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In vitro germination of wheat pollen on raffinose medium

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SUMMARY

Wheat pollen gave up to 81·7 % germination when cultured on a 0·7 % agar medium containing 100 mg l^{-1} H_3BO_3, 300 mg l^{-1} $CaCl_2.2H_2O$ and 0·75 M raffinose. On this medium at 25 °C, germination started within 5 min and tubes reached a mean maximum length of 200 μm after 1 h. Maltose supported 27·9 % germination, while sucrose gave at most 6·8 %. Pollen on media with fructose, galactose, dextrose or lactose failed to germinate.

Key words: *Triticum aestivum* (wheat), pollen germination *in vitro*, raffinose.

INTRODUCTION

It has been known for many years that boron is an essential ingredient in media for pollen germination of most species (Vasil, 1964) but pollen from some cereal species fails to germinate on a simple solution of only sucrose and H_3BO_3. The discovery that calcium must also be present to achieve maximum *in vitro* germination for pollen sown in low density (Brewbaker & Kwack, 1963) stimulated the development of an appropriate medium for maize, and there have been extensive studies of that species (Cook & Walden, 1967; Pfahler, 1967; Pfahler *et al.*, 1982). Recently, Kariya (1989) reported successful germination of rice pollen. However, there are no published reports of wheat pollen germination *in vitro*. This paper aims to establish a method for culture of wheat pollen which will allow further work on seed set in wheat cultivars susceptible and resistant to boron deficiency.

MATERIALS AND METHODS

Plant material

Seeds of *Triticum aestivum* L. (wheat) cultivars Sonora 64, UP 262, Hartog, Halberd, Vulcan and Eradu were obtained from Dr M. Mackay, Australian Winter Cereals Collection, Tamworth, New South Wales. In experiments to be reported elsewhere these lines are being investigated for tolerance or susceptibility to boron. Seeds were sown 6 per 140 mm pot in a soil mix of 2 parts coarse sand:2 composted jarrah bark:1 peat. One gram of slow-release fertilizer was used as a basal dressing followed by liquid fertilizer (20 ml l^{-1} Spring®) each week after week 3, at a rate of 150 ml per pot. Seed was sown at 2-wk intervals from March to April, in a glasshouse heated at night so the minimum temperature was kept to above 10 °C. The daily maximum during the flowering period was 20–32 °C. Pollen was shaken directly into dishes of agar medium, as soon as possible after the anthers were extruded from the glumes. This was usually between 07.30 and 08.30 h. Pollen grains soon fell from the anthers, and those remaining in the anthers 3–4 h after anther emergence had low germination under optimum culture conditions (data not shown).

Media

All media were prepared using distilled water, and contained 0·7 % agar dissolved by boiling briefly in a microwave oven. Medium was poured into 5 cm diameter plastic Petri dishes at approximately 4 ml per dish. Medium was stored at 25 °C until use, and if it was to be stored for longer than 12 h, it was poured into sterile dishes; for medium used within 12 h, non-sterile dishes could be used. After sowing with pollen, open dishes were placed on moist filter paper in a tray covered with plastic film, at 25 °C.

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For different experiments media were prepared with various levels of H_3BO_3, $CaCl_2.2H_2O$, and the sugars: sucrose, fructose, dextrose, galactose, lactose, maltose and raffinose. All chemicals were of AR grade.

Scoring

Pollen was observed under the microscope at × 100 magnification and was recorded as germinated if the pollen tube was longer than half the diameter of the grain. Burst pollen was also scored and note made of the occurrence of double pollen tubes and sterile grains. In calculations of percentage germination, the germinated grains were taken as a percentage of the total of: grains germinated, grains intact but ungerminated, and burst grains. Sterile grains were not included in the total but were only ever at 0·3–4·8 %. Pollen grains in three randomly chosen fields were scored in each of five sectors on each plate. In most experiments, pollen germination was scored after 2–4 h, but in some experiments dishes were scored at shorter intervals up to 4 h. When lengths of pollen tubes were recorded, dishes were scored after 4 h. The lengths of pollen tubes were recorded after drawing germinated pollen from randomly selected fields (to a total of 30 tubes for each treatment, or time), using an Olympus drawing apparatus (BH 2-DA). Tube length was then calculated by using a piece of damp cotton thread to measure the drawing, and the length calculated from the measured value.

RESULTS

In media with 100 mg l^{-1} H_3BO_3 and 300 mg l^{-1} $CaCl_2.2H_2O$, and with sucrose between 0·3 and 0·9 M, pollen gave low and variable germination with a maximum of 6·8 % germination in 0·75 M sucrose (Table 1). Modification of the concentrations of boron and calcium did not significantly affect the level of germination (Table 2).

In media with 100 mg l^{-1} H_3BO_3 and 300 mg l^{-1} $CaCl_2.2H_2O$, raffinose and maltose were the only sugars that supported germination, with raffinose being significantly better than maltose. The other sugars, dextrose, fructose, lactose, and galactose did not stimulate germination, but pollen burst on these media (Table 3). Different cultivars showed different levels of pollen germination on media with the optimum level of raffinose (0·75 M) (Table 3).

On medium with 0·75 M raffinose, pollen which burst did so within 5 min of the cultures being set up (Fig. 1). Pollen germination occurred over the first 10 min and pollen tube growth continued for 45 min

Table 1. *Response of pollen of wheat cvs Hartog and Vulcan to different concentrations of sucrose in media with 100 mg l^{-1} H_3BO_3 and 300 mg l^{-1} $CaCl_2.2H_2O$. Data are means for samples from 3 replicate dishes*

	Vulcan			Hartog		
Sucrose (M)	Germinated (%)	Ungerminated intact (%)	Burst (%)	Germinated (%)	Ungerminated intact (%)	Burst (%)
0·30	—	—	—	0·7	49·0	50·3
0·45	0·9	48·0	51·2	2·5	79·4	18·1
0·60	2·5	70·9	26·6	6·7	79·5	14·3
0·75	2·0	78·3	19·7	6·8	76·4	17·6
0·90	0	72·1	27·9	1·0	84·5	14·5

Table 2. *Response of pollen of wheat cultivars Sonora 64 and UP 262, on media with 0·75 M sucrose and different concentrations of H_3BO_3 and $CaCl_2.2H_2O$. The experiment was repeated 3 times on different days and the mean percentages are given*

		Sonora 64			UP 262		
H_3BO_3 (mg l^{-1})	$CaCl_2.2H_2O$ (mg l^{-1})	Germinated (%)	Ungerminated (%)	Burst (%)	Germinated (%)	Ungerminated (%)	Burst (%)
50	150	0·6	75·3	24·1	1·3	43·8	54·9
50	300	6·3	60·0	33·7	1·6	54·1	44·3
50	600	1·6	61·8	36·6	1·8	54·3	43·9
100	150	6·7	49·5	43·8	0·9	50·3	48·8
100	300	1·0	68·2	18·0	3·4	49·5	47·1
100	600	1·0	69·6	29·4	1·1	60·1	38·8
150	150	1·5	42·6	55·9	0	40·5	59·5
150	300	2·8	52·7	44·6	0·4	45·6	54·0
150	600	1·0	79·1	19·9	0·5	53·6	45·9

Table 3. *Response of pollen of wheat cultivars to media with 100 mg l^{-1} H_3BO_3 and 300 mg l^{-1} $CaCl_2.2H_2O$ with different sugars. Means are from 15 samples on each plate*

Sugar	M	Germinated (%)	Ungerminated (%)	Burst (%)
		Hartog		
Raffinose	0·45	6·6	11·5	82·0
	0·60	52·1	8·7	39·2
	0·75	71·4	2·3	26·3
	0·90	53·9	20·9	25·3
Dextrose	0·45	0	48·6	51·5
	0·60	0	55·2	44·8
	0·75	0	68·2	31·8
	0·90	0	73·2	26·8
		Eradu		
Raffinose	0·45	7·9	14·8	77·3
	0·60	28·0	33·7	38·4
	0·75	56·2	9·1	34·7
	0·90	20·9	45·6	33·5
		Halberd		
Maltose	0·45	0·6	12·1	87·3
	0·60	5·0	32·6	62·4
	0·75	26·1	25·3	48·7
	0·90	11·1	58·3	30·6
	1·05	0·7	58·5	40·8
		Vulcan		
Raffinose	0·45	2·3	10·2	87·5
	0·60	26·4	2·6	71·0
	0·75	81·7	4·6	13·8
	0·90	12·1	48·9	39·1
Maltose	0·45	0	7·8	92·2
	0·60	13·2	7·1	79·8
	0·75	26·3	13·4	60·3
	0·90	27·9	46·9	25·2
Dextrose	0·45	0	2·7	97·4
	0·60	0	0·6	99·4
	0·75	0	5·0	95·0
	0·90	0	56·1	44·0
Lactose	0·45	0	0	100·0
	0·60	0·4	4·0	95·6
	0·75	0	21·8	78·2
	0·90	0	30·8	69·2
Fructose	0·75	0	67·2	32·8
Galactose	0·75	0	90·5	9·6
Galactose, fructose dextrose	0·25 Each	0	6·6	93·5

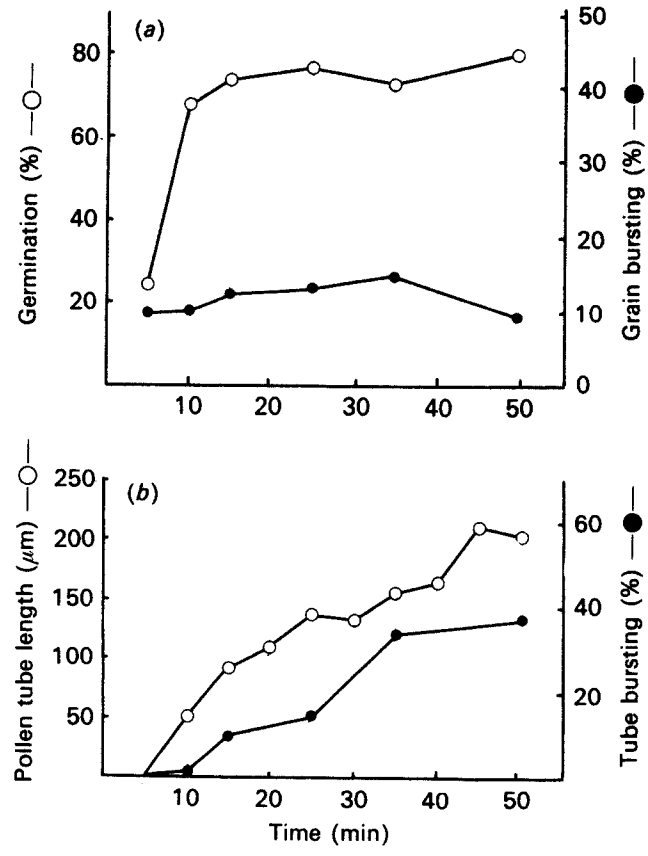

Figure 1. Time-course of wheat cultivar Vulcan pollen response on medium with 100 mg l^{-1} H_3BO_3, 300 mg l^{-1} $CaCl_2.2H_2O$ and 0·75 M raffinose. (*a*) pollen germination and grain bursting; (*b*) pollen tube growth and tube bursting.

to reach a mean maximum of 200 μm for cv. Vulcan (Fig. 1). The number of burst pollen tubes reached a plateau of 35 % after 35 min. Double pollen tubes were occasionally seen. From pollen germinating on raffinose media there were double tubes from between 0·8–4·3 % of the total grains present in a sample.

The effect of different levels of boron in media containing 0·75 M raffinose and 300 mg l^{-1} $CaCl_2.2H_2O$ showed that the optimum level was 100 mg l^{-1} H_3BO_3 (Table 4). Data for pollen tube length and percentage germination (arcsine transformed), were analysed statistically (ANOVA) and it was found that there were significant differences between cultivars and between boron levels, and also a significant interaction.

Experiments were repeated on different days, and the actual level of percentage germination differed between days of collection, and was much lower from secondary than from primary tillers (data not shown). For experimental purposes only pollen from primary tillers was used.

DISCUSSION

Wheat pollen from newly emerged anthers is capable of *in vitro* germination. The importance of collection of pollen directly from freshly extruded anthers was also stressed by Kariya (1989) who was successful in germinating rice pollen. Wheat pollen will germinate on media containing raffinose or maltose. Although maltose supports less germination than raffinose, it is one tenth the price of raffinose. The pollen tubes on raffinose medium grow to a maximum mean length of around 200 μm which is comparable to the length reached by rice pollen *in vitro* (Kariya, 1989). The speed of germination is similar to that reported for rice (Kariya, 1989) and maize (Cook & Walden, 1965).

We have shown that sucrose supports little germination of wheat pollen but it has been reported to

Table 4. *Response of wheat pollen cultivars Sonora 64, Vulcan, Eradu, Halberd to media with different levels of H_3BO_3 and with 300 mg l^{-1} $CaCl_2.2H_2O$ and 0·75 M raffinose*

H_3BO_3 (mg l^{-1})	Germinated (%)	Ungerminated (%)	Burst (%)	Tube Length (μm)
		Sonora 64		
0	0·2a	32·0	67·9	
25	22·4b	45·2	32·4	120·1a
50	35·5cd	27·4	37·1	87·1b
75	28·7bc	21·5	49·8	86·6b
100	41·9d	17·2	40·9	75·0b
125	12·6e	33·0	54·3	79·0b
		Eradu		
0	2·8a	31·4	65·9	
25	39·7c	29·1	31·6	64·7a
50	42·7cd	23·6	33·7	78·0bc
75	38·6c	20·7	40·7	80·3b
100	50·6d	16·5	32·9	65·8ac
125	26·1b	34·5	39·4	54·2a
		Vulcan		
0	0·2a	27·8	72·0	
25	50·3b	21·9	27·8	150·3a
50	57·7b	23·9	18·4	237·6b
75	53·5b	23·2	23·3	196·9b
100	63·7b	19·0	17·3	234·0b
125	42·1c	30·1	27·8	118·1c

Means are from 15 samples from each of 2 plates. Data for % germination were arcsine transformed then analysis of variance performed on data for each cultivar. Within each column and for each cultivar data that are significantly different are indicated by different letters. Tube length data were also analysed with analysis of variance.

be effective for germination of rice and maize pollen (Kariya, 1989; Cook & Walden, 1965). However, without giving exact data, Cook & Walden (1965) reported that for maize, raffinose was equally as effective as sucrose and lactose also gave good germination. Dextrose was reported by Vasil (1960) to give higher germination than sucrose for *Pennisetum typhoideum* L. C. Rich., but pollen tubes were shorter.

Sucrose has been shown to be inhibitory to callus development and androgenesis in wheat and barley anther cultures whereas maltose supported a high level of callus induction and regeneration of green shoots (Hunter, 1987; Orshinsky *et al.*, 1990). It was shown that maltose supported a higher level of wheat pollen germination, than did sucrose, but that it was not as effective as raffinose.

The availability of a pollen germination medium opens the possibility for experiments on *in vitro* physiology of tube growth, and may have use in experiments on pollen mediated gene transfer.

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