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Cost of Propagating Broccoli Plants through Tissue Culture[1]

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Abstract. **Costs were estimated for vegetatively producing plants of broccoli (*Brassica oleracea* L. (Italica group) through tissue culture with 3 culture stages and growing the plants 7 weeks in the greenhouse to field transplant size. The analysis was based on producing 11,000 finished transplants per week. Unit costs were estimated at 11.6¢ for tissue culture production of each plantlet and an additional 3.8¢ greenhouse cost for growing to transplant size.**

A format for estimating and calculating costs for tissue culture propagation of plants has not been published although it is an important consideration in commercial production. The information for this paper was collected as part of the pilot study phase of commercially producing broccoli transplants for hybrid seed production. The objective of this report is to identify the costs and develop a format to calculate unit costs for tissue-cultured plants. This analysis includes the costs of operations through the transplant stage. A basic assumption is that the laboratory will be continually operated by propagating a mixture of crops. For ease in this example, broccoli is the only crop being produced during this time period and 100% of the facility and labor is being charged to broccoli.

The initial propagules for Stage 1 are flower buds from broccoli stock plants (Table 1). After 5 weeks in culture, the shoots that arise are excised and subcultured on Stage 2 medium with a 5-fold increase in 5 weeks. The shoots are subcultured again to obtain another increase of shoots. These shoots are then transferred to the Stage 3 conditions for 3 weeks for root development and hardening of the plantlets. The plantlets were planted into Todd Planting Flats (Model 150) and grown in the greenhouse to transplant size in 7 weeks (Stage 4).

The no. of plants that can be produced per week was determined by the maximum no. of cultures that 4 tissue culture technicians can explant in 1 week (Table 2). This included explanting a sufficient no. of cultures in each propagation stage during the week to satisfy the final weekly production goal of 11,000 finished plants, and media preparation and cleanup. In addition of the 4 tissue culture technicians, other employees included in this example are a supervisor and a greenhouse technician.

To determine unit costs of producing broccoli, the following assumptions

Table 2. Calculation of labor requirements for explanting operations by stage of propagation.

Stage	No. of explants	Rate of explanting per hr	Hr of labor
I	147	63	2.3
II Cycle 1	440	63	7.0
II Cycle 2	2,200	63	34.9
III	11,000	125	88.0
	Total		132.2

Table 1. The 4 stages of broccoli propagation and the no. of explants to be cultured or transplanted to produce 11,000 per week.

Stage	Weeks duration	Type of propagule	Propagules required	Fold increase
I	5	Flower buds	147	3
II Cycle 1	5	Shoots	440	5
II Cycle 2	5	Shoots	2,200	5
III	2	Shoots	11,000	1
IV	7	Rooted plantlets	11,000	1

Table 3. Estimated costs for producing 11,000 tissue cultured broccoli plantlets.

A. Wage and salary cost		
1. Explanting labor (132.2 hr[z] @ $3.60)	$475.92	
2. Media preparation & cleanup labor (27.8 hr @ $3.60)	100.08	
3. Production supervision and administration ($20,500/yr)	394.23	
Total wage and salary cost		$ 970.23
B. Media cost (using premixed salts)		
1. Stage 1 and 2 media (2787 explants × 25 ml/explant = 70 liters @ $.717)	$ 50.19	
2. Stage 3 media (11,00 explants × 1 culture jar/27 explants = 407 culture jars × 75 ml/culture jar = 31 liters @ $.615)	19.07	
Total media cost		69.26
C. Culture vessel cost		
1. 25 × 150 mm culture tubes and plastic caps (2787 @ .10¢/unit = $278.70 amortized over twenty 5-week cycles @ 9% interest)	$ 15.24	
2. Culture jars (407 @ 5¢/unit = $20.35 amortized over ten 2-week cycles @ 9% interest)	2.07	
Total culture vessel cost		17.31
D. Equipment and laboratory cost		
1. Equipment ($10,000 amortized over 260 weeks @ 9%)	$ 47.79	
2. Laboratory facility ($50,000 amortized over 1040 weeks @ 9%)	$103.71	
Total equipment and laboratory cost		$ 151.50
E. Overhead costs		
1. Real estate tax ($50,000 @ 2.5% = $1250/52 wk)	$ 24.04	
2. Utilities (electricity, water, sewer, telephone)	22.00	
3. Office expenses	20.00	
Total miscellaneous cost		66.04
Total tissue culture cost		$1274.34

[z]See Table 2.

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were made. Laboratory technicians earning $3.60 per hr (including fringe benefits), could asceptically plant 63 Stage 1 and Stage 2 propagules per hr in culture tubes and could plant 125 Stage 3 propagules per hr in quart jars. Media costs were based on premixed Murashige and Skoog inorganic salts and costs of each additional constituent added to each medium. Media volume was 25 ml per culture tube and 75 ml per quart jar. It was assumed that 1 technician working 27.8 hr per week could prepare the weekly media supplies and keep the laboratory and glassware clean. The culture vessel costs were calculated for the no. required per unit and were amortized over the average useful life. The laboratory supervisor's annual salary was assumed to be $20,500 (including fringe benefits) and was allocated based on 1 week for 11,000 plants.

The equipment cost of $10,000 was based on estimates of several commercial laboratories that have purchased and used equipment and have used ingenuity in trimming costs. The equipment costs were amortized over 5 years. The laboratory facility cost was estimated at $50,000 and was amortized over 20 years. Tissue culture overhead costs were estimated by checking costs for local utilities and taxes.

Equipment, laboratory, and overhead costs were allocated at the rate of 1 week for 11,000 plants. This allocation was made on a weekly production basis, instead of a cost per unit area (as ft^2) of growth room, since culture room space is a minor cost to the total operation. Furthermore, space requirement will change as both the multiplication factor and the length of time in culture room are variable for each crop under consideration.

For the greenhouse phase of the operation, it was estimated that 275 broccoli plantlets can be transplanted per hr into planting flats. The no. of flats and amount of soil mix required were based on 120 cells per flat. The bench charge assumed was $1.61/m^2$ (15¢ per ft^2) per week.

The cost analysis shows labor costs to be a significant factor in the total tissue culture production costs. It is apparent that labor saving alterations will reduce production costs. Elimination of Stage 3 would reduce the tissue culture costs 3¢ per unit. Another important factor in reducing unit costs is to increase the no. of propagules multiplied in each culture passage.

The cost of broccoli propagation will be affected by various factors including size of operation, labor requirements and wages, equipment and laboratory investment, and greenhouse expenses. Because of the differences, costs should be budgeted for each situation. This study provides a guide for estimating broccoli propagation costs.

Table 4. Estimated greenhouse costs for producing 11,000 finished tissue cultured broccoli to transplant size.

A. Wages for transplanting (11,000 plantlets transplanted @ 275/hr = 40 hr @ $3.60)		$144.00
B. Material costs		
1. Flats[z] (11,000 cells x 1 flat/120 cells = 92 flats @ $2.09 = $192.28 amortized over twenty 7-wk cycles @ 9% interest	$10.88	
2. Soil mix required 0.56 m^3 @ $35.71/$m^3$ = 20 cu ft @ $1)	20.00	
Total material costs		30.88
C. Bench charge[y] (11,000 cells x 48 cells/.09m^3 = 21.4 m^2 @ 1.61/m^2 per week x 7 weeks =		241.18
Total greenhouse cost		$416.06

[z]Todd Planting Flats, Model 150.

[y]Includes depreciation, interest, repairs, taxes, insurance, utilities, indirect labor, and overhead costs for the use of the greenhouse. *Note:* 1 sq ft = .09 m^2; 1 cu ft = .028m^3.

Table 5. Summary of unit costs producing broccoli to transplant stage based on 11,000 plants produced per week.

I. Tissue culture production		
A. Wage and salary cost	8.8¢	
B. Media cost	0.6	
C. Culture vessel cost	0.2	
D. Equipment and laboratory cost	1.4	
E. Overhead cost	.6	
Total tissue culture cost		11.6¢
II. Greenhouse production		
A. Labor wages	1.3¢	
B. Flats and soil mix cost	0.3	
C. Bench charge	2.2	
Total greenhouse cost		3.8
Total cost per broccoli transplant		15.4¢

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In Vitro Propagation of Horseradish with Leaf Pieces

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Horseradish (*Armoracia lapathifolia* Gilib. Cruciferae) is one of the few horticultural crops propagated as true root cuttings. This perpetuates many viruses and root borne diseases causing yield reductions (2) as growers take propagation material from production fields. Multiplication of new and virus-free cultivars takes time by root cuttings and stock is subject to reinfection from existing cultivars and other members of the Cruciferae. Wurm (3) found the hypocotyl and stem of seedlings of horse-radish would form adventitious shoots. We have found that whole leaf cuttings of horseradish develop roots under mist and subsequently generate shoots from the base of the petiole. However, while small pieces of leaf blade die before generating plants under mist, these pieces can produce plants under sterile conditions by *in vitro* techniques as follows:

Mature leaves, 15-20 cm in length are excised, carefully rolled and immersed in a solution of 0.5% sodium hypochlorite (10% chorox), 0.1% polyoxyethylene sorbitan (Tween 20) for 15 min. Leaves are rinsed with sterile distilled water several times and sectioned on filter paper moistened with 0.1% citric-0.1% ascorbic acid. Roughly triangular-shaped pieces 1 cm across appear to be better than a 1 cm leaf disc. Smaller pieces proliferate with difficulty and petiole slices 1-2 mm in length do not proliferate at all.

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