Theor. Appl. Genet. 60, 77-79 (1981)

Float Culture of Wheat Anthers

Y. Henry and J. De Buyser
ADAR, Laboratoire d'Amélioration des Plantes, Université Paris-Sud, Orsay (France)

Summary. Experiments on wheat anther culture in liquid media either synthetic or with potato extract show that it is possible to obtain as many embryos as when using solid potato extract medium. In liquid media young embryos or 14-day old induced anthers can differentiate green plants for regeneration. Glutamine is effective in culturing anthers and can replace potato extract in the medium.

Key words: Anther culture – Liquid medium – Glutamine

Introduction

In vitro anther culture has progressed considerably and today more than 150 species have been found which can produce pollen plants. Most of these have been obtained by anther culture on agar media, although some results with liquid media have been published on *Nicotiana* and *Paeonia* (Sunderland and Roberts 1976; Wernicke 1976; Wernicke and Kohlenbach 1977).

In our work we used float anther culture of winter wheat for embryo production as well as for plant regeneration (Craig 1974). We studied comparatively the effect of glutamine (Wernicke and Kohlenbach 1977; Horner and Pratt 1979) and potato extract (Chuang et al. 1978) respectively in the medium. We also transferred anthers previously grown 14 days on solid medium onto liquid media.

Material and Methods

Experiments were carried out on greenhouse plants of winter wheat (*Triticum aestivum* L.) which had been vernalized in a cold chamber (8 weeks at 5-7°C) and grown during the Spring under suitable conditions. Before removal of the anthers, the spikes were subjected to temperature stress, 5 to 7 days at 3°C, to increase pollen induction frequency and spontaneous diploidization (Amssa et al. 1980). The anthers from one spike, containing uninucleate microspores, were aseptically excised and combined, then floated on the surface of 5 ml aliquots of liquid medium in plastic Petri dishes (50 mm wide, 20 mm deep). Anthers were inoculated on the Chinese potato 2 medium (Chuang et al. 1978) with different modifications (Table 1).

Dishes inoculated with anthers were sealed with 'Cellofrais' and incubated in a 16 h light room. The numbers of productive and unproductive spikes and anthers between 4 and 6 weeks were recorded.

Embryos obtained here after about four weeks, or in other experiments after two weeks of culture, were plated on different modifications of the Chinese regeneration potato medium (Chuang et al. 1978) (Table 1). For embryo cultures, a volume of 2-3 ml of medium per dish allowed adequate conditions for plantlet development (Jensen 1976).

Table 1. Culture media

	Anther						Regeneration		
	G_1	G_2	G_3	G_4	P1	P	R_1	R_2	R_3
Basal medium	Chinese anther medium						Chinese reg. med.		
Agar (g/l)	0	0	0	0	0	7	7	0	0
Potato extract (ml)	0	0	0	0	100	100	50	50	50
Glutamine (g/l)	0.2	0.5	1	2	0.2	0.2	0.05	0.05	0.05
Zeatine (mg/l)	0	0	0	0	0	0	0	0	0.05

0040-5752/81/0060/0077/$ 1.00

Results

Anther Culture on Liquid Media

Anthers simply floated, without support, on the surface of the liquid medium, produce embryos, but these appear some days later than on solid medium. The potato liquid medium (P1) is less effective than the same with agar (P) (Table 2). In the best medium (containing 0.5 g/l glutamine) more than 75% of the spikes were embryogenic, a higher concentration of glutamine is toxic for androgenesis. Although liquid media can increase the number of responding spikes, the total yield is not better than with the use of solid potato medium. In our culture conditions, potato extract can only be replaced by optimal glutamine concentration.

Plant Regeneration in Liquid Media

Regeneration can occur on different media (Table 3), however zeatine (0.05 mg/l) seems to significantly decrease the rate of plantlet production. Our results show that there is no difference between agar or liquid potato regeneration medium. However, the liquid R_2 medium is preferable to the R_1 medium since regeneration in the first occurs two weeks earlier and produces better root systems.

Anther Transfer on Liquid Medium

In transfer experiments (Table 4), anthers were plated on potato medium (P) for the first fourteen days are then transferred to regeneration R_3 medium. Preliminary experiments showed that transfer cannot take place before twelve days. In float cultures, embryos can develop and regenerate green plantlets in the anthers or free in the liquid medium. Transferring the anthers does not increase embryogenesis but does increase significantly the regeneration of plants.

Preliminary experiments made in the laboratory of C. Nitsch on wheat pollen cultures show that it is possible to obtain 1-2 mm embryos if the pollen suspension is made with R_3 medium and if anthers which have been cultured 12-14 days on solid potato medium are used.

Table 2. Anther culture with different media

	Liquid					Agar
Media	G_1	G_2	G_3	G_4	P1	P
Inoculated spikes (1)	106	123	103	165	90	352
Embryogenic spikes (2)	56	94	41	42	34	212
2/1	0.53	0.76	0.40	0.25	0.38	0.60
Embryogenic anthers (3)	109	199	73	70	70	592
3/2	1.95	2.12	1.78	1.67	2.06	2.79

Table 3. Plant generation

Media		R_1	R_2	R_3
Embryos	(4)	1023	1148	9782
Green Plants	(5)	65	74	288
	5/4	0.06	0.06	0.03

Table 4. Transfer of anthers

		Number	Frequency
Inoculated spikes	(1)	106	
Embryogenic spikes	(2)	49	2/1 = 0.46
Embryogenic anthers	(3)	92	3/2 = 1.88
Embryos	(4)	212	4/3 = 2.30
Green plants	(5)	26	5/4 = 0.12

Discussion

Liquid media proved to be efficient for androgenesis. In one experiment without agar and without potato extract it was possible to reach the yield obtained on agar potato medium. Another advantage of the synthetic medium is that it is more reliable than an extract. Indeed, we could observe that the effect of potato extract in the medium varies with the variety of the potato and with the season.

We believe that some amino acids (Nitsch and Godard 1979) are very important in androgenesis. This is known for embryo culture and Jensen (1976) uses a medium containing 11 amino acids for barley haploid embryo culture. Potato tuber contains many amino acids, glutamine and asparagine. Therefore, it is understandable that glutamine added in the medium can at least partially replace the potato extract. Glutamine can act as nitrogen source and it will be interesting to see if it could replace the mineral nitrogen source as NO_3^- and NH_4^+ in liquid media. Asparagine must also be tested either alone or with glutamine.

Another important point in wheat anther culture is the need of 2-4 D during the induction period : if 2-4 D is absent from the medium before 12 days of culture, no embryogenesis occurs. A new step will be reached for wheat haploid production when it will be possible to culture free pollen. This would make easy to modify the medium during the 'in vitro' culture period. In other words, the manipulation of the successive media necessary to induce and regenerate the plants could be achieved without injury for the young embryos.

Acknowledgement

The authors wish to thank Dr. Colette Nitsch in whose laboratory the pollen culture experiments were carried out. Thanks are also due to Thierry Guenegou for his expert technical assistance.

Literature

Amssa, M.; De Buyser, J.; Henry, Y. (1980): Origine des plantes diploides obtenues par culture in vitro d'anthères de Blé tendre (*Triticum aestivum* L.): influence du prétraitement au froid et de la culture in vitro sur le doublement. C.R. Acad. Sci. (Paris) 290, 1095-1097

Chuang, C.-C.; Ouyang, T.-W.; Chia, H.; Chou, S.-M.; Ching, C.-K. (1978): A set of Potato media for Wheat anther culture. In: Proc. China-Australia Plant Tissue Culture Symp., Peking, pp. 51-66. Peking: Science Press

Craig, L. (1974): Haploid plants (2n = 21) from in vitro anther culture of *Triticum aestivum* L. Can. J. Genet. Cytol. 16, 697-700

Horner, M.; Pratt, M.L. (1979): Amino Acid Analysis of in vitro and androgenic anthers of *Nicotiana tabacum*. Protoplasma 98, 279-282

Jensen, C.J. (1976): Barley monoploids and doubled monoploids: techniques and experience. In: Barley Genetics 3 (ed. Gaul, H), pp. 316-345. München: Thiemig.

Nitsch, C.; Godard, M. (1979): The role of hormones in promoting and developing growth to select new varieties in sterile cultures. In: Plant Regulation and World Agriculture (ed. Scott, T.K.), pp. 49-62. New York: Plenum Publ. Co.

Sunderland, N.; Roberts, M. (1976): Anther and pollen culture. John Innes Institute, Ann. Rep. 67, pp. 60-65. Norwich: John Innes Institute

Wernicke, W. (1976): Investigations on liquid culture medium as a means of anther culture in *Nicotiana*. Z. Pflanzenphysiol. 79, 189-198

Wernicke, W.; Kohlenbach, H.W. (1977): Versuche zu kultur isolierter Mikrosporen von *Nicotiana* und *Hyoscyamus*. Z. Pflanzenphysiol. 81, 330-340

Received February 9, 1981
Accepted March 17, 1981
Communicated by G. Wenzel

Y. Henry
J. De Buyser
A.D.A.R., Laboratoire d'Amélioration des Plantes
Bat. 360, Université Paris-Sud
91405 Orsay cedex 05 (France)