Cotton Fiber as a Substitute for Agar Support in Tissue Culture

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Abstract. Cotton fibers were tested as a substitute for agar in tissue culture. The cost of agar has prompted us to search for an alternative more economical medium support. Effectiveness as a medium support was evaluated in terms of callus maintenance and shoot organogenesis using *Artemisia*, *Agrostis*, and *Taxus*. *Taxus* and *Agrostis* calli cultivated on liquid media with cotton fiber as medium support (25 ml of medium per gram of cotton) grew better than calli on agar (0.8% w/v). There were no significant differences in shoot organogenesis of *Artemisia* and *Agrostis* grown in 25 ml of medium per gram of cotton from those grown in agar medium.

Although most plant tissues can grow well in liquid media, a semisolid medium is recommended for use under some circumstances. Since 1943, agar has been widely used as a tissue culture support (White, 1943). According to Romberger and Tabor (1971), contaminants present in agar can be released and affect the culture of plant tissues. Starch copolymer (Cook, 1977), fabric tissue (100% polyester) (Cheng and Voqui, 1977), filter paper platforms (Horsch et al., 1980), glass fiber (Tabor, 1981), and gellan gum (Eyre and Caswell, 1991) have been reported as alternatives to agar. In Brazil, agar support is an expensive component of tissue culture medium. In the present study, rolled cotton fiber was tested as a possible substitute for agar because it is readily available and at a low cost (U.S. \$19.00/kg). To our knowledge, cotton has not been reported as a medium support for plant tissue cultures. The objectives of this research were to compare agar and cotton support for callus maintenance and clonal propagation using several species (*Artemisia annua* L., *Agrostis stolonifera* var. *palustris* Farwell, *Taxus ×media* Rehd cv. *Hicksii*).

Materials and Methods

Callus induction and maintenance. Callus initiation and growth of *Agrostis* were according to Blanche et al. (1986). Seeds were surface-disinfected in 0.5% NaOCl (10% v/v Clorox) solution with four drops of Tween 20 for 20 min. Treated seeds were rinsed in sterile distilled water and then cultured on MS salts (Murashige and Skoog, 1962), 3% (w/v) sucrose, 4.5 μM 2,4-dichlorophenoxyacetic acid (2,4-D), 0.8% (w/v) Sigma type E agar (Sigma Chemical Co., St. Louis). The medium was adjusted to pH 5.7. Cultures were incubated in darkness and subcultured four times (8-week intervals) before the experiment.

Agrostis callus (200 to 250 mg/vessel) was cultured on 25, 16.7, and 10.9 ml medium per gram of rolled cotton (Johnson and Johnson, Skillmann, N.J.) and 0.8% (w/v) type E agar and incubated in darkness. Cotton used in this study was 100% pure, sterile, nonchlorine-bleached rolled fiber manufactured according to USP specifications. Cotton fiber (1.2 g) was compressed, placed in glass culture vessels (4 cm diameter $\times$ 9.5 cm high, baby food jars with Magenta B cap), and sterilized. Sterile medium (30, 20, or 13 ml) was poured into vessels containing the sterilized cotton fibers. Agar-amended sterile medium (25 ml) was dispensed into each vessel. Callus was transferred to cotton- or agar-supported medium. The experiments were replicated five times in a completely randomized design. This experiment was conducted twice. Growth was mea-

sured 4 weeks later as fresh weight increase.

Callus growth in *Taxus* was modified from Fett-Netto et al. (1992). Young stem segments of *Taxus ×media* cv. *Hicksii* were collected from 26.5-liter potted plants that were 1.0 m tall (Cottage Gardens, Lansing, Mich.). In July 1993, stem segments (6 to 8 mm long) were collected and surfaced disinfected with 1.03% NaOCl [20% (v/v) Clorox (Clorox Corp., Cleveland)] solution, plus four drops of Tween 20, for 20 min. Treated explants were rinsed three times in sterile distilled water and cultured in MS medium with 4.5 μM 2,4-D. The pH was adjusted to 5.7. The cultures were incubated under fluorescent lights with photon flux of $\approx 52 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for a 16-h photoperiod at $25 \pm 2^\circ\text{C}$. Calli started to develop on the cut portions of the explants not immersed in the medium. Apparently, the calli developed from the cambium and outer parenchyma stem cells. Cultures were subcultured before the study.

Taxus callus growth was maintained on B5 salts (Gambor et al., 1968) supplemented with 18.1 μM 2,4-D, 4.6 μM kinetin, and 1% (w/v) polyvinylpyrrolidone (PVP-10) (Fett-Netto et al., 1992). *Taxus* callus (270 to 305 mg/vessel) was then subcultured to this medium using cotton and agar support previously described. The cultures were incubated for 30 days. Callus growth was measured by fresh weight increase. Each treatment had five replications and the experiment was conducted twice.

Shoot organogenesis. Nodal and apical segments (3 to 5 mm) were collected from *Artemisia* plants cultivated in the Medicinal Plant Garden at the Research Institute of Pharmaceutical Sciences, Univ. of Mississippi. The explants were surface-disinfected in 1.03% NaOCl [20% (v/v) Clorox] solution plus four drops of Tween 20 for 10 min and then rinsed three times in sterile distilled water.

Shoot cultures in *Artemisia* were modified from Nair et al. (1986). The explants were cultured on MS salts, with 30 g of sucrose, 2.3 μM kinetin, 1.1 μM indole-3-acetic acid (IAA), and cotton (25, 16.7, or 10.9 ml medium per gram of cotton) and agar support. The pH was adjusted to 5.8. Cultures were incubated for 30 days under the same conditions described previously. After the incubation period, the number of microshoots derived from the axillary buds was recorded. *Artemisia* microshoots were rooted in MS medium to confirm viability. Each treatment was replicated four times and the experiment was conducted three times.

Nodal segments (3 mm) of differentiated plantlets were excised from shoots derived from *Agrostis* calli (Blanche et al., 1986). Explants were cultured on MS half-strength salt medium with cotton (25, 16.7, or 10.9 ml medium per gram of cotton) and 0.8% agar as medium support, and 30 g of sucrose. The pH was adjusted to 5.7. The cultures were incubated under the same environment conditions as *Artemisia* shoot cultures. After an incubation period of 30 days, the number of shoots was recorded. All analyses on callus fresh weight increases in *Agrostis* and *Taxus* and shoot cultures of *Artemisia* and *Agrostis* were performed using SAS (1985).

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Results and Discussion

Growth of *Agrostis* and *Taxus* calli on cotton support with 25 ml medium per gram of cotton was superior to that on agar (Table 1). The higher amounts of callus growth of *Taxus* and *Agrostis* on cotton may be due to better nutrient diffusion on cotton than agar, and/or due to agar impurities, as suggested by Romberger and Tabor (1971). However, the refinement of type E agar used in this study lessens the potential for callus injury due to impurities.

Microshoot formation of *Agrostis* and *Artemisia* was similar whether cotton at 25 ml medium per gram or agar was used as support for the explants. Both species was affected by the degree of cotton saturation (Table 1). For *Agrostis* shoot culture, there was no statistical difference between agar-supported cultures and cotton support. However, when 10.9 ml of medium was added to cotton, the number of shoots induced decreased by more than half. Shoot formation in *Artemisia* was significantly reduced with less medium applied on cotton.

In terms of callus growth and microshoot proliferation, cotton was a suitable substitute for agar for tissue culture of *Taxus*, *Agrostis*, and *Artemisia*, provided sufficient medium was applied to the cotton. Root induction on *Artemisia* and *Agrostis* was unaffected by medium support. Presence of roots was noticed in microshoots of both species after 30 days of culturing on either support. According to Cheng and Voqui (1980), the use of fabric tissue (100% polyester) facilitates periodic changes of the medium without transfer of the cultured tissues, and this also may be true for cultures using cotton support. Minimal disturbance of callus culture and reproducibility were the basis of the filter paper growth assay technique of Horsh et al. (1980). This study

Table 1. Cotton or agar as supports for callus growth and shoot formation in cultures of *Agrostis*, *Taxus*, and *Artemisia*.

Genus	Support			Dunnnett's ($P \leq 0.05$)
	Cotton (ml of medium/g of cotton)		Agar (% w/v)	
	10.9	16.7	25	0.8
<i>Callus growth</i>				
<i>Increase in fresh weight (g/culture)</i>				
<i>Agrostis</i>	0.20*	0.48	0.64*	0.41
<i>Taxus</i>	0.10*	0.39	0.78*	0.37
<i>Organogenesis</i>				
<i>No. shoots/explant</i>				
<i>Agrostis</i>	2.0	5.0	4.7	4.2
<i>Artemisia</i>	1.1*	4.4*	9.2	9.8

*Indicates cotton treatments that differ from agar treatment at $P \leq 0.05$ using Dunnnett's test.

shows that cotton can be used for callus growth and very precise data also may be obtained with the same benefit. However, each plant species and use should be tested to verify advantages or disadvantages.

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