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Embryo yield in wheat anther culture is influenced by the choice of sugar in the culture medium

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SUMMARY. The effect of employing different sugars in wheat anther culture has been investigated using four Spring wheat cultivars. The most responsive cultivar, Orofen, gave a three to four-fold increase in embryo yield when maltose was used in place of sucrose, with 50 embryos being produced for every 100 anthers cultured. Measurement of sugar concentrations in the culture media indicated that sucrose was more rapidly hydrolysed than maltose. However, neither the osmotic potential of the medium nor the concentration of glucose appeared to be critical factors in determining embryo yield.

Keywords: Triticum aestivum - Anther culture - Sugars - Wheat

INTRODUCTION

Since the initial discovery that immature pollen could be induced to divide and form plantlets when anthers were removed from the plant and cultured aseptically (Guha and Maheshwari 1966), sucrose has generally been used as the major carbohydrate source in the culture medium.

For wheat anther culture, improvements and modifications to sucrose based culture media have resulted in a significant increase in yields of pollen-derived plants (Ouyang et al. 1973; de Buyser and Henry 1980; Liang et al. 1982; Chu and Hill 1988).

Following reports that the frequency of embryo production in cultured barley anthers can be increased by modifying the sugar content of the medium (Sorvari and Schieder 1987; Hunter 1988; Hunter et al. 1988; Finnie et al. 1989), this study sought to determine the effect of substituting maltose or other sugars for the sucrose in a liquid medium (Datta and Wenzel 1987) developed for wheat anther culture.

MATERIALS AND METHODS

Plant material. The Spring wheat (Triticum aestivum L. em Thell) cultivars Orofen, Chris, Atys and Kite were used in this study. Plants were grown in a lightly shaded glasshouse with a maximum temperature of 25°C. Tillers were harvested in spring and early summer when the tip of the inflorescence could be felt between the base of the flag leaf and the leaf immediately below it. In some cases the tillers were wrapped in foil and subjected to a period of cold pretreatment (+4°C) of up to 15 days prior to dissection. Anthers removed from primary and secondary florets located close to the middle of the inflorescence were macerated in 4% (w/v) acetocarmine in 50% (v/v) acetic acid and examined microscopically. If a high proportion of developing pollen grains were judged to be at the uninucleate stage, the remaining anthers in the inflorescence were used for culture.

Anther culture. Filter-sterilised media for anther culture were based on WM2S (Datta and Wenzel 1987) and all contained N6 inorganic salts and vitamins (Chu et al. 1975), 10% (w/v) Ficoll Type 400-DL (Sigma Chemical Co.), 0.5% (w/v) myo-inositol, 0.5% (w/v) glucose, 160 mg/l L-glutamine and 5 mg/l 2,4-dichlorophenoxyacetic acid. The media differed only in their sugar contents as described in Table 1.

Table 1: Concentrations of sugars included in liquid WM2-based media for wheat anther culture

Medium	sucrose	maltose	melibiose
WM2S	0.175M	-	-
WM2MA	-	0.175M	-
WM2MAS	0.030M	0.145M	-
WM2ME	-	-	0.175M

Medium	glucose	fructose
WM2FG	0.175M	0.175M
WM2GG	0.350M	-

Maltose (Sigma Grade I), fructose (Cell culture tested) and melibiose were obtained from the Sigma Chemical Co., and sucrose from BDH Chemicals, Australia Pty. Ltd. Glucose (Analytical grade) was obtained from Ajax Chemicals (Sydney). Sugar assays were performed using a Boehringer Mannheim kit (sucrose/D-glucose/D-fructose, UV method).

Anthers from a single inflorescence were

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randomly distributed between the different media used in each experiment, in 35mm petri dishes containing 1.0 ml of medium. Care was taken not to bias any dish in favour of anthers from a particular region of the inflorescence. Between 20 and 40 anthers were placed in each dish; the dishes were sealed with Parafilm and incubated in the dark at 25°C for 6 to 8 weeks after which time each dish was scored for number of embryos, by inspection under a dissecting microscope. Dishes were retained for a further two weeks and rescored to ensure late developing embryos were included in the final scores.

RESULTS AND DISCUSSION

The four cultivars used in this investigation showed a wide variation in response to anther culture from almost no embryos in the cultivar Kite to up to 50 embryos per 100 anthers in Orofen. The results presented in Table 2 indicate that replacing the sucrose in the medium with maltose gave a marked increase in the frequency of embryo production in all three responsive cultivars. Partial replacement of the sucrose with maltose (WM2MAS) was less effective. In contrast with results obtained in barley anther culture in starch

Table 2: Frequencies of embryo production in cultured anthers of four cultivars of spring wheat

Cultivar	Number of anthers cultured	Embryos/100 anthers WM2S	WM2MA	WM2MAS	WM2ME
Orofen	1130	13	50	15	1
Chris	348	3	36	17	1
Atys	808	5	10	11	0
Kite	1427	0	0	1	0

media (Sorvari and Schieder 1987) melibiose was a poor substitute for sucrose in this study of wheat anther culture in liquid medium.

To determine whether the embryo production frquency was correlated with a reduced hydrolysis of the disaccharide component of the medium, the concentrations of sucrose, fructose and glucose in the culture media WM2S and WM2MA both with and without anthers were measured at intervals over a 3 week period. The results shown in Fig.1 indicate that in WM2S medium containing anthers, sucrose was rapidly hydrolysed to yield glucose and fructose; after 3 weeks no sucrose could be detected. In WM2S without anthers the rate of hydrolysis of sucrose is much lower, suggesting that most of the sucrose hydrolysis observed during the anther culture is mediated by enzymes provided by the plant material. In the case of WM2MA, maltose hydrolysis (as measured by glucose production) was below detectable limits whether or not anthers were present in the medium.

These results suggested that the improved anther culture response in the maltose based medium might be attributable to the reduced breakdown of the disaccharide. However, this hypothesis was not supported by the results of a further experiment. Here anthers from the cultivar Orofen were cultured in media

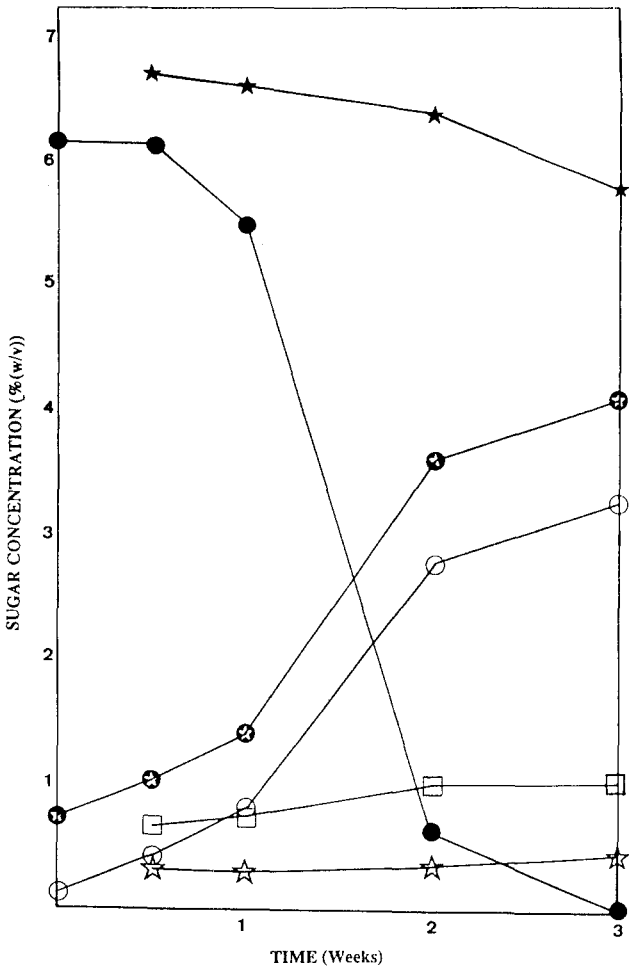

Figure1: Sugar concentrations in WM2S medium incubated at 25°C with and without anthers over a three week period.

where sucrose was replaced by maltose (WM2MA) or by the expected hydrolysis products of sucrose (WM2FG) and maltose (WM2GG) in concentrations corresponding to complete hydrolysis of the disaccharides. The results (shown in Table 3) show once again that the maltose based medium gives a higher frequency of embryo production than the sucrose based medium. However, a high level of embryo production was also observed in WM2GG, the medium corresponding to WM2MA after complete hydrolysis of the maltose to glucose. A relatively low frequency of embryo production was observ-

Table 3: Frequencies of embryo production in cultured anthers of Orofen. Results from two separate experiments are shown

Experiment No.	Number of anthers cultured	Embryos/100 anthers WM2S	WM2MA	WM2FG	WM2GG
1	2078	18	49	16	40
2	601	15	32	12	21

ed in the medium containing glucose and fructose in equimolar amounts (WM2FG). These results suggest that sensitivity to fructose is a more important factor than either the osmotic potential of the medium or the concentration of glucose.

The regenerative capacity of Orofen embryos produced in the different media was tested by transferring them to plates of R1 regeneration medium (Datta and Wenzel 1987) immediately after scoring. Embryos from all four media showed similar capacities to regenerate, yielding plantlets at a frequency of 32%, of which approximately 30% were green, the rest being albino. The low regeneration frequency observed may result from the embryos being left in the induction medium for up to 10 weeks. In a previous experiment (unpublished data) using the same cultivar (Orofen) in which embryos were placed on regeneration medium, about one third of the green plants obtained were fully fertile, indicating that spontaneous chromosome doubling had occurred.

It is concluded that substantial improvements in the production of pollen-derived embryos can be achieved when maltose is substituted for sucrose in liquid anther culture medium. The concentration and composition of the sugar components should be considered as critical variables in establishing an optimised procedure for the induction of androgenesis in wheat.

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