Optimizing *in vitro* Growth Conditions for *Magnolia* 'Ann'

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Index Words: cytokinins, basal salts, *Magnolia* 'Ann', phenolic binding agents, polyploidy, micropropagation

Significance to Industry: *Magnolia* (*M. liliiflora* 'Nigra'× *M*. *stellata* 'Rosea') 'Ann' (NA 28344; PI 326570) is a member of the 'Little Girl' series of magnolias that have become widely popular. *Magnolia* 'Ann' is characterized by a desirable combination of traits including prolific and remontant flowering and a shrubby form. Due to difficulties in propagating magnolias from cuttings or grafting, development and optimization of *in vitro* propagation methods would be desirable. *Magnolia* 'Ann' is also a triploid, interspecific hybrid that is reportedly sterile (22). *In vitro* chromosome doubling may be an approach to develop allopolyploids with restored fertility (4) and provide an opportunity to use this cultivar in future breeding programs. To enhance the ornamental qualities of 'Ann', micropropagation protocols were developed as a platform for propagation and future ploidy level manipulation. Murashige and Skoog basal medium (MS)(17) supplemented with 2 µM benzylamino purine (BAP) provided high shoot proliferation, while Lloyd and McCown Woody Plant Medium (WPM)(8) containing charcoal may be used to produce elongated plantlets more suitable for rooting and *ex vitro* establishment.

Nature of Work: Tissue culture is a useful tool for propagation and plant breeding. *In vitro* protocols provide a foundation for ploidy level manipulation and allow for the rapid propagation of valuable cultivars. Previous *in vitro* propagation studies on *Magnolia* have focused on endemic species for conservation, including *M. acuminata* var. *cordata* (15,16), *M. dealbata* (12), *M. denudata* (1), *M. fraseri* (15,16), *M. macrophylla* (13,16), *M. obovata* (7), *M. officinalis* (21), *M. pyramidata* (14,16), *M. sieboldii* (9), *M. sinicum* (5), and *M. virginiana* (15,16). However, little work has been done on micropropagation of ornamental *Magnolia* taxa with the exception of *M.* × *soulangiana M.* (6,11), *M. grandiflora* (18,19) and *M. delavayi* (10). These studies have indicated that media composition and plant growth regulators are important factors influencing the *in vitro* propagation of Magnolia. Shoot proliferation in Magnolia during micropropagation has been reported to be difficult due to the high content of phenolic substances (5, 18). Therefore, the objectives of this study were to evaluate a range of basal media

compositions, phenolic binding agents, and cytokinins in a series of experiments to optimize *in vitro* growth conditions for 'Ann'.

'Ann' stock cultures were maintained on MS basal salts and vitamins, 2 µM BAP, 30 g/l sucrose, 0.1 g/L myo-Inositol, 0.1 g/L MES monohydrate, and solidified with 0.8% agar at the N.C. State Mountain Horticultural Crops Research and Extension Center (MHCREC) in Mills River, N.C. Cultures were maintained at 23 °C (73°F) under a 16h photoperiod.

The effect of basal media composition was tested with five basal salt compositions and vitamins (MS, ½ MS, WPM, Blaydes Modified Basal Medium (Blaydes)(2), and Driver and Kuniyuki basal salt mixture (DKW)(3) in factorial combination with phenolic binding agents (none, 1 g/l polyvinylpyrrolidone (PVP)(12), or 1 g/l charcoal). All media were supplemented with 30 g/l sucrose, 2 µM BAP, 0.1 g/L myo-Inositol, 0.1 g/L MES monohydrate, and solidified with 0.8% agar. The experiment consisted of six replicates (jars) per treatment and five subsamples (subcultured explants) per replicate arranged in a completely randomized design. After eight weeks, data were collected on shoot number, shoot length, root number, fresh weight, and dry weight.

In a separate experiment, the effect on plant growth of three cytokinins; BAP, meta-topolin (mT), and 6-(γ,γ-dimethylallylamino) purine (2iP)(11) at three concentrations (2, 4, and 8 µM) with or without 1 g/l charcoal was evaluated in a completely randomized design with a factorial arrangement of treatments. Based on the results of the first experiment, basal media consisted of MS basal salts and vitamins, 30 g/l sucrose, 0.1 g/L myo-Inositol, 0.1 g/L MES monohydrate, and solidified with 0.8% agar. The experiment consisted of six replicates (jars) per treatment and five subsamples (subcultured explants) per replicate, arranged in a completely randomized design. After eight weeks, data were collected on shoot number, shoot length, root number, root length, fresh weight, and dry weight. Data for both studies were subjected to analysis of variance (Proc GLM, SAS version 9.1; SAS Institute, Cary, NC). Means separations were based on LSD.

Results and Discussion: In the first experiment, the influence of media composition and phenolic binding agents on plant growth were examined. Media composition, phenolic binding agents and their interaction had a significant effect on shoot number, root number, fresh weight and dry weight (Table 1). In general the number of shoots produced per explant was lower on both Blaydes media and media supplemented with activated charcoal. Fresh weight was significantly lower on Blaydes and WPM medium as well as media supplemented with activated charcoal, while phenolic binding agents (PVP and charcoal), as well as WPM and DKW reduced dry weight. Rooting increased on WPM media and media containing charcoal. There was no interaction between media composition and phenolic binding agents on shoot length. Even though shoot length was less on Blaydes media overall, shoots were significantly longer on all media containing charcoal (Table 1). Reduced shoot proliferation and increased shoot elongation and

rooting in response to charcoal have been found for *Acacia mearnsii* and *Anacardium occidentale* (cashew) (20).

In the second experiment, the influence of cytokinins, cytokinin concentration and charcoal was examined. There was a significant interaction between cytokinin and charcoal that influenced shoot number, shoot length and fresh weight, while a complex interaction between cytokinin, cytokinin concentration and charcoal affected dry weight (Table 2). In general, shoot number was higher on media containing BAP, regardless of concentration, and lower on media containing charcoal. Interestingly, mT and 2iP did not promote shoot proliferation. Meta-topolin has been reported to produce longer, greener and less hyperhydrated shoots and may be an alternative cytokinin to BAP (23). Unexpectedly, cytokinin concentration did not have a significant effect on shoot number. Similar to the first experiment, charcoal significantly promoted root formation.
This study demonstrated that there are several different media components that interact to influence in vitro growth of 'Ann'. Based on the results, MS media supplemented with BAP provides high shoot proliferation, while WPM containing charcoal may be used to produce elongated plantlets more suitable for rooting and *ex vitro* establishment. Further studies are required to optimize rooting media. Protocols developed in this study will be used in future experiments focused on the development of allopolyploids to restore fertility.

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Table 1. Summary of means for growth responses to different in vitro culture media and phenolic binding agents.

Media	Phenolic Binding Agent	Shoot Number[1]	Shoot Length (mm)[1]	Root Number[1]	Fresh Weight (g)[1]	Dry Weight (g)[1]
MS	none	3.2±0.2^{A}	17.2±1.8^{C}	0.2±0.07^{DE}	5.6±0.60^{B}	0.56±0.04^{AB}
	PVP	2.8±0.3^{AB}	20.0±2.8^{BC}	0.1±0.10^{DE}	4.8±0.45^{B}	0.58±0.04^{AB}
	Charcoal	1.1±0.1^{C}	24.2±1.7^{AB}	0.6±0.03^{BC}	2.8±0.22^{CD}	0.42±0.03^{BC}
½ MS	none	2.8±0.2^{AB}	24.4±2.6^{AB}	0.3±0.14^{CDE}	8.1±1.21^{A}	0.69±0.08^{A}
	PVP	2.6±0.2^{AB}	19.9±1.1^{BC}	0.1±0.04^{DE}	4.6±0.35^{BC}	0.53±0.04^{B}
	Charcoal	1.1±0.1^{C}	22.2±1.7^{B}	0.3±0.08^{CDE}	2.1±0.24^{D}	0.35±0.03^{C}
WPM	none	2.3±0.2^{AB}	19.9±1.9^{BC}	0.7±0.20^{B}	2.4±0.38^{D}	0.39±0.06^{BC}
	PVP	2.6±0.3^{AB}	16.5±1.6^{C}	0.4±0.19^{BCD}	1.9±0.57^{D}	0.33±0.05^{D}
	Charcoal	1.2±0.1^{C}	22.4±1.3^{B}	1.8±0.16^{A}	2.4±0.16^{D}	0.42±0.03^{BC}
DKW	none	2.6±0.4^{AB}	22.7±3.0^{B}	0.1±0.04^{DE}	4.9±1.07^{B}	0.50±0.04^{B}
	PVP	2.9±0.4^{A}	19.0±1.0^{BC}	0.0±0.00^{E}	4.2±0.79^{BC}	0.49±0.08^{BC}
	Charcoal	1.1±0.1^{C}	30.1±4.1^{A}	0.7±0.20^{BC}	3.0±0.53^{C}	0.29±0.04^{D}
Blaydes	none	1.2±0.2^{C}	8.2±2.1^{D}	0.3±0.12^{CDE}	2.2±0.24^{D}	0.62±0.09^{AB}
	PVP	1.5±0.2^{C}	13.6±3.2^{CD}	0.1±0.11^{DE}	2.2±0.44^{D}	0.45±0.06^{BC}
	Charcoal	0.9±0.1^{C}	14.2±1.3^{CD}	0.6±0.10^{BC}	0.9±0.16^{D}	0.47±0.04^{BC}
Analysis of Variance[2]						
Media		**	**	**	**	**
PBA		**	**	**	**	**
Media x PBA		*	NS	**	**	*

[1]Values represent means ± SEM. Means followed by different letters within columns are significantly different, P<0.05.

[2]NS, *, **: Nonsignificant or significant at p=0.05 and 0.01, respectively. PBA=Phenolic Binding Agent.

Table 2. Summary of means for *in vitro* growth responses to different concentrations of cytokinins and phenolic binding agents.

Cytokinin	Conc. (µM)	Phenolic Binding Agent	Shoot Number[1]	Shoot Length (mm)[1]	Root Number[1]	Fresh Weight (g)[1]	Dry Weight (g)[1]
BAP	2	None	2.64±0.4 ^{A}	18.6±1.9 ^{ABC}	0.00^{B}	1.00±0.0 ^{D}	0.23±0.03^{A}
		Charcoal	1.00±0.0 ^{C}	18.2±1.0 ^{ABC}	1.40±0.5^{A}	1.33±0.1 ^{D}	0.18±0.01^{AB}
	4	None	2.40±0.1 ^{A}	19.2±0.8 ^{ABC}	0.00^{B}	1.00±0.0 ^{D}	0.2±0.02^{A}
		Charcoal	1.08±0.1 ^{C}	17.0±0.7 ^{BCD}	0.60±0.2^{AB}	1.34±0.2 ^{D}	0.17±0.02^{B}
	8	None	2.40±0.2 ^{A}	22.5±0.7 ^{A}	0.00^{B}	1.00±0.0 ^{D}	0.19±0.02^{A}
		Charcoal	1.15±0.1 ^{BC}	18.9±1.7 ^{ABC}	1.00±0.6^{A}	1.37±0.2 ^{CD}	0.14±0.01^{BC}
mT	2	None	1.40±0.2 ^{BC}	13.6±1.7 ^{D}	0.00^{B}	2.00±0.0 ^{BC}	0.11±0.01^{C}
		Charcoal	1.25±0.2 ^{BC}	19.7±2.2 ^{AB}	0.75±0.5^{AB}	1.99±0.3 ^{BC}	0.20±0.02^{A}
	4	None	1.33±0.2 ^{BC}	15.4±1.8 ^{CD}	0.00^{B}	2.00±0.0 ^{BC}	0.16±0.03^{BC}
		Charcoal	1.06±0.1 ^{C}	16.7±3.0 ^{BCD}	0.33±0.3^{B}	1.65±0.4 ^{BC}	0.13±0.01^{BC}
	8	None	1.55±0.3 ^{B}	14.9±1.4 ^{CD}	0.00^{B}	2.00±0.0 ^{BC}	0.14±0.03^{BC}
		Charcoal	1.13±0.1 ^{BC}	15.5±0.1 ^{CD}	0.00^{B}	2.33±0.3 ^{AB}	0.13±0.01^{BC}
2iP	2	None	1.06±0.1 ^{C}	19.6±2.5 ^{ABC}	0.00^{B}	3.00±0.0 ^{A}	0.21±0.04^{B}
		Charcoal	1.10±0.1 ^{BC}	17.9±2.8 ^{ABCD}	1.00±0.4^{A}	1.84±0.4 ^{BC}	0.18±0.02^{A}
	4	None	1.12±0.1 ^{BC}	13.9±1.7 ^{D}	0.00^{B}	3.00±0.0 ^{A}	0.13±0.01^{BC}
		Charcoal	1.20±0.1 ^{BC}	19.0±0.7 ^{ABC}	0.83±0.2^{AB}	2.01±0.2 ^{B}	0.22±0.02^{A}
	8	None	1.06±0.1 ^{C}	20.7±1.8 ^{AB}	0.67±0.7^{AB}	2.37±0.6 ^{AB}	0.13±0.02^{BC}
		Charcoal	1.00±0.0 ^{C}	17.9±1.2 ^{ABCD}	0.00^{B}	3.00±0.0 ^{A}	0.23±0.02^{A}
Analysis of Variance[2]							
Cytokinin			**	*	NS	**	**
Conc.			NS	NS	NS	NS	NS
PBA			**	NS	**	NS	NS
Cytokinin x Conc.			NS	NS	NS	NS	NS
Cytokinin x PBA			**	*	NS	**	**
Conc. x PBA			NS	NS	NS	*	NS
Cytokinin x Conc. x PBA			NS	NS	NS	NS	**

[1]Values represent means ± SEM. Means followed by different letters within columns are significantly different, P<0.05.

[2]NS, *, **: Nonsignificant or significant at p=0.05 and 0.01, respectively. Conc. = Concentration; PBA=Phenolic Binding Agent.