Short Communication

Effect of 2,4-Dichlorophenoxyacetic Acid on the Cytokinin Requirement of Soybean Cotyledon and Tobacco Stem Pith Callus Tissues

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Studies pertaining to the detection, isolation and chemical characterization of naturally occurring and synthetic cytokinins have been aided by the utilization of cultured callus tissues derived from soybean cotyledons (6, 9, 10, 11, 12) and tobacco stem pith (7, 8, 13, 14). Both tissues, when exposed to a specific and defined chemical milieu, require kinetin or another similarly active 6-substituted aminopurine for continual growth and maintenance in culture (10). It has also been reported that numerous structural variants of phenylurea stimulate cell division in isolated stem pith of tobacco (2). Although an auxin such as indole-3-acetic acid (IAA) or α-naphthalene acetic acid (NAA), is a necessary constituent of the basal medium for optimum growth, the auxin does not elicit significant callus proliferation in the absence of a cytokinin (10). In the soybean callus, however, the specific auxin used appears to be quite important with respect to the nature of the cytokinin-auxin mediated growth response. For example, adenine is known to exhibit slight cytokinin activity, no activity or even inhibition of callus growth when supplied in combination with IAA (10, 11). On the other hand, Miller (11) has recently demonstrated that adenine will consistently stimulate soybean callus growth when NAA is substituted for IAA. In essence, a highly effective auxin seems to be a requirement for the expression of cytokinin activity by adenine. In view of these results, it seemed reasonable to assume that an auxin even more effective than NAA might stimulate the callus proliferation in the absence of any added cytokinin. 2,4-D meets this requirement and is known to stimulate growth of soybean root cells cultures (4) and has been used without added cytokinins in the culture of other tissues (3, 5, 15, 16, for examples).

The tissues examined were originally derived from stem pith of tobacco (Nicotiana tabacum, var. Wisconsin No. 38) and from cotyledons of soybean (Glycine max, var. Acme). In this laboratory, stock cultures have always been maintained on a sucrose-salt-vitamin basal medium (10) containing 0.5 mg/l kinetin and either 5.0 mg/l IAA (tobacco callus) or 2.0 mg/l NAA (soybean callus). The stock cultures were routinely subcultured on the same medium once a month for several years without observable changes in growth pattern or physical characteristics.

For the experiments 3 small clumps of callus stock were planted in each 125 ml Erlenmeyer flask containing 50 ml of test medium which was solidified with 1% (w/v) Bacto-agar. The tissues were grown at 27°C and constantly exposed to fluorescent lighting at about 40 ft-c for a period of 4 weeks (soybean callus) or 5 weeks (tobacco callus). At the completion of each experiment the tissues were removed from the agar, blotted briefly, and subjected to standard fresh weight determinations.

Effect of 2,4-D and Kinetin on Soybean Callus Growth. Over a concentration range of 0.5 to 10.0 mg/l, 2,4-D in the basal medium alone stimulated callus growth (fig 1). The optimum growth response appears to be near the highest concentration used (10.0 mg/l). Although not reflected in the mean fresh weight, at this 2,4-D concentration there was some variability with occasional inhibition of several tissues pieces. Therefore, a conservative estimate of the 2,4-D concentration for optimum growth is between 5.0 and 10.0 mg/l.

Various concentrations of 2,4-D (0.005–0.5 mg/l) in combination with kinetin at 1 concentration (1.0 mg/l) elicited dramatic callus proliferation (fig 1). In fact, several concentrations of 2,4-D with the 1 kinetin concentration were considerably better than the optimum concentration of 2,4-D alone. In comparing the 2 curves, the optimum concentration of 2,4-D for callus growth is much lower when kinetin is present. Kinetin, however, is severely inhibitory when the 2,4-D concentration is high. Thus, the same general pattern as previously reported for adenine and NAA interaction seems to be involved here except that 2,4-D stimulates significant callus growth in the absence of even a poor exogenous cytokinin. For optimum growth, however,
a low concentration of an effective cytokinin is necessary.

Stock cultures of soybean callus, grown on kinetin and NAA containing medium, normally appear as white and dense compact masses of callus which result from cell division and cell enlargement. Conversely, tissues grown on 2,4-D were yellow, and the newly formed cells were relatively larger and more watery. Nevertheless, superficial and microscopic examinations revealed that the callus growth response to 2,4-D was due to cell division as well as enhanced cell enlargement. We have not performed any statistical studies on the relative sizes of the cells. Therefore, exactly what the shifts in optimum 2,4-D concentrations really represent is not clear. However, the soybean tissue has been maintained through 9 subcultures on basal medium containing only 2,4-D (5 or 10 mg/l) and continues to grow when subcultured. Cell division is obviously occurring at a good rate. Tissue aliquots taken from the 2,4-D stock at monthly intervals still respond normally to a combination of kinetin and NAA and do not grow on basal medium containing only NAA; the results with 2,4-D therefore do not represent the selection of a unique strain of cells with altered growth substance requirements.

**Tobacco Callus Tissue.** The results of a typical experiment using tobacco callus grown from small transplants (approx. 40 mg fr wt) on 2,4-D in the absence and presence of kinetin are presented in table I. Unlike the soybean callus, the tobacco tissue response is somewhat complicated by the stimulation of budding and formation of plantlets by kinetin. A comparison of fresh weights of callus with no buds with budding callus would be misleading. Therefore, it is difficult to evaluate whether a shift in optimum concentration of 2,4-D occurred. Nevertheless, 2,4-D alone and at relatively low concentration (0.5 mg/l) did stimulate new cell formation and dramatic cellular enlargement. Microscopic examination revealed that many of the newly formed cells of the callus grown on 2,4-D appeared to be larger than those cells of callus normally grown on medium containing kinetin and equivalent levels of IAA or NAA.

**Table I. Response of Tobacco Callus Tissue to 2,4-D in the Presence or Absence of Kinetin**

The values given represent an average of 30 pieces of callus grown for 35 days at the temperature and light regime indicated in the text.

<table>
<thead>
<tr>
<th>2,4-D mg/l</th>
<th>No. of pieces with buds</th>
<th>No. of pieces callus only</th>
<th>Callus only fr wt mg/piece</th>
</tr>
</thead>
<tbody>
<tr>
<td>With no kinetin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.005</td>
<td>4</td>
<td>25</td>
<td>53</td>
</tr>
<tr>
<td>0.05</td>
<td>5</td>
<td>30</td>
<td>59</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>30</td>
<td>99</td>
</tr>
<tr>
<td>5.0</td>
<td>0</td>
<td>30^2</td>
<td>143</td>
</tr>
<tr>
<td>10.0</td>
<td>0</td>
<td>29^4</td>
<td>61</td>
</tr>
<tr>
<td>With kinetin (1.0 mg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.005</td>
<td>30</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>27</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>3</td>
<td>28^3</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>0</td>
<td>30^4</td>
<td></td>
</tr>
</tbody>
</table>

1. Each piece a mixture of loose and compact callus, but all in healthy condition.
2. Callus loose and dark in appearance with newly formed white cell clumps at the periphery.
3. Callus dark brown, only very slight growth.

The results of this study support the initial assumption that a highly effective and chemically stable auxin, in the absence as well as in combination with a conventional cytokinin, would stimulate soybean cotyledon and tobacco stem pith callus growth in culture. The 2,4-D concentration required for optimum soybean callus growth is significantly lower when kinetin is present. Kinetin, however, is severely inhibitory when the 2,4-D concentration is high. This pattern is similar to that previously reported for adenine and NAA interaction (11).
Unlike NAA, however, 2,4-D stimulates callus growth in the absence of even a poor exogenous cytokinin.

A possible explanation of the above results is that 2,4-D primarily acts as an auxin and secondarily stimulates cytokinin synthesis. Another possibility is that cytokinins are not necessarily involved in cell division but cause their effects by increasing the sensitivity of a tissue to the occurrence of auxin-stimulated reactions. In other words, the auxin reactions are the truly critical ones in cell division and their effectiveness can be increased either by the metabolic modifications caused by the cytokinins or by the use of more efficient auxins. A third suggestion is that 2,4-D itself may directly act as a cytokinin and also as an auxin. A difficulty with this idea is that 2,4-D alone does not stimulate bud formation in the tobacco callus and in this respect does not appear to be operating as a cytokinin. The second possibility deserves serious consideration since it is already known that, for the expression of activity, the weak cytokinins such as adenine, methyladene and ethyladenine require the presence of an efficient auxin such as NAA and in higher quantities than is needed with the most effective cytokinins (11). The results of this study further support this suggestion since a very effective auxin (2,4-D) stimulated a small but significant growth response in the absence of exogenous cytokinins and elicited considerably greater growth at lower concentrations in the presence of an effective cytokinin.

The question as to whether the elimination of a cytokinin requirement by 2,4-D is a general phenomenon is still open since existing evidence indicates that an external source of auxin is not needed at all for the proliferation of some callus cultures. For example, exogenous auxin is not required for the growth of *Picea glauca* callus (17) or as demonstrated by Braun and Wood (1), the hormone can be effectively substituted for by a combination of 4 salts, KCl, NaNO₃, NaH₂PO₄ and (NH₄)₂SO₄, in the growth medium of *Vinca rosea* tissues. But, the capacity of the *Vinca* callus cells to synthesize auxin appears to be activated by an unknown mechanism involving the added salts (1). It is generally believed, moreover, that most normal cell types require auxin for growth. The apparent auxin-independent growth of some tissues, therefore, may merely reflect hormonal biosynthesis. Nevertheless, it is possible that the elimination of a cytokinin requirement by 2,4-D is not a general phenomenon. The effect of 2,4-D on the soybean and tobacco tissues, however, may be of potential importance and deserves serious consideration in future investigations relating to the nature of auxin-cytokinin interactions.

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**Literature Cited**


