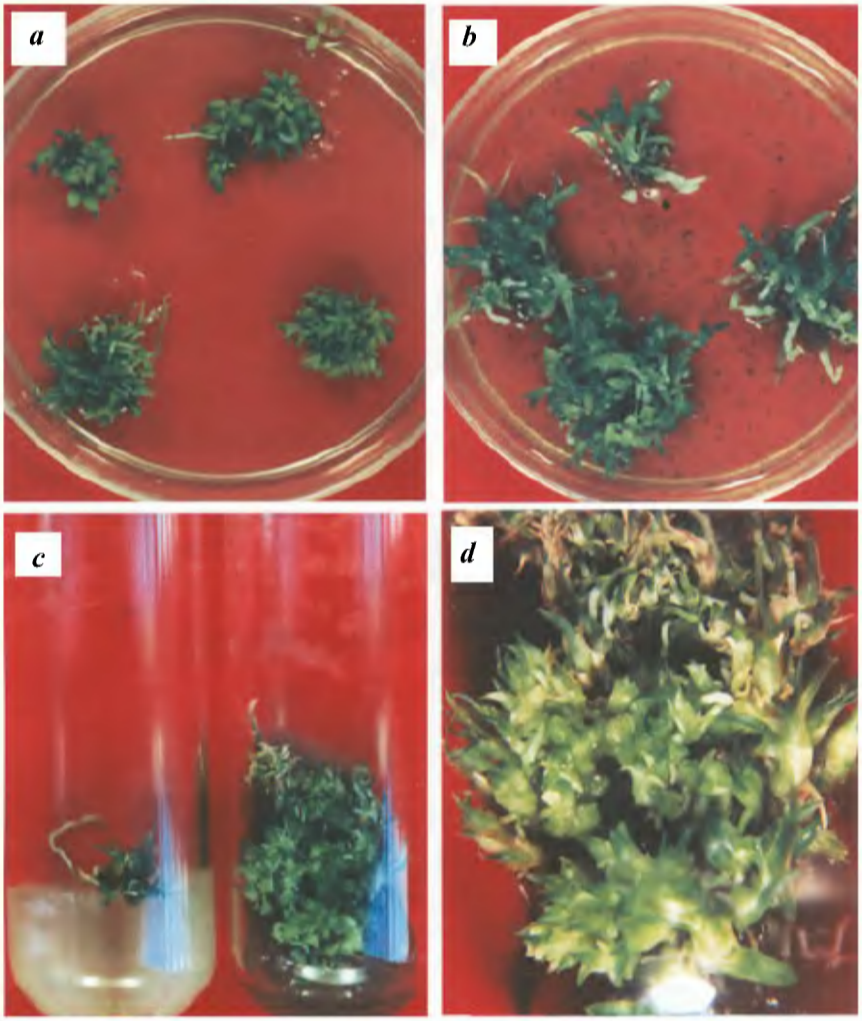

Figure 1 ***a–d.*** Shoots developed from PLBs of *Dendrobium chrysotoxum* after 90 days of culture on agar (***a***, 1X) and isubgol (***b***, 1X) gelled media, and agar (left) and guar gum (right) gelled media (***c***, 1.5X). ***d***, A magnified view of PLBs differentiated on guar-gelled medium (3.5X).

Table 2. Comparative cost of different gelling agents used for plant tissue culture media

Gelling agent	Cost (Rs/g)	Concentration used (%)	Cost/l (Rs)
Agarose (Sigma)	38610/500	0.9	695
Agar (Difco-bacto)	4950/454	0.9	98
Agar* (Qualigens)	950/500	0.9	17
Alginate (sodium salt, Sigma)	3285/500	0.5–2	131 at 2%
Carrageenan (Sigma)	3780/500	1	76
Ficoll (Sigma)	34155/500	10–40	27324 at 40%
Guar gum* (HiMedia)	120/500	3–4	7.2 at 3%
Gum Katira	150/500	3	9
Isubgol* (Telephone brand)	120/500	3	7
Phytagel (Sigma)	4770/500	0.3–0.5	48 at 0.5%
Starch (Tapioca)	15/500	10	3

*Used in the present study.

even if the media are left un-inoculated[12]. Upon autoclaving starch yields sugars, which have their own effects on cultures, osmotic or metabolic. Agarose, a component and purification product of agar is superior to agar in gelling ability and transparency[13]. However, because of its high cost, agarose cannot be used for routine experiments and in plant tissue culture industry. Carrageenan and alginates, because of their gelling ability only in the presence of specific ions are not suitable substitutes of agar[14,15]. Ficoll does not form a gel, but makes the medium viscous causing floatation of explants[16]. Moreover, at the used concentration, the cost per litre of medium is higher than even agarose. Gelrite has many desirable properties, but causes hyperhydricity and vitrification in some cases[17]. Therefore, though not a perfect replacement for agar, it

has found wide acceptance[17,18]. But for its higher melting point (70.6°C), which necessitates quick dispensing[8], isubgol is a cost-effective gelling agent with desirable properties[9]. Gum Katira, like ficoll, does not form a firm gel at 3%, but the explants remain on the surface if left undisturbed. In transparency, Gum Katira-gelled medium is comparable to the liquid medium. Therefore, it can be an excellent gelling agent for experiments requiring regular observations of cells, tissues or organs growing inside the medium[9].

As cost of isubgol and guar gum per litre of the medium is about 2.5–13 times less than different brands of agar (Table 2), these are two highly cost-effective gelling agents, which could be used for reducing the cost of *in vitro*-propagated orchids plants. Both being of plant origin, are biodegradable and do not pose any threat to the environment on being dispensed-off after use. Moreover, both *P. ovata* and *Cyamopsis tetragonoloba*, sources of isubgol and guar gum respectively, are easily cultivated and therefore, their increased demands can be easily met. However, media gelled with isubgol or guar gum, require quick adjustment of pH and dispensing because of their higher gelling point.

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ACKNOWLEDGEMENT. We thank Prof. S. P. Vij, Department of Botany, Panjab University, Chandigarh for providing cultures of *Dendrobium chrysotoxum*.

Received 19 June 2004; revised accepted 14 September 2004

Spectral attenuation models in the Sikkim Himalaya from the observed and simulated strong motion events in the region

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Strong motion recordings by the Sikkim Strong Motion Array (SSMA) of 80 events with good signal-to-background noise ratio (≥ 3) for magnitude between 3 to 5.6, have enabled estimation of source model and site response, and also the simulation of spectral acceleration for moderate-to-large earthquakes with $6 \leq M_W \leq 8.3$. A combined simulated and recorded data set have ultimately been utilized for deriving the spectral attenuation laws incorporating the local site conditions, namely, topographic effect and site response, with and without shear wave velocity in addition to the normal earthquake parameters, viz. moment magnitude (M_W) and source-to-site distance (r). The spectral attenuation laws developed in this study have been found to be appropriate for predicting free-field horizontal Peak Ground Acceleration (PGA) for earthquakes of $3 \leq M_W \leq 8.3$ and for the sites with distances up to 100 km from the source in the Sikkim Himalaya. The estimated spectral acceleration through these attenuation laws mimic the mean spectral acceleration simulated using Brune's source model.

THE Sikkim Himalaya located in the northeastern Indian Peninsula, is seismically one of the six most active regions

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