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ANTHER CULTURE OF *SOLANUM TUBEROSUM* L.

J. M. DUNWELL and N. SUNDERLAND

John Innes Institute, Norwich, England

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SUMMARY

Embryoid formation was induced in pollen of *Solanum tuberosum*, cv. Pentland Crown, Maris Piper and Record, by culturing anthers at or just prior to the first pollen mitosis. The composition of the medium was not critical in respect of either nutrients or hormones. In the presence of relatively high hormone levels, callus formation also occurred from somatic tissues of the anthers.

INTRODUCTION

Solanacaeous plants have proved to be especially amenable to the technique of anther culture. The cultivated potato is, however, a notable exception. LABIB & MELCHERS (1972) have reported through the Haploid Information Service on an extensive but abortive survey of 14 varieties cultured under a wide range of conditions. KOHLENBACH & GEIER (1972) also tested two other varieties without success, but observed the formation of callus from somatic tissues; in one instance, the callus gave rise to adventitious embryos. In another series of experiments bearing on the problem, IRIKURA & SAKAGUCHI (1972) attempted to induce pollen growth in several dihaploid *tuberosum* lines. Here again, there was no direct evidence of pollen growth, but a few anthers showed root differentiation which may have been of pollen origin. These latter workers did, however, succeed in raising haploid plantlets from pollen in the tuberous diploid species, *S. verrucosum* SCHLECHTD.

During the past two years, we have tested anthers of numerous pollen-fertile varieties and unnamed genotypes, and in some have observed the formation of pollen embryoids. This paper deals with our findings on three cultivars, Pentland Crown, Maris Piper and Record.

MATERIALS AND METHODS

Tubers of Pentland Crown were supplied by the Scottish Plant Breeding Station, Roslin, and of Maris Piper and Record by the Plant Breeding Institute, Cambridge. Plants were cultivated in P1 compost in the John Innes glasshouses, and grown with tubers exposed. Stolons were removed as they developed in order to prolong the flowering period. Two series of plants were examined. The first flowered during April and May 1972. Probably because of a cold spring many of the flowers produced were abnormal; they either abscissed soon after emergence or opened prematurely. Flowering also ceased after the first flush despite the removal of stolons. The second planting produced more favourable plants and these gave a sequence of flowers from late June to early September.

Immature flower buds were surface-sterilised for 20–30 min in a 0.12% solution

of sodium hypochlorite containing 0.1 % Tween 20, and rinsed in sterile water. After removal of the perianth, anthers were carefully dissected away and inoculated in pairs into 30 ml plastic tubes each containing 10 ml of medium. In order to assess the developmental state of the pollen, the fifth anther in each bud was squashed in aceto-carmine as described by SUNDERLAND & WICKS (1971).

For many of the cultures, the basal media (all components except hormones) recommended by GUHA & MAHESHWARI (1967) (GM) and MURASHIGE & SKOOG (1962) (MS) were used, but those of BLAYDES (1966) (B), BOURGIN & NITSCH (1967) (H), SCHENK & HILDEBRANDT (1972) (SH) and WHITE (1963) (W) were also tried. The basal media were solidified with agar and used alone or in conjunction with either kinetin or an auxin, 1-napthylacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D) or p-chlorophenoxyacetic acid (PCA) in varying concentrations. In some instances, more than one auxin was included; in others, the sucrose level was used at varying concentrations up to 20 per cent w/v, and in others coconut milk was used instead of, or in addition to, kinetin. All constituents were sterilised by autoclaving. The pH was adjusted in all cases to 5.9 before autoclaving.

Cultures were incubated at 25 ± 2 °C in a room dimly-lit by fluorescent tubes; some were also kept in boxes in the same room to exclude light. Cultures were harvested after 6–12 weeks and examined for the presence of multicellular grains or pollen embryoids as described by SUNDERLAND & WICKS (1971).

RESULTS

Anthers were cultured at meiotic, microspore and young pollen grain stages as in the procedure adopted for tobacco (SUNDERLAND & WICKS, 1969). Not enough anthers were found to contain microspores undergoing the first pollen mitosis to constitute a separate category; such anthers were therefore included in the microspore category. The third category consisted of those anthers in which the first mitosis had just been completed and differentiation of generative and vetetative cells had commenced. Older anthers were not tested (see SUNDERLAND, 1971). Data obtained with each cultivar in both the spring and summer plants were as shown in Tables 1 and 2.

Table 1. Date for spring planting 1972. A = Number of anthers cultured in each variety; B = number producing pollen embryoids; C = number producing callus. Figures in brackets are for dark-grown cultures.

Stage and petal length	Variety	A		B		C	
Meiosis	Pentland C.	4	(12)	0	(0)	2	(0)
3–4.5 mm	Maris P.		(8)		(0)		(1)
Microspore	Pentland C.	88	(120)	0	(1)	4	(6)
4.5–7 mm	Maris P.	98	(76)	1	(2)	6	(9)
	Record	148	(88)	3	(1)	2	(2)
Young pollen	Pentland C.	34	(16)	0	(0)	1	(0)
grains	Maris P.	40	(28)	1	(0)	4	(2)
7–8 mm	Record	30	(28)	0	(0)	1	(0)

Table 2. Data for summer planting 1972. A = Number of anthers cultured in each variety; B = number producing pollen embryoids; C = number producing callus. Light-grown cultures only.

Stage and petal length	Variety	A	B	C
Meiosis	Pentland C.	44	0	5
3–4.5 mm	Record	8	0	2
Microspore	Pentland C.	232	31*	2
4.5–7 mm	Maris P.	120	3	12
	Record	126	1	2
Young pollen	Pentland C.	121	3	1
grains	Maris P.	22	0	2
7–8 mm	Record	60	0	1

*Includes one anther producing a plantlet and three producing roots only.

Embryoid formation

Embryoid formation was largely restricted to anthers cultured at the microspore stage, though it also occurred in an occasional anther of the young pollen grain stage. Embryoids were not observed in any anther cultured at the meiotic stage. Light was not essential for induction (Table 1). With Maris Piper and Record, the frequency of embryoid formation was low in both series of plants, between 1 to 2% of the total number of anthers cultured. With Pentland Crown, on the other hand, there was a marked difference in frequency between the two series; in the first, embryoids were observed in only one anther, whereas in the second they occurred in slightly more than 13% of the anthers cultured (microspore stage). This difference may have been due to the healthier condition of the summer plants.

There was also a difference in embryoid size between the two series. In the first, cell numbers varied with up to a maximum of about 16 cells (Fig. 1A). The staining capacity of the embryoids was poor indicative of cell degeneration. Since the anthers had been in culture for about 3 months when examined, this degeneration suggested that division had commenced early in culture and then ceased. In the second series, embryoids were larger and contained many cells (Fig. 1B); some again showed signs of degeneration but others stained well and might have been capable of further growth. In one anther of Pentland Crown, a plantlet emerged after about 3 weeks of culture, and in three others roots emerged from the pollen sacs. Root-tip squashes revealed mitoses consisting of the gametic number of chromosomes.

Callus formation

Callus formation occurred in all three varieties, with probably a slightly greater frequency in Maris Piper. There was also a greater frequency of callus formation in anthers cultured at the microspore stage. Most of the calluses were derived from somatic tissues, either from the cut end of the filament or from connective tissues. In some instances, anthers produced both somatic callus and pollen embryoids. In a few instances, the callus emerged directly from the pollen sac, and though these may have been of pollen origin they grew very slowly and chromosome counts were not attempted. The form, texture, growth-rate and colour of the calluses varied with the

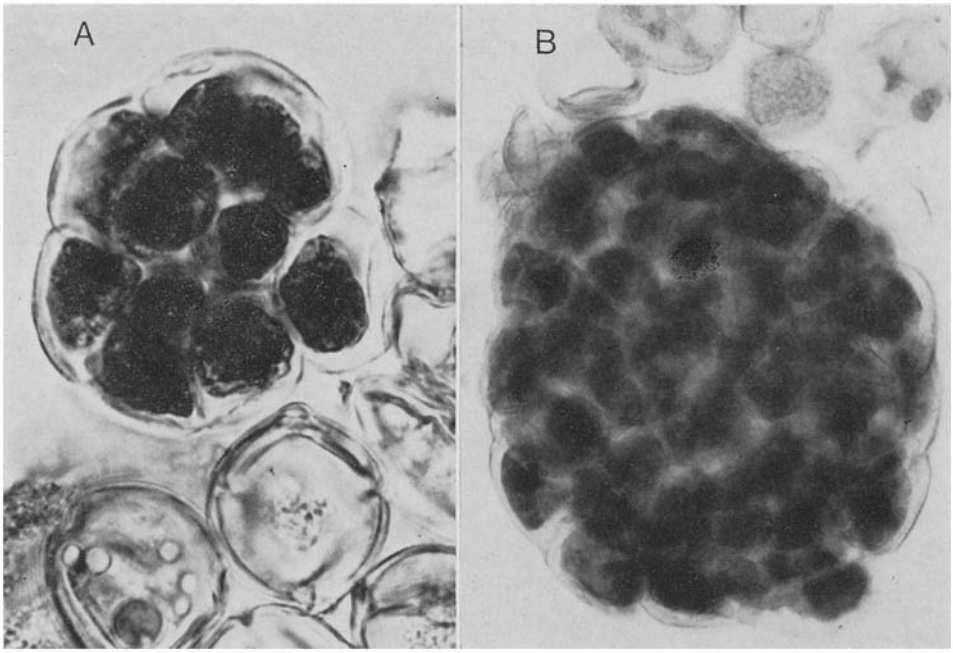

Fig. 1. Pollen embryoids of *Solanum tuberosum*. A: Maris Piper, longest diameter approximately 40 μm. B: Pentland Crown, 130 μm. Degenerating pollen grains can also be seen.

composition of the culture medium. Most of the calluses proliferated without organised growth. However, a few showed root differentiation, and in two instances, one in Pentland Crown and the other in Record, a plantlet grew out from the callus soon after its formation (cf. KOHLENBACH & GEIER, 1972).

Culture medium

The composition of the media in which embryoid and callus formation occurred was as shown in Table 3. Choice of media was to some extent influenced by preliminary results obtained in 1971 on Pentland Crown. These showed that embryoid formation could be induced in MS or GM nutrients supplemented with kinetin (or coconut milk) and an auxin in concentrations greater than 1.0 mg/l. The present data revealed that the earlier results were misleading; embryoids were certainly produced in the presence of high hormone levels, but kinetin (or coconut milk) was not necessary for induction, and auxins only in low concentrations. Auxin concentrations greater than 1.0 mg/l were undesirable in that they induced growth in somatic tissues as well as in the pollen.

DISCUSSION

The data of Table 2 indicate that embryogenesis is inducible in potato pollen by culturing anthers at or just prior to the first pollen mitosis. The peak response was

Table 3. List of basal media and supplements in which growth occurred. A = pollen embryoids; B = roots only; C = roots plus shoot; D = callus formation; E = callus plus roots; F = callus plus roots and shoots. NAA = 1-napthylacetic acid; 2,4–D = 2,4– dichlorophenoxyacetic acid; PCA = p-chlorophenoxyacetic acid; K = kinetin; CM = coconut milk; S = sucrose.

Basal Medium	Auxin	concentration (mg/l)	Other components	A	B	C	D	E	F
Murashige	NAA	0.01				×			
& Skoog		0.01	K 0.01 or 10.0	×					
		0.1		×					
		0.1	K 1.0 or 10.0	×					
		1.0		×					
		1.0	K 0.01, 0.1 or 1.0	×					
		2.0	K 0.1	×			×		
		2.0	K 2.0, 5.0 or 5.0 + S 6%				×		
		2.0 + 2,4-D 2.0	K 2.0 or 5.0				×		
		5.0	K 5.0 or CM 10%				×		
		5.0 + 2,4-D 2.0	K 5.0				×		
		10.0					×		
		10.0	K 1.0 + CM 10% or K 2.0				×		
		10.0	K 10.0	×					
		10.0	K 2.0 + S 5, 10 or 20%	×			×	×	
		10.0	K 5.0 + S 5, 10 or 20%	×			×	×	
	2,4-D	0.001		×	×				
		0.1	K 1.0	×					
		0.1	CM 10% + S 10%				×		
		1.0	CM 20%	×					
	PCA	0.01	K 1.0 or 10.0	×					
		0.1	K 0.1		×				
		0.1	K 0.01, 1.0 or 10.0	×					
		1.0	K 0.1						×
		1.0	K 1.0 or 10.0	×					
		1.0	K 1.0 + S 5%				×*		×
		10.0	K 0.1, 1.0 + CM 10% or K 10.0	×					
Guha & Maheshwari			K 5.0 + CM 10%	×					
	NAA	1.0	K 2.0				×		
		2.0	K 1.0, 2.0, 5.0 or 10.0	×			×		
		2,0	K 0.1 + CM 10%	×			×*		
		10.0					×		
		10.0	K 2.0 or 5.0				×		
	2,4-D	1.0	K 0.1				×		
		2.5	CM 20%	×					
Schenk & Hildebrandt	NAA	2.0 + 2,4-D 2.0	K 5.0	×			×		
		10.0	K 5.0				×		
Bourgin & Nitsch			K 2.0 + CM 10%	×					
Blaydes	NAA	5.0	K 0.03	×					
White	2,4-D	5.0	K 0.5 + CM 10%				×		

*Callus emerged from pollen sac.

obtained in those anthers whose pollen was about to enter mitosis, and the response declined sharply thereafter with increasing anther age. In this, potato appears to differ slightly from the model system (tobacco) in which the peak response occurs in anthers inoculated just after the first mitosis, and in which the response declines relatively slowly with anther age (SUNDERLAND 1973). In other words, the stage-requirement in potato is very critical. We do not know of any morphological character by which this critical point can be assessed in order to obviate direct cytological examination of the pollen. Embryogenesis occurred in anthers taken from buds in which the distance from base to apex of the closed petals measured from 6 to 7 mm that is, towards the end of the microspore stage defined in Table 2. Petal length can therefore be a partial guide in the selection of appropriate anthers, but it must be borne in mind that potato flower buds are relatively small, and that a small change in petal length may involve a large change in the developmental state of the pollen.

Medium-composition is less critical for induction than anther age. Indeed, medium requirements do not appear to be very different from those in the tobacco system, in which induction occurs in the absence of exogenous hormones (NITSCH, 1970). The principal difference between the two systems is one of quantity. Whereas the number of pollen embryoids produced per anther in tobacco may run into thousands (SUNDERLAND & WICKS, 1971), the number produced in potato is small, on average less than five. In tobacco, relatively few embryoids survive and develop into functional plantlets; on average from 15–20 per anther (SUNDERLAND, 1971). This represents a 'survival' rate no greater than about 0.05 % of the total pollen population in the anther. The potato system appears to be similar in showing a low 'survival' rate.

The fact that a pollen plantlet was obtained in the present experiments (which involved relatively small numbers of plants and cultures) encourages us to believe that with refinements, anther culture can offer to potato breeders an alternative and effective method for the production of haploid and dihaploid plants. This belief has been strengthened by more recent experiments carried out on fieldgrown plants, in which culture was restricted to anthers in the late microspore stage and to MS-medium supplemented with either NAA 0.01, PCA 0.01 or 2,4-D 0.001 mg/l. In one unnamed genotype over 60 % of anthers cultured produced embryoids.

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REFERENCES

BLAYDES, D. F., 1966. Interaction of kinetin and various inhibitors in the growth of soybean tissues. Physiologia Pl. 19:748–753.

BOURGIN, J. P. & J. P. NITSCH, 1967. Obtention de *Nicotiana* haploides à partir d'étamines cultivées in vitro. Ann. Physiol. vég. 9:377–382.

GUHA, S. & S. C. MAHESHWARI, 1967. Development of embryoids from pollen grains of *Datura* in vitro. Phytomorphology 17:454–461.

IRIKURA, Y. & S. SAKAGUCHI, 1972. Induction of 12-chromosome plants from anther culture in a tuberous *Solanum*. Potato Res. 15:170–173.

KOHLENBACH, H. W., & T. GEIER, 1972. Embryonen aus in vitro kultivierten Antheren von *Datura meteloides* Dun., *Datura wrightii* Regel und *Solanum tuberosum* L. Z. PflPhysiol. 67:161–165.

MURASHIGE, T. & F. SKOOG, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Pl. 15:473–497.

NITSCH, J. P., 1970. Experimental androgenesis in *Nicotiana*. Phytomorphology 19:389–404.

SCHENK, R. U. & A. C. HILDEBRANDT, 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Can. J. Bot. 50:199–204.

SUNDERLAND, N., 1971. Anther culture: a progress report. Sci. Prog. Oxf. 59:527–549.

SUNDERLAND, N., 1973. Pollen and anther culture. In: H. E. Street (Ed.), Plant tissue and cell culture, Ch. 9. Blackwell, Oxford.

SUNDERLAND, N. & F. M. WICKS, 1969. Cultivation of haploid plants from tobacco pollen. Nature, Lond. 224:1227–1229.

SUNDERLAND, N. & F. M. WICKS, 1971. Embryoid formation in pollen grains of *Nicotiana tabacum*. J. Exp. Bot. 22:213–226.

WHITE, P. R., 1963. The cultivation of animal and plant cells, 2nd ed. Ronald Press Company, New York.