tions of old rats by cytochrome c administration may be due to the increase of lipid catabolism.

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Preferential Growth of Haploid Plant Cells *in vitro*

HAPLOIDS from higher eukaryotes are of great importance for genetic analysis[1]. The observation of Morpurgo[2] that haploid segregants can be obtained from diploid lines of *Aspergillus nidulans* by parafluorophenylalanine (PFP) treatment suggests that defined chemical manipulations may be useful in generating and maintaining haploid cells in cultures of higher plants. Parafluorophenylalanine has been found to induce haploidization not only in some species of *Aspergillus*, but also in some other genera of fungi such as *Ustilago*[3].

To isolate haploids from higher plants is laborious. The technique of another culture[4,5] has been used to circumvent these difficulties but it has been observed that anther culture invariably leads to calluses composed of cells of different levels of ploidy[6-9] and also that only a few species of plants respond to the culture technique by producing haploids[10-13]. This limits the recovery and handling of haploid cell lines from anther cultures. Because the generation and maintenance of haploid cell lines are of central importance in the study of somatic cell genetics, we are attempting to define the conditions required to maintain haploidy in cultured cells.

Table 1 Growth of Haploid and Diploid Cells on Medium containing Varying Concentrations of PFP

Dose of PFP (μg/ml.)	Weight of initial inoculum (g)		Weight of callus after 6 weeks (g)		Fold increase	
	Haploid	Diploid	Haploid	Diploid	Haploid	Diploid
0	0.8	0.8	13.8	12.9	17	16
1	0.8	0.8	15.4	7.8	20	10
5	0.8	0.8	13.9	6.7	17	8
7	0.8	0.8	13.0	6.2	16	5
9	0.8	0.8	15.1	1.1	19	1.5
15	0.8	0.8	13.9	1.0	17	1

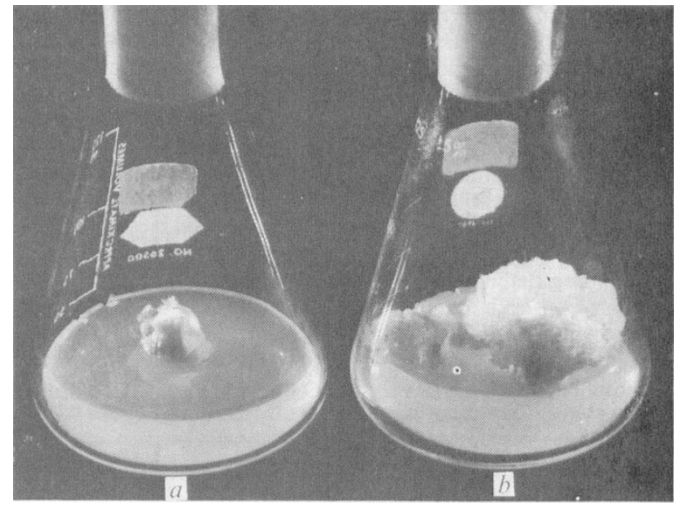

Fig. 1 *a*, Diploid callus on 9 μg/ml. of PFP showing practically no growth after 6 weeks. *b*, Haploid callus on 9 μg/ml. of PFP showing vigorous growth.

Here we describe the successful utilization of PFP to maintain preferentially the haploid state in cells from higher plants grown in culture. Calluses derived from the pith of cytologically identified haploid and diploid plants of *Nicotiana tabacum* (var. Havana Wisconsin 38, $2n=48$) were inoculated on the standard tobacco medium of Linsmaier and Skoog[14] containing 4 p.p.m. IAA and 1 p.p.m. kinetin. Various concentrations of PFP were added to the medium and the cultures were kept in complete darkness at 28° C. The results (Table 1) show that the growth of diploid cells is progressively inhibited at increasing levels of PFP while the growth of haploid cells is unaffected by the presence of PFP. Specifically, at a level of 9 μg/ml. of PFP the growth of diploid callus is inhibited and at 15 μg/ml. the diploid callus turns black and dies. The vigorous growth of the haploid callus is not affected by these concentrations of PFP. Fig. 1 shows haploid callus growth and diploid inhibition on 9 μg/ml. PFP after 6 weeks of growth. Cytological observations have confirmed that the haploid state of the tissue is maintained during the PFP treatment.

Our work demonstrates that it is possible to maintain stable cultures of haploid cells, and to select preferentially haploid cells from mixed populations of cells of varying ploidy. Further work on the applicability of the PFP technique to other species and the preferential recovery of haploid cells *in vitro* is being pursued in this laboratory.

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