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Micropropagation of kauri (*Agathis australis* (D.Don.) Lindl.): *in vitro* stimulation of shoot and root development and the effect of rooting hormone application method

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Abstract

Kauri (*Agathis australis* (D.Don.) Lindl.) is a coniferous forest species endemic to New Zealand. This unique resource is currently under threat from *Phytophthora* taxon *Agathis* infection that causes kauri die-back. This situation highlights the need not only for reliable clonal propagation methodologies to amplify genotypes exhibiting disease resistance but also the development of protocols that could be used for mature material for *ex situ* conservation of important genotypes. Understanding the viability of previously stored seed for culture initiations is also critical if trees subsequently die.

Six agar-based culture media were compared for their effects on shoot production from *in vitro* germinated mature zygotic kauri embryos. All root and some hypocotyl tissue was removed from the germinated embryos prior to initiation onto the culture medium. The number of shoots produced was highest (10 per embryo) in a full-strength, modified Quiorin and Lepoivre medium containing 3.5 g L^{-1} activated charcoal.

Four treatments incorporating use of rooting hormones were compared for their effects on root development from shoots produced *in vitro*. The dipping of stem ends in rooting powder containing talc plus 2% indole-3-butyric acid before transfer to potting mix stimulated root development in 68% of the shoots. Only 5 – 14% of shoots maintained in agar-based cultures with added rooting hormones before transfer to potting mix produced roots. Rooted plants continued to grow vigorously when transferred to standard nursery conditions. Variability among seed sources was high and there was no evidence that genotype influenced *in vitro* production of either shoots or roots.

Keywords: *Agathis australis*; organogenesis; root initiation; tissue culture.

Introduction

Kauri (*Agathis australis* (D.Don.) Lindl.) is known throughout the world as an iconic New Zealand coniferous forest species. Information about its history, cultural significance, timber properties and growth potential has been collected and reviewed by Steward and Beveridge (2010). Desirable features include: naturally good stem form; sapwood with a degree of stiffness comparable to that of Douglas-fir (*Pseudotsuga menziesii* (Mirbel) Franco); and tolerance to a wide range of site types, including marginal land. Growth models based on recent and historical data suggest that kauri is a more productive and commercially viable species than was previously thought (Steward & Beveridge, 2010). Research is now aimed at the improvement of field establishment techniques and enhancement of early growth rates.

Traditionally, kauri has been propagated by seed. Seedlings can be raised in containers or nursery beds as bare-rooted seedlings. Vertical taproots are

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damaged by mechanical undercutting and care needs to be taken to ensure adequate proliferation of lateral feeding roots occurs (Bergin & Steward, 2004). Kauri plants have been raised from cuttings but this method is considered to be too expensive to be adopted on a large scale (Barton, 1994). Seed has conventionally been sown as soon as possible after collection (February – March) to ensure maximum viability and to take advantage of warm conditions. Little is known about the continued viability of stored seed.

Tissue culture technology offers opportunities for the generation and use of clonal material for examination of site x genotype interactions that could increase the efficiency of planting programmes.

Research on embryogenesis and organogenesis of kauri began in the early 1990s. Methods were developed for stimulation of callus initiation from one- and two-year-old kauri cones and organ initiation from shoot tips derived from mature trees and three-year-old seedlings (J. Aitken-Christie et al., 1991, unpublished data). At that time, little progress was made in organogenesis and emphasis shifted to the study of embryogenesis. We are not aware of any published reports of the successful germination of somatic embryos from any genera of the Araucariaceae (*Araucaria*, *Agathis* and *Wollemia*).

Burrows et al. (1988) reported successful organogenesis from tissue derived from two-year-old seedlings of Queensland kauri (*Agathis robusta* (C.Moore) F.M.Bailey) and a range of *Araucaria* species. *In vitro* rooting of *Agathis robusta* was found to be possible but the success rate was only 5 – 20%. Although growth and multiplication were slow, the authors considered that their techniques were an improvement on current vegetative propagation procedures. They showed that newly initiated shoots derived from isolated stem segments of two-year-old *A. robusta* seedlings were less vigorous than those still in contact with a field-grown stem segment. This indicates that the culture medium was deficient in some factor provided by the parent plant.

In the study described here, culture methods known to be successful with kauri and other tree species were used as a basis for trials in which the effects of different media formulations could be compared. The aim was to increase the rates of shoot and root initiation; to monitor the early growth of resulting plantlets *in vitro*; and to observe the effects of transfer to standard nursery conditions.

Materials

Seed sources

Open-pollinated seed was collected from kauri trees growing at two sites in Northland, New Zealand. Seed was stored at 4 ºC prior to preparation for tissue culture. Seed from trees A, B and C (Hokianga Region) was collected in March 2007 and stored for approximately three months prior to use. Seed from Tree D (Waipoua Forest) had been collected in February 2006 and was stored for 9 months before use. Seed from Trees E and F (Hokianga), collected in March 2006, was used after 4 weeks' storage. Seeds were stored in different locations prior to use.

Media formulations

Shoot-initiation and early growth media

Six agar-based media were prepared and used for kauri-shoot initiation and early growth rate. Variants of a medium ("LP") described by Quoirin and Lepoivre (1977) were compared with a medium ("MS") described by Murishige and Skoog (1962). All six media were modified by the addition of charcoal (ch) at a rate of 3.5 g L^{-1}:

1. Full-strength LPch
2. Full-strength LPch + L-glutamic acid (1.0 g/L) + casein (0.05 g/L)
3. Half-strength LPch
4. Half-strength LPch + L-glutamic acid (1.0 g/L) + casein (0.05 g/L)
5. Half-strength MSch
6. Half-strength MSch + L-glutamic acid (1.0 g/L) + casein (0.05 g/L)

Full- and half-strength basal salt strength formulations of LP media (Hargreaves & Menzies, 2007) were tested. Only half-strength MS media (Burrows et al., 1988) were tested as earlier research had shown full-strength MS to be toxic for kauri (K. Gough, unpublished data).

Casein is widely used as a media supplement to provide a supply of organic nitrogen. The major component of casein is L-glutamic acid but casein also contains a range of amino acids (Ellinger & Boyne, 1965) that may assist plant growth.

Casein is a natural product so its composition may vary for a range of reasons. Therefore, only a low level of casein was added in each case. Instead, pure L-glutamic acid was added in order to provide a supply of organic nitrogen in a specific and reproducible manner. Pure L-glutamic acid has been found to be beneficial for elongation of *Wollemia nobilis* organogenic shoots (unpublished, K. Gough).

Full details of media used for these experiments are given in Appendix 1.

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Root-initiation Treatments

Four treatments were tested:

I. Control, i.e. culture in GD medium (Hargreaves & Menzies, 2007) containing 1 mg L^{-1} indole-3-butyric acid (IBA) and 0.5 mg L^{-1} 1-naphthalene acetic acid (NAA).

II. Culture in half-stength LP medium containing 1 mg L^{-1} IBA and 0.5 mg L^{-1} NAA.

III. The end of each stem was cut and dipped in rooting powder "Powder 1" (talc containing 1% IBA).

IV. The end of each stem was cut and dipped in rooting powder "Powder 2" (talc containing 2% IBA).

Full details of media and rooting powder compositions used for these experiments are provided in Appendix 1.

Potting Compost

Composition of the potting mix used is given in Appendix 2.

Methods

General sterilisation and culture techniques

Seed was soaked in water overnight and then immersed in 50% Chlorodux solution (5% available chlorine; Chlorogene Supplies, Lower Hutt, New Zealand) containing a few drops of surfactant (Silwett L-77; Helena Chemical Company, Tennessee, USA). After 20 minutes on an orbital stirring table, the seed suspension was drained and rinsed twice with sterile water on a laminar flow bench. Zygotic embryos were then removed aseptically and placed on shoot initiation media in Petri dishes (9 cm x 2.5 cm). The dishes, each containing five embryos, were placed in an illuminated incubator (photosynthetic photon flux density: 80 µE m s^{-1}) with a photo-period of 16 h light at 24 ºC and 8 h dark at 18 ºC. Shade cloth placed over the dishes reduced light intensity by approximately 50% during the first two weeks. Following germination, the radicle was removed from each embryo and the hypocotyl with cotyledons and epicotyl transferred to a 180 mL white-lidded glass jar (Australian Glass Company, Auckland, New Zealand) containing 30 mL of the appropriate initiation medium. Developing shoots were transferred to fresh jars of medium at 6 – 8 week intervals (3 – 4 shoots per jar). When approximately 3 cm in height, a basal portion (1 cm) was removed and used for initiation of new shoots. Shoot tips and basal segments were maintained in the same medium (Figure 1). When more than four basal segments had been obtained, the clonal material was transferred to 600 mL clear-lidded glass Agee jars (Australian Glass Company, Penrose, Auckland) each containing 80 mL of medium (8 – 12 shoots per jar).

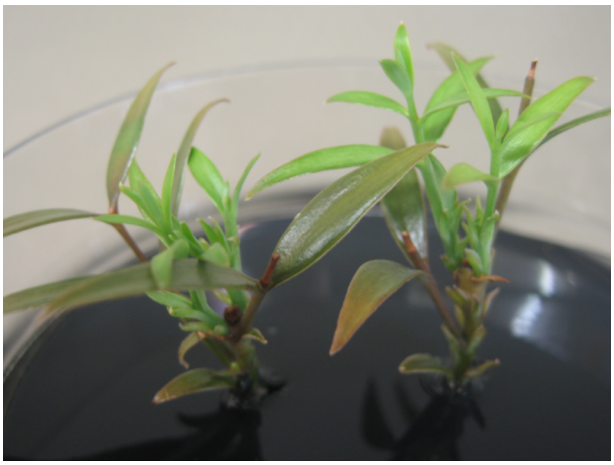

FIGURE 1: Kauri shoots elongating from stem segments.

Germination and early epicotyl development of Seedlot A

Thirty embryos from Seedlot A were transferred to each of the six shoot-initiation media using techniques described above. Roots were abscised prior to transfer to test media. Shoot development was assessed after 6 weeks.

Shoot multiplication of Seedlots A, B and C

Groups of 30 embryos from Seedlot A, 15 or 16 embryos from Seedlot B and five or six embryos from Seedlot C were transferred to each of the six shoot-initiation media using the methods described above. After *in vitro* germination, hypocotyls (including cotyledons and the developing epicotyls) were transferred from petri dishes to jars. Following elongation, shoots could be divided into stem segments with leaves and developing shoots, and these were transferred to fresh media every 8 – 12 weeks. At the time of each transfer, the number of shoots derived from each embryo and leaf characteristics (colour and resin formation) were noted.

Where possible, data were tested by ANOVA (Sokal & Rohlf, 1981) and treatment means were compared using Tukey's Multiple Range Test option. The assumptions for a valid analysis of variance were tested prior to analysis and were not violated. The data were normally distributed and so no transformation was required.

Root-initiation rate and early growth

Embryos derived from Seedlots A, D, E, and F were first germinated on shoot-initiation medium No. 3. When the shoots were approximately 3-cm long, they were cut at their base. This was done immediately

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before replications of seven shoots were subjected to one of four root-initiation treatments. Each treatment was replicated three times for Seedlots A and E and four times for Seedlots D and F.

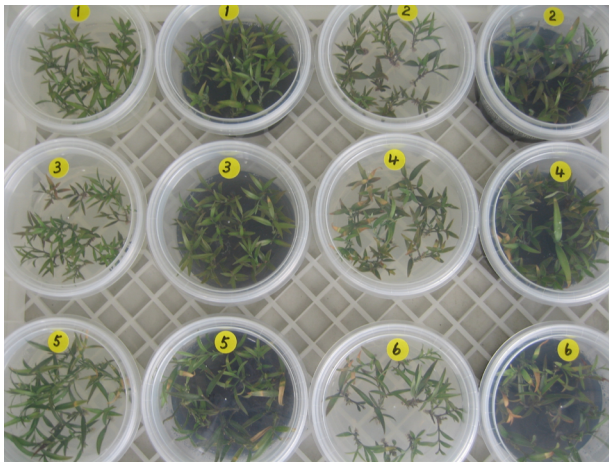

FIGURE 2: Kauri shoots recently transferred to root-initiation media. Light-coloured medium = Treatment I (Control). Dark-coloured medium = Treatment II.

At the end of the 14-day treatment period, shoots from Treatments I and II were transferred to potting mix in propagation trays. Shoots undergoing either Treatment III or IV were prepared at the same time.

All cultures were maintained at a temperature of 22 – 26 ± 2 ºC and high humidity (approximately 80 – 90% by hand misting). Two weeks after transfer to potting mix, shoots were gradually hardened off to ambient (40 – 50%) humidity conditions.

Every two weeks, a random sample of shoots was carefully lifted from the potting mix using forceps. Root development was observed before the shoots were equally carefully replaced into the potting mix. Final observations were made after 10 weeks. Rooted shoots were then transferred to plastic 6.5-cm diameter and 9-cm height pots and moved to standard nursery conditions.

Where possible, data were tested by ANOVA (Sokal & Rohlf, 1981) and treatment means were compared using Tukey's Multiple Range Test option. The assumptions for a valid analysis of variance were tested prior to analysis and were not violated. The data were normally distributed and so no transformation was required.

Results

All seedlots produced viable cultures irrespective of storage period. The number of shoots available per genotype determined which experiments they were used for.

Effect of medium formulation on germination and early epicotyl development of Seedlot A

All of the embryos transferred to initiation media produced epicotyls within six weeks (Table 1). Those in medium No. 4 (half-stength LPch + L-glutamic acid 1.0 g/L + casein 0.05 g/L) showed the best development.

Effect of medium formulation on shoot multiplication for Seedlots A, B and C

The composition of initiation medium had a significant effect on the number of shoots produced from each seedling ($p < 0.05$). An average of 8 – 10 shoots per seedling was produced in Medium No. 1 and Medium No. 3 compared with 1 – 2 shoots in Medium No. 5 (Table 2). Addition of L-glutamic acid and casein reduced the number of shoots produced in Medium No. 1 but had no effect on productivity in Medium No. 3 or Medium No. 5. All three seedlots tested here were collected from the same area at the same time but came from different trees. At this level, seed source had no significant effect on the mean number of shoots produced per seedling (Table 3).

Shoots maintained on Medium No. 5 media developed resin droplets on the foliage, irrespective of seedlot. Foliage browning was apparent 2 – 4 weeks after transfer to all media except full-strength LPch Medium (No. 1).

TABLE 1: Development of embryos from Seedlot A after *in vitro* culture for 6 weeks.

Shoot-initiation Medium[1]				Numbers of embryos		
No.	**Type**	**Strength**	**L-glutamic acid & casein added**	**Cotyledons only**	**Epicotyls emerged**	**Epicotyls elongated**
1	LPch	Full	No	12	18	0
2	LPch	Full	Yes	7	20	3
3	LPch	Half	No	1	21	8
4	LPch	Half	Yes	0	12	18
5	MSch	Half	No	6	18	6
6	MSch	Half	Yes	1	23	6

[1] *See Appendix 1 for full details of medium composition*

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TABLE 2: Effect of culture medium on shoot productivity (Seedlots A, B and C).

Shoot-initiation Medium[1]				Number of embryos	Number of shoots/ hypocotyl[2]
No.	Type	Strength	L-glutamic acid & casein added		
1	LPch	Full	No	51	10.33 a
2	LPch	Full	Yes	50	5.66 b
3	LPch	Half	No	51	8.43 ab
4	LPch	Half	Yes	50	7.73 ab
5	MSch	Half	No	51	2.00 c
6	MSch	Half	Yes	52	0.69 c

[1] See Appendix 1 for full details of medium composition
[2] Values followed by the same letter do not differ at p < 0.05 (Tukey's Multiple Range Test).

Comparison of four test treatments on kauri root initiation rate and early growth

Of the 392 shoots used for the root initiation study, 97% survived for at least 10 weeks. Some foliage browning was observed among those derived from Seedlots D, E and F but not Seedlot A.

The percentage of shoots producing roots was higher ($p < 0.05$) after Treatment III (41%) or Treatment IV (68%) than after Treatment II (5%) (Table 4). The effect of either Treatment II or III was not significantly different from that of the control Treatment I (14%) however. Seedlots differed in origin, collection date and storage time but there was no significant effect of seedlot on root development, Table 4.

Shoots transferred to agar media containing rooting hormones (Treatment II) tended to produce a large callus. Shoots treated with a rooting-powder dip (Treatments III or IV) developed root initials in the cambium and had vascular connections from within the cambial layer. Each rooting shoot produced 1 – 4 strong and relatively thick roots that were vertically, rather than laterally orientated (Figure 3). Following transfer to the nursery propagation facility, rooted plants grew well irrespective of treatment. However, plants that developed after rooting-powder treatment had longer lateral branches than naturally germinated kauri seedlings. No plagiotrophism was observed, and plants remained strongly orthotropic. Plants propagated by tissue culture methods were found to assume the characteristics of seedlings of a similar age after 10 weeks under standard nursery conditions (Figure 4). The original foliage, formed *in vitro*, lacks the cuticular wax of material grown *in situ* under natural conditions. *In vitro* foliage probably also has differences in chloroplasts due to the low-light, high-humidity and gaseous environment present in sealed vessels. It is the new foliage that forms concurrently with root generation and hardening to ambient conditions that continue the growth of the plant while the original foliage, formed *in vitro*, dies back.

TABLE 3: Effect of seedlot on shoot productivity.

Seedlot	Number of hypocotyls	Number of shoots/ hypocotyl[1]
A	180	5.58 a
B	94	5.84 a
C	31	6.97 a

[1] Values followed by the same letter do not differ at p < 0.05 (Tukey's Multiple Range Test).

Discussion

Culture methods used in this investigation can be expected to stimulate the production of 10 healthy shoots from a single kauri embryo. Of these shoots, 6 – 7 can be expected to form roots and develop into vigorous plants. The 68% success rate in root development is not as high as the 80% reported by Burrows et al. (1988) for *Agathis robusta*. On the other hand, it represents a major improvement compared with standard nursery propagation methods in which a maximum of one seedling develops from each embryo.

Preest (1979) drew attention to the fact that collection of kauri seed is difficult, hazardous and, therefore, expensive. In terms of propagation, these factors may be offset to some extent by the abundance and regularity of seed crops, the high germination rate (88% in fresh seed) and the fairly rapid rate of juvenile growth. Even so, Preest considered that collected seed material should be preserved and conserved. The culture methods described here offer methods for more efficient use of collected material. They could be of special use to Maori wishing to propagate material from notable parent trees.

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TABLE 4: Percentage of shoots with developing roots 10 weeks after transfer to potting medium.

Seedlot	Replicate	Root-initiation Treatment				Seedlot mean[1]
		I	II	III	IV	
A	1	14	0	43	57	33*
	2	0	0	57	71	
	3	0	43	43	71	
D	1	29	0	14	57	26*
	2	0	14	29	57	
	3	0	14	14	100	
	4	0	0	0	86	
E	1	0	0	29	43	29*
	2	0	0	71	71	
	3	29	0	43	57	
F	1	29	0	43	57	40*
	2	29	0	57	86	
	3	29	0	100	86	
	4	43	0	29	57	
Treatment mean[2]		**14 bc**	**5 c**	**41 ab**	**68a**	

[1] Seedlot means were not significantly different at p ≤ 0.05 (Tukey's Multiple Range Test)

[2] Treatment means followed by the same letter do not differ at p < 0.05 (Tukey's Multiple Range Test).

Knowledge about the history of the seed collected is likely to add to its value. The wide variability observed among the seedlots used in this investigation suggests that growth characteristics may also vary. The culture methods described here offer scope for the cloning of selected genotypes and propagation of characteristics likely to enhance performance in plantations.

Burrows et al. (1988) showed that newly formed shoots of *Agathis robusta* were more vigorous while still in contact with the field-grown stem segment than when isolated. This indicates that it may be possible to supplement media in a way that provides more of the stimulatory factors present in the parent tissue. The difference in shoot initiation and early growth rates shown here suggest that kauri development is sensitive to culture conditions. Further work will be needed to investigate specific requirements.

FIGURE 3: Root formation from tissue-cultured shoots, showing multiple vertical root formation 10 weeks after treatment.

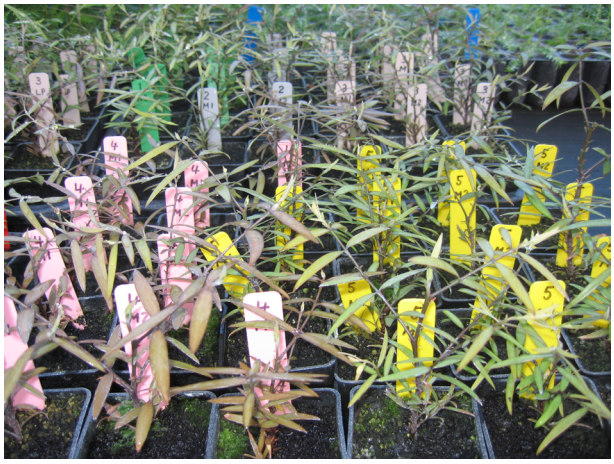

FIGURE 4: Rooted plants showing good growth following 10 weeks in the nursery propagation facility.

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It may also be worthwhile to investigate the effect of increased temperature during tissue culture since Burrows et al. (1988) reported an almost linear increase in shoot length at temperatures ranging from 22 ºC (2.8 mm) to 30 ºC (6.5 mm).

Slow growth observed in young kauri plants may be attributable in part to the time required for the development of effective root systems. Kauri seedlings, whether potted or bare-rooted, are slow to form balanced fibrous root systems (Morrison & Lloyd, 1972; Bergin & Steward, 2004) and this could account for observations of slow seedling establishment in plantations and naturally established stands (Steward & Beveridge, 2010). Plants raised by tissue culture methods may establish more successfully than seedlings if earlier development of vigorous root systems confers an advantage.

Although the culture of kauri plants from non-germinated excised zygotic embryos is possible, shoot multiplication is a slow process. There is little doubt that shoot initiation and growth rate would respond to further modification of media formulation. Different plant growth regulators could be evaluated, and it would be worthwhile to test them on clonal material. Further investigation of *in vitro* root initiation using methods developed for *Agathis robusta* might be useful for the development of pathogen sensitivity screening tests and for long-term *ex situ* conservation of kauri propagation material.

Conclusions

Media containing LP basal salts at either half- or full strength were better for sustained shoot elongation of *Agathis australis* than half-strength MS media.

Rooting powder containing 1 or 2% IBA was beneficial for rooting *in vitro* grown *Agathis australis* shoots. This method was also simpler and more cost effective than a two-week transfer to GD rooting medium.

There were no significant differences between seedlot means for either shoot production or rooting.

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APPENDIX 1: Composition of each treatment used

Treatment Use			Shoot initiation and maintenance						Root initiation			
Treatment Type			**Solid medium**						**Solid medium**		**Powder dip**	
Treatment no.			**1**	**2**	**3**	**4**	**5**	**6**	**I**	**II**	**III**	**IV**
Treatment Name			**LPch**	**LPch+ L-glutamic acid + casein**	**½LPch**	**½LPch + L-glutamic acid + casein**	**½MSch**	**½MSch + L-glutamic acid + casein**	**CONTROL: GD + rooting hormones**	**½LP + rooting hormones**	**Powder 1**	**Powder 2**
Component type	**Component name**	**Component structure**	**Concentration (mg/L)**						**Concentration (mg/L)**		**%**	
Major elements	Potassium nitrate	KNO_3	1800	1800	900	900	950	950	1000	900	-	-
	Potassium chloride	KCl	-	-	-	-	-	-	300	-	-	-
	Potassium dihydrogen phosphate	KH_2PO_4	270	270	135	135	85	85	-	135	-	-
	Calcium nitrate	$Ca(NO_3)_2 \cdot 4H_2O$	1200	1200	600	600	-	-	-	600	-	-
	Calcium chloride	$CaCl_2 \cdot 2H_2O$	-	-	-	-	220	220	150	-	-	-
	Ammonium nitrate	NH_4NO_3	400	400	200	200	825	825	-	200	-	-
	Ammonium sulphate	$(NH_4)_2SO_4$	-	-	-	-	-	-	200	-	-	-
	Magnesium sulphate	$MgSO_4 \cdot 7H_2O$	360	360	180	180	185	185	250	180	-	-
	Sodium dihydrogen phosphate dihydrate	$NaH_2PO_4 2H_2O$	-	-	-	-	-	-	100	-	-	-
	Di-Sodium hydrogen phosphate dihydrate	Na_2HPO_4	-	-	-	-	-	-	3	-	-	-
Minor elements	Zinc sulphate	$ZnSO_4 \cdot 7H_2O$	8.60	8.60	8.60	8.60	8.60	8.60	1.00	8.60	-	-
	Boric acid	H_3BO_3	6.20	6.20	6.20	6.20	6.20	6.20	5.00	6.20	-	-
	Manganous sulphate	$MnSO_4 \cdot 4H_2O$	20.0	20.0	20.0	20.0	22.3	22.3	20.0	20.0	-	-
	Cupric sulphate	$CuSO_4 \cdot 5H_2O$	0.25	0.25	0.25	0.25	0.025	0.025	0.20	0.25	-	-
	Potassium iodide	KI	0.08	0.08	0.08	0.08	0.83	0.83	1.00	0.08	-	-
	Sodium molybdate	$Na_2MoO_4 \cdot 2H_2O$	0.25	0.25	0.25	0.25	0.25	0.25	0.20	0.25	-	-
	Cobaltous chloride	$CoCl_2 \cdot 6H_2O$	0.025	0.025	0.025	0.025	0.025	0.025	0.20	0.025	-	-

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APPENDIX 1: continued

Treatment Use			Shoot initiation and maintenance						Root initiation			
Treatment Type			**Solid medium**						**Solid medium**		**Powder dip**	
Treatment no.			**1**	**2**	**3**	**4**	**5**	**6**	**I**	**II**	**III**	**IV**
Treatment Name			**LPch**	**LPch+ L-glutamic acid + casein**	**½LPch**	**½LPch + L-glutamic acid + casein**	**½MSch**	**½MSch + L-glutamic acid + casein**	**CONTROL: GD + rooting hormones**	**½LP + rooting hormones**	**Powder 1**	**Powder 2**
Component type	**Component name**	**Component structure**	**Concentration (mg/L)**						**Concentration (mg/L)**		**%**	
Vitamins	Thiamine HCl	$C_{12}H_{17}ClN_4OS{\cdot}HCl$	0.4	0.4	0.4	0.4	0.1	0.1	5.0	0.4	-	-
	Nicotinic acid	$C_6H_5NO_2$	-	-	-	-	0.5	0.5	5.0	-	-	-
	Pyridoxine HCl	$C_8H_{11}NO_3{\cdot}HCl$	-	-	-	-	0.5	0.5	0.5	-	-	-
Iron-containing compounds	Ethylenediamine-tetra-acetate disodium dihydrate	$C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$	40	40	40	40	40	40	40	40	-	-
	Ferrous sulphate heptahydrate	$FeSO_4{\cdot}7H_2O$	30	30	30	30	30	30	30	30	-	-
Rooting Hormones	3-Indolebutyric acid (IBA)	$C_{12}H_{13}NO_2$	-	-	-	-	-	-	1.0	1.0	1.0	2.0
	1-Naphthalene acetic acid (NAA)	$C_{12}H_{10}O_2$	-	-	-	-	-	-	0.5	0.5	-	-
Fertiliser	Thrive[1]		-	-	-	-	-	-	-	-	5	10
Fungicide	Captan[2]		-	-	-	-	-	-	-	-	5	10
Other ingredients	Sugar	$C_{12}H_{22}O_{11}$	30000	30000	30000	30000	30000	30000	20000	30000	-	-
	Myo-inositol	$C_6H_{12}O_6$	1000	1000	1000	1000	1000	1000	1000	1000	-	-
	Bacto agar	$(C_{12}H_{18}O_9)_n$	7500	7500	7500	7500	7500	7500	7500	7500	-	-
	Charcoal[3]	C	3500	3500	3500	3500	3500	3500	-	-	-	-
	L-glutamic acid	$C_5H_9NO_4$	-	1000	-	1000	-	1000	-	-	-	-
	Casein	variable	-	50	-	50	-	50	-	-	-	-
	Talc	$Mg_3Si_4O_{10}(OH)_2$	-	-	-	-	-	-	-	-	89	78

[1] *Thrive is a foliar fertiliser produced by Yates Ltd.*
[2] *Captan is a fungicide produced by Jiangsu Alpha Hawk Chemicals Industrial Corp.*
[3] *Activated charcoal (Merck).*

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APPENDIX 2: Composition of potting mix

The potting mix used was prepared using a range of components as follows:

Major component	%
Fibre Medium Screened[1]	40.0
Pacific Pumice (3mm)	20.0
Perlite C4	20.0
Scobie Peat	20.0

[1] *Daltons cutting mix (with no fertilisers)*

Each cubic metre of basic mixture above was supplemented with the following additives:

Additive	Kg m^{-3}
Dolomite	3.0
Gypsum Fine	2.0
Wetting Agent (Hydraflo II G[1])	1.0
Lime	1.0

[1] *Scotts, Australia*

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