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# Chromosome aberration and ploidy equilibrium of *Vicia faba* in tissue culture

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**Summary.** Different phytohormone concentrations induced different fequencies of various chromosome aberrations in calli of *Vicia faba*. NAA 10 ppm plus KT 2.5 ppm produced more haploids and NAA 30 ppm plus NAA 7.5 ppm produced more tetraploids and breakage. The relationship among the aberrations was analyzed. The hypothesis of ploidy equilibrium was established. The chromosome doubling rate and reduction rate of each treated group were calculated in relation to the observed data and the hypothesis. The frequency of tetraploids and breakage are correlated with each other. The frequency of total aberrations is linearly correlated with that of micronucleus formation. The regression equation is $x = 31.92 + 10.67\,y$.

**Key words:** *Vicia faba* – Callus – Chromosome aberration – Somatic reduction – Ploidy equilibrium

## Introduction

Chromosome aberrations often occur in plant tissue culture. Study on and control over their type and frequency will not only promote their application to agriculture but may also provide a way of isolating aberrant cell lines which are useful in somatic genetics. Many authors have worked on this (Kunach 1984). Most reports have focused on polyploidy and aneuploidy (Melchers 1965; Orton 1980); only a few concerned somatic reduction. Huskins (1948) demonstrated somatic reduction for the first time. Later, Huskins and Cheng (1950) reported on *Allilium cepa*. The work with cultured cells was first reported by Mitra and Steward (1961), while D'Amato (1980) described the process in cultured cells and discussed this problem. A lot of work (Venkeleswaran 1963; Yamane 1975; Cionini 1978; Hesemann 1980) was done with *Vicia faba* owing to its large chromosomes and small chromosome complement, but none of the publications above involved the relationship among different aberrations. Shen et al. (1985) reported their work with the root tip of *Vicia faba* and discovered the linear correlation of aberration frequency with micronucleus frequency. Similar work in tissue culture has not yet been reported.

## Materials and methods

Calli induced from the attachment point of cotyledons of *Vicia faba* were subcultured in MS medium with NAA 2 ppm and KT 5 ppm for 4 weeks and then transferred into the media containing different combinations of NAA and KT (Table 1). After a 4 day culture, the calli were pretreated with 0.1% colchicine for 2 h, fixed with carnoy's fluid for 24 h, hydrolyzed with 1 N HCl for 3 min and stained with carbal fuchsine for 20 min. The preparations were made by squashing. Both mitotic cells and interphase cells were examined.

## Results

The aberrations observed are haploid, tetraploid, breakage and micronucleus formation (Fig. 1 a–d). Karyotype analysis shows that the haploid has one complete chromosome set (Fig. 1 e). Aberrations occurred in all the media used (Table 1). Medium 1 is the control group which has no phytohormone at all, so its aberrations can be considered produced by callus induction and subculture. The highest frequency of haploids is 13.8% which is caused by Medium 5 (NAA

**Table 1.** Effects of phytohormone on chromosome aberration

| Medium no. | Hormone concentration (ppm) | | No. of observed cell | Normal cell | | Aberrant cell | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Haploid | | Tetraploid | | Breakage | | Others | | Total | |
| | NAA | KT | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| 1 | 0 | 0 | 110 | 56 | 50.9 | 7 | 6.4 | 25 | 22.7 | 0 | 0 | 22 | 20.0 | 54 | 49.1 |
| 2 | 1 | 0.25 | 137 | 64 | 46.7 | 8 | 5.8 | 33 | 24.1 | 1 | 0.73 | 31 | 22.6 | 73 | 53.3 |
| 3 | 2 | 0.5 | 275 | 142 | 51.6 | 20 | 7.3 | 65 | 23.6 | 9 | 3.3 | 39 | 14.2 | 133 | 48.4 |
| 4 | 5 | 1.25 | 186 | 80 | 43.0 | 15 | 8.1 | 48 | 25.8 | 4 | 2.2 | 39 | 21.0 | 106 | 57.0 |
| 5 | 10 | 2.5 | 240 | 97 | 40.4 | 33 | 13.8 | 64 | 26.7 | 6 | 2.5 | 40 | 16.7 | 143 | 59.6 |
| 6 | 20 | 5 | 222 | 88 | 39.6 | 21 | 9.5 | 60 | 27.0 | 20 | 9.0 | 33 | 14.9 | 134 | 60.4 |
| 7 | 30 | 7.5 | 118 | 9 | 7.6 | 4 | 3.4 | 91 | 77.1 | 11 | 9.3 | 3 | 2.5 | 109 | 92.4 |
| 8 | 40 | 10 | 253 | 58 | 22.9 | 9 | 3.6 | 133 | 52.6 | 19 | 7.5 | 34 | 13.4 | 195 | 77.1 |
| 9 | 50 | 12.5 | 104 | 38 | 36.5 | 3 | 2.9 | 28 | 26.9 | 13 | 12.5 | 22 | 21.2 | 66 | 63.5 |
| 10 | 100 | 25 | 179 | 77 | 43.0 | 2 | 1.1 | 47 | 26.3 | 20 | 11.2 | 33 | 18.4 | 102 | 57.0 |

**Table 2.** Effects of phytohormone on micronucleus formation and the correlated frequency of chromosome breakage

| Medium no. | Phytohormone concentration (ppm) | | Interphase cells | Micronucleus frequency | | Corrected frequency of breakage |
|---|---|---|---|---|---|---|
| | NAA | KT | | No. | % | |
| 1 | 0 | 0 | 641 | 11 | 1.72 | 0 |
| 2 | 1 | 0.25 | 1346 | 23 | 1.71 | 0.96 |
| 3 | 2 | 0.5 | 1033 | 20 | 1.94 | 4.29 |
| 4 | 5 | 1.25 | 979 | 30 | 2.04 | 2.90 |
| 5 | 10 | 2.5 | 1232 | 45 | 3.65 | 3.41 |
| 6 | 20 | 5 | 1133 | 33 | 2.91 | 2.47 |
| 7 | 30 | 7.5 | 1391 | 70 | 5.03 | 40.7 |
| 8 | 40 | 10 | 1023 | 45 | 4.40 | 15.8 |
| 9 | 50 | 12.5 | 1258 | 28 | 2.23 | 17.1 |
| 10 | 100 | 25 | 1110 | 26 | 2.34 | 15.2 |

10 ppm, KT 2.5 ppm) and that of tetraploid is 77.1% which is caused by Medium 7 (NAA 30 ppm, KT 7.5 ppm). Superficially, the frequency of breakage found in Medium 9 (NAA 50 ppm, KT 12.5 ppm) is the highest but it needs to be corrected because the frequencies of breakage were calculated without taking in the breakage of tetraploids. The corrected frequencies are given in Table 2 and were obtained from the following formula:

$$\text{Corrected frequency} = \frac{\text{Breakage}}{\text{Total} - \text{tetraploid}}.$$

The true highest frequency of breakage is caused by Medium 7 according to the data in Table 2.

Figure 2 shows the relationships between haploids, tetraploids and breakage. The changing pattern of tetraploid frequency with phytohormone concentration is somewhat like that of breakage frequency but we have no knowledge about this relationship. The frequency of haploids is correlated with that of tetraploids. The tetraploid frequency of Medium 7 is the highest while its haploid frequency is very low and the haploid frequency of Medium 5 is the highest while its tetraploid frequency is relatively low. This correlation, however, is not a linear one. In order to reveal the relationship between haploids and tetraploids, another experiment was designed in which the material was not pretreated with colchicine before the preparations were made. The data is given in Table 3. In the second experiment there were two kinds of haploid metaphase and two kinds of tetraploid metaphase. One was normal (Fig. 1 f, g) and the other was undergoing chromosome doubling (Fig. 1 h, i). Thus the chromosome doubling rate of both ploidy in the present cell cycle can be calculated as

$$\text{Doubling rate of haploid, } K_d = \frac{\text{Doubling haploid}}{\text{Total haploid}}$$

$$\text{Doubling rate of diploid, } K_h = \frac{\text{Doubling diploid}}{\text{Total diploid}}.$$

The data in Table 4 shows that the $K_d$ and $K_h$ of each medium are almost the same. This has been confirmed by the *t*-test. The difference between all pairs of K were not significant statistically. Indeed, we have no reason to think that haploids or diploids can more easily double their chromosomes in the same cell population than the other, according to the endomitosis theory. This holds that chromosome doubling is mainly due to the separation of sister chromotids without the formation of a functional spindle (Sunderland 1976).

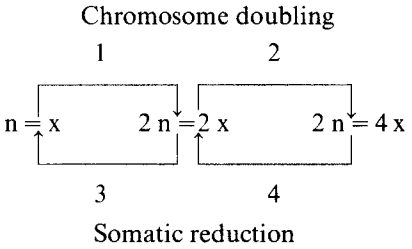
The occurrence of haploids in diploid culture implies that there was somatic reduction in the population. Four different mechanisms of somatic reduction were reported: nucleus fragmentation followed by mitosis (D'Amato 1980), the segregation of homologous chromosomes (Huskins and Cheng 1950), multipolar

**Table 3.** Effects of phytohormone on chromosome aberration

| Medium no. | Hormone concentration (ppm) | | Total cells observed | Diploid | | | | Haploid | | | | Tetraploid | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NAA | KT | | Normal | Doubling | Sum | % | Normal | Doubling | Sum | % | No. | % |
| 1 | 0 | 0 | 1270 | 854 | 57 | 911 | 71.7 | 41 | 3 | 44 | 3.46 | 315 | 24.8 |
| 2 | 1 | 0.25 | 577 | 401 | 7 | 408 | 70.7 | 18 | 0 | 18 | 3.12 | 151 | 26.2 |
| 3 | 2 | 0.5 | 996 | 681 | 10 | 691 | 69.4 | 45 | 1 | 46 | 4.62 | 296 | 26.0 |
| 4 | 5 | 1.25 | 1030 | 673 | 23 | 696 | 67.6 | 49 | 2 | 51 | 4.95 | 283 | 27.5 |
| 5 | 10 | 2.5 | 843 | 488 | 27 | 515 | 61.1 | 83 | 4 | 87 | 10.3 | 241 | 28.6 |
| 6 | 20 | 5 | 1123 | 683 | 38 | 721 | 64.2 | 71 | 4 | 75 | 6.68 | 327 | 29.1 |
| 7 | 30 | 7.5 | 1359 | 137 | 186 | 323 | 23.8 | 18 | 25 | 43 | 3.16 | 993 | 73.1 |
| 8 | 40 | 10 | 796 | 161 | 151 | 312 | 39.2 | 17 | 12 | 29 | 3.64 | 455 | 57.2 |
| 9 | 50 | 12.5 | 1008 | 665 | 42 | 707 | 70.1 | 7 | 0 | 7 | 0.69 | 294 | 29.2 |
| 10 | 100 | 25 | 751 | 509 | 24 | 553 | 70.9 | 6 | 0 | 6 | 0.80 | 212 | 28.2 |

spindle (Sunderland 1976) and somatic pairing (Mitra and Steward 1961). Nucleus fragmentation was observed in the second experiment (Fig. 1j). This seems, however, not to be the sole mechanism or at least not to be the main mechanism as the haploid frequency is too high to be a result of nucleus fragmentation.

The equation of ploidy equilibrium can be deduced from the above observation, calculation and analysis. The observed frequencies of haploid, diploid and tetraploid resulted from the equilibrium of four processes – the changes of haploid into diploid, diploid into tetraploid, diploid into haploid and tetraploid into diploid:




The last process can be neglected as it was so rare as not to be seen in 1000s of cells, though it would occur theoretically.

Let $h_0$, $d_0$ and $p_0$ stand for the frequencies of haploids, diploid and tetraploids in the control group; $h_n$, $d_n$ and $p_n$ for those in the treated group. Then the doubling rate of diploid, $K'_d$ can be calculated in the following way:

$$K'_d = \frac{p_n - p_0}{d_0}.$$

The $K'_d$ values of both experiments are given in Table 3. Apparently, there is no statistically significant differences between $K'_d$ and $K_d$ in both experiments.

As the increased frequency of haploids, $\Delta h = h_n - h_0$, is affected by Processes 1 and 3, it can be resolved into two parts: the reduction part, $\Delta h_r$, and the doubling part, $\Delta h_d$, i.e. $\Delta h = \Delta h_r - \Delta h_d$.

$$\because \quad K'_d = K_d = K_h$$

$$\therefore \quad \Delta h_d = K'_d \cdot h_0$$

$$\therefore \quad h_n = h_0 + \Delta h$$

$$= h_0 + \Delta h_r - h_0 \frac{p_n - p_0}{d_0}$$

$$\Delta h_r = h_n - h_0 + h_0 \frac{p_n - p_0}{d_0}.$$

Finally, the somatic reduction rate is

$$R = \frac{\Delta h_r}{d_0}.$$

The R values of the two experiments are presented in Table 3. Comparison of this data indicated that the reduction rates of most medium are stable in the two experiments. Differences between the R values of Experiment 1 and those of Experiment 2 are not significant statistically. Similarly, the doubling rate, $K'_d$ is also stable. Furthermore, $K'_d$ almost equals $K_d$ and $K_h$ in the second experiment. Thus we can not refuse the hypothesis of ploidy equilibrium.

The interphase micronucleus was also observed and counted in the first experiment. The process of its formation is just like that in noncultured cells (Fang 1981), i.e. it originated from chromosome breakage (Fig. 1k). The frequencies are given in Table 2. Figure 3 shows the relation between the frequency of total aberrations and that of the micronucleus. It is clear that there is a positive linear correlation between them. The regression equation is: $x = 31.92 + 10.67\,y$

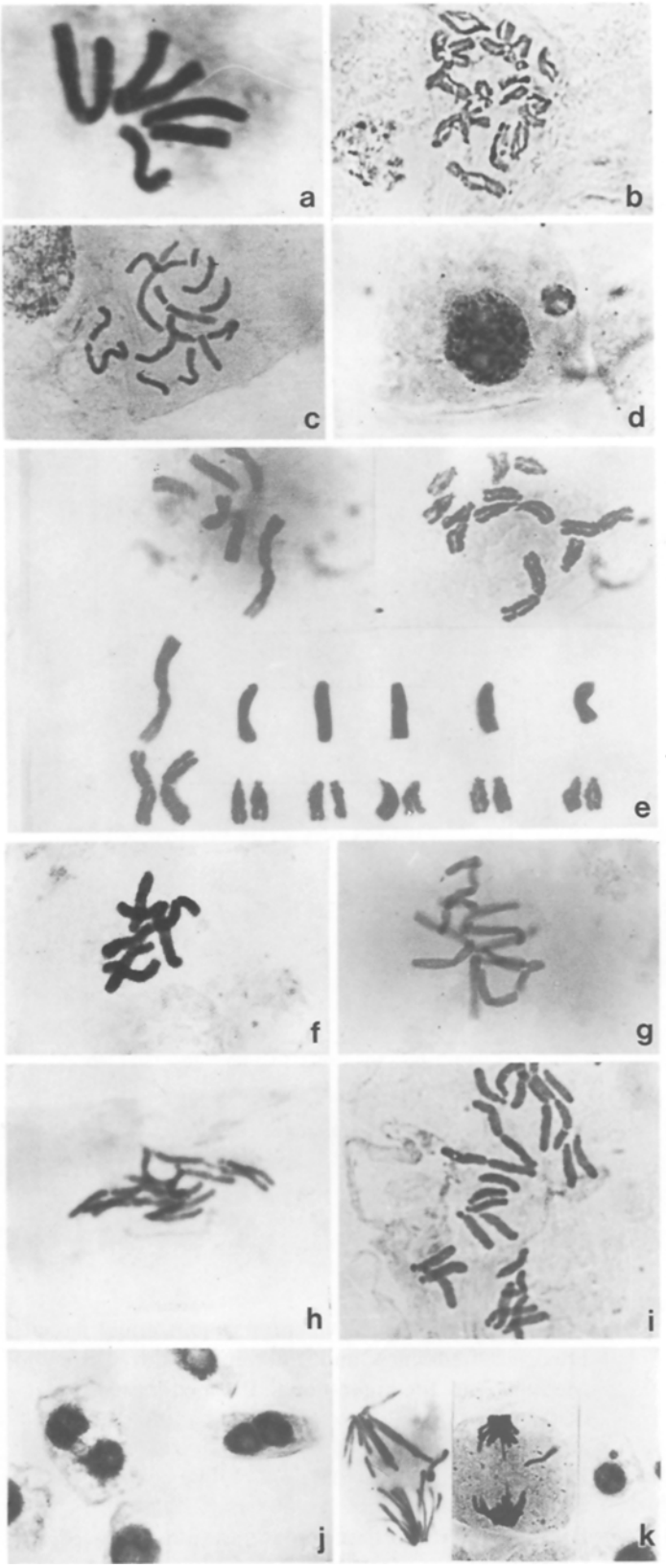



**Fig. 1.** **a** Haploid metaphase; **b** tetraploid metaphase; **c** chromosome breakage; **d** micronucleus; **e** karyotype of haploid and diploid; **f** normal haploid (without colchicine pretreatment); **g** normal diploid (without colchicine pretreatment); **h** chromosome doubling of haploid (without colchicine pretreatment); **i** chromosome doubling of diploid (without colchicine pretreatment); **j** nucleus fragmentation; **k** formation of micronucleus

**Table 4.** Comparison of somatic reduction rate and chromosome doubling rate in the two experiments

| Medium no. | Experiment 1 | | Experiment 2 | | | |
|---|---|---|---|---|---|---|
| | Reduction rate R (%) | Doubling rate $K'_d$ (%) | Reduction rate R | Doubling rate $K'_d$ (%) | Doubling rate of haploid $K_h$ (%) | Doubling rate of diploid $K_d$ (%) |
| 1 | | | | | 6.82 | 6.26 |
| 2 | −0.367 | 2.12 | −0.272 | 1.95 | 0 | 1.72 |
| 3 | 1.83 | 1.55 | 1.70 | 1.67 | 2.17 | 1.45 |
| 4 | 2.48 | 4.09 | 2.30 | 3.63 | 3.92 | 3.33 |
| 5 | 10.8 | 5.64 | 9.88 | 5.30 | 4.60 | 5.24 |
| 6 | 5.02 | 5.92 | 4.79 | 6.00 | 5.33 | 5.27 |
| 7 | 2.69 | 76.7 | 2.83 | 67.4 | 58.1 | 57.6 |
| 8 | 2.48 | 42.0 | 2.43 | 45.2 | 41.4 | 48.4 |
| 9 | 4.40 | 5.92 | −3.57 | 6.14 | 0 | 5.94 |
| 10 | 6.49 | 5.08 | −3.49 | 4.74 | 0 | 4.34 |

R: Reduction rate; $R = \frac{\Delta h_r}{d_0}$: see text. $K_d, K_h$: Chromosome doubling rate of diploid and haploid: $K_d = \frac{\text{Doubling diploid}}{\text{Total diploid}}$, $K_h = \frac{\text{Doubling haploid}}{\text{Total haploid}}$. $K'_d$: Chromosome doubling rate; $K'_d = \frac{p_n - p_0}{d_0}$; see text

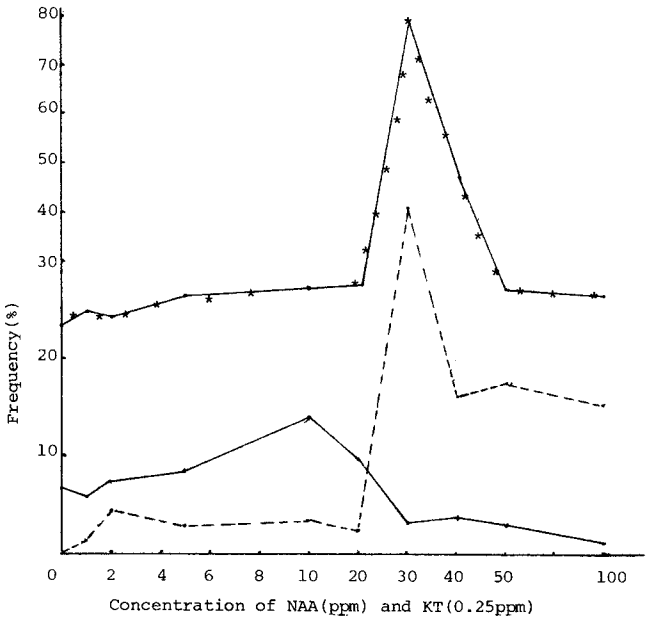



**Fig. 2.** Relationship among haploid, tetraploid and breakage frequencies. —— haploid; - - - breakage; −∗− tetraploid

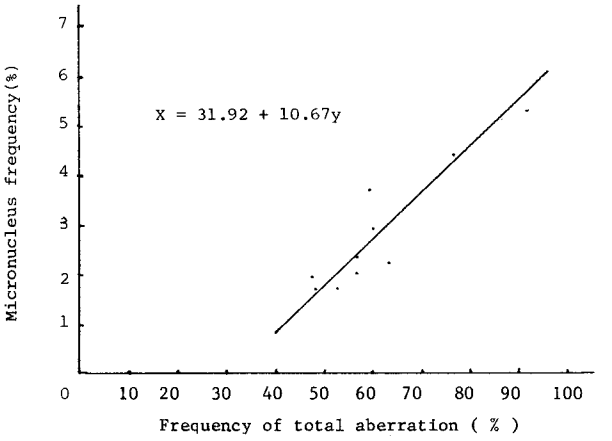



**Fig. 3.** Regression of micronucleus formation and total aberration

while x represents the frequency of total aberrations and y represents that of micronucleus formation. This result is similar to that from noncultured cells (Shen et al. 1985).

In conclusion, the haploid frequency and somatic reduction rate of Medium 5 are the highest ones and the tetraploid frequency, chromosome doubling rate, breakage frequency and micronucleus frequency of Medium 7 are the highest of all the media used.

## Discussion

Different phytohormone concentrations induced different frequencies of various chromosome aberrations. NAA 10 ppm plus KT 2.5 ppm, especially, induced more haploids and NAA 30 ppm plus KT 7.5 ppm in-

duced more tetraploids. This result implies that for a callus composed of more haploids or tetraploids, one can screen the culture conditions. Using also the single-cell cloning technique, aberrant cell lines may be obtained. The mechanism of the effect of phytohormone, however, needs more effort. The hypothesis of ploidy equilibrium provides a mathematical method for such screening and for analysing the relationship between haploids and tetraploids, although the hypothesis needs more support.

The frequency of micronuclei is much easier to count in the interphase than those of other aberrations in metaphase or anaphase. Since the frequency of total aberration is linearly correlated with that of the micronucleus, the latter can be used as an indicator for detecting the former. The conditions in which micronucleus frequency is lower will cause fewer aberrations and vice versa. This indicator may be useful in the study of chromosome stability in plant tissue culture.

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