INCREASED GROWTH OF HYBRID SPRUCE BUD CULTURES ON STATIONARY LIQUID MEDIUM

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Abstract

The effects of using liquid medium to increase growth of bud cultures of Spartan spruce (Picea glauca X P. pungens) were examined. The goals of the study were to improve the survival and elongation frequencies of the cultured buds, and to develop a culture system that could be used to initiate cultures from mature spruce trees during any season of the year.

All cultures were initiated with buds excised from lateral branches of 10 year-old Spartan spruce. Bud scales were removed under a disecting microscope, and the upper two-thirds of the buds were cultured on both agar-solidified and liquid medium containing 10-4 M IAA and 10-6 M kinetin. No surface sterilization techniques were used. After four weeks all cultures were evaluated for average weight, needle elongation frequency, lateral shoot development, shoot elongation, and callus induction frequency. Cultures on liquid medium exceeded those on agar-solidified medium for all traits. Basal callus formation has occurred on these shoots, but no actual roots have been observed.

Additional keywords: Tissue culture, mature spruce, shoot growth.

INTRODUCTION

The development of efficient culture systems for cloning mature spruce tissue has been extremely slow. Some progress has been made at culturing immature spruce (Campbell and Durzan 1975, 1976), however to date, no process for reproducing intact trees from mature tissue has been developed. Several problems have impeded the development of a culture system for mature spruce. One problem is isolating tissue that can be cultured at any time of year without contamination. Another major problem is sustaining growth and shoot elongation in the cultured tissue. Finally, it has thus far been impossible to regenerate roots on shoots derived from mature tissues.

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Stationary liquid medium has been demonstrated to be superior for the culture of many species. Included in these species are Nicotiana Wernicke and Kohlenbach, 1976; Sunderland and Roberts, 1977), Arabidopsis (Keathley and Scholl, 1982) Brassica (Lichter, 1981), Petunia (Mitchell et al., 1981), barley (Kao, 1981), and wheat (Wilson, 1977).

The physiological basis of the success of such cultures is uncertain. In some instances the agar used in solid medium may inhibit culture growth (Kohlenbach and Wernicke, 1978). The medium in the region of the cultured tissue is also less likely to become depleted of nutrients and plant growth regulators with liquid medium. Finally, metabolic by-products will not build up in the region of the cultured tissue as quickly in liquid medium as they will on agar-solidified medium. In any event, the use of liquid medium appears to be advantagous in some plant tissue culture systems.

In this study the use of liquid medium for the culture of dormant buds from hybrid spruce (Picea glauca X P. pungens) was examined. The goals of the study were to improve the frequency of elongation, degree of elongation, and survival frequency of the cultured buds, and to develop a culture system that could be used to initiate cultures from mature spruce trees during any season of the year.

MATERIALS AND METHODS

Plant Material

All cultures were initiated with buds excised from lateral branches of 10 year-old hybrid spruce (Picea glauca X P. pungens). Branches were collected in July, after bud-set had occurred. Bud scales were removed under a directing microscope, and the unelongated shoot was placed on culture medium. No surface sterilization techniques were applied to the tissue.

Culture Medium

The culture medium was DBM-1 (Gresshoff and Doy, 1972) augmented with 10-6 M kinetin and 10-4 M indole-3-acetic acid (IAA). All medium was adjusted to pH 5.8 and filter sterilized. The solid medium contained 8.0 grams of agar per liter. One hundred and twenty buds (Three samples of size 40) were cultured on each type of medium. Shell vials (21 X 70 mm) were used for cultures on liquid medium, and test tubes (18 X 150 mm) were used for cultures on solid medium. In both cases 5 ml of medium were used.

Culture Conditions

Cultures were kept at 24 degrees centigrade, 80 percent relative humidity, and under constant illumination. After four weeks the cultures were scored for frequency of shoot elongation, average weight, frequency of buds showing needle elongation, frequency of callus formation at the base of the bud, and the frequency of buds with lateral shoots developing.

Data Analysis

The data were analyzed using standard statistical procedures for the analysis of variance. All frequency data were transformed using the arcsin transformation for porportions prior to analysis to minimize the inequalities of error variance that occur in frequency data (Snedecor and Cochran, 1976).

RESULTS

Callus Induction

Callus growth occurred at the basal end of the buds and along some of the elongating needles. Callus tissue on the needles was generally white and quickly became necrotic. The callus induction frequency was significantly higher for cultures on liquid medium (table 1). In addition, callus tissue grown on liquid medium was green and firm, whereas callus induced on solid medium was brown and necrotic.

Shoot Elongation

The frequency of buds that elongated was equal on both types of medium (table 1). The actual degree of elongation was not measured due to the increased probability of contamination during measurement. Shoots produced in liquid medium were longer than those grown on solid medium. Although lengths were not measured, the increased growth of the cultures on liquid medium is clearly demonstrated by the differences in average weight (table 1).

Lateral Shoots

In 38 percent of the cultures grown in liquid medium multiple shoots were induced in the callus region at the basal end of the explant. No additional shoots grew in the cultures on solid medium. This, along with the elevated levels of growth for the other parameters that were measured, indicates the possibility of increased meristematic activity in cultures grown in liquid medium.

Needle Elongation

One surprising result of this study was the observation that needle and shoot elongation can occur independently. Needle elongation occurred at a higher frequency in cultures in liquid medium (table 1), but on both types of medium there were buds where the needles elongated and the bud did not. There were also cases on the solid medium where the bud elongated but the needles did not (86 percent of the buds elongated, but only 22 percent showed signs of needle elongation). Along this same line, in three cultures (one on liquid medium and two on solid medium) growth in length was observed without any noticeable increase in diameter.

Average Weight

The differences in average weight (table 1) best delineate the differential growth rates that were obtained with these two types of medium. After four weeks of growth the shoots on liquid medium had an average weight of 235 mg (max. 286 mg, min. 155 mg), and those on solid medium had an average weight of only 5 mg (max. 6 mg, min. 3 mg). This nearly fifty-fold increase in growth clearly shows the advantage of using liquid medium for cultures of this type.

Table 1. Measurements of hybrid spruce bud culture growth on solid and liquid medium.

	Medium Type	
	Solid	Liquid
Callus Induction Frequency	48	93**
Shoot Elongation Frequency	86	90
Percent with Lateral Shoots	0	38**
Needle Elongation Frequency	22	100**
Average Weight (mg.)	5	235**

** Mean square significant at the 0.01 level of probability.

DISCUSSION

Cultures grown on liquid medium equalled or exceeded those grown on solid medium for all traits that were measured (table 1). Although both culture types resulted in a high frequency of buds showing some elongation (90% on liquid and 86% on solid), the cultures on liquid medium showed substantially greater growth. The reason for this is not clear, but the possibility that it was caused simply by the increased availability of fluid for cell enlargement can be ruled out, since the observed increase in lateral shoot and callus growth both indicate increased meristematic activity in buds cultured in liquid medium. Whether this is due to increased availability and constancy in the levels of the plant growth regulators near the explant, a slower build-up of metabolic by-products in the region of the explant, or the absence of growth inhibiting impurities from the agar when liquid medium is used could not be determined. In any case, the growth of explants in liquid medium was significantly better than the growth obtained using agar-solidified medium, and the use of liquid medium is recommended for future studies aimed at developing micropropagation systems for mature spruce.

The most intriguing aspect of this study for future consideration is the indication that height, diameter, and needle growth may be regulated by separate pathways. Further study of this could result in refined methods for using hormone treatments to manipulate and regulate the growth of seedlings in greenhouses.

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