Selection for diploid cells in suspension cultures of *Haplopappus gracilis*

CHANGES in the number and structure of chromosomes of plant cells cultured *in vitro* are of common occurrence[1], and cell populations devoid of diploid karyotype have been reported[2-4]. The chromosome number is known to become variable even in clones derived from single cells[5,6]. In the case of *Picea glauca* Voss. ($n = 21$) callus cultures, a positive correlation between growth rate and chromosome number was observed[7], which indicates a selection for polyploid cells. Only diploid cells were, however, observed in mitosis in suspension cultures of *Haplopappus gracilis* (Nutt.) Gray ($n = 2$) (ref. 2), *Medicago sativa* L. ($n = 16$) (ref. 8) and *Crepis capillaris* L. Wallr. ($n = 3$) (ref. 9). This could result from polyploid cells dividing less frequently than diploid cells, as diploid and polyploid cells differ physiologically. A strong selection for diploid cells was observed[10] in *Vicia hajastana* Grossh. ($n = 5$) suspension cultures initiated from mature seeds. It therefore seemed likely that cell cultures of other plant species with a low chromosome number, $n = 7$ or less, would show a selection for diploid cells.

Callus cultures were initiated from hypocotyl segments of 3-d-old seedlings of *H. gracilis* on agar B5 medium[11] containing 4.5×10^{-6} M 2,4-dichlorophenoxyacetic acid (2,4-D) The calli were kept in a culture room at constant temperature (27-28° C) and light (2,000 lx). Subculturing was carried out every 3 weeks. After 122 d in culture, some cells were transferred to liquid B5 medium with 2,4-D at the same concentration. The suspension cultures were subcultured every 3 d, and were agitated by a gyratory shaker at 150 r.p.m. The cytological technique was essentially that of Singh *et al.*[10].

An increase in the frequency of aneuploid cells was observed after 94 d in culture; a drastic decrease subsequently occurred. The frequency of diploid cells increased consistently whereas that of tetraploid cells steadily declined (Table 1). Anaphase analyses revealed a number of anomalies, such as bridges, fragments, unequal distribution of chromatids and laggards in low frequencies (1–3%). Chromosomes with altered morphology were also observed at metaphase. These anomalies could have been induced by 2,4-D, or some other factor, present in the culture medium, and would lead to the production of aneuploid cells from diploid and polyploid cells. Furthermore, endoreduplication is likely to occur in plant cells cultured *in vitro*[12,13]. The increase in the frequency of diploid cells under such a condition, therefore, clearly shows a strong selection for such cells. A similar selection for diploid cells was observed in suspension cultures of *H. ravenii* ($n = 4$), a closely related species, initiated from stem segments[14].

Table 1 Frequencies (%) of different cell types in two suspension cultures (A and B) of *H. gracilis*

Chromosome class	Days in culture				
	7	14	94	175	258
Hypodiploid (<4)					
Culture A	3.0	1.1	5.4	0.8	—
B	2.0	2.1	2.9	0.7	1.4
Mean	2.5	1.6	4.3	0.7	0.7
Diploid ($2n = 4$)					
Culture A	52.0	58.7	60.8	82.4	92.0
B	55.0	55.2	57.1	86.7	93.8
Mean	53.5	56.9	59.2	84.7	92.9
Hyperdiploid (5)					
Culture A	4.0	1.1	13.8	7.6	2.5
B	1.0	1.0	22.9	5.3	1.4
Mean	2.5	1.1	17.9	6.5	1.9
Hypotetraploid (6 and 7)					
Culture A	6.0	9.8	3.1	—	0.6
B	3.0	8.3	3.8	0.7	1.4
Mean	4.5	9.1	3.4	0.4	1.0
Tetraploid ($4n = 8$)					
Culture A	35.0	28.3	13.8	9.2	4.9
B	32.0	29.2	9.5	6.7	2.1
Mean	33.5	28.7	11.9	7.8	3.6
Hypertetraploid (>8<16)					
Culture A	—	1.1	2.3	—	—
B	1.0	2.1	3.8	—	—
Mean	0.5	1.5	3.0	—	—
Octaploid ($8n = 16$)					
Culture A	2.0	—	0.8	—	—
B	4.0	2.1	—	—	—
Mean	3.0	1.1	0.4	—	—
No of cells observed					
Culture A	100	92	130	131	163
B	100	96	105	150	146
Total	200	188	235	281	309

The cytogenetic behaviour of *H. gracilis* cells has been investigated previously[2,15-18]. In some studies, a systematic analysis of the changes in the frequencies of various karyotypes during the *in vitro* culture was not made[2,16,17], but where such an analysis was made[15,18], an increase in the frequency of polyploid cells was observed. Since agar media were used[15,18], these findings do not necessarily contradict the results reported here. An increase in the frequency of polyploid cells was observed when *H. gracilis* cells were cultured on agar B5 medium[19]. Only diploid cells were observed in a suspension culture of *H. gracilis*, which was interpreted to indicate a selection for diploid cells[2].

The distribution of the two chromosomes of *H. gracilis* in the aneuploid cells was non-random; chromosome I was present more often than expected. In fact, almost all of the cells with five chromosomes had an extra chromosome I. A similar non-random distribution of chromosomes has been observed in suspension cultures of *V. hajastana*[10]. Since both chromosome I and II seem to be equally susceptible to mitotic irregularities, it seems that cells possessing an extra chromosome I were at a selective advantage over those possessing an extra chromosome II.

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Lack of permeability of mouse placenta to maternal and foetal cells

IN man, foetal leukocytes circulate in the maternal blood in most pregnancies and are occasionally detected up to 1 yr after delivery[1–5]. On the other hand, maternal white blood corpuscles have seldom been found in the foetal