Under glasshouse conditions, this generally consists of an unsealed bench, on top of which is placed a layer of felt. The felt is kept wet by application of water once or twice a day.

The plants are watered when they are stood upon the felt, this establishes a column of water from the growing medium into the felt. As tension is increased on the water in the growing medium, water is drawn from the felt into the medium to maintain container capacity.

Outdoors, beds of fine sand, over plastic film, have been used to achieve the same results. The sand must be a type which can hold enough water to supply the plants.

Provided the benches or sand beds are not sealed, water can be applied easily without sophisticated controls. We use a time clock to operate a solenoid valve and apply water for 20 minutes, night and morning in summer, once per day in winter. Any excess, above what the reservoir will hold, simply drains away.

When using capillary watering it must be remembered that the normal water flow is reversed so that there can be a build up of salts in the container. To overcome this problem, we water from above the container once per week, using enough water to flush any build up of salts out of the container.

Capillary watering has been shown to give considerable increases in growth when compared with conventional watering techniques. Many nurserymen remain convinced that their existing watering techniques are perfectly adequate. If you are in this group, perhaps you owe it to yourself to try capillary watering on a small area of your nursery.

CLONAL PROPAGATION OF WOODY PLANTS USING TISSUE CULTURE, WITH SPECIAL REFERENCE TO APPLES

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Abstract. A critical review of the published papers on plantlet regeneration of woody species shows that very few reported systems are ideal for clonal propagation. Some depend on use of embryonic, juvenile and endosperm tissues, or on tissues such as the nucellus of *Citrus* that show unusual properties. In others plantlets regenerated from callus have poor vascular connection between roots and shoots and die on transplanting to pots.

Culture methods that induce multiple shoot production from excised shoot tips or axillary buds, and the subsequent rooting of these shoots without the involvement of excessive callus offer greatest potential. One such

method, that developed by Jones and coworkers (37) for apple, is described and is being applied to apple rootstocks in quarantine in Western Australia. The question of the role of the phenolic compound phloroglucinol is discussed.

INTRODUCTION

Relatively simple methods of micropropagation have been developed for many herbaceous plants and these techniques are suitable for commercial application (25,50,53). However there are few reports of success for woody angiosperms — trees, shurbs, creepers and vines.

Assessment of the desirable attributes of woody plants usually cannot be made until the plant is mature. In some species, by the time the plant reaches this stage of growth, vegetative propagation by conventional methods is slow or difficult, if not impossible. Thus propagation using *in vitro* methods would be most useful. It is possible to assemble an impressive list of woody species for which there are reports of organogenesis or embryogenesis *in vitro* (Table 1). However, when one examines the cited papers in detail it becomes clear that in many cases the tissues or methods used are not suitable for large scale clonal propagation. Also, there are very few examples in which the plantlets survive transfer from the culture tubes to pots.

Embryo culture of woody species is possible, but examples are not included in Table 1, for while embryos raised in this way may be valuable hybrids that would otherwise abort, the method does not result in clonal propagation.

Several of the examples in Table 1, e.g. *Ilex*, *Prunus*, *Ulmus* and *Azadirachta* involve regeneration of plantlets from callused embryos or seedling tissues. While such cultures may give us some clues on how to handle the species *in vitro*, the regeneration *in vitro* may be much easier when material is derived from seedling or juvenile explants than that from explants of mature plants (50).

Similarly, although endosperm cultures may give rise to plantlets in woody species of the Loranthaceae and Santalaceae, the triploidy or higher ploidy of this tissue results in undesirable polyploid plants.

Cultures of nucellar tissue of *Citrus* and related genera have been used successfully for plantlet regeneration, but unfortunately the development embryoids from nucellar tissue has not yet been induced in species outside the Rutaceae.

Meristem cultures may provide an efficient way of eliminating viruses, as in *Musa*, *Manihot* and *Ribes* (Table 1) but generally yield only one plantlet per explant and are time consuming to set up.

The systems most useful for propagation utilize relatively large explants, which in culture yield many plantlets per explant. A high yield per explant can be achieved in two ways. Either the explant can be induced to form a callus on which shoots and roots or embryoids are subsequently differentiated or, alternatively, the buds on the explants can be induced to proliferate with little or no callusing, and the multiple shoots then excised and individually rooted.

The first possibility, involving the use of callus, has been successful in several cases, *Coffea*, *Citrus* and *Populus*, (Table 1), but it has several disadvantages. Cells proliferating to form a callus frequently undergo cytological changes. These may result in abnormal plants being regenerated and probably contribute to a decline in regeneration potential over time, which is usually observed. A further difficulty is that, although roots and shoots may arise from the same piece of callus, there may be little or no vascular connection between them and this has predictably disastrous consequences for the young plant on pricking out to pots.

The culture methods that utilize large explants and result in numerous rooted shoots offer most potential for clonal propagation. The species for which such techniques have been developed include *Bougainvillea*, *Rubus*, *Eucalyptus* and *Malus* (Table 1).

The system of culturing apple shoot tips has been developed by O.P. Jones and colleagues at East Malling Research Station. I have investigated the use of their methods for propagation of apple rootstocks of interest to Western Australian growers. New material can be brought into Australia through quarantine in very limited amounts, and the methods of rapidly multiplying material in tissue cultures can be exploited while the "parent" plant is still held in quarantine. Thus when the line is cleared from quarantine, many plants are available to growers.

PROBLEMS IN ESTABLISHING APPLE TISSUE AND ORGAN CULTURES

1. Sterilization. Apple shoots are killed or damaged by common surface sterilants, and a 2-step sterilization procedure is necessary (30,37). Shoots (1.5 - 2 cm long) bearing small leaves are first dipped into wetting agent (0.01% Tween 80) then in sodium hypochlorite (0.14%) for 1 min, then washed 3 times in sterile water. They are placed on culture medium overnight, and next day they can tolerate 40 mins. in 0.4% sodium hypochlorite. Using this sterilization method, I obtain around 50% uncontaminated shoots, though different batches give 0 - 100% uncontaminated. Bud material is more resistant to

Table 1. Woody Trees, Shrubs, Climbers, Vines and Parasites which regenerate shoots and/or roots in culture.

Species	Explant	Regeneration	Survival After Transfer to Soil	Reference
MONOCOTYLEDONS				
MUSACEAE				
Musa sapientum	Meristem	shoots, roots	Yes	5
M. acuminata (Syn., *M.* cavendishii	Meristem	shoots	—	50
PALMAE				
Elaeis guineensis	Meristem, Embryo	callus, embryoids leaves, roots	?	56,57
DICOTYLEDONS				
ACTINIDEACEAE				
Actinidia chinensis	stem and shoots	shoots	Yes	24
AQUIFOLIACEAE				
Ilex aquifolium	embryo	embryoids from cotyledons — plantlets	No	26
EUPHORBIACEAE				
Hevea brasiliensis		embryos, shoots	?	53
Jatropha panduraefolia		callus, shoots, roots	No	64
Manihot esculentum	stem, tip, meristem	(callus) shoots, roots	Yes	40
Putranjiva roxburghii	endosperm	callus — shoots, roots	No	65
Ricinus communis	endosperm	callus — embryoids	No	60
GROSSULARIACEAE				
Ribes grossularia	meristem	single shoot	Yes	35
LEGUMINOSAE				
Acacia koa	stem	callus — shoots, root	?	63

Table 1. (Continued)

Species	Explant	Regeneration	Survival After Transfer to Soil	Reference
LORANTHACEAE				
Dendrophthoe falcata	endosperm	callus — shoots, haustoria	No	52
Nuytsia floribunda	embryo	callus — shoots, roots	No.	51
Scurrula pulverulenta	endosperm	callus — shoots, haustoria	No	6
Taxillus vestitus	endosperm	callus — shoots, haustoria	No	52
T. cuneatus	endosperm	callus — shoots, haustoria	No	52
MELIACEAE				
Azadirachta indica	mature embryo	callus — shoots, roots rare	No	59
MORACEAE				
Broussonetia kazinoki	stem	callus, shoots or roots	No	53
Morus alba		callus — shoots		21
MYRTACEAE				
Eucalyptus bancroftii	seedling nodal stem pieces	shoots, roots		13,20
E. citriodora	lignotubes	(callus)shoots, roots	?	2
E. deglupta	seedling nodal stem pieces	shoots, roots		13

Table 1. (Continued)

Species	Explant	Regeneration	Survival After Transfer to Soil	Reference
MYRTACEAE (Continued)				
E. ficifolia	stem tips	shoots, roots	Yes	19
	nodal stem pieces, from mature trees	multiple shoots		
E. grandis	nodal stem pieces from seedling and mature trees	shoots, roots	Yes	13,14,20
NYCTAGINACEAE				
Bougainvillea glabra	stem tips	shoot proliferation from callused base	Yes	12
ROSACEAE				
Malus sylvestris	endosperm	callus shoots, roots	—	47
	seedling shoot tips	(callus)shoot proliferation, roots	No	1
	mature plant shoot tips	(callus)shoot proliferation roots	Yes	3,32,37
	embryo	(callus) shoot proliferation roots	—	48
Prunus dulcis (Syn.: *P. amygdalis*)	seedling	callus — shoots, roots	No	43
Pyrus communis	embryo	(callus) shoots, roots	—	48

Table 1. (Continued)

Species	Explant	Regeneration	Survival After Transfer to Soil	Reference
ROSACEAE (Continued)				
Rosa multiflora	meristem, stem tip	shoots, roots	?	17
Rubus fruiticosus	stem tip	shoot proliferation, roots	Yes	8
RUBIACEAE				
Coffea arabica	stem	callus — shoots	No	46,62
C. canephora	stem	callus — embryoids plantlets	Yes	46,66
RUTACEAE				
Citrus aurantifolia	ovules	callus-embryoids	No	45
	nucellus	callus - shoots roots		
C. maximum (Syn.: C. grandis)	stem, leaf	callus - shoots roots	Yes	11
C. microcarpa	ovules nucellus	callus-embryoid plantlets	No	58
C. paradisi	ovules nucellus	callus-embryoids	Yes	42
C. sinensis	nucellus	callus-embryoids plantlets	Yes	9,42
	stem, leaf	callus — shoots shoot proliferation, roots	Yes	11
Citrus (other species)	nucellus or seedling stem	embryoids	—	10,50

Table 1. (Continued)

Species	Explant	Regeneration	Survival After Transfer to Soil	Reference
RUTACEAE (Continued)				
Eremocitrus glauca	nucellus	embryoids	—	50
Fortunella crassifolia	nucellus	embryoids	—	50
Fortunella japonica (Syn.: *C. mandurensis*	seedling stem	callus - shoots roots	Yes	22
Microcitrus australasica	nucellus	embryoids	—	50
Microcitrus warburgiana	nucellus	embryoids	—	50
Poncirus trifoliata	nucellus	embryoids	—	50
SALICACEAE				
Populus tremuloides	stem sections	callus - shoots roots	Yes	72,73
P. trichocarpa	catkin primordia	callus - female structures callus - shoots, roots	No	4
P. nigra		callus — shoots, - roots (never together)	No	21
	internodes	callus - shoots, roots	No	69
SANTALACEAE				
Exocarpus cupressiformis	endosperm	callus - shoots	No	38
Leptomeria acida	endosperm	callus - shoots	No	38
ULMACEAE				
Ulmus americana	seedling	callus, shoots	No	15

sterilizing than are growing shoots. The outer scales are removed and stem segments bearing buds are given 15 minutes in 0.5% sodium hypochlorite and then washed three times (16). However, using the culture methods of Jones *et al.* (37) I have found that explants of dormant axillary buds on short lengths of stem are satisfactory, but growth over the first five weeks or so is far slower than for explants of growing shoots.

2. Browning of explants. Apple tissues frequently turn brown in culture due to oxidation of polyphenols. This can be overcome by the use of polyvinylpyrrolidine (PVP) (70,29) and after 4 - 8 weeks in culture, PVP can be omitted when subculturing. In other species, various other anti-oxidants have been used to prevent browning of tissue and media: [*Eucalyptus* (19) *Coffea* (46)], or, an expensive alternative, the tissue has been changed to new media each day: *Rubus* (8). The browning of apple shoot tips sterilized by the 2-step method of Jones (37) is negligible.

3. Regeneration of shoots. There are several reports in which apple callus or organ cultures have produced sporadic shoots but in which rooting was difficult (16,18,31,47,48,70). The discovery by Jones and colleagues (32,33,37) of the effect of phloroglucinol on shoot proliferation and rooting in culture has made micropropagation of applies a viable proposition.

DEVELOPMENT OF THE METHOD OF PROPAGATION

Jones was examining the growth promoting substances found in apple xylem sap, and detected a growth promoting substance whose effect was enhanced by IAA at concentrations too low for the IAA to be effective by itself (36). This growth promoting substance had characteristics of a phenolic, phloroglucinol. Phloroglucinol or phloretic acid (2 breakdown products of phloridzin) in association with auxin were found to more than double the proportion of 'M 7' apple rootstock which rooted *in vitro* (33). Phloridzin itself and other structurally related compounds (caffeic acid, catechol, pyrogallol) were ineffective.

Jones (32) then reported an impressive proliferation of shoots in cultured 'M7' and 'M26' apple shoot tips when cultured with benzylaminopurine, gibberellin, auxin and phloroglucinol or phloridzin. The new shoots grow from the axillary buds of the original explant; they do *not arise de novo* from callus. After some 12 weeks in culture up to 38 shoots 2-5 cm long arise from each single shoot.

In a paper in 1977, Jones *et al.* combined the rooting and shoot proliferation methods they had devised; theoretically, the following method can yield 60,000 shoots from a single shoot

tip of some lines in 8 months (37). Shoot tips 1.5 - 2 cm long are cultured for 4 weeks in 10 ml medium, after which each tip has produced 2 - 5 shoots. Single shoots are transferred to 125 ml medium in 250 ml flask for further 8 weeks, by when each shoot has produced 2 - 42 shoots, 2 - 5 cm long. Shoots are cut off and placed in rooting medium, and within 6 weeks 97% of shoots have produced roots. Rooted shoots are transferred to glasshouse pots with 85% survival.

I have been using this method with trivial alterations — I use 100 ml of media in screw cap bottles rather than the 250 ml flasks. Also I find a forest of shoots difficult to handle so I prefer to sub-culture shoots after 4 weeks, when each clump has 6 - 10 shoots. Mullins *et al.* (49) have also used much the same technique as Jones except that they have attempted to induce roots on shoots under non-sterile conditions.

Rootstocks I am using are 'MM 104' and 'M 109', in which shoot proliferation is similar to that in the stocks 'M 26' and 'M 7' used by Jones. Rooting is only successful for 50% of shoots in my cultures. Other lines, e.g. 'M 106' and 'Merton 793', do not proliferate as readily as 'M 26', and preliminary experiments have not yet revealed an appropriate combination of hormones.

Jones (37) has reported that 'M 27' behaves in culture as well as 'M 26'. Interestingly, they report that 'Pixy' plum rootstock and 'F12/1' cherry rootstock respond well to media containing phlorglucinol, though alteration of the cytokinin level may be required. I am attempting to use similar methods to propagate cherry lines in quarantine in Western Australia.

Effects of phloridzin, phloroglucinol and phloretic acid, *in vivo* and *in vitro*. Phloridzin is a phenolic restricted to *Malus* despite some early erroneous reports of its occurrence in other species of Rosaceae (28,71). In apples it occurs in bark, root, fruit skin, seed coat, embryo and leaves, where it may rise to 3-7% dry weight (32). Its actual role in *Malus* is something of a mystery as it seems not to be involved in resistance to disease as was earlier suggested (27). Its concentration is not connected with seasonal growth and is much the same in both standard and dwarfing rootstocks (28,55) though callus from a dwarfing rootstock, 'EM IX', was found to contain more phenolics than callus from a semistandard stock, 'EM XIII' (44). Its presence in seed coats and embryos is thought to be only indirectly related to after-ripening and seed dormancy (7).

In apple plants two breakdown products of phloridzin, phloroglucinol and phloretic acid can be detected in the xylem sap. In the presence of auxin, both phloroglucinol and phloretic acid markedly increase rooting of shoots while phloridzin itself is ineffective (33). For other species, some phenolics enhance

the effect of auxin on rooting and their action was attributed to suppression of auxin oxidation (41). This does not appear to be the case for apple rooting (33).

In cultured apple shoots (again in the presence of auxin) both phloridzin and phloroglucinol stimulate shoot proliferation, but closely related compounds such as caffeic acid, catechol and pyrogallol are ineffective. Also, the phenolics found in other genera, such as arbutin in *Pyrus*, or salicin in *Populus*, do not have a stimulatory effect on *Malus*. Arbutin does, however, cause a growth increase in pear cultures (32,33,-34). Thus Jones has suggested that the effect of the glycoside may be to promote growth only in the tissues in which it occurs naturally. This does not seem to be the case, however, as while phloridzin is indeed inhibitory for many plant and animal systems (23,39,61,67,68), it is also reported to have a stimulatory effect on photosynthesis in genera other than *Malus* (see reference 23). The effect of phloroglucinol too, is not confined to *Malus* as, in the presence of auxin, it promotes shoot proliferation in cherries and plums and oat mesocotyl growth (37,54).

An investigation of the mechanism of action of phloridzin and phloroglucinol may be interest *per se*, as well as possibly giving further information on the mechanism of sucrose uptake and utilization by in vitro plant cultures, and the role of auxin.

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