Effect of Gibberellic Acid on Crown Gall Tumor Induction in Aging Primary Pinto Bean Leaves

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ABSTRACT

Gibberellic acid was tested for its effect on tumor induction by Agrobacterium tumefaciens in primary pinto bean (Phaseolus vulgaris) leaves in various stages of development. The hormone was found to promote tumor induction in partially aged leaves but did not effect tumor induction in very young leaves or in fully matured leaves. It is suggested that the natural loss of susceptibility to tumor induction in maturing pinto bean leaves is associated with a concomitant loss of endogenous gibberellins and/or a sensitivity to gibberellins.

Agrobacterium tumefaciens is well known for its ability to induce autonomous tumors on the roots, stems, and leaves of a variety of plant species. Lippincott and Heberlein (8) observed that the primary leaves of pinto beans are most susceptible to tumor induction by A. tumefaciens immediately after opening (approximately 180–190 hr after sowing) and thereafter undergo a rapid decline in their susceptibility to the bacterium. As such leaves are apparently healthy, actively growing, and in the process of expansion, it is intriguing that fewer tumors should develop when more mature leaves are inoculated.

Plant hormones are known to play a vital regulatory role in the growth and development of plant organs which in turn could have a major effect on an organism's susceptibility to the pathogenic properties of the bacterium. Khalifa and Lippincott (3) observed that the number of tumors induced by a fixed concentration of A. tumefaciens on primary pinto bean leaves could be enhanced by topical application of certain plant hormones. In light of these findings, an effort was made to determine if plant hormones play a role in the rapid decline in the bean leaf's susceptibility to tumor induction.

MATERIALS AND METHODS

Bacteria and Culture Media. Agrobacterium tumefaciens strain B6M was used throughout the course of these experiments. The bacterium was grown in broth medium containing 0.8% nutrient broth (Difco) supplemented with 0.5% sucrose and 0.1% yeast extract (Difco).

Pinto Bean Leaf System. The pinto bean (Phaseolus vulgaris var. Pinto) leaf assay used in this study was the same as that described by Lippincott and Heberlein (8). Stationary phase cultures of the bacterium containing approximately $3.5 \times 10^6$ colony forming units per milliliter were used to inoculate the leaves. After drying, inoculated leaves received a single hormone treatment. Gibberellic acid (GA$_3$, Eastman Chemical Company) dissolved in 0.05 M phosphate buffer (pH 6.9) was spread over the upper surface of the inoculated leaves with the aid of a pipette. Leaves were examined for number of tumors 7 days after inoculation.

RESULTS

Several growth substances were examined for their effect on tumor induction when applied to leaves inoculated with A. tumefaciens at different stages of development. Phosphon D (2,4-dichlorobenzyltributylphosphonium chloride) and B-9 (N-dimethylaminosuccinamic acid) had no effect. Indole-3-acetic acid, kinetin, and gibberellic acid enhanced the number of tumors formed per leaf as previously observed by Khalifa and Lippincott (3). This enhancement was restricted to leaves that were in an intermediate stage of development. Since the enhancement effect was most pronounced after gibberellic acid treatment, it was selected for further study.

Table I shows that there is a sharp decline in the mean number of tumors that develop per leaf with increasing leaf age. Stage I leaves yielded the maximum number of tumors, while stage II leaves had significantly less than stage I and stage III leaves had only a negligible number. Gibberellic acid increased the tumor number when applied to inoculated stage II leaves but not when applied to either stage I or stage III leaves. The restoration of susceptibility to crown gall tumor induction by gibberellic acid in stage II was found to increase with increasing concentration. The concentration of gibberellic acid providing maximum restoration of susceptibility varied systematically for concentrations between 1 $\mu$g/ml to 100 $\mu$g/ml. The number of tumors on the 100-$\mu$g/ml treated leaves was typically less than that on the 10-$\mu$g/ml treated leaves but was significantly more than on the control leaves. Restoration of susceptibility to tumor induction by gibberellic acid was observed in all four experiments which involved pinto bean plants derived from separate sowings.

Gibberellic acid treatment promoted leaf expansion and elongation of the first internode. This effect was proportional to the concentration of gibberellic acid applied with 100 $\mu$g/ml being most effective (highest concentration tested). This phenomenon was observed in plants of all stages but was more pronounced in stages I and II than in stage III plants.

DISCUSSION

The results presented in this paper confirm earlier observations: that of Lippincott and Heberlein (8) that primary pinto bean leaves rapidly lose their potential to form tumors and...
after a high point approximately 180 hr after the sowing of
seeds, and that of Khalifa and Lippincott (3), Khalifa and Nur
(4), and Khalifa and Yousif (5) that gibberellic acid promotes
crown gall tumor induction. An interesting point that emerges
from this investigation is that promotion of tumor induction by
gibberellic acid is restricted to leaves in an intermediate
stage in their development. Gibberellic acid fails to enhance
tumor number in primary pinto bean leaves when inherent sus-
ceptibility to tumor induction is either very high, as in young
leaves, or very low, as in fully mature leaves. Hence, both
susceptibility to tumor induction and effectiveness of exogenous
gibberellic acid to restore susceptibility is dependent upon the
developmental stage of the leaves.

The fact that gibberellic partially restores susceptibility to
tumor induction in moderately aged leaves, but not in fully
mature leaves, suggests that it may act by prolonging juvenility.
A critical concentration of juvenile cells may be required for
transformation. Leaf cell maturation and loss in cell division
capacity could be associated with a change in the level of en-
dogenous gibberellic and/or a loss in the sensitivity to the
hormone. Under this regime stage I leaves would have a high
gibberellic content and high sensitivity, stage II leaves mod-
erate gibberellic content and moderate sensitivity, and stage
III leaves little or no gibberellic and little or no sensitivity. It
is well established that young tissues of various species of Phase-
olus including P. vulgaris are rich in gibberellics (2, 6, 10) and
that gibberellic can restore juvenility in a variety of plant spe-
cies (1, 9).

It is also possible that gibberellic restores susceptibility to
tumor induction in moderately aged leaves by acting directly
on the wound-healing cell division processes which appear to be
essential for tumor induction. In Kalanchöe daigremontiana
maximum susceptibility to A. tumefaciens has been shown to
occur just prior to wound induced cell division (7). A direct
relationship between wound healing and gibberellic in P.
vulgaris, however, has yet to be established.

Table I. Effect of Gibberellic Acid on Crown Gall Tumor
Induction in Primary Pinto Bean Leaves at Different
Stages of Maturity

<table>
<thead>
<tr>
<th>Hormone Treatment</th>
<th>No. of Tumors/Leaf (± SEM) Inoculated with A. tumefaciens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stage I</td>
</tr>
<tr>
<td>Control</td>
<td>149.0 ± 9.9</td>
</tr>
<tr>
<td>1</td>
<td>148.6 ± 10.5</td>
</tr>
<tr>
<td>10</td>
<td>148.0 ± 7.1</td>
</tr>
<tr>
<td>100</td>
<td>133.0 ± 12.5</td>
</tr>
</tbody>
</table>

LITERATURE CITED

factors on the initiation and growth of crown-gall tumors on primary pinto
Phytopathol. et Phytoprotec. 29-37.
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in Hedera helix as studied by deoxyribonucleic acid-ribonucleic acid hy-