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Production of auxotrophic mutants in ferns

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1. INTRODUCTION

The primary aim of this work was to develop a model system for selecting auxotrophic mutants in higher plants. Pteridophytes offer a unique experimental material for the study of plant genetics. They possess haploid spores and gametophytes amenable to techniques used in handling fungal ascospores and cell clones. The fern species *Todea barbara* (L.) and *Osmunda cinnamomea* (L.) were chosen for these experiments because their low chromosome numbers indicated that they were true haploids, and because their life-cycles can be completed in sterile culture. Of many selective techniques employed, the one modelled after a system designed by Puck & Kao (1967) for use with mammalian tissue culture cells met with success. This technique takes advantage of the light sensitivity characteristics of DNA that has incorporated 5-bromodeoxyuridine (BUdR).

2. METHODS AND RESULTS

Observation had indicated that many mutagen-treated spores would undergo germination and several cell divisions before dying. Since my earlier attempts at direct spore selection had failed, experiments were focused on gametophytes consisting of from 3 to 4 prothallus cells, under the assumption that the growth-limiting compound was present in the spore at a level which would allow several cell divisions.

In order to demonstrate that it is possible to isolate various auxotrophic types, the following experiment was performed. Spores of *T. barbara* were treated with a 1 % solution of ethyl methanesulphonate in distilled water for 1½ h at 25 °C. Spore survival was 47 %. Spores were then sterilized with a 5 % Clorox–½ % Tween 80 solution, washed 3 times with sterile distilled water and placed in a minimal medium of Knop's inorganic salt solution supplemented with 1 % sucrose at a density of about 100 surviving spores per ml. Cultures were maintained in cool white light of 400–450 ft candles intensity with a 16 h light, 8 h dark cycle at 25 ± 1 °C. Under these conditions spores germinate and produce a 3- to 4-celled sporeling after 5 days. The medium was then supplemented with BUdR to a concentration of 10^{-5} M and the cultures were held in the dark for 48 h. At the end of the dark period sporelings were removed from the BUdR medium, washed twice in Knop's salt solution, and plated on minimal medium supplemented with 200 mg/l. yeast extract and 500 mg/l. vitamin-free casein hydrolysate and solidified by the addition of 0·8 % agar. These cultures were placed 30 cm from a bank of three cool white fluorescent lamps. Survival after 3 weeks was 1·36 % of the BUdR treated sporelings. Normal-appearing plants were selected and screened for growth on minimal and various supplemented media following the procedure of Holliday (1956). Thirty-three auxotrophic mutants were obtained from a sample of 200 plants. The data are summarized in Table 1. Growth is expressed as the percentage of increase in wet weight in a 3-week period.

The following modification of the described procedure proved to be a convenient method for the selection of large numbers of specific mutant types. Spores of *O. cinnamomea* were carried through the same experimental protocol outlined above; however,

at the end of the dark period in BUdR the spores were washed twice and plated on an unsupplemented salt solution solidified with 0·8 % agar. These cultures were placed 30 cm from a bank of cool white fluorescent lamps. Survival after 3 weeks was 0·21 % of the BUdR treated sporelings. These surviving plants were picked off the medium and

Table 1. *Auxotrophic mutants of* Todea barbara*

Isolation number	Required supplement	Increase in wet weight in 3 weeks (%)	
		Minimal medium	Minimal medium plus required supplement
12	Adenine	−8	+117
89	Cytosine	−13	+76
144	Biotin	+111	+209
82	Choline	−9	+188
25	Inositol	+3	+170
86	Nicotinic acid	+5	+162
138	Nicotinic acid	−8	+115
18	Para-amino benzoic acid	−21	+159
48	Para-amino benzoic acid	−15	+106
53	Riboflavin	−27	+131
43	Thiamine	−1	+93
104	Thiamine	0	+149
186	Thiamine	−17	+130
99	Arginine	+1	+132
115	Arginine	−4	+96
160	Arginine	−7	+110
6	Arginine or	−1	+152
	Ornithine	+10	+167
69	Cysteine	−2	+81
16	Cysteine or	−9	+140
	Methionine	−16	+127
129	Cysteine or	−5	+81
	Methionine	−23	+98
4	Histidine	0	+107
135	Histidine	0	+105
36	Isoleucine	+3	+108
179	Leucine	−4	+121
197	Lysine	−12	+72
121	Methionine	−2	+125
181	Methionine	−1	+89
61	Phenylalanine	−10	+101
105	Proline	−18	+217
153	Threonine	+24	+147
50	Tryptophane	+1	+145
39	Valine or	+7	+129
	Isoleucine	0	+108
147	Valine or	+6	+91
	Isoleucine	−7	+85

* Initial weight of tissue for growth tests approximately 50 mg. Auxotrophic supplements were supplied in the following amounts: (1) all amino acids, 100 μg/ml; (2) all nucleic acid bases, 50 μg/ml; (3) i-inositol, 4·0 μg/ml; (4) choline chloride, 1·0 μg/ml; (5) thiamine HCl, 0·4 μg/ml; (6) para-amino benzoic acid, riboflavin, pyridoxine HCl, nicotinic acid and folic acid, 0·2 μg/ml. Vitamins were filter-sterilized and added to previously autoclaved medium.

discarded. The cultures were then supplemented with a specific chemical which would allow the desired auxotrophic type to grow. Growing plants were selected after 3 weeks and characterized according to their growth on minimal and supplemented medium. In an experiment designed to isolate uracil requiring mutants, the minimal medium was supplemented with 100 μg/ml. uracil. Of 20000 BUdR treated sporelings which did not grow on minimal medium, seven grew upon addition of uracil. Further tests verified that three of these required uracil for growth.

3. DISCUSSION

Evidence from mammalian tissue culture cells (Puck & Kao, 1967) suggests that the selective system works because BUdR is incorporated into the DNA of the chromosomes of metabolizing cells. Auxotrophs have presumably slowed their metabolism after several cell divisions so that the amount of BUdR incorporated into their chromosomes is much less extensive than non-auxotrophs. Incorporation of BUdR causes DNA to become light-sensitive in the near visible region of the spectrum (Stahl *et al.* 1961). Exposure to near-visible radiation produces large numbers of chromosomal aberrations and death in cells that have incorporated BUdR, while cells that did not incorporate BUdR are impervious to the killing action of near-visible radiation (Puck & Kao, 1967).

These experiments demonstrate that it is possible to obtain auxotrophic mutants in higher plants. Production of biochemical mutants will provide a powerful tool for genetics and physiology when combined with recent advances in plant cell culture, such as the production of haploid plants (unpublished observations; Guha & Maheshwari, 1966; Nakata & Tanaka, 1968; Nitsch & Nitsch, 1969) and of whole plants from single cells (Vasil & Hildebrandt, 1965).

SUMMARY

A method has been devised for the selection of auxotrophic mutants in higher plants. The method depends upon the incorporation of BUdR into the DNA of non-auxotrophic cells and upon its lack of incorporation into the DNA of auxotrophic cells. A wide range of auxotrophic types were recovered.

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