The Role of Lectins in Cell Division of Tissue Cultures of Soybean and Tobacco

INDRA K. VASIL and DAVID H. HUBBELL

Received April 10, 1977 · Accepted May 5, 1977

Summary

Lectins, phytohemagglutinins, have been reported to act as mitogenic agents in animal and plant tissue cultures. We have tested the effect of three different lectins (soybean agglutinin, phytohemagglutinin-M, and wheat germ agglutinin) on the growth of tissue cultures of soybean root segments and tobacco with explants. In no case any significant improvement in growth over controls could be demonstrated, except in the case of wheat germ agglutinin, which appears to enhance the growth of tobacco pith explants in the presence of an auxin and a cytokinin.

Key words: cell division, lectins, plant tissue culture.

Introduction

Certain proteins that possess the ability to agglutinate plant and animal cells are widely distributed in nature, particularly in the seeds of legumes and in some invertebrates (BOYD, 1970; SHARON and LIS, 1972). These are known by the generic name of lectins, and those isolated from plants are commonly termed phytohemagglutinins (PHA). Lectins are known to bind specifically to the cell membrane and are being used increasingly in studies of the structure and function of surface membranes.

There have been only a few studies of the effects of lectins on plants. PHA has been reported to induce parthenocarpic fruit set in a male-sterile mutant of tomato, and in the Bartlett pear (BANGERTH et al., 1972). NAGL (1972 a, b) found a temporary stimulation of growth and mitotic activity in Phaseolus coccineus and Allium cepa. Concanavalin A (Con A) agglutinates plant protoplasts in the same manner as animal cells (GLIMELIUS et al., 1974), while Con A as well as PHA stimulate germination of lily pollen (SOUTHWORTH, 1975). A weak and insignificant mitotic effect of PHA on tomato callus cells has also been reported (LEVENKO and KIFORAK, 1975).

Legume lectins interact with Rhizobium (HAMBLIN and KENT, 1973; BOHLOOL and SCHMIDT, 1974; DAZZO and HUBBELL, 1975 a, b), and recent evidence indicates that the lectins participate in the determination of host specificity in the Rhizobium-legume symbiosis by binding specifically to surface polysaccharides of the appropriate infective Rhizobium cells and allowing specific adsorption of these cells to the legume root surface (BOHLOOL and SCHMIDT, 1974; DAZZO and HUBBELL, 1975 b). HUBBELL

(personal communication) has recently speculated that the reported mitogenic activity of lectins (ROBBINS, 1964; DOUGLAS et al., 1969; NAGL, 1972a) may play a key role in the nodulation of legume roots, and the lectin may actually enter legume root cells by virtue of its adsorption to the bacteria. The present report describes a study of the possible effect of lectins on the growth of plant tissues in vitro.

**Materials and Methods**

Seeds of soybean (*Glycine max*) were sterilized by a 2 min immersion in ethanol, followed by 10 min in 10% chloroform. The seeds were then rinsed with sterile distilled water, placed in hydrogen peroxide for 3-4 minutes, and rinsed finally with six changes of sterile distilled water. The sterilized seeds were placed on moistened filter paper in Petri dishes at 25 °C in the dark for 3 days, or until the roots were about 3 cm long. The terminal 0.3 cm of each root was discarded, and 2-3 segments, each 3 mm thick, were isolated from the cut end of each root. Ten root segments were placed in each Petri dish, and there were a total of 20 segments for each treatment.

Tobacco (*Nicotiana tabacum* Wis. 38) stem pieces were sterilized for 15 min in 10% chloroform and rinsed several times with sterile distilled water. The stem pith was then extracted with a sterile canula (3 mm diam) and cut into 5 mm long segments. Ten pith segments were placed in each Petri dish, and there were 20 segments for each treatment.

Soybean root segments were cultured on B-5 medium (GAMBORG et al., 1968) containing 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 10^-8 M kinetin, 2% sucrose, and 1% agar. Tobacco pith segments were cultured on MURASHIGE and SKOOG's (1962) medium containing 0.04 mg/l kinetin, 4 mg/l indole-3-acetic acid (IAA), 100 mg/l inositol, 2% sucrose, and 1% agar. All cultures were incubated at 25 °C in the dark. All experiments were repeated twice.

Lectins were dissolved in 0.01 M sodium phosphate buffer (pH 7.4), sterilized through a 0.45 μm Millipore filter, and then added to sterilized and warm agar medium. Soybean agglutinin (Miles Laboratories, Kankakee, Illinios) was used for soybean root segments, and phytohemagglutinin-M (Calbiochem, La Jolla, Calif.) prepared from *Phaseolus vulgaris* and wheat germ agglutinin (Miles Laboratories) was used for tobacco stem pith cultures.

Growth of the cultured root or stem pith segments took place by cell proliferation and callus formation, and was determined by removing two pieces from each treatment, blotting on filter paper, and determining the fresh weight. Significant increases in cell number were also noted but these were highly variable, and hence of little value for comparative analysis.

**Results and Discussion**

**Soybean**

Fresh weight of soybean root explants taken from various media after 2, 4, 8, 16 and 32 days of growth clearly shows that growth by cell division and callus formation occurred only in the presence of 2,4-D and kinetin (Table 1). There is no growth in the absence of the plant growth substances, with or without the addition of various concentrations of soybean agglutinin.

**Tobacco**

The growth of tobacco pith explants in media with or without phytohemagglutinin-M (PHA) or wheat germ agglutinin (WGA) is shown in Tables 2 and 3. Here
Table 1: Growth (fresh weight in mg) of soybean root segments on B-5 medium. Average fresh weight of starting explants was 23.3 mg.

<table>
<thead>
<tr>
<th>Days in Culture</th>
<th>No SBA</th>
<th>SBA (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OH +H</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>OH</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>OH</td>
<td>33</td>
</tr>
<tr>
<td>8</td>
<td>OH</td>
<td>30</td>
</tr>
<tr>
<td>16</td>
<td>OH</td>
<td>54</td>
</tr>
<tr>
<td>32</td>
<td>OH</td>
<td>24</td>
</tr>
</tbody>
</table>

SBA = soybean agglutinin
OH = no auxin and no cytokinin
+H = 1 mg/l 2,4-D and 10⁻⁶ M kinetin

Table 2: Growth (fresh weight in mg) of tobacco stem pith explants on Murashige and Skoog's medium. Average fresh weight of starting explants was 67.5 mg.

<table>
<thead>
<tr>
<th>Days in Culture</th>
<th>No PHA</th>
<th>µg/ml PHA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OH +H</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>OH</td>
<td>92</td>
</tr>
<tr>
<td>8</td>
<td>OH</td>
<td>101</td>
</tr>
<tr>
<td>16</td>
<td>OH</td>
<td>153</td>
</tr>
<tr>
<td>32</td>
<td>OH</td>
<td>208</td>
</tr>
</tbody>
</table>

PHA = phytohemagglutinin-M
OH = no auxin and no cytokinin
+H = 4 mg/l indole-3-acetic acid and 0.04 mg/l kinetin

Table 3: Growth (fresh weight in mg) of tobacco stem pith explants on Murashige and Skoog’s medium. Average fresh weight of starting explants was 58 mg.

<table>
<thead>
<tr>
<th>Days in Culture</th>
<th>No WGA</th>
<th>µg/ml WGA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OH +H</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>OH</td>
<td>88</td>
</tr>
<tr>
<td>8</td>
<td>OH</td>
<td>78</td>
</tr>
<tr>
<td>16</td>
<td>OH</td>
<td>155</td>
</tr>
<tr>
<td>32</td>
<td>OH</td>
<td>175</td>
</tr>
</tbody>
</table>

WGA = wheat germ agglutinin
OH = no auxin and no cytokinin
+H = 4 mg/l indole-3-acetic acid and 0.04 mg/l kinetin

again, as with soybean root segments, growth by cell proliferation and callus formation took place only in the presence of IAA and kinetin, with or without the added PHA or WGA.

Data on cell counts from soybean as well as tobacco cultures generally agreed with the fresh weight determination, but owing to the inconsistent nature of the cell counts these are not presented here.

The evidence from the above experiments shows that none of the lectins used – soybean agglutinin, PHA, or WGA – increase or induce any significant cell division activity in cultured soybean root and tobacco pith segments. Wheat germ agglutinin appears to improve the growth of tobacco stem pith segments, in the presence of an auxin and a cytokinin (Table 3). The slight and transient increase in mitotic activity reported by Nagl (1972 a, b) with PHA might be due to the presence of contaminants – possibly plant growth substances – in the relatively impure lectin preparations. He observed stimulation of germination and early seedling growth in Phaseolus coccineus and enhancement of root growth in Allium cepa; both of these processes largely involve cell enlargement and elongation, and can take place without much accompanying cell division activity. As the enhancement of growth in both instances took place only during the early phases of growth and lasted for relatively short periods of time, it could also have been caused by an enhanced uptake of water and nutrients due to the changed nature of the plasma membrane resulting from the binding or absorption of the lectin to these membranes. Southworth (1975) has reported stimulation of lily pollen germination by Con A and PHA, largely caused by the shortening of the lag period before germination. It is important to note that pollen germination also involves elongation of the pollen tube following hydration, and is not dependent on cell division activity. It would appear more likely, therefore, that lectins cause the reported effects on plant cell growth (expansion growth) by binding or adsorbing to the plasma membrane, rather than by inducing mitotic activity. In cases where growth is largely by cell division activity, as in the systems used in the present study, the addition of lectins to the nutrient media does not result in any growth improvement.

Acknowledgements

This research was supported by National Science Foundation grant no. INT 76–17525 and DEB 75–14043. Ms Jane Cundiff provided excellent technical assistance.

References


Sharon, N. and H. Lis: Science 177, 949 (1972).

Professor Dr. Indra K. Vasil, Department of Botany, University of Florida, Gainesville, Florida 32611, U.S.A.