Chromosomal Behaviour in Cultures of Vicia faba

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Vicia faba L. a classical experimental plant with a pair of marker chromosomes has been a suitable material for cytogenetical investigations from a long back. Earlier workers relied on undefined nutrient media for initiation of callus masses which was supplemented with yeast extract and coconut milk (Venkateswari 1962). Grant and Fuller (1968) reported less than 1% success in establishing culture with this type of media. Mitchell and Gildow (1975) suggested Schenk and Hildebrandt's defined media and in our investigation best result was obtained in this media. Regarding chromosomal behaviour in the tissue grown in vitro a little amount of work has been done. It is well documented that during the culture of many plant species cells with variously altered chromosome complements appeared particularly in culture lines. Plant tissue culture has been done generally with a view to regenerate normal plant from the cultured cells. This regeneration has some relationship with the chromosome behaviour during culture. The stability of chromosome is thus a prerequisite step in the differentiation or regeneration of plants. The present investigation has been undertaken to initiate callus tissues to study the changes in chromosome number and structure progressively from the time of initiation to one year old cultures.

Materials and methods

Seeds of Vicia faba L. early long pod of Suttons were surface sterilized with 0.1% HgCl₂ for 20 minutes, washed thoroughly with sterile distilled water and incubated aseptically in sterile water for overnight. Excess water was removed and seeds left in dark for 6-8 hours for maximum growth of the embryo. Seed coats and cotyledons were removed aseptically and embryo taken, dipped in absolute alcohol. The hypocotyl segments of the embryo (3-5 mm long) incubated in different media. The nutrient media tested for initiation of callus were Murashige and Skoog (MS), Gamborg (B₅), Schenk and Hildebrandt (SH), pH in each case was adjusted to 5.5 before autoclaving. For cytological study, the tissue was fixed in Carnoy's solution for overnight at 4-8°C. The small pieces of tissues were stained with 2% 9:1 orcein: (N) HCl mixture for 2-3 hours and squashed in 45% acetic acid.

Observations

After many trials in different media success in initiating growth of callus of Vicia faba was obtained in mod. B₅ and mod. M. SH. The best result was obtained
Figs. 1–6. 1, in vitro germinated seedling of *Vicia faba* showing "blackening" of epicotyl region. 2, a normal diploid cells with 12 chromosomes from 6 weeks old culture. 3, an aneuploid metaphase plate having 14 chromosomes showing a structurally altered chromosomes. 4, 36 weeks old culture showing one fragment. $2n = >18 + 1f$. 5, 54 weeks old culure showing three fragments. $2n = 48 + 3f's$. 6, an octaploid cell from 30 weeks old culture showing three altered chromosomes. $2n = 48$. 
in SH media when it was supplemented with 5 mg/lit nicotinic acid, 5 mg/lit pyridoxin HCl, 10 mg/lit thiamine HCl, 1 gm/lit of mesoinostol, riboflavin .2 mg/lit, calcium pantothenate 1 mg/lit, aminobenzoic acid .02 mg/lit, biotin .01 mg/lit, 24D-.5 mg/lit, KI-.1 mg/lit. In Vicia faba one barrier in maintaining cultures is the blackening of tissues. In the field also, the blackening of shoots occur after growth of young seedlings and the plant ultimately dies (Fig. 1). After many trials this has been overcome but the cause is yet to be determined. Proliferation of tissues starts within 10 days of inoculation and is better in dark light at 22–25°C with a maximum humidity of 55%. Friable creamish white tissues were produced within one month of culture. Callus was maintained by subculturing in the same medium at an interval of 6 weeks. The concentration and combination of hormones were changed from 24D-.5 mg/lit and Kinetin .1 mg/lit to 24D-.25 mg/lit, NAA-1 mg/lit and KI-.1 mg/lit for maintaining good growth as well as for reducing the effect of 24D on chromosomes.

For all cytological experimentation cultures were fixed within 7 days after inoculation when growth is in the exponential phase. Cytological studies were

Figs. 7-10. 7, a clumped metaphase plate showing a single structurally altered chromosome from 36 weeks old culture. 2n=>12<18. 8, an octaploid cell from 54 weeks old culture tissue having two altered chromosomes. 2n=48. 9, a well-scattered tetraploid metaphase plate from 24 weeks. 2n=24. 10, an octaploid cell from 24 weeks old cultures. 2n=48.
made in cultures starting from its initiation to 54 weeks old cultures. From 18 weeks onward most of the nuclei remain in an active state of division. Most of the cells show normal chromosome number with 2n=12 from the callus tissue of 6 and 12 weeks (Fig. 2). The normal *Vicia faba* has the chromosome number 2n=12 with one pair of long chromosomes having secondary constriction sometimes called ‘M’ chromosome and 5 pairs of short and subterminal chromosomes. The chromosomes in the metaphase stage from the cells in culture in some cases appeared much condensed and scattered like the cells undergoing treatments with pretreating agents. Chromosomal preparations from 18 weeks onward showed different levels of ploidy in all the passages but the ratio of diploid and polyploid cells varied. In 6 and 12 weeks old cultures aneuploid condition closer to diploid (Fig. 3) was found. Laggards anaphase bridge, binucleate condition, multipolarity were not found in *Vicia faba* culture tissues. However, the fragments were occasionally found in older cultures (Figs. 4 and 5). The interesting feature in the present investigation is that the new chromosome types i.e., submedian chromosomes were noted from the initial passage to 1 year old callus tissues (Figs. 3, 6, 7, 8). This number varies from 1 to 3 in each cell. It has already been mentioned that in normal *Vicia faba* cells there were no chromosomes with submedian constriction. The range of variation in chromosome number (Figs. 2–10) and the percentage of diploid and polyploid cells observed are represented in a tabular form.

**Table 1**

<table>
<thead>
<tr>
<th>Age of the callus in week</th>
<th>No. of metaphase</th>
<th>Diploid number</th>
<th>More than diploid number</th>
<th>Percentage of diploid</th>
<th>Percentage of more than diploid, aneuploid to octaploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>15</td>
<td>15</td>
<td>—</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>18</td>
<td>13</td>
<td>5</td>
<td>72.2</td>
<td>27.8</td>
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<tr>
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<td>16</td>
<td>12</td>
<td>4</td>
<td>80</td>
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<td>7</td>
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<td>25</td>
<td>51.9</td>
<td>48.1</td>
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<tr>
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<td>47</td>
<td>24</td>
<td>23</td>
<td>51.1</td>
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</tbody>
</table>

**Discussion**

Chromosomal stability is an essential prerequisite for maintenance of genetically defined material and specially to those involving plant regeneration. But most of the plant species in culture exhibit chromosomal instability both structurally and numerically. Venkateswaran (1963) demonstrated that *Vicia faba* liquid cultured cells display a diversity of cytological condition and nuclear behaviour including anaphasic and telophasic bridge, laggards, rearrangements, structural changes by breakage and reunion of chromosome and changes in levels of ploidy. Drazena *et al.* (1978) demonstrated the presence of an extra chromosome and various structural changes in *Vicia faba* with the help of Giemsa C-banding technique.

In our observation also numerically and structurally changed chromosomes
are noted but not the other types of abnormalities. The abnormalities were due to the influence of culture medium where various types of complex growth substances were present (Venkateswaran 1963, Torrey 1959). In old cultures different workers have noted a high variation in chromosome number which are highly aneuploid. Similar aneuploid number is also found in our present findings which may be due to the abnormalities of the mitotic spindle under cultural conditions. From the very beginning of the culture we noted a new type of chromosome complement with submedian constriction and it was present almost in all passages. The origin of this new type of chromosome might not be due to the result of union between the long chromosome pair but can be accounted for by breaks in a chromosome and their structural rearrangements. Similar types of structural changes in chromosomes were reported by Therman (1956) in crown gall tissue of *Vicia faba*. Again these types of variation were found following irradiation with X-ray and other chemical treatments. It is possible that some component in the culture medium have induced some break in chromosomes and then different types of reunions between the chromosome fragments lead to new chromosome complements in the cells. The persistence of new chromosome complements in the cultured cells may lead to the establishment of a new cell line in culture which will help as a marker in any fundamental studies.

**Summary**

Culture tissues were established from hypocotyl segments of *Vicia faba*. Chromosomal analysis from initiation to 1 year old culture was done. Only numerically and structural variation in chromosomes were found without any spindle disturbances. Another significant feature was the occurrence of new chromosome complement i.e. submedian type in this culture.

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**References**


