

[10.1071/BT22116](https://doi.org/10.1071/BT22116)

Australian Journal of Botany

Supplementary Material

Asymbiotic germination of *Prasophyllum* (Orchidaceae) requires low mineral concentration

Marc Freestone^{A,B,*}, Celeste Linde^B, Nigel Swarts^C, and Noushka Reiter^{A,B}

^ARoyal Botanic Gardens Victoria, Science Division, Corner of Ballarto Road and Botanic Drive, Cranbourne, Vic. 3977, Australia.

^BEcology and Evolution, Research School of Biology, The Australian National University, Canberra, ACT 2600, Australia.

^CTasmanian Institute of Agriculture, The University of Tasmania, Sandy Bay, Tas. 7005, Australia.

*Correspondence to: Marc Freestone Royal Botanic Gardens Victoria, Science Division, Corner of Ballarto Road and Botanic Drive, Cranbourne, Vic. 3977, Australia Email: marc.freestone@gmail.com

Supplementary Table S1. Composition of nutrient media for the broad spectrum germination trial. Each of the 81 media are defined by a four-letter coding system (first letter = minerals, second = auxins, third = cytokinins, fourth = growth factors + amino acids + sucrose), e.g. the medium LMHL contains the L column of minerals, M column of Auxins, H column of cytokinins, L column of growth factors + amino acids, and L column of sucrose (de Fossard, 1985).

Component	Minerals			Auxins			Cytokinins			Growth factors + amino acids		
	L	M	H	L	M	H	L	M	H	L	M	H
NH ₄ NO ₃ (mmol/L)	5	10	20	-	-	-	-	-	-	-	-	-
KNO ₃ (mmol/L)	-	10	20	-	-	-	-	-	-	-	-	-
KH ₂ PO ₄ (mmol/L)	0.1	-	-	-	-	-	-	-	-	-	-	-
NaH ₂ PO ₄ (mmol/L)	-	1.0	2.0	-	-	-	-	-	-	-	-	-
KCl (mmol/L)	1.9	-	-	-	-	-	-	-	-	-	-	-
CaCl (mmol/L)	1.0	2.0	3.0	-	-	-	-	-	-	-	-	-
MgSO ₄ (mmol/L)	0.5	1.5	3.0	-	-	-	-	-	-	-	-	-
H ₃ BO ₃ (mmol/L)	10	50	150	-	-	-	-	-	-	-	-	-
MnSO ₄ (mmol/L)	10	50	100	-	-	-	-	-	-	-	-	-
ZnSO ₄ (mmol/L)	1	20	40	-	-	-	-	-	-	-	-	-
CuSO ₄ (mmol/L)	0.01	0.1	1.5	-	-	-	-	-	-	-	-	-
Na ₂ MoO ₄ (mmol/L)	0.01	0.1	1.0	-	-	-	-	-	-	-	-	-
CoCl ₂ (mmol/L)	0.1	0.5	1.0	-	-	-	-	-	-	-	-	-
KI (mmol/L)	0.5	2.5	5.0	-	-	-	-	-	-	-	-	-
FeSO ₄ (mmol/L)	10	50	100	-	-	-	-	-	-	-	-	-
Na ₂ EDTA (mmol/L)	10	50	100	-	-	-	-	-	-	-	-	-
Na ₂ SO ₄ (mmol/L)	40	450	650	-	-	-	-	-	-	-	-	-
Indole-3-acetic acid (μmol/L)	-	-	-	0.1	1	10	-	-	-	-	-	-
Indole-3-butyric acid (μmol/L)	-	-	-	0.1	1	10	-	-	-	-	-	-
1-Naphthaleneacetic acid (μmol/L)	-	-	-	0.1	1	10	-	-	-	-	-	-
2-Naphthoxyacetic acid (μmol/L)	-	-	-	0.1	1	10	-	-	-	-	-	-
Kinetin (μmol/L)	-	-	-	-	-	-	0.1	1	10	-	-	-
6-Benzylaminopurine (μmol/L)	-	-	-	-	-	-	0.1	1	10	-	-	-
Inositol (μmol/L)	-	-	-	-	-	-	-	-	-	100	300	600
Nicotinic acid (μmol/L)	-	-	-	-	-	-	-	-	-	4	20	40
Pyridoxine.HCl (μmol/L)	-	-	-	-	-	-	-	-	-	0.6	3	6
Thiamine.HCl (μmol/L)	-	-	-	-	-	-	-	-	-	0.1	2	40
Biotin (μmol/L)	-	-	-	-	-	-	-	-	-	0.04	0.2	1.0
D-Ca-Pantothenate (μmol/L)	-	-	-	-	-	-	-	-	-	0.2	1	5
Riboflavin (μmol/L)	-	-	-	-	-	-	-	-	-	0.1	1	10
Ascorbic acid (μmol/L)	-	-	-	-	-	-	-	-	-	0.1	1	10
Choline Chloride (μmol/L)	-	-	-	-	-	-	-	-	-	0.1	1	10
Cysteine.HCl (μmol/L)	-	-	-	-	-	-	-	-	-	10	60	120

Glycine (μmol/L)	-	-	-	-	-	-	-	-	-	0.5	5	50	S U C R O S E
Sucrose (mmol/L)	-	-	-	-	-	-	-	-	-	6	60	120	
Agar (g/L)	-	-	-	-	-	-	-	-	-	8	8	8	
Streptomycin sulfate (g/L)	-	-	-	-	-	-	-	-	-	0.05	0.05	0.05	

Supplementary Table S2. Presence (1) or absence (0) of stage 5 germination of *Prasophyllum frenchii* in the broad spectrum experiment after 12 months. ‘-’ denotes a contaminated replicate (not measured). Treatment names are as follows: the first letter represents the concentration of the mineral component (‘L’ = Low, ‘M’ = Medium, ‘H’ = High), the second letter the concentration of the auxins component, the third letter the concentration of the cytokynins component and the fourth letter the concentration of the sucrose plus growth factors plus amino acids component as per de Fossard *et al.* (1974; 1985).

Media	Replicate #				Media	Replicate #			
	1	2	3	4		1	2	3	4
LLLL	0	0	-	-	MMMh	0	0	0	1
LLLM	1	0	1	-	MMHL	0	0	0	0
LLLH	0	0	-	-	MMHM	0	0	0	-
LLML	0	0	0	0	MMHH	0	0	0	-
LLMM	1	1	1	-	MHLL	0	0	-	-
LLMH	1	1	0	-	MHLM	0	0	0	0
LLHL	0	0	-	-	MHLH	0	0	0	0
LLHM	0	0	0	0	MHML	0	0	0	0
LLHH	0	0	0	0	MHMM	0	0	0	0
LMLL	0	0	0	-	MHMh	0	0	-	-
LMLM	0	0	0	0	MHHL	0	0	0	-
LMLH	0	0	0	0	MHHM	0	0	-	-
LMMML	0	0	0	0	MHHH	0	0	0	-
LMMM	0	0	0	-	HLLL	0	0	0	-
LMMH	0	0	0	-	HLLM	0	-	-	-
LMHL	0	0	0	0	HLLH	0	0	0	0
LMHM	0	0	0	0	HLML	0	0	0	-
LMHH	0	0	-	-	HLMM	0	0	0	-
LHLL	0	0	0	0	HLMH	0	0	0	0
LHLM	0	0	0	0	HLHL	0	-	-	-
LHLH	0	0	-	-	HLHM	0	0	0	-
LHML	0	0	0	0	HLHH	0	0	0	0
LHMM	0	0	0	0	HMLL	0	0	0	0
LHMH	0	0	0	-	HMLM	0	0	0	-
LHHL	0	0	0	0	HMLH	0	0	0	0
LHHM	0	0	0	-	HMML	0	0	0	-
LHHH	0	0	0	0	HMMM	0	0	0	0
MLLL	0	0	0	-	HMMH	0	0	0	0
MLLM	0	0	0	-	HMHL	0	0	0	0
MLLH	0	0	0	0	HMMH	0	0	-	-
MLML	0	-	-	-	HMMH	0	0	0	0
MLMM	0	0	0	0	HLLL	0	0	0	-
MLMH	0	-	-	-	HLLM	0	0	0	0
MLHL	0	0	0	0	HLLH	0	0	-	-
MLHM	0	0	0	-	HLML	0	0	0	0
MLHH	0	0	0	0	HHMM	0	0	0	-

MMLL	0	0	0	0	HMH	0	0	0	0
MMLM	0	0	0	0	HHHL	0	0	0	0
MMLH	0	0	0	-	HHHM	0	0	0	-
MMML	0	0	0	-	HHHH	0	-	-	-
MMMM	-	-	-	-					

Supplementary Table S3. Modelled mean leaf length increase in the replate media trial, standard error, and the summary statistics of linear mixed effects model output with W9+C set as the reference group. '+C' = the addition of 5 % coconut water; '+B' = the addition of 5 % banana pulp. The variance of the random effect (jar) was 27.93 compared to a residual variance of 327.13, indicating that there was more variance among jars than within jars.

Media	Mean (mm)	SE (mm)	t-value	Pr(> t)
W9+C	35.8	3.9	-	-
W9+B	33.9	5.0	-0.37	0.709
W9.2+C	30.2	5.5	-1.02	0.310
W3+C	28.9	5.4	-1.28	0.203
W2.5+C	28.6	6.2	-1.16	0.249
W9+C+B	26.3	5.6	-1.72	0.088
W3+B	26.2	5.2	-1.83	0.070
W1+C	23.9	4.9	-2.42	0.018
W2.5+B	23.8	5.6	-2.16	0.033
W9.2+B	23.1	5.6	-2.27	0.026
W9.5+C	22.7	5.1	-2.57	0.012
W1+C+B	21.2	5.2	-2.82	0.006
W9.5+B+C	21.1	5.5	-2.69	0.008
W9.5+B	18.3	5.1	-3.44	>0.001
W9.2+B+C	15.5	6.6	-3.06	0.003
W1+B	14.1	5.0	-4.30	>0.001
W3+C+B	13.7	5.8	-3.85	>0.001
LLLM+C	12.1	5.9	-3.99	>0.001
1/2MS+C	11.1	5.5	-4.47	>0.001
1/2MS+C+B	9.8	5.7	-4.54	>0.001
LLLM+C+B	7.0	5.7	-5.07	>0.001
LLLM+B	6.9	5.8	-5.01	>0.001
W2.5+B+C	6.4	6.5	-4.49	>0.001
1/2MS+B	5.9	5.8	-5.16	>0.001

Supplementary Table S4. Modelled mean root length in the replat media trial, standard error, and the summary statistics of linear mixed effects model output with W9.5+C set as the reference group. ‘+C’ = the addition of 5 % coconut water; ‘+B’ = the addition of 5 % banana pulp. The variance of the random effect (jar) was 0.74 compared to a residual variance of 22.47, indicating that there was more variance among jars than within jars.

Media	Mean	SE	t-value	Pr(> t)
W9.5+C	10.3	0.7	-	-
W1+C	9.4	1.0	-0.89	0.380
W9+C+B	8.9	1.2	-1.14	0.257
W9+B	8.8	1.0	-1.50	0.140
W9.2+C	8.7	1.2	-1.31	0.192
W9.2+B	8.5	1.2	-1.48	0.145
W9.5+B	8.1	1.0	-2.07	0.043
W9+C	7.8	1.2	-2.09	0.039
W3+C	7.8	1.1	-2.22	0.029
W3+B	7.0	1.1	-3.09	0.003
W1+C+B	7.0	1.1	-3.13	0.002
W2.5+C	6.8	1.4	-2.49	0.014
W2.5+B	6.7	1.2	-3.04	0.003
W9.5+B+C	6.5	1.2	-3.26	0.001
W1+B	6.1	1.0	-4.10	>0.001
1/2MS+C	5.4	1.2	-4.11	>0.001
W9.2+B+C	4.8	1.5	-3.78	>0.001
LLLM+B	4.7	1.2	-4.51	>0.001
W3+C+B	4.2	1.2	-4.82	>0.001
1/2MS+B	3.8	1.3	-5.18	>0.001
LLLM+C	3.6	1.3	-5.24	>0.001
1/2MS+C+B	3.5	1.2	-5.49	>0.001
W2.5+B+C	3.0	1.5	-5.01	>0.001
LLLM+C+B	2.8	1.2	-6.14	>0.001

Supplementary Table S5. Percentage of seedlings with green leaves at the conclusion of the replant media trial, and the summary statistics of general linear model output with W3+C set as the reference group.

Media	% seedlings with green leaves	z-value	Pr(> z)
W3+C	91.9	-	-
W1+B	88.9	-0.5	0.6
W9.2+B	84.2	-1.0	0.3
W9.5+B	79.7	-1.6	0.1
W9+C+B	74.2	-1.9	0.1
W3+B	71.4	-2.2	0.0
W9+C	68.8	-2.3	0.0
W9+B	67.1	-2.6	0.0
W9.5+C	66.7	-2.6	0.0
W1+C+B	64.7	-2.7	0.0
W9.5+B+C	55.9	-3.2	0.0
W3+C+B	48.0	-3.5	0.0
W9.2+B+C	47.1	-3.3	0.0
W2.5+C	42.1	-3.6	>0.001
W2.5+B	40.0	-4.0	>0.001
W9.2+C	39.4	-4.1	>0.001
1/2MS+C+B	37.0	-4.1	>0.001
1/2MS+B	32.0	-4.3	>0.001
W1+C	21.6	-5.6	>0.001
1/2MS+C	19.4	-5.1	>0.001
LLLM+B	13.8	-5.3	>0.001
W2.5+B+C	12.5	-4.5	>0.001
LLLM+C	4.0	-4.7	>0.001
LLLM+C+B	3.6	-4.8	>0.001