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EFFECTS OF *p*-FLUOROPHENYLALANINE (PFP) ON THE GROWTH OF CELL LINES DIFFERING IN PLOIDY AND DERIVED FROM *NICOTIANA SYLVESTRIS*

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SUMMARY

The growth inhibitory activity of *p*-fluorophenylalanine (PFP) (concentration range 25—75 μg/ml) has been tested on callus initiation and early callus growth and on suspension cultures derived from one diploid and three haploid plants of *Nicotiana sylvestris*. All the culture lines contained cells at more than one level of ploidy; the cultures of haploid origin contained a high level of haploid cells. Under conditions of partial growth inhibition by PFP there was, despite the reduced sensitivity of the cultures of haploid origin, no preferential growth of haploid cells in these cultures. Genotype not ploidy level determined sensitivity to growth inhibition by PFP.

INTRODUCTION

Gupta and Carlson [1] reported that PFP at a concentration of 9 μg/ml was strongly inhibitory to the growth of a callus derived from a diploid plant of *Nicotiana tabacum* (var. Havana Wisconsin 38) and without effect on a callus derived from a haploid plant of the same variety. From this observation they claimed that PFP could be used to select preferentially the haploid cells from a mixed population of cells varying in ploidy. We have now examined this possibility with *Nicotiana sylvestris* Speg. and Comes (2n = 24) using cell lines of differing ploidy derived from haploid and diploid plants.

MATERIALS AND METHODS

Plants

Haploid plants were of anther origin [2]. Their ploidy was checked by pretreating root tips for 1.5 h in 0.05% colchicine, fixation in 50% aqueous formic acid, Feulgen staining and counting chromosomes in squash preparations.

Abbreviation: PFP, *p*-fluorophenylalanine.

Diploid plants were from the same seed sample as those used to generate the haploids by anther culture.

Callus initiation and culture of cell suspensions

Callus was initiated from petioles of haploid and diploid plants which had reached the rosette form (diam. rosette 30—40 cm). Petioles were surface sterilised in 20% "Domestos" for 10 min, cut into 2—3-mm segments and placed on Linsmaier and Skoog medium [3] containing 1 g/l Difco casein hydrolysate, 0.4 mg/l 2,4-D and 0.03 mg/l kinetin and solidified with 0.8% "Oxoid" Ionagar No. 2. The calluses were sub-cultured or used in initiating cell suspension cultures after 5 weeks.

Cell suspensions were cultured in the above medium with the Ionagar omitted and incubated at 25° in continuous fluorescent light on a horizontal platform shaker at 120 rev./min. After the initiation passage, suspensions were sub-cultured every 21 days using sufficient suspension to give an initial cell density of 10^5 cells/ml. Cell numbers were determined as previously described [4].

DNA estimation and chromosome counts

Squash preparations of the cultures (after 21 days' incubation) were prepared after fixation in 50% aqueous formic acid, hydrolysis with 1 *N* HCl at 60° for 12 min and Feulgen staining. For standardisation, root tips from diploid plants were always processed with the sample of cell suspension and examined as squash preparations. DNA values were obtained using a Vickers M85 scanning microdensitometer.

50 interphase nuclei were scored for the root tip preparations and gave two sharp peaks corresponding to the G1 and G2 phases of the cell cycle. 200 interphase nuclei from 4—5 slides were scored for each cell culture. The DNA values ranged from 15 to 500 arbitrary units and these values were grouped into 30 classes defined by dividing $\log_{10}500 - \log_{10}15$ into 30 equal spacings; the antilogs setting the class limits for the densities recorded.

Chromosome counts on cell suspensions (samples taken on the 10th day of incubation) were performed as described above for checking the haploidy of the anther culture derived plants.

RESULTS AND DISCUSSION

Preliminary experiments showed that callus or cell suspension cultures of both diploid and haploid origin from *Nicotiana sylvestris* were insensitive to a concentration of PFP of 10 μg/ml but showed significant growth inhibitions within the concentration range 25—75 μg/ml.

Four cell lines were involved in the main series of experiments. One (D) from a diploid plant and three (H6, H7 and H9) from separate haploid plants derived from anthers from different diploid plants.

The calluses used to initiate the suspension cultures were initiated in the

absence of PFP, but the effect of PFP upon callus initiation and growth of callus on the petiole explants was also tested with the diploid and two of the haploid plants (H6 and H7). In the presence of 37.5 μg/ml PFP, callus was only initiated on 4 out of 7 diploid petioles, was strongly inhibited in its growth and turned brown. At higher concentrations the number of petioles initiating callus was further reduced and growth still more strongly inhibited. With 37.5 μg/ml PFP all petioles from the haploid plants initiated callus and the callus growth was healthy and uninhibited. With 50 μg/ml, callus initiation and callus growth was inhibited with petioles from one of the haploid plants (H6). Significant inhibition only occurred with 75 μg/ml with the second haploid (H7). This contrast between H7 and D is shown in Fig. 1. Callus initiation and growth from petioles of the haploid plants was therefore more resistant to inhibition by PFP than that from the diploid plant although the haploids differed in their sensitivity. This observation, although effected at a much higher level of PFP than that used by Gupta and Carlson [1] was similar to that reported for callus growth in *Nicotiana tabacum*.

Cell suspension cultures were initiated from the four culture lines in the culture medium without PFP and then tested for their sensitivity to PFP inhibition after one or two such culture passages in the absence of PFP (*i.e.* tested in Passage 2 and Passage 3). The effect of PFP on growth was assessed by cell counts after 21 days of incubation (no further significant increases in cell number occurred when incubation was extended for a further 7 days). The data were expressed as % inhibition of final cell number (Fig. 2) or in terms of actual final cell densities (cell number $\cdot 10^{-6}$/ml) (Fig. 3). These experiments showed that the four lines differed significantly in their sensitivity to inhibition by PFP. The line of diploid origin (D) was most sensitive, line H9 least sensitive, and lines H6 and H7 of intermediate sensitivity.

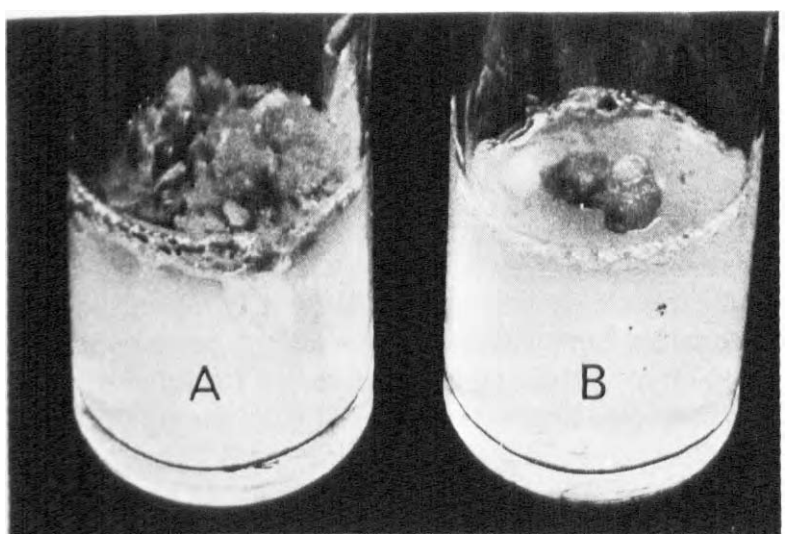

Fig.1. Callus growth from petioles on medium containing 37.5 mg/l PFP. A, Haploid (H7); B, Diploid (D).

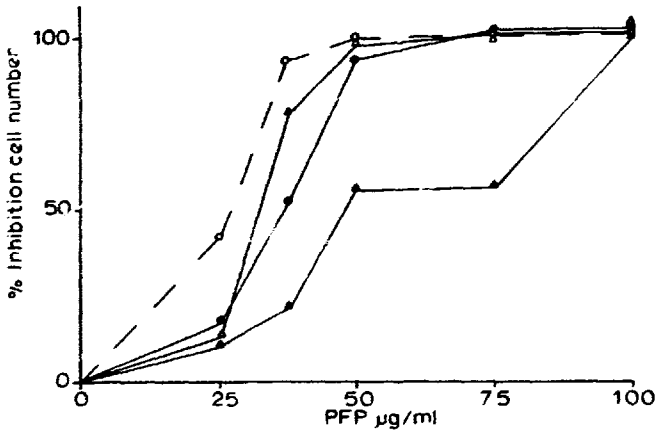

Fig. 2. Percentage inhibition of growth by PFP incorporated into the third suspension passage; control suspensions initiated and grown for three passages in the absence of PFP. Growth was assessed by cell number/ml after 21 days' incubation. Key: Diploid-derived strain D (O); haploid-derived strains H6 (•), H7 (△) and H9 (▲).

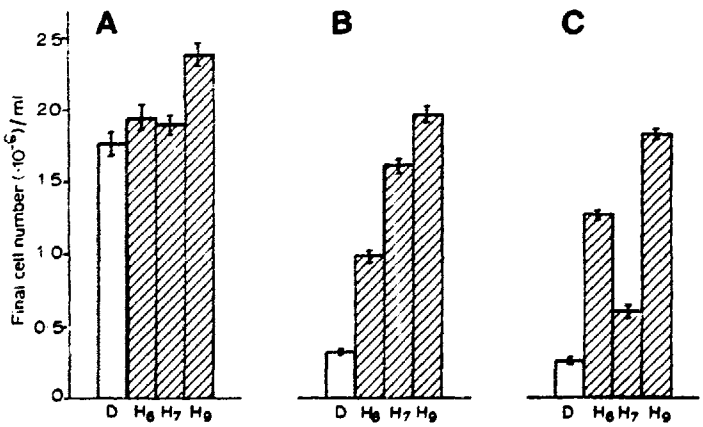

Fig. 3. Values for cell number per ml culture after 21 days' incubation. A, After two suspension passages in the absence of PFP. B, After a passage in 37.5 mg/l PFP following the first suspension passage. C, After a passage in 37.5 mg/l PFP following the second suspension passage. The vertical lines equal twice the standard errors plotted with the means as midpoints.

All the Feulgen staining density profiles obtained by microdensitometry possessed at least three peaks indicating that cells of at least two ploidy levels were present, and that the haploid lines differed in the extent to which they had undergone polyploidisation in culture (this is illustrated for lines D, H7 and H9 in Fig. 4. These profiles were in good agreement with the ploidy distributions obtained by chromosome counts (Table I). When all four of these lines were grown in the presence of PFP no significant changes in ploidy composition occurred (Fig. 4). If haploid cells in the H lines had grown preferentially in the presence of the inhibitory levels of PFP the first two density peaks (corresponding to haploid G1 and haploid G2 + diploid G1 respectively)

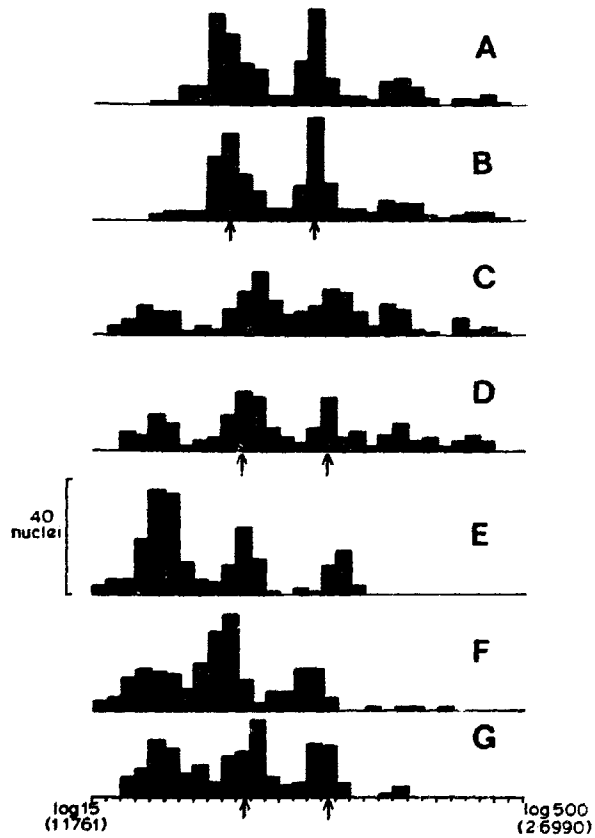

Fig. 4. Microdensitometer profiles for third passage cells grown in the absence and presence of PFP. These suspensions were previously cultured in the absence of PFP. Strain D: A, no PFP; B, 37.5 mg/l PFP*. Strain H7: C, no PFP; D, 37.5 mg/l PFP. Strain H9: E, no PFP; F, 37.5 mg/l PFP; G, 50 mg/l PFP. Arrows indicate diploid G1 and G2 DNA levels for root tip nuclei.

TABLE I

CHROMOSOME COUNTS FOR THE CULTURE LINES D AND H9

Values are percentages of nuclei scored at each ploidy level.

Culture line and culture passage	Chromosome number				
	n	2n	>2n	4n	8n
D, Passage 2	0	62		36	2
H9, Passage 3	64	34	2		
H9, Passage 3 in presence of 37.5 μg/ml PFP	52	36	12		

* This culture was strongly inhibited in growth by PFP.

should have increased at the expense of the higher density peaks. It is also clear that in the presence of PFP (particularly at the higher levels of 50 and 75 μg/ml) diploid cells in the haploid derived lines were able to grow under conditions when all cells of the diploid derived line were inhibited. Whereas, diploid cells in division were observed in the presence of 37.5 μg/ml PFP with the haploid derived line H9 (Table I), no dividing cells could be observed in preparations from culture line D in this medium. The differing but higher resistances of the haploid derived cell lines to PFP appears to reflect differences in their genotype (genetic segregation during pollen mother cell meiosis) and not to be related to their differences in ploidy composition as such. Genotype not ploidy level determines sensitivity to growth inhibition by PFP.

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