Cytogenetic Effect of Plant Tissue Culture Medium with Certain Growth Substances on *Allium sativum* L. Meristem Root Tip Cells

**J. DOLEŽEL and F. J. NOVÁK**

Institute of Experimental Botany, Czechoslovak Academy of Sciences

**Abstract.** The effect of plant tissue culture medium with different concentrations and combinations of growth regulators (kinetin, indol-3-ylacetic acid, 2,4-dichlorophenoxyacetic acid) was evaluated on mitosis of *Allium sativum* meristem root tip cells. Different combinations of growth regulators at low concentrations had no effect on induction of mitotic aberrations or inhibition of mitotic activity. Inhibition of mitotic activity, a tendency to chromosome stickiness and clumping and a slight increase in the frequency of mitotic aberrations were observed at higher concentrations. It may be proposed that plant tissue culture media have no direct effect on induction of mitotic aberrations in plant tissue cultures *in vitro*.

Continuing progress in plant cell, tissue and organ culture technologies is making them more and more useful in the improvement and commercial production of plants as well as in research. Genetic variability has been reported for most genera cultured *in vitro* (SKIRVIN 1978, NOVÁK 1978). This may be useful in plant improvement (LARKIN and SCOWCROFT 1981, NOVÁK 1981) but at the same time it seems impossible to use dedifferentiated culture for clonal propagation, genome preservation and mutation induction (CHALEFF 1981). The variability in chromosome number (D'AMATO 1977, 1978, BAYLISS 1980, CONSTANTIN 1981) has been most extensively studied in the *in vitro* cultures though its causes remain obscure. Some authors suggest that certain plant growth regulators, especially 2,4-dichlorophenoxyacetic acid, may induce mitotic aberrations, endomitosis or endoreduplication in cultivated cells (SHAMINA 1966, GHOSH and GADGIL 1979, CHEN and CHEN 1980). However, BAYLISS (1980) has recently suggested that it disorganizes growth of callus cultures, and has not found any direct effect of plant growth regulators that leads to mitotic aberrations.

The aim of the present study was to study the effect of plant tissue culture medium with different combinations and concentrations of growth regulators on induction of mitotic aberrations in meristem root tip cells of *Allium sativum*. The same system was tested at the level of garlic callus tissue (DOLEŽEL and NOVÁK 1984a) and *Tradescantia* stamen hairs to check frequency of somatic mutations (DOLEŽEL and NOVÁK 1984b).

Received October 10, 1983; accepted January 30, 1984

* Address: Sokolovská 6, CS-772 00 Olomouc, Czechoslovakia.
MATERIAL AND METHODS

Bulbils of *Allium sativum* cv. "Bzenecký paličák" of the uniform size and age were used for the present cytological studies. The bulbils were allowed to germinate in moist Perlite. The bulbils were ready to transfer to 400 ml beakers with test solutions after the roots had reached the length of about 1.5–2.5 cm. Before transfer the shoots and out-coatings of the bulbils were removed. The bulbils were then placed on the top of the beakers by means of carriers (10 bulbils per treatment) so that only the roots were immersed. The treatment took place in dark at 22 °C. Test solutions were continuously aerated and changed daily. For preparation of slides the commonly used Feulgen squash technique was used without any pretreatment. One root tip was taken from each of 10 bulbils per variant.

In the first part of the experiment the frequency of anaphase aberrations was evaluated. Roots were cultivated for 24 h in plant tissue culture medium BDS (Dunstan and Short 1977) without agar and sucrose; the hormonal composition was as follows: (i) BDS without growth regulators, (ii) BDS + + 5.0 μM 2,4-D, (iii) BDS + 5.0 μM KIN, (iv) BDS + 5.0 μM IAA, (v) BDS + 5.0 μM 2,4-D + 5.0 μM KIN, (vi) BDS + 5.0 μM 2,4-D + 10.0 μM KIN + 10.0 μM IAA, (vii) control — distilled water. In all cases the pH of the media was adjusted to 5.5. After 24 h treatment the roots were washed in distilled water and the carriers with bulbils were transferred to the beakers with Hoagland's nutrient solutions. The roots were fixed after 8, 24 and 48 h of the culture.

The second part of this work was centered on the effect of continuous cultivation in the medium BDS without agar and sucrose with different concentrations of 2,4-D, KIN and IAA on the frequency of anaphase aberrations, mitotic index and duration of metaphase stages. Roots were continuously cultured under the standard conditions in the following solutions: (i) BDS without growth regulators, (ii) BDS + 5.0 μM 2,4-D, (iii) BDS + 50.0 μM 2,4-D, (iv) BDS + 500.0 μM 2,4-D, (v) BDS + 5.0 μM KIN, (vi) BDS + + 16.0 μM KIN, (vii) BDS + 50.0 μM KIN, (viii) BDS + 5.0 μM IAA, (ix) BDS + 50.0 μM IAA, (x) BDS + 500.0 μM IAA. In all cases pH of the media was adjusted to 5.5. Roots were fixed after 6, 12, 24 and 48 h of the culture.

In both parts of this work counts were made of the frequency of anaphase aberrations (acentric fragments, chromosome bridges, side-arm bridges, lagging chromosomes, multipolar spindles) and mitotic indices using 500 and 2000 cells, respectively, as recommended by Grant (1982).

RESULTS

The frequencies of aberrant anaphases in meristem root tip cells after 24-h culture in the medium BDS with different hormonal composition are summarized in Table 1. Mean frequency [%] of aberrant anaphases in the control was 1.37 ± 0.28 during the whole experiment. There were statistically non-significant differences (at P = 0.50) in the frequencies of aberrant anaphases between all variants tested and the control. Consequently, under the

Abbreviations used: 2,4-D = 2,4-dichlorophenoxyacetic acid; IAA = indol-3-ylacetic acid; KIN = kinetin.
TABLE 1
Mean frequencies [%] of aberrant anaphases in meristem root tip cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Recovery time</th>
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<tbody>
<tr>
<td></td>
<td>4 h (x ± S. E.)</td>
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<tr>
<td>BDS</td>
<td>1.22 ± 0.49</td>
</tr>
<tr>
<td>BDS + 5.0 μM 2,4-D</td>
<td>1.56 ± 0.58</td>
</tr>
<tr>
<td>BDS + 5.0 μM KIN</td>
<td>1.37 ± 0.57</td>
</tr>
<tr>
<td>BDS + 5.0 μM IAA</td>
<td>1.17 ± 0.42</td>
</tr>
<tr>
<td>BDS + 5.0 μM 2,4-D</td>
<td>1.33 ± 0.53</td>
</tr>
<tr>
<td>BDS - 5.0 μM KIN</td>
<td>1.37 ± 0.57</td>
</tr>
<tr>
<td>BDS - 5.0 μM 2,4-D</td>
<td>1.33 ± 0.53</td>
</tr>
<tr>
<td>BDS + 10.0 μM KIN</td>
<td>1.46 ± 0.45</td>
</tr>
<tr>
<td>BDS + 10.0 μM IAA</td>
<td>1.64 ± 0.61</td>
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</table>

conditions described, the culture medium BDS either without or with growth regulators as used in plant tissue culture has no effect on the induction of anaphase aberrations in meristem root tip cells of *Allium sativum*.

Fig. 1 shows the effect of different concentrations of growth regulators (2,4-D, KIN, IAA) in the medium BDS on mitotic activity during continual cultivation. While 2,4-D at 5.0 μM had no profound effect, the level 50.0 μM strongly inhibited mitotic activity, the inhibition being even more pro-

![Fig. 1](image-url)

**Fig. 1.** Mitotic index evolution (in % of control) of *A. sativum* root meristems in the presence of plant tissue culture medium BDS with 2,4-D, IAA or KIN in different concentrations.

![Fig. 2](image-url)

**Fig. 2.** Frequency of metaphase stages (in % of control) in *A. sativum* root meristems in the presence of plant tissue culture medium BDS with 2,4-D, IAA or KIN in different concentrations. Evaluation of the frequency of metaphase stages in the presence of 500 μM 2,4-D or 500 μM IAA was impossible due to a low mitotic index.
### Table 2

Mean frequencies [%] of aberrant anaphases in meristem root tip cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of treatment</th>
<th>6 h (X ± S.E.)</th>
<th>12 h (X ± S.E.)</th>
<th>24 h (X ± S.E.)</th>
<th>48 h (X ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDS + 5.0 μM 2,4-D</td>
<td>1.47 ± 0.44</td>
<td>1.55 ± 0.63</td>
<td>1.02 ± 0.24</td>
<td>1.46 ± 0.63</td>
<td></td>
</tr>
<tr>
<td>BDS + 50.0 μM 2,4-D</td>
<td>1.06 ± 0.47</td>
<td>1.67 ± 0.63</td>
<td>1.26 ± 0.49</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>BDS + 500.0 μM 2,4-D</td>
<td>2.02 ± 0.93</td>
<td>-</td>
<td>-</td>
<td>1.46 ± 0.63</td>
<td></td>
</tr>
<tr>
<td>BDS + 50.0 μM IAA</td>
<td>1.37 ± 0.58</td>
<td>1.31 ± 0.31</td>
<td>1.49 ± 0.63</td>
<td>1.69 ± 0.52</td>
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<tr>
<td>BDS + 500.0 μM IAA</td>
<td>1.18 ± 0.56</td>
<td>1.37 ± 0.50</td>
<td>1.48 ± 0.28</td>
<td>1.66 ± 0.54</td>
<td></td>
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<tr>
<td>BDS + 5000.0 μM IAA</td>
<td>1.05 ± 0.46</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
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<tr>
<td>BDS + 5.0 μM KIN</td>
<td>1.39 ± 0.55</td>
<td>1.51 ± 0.62</td>
<td>1.22 ± 0.65</td>
<td>1.57 ± 0.71</td>
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<tr>
<td>BDS + 16.0 μM KIN</td>
<td>1.43 ± 0.58</td>
<td>1.02 ± 0.39</td>
<td>1.65 ± 0.27</td>
<td>1.31 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>BDS + 500.0 μM KIN</td>
<td>1.70 ± 0.69</td>
<td>1.18 ± 0.44</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Control (BDS)</td>
<td>1.22 ± 0.82</td>
<td>1.09 ± 0.35</td>
<td>1.58 ± 0.42</td>
<td>1.46 ± 0.45</td>
<td></td>
</tr>
</tbody>
</table>

* Evaluation of anaphases was impossible due to a low mitotic index.
** Evaluation of anaphases was impossible due to high tendency to chromosome stickiness and clumping.

Announced at 500.0 μM. At this concentration the mitotic index decreased to 10% of the control (BDS without growth regulators) after 12-h culture. IAA had a light inhibitory effect. A decrease in mitotic activity was observed at the highest concentration only (500.0 μM). On the other hand, a slight increase in the mitotic activity was noted at 5.0 μM and 50.0 μM. Kinetin inhibited mitotic activity at as low a level as 16.0 μM. The inhibition was rather strong at 50.0 μM and as a result the mitotic index equalled 14% of the control after 48-h culture.

The frequencies of aberrant anaphases observed in the course of a continual cultivation are summarized in Table 2. Mean frequency [%] of aberrant anaphases in the control was 1.34 ± 0.38 during the whole experiment. Despite a slight increase noted for 6 h treatment with 500.0 μM 2,4-D there were statistically nonsignificant differences (at P = 0.50) between all variants tested and the control. Except for 24- and 48-h treatment with 500.0 μM IAA there were no other disturbances of mitotic division observed, besides anaphase aberrations. A strong tendency to chromosome stickiness and clumping was noted at 500.0 μM IAA which made the evaluation of anaphase stages impossible.

Interference of growth regulators with a normal function of the mitotic spindle should change mitotic phase proportions. Consequently, relative length of the metaphase could be a sensitive measure of such interference. Fig. 2 shows that across all variants relative length of the metaphase ranged from 60% to 120% of that in the control one (BDS without growth regulators) except for the treatments with 500.0 μM 2,4-D and 500.0 μM IAA for more than 12 h, where evaluation of mitotic phase proportions was impossible because of a low mitotic index. The treatment with IAA, KIN, 2,4-D at concentrations of up to 50.0 μM thus had no effect on accumulation of metaphase stages in meristem root tips of *Allium sativum*.
Our observations show that exogenous IAA at the concentrations 5 μM and 50 μM has a slight stimulatory effect on mitotic activity of meristematic root cells of *A. sativum*. This is in agreement with the results of Ryland (1948) and Sen (1974). On the other hand, Mc Manus (1959) observed a decrease in mitotic activity of meristematic root cells of *A. cepa* after the treatment with IAA at concentrations as low as 1 ppm (5.7 μM) and strong inhibition at 10 ppm IAA (57 μM). Cortés et al. (1980) found that IAA at the concentration of 0.1 μM strongly inhibited mitosis in meristematic root cells of *A. cepa*.

None of the authors cited observed an increase in the frequency of mitotic aberrations which is in good agreement with our results, except for the treatment with 500 μM IAA having induced chromosome stickiness and clumping.

In our experiment, the treatment with kinetin at 5 μM had no effect on mitotic activity of meristem root tip cells of *A. sativum*. Nevertheless, kinetin inhibited mitosis at the concentration 16 μM and especially 50 μM. These results are in close agreement with those of Guttman (1956) and Mc Manus (1959).

The treatment with kinetin caused no increase in the frequency of mitotic aberrations. This is in satisfactory agreement with the data of Mc Manus (1959) and Sen (1974) but Guttman (1956) observed induction of polyploidy as a result of a lack of synchronization between nuclear division and cytokinesis after the treatment with kinetin at the concentrations 3 ppm (14 μM) and 5 ppm (23 μM).

We have found higher concentrations of 2,4-D to inhibit mitotic activity which is in agreement with the results of other authors (Ryland 1948, Sen 1974, Sikka and Sharma 1976).

The ability of 2,4-D to induce mitotic and chromosomal aberrations has often been reported regardless of its concentration. Most of the authors studied the effect of 2,4-D at high (herbicidal) concentrations. At these concentrations 2,4-D induces disturbances of mitotic spindle, restitution mitoses, chromosomal bridges in anaphase and chromosome stickiness (Ryland 1948, Nygren 1949, Mohandas and Grant 1972, Sikka and Sharma 1976). It is not possible to directly compare our results with those of the authors cited as we have tested the effect of low concentrations of 2,4-D. The concentrations 5 μM and 50 μM of 2,4-D did not induce a significantly higher number of mitotic aberrations as compared to the control. On the other hand Fiskesjö et al. (1981) reported that 2,4-D at the concentration 4.5 μM resulted in a strong tendency to chromosome stickiness in 45% mitoses of *A. cepa* root tip cells. This difference is difficult to explain but one may suggest different sensitivity of *A. cepa* in comparison with *A. sativum* and moreover the effect of different experimental conditions, such as temperature, length of treatment, pH and oxygen concentration of the solutions (Kihlman 1966).

It should be taken into account that it is not possible to simply compare the effect of growth regulators on cells of an organized tissue *in situ* and on those of an unorganized callus tissue *in vitro*. It may be agreed that growth regulators tested (IAA, KIN, 2,4-D) at low concentrations — as used in plant tissue culture media — are likely to have no effect on induction of mitotic aberrations.
Acknowledgement

The authors wish to express their thanks to Miss J. Potomková for revision of the English manuscript.

REFERENCES


