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Pollen Callus Culture in *Triticum aestivum*

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Summary. Pollen shed between 4–8 d from anthers of *Triticum aestivum* cultured in liquid medium gave rise to calluses. Tillers were harvested at the mid-to late-unicellular pollen stages and chilled for 8 d at 4–5 °C before the anthers were dissected out. Pollen cultures gave about 6 times as many calluses on a per anther basis as anthers cultured on solid medium. With the most productive of 5 cultivars tested, pollen culture results in roughly one callus for each anther used, though the calluses formed by pollen culture were less productive for the regeneration of shoots than calluses derived from anthers cultured on solid medium. The ratio of green to albino shoots is roughly 1 : 1 for anther cultures but considerably less for pollen cultures.

Key words: *Triticum aestivum* – Pollen – Anther culture – Callus – Regeneration – Plantlets

Introduction

The production of plants in vitro from pollen mechanically isolated from anthers has been attempted in various cereals: barley (Sunderland 1978), rice (Chen et al. 1980), rye (Wenzel et al. 1975) and wheat (Wei 1980). While in some instances, calluses were obtained from the isolated pollen in low frequency, regeneration of plantlets from the callus was unsuccessful. However, following the procedure recommended by Sunderland and Roberts (1977) in which pollen cultures were initiated with pollen shed from tobacco anthers floated on liquid medium, Chen et al. (1980) succeeded in culturing rice pollen to give both calluses and plantlets. In view of the ease with which pollen can be separated from floating anthers we have used this method since 1979 to test the feasibility of pollen culture with wheat. We describe here in the conditions required for callus formation and plant regeneration from wheat pollen shed from anthers in float culture.

Materials and Methods

Experiments were carried out with five cultivars of *Triticum aestivum* L: 'Beijing 8', 'Ou rou', 'Yang mai 1', 'Nong lin 61' and 'Ai gan hong'. Spikes were collected from plants grown in pots outdoors during the normal growing season.

Pollen Stages and Cold Pretreatment of Anthers

For callus production from pollen of anther cultures, wheat cultures need to be initiated with anthers at the mid-unicellular stage (Ouyang et al. 1973). As callus formation was enhanced by a cold pretreatment given prior to culture of the anthers (Sunderland and Roberts 1977), in the present investigation the spikes, with anthers at both mid- and late-unicellular stages, were subject to a cold-stress at 4–5 °C for 8 d.

Culture of Anthers

Anthers from one spike, containing mid- and late-unicellular microspores, were aseptically excised and combined, then floated on the surface of 3 ml aliquots of medium in glass petri dishes (60 mm × 15 mm). The dishes were sealed with parafilm and incubated at 25–27 °C in darkness. Three different basal media were used with each containing 5% W/V sucrose and 2–3 mg/l 2,4-dichlorophenoxyacetic acid. They were the potato-2 medium (Chuang et al. 1978) and two chemically defined media: N_6 (Chu 1978) and C_1 media (Chen et al. 1979). Following the recommendation by Nitsch (1974) for the culture of isolated tobacco pollen, each medium was supplemented with 800 mg/l glutamine, 100 mg/l serine and 5000 mg/l m-inositol. The complete media were adjusted to pH 5.8 with NaOH and sterilized with millipore filters (pore 0.45 μ).

Culture of Shed Pollen

Anthers were preincubated in the media, removed from the dishes and discarded after varying time periods. The medium with shed pollen was then centrifuged in sterile tubes to pellet the pollen. The pellets were washed twice, resuspended in the same medium, and cultured in darkness with pollen densities of about 4×10^5 grain/ml.

Culture of Pollen Calluses

Calluses stopped growing and turned brown if left for more than 28 d in the floatation medium. They also lost the capacity of regeneration. To keep them growing actively, calluses were

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transferred after 20 d to a growth medium which comprised the same basal constituents as the original ones but with the Nitsch's (1974) supplements replaced by 250 mg/l lactal albumin hydrolysate. Kinetin was also included at 0.3–0.5 mg/l. The cultures were incubated at 25–27 °C and illuminated for 10 h each day by daylight lamps at an intensity of 2 K lux.

Regeneration of Plantlets

Vigorously growing calluses 2–3 mm in diameter were transferred to an agar medium comprising the basal constituents of MS medium (Murashige and Skoog 1962) containing 0.3 mg/l indol-3yl-acetic acid (IAA) and 1.0 mg/l kinetin. Green shoots which developed in this medium were subsequently transferred to a rooting medium comprising half strength MS constituents: 2% (W/V) sucrose, 0.5 mg/l IAA and 0.1 mg/l kinetin. Cultures for regeneration were incubated as described above for callus growth.

Results

Callus Formation

Pollen collected from anthers floated for up to 3 d failed to give callus when re-incubated in fresh medium. However, calluses were obtained with pollen samples collected after 4 d or longer. Most of the pollen was shed between 4–8 d. With a 4–8 d fraction better callus yields were obtained in the supplemented C_1 medium than with either potato-2 medium or N_6 medium (Table 1).

Table 1. Comparisons between the effects of C_1 medium potato-2 medium and N_6 medium

Cultivars	Medium for callus induction	Number of		%
		Anthers inoculated	Pollen calluses produced[a]	
'Ou rou'	C_1	60×3	156	86.7
	Potato-2	60×3	132	73.3
	N_6	60×3	134	74.4
'Beijing 8'	C_1	60×3	57	31.7
	Potato-2	60×3	38	21.1
	N_6	60×3	35	19.4

[a] According to the counting made on the 25th day after inoculation

With this supplemented C_1 medium, multicellular pollen grains could be discerned under a dissecting microscope after about 16 d while calluses became visible within 21 d. Pollen cultures gave greater callus yields than anther cultures on agar medium (Table 2). Yields were in general about 6 times greater for all the cultivars tested. The cultivar 'Ou rou' gave the best callus yields, with roughly one callus formed from each anther cultured. About 30 anthers at the same stage could be obtained from one wheat spike. Hence with the cultivar 'Ou rou' it was possible to achieve callus yields amounting to 30 for every spike used.

Plant Regeneration

Both green and albino shoots were produced from calluses derived from the shed pollens. This was in accord with previous findings for calluses from anther cultures (Chuang 1978). The ratio of green to albino shoots was 1 : 6 for the 'Yang mai 1' cultivar and 1 : 3 for the 'Beijing 8' cultivar. Less than 10% of the calluses gave green shoots (Table 3). In contrast, calluses derived from anther cultures on agar medium gave greater yields of green shoots, roughly 3 times as many, but fewer albinos. The ratio for the anther culture derived calluses was roughly 1 : 1 for the 'Yang mai 1' cultivar and 2 : 1 for 'Beijing 8' cultivar. It is highly improbable that the better productivity of green plantlets in anther culture was due to some calluses deriving from somatic tissues of the anther. The frequencies of plantlets with roots roughly was 90%.

Discussion

The data are consistent with those for rice (Chen et al. 1980), i.e. pollen cultures are more productive than

Table 2. Comparison between the efficiencies of the isolated pollen culture in liquid medium and anther culture on agar medium

Cultivars	Medium	Numbers of anthers inoculated	Anther culture on agar medium (I)		Isolated pollen culture in liquid medium (II)		Ratio II/I
			Number of calluses	%	Number of calluses	%	
'Beijing 8'	C_1	60×3	8	4.4	57	31.7	7.1
'Ou rou'	C_1	60×3	24	13.3	156	86.7	6.5
'Nong lin 61'	C_1	60×3	7	3.9	41	22.8	5.8
'Yang mai 1'	C_1	60×3	9	5.0	56	31.1	6.2
'Ai gan hong'	C_1	60×3	3	1.7	14	7.8	4.7

Table 3. Comparison between the callus differentiation of the isolated pollen culture and anther solid culture

Cultivars	Anther solid culture				Isolated pollen culture			
	Number of			Total frequency (%)	Number of			Total frequency (%)
	Caluses	Green shoots (%)	Albino shoots (%)		Calluses	Green shoots (%)	Albino shoots (%)	
'Yang mai 1'	45	14 (31.1)	15 (33.3)	64.4	29	2 (6.9)	12 (41.1)	48.3
'Beijing 8'	56	17 (30.3)	9 (16.0)	46.3	34	3 (8.9)	9 (26.5)	35.4

anther culture on agar media, though wheat anther culture on agar medium offers better opportunities for the production of green plants. The reason for this is to be understood. From observations on rice, Chen et al. (1980) suggested that the pollen grains liberated from rice anthers during less than 10 days differentiated only albino plants. Calluses derived from the 10–14 d fraction of pollen grains liberated from rice anthers during culture, differentiated into some green plantlets though most of regenerated plants were albino plants too. There is an increase in the ratio of green to albino plants with increasing the time of anther preinoculation. In wheat Henry and Buyser (1981) have shown that in liquid media young pollen embryos or induced anthers which were cultured on the agar medium for 14 d, then removed to a liquid media could differentiate green plants. In rice Genovesi et al. (1979) reported a decrease in the ratio of green to albino plants with increasing developmental stage of the anthers cultured.

The shed pollen grains from anthers inoculated less than four days are unable to proliferate into multicellular units and calluses. It is possible that these pollen grains were devoid of the ample nurse substances provided by the anther tissues. Therefore the isolated pollen grains remained at the uninuclear stage.

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