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Tomato Anther Callus Production: Solidi@ing Agent and Concentration Influence Induction of Callus

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Abstract. **Anthers from. three tomato cultivars, 'L-680A', 'Ailsa Craig', and 'Licato', were plated on DBM1 medium solidified with one of four solidifying agents, Bacto-agar, Gelrite, Noble agar, or Phytagar, to evaluate their ability to promote initiation and growth of tomato anther callus. The optimum concentration of each solidifying agent was compared with a liquid control. Optimum levels of the various solidifying agents were (in g·liter^{-1}) Phytagar, 5; Gelrite, 3; Noble agar, 6 and Bacto-agar, 8. Both the number and diameter of calluses were affected by type of solidifying agent and anther genotype. Significant interactions were also found between tomato cultivars and solidifying agent. Noble agar produced good results with 'L-680A' and 'Ailsa Craig', but not with 'Licato'. Bacto-agar reduced the number and size of callus by 38% when compared with the liquid treatment and by 42% when compared with the best agar treatment (Noble agar).**

In an attempt to optimize the production of tomato another-derived callus, we noted seemingly significant improvement when the concentration and type of solidifying agent were modified. We became interested in quantifying the increased performance that might be obtained when the type of solidifying agent was varied.

Solidifying agents are used to prepare semisolid plant tissue culture media. Semisolid media provide a fixed anchorage that determines polarity and vertical growth for plant tissue (Dunwell, 1986). Other advantages of using solidifying agents are their stability at all feasible incubation temperatures, their inability to be digested by plant enzymes, and the absence of reaction with media constituents (George and Sherrington, 1984). However, it has been shown that agars may contain inhibitory substances for pollen embryo production. For *Nicotiana tabacum*, a liquid medium has been shown to be significantly better than any agar-solidified medium (Kohlenbach and Wernicke, 1978).

Agar concentrations for tissue culture media have ranged from < 3 to 15 g·liter^{-1} (Debergh, 1983; George and Sherrington, 1984; Miller and Murashige, 1976; Singha, 1982), depending on the type. Gelrite, a hydrocolloid gellan gum, is used to solidify media at concentrations of 1.7 g·liter^{-1} and up. For tomato anther culture, the most commonly used solidifying agent has been Difco Bacto-agar at concentrations of 7 to 8 g-liter^{-1} (Cappadocia and Sree-Ramulu, 1980; Dao and Shamina, 1978; Gresshoff and Doy, 1972; Zamir et al., 1980); however, no studies have determined the effects of different solidifying agents and concentrations on tomato anther callus production.

This study was undertaken to determine the effects of gelling agent type and concentration on the induction and growth of tomato anther callus. This work was divided into two parts. First, the gelling agents were evaluated separately across a range of concentrations for their ability to induce healthy callus and to support rapid callus growth. Subsequently, optimum concentrations of each solidifying agent were compared using the same cultivars.

Materials and Methods

The DBM1 medium of Gresshoff and Doy (1972), supplemented with 2 mg l-naphthaleneacetic acid (NAA)/liter and 5 mg N-(2-furanyl-methyl) -lH-purin-6-amine (kinetin)/liter, was solidified with one of four solidifying agents, Noble, Bacto, Phytagar, or Gelrite. Gelling agent concentrations were chosen on the basis of recommendations by the producers and the literature (Table 1). Media pH was adjusted to 5,8 with 0.1 N NaOH before solidifying agents were added. Each medium was autoclave for 18 min at 121C and 107,873 Pa (1.1 kg-cm-z).

Greenhouse-grown plants were watered as needed and fertilized once per week. Flower buds (2 to 4 mm) were harvested in the morning. Each 1.5- to 1.8-mm anther contained pollen mother cells at the beginning of prophase I (leptotene). They were immediately surface-sterilized with a 15- to 20-sec dip in 70% ethanol followed by immersion in 0.5% sodium hypochlorite for 5 min. Buds were rinsed four times with sterilized Type I water. Five anthers from each bud were plated on a petri dish [6 x 1.5 cm) containing 10 ml of medium. The dishes were wrapped with Parafilm and placed in darkness in a growth chamber at 26 ± 1.5C for 4 weeks. After dark treatment, each plate was exposed to 4 weeks of 16-hr photoperiod (88 µmol·s^{-1}m^{-2}) provided by cool-white fluorescent lamps.

For each gelling agent, an experiment was designed as a split plot, where tomato cultivars were assigned the main plots and gelling agent concentrations the split plots. At the end of 8 weeks, the number of anthers with callus and the diameter of

Table 1. Solidifying agents and concentrations used for evaluating tomato anther callus induction and growth.

Solidifying agent	Source[z]	Individual experiments: Agar concn (g·liter^{-1})				No. replications
Bacto-agar	Difco	4.0	6.0	8.0	10.0	12
Phytagar	Gibco	3.0	5.0	7.0	9.0	13
Noble	Difco	4.0	6.0	8.0	10.0	11
Gelrite	Kelco	1.0	2.0	3.0	4.0	12

[z]Difco, Detroit; Gibco, Grand Island, N. Y.; Kelco, San Diego.

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observed callus were recorded. Callus diameter was recorded by taking the mean of the polar and equatorial diameters observed in a reticale-equipped Nikon (Chiyoda-Ku, Tokyo) SMZ-10 stereoscopic microscope. Data were analyzed by analysis of variance techniques. Contaminated plates were represented by missing-value calculations (Steel and Torrie, 1980).

An experiment was conducted to determine which of the optimum solidifying agent concentrations produced significantly more anther callus than the others. The effect of a liquid treatment was measured to determine the net effect of gelling agent on callus growth. This treatment consisted of 2 ml of autoclave DBM1 medium added to each petri dish (6 *x* 1.5 cm).

Results and Discussion

Each of the tomato cultivars exhibited significantly different rates of callus initiation and growth, as measured by changes in callus diameter.

Noble agar. Increased concentrations of Noble agar significantly reduced the number of anthers that callused, but had no effect on callus diameter (Tables 2 and 3). Treatments using 10 g agar/liter produced 27% less callus than did treatments using 4 or 6 g agar/liter. No significant interactions were observed. In general, both 'L-680A' and 'Ailsa Craig' responded well. Low concentrations (4 to 6 g·liter^{-1}, of solidifying agent produced healthy, transparent-white 'L-680A' callus and yellow 'Ailsa Craig' or 'Licato' callus.

Bacto-agar. Increased solidifying agent concentration significantly increased callus formation when a range of 4 to 8 g solidifying agent/liter was used; significantly fewer (29%) calli were produced when 10 g·liter^{-1} was used in the media (Tables 4 and 5). This result is in agreement with Gresshoff and Doy (1972) and Zamir et al. (1980), who used 8 g Bacto-agar/liter in tomato anther culture systems. Callus growth was not significantly affected as solidifying agent concentration increased.

Table 2. Noble agar concentration effects and mean squares (MS) for number and diameter of tomato anther callus.

Source of variation	df	Callus			
		No.		Mean diam	
		MS	F	MS	F
Replication	10	1.58	0.85	0.78	*1.30*
Cultivar (C)	2	51.96	28.08**	12.99	21.65**
Error a	20	1.85	1.62	0.60	1.27
Solidifying agent concn (A)	3	5.75	5.04**	0.30	0.64
A × C	6	1.34	1.17	0.24	0.64
Error b	90			0.47	

**F test significant at *P = 0.01*.

Table 3. Tomato cultivar and Noble agar concentration effects on number of anther-derived calli.[z]

Cultivar	Concn (g·liter^{-1})[y]				Mean[x]
	4.0	6.0	8.0	10.0	
L-680A	4.18	4.36	3.73	3.09	3.84
Ailsa Craig	3.36	3.18	3.27	2.09	2.98
Licato	1.91	1.91	1.27	1.64	1.68
Mean[w]	3.15	3.15	2.76	2.27	

[z]Five anthers per dish.
[y]Dunnett 0.05: nonsignificant.
[x]Dunnett 0.05: 0.59, cv% = 48.0.
[w]Dunnett 0.05:0.55, cv% = 37.6.

Table 4. Bacto-agar concentration effects and mean squares (MS) for number and diameter of tomato anther callus.

Source of variation	df	Callus			
		No.		Mean diam	
		MS	F	MS	F
Replication	11	3.94	3.42	3.32	4.74
Cultivar (C)	2	38.98[z]	33.89**	8.86	12.66**
Error a	22	0.83		0.70	1.01
Solidifying agent concn (A)	3	3.72	3.23*	0.51	0.73
A × C	6	0.82	0.71	1.03	1.47
Error b	98[y]	1.22[z]		0.70	

[z]Pooled error (=1. 15) was used to obtain F because Error b > Error a.
[y]df reduced from 99 to 98 because of missing values.
*,**F test significant at *P* = 0.05 or 0.01, respectively.

Table 5. Tomato cultivar and Bacto-agar concentration effects on number of anther-derived calli.[z]

Cultivar	Concn (g·liter^{-1})Y				Mean[x]
	4.0	6.0	8.0	10.0	
L-680A	3.42	3.17	3.50	2.71	3.20
Ailsa Craig	1.83	2.25	2.33	1.92	2.08
Licato	1.08	1.33	2.17	1.08	1.41
Mean[w]	2.11	2.25	2.67	1.90	

[z]Five anthers per dish.
[y]Dunnett 0.05: nonsignificant.
[x]Dunnett 0.05:0.38, cv% = 40.9.
[w]Dunnett 0.05:0.55, cv% = 47.1.

Table 6. Gelrite concentration effects and mean squares (MS) for number and diameter (in millimeters) of tomato anther callus.

Source of variation	df	Callus			
		No.		Mean diam	
		MS	F	MS	F
Replication	11	1.97	1.46	0.35	1.03
Cultivar (C)	2	10.63	7.87**	10.15	29.85
Error a	22	1.21[z]		0.31[y]	
Gelrite concn (G)	3	7.04	5.21**	0.76	2.23
C × G	6	1.34	0.99	1.04	3.06
Error b	99	1.39[z]		0.36[y]	

[z]Error a and b pooled (= 1.35) because Error b > Error a.
[y]Error a and b pooled (=0.34) because Error b > Error a.
*,**F test significant at *P* = 0.05 or 0.01, respectively.

Table 7. Tomato cultivar and Gehite concentration effects on number of anther-derived calli.[z]

Cultivar	Concn (g·liter^{-1})[y]				Mean[x]
	1.0	2.0	3.0	4.0	
L-680A	3.25	3.42	3.83	3.33	3.46
Ailsa Craig	3.58	3.00	3.58	2.08	3.06
Licato	2.83	2.33	3.00	1.92	2.52
Mean[w]	3.22	2.91	3.47	2.44	

[z]Five anthers per dish.
[y]Dunnett 0.05: nonsignificant.
[x]Dunnett 0.05:0.45, cv% = 18.2.
[w]Dunnett 0.05:0.58, cv% = 39.1.

Gelrite. Gelrite at 3 g-liter-l produced significantly more callused anthers than at 4 g·liter-l (Tables 6 and 7). Callus diameter was not significantly affected by increased Gelrite concentration, but the cultivar x Gelrite interaction was significant (Table 6). 'L-680A' callus diameter increased with increasing Gelrite concentration. Fifty percent more callus was produced from 'L-680A' than the other cultivars. In contrast, anther callus from 'Ailsa Craig' or 'Licato' increased in diameter when Gelrite at 1 or 3 g-liter-l was used in the medium and showed reduced growth when 2 or 4 g·liter-1 was used (Table 8). In other studies (data not shown), Gelrite concentrations higher than 5 g·liter-1 substantially increased medium pH. Therefore, additional HC1 would be required to ensure a medium pH of 5.8. Gelrite at 3 g·liter-1 is optimum for use with tomato anther culture. This concentration has also been reported to be favorable for tissue culture (Kyte, 1983).

Phytagar. Nine grams per liter of Phytagar produced significantly fewer calli than did any of the other Phytagar concentrations (Tables 9 and 10). No differences were observed among Phytagar at 3, 5, and 7 g·liter -1. Anther callus was formed on 66% of the 'L-680A' anthers, 48% of 'Ailsa Craig' anthers, and 22% of 'Licato' anthers. The Phytagar–DBMl medium combination produced very small 'Licato' callus that grew more slowly than similar callus on other treatments. Phytagar at 5 g·liter-1 appeared to be the optimum concentration for tomato anther culture.

Evaluation of solidifying agent and optimum concentration. Because most tomato anther culture studies have used media solidified with a solidifying agent (e.g., Bacto-agar), we anticipated that the liquid treatment would induce fewer calli than Bacto-agar (8 g·liter-1). Instead, the Bacto-agar treatment produced 38% fewer anthers with callus when compared with liquid culture and 42% fewer than Noble agar at 6 g·liter-1 (Tables 11 and 12). No significant differences were found among other gelling agents and the liquid control. The dramatic reduction in the number of 'L-680A' calli produced on Bacto-agar, coupled with an increase in performance on Phytagar when compared with Gelrite, contributed to a significant cultivar × solidifying agent concentration interaction. Callus growth, as measured by

Table 8. Tomato cultivar and Gelrite concentration effects on tomato anther callus diameter (in millimeters).2

	Concn (g·liter -1)[y]				
Cultivar	1.0	*2.0*	*3.0*	*4.0*	Mean[x]
L-680A	1.56	2.11	2.36	2.66	2.17
Ailsa Craig	1.54	1.36	1.43	1.37	1.42
Licato	1.35	1.23	1.48	1.25	1.33
Mean[w]	1.48	1.57	1.76	1.78	

[z]Mean diameter of the calli in a dish.
[y]Dunnett 0.05: 0.58.
[x]Dunnett 0.05:0.23, cv% = 16.9.
[w]Dunnett 0.05: nonsignificant, cv% = 37.2.

Table 9. Phytagar concentration effects and mean squares (MS) for number and diameter (in millimeters) of tomato anther callus.

		callus			
		No.		Mean diam	
Source of variation	df	MS	F	MS	F
Replication	12	1.13	0.07	0.32	0.82
Cultivar (C)	2	60.01	35.93*	7.03	18.02**
Error a	24	1.67	1.70	0.39	1.56
Solidifying agent concn (A)	3	6.15	6.27**	0.14	0.56
C × A	6	0.65	0.66	0.28	1.12
Error b	106[y]	0.98		0.25	

[z]df reduced from 108 to 106 due to missing values.
*,**F test significant at *P* = 0.05 or 0.01, respectively.

Table 10. Tomato cultivar and Phytagar concentration effects on number of anther-derived calli.[z]

	Concn (g·liter-1)[y]				
Cultivar	*3.0*	*5.0*	*7.0*	*9.0*	Mean[x]
L-680A	3.54	3.77	3.31	2.69	3.33
Ailsa Craig	2.92	2.72	2.31	1.77	2.44
Licato	1.06	1.61	1.15	0.92	1.19
Mean[w]	2.51	2.72	2.26	1.79	

[z]Five anthers per dish.
[y]Dunnett 0.05: nonsignificant.
[x]Dunnett 0.05: 0.51, cv% = 55.8.
[w]Dunnett 0.05:0.47, cv% = 42.3.

Table 11. Solidifying agent effects and mean squares (MS) for number and diameter (in millimeters) of tomato anther callus.

		Callus			
		No.		Mean diam	
Source of variation	df	MS	F	MS	F
Replication	11	1.56	0.68	0.27	0.33
Cultivar (C)	2	77.29	33.90**	31.68	38.17**
Error a	22	2.28	2.13	0.83	2.02
Solidifying agent (A)	4	5.18	4.84**	1.54	3.76**
A × C	8	3.86	3.61**	1.78	4.34**
Error b	130	1.07		0.41	

**F test significant at *P = 0.01.*

Table 12. Tomato cultivar and gelling agent effects on number of anther-derived calli.[z]

	Gelling agent and concn (g·liter-1)[y]					
Cultivar	Noble 6.0	Phyta 5.0	Gelrite 3.0	Liquid	Bacto 8.00	Mean[x]
L-680A	4.00	3.83	3.17	3.17	1.58	3.15
Licato	1.17	1.60	1.83	2.00	1.42	1.60
Ailsa Craig	1.17	0.85	1.17	0.75	0.67	0.91
Mean[w]	2.11	2.09	2.05	1.97	1.22	

[z]Five anthers per dish.
[y]Dunnett 0.05: 0.96.
[x]Dunnett 0.05: 0.53, cv% = 80.6.
[w]Dunnett 0.05:0.53, cv% = 54.2.

Table 13. Tomato cultivar and gelling agent effects on tomato anther callus diameter (in millimeters).[z]

	Gelling agent and concn (g·liter-1)[y]					
Cultivar	Gehite 6.0	Noble 5.0	Phyta 3.0	Liquid	Bacto 8.00	Mean[x]
L-680A	2.09	2.57	2.04	1.66	0.87	1.87
Licato	0.95	0.59	0.78	0.82	0.84	0.80
Ailsa Craig	0.58	0.39	0.43	0.37	0.29	0.29
Mean[w]	1.18	1.18	1.08	0.95	0.70	

[z]Mean diameter of the calli in a dish.
[y]Dunnett 0.05: 0.88.
[x]Dunnett 0.05: 0.29, cv% = 89.9.
[w]Dunnett 0.05; 0.29, cv% = 61.9.

callus diameter, was also significantly affected by gelling agent treatment (Table 13). Calli on Bacto-agar grew slower than those on any other solidifying agent and grew significantly worse (40% to 42%) than on any other semisolid medium. No significant differences were found between callus growth on liquid medium and growth on Phytagar, Noble agar, or Gelrite. However, significantly increased growth of 'L-680A' calli on these three media contributed to a significant cultivar x solidifying agent interaction.

We conclude that tomato anther culture systems may not require solidified culture media; however, gelling agents and concentration should be evaluated to determine their net affect. Clearly, using Noble agar, Phytagar, or Gelrite at optimized rates could lead to a 42% increase in calli formation and growth when compared to optimum rates of Bacto-agar.

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