

# MICROPROPAGATION OF AUSTRALIAN NATIVE GREVILLEAS

**Dr. Josekutty Puthiyaparambil, MS, PhD**

**Manager, Tissue Culture Lab**

**Birdwood Nursery, Woombye, Australia, QLD 4559**

**E mail:** [jose@birdwoodnursery.com.au](mailto:jose@birdwoodnursery.com.au)

[josekutty964@gmail.com](mailto:josekutty964@gmail.com)

**Tel: +61 478 793 110**



**IPPS**

Sharing Plant Production Knowledge Globally

Presented at the 57th Annual Meeting of  
the Western Region of North America –  
International Plant Propagators' Society,  
October 19-22, 2016, Tempe, Arizona, USA

# GREVILLEAS

**Member of family Proteaceae**

**Three groups:**

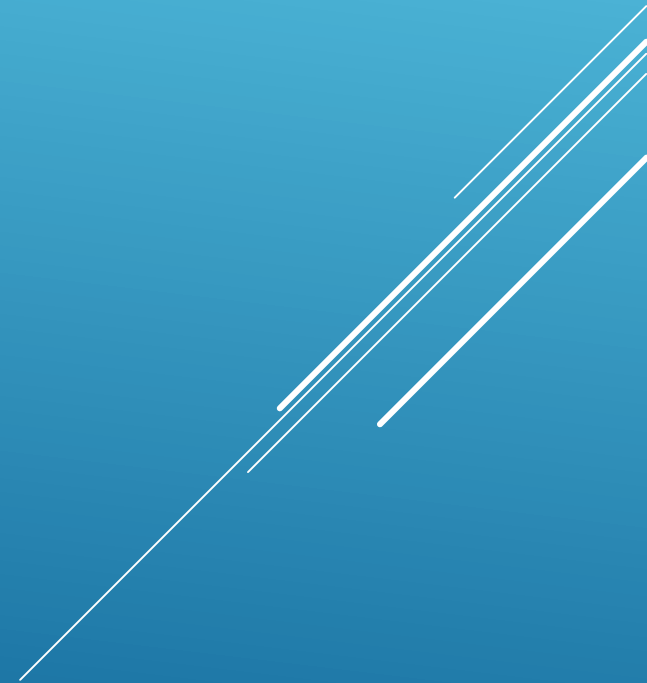
**The Banksia Group, the Rosmarinifolia Group,  
and the Toothbrush Group.**

**Many hybrids are also available.**

**Named after Charles Francis Greville**



**G. 'Honeygem'**: hybrid between *G. pteridifolia* and *G. banksii*



# IMPORTANCE

- Australian native, woody, flowering ornamentals with attractive flowers and foliage
- Grevilleas with nectar attract pollinators and native birds
- Excellent native garden plant, ground cover or shade tree
- Used in Aboriginal medicine, in food, and for making tools



Noisy minor on *G. superb*



Oleander butterfly on *Grevillea*



Honey bees on *G. moonlight*

*Grevillea* for food (e.g. *G. annulifera*, *G. heliosperma*), medicine (e.g. *G. striata*, *G. pyramidalis*), *G. pteridifolia* and *G. striata* were also used to make tools (Olde and Marriott, 1994).

# IMPORTANCE

- Many species and hybrids with different plant forms and a range of flowers available to suit everyone's interest
- A few timber species, distributed in a few countries ( in QLD, Australia *G. robusta* and *G. baileyana* -common)
- <http://anpsa.org.au/grev3.html>



*G. robusta*



*G. wilkinsonia*



*G. pteridifolia*

The Grevillea Book, Vol. 1-3. Olde and Marriott (1994)

# ADAPTABILITY

Grevilleas love sunshine and well drained light soil that is low in phosphate.



G. 'Ned Kelly'

# PROPAGATION

Seed propagation is ok for straight species

Fairly easy (Could be selling around \$ 3.0 per plant)

Vegetative propagation, a must for hybrids

1. Semi hardwood cuttings

Difficult due to low rooting rates in many cases (\$ 3.0 -10.0)

2. Grafting (Silky Oak – Best root stock)

Very difficult in many cases (\$15 -\$ 50 or more per plant)

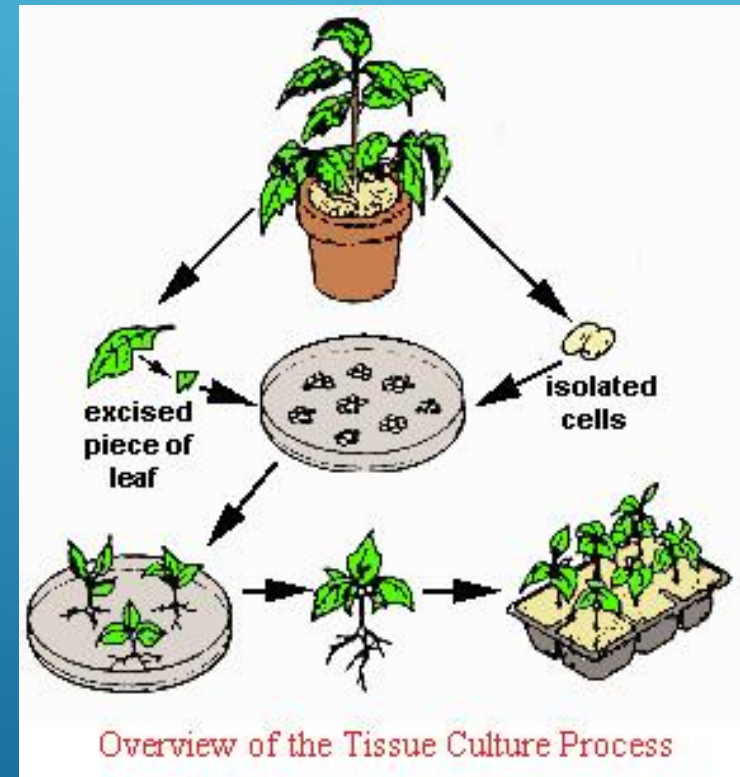


# PLANT TISSUE CULTURE - WHY?

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
- Produce large numbers in a small space and time
- Freedom from seasonality of production
- Produce clean / disease-free plants
- Less expensive compared to grafted grevilleas
- Induce juvenility
- Accelerate maturity & early flowering





# WHY TISSUE CULTURE GREVILLEAS?

- **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**
- **Reduce cost of production**
- **Overcome quarantine barrier for export**



# MAJOR STEPS IN GREVILLEA TISSUE CULTURE

- **Initiation**
- **Multiplication**
- **Rooting**
- **Acclimatization / Hardening**



# INITIATION

Grevillea initiation can take place in different media like MS medium (Offord and Tyler, 1998), WPM medium (Bunn et al 1992), or half-strength MS medium with  $1/10^{\text{th}}$   $\text{KH}_2\text{PO}_4$  and supplemented with low levels of cytokinin alone (2.0-5.0  $\mu\text{M}$  BAP) or a combination of NAA or IBA and BAP at ratios 1:5 to 1:10 with the range of BAP being 5.0-10.0  $\mu\text{M}$ . 2iP was also useful. 16-hr photoperiod at 50-100  $\mu\text{mol m}^{-2}\text{s}^{-1}$  light is adequate.

It can take 1-3 months for initiation.

Most significant problem at initiation step is contamination. Pubescent nature and /or the waxy stem harbors a lot of contaminants. Combination disinfection treatments with ethanol followed by bleach works better than a single treatment. However, tissue death is an issue with some of the species and hybrids during decontamination of explants.



Variety of contaminants

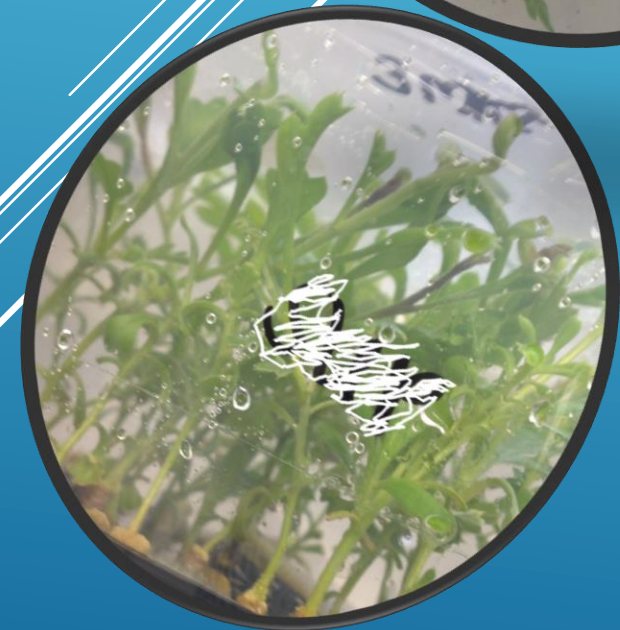
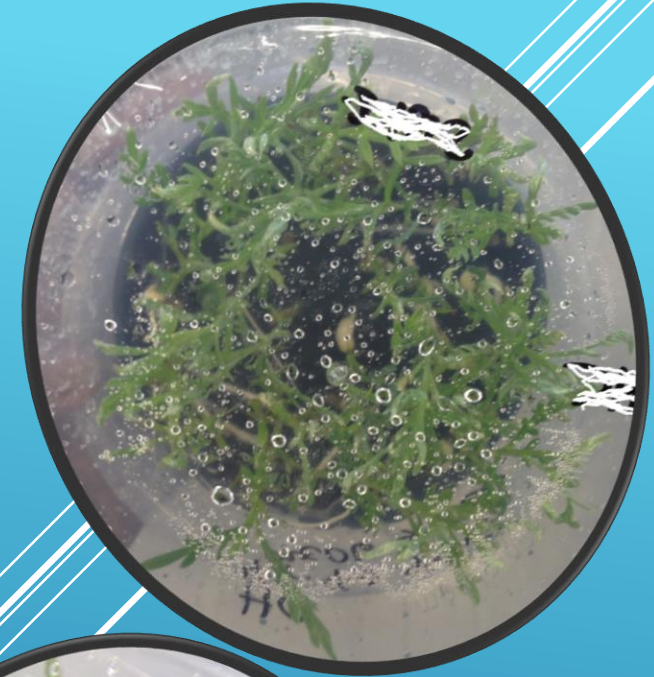
# MULTIPLICATION

WPM + 5  $\mu\text{M}$  Kin + 0.5  $\mu\text{M}$  BAP – shoot multiplication (Bunn et al. 1992)

$\frac{1}{2}$  MS + 10  $\mu\text{M}$  BAP + 0.5  $\mu\text{M}$  IBA (adv. shoots on leaf explants of *G. scapigera* (Bunn et al. 1992)

$\frac{1}{2}$  MS and WPM was helpful along with 1-4  $\mu\text{M}$  BAP and 0.01-0.02 NAA in the case of some grevilleas

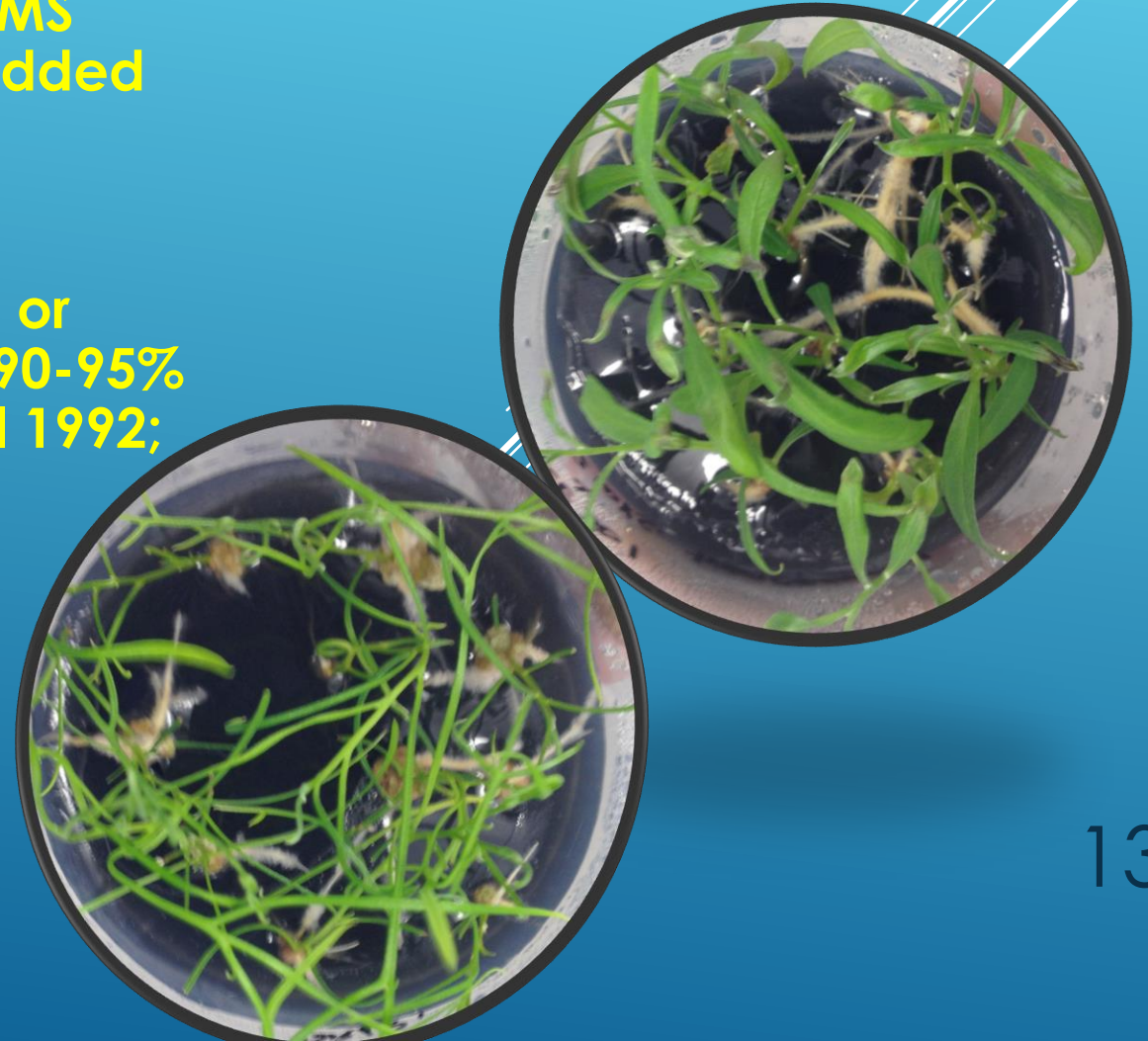
17 species of grevilleas multiplied on MS medium containing 1.0- 1.5  $\mu\text{M}$  BAP alone (Offord and Tyler 1998).



# ROOTING

In vitro rooting is reasonably easy in  $\frac{1}{2}$  MS Medium containing 5.0 –10.0  $\mu\text{M}$  IBA. Added charcoal (0.5 -2.0 g/l) also is helpful.

Ex vitro rooting with IBA powder (1g/Kg or 3g/Kg), fogged glasshouse condition (90-95% humidity) gave good results (Bunn et al 1992; Offord and Tyler 1998)



# ACCLIMATIZATION

- In vitro rooted Grevilleas acclimated in greenhouse with fogging initially, but misting two weeks after deflasking.
- Ex vitro rooted Grevilleas rooted and acclimated in a fogged glass house (Bunn et al 1992; Offord and Tyler 1998).
- Porosity of potting mix is critical for easy hardening

# Acclimatized Grevilleas



'Moonlight'



'Longistyla'



'Superb'



'Orange Marmalade'



# Leaf shape transition with maturity





# PROPAGATABILITY INDEX

Propagatability index (PI) is the product of success rates at initiation x multiplication x rooting x acclimatization

$PI = I \times M \times R \times A$ , where  $I$  = % success rate at initiation,  
 $M$  = multiplication rate per month,  $R$  = rate of rooting,  
 $A$  = rate of establishment at hardening stage

For example  $(I) 0.50 \times (M) 4 \times (R) 0.90 \times (A) 0.80 = (PI) 1.44$

In general, tissue culture of a species in demand with a PI over 0.70 is commercially viable.





Thank  
you!

