TISSUE CULTURE STUDIES OF NINE PEA CULTIVARS

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A large number of plant cells can be screened for herbicide resistance or tolerance in a relatively small space and a short period of time using tissue culture systems. However, the proper nutrient, vitamin, and hormone concentrations needed to induce callus growth and cell proliferation are not known for many crop species (3,4,5). Jacobsen et al. (5,6,7) reported that callus formation, regeneration, and *in vitro* differentiation of peas (*Pisum sativum* L.) in tissue culture are not only medium specific, but also genotypic specific. The purpose of the present experiment was to develop media for good callus formation and cell proliferation of several pea cultivars in suspension culture.

Nine pea cultivars were used: 'Scout', 'Paloma', 'Alaska', 'Garfield', and 'Latah' (spring-types), and 'Frogel', 'Glacier', 'Melrose', and 'Common' (winter-types). Fifty seeds of each cultivar were surface sterilized for 5 min in 0.5% sodium hypochlorite and then were rinsed three times with sterilized, distilled water. Seeds of each cultivar were placed in sterile petri dishes on Whatman #1 filter paper moistened with 5 ml of sterile water. Petri dishes were wrapped with aluminum foil and placed in a growth chamber for 3 d at 24C. Seedlings then were surface sterilized for 5 min with 5% sodium hypochlorite and triple rinsed with sterile, millipore filtered water. Using aseptic techniques, the cotyledons, root, and shoot were removed from each seedling leaving only the cotyledonary node. Three cotyledonary nodes of each cultivar were transferred into 125 ml Erlenmeyer culture flasks containing 25 ml of liquid medium and/or into separate culture bottles containing 25 ml of Blaydes' medium solidified with 8 g/l of agar (Table 1). Growth regulators were used at the following rates: picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxylie acid) at 0.1 mg/l, 2,4-D (2,A-dichlorophenoxy acetic acid) and NAA (naphthylacetic acid) at 1.0 mg/l, and kinetin at 0.5 mg/1. All media were supplemented with 1.0 mg/l of nicotinic acid, 1.0 mg/l of pyridoxine-HCl, 10.0 mg/l of thiamine-HCl, and 100 mg/l of inositol. Liquid cultures were placed on an orbital shaker (125 rpm) and incubated at 24C in the dark. Fresh medium was added every 15 to 20 d until cells could be inoculated onto proliferation medium. Callus cultures were incubated in the dark at 24C.

Table 1. Pea cultivars tested in different media and combinations of growth regulators used to induce callus formation.

Blaydes' solid		B5 liquid		MS liquid	
kinetin + 2,4-D	NAA	2,4-0	picloram + kinetin	picloram	picloram +• kinetin
Glacier	Alaska	Alaska	Alaska	Garfield	Latah
Melrose	Latah	Latah	Glacier	Alaska	Paloma
Common	Garfield	Garfield	Garfield		Scout
Alaska	Melrose	Glacier	Common		Common
Latah	Common	Mel rose	Mel rose		Alaska
Frogel	Glacier				
Scout					

After the cultures were established on induction media (15 to 45d depending on cultivar), subcultures were transferred to proliferation media. Cultures initiated on Blaydes' solid medium (1) were subcultured to Blaydes' liquid medium that contained 0.5 mg/l kinetin and 1.0 mg/l 2,4-D or picloram plus kinetin were subcultured to B5 media, which contained kinetin at 0.5 and NAA or 2,4-D at 1.0 mg/l. Cultures initiated on MS liquid medium with picloram plus kinetin were transferred to MS medium that contained 1.0 mg/l of NAA and 0.5 mg/l of kinetin. Inoculum size from Blaydes' solid medium varied from the original explant to 100 mg of callus depending upon the cultivar. Ten ml aliquots of liquid media containing from 0.2 to 1.0 ml of packed cells were used to inoculate liquid media. Addition of casein hydrolysate at 2 g/l to proliferation media was evaluated for increased cell proliferation. The use of glucose versus sucrose also was compared with the cultivar Glacier in the B5 proliferation medium with 1.0 mg/l NAA and 0.5 mg/l kinetin. Visual evaluations of callus formation and/or cell suspension formation were made every 15 d and were rated from no formation (-) to good formation (+++).

Table 2. The effect of media on induction and proliferation among pea cultivars.

Cultivar	Induction	Proliferation	
	Blaydes' solid	Blaydes' liquid	
Glacier	-*		
Garfield	++	+	
Latah	++	-	
Scout	+++	-	
Common	-		
Frogel	+++	+++	
Melrose	+++	+++	
Alaska	-		
	B5/2.4-D	B5/2.4-D+K**	B5/NAA+K
Alaska	-		
Latah	-		
Garfield	-		
Glacier	++	++ ++	
Melrose	+	+ -	
	B5/P***+K	B5/2.4-D+K	B5/NAA+K
Alaska	-		
Common	-		
Garfield	+	+	+
Glacier	+++	++ +++	
Melrose			
	MS/P + K		MS/NAA+K
Alaska	-		
Common	++		-
Latah	-		
Pa 1oma	-		
Scout	+		-

* no callus growth, + poor callus growth, ++ fair callus growth, +++goodcallusgrowth.
**K = kinetin
***P=picloram.

None of the media and hormone combinations used in this experiment induced callus in all 9 cultivars. The best callus growth was initiated on Blaydes' solid medium for five of eight cultivars (Table 2). However, only three of the five cultivars proliferated in Blaydes' liquid medium. Callus was not induced on B5 medium with NAA, but some shoots (not more than 1 per explant) and roots were induced on Melrose and Glacier (data not presented). Callus growth was induced in the cultivars Glacier and Melrose with B5 liquid and 2,4-D. Callus proliferation of Glacier was fair when cells were transferred from B5 with 2,4-D to B5 with kinetin and 2,4-D or NAA. B5 plus picloram and kinetin induced good callus growth in Glacier, and when these cells were transferred, proliferation was fair on B5 with 2,4-D plus kinetin and good on B5 with NAA plus kinetin. Glacier formed some embryoid-like structures in the B5 medium with picloram and kinetin. MS medium with picloram and kinetin induced some callus in Common and Scout, but neither cultivar proliferated on MS with NAA plus kinetin (Table 2). Callus growth was not induced in Alaska or Garfield on MS medium with picloram. There were no differences in callus growth of Glacier in glucose or sucrose B5 medium (data not presented). Two grams of casein hydrolysate/l added to the proliferating media increased cell growth in all cultivars that were successfully proliferated (data not presented).

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