In vitro Grevilleas©

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INTRODUCTION

The genus is named after Charles Francis Greville and is predominantly Australian with some species from neighbouring countries. Grevilleas are a member of the family *Proteaceae*. There are three groups; the Banksia Group, the Rosmarinifolia Group, and the Toothbrush Group. From these groups there are also a number of interspecific hybrids.

Importance

The plants are a range of prostrate shrubs to trees mostly woody natives. Flowering ornamentals with attractive flowers and foliage and those with nectar attract pollinators and native birds. They make excellent native garden plants from ground covers to shade trees. They have a range of flowers available to suit everyone's interest.

There are many species and hybrids with different plant forms and a range of flowers available to suit everyone's interest. There also a few varieties that are commercial timber species.

Adaptability

Grevilleas will grow in most environments and love sunshine and well drained light soil low in phosphates.

PROPAGATION

Seed propagation is ok for straight species. Generally it is fairly easy and young plants could be selling around $2.00 each. Genetic variation not of any concern.

Vegetative propagation is a must for hybrids due to sterility and need to maintain consistency of appearance. Need to be aware of seasonal issues. Can be done in three ways: cutting, grafting, and micropropagation.

Semi-hardwood cuttings

Difficult due to low strike rates in many cases as low as 10%. Will translate to $3.00-$10.00 per young plant.

Grafting

Very difficult to do which in many cases results in $15 or more per plant. Silky oak (*Grevillea robusta*) is considered to be the best root stock.

Micropropagation or plant tissue culture

This is a technique of growing isolated organs/tissues and cells of plants in a defined nutrient medium under controlled conditions of light, temperature, and humidity.

Advantages of tissue culture:

- Rapid cloning (clones are identical plants)/uniformity
- Production of large numbers in a small space and time
- Freedom from seasonality of production
- Production of clean/disease free plants
- Less expensive in many cases, compared to grafted plants
- Induce juvenility
- Accelerate maturity and early flowering

Why tissue culture grevilleas? As detailed above normal vegetative propagation is difficult and results in expensive young plants. Tissue culture gives the following benefits:

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- Fast and reliable multiplication/cloning
- Avoid segregation of hybrids
- Generate clean/disease free plants
- Induce juvenility for accelerated cutting production
- Uniformity of the plantlets
- Early flowering
- Reduces cost of production

Major steps in tissue culture production of grevilleas. There are basically four major steps in micropropagation of grevilleas: initiation, multiplication, rooting, and acclimatization/hardening.

1. Initiation.

First step is to initiate the plants. This involves getting micro cuttings clean, sterile, and stable on the agar. The agar or growing medium is very important. *Grevillea* initiation can take place in different media like MS Medium (Offord and Tyler, 1998), WPM medium (Bunn and Dixon, 1992), half strength MS medium with $1/10^{th}$ KH_2PO_4 supplemented with low levels of cytokinin alone (BAP 2.0-5.0 µM) or a combination of NAA or IBA and BAP at ratios 1:5 to 1:10 in the range of BAP (5.0-10.0 µM); 2iP was also useful. A 16-h photoperiod at 50-100 µmol m^{-2} s^{-1} light is adequate.

It can take 1-3 months before initiation takes place. The most significant problem at initiation step is contamination. The pubescent nature and/or the waxy stem harbours a lot of contaminants. These can be a major issue in the clean sterile growing environment. Combination disinfection treatments with ethanol followed by bleach worked better than a single treatment. However, tissue death is an issue with some of the species and hybrids during the decontamination of explants. There is a large variety of contaminants that need to be removed or at least controlled.

2. Multiplication.

Once the plants are stable and growing on the agar they can be put into multiplication mode whereby the number of units rapidly increases.

Again the make-up of the agar or growing media is a key issue. Grevillea multiplication can take place in different media like WPM + 5 µM Kin + 0.5 µM BAP — shoot multiplication (Bunn and Dixon, 1992), or ½ MS + 10 µM BAP + 0.5 µM IBA (adventitious shoots on leaf explants of *G. scapigera* (Bunn and Dixon, 1992). Also ½ MS and WPM was helpful along with 1-4 µM BAP and 0.01-0.02 NAA in the case of some grevilleas. Seventeen species of grevilleas have been multiplied on MS medium containing 1.0-1.5 µM BAP alone (Offord and Tyler, 1998).

3. Rooting.

Once the plants have multiplied two or three fold some are put into a rooting agar and some back into multiplication. Those on rooting will produce small agar specific roots. In vitro rooting is reasonably easy in ½ MS medium containing 5.0-10.0 µM IBA. In some cases added charcoal (0.5-2.0 g L^{-1} also is helpful. Sometimes the new cuttings bypass this stage in the laboratory. This is referred to as ex-vitro rooting.

Ex vitro rooting with IBA powder at 1 g kg^{-1} (1000 ppm) or 3 g kg^{-1} (3000 ppm) in a fogged glasshouse at 90-95% humidity, gave good results (Bunn and Dixon, 1992; Offord and Tyler, 1998).

4. Acclimatisation.

The hardest and most risky part of the process is the acclimatisation or hardening off where the young plants are weaned off agar and removed from the moist controlled atmosphere of the laboratory and “taught” to grow in a normal medium in regular greenhouses. In vitro rooted grevilleas acclimatised in greenhouse with fogging initially but misting after 2 weeks from deflasking. High porosity of the potting mix is critical for success.

The propagatability index

The question arises on whether the selected taxon of *Grevillea* is better by tissue culture or conventional means. To assist in this there is the propagatability index (PI). This is the product of success rates at each stage an equals: [Initiation (I)] × [multiplication M)] × [rooting (R)] × [acclimatization (A)] or (PI = I×M×R×A) where I = success rate at initiation, M = multiplication rate per month × R = Rate of rooting and A = rate of establishment at hardening stage. For example: cultivar "A": I = 0.50, M = 4.0, R = 0.90, A = 0.80 which gives 0.5×4.0×0.9×0.8 = 1.44; the PI is 1.44. In general, tissue culture of a species in demand with a PI over 0.70 is commercially viable.

CONCLUSION

Grevilleas can be done by micropropagation although not all cultivars have a success rate that is commercially viable.

Literature cited

Bunn, E., and Dixon, K.W. (1992). In vitro propagation of the rare and endangered *Grevillea scapigera* (*Proteaceae*). HortScience *27*, 261–262.

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