Cost of Production in Commercial Micropropagation and Research Strategies

R.A.de Fossard

Xarma Pty.Ltd., Eagle Heights, Queensland 4271

Research strategies in commercial micropropagation essentially require a thorough understanding of the components in the cost of production allied with a dissection of the processes involved in the technique (de Fossard, 1976, 1990, 1993). The major component is overheads and the first task is to distribute these costs to various parts of the laboratory. The laboratory can be conveniently divided into three main areas—the preparation area, the inoculation area and the incubation area.

COSTS OF PRODUCTION

The Preparation Area. This is concerned with the preparation, dispensation, sterilization, and storage of culture media. The preparation area "sells" vessels of sterilized culture media to the inoculation area. How much it "sells" each vessel for depends on the effective utilization of this area—on whether, if it has a capacity to produce 500 vessels per day that it, in fact, produces 500 vessels per day. Under-utilization of this part of the facility can double if not triple the cost of production per vessel.

The Inoculation Area. This area "buys" sterilized media in culture vessels from the preparation area and places plant material in them aseptically. The costs in this area reflect three cultural properties, multiplication rate of the species concerned, inoculation rate, and the number of cultures which can be grown in each vessel. All three properties are worthy objects for the research worker. The multiplication rate of a species is the number of useable pieces a culture can be divided into at the end of its incubation cycle. The inoculation rate is the number of pieces an inoculator can "plant" on fresh medium per hour or per shift. The inoculation rate can be a reflection of the inoculator's performance and attitude; alternatively, it is a reflection of the type of growth achieved by the "mother" culture, for example, whether there is a lot of root development. Number of cultures grown in each vessel is mainly dependent on the type of growth achieved by a species.

The Incubation Area. This area consists of various systems to expose cultures to light and temperature for periods of growth between inoculation (the beginning of one growth cycle) and division and/or planting out (at the end of the growth cycle). The cost of incubating cultures is calculated by distributing overheads on a ft² or m² per week basis, calculating how many culture vessels fit into these unit areas, how many cultures are in each vessel, and multiplying this cost by the number of weeks in the growth period for the species in question. Cultures needing only four weeks incubation would clearly cost less than those needing longer. These incubation costs must be worked out before the cost of different multiplication rates can be calculated because this gives the cost of the "mother" vessel whose cultures are to be divided into 2, 3, 4, or more pieces.

The Final Cost. The final cost of producing cultures ready for planting-out is mainly a combination of multiplication rate, inoculatin rate, number of cultures per vessel, and incubation costs. But that is not the end of the story—there are two types of losses which need to be accommodated. One type is the technical or biological losses, cultures discarded because of microbial contamination and/or poor growth. The second type is the "saleable-but-not-sold" loss—everything goes to plan, nice cultures are produced but the market disappears! Needless to say, all costs associated with the production of cultures have to be borne by the cultures which are finally sold (Tables 1 to 6).

Notes on Tables 1 to 6. In Table 1, more than 60% of the total cost of production of a vessel of sterilized culture medium is attributable to overheads. These data were derived from a laboratory producing an average of 500 vessels of media per week although its capacity is to produce 1000 vessels per day. However, in Table 2, we see that the cost of a vessel of medium amounts to only 16% of the total cost of production.

Table 1. Cost components of culture medium, inoculation, and incubation.

Sterilized culture medium per vessel:		
Chemicals	2.8¢	3.6%
Gelling agent	2.6 m c	3.4%
Vessel	10.5 c	13.9%
Fuel	0.5 c	0.7%
Labour	11.9¢	15.7%
Overheads	47.8¢	62.8%
TOTAL	75.8¢	100.0%
Inoculation costs per 8-hour shift:	\$100.76	
Incubation costs:		
Total weekly costs	\$577.15	
Used capacity (80% Total)	5760	vessels

Tables 3 to 5 illustrate some of the "dynamics" of cost of production and its relationship with multiplicaton rate, inoculation rate, and number of cultures per vessel. We see from Tables 3 and 4 that there are quite large reductions in sale price when we achieve an increase in multiplicaton rate from $\times 2$ to $\times 3$, but the reductions are less, though still desirable, as we achieve $\times 4$ and $\times 5$. The story is similar with inoculation rate improvements and planting more cultures per vessel.

It is worth noting, from Table 2, that the cost of cultures before and after adjustment for losses and profit mark-up are:

- 21.2¢ per culture at the laboratory door (before losses are accommodated);
- 23.5¢ per culture after 10% technical losses are accommodated;
- 31.4¢ per culture after 25% "not sold" losses are accommodated; ·
- 36.1¢ per culture after addition of 15% profit mark-up.

Table 2. Cost of cultures before and after adjustment for losses and profit mark-up.

Cost of production for a species with:

Inoculation rate No cultures/vessel Multiplication rate		150 vessels 15 4	per 8	hours		
Incubation period		8 weeks				
Cost of culture vessel	5.1¢	2	3.9%	21.5%	16.1%	
Cost of inoculation	4.5 m c	2	12%	19.0%	14.3%	
R&D levy:1¢ per culture	1.0¢		4.7%	4.3%	3.2%	
Cost of incubation	5.3¢	2	5.2%	22.7%	17.0%	
Cost: Parent culture	5.3¢	2	5.0%	22.5%	16.9%	
SUB-TOTAL	21.2¢	10	0.0%	90.0%	67.5%	
10% Technical loss	2.4¢			10.0%	7.5%	
SUB-TOTAL	<u>-</u>	23.5¢		100.0%	75.0%	
25% "Not-sold" loss	7.8¢				25.0%	
SUB-TOTAL	<u>.</u>	31.4¢			100.0%	
15% Profit mark-up	4.7¢					
SALE PRICE		36.1¢				

Table 3. Sale price of cultures as affected by multiplication rate and number of cultures per vessel.

Muliplication rate	No. cultures per vessel	Sale price per culture (¢)	Difference (¢)
2	10	79.4	
3	10	59.6	19.9
4	10	53.0	6.6
5	10	49.7	3.3
2	15	54.1	
3	15	40.6	13.5
4	15	36.1	4.5
5	15	33.8	2.3

Table 4. Sale price of cultures as affected by inoculation rate and number of cultures per vessel.

Inoculation rate	No. cultures per vessel	Sale price per culture (¢)	Difference (¢)
75	10	68.2	
150	10	53.0	15.3
225	10	47.9	5.1
300	10	45.3	2.5
75	15	46.2	
150	15	36.1	10.2
225	15	32.7	3.4
300	15	31.0	1.7

Table 5. Sale price of cultures as affected by number of cultures per vessel.

Inoculation rate:	150	vessels per 8 hours	
Multiplication rate:	4		
No. cultures per vessel		Sale price per culture (¢)	Difference (¢)
5		103.7	
10		53.0	50.7
15		36.1	16.9
20		27.6	8.4

Although the labour component (Table 6) is less than 60% in the example given here, there is probably a 50% to 70% range depending on how a laboratory is run and what is classified as direct labour.

MICROCUTTINGS

Tables 1 to 6 are based on the situation where all the cultures at the end of an incubation period leave the laboratory and are "sold" to the planting-out facility for 36.1¢ per culture (\$5.42 per vessel). In the planting out facility, each culture may either be divided into four "units" at the time of deflasking or be divided into four after acclimatization—or it may be decided not to divide it at all so as to achieve a "bushy" habit—much depends on the species and the type of "conditioning" achieved in the laboratory.

Another option may be available to the laboratory and this is to persuade the cultures to be harvested as microcuttings. Cultural conditions are varied so that from a basal mass of tissue several shoots arise and, when these are more than

about 2.5 cm long, they are cut off in the laboratory (and sent to the planting out facility) and the base of the culture is divided and used for new cultures.

Table 6. Direct labour costs as a	percent of cost of production.	
Cost of production for a species wi	th:	
Inoculation rate	150 vessels per 8 hours	
No.cultures/vessel	15	
Multiplication rate	4	
Incubation period	8 weeks	
Labour component		
Cost of culture vessel	15.8%	
Cost of inoculation	98.5%	
R&D levy:1¢ per culture		
Cost of incubation	61.3%	
Cost: Parent culture	53.2%	
TOTAL	53.1%	

Table 7 gives some of the cost of production figures for a cultural situation similar to the one used in Table 2 with the exception that from one to three microcuttings per culture have been obtained.

Thus if only one microcutting per culture is obtained from "mother" cultures, in Table 7, its sale price is 27ϕ whereas if three microcuttings per culture were derived from each "mother" culture the sale price per microcutting would be 9.0ϕ .

Table 8 illustrates the situation with higher multiplication rates in which two basal pieces of culture are subbed irrespective of multiplication rate so that with higher multiplication rates an increasing number of microcuttings are derived from each "mother" culture with corresponding reductions in sale price per microcutting.

COSTS IN THE PLANTING OUT FACILITY

This gets us to the stage where either microcuttings or vessels with cultures leave the laboratory. The success of the whole exercise depends on getting these cultures and microcuttings to become self-sustaining, photosynthesizing plants and, as far as cost of production is concerned, technical or biological losses in the planting out or acclimatization facility may become a major factor.

Another important factor is the deflasking rate, the number of cultures that are removed from a vessel and placed in a propagating mix per hour or per shift. The deflasking rate is, like the inoculation rate, partly a reflection of the aptitude of the person concerned and partly a reflection of the type of growth of the cultures, cultures with a lot of root development taking longer to deflask and "sow"

than **Table 7.** Cost of production of microcuttings (MC) as affected by number of MCs per culture and number of pieces for subculturing (SUB).

Cost of production for a species with:

Inoculation rate	150 vessels per 8 hours	
No.cultures/vessel	15	
Multiplication rate	4	
Incubation period	8 weeks	
Cost of culture vessel	\$0.76	
Cost of inoculation	\$0.67	
R&D levy:1¢ per culture	\$0.15	
Cost of incubation	\$0.80	
Cost: Parent culture	\$0.79	
SUB-TOTAL	\$3.18	

	<u>.</u>		Nu	mber of	units for	r*		
	SUB 1	MC 3	SUB 2	$rac{ ext{MC}}{2}$	SUB 3	MC 1	SUB 4	MC 0
Cost of culture	•							
vessel Cost of	\$0.76		\$0.76		\$0.76		\$0.76	
inoculation R&D levy	\$0.67		\$0.67		\$0.67		\$0.67	
1¢ per culture Cost of	\$0.15		\$0.15		\$0 15		\$0.15	
incubation Cost: Parent	\$0.80		\$0.80		\$0.80		\$0.80	
culture	\$0.79	\$2.38	\$1.59	\$1.59	\$2.38	\$0.79	\$3.18	\$0.00
EQUALIZING COSTS	\$0.00	\$0.00	-\$0.79	\$0.79	-\$1.59	\$1.59	-\$2.38	\$0.00
SUB-TOTAL	\$3.18	\$2.38	\$3.18	\$2.38	\$3.18	\$2.38	\$3.18	\$0.00
10% Technical								
loss 25% "Not- sold"	\$3 53	\$2.65	\$3.53	\$2.65	\$ 3 5 3	\$2.65	\$3.53	\$0.00
loss 15% Profit	\$4.70	\$3.53	\$4.70	\$3.53	\$4.70	\$3.53	\$4.71	\$0.00
mark-up	\$5.41	\$4.06	\$5.41	\$4.06	\$5.41	\$4.06	\$5.42	\$0.00
SALE PRICE	36.1¢	9.0¢	36.1¢	13.5¢	36.1¢	27.0¢	36.1¢	\$0.00

rootless cultures. In Table 9, the unit cost of deflasking varies from 4.0¢ (with 2000 units/shift—the whole culture situation) to 1.6¢ (with 5000 units/shift—the microcutting level).

With whole cultures, there is also the question of whether these are to be divided at the time of deflasking or after acclimatization if, indeed, they are to be divided at all. Table 10 illustrates three main options yielding sale prices for: undivided cultures, divided cultures, and microcuttings. A 35ϕ culture has a sale price of 75ϕ after 4 weeks acclimatization whereas a divided culture would sell for 30ϕ . In contrast, a 15ϕ microcutting would sell for 37ϕ but if its cost were about 5ϕ (see Table 8) at the laboratory door, it would sell, after 4 weeks of acclimatization, for about 20ϕ . A further difference in options with "whole" cultures (not applicable to microcuttings) is to place the vessels in the acclimatization area for a period **prior** to deflasking—an additional cost but one that may be "rewarded" with higher survival rates in this facility.

Table 8. Sale price of microcuttings as affected by multiplication rate with two pieces per culture for subculturing.

Inoculation rate	150 vessels	per 8 hours	
No. cultures/vessel	15	per o nours	
Incubation period	8 weeks		
Multiplication rate	No. pieces/culture for subbing	No. microcuttings per culture	Sale price per microcutting (¢)
4	2	2	13.5
6	2	4	6.8
8	2	6	4.5
10	2	8	3.4

RESEARCH STRATEGIES IN COMMERCIAL MICROPROPAGATION

In my view, what matters in commercial micropropagation is the achievement of a high success rate in the planting out facility. This will depend not only on practices in this facility but also on the "conditioning" of the cultures in the laboratory.

So now we are at the point where we can develop strategies for commercial micropropagation. We look first at the planting-out end of things and make a few sweeping generalizations:

Microcuttings have the potential to be the most cost effective type of unit to "sell" to the planting out facility. The rate of "sowing" microcuttings is much higher than most other types of culture and the laboratory does not lose its "mother" cultures. These microcuttings are harvested in the laboratory and the bases of the cultures are divided for the next cycle of growth. ■ If a species is not suitable for microcuttings, then aim to produce multi-shooted cultures which can be subdivided either at the time of deflasking or after acclimatization.

Table 9. Cost of production of established plants as affected by a variable deflasking rate and establishment period.

Number of "units" per culture	4	
Deflasking labour costs/hour	\$10.00	
Number of hours for deflasking	8	
Total cost of deflasking	\$80.00	
Number of cultures or "units" deflasked	2000	5000
Cost of deflasking per culture or "unit"	4ϕ	1.6¢
Cost seed tray + mix	118.3¢	
Number microunits/tray	56	
Thus, cost tray + mix/microunit	2.1¢	
Overheads/m ² /year	\$191.48	
Number seed trays/m2 area	9	
Thus, overheads/seed tray/week	40.9¢	
Number microunits/tray	56	
Thus, overheads/microunit/week	0.7¢	
Number weeks establishment stage	48	
Thus, overheads/microunit due to establishment	2.9¢	5.8¢
Plant losses (%) during establishment stage	10	
Saleable-but-not-sold loss (%) factor	25	
Profit (%) mark-up	15	

Research leading to increases in multiplication and inoculation rates is cost effective only up to a certain point and all experiments should include an evaluation of the performance of cultures on planting out. If we move acclimatization success rates to the top of our experimental objectives, then our strategies would include experimentation in the laboratory and in the planting out facility. In the laboratory, treatments to increase food reserves in the cultures (for example, by giving them higher concentrations of sucrose in the medium) and to "harden" cultures (for example, by exposing them to lower humidity) could be tested. In the planting out facility, cultures could be given various periods of exposure to a greenhouse environment while they are still in the culture vessel, prior to deflasking—this could include removal of the lids of the vessels. Published research mainly comes from universities and research institutes and much of it is difficult to reproduce in other similar laboratories let alone commercial laboratories. So commercial laboratories must expect to do their own research. Part of the problem about published work is that either important details (such as the constitution of culture media) are excluded following editorial constraints or that the authors may not know that one or more aspects of their technique were important factors in their results. However, another reason is that much of this institutional work is done in test tubes rather than in the types of larger vessels

used, on economical grounds, by commercial laboratories. It is important to realise that the type of vessel, the type of closure, and the volume of medium per vessel can influence cultural responses—quite apart from the use of expensive controlled-environment cabinets in institutions, compared to incubation areas in commercial laboratories. Another detail often omitted from published work is planting out details of relevance to nurseries.

Table 10. Sale price of undivided cultures, divided cultures and microcuttings after establishment in seed trays for 4 and 8 weeks.

			Undivided micro-unit			
No. weeks	20	. 25	30	35	40	45
4	49	58	67	75	84	92
8	54	63	71	80	89	97
	D		ures: mult micro-unit	_	rate = ×	4
	20	25	30	35	40	45
		oo				<u> </u>
	E1	fective cos	t (cents) p	er unit afte	er divisio	n
No. weeks	5	fective cos 6.25	t (cents) po	er unit afte 8.75	er division	n 11.25
			-	- · · · ·		
4	5	6.25	7.5	8.75	10	11.25
No. weeks	24	6.25 26 31	7.5 28	8.75 30 35	10 32 37	11.25 35
4	24	6.25 26 31	7.5 28 33	8.75 30 35	10 32 37	11.25 35
4	5 24 29	6.25 26 31 Microcu	28 33	8.75 30 35 ro-units (c	10 32 37 ents)	35 40

CONCLUSIONS

This paper has dealt with the cost of production of plants by micropropagation and how such studies influence research directions. A similar study with seeds, cuttings, and other propagules would lead to similar approaches and yield similar complex managerial decisions (de Fossard, 1992). Attempting to lower

cost of production is a controllable, worthwhile aim, reducing technical losses is another worthwhile aim but minimizing the "saleable-but-not-sold" losses requires rather different skills and decision-making.

REFERENCES

deFossard, R.A. 1976. Tissue culture for plant propagators. Xarma, Eagle Heights, Queensland.

deFossard, R.A. 1990. Micropropagation. Xarma, Eagle Heights, Queensland.

deFossard, R.A. 1992. Nursery costs. Xarma, Eagle Heights, Queensland.

deFossard, R.A. 1993. Plant tissue culture propagation. Xarma, Eagle Heights, Queensland.