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In vitro propagation of Australian Proteaceae (*Conospermum*)

Eric Bunn, Kings Park and Botanic Garden, Kings Park West Perth 6005

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Abstract

In vitro methods are investigated for four species of the Australian genus *Conospermum* (smokebush): *C, fioribundum, C. incurvum, C. stoechadis* and C. *triplinervum.* Shoots are multiplied on a 1/2 MS medium supplemented with 5u.M kinetin and 0.5 μ,M BA. Multiplication rates vary between species but addition of cytokinins effectively doubles or trebles the multiplication rate. Shoots are elongated by alternating incubation periods on media without plant hormones or containing up to 3μ.M GA, Elongated shoots are then rooted on medium containing auxins (10 μ.M NAA or 5μ.M NAA + 5μ,M IBA for best results with *Conospermum stoechadis* and C. *triplinervum).* Rooted shoots are acclimatized before transfer to pasteurized potting medium (coarse sand, peat; perlite in equal proportions), resulting in a creditable survival rate for *Conospermun triplinervum, C. stoechadis* and C. *incurvum.*

Abbreviations

MS = Murashige &Skoog (1962) medium, K = kinetin, BAP = 6-benzylaminopurine, GA = gibberellic acid, PGR = plant growth regulators, NAA = naphthalene acetic acid, IBA = indolebutyric acid.

Summary

Viable tissue cultures of four western Australian smokebush species, *Conospermum fioribundum,* C, *incurvum, C. stoechadis* and *C. triplinervium* are established from shoot tips of cuttings from wild collected mature plants, Sterilization procedures (Table 1a) are broadly successful but variable with species and the time of year when explant material is collected (Table 1 b),

Table 1a Sterilization procedure for *Conospermum*** shoots

Wash in running tap water for hours
Rinse in 1% v/v tween-80 for 2 minutes
Sterilize in 1%v/v NaOCI for 5 -7 minutes
Wash in 3 changes of sterile distilled water
Cut material into nodal or apical segments
Place explants in media
Keep cultures in darkness for 7 days
Move sterile cultures to standard culture conditions after 7 days
Subculture onto fresh media monthly

***Conospermum fioribundum, C. incurvum, C. stoechadis, C. triplinervum*

Table 1b. Percentage of sterile viable explants resulting in successful initiation of cultures of *Conospermum* species from seasonally collected from wild plants.

Species	Autumn	Winter	Spring	Summer
C. floribundum				70
C. incurvum			36	
C. triplinervum		16		
C. stoechadis			20	

Shoot cultures are multiplied on 1/2 MS (1962) medium supplemented with 500 μM myo- inositol, 3μ,M each thiamine and pyridoxine hydrochloride and nicotinic acid, 60 mM sucrose, 50O μM MES buffer, pH 6.0, 9 g/L agar, 2.5μ.M Kinetin + 0.25 μM BAP, or 5μM kinetin + 3μM GA. Initially tubes were used, then 250 ml, glass jars with aeration holes covered with a medical (autoclavable) porous tape or teflon microfilter self-adhesive discs. Shoot cultures are incubated at 25ºC with 16 hours light and 8 hours of darkness on a daiiy cycle. Shoot multiplication has been estimated (Table 2) in vitro in basal (1/ 2 MS no growth regulators) and plant hormone supplemented media and indicates that cytokinins (kinetin or BAP).are essential to obtain shoot proliferation. The addition of 3 μM GA to the medium stimulates greater shoot elongation in all species than basal medium.

Table 2. Empirical in vitro growth performance of *Conospermum* species

Species	Medium:PGR(μM)	Multiplication rate/month
C. floribundum	1/2MS	x1-2
	1/2MS:K(2.5)+BAP(0.25)	x3
	1/2MS:K(5)+GA(3)	x3
C. incurvum	1/2MS	x1-2
	1/2MS:K(2.5) + BAP(0.25)	x2-3
	1/2MS-K(5) + GA(3)	x2-3
C. triplinervum	1/2MS	x1-2
	1/2MS:K(2.5) + BAP(0.25)	x4
	1/2MS:K(5)+GA(3)	x4
C. stoechadis	1/2MS	x1-2
	1/2MS:K(2.5)+BAP(0.25)	x3
	1/2MS:K(5)+GA(3)	x5

MS=Murashige &Skoog (1962) medium, K=kinetin, BAP=6-benzylaminopurine, GA=gibberellic acid, PGR-plant growth regulators

Root induction of suitable shoots indicates that 87 % of shoots of *Conospermum tnplinervum* initiate roots when incubated on medium containing 10 μM NAA and up to 93% with a combination of 5μM NAA and 5 μM IBA. Results with C. *stoechadis* are less impressive but 40% of shoots initiated roots on medium supplemented with 10 μM NAA. Transfer of rooted shoots to pasteurized potting mixture (peat: perlite : coarse sand) has been successful but variable and further refinement is necessary to increase survival rates. Rooted shoots of *Conospermum incurvum* rooted on basal medium (1/2 MS, no growth regulators) have been successfully established in potting mixture.

Further work planned to finalize this study includes: repeating root induction experiments for alt species to obtain an optimal root induction medium and refining transfer to soil procedures to maximise plantlet survival.