Physiological Comparisons of Pith Callus With Crown-Gall and Genetic Tumors of Nicotiana glauca, N. langsdorffii, and N. glauca-langsdorffii Grown in Vitro. 1. Tumor Induction and Proliferation

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Abstract. Agrobacterium tumefaciens B-6 and T-37 strains, inoculated into Nicotiana glauca, N. langsdorffii, and their interspecific hybrid, which forms genetic (spontaneous) tumors as well, initiate amorphous tumors from the B-6 strain and organoid tumors (aberrant roots, stems, and buds) from the T-37 strain. In the hybrid, the critical point was to induce crown gall tumors at the site of wounding and not spontaneous genetic tumors. To succeed, this inoculation had to be made at a very early (5-6 leaf stage of development). It is observed that genetic organoid tumors readily formed at the nodes following flowering or leaf abscission. Furthermore, it was noted that genetic tumor derivatives are obtainable from hybrid pith callus or hybrid seedlings cultured in vitro.

A marked difference in stem elongation was observed in hybrid tobacco plants inoculated with different strains of Agrobacterium tumefaciens or distilled water, and uninoculated controls. Inoculation of hybrid plants with the B-6-G2 avirulent strain of Agrobacterium tumefaciens stimulated stem elongation over controls, the T-37 inoculated stems were slightly stunted, and the B-6 inoculated stems were quite stunted and succumbed at an early age.

The objective of the forthcoming series of experiments is the establishment in vivo of genetic tumors and Agrobacterium tumefaciens (strains B-6 and T-37) induced tumors in the amphidiploid hybrid, Nicotiana glauca-langsdorffii. The induction of these crown gall tumors is a critical procedure because the amphidiploid readily responds at the sites of wounding by the formation of genetic tumors (1,3). The establishment of an experimental tissue system of this sort allows for a comparative evaluation of the degree of symmetry between the crown gall tumor and genetic tumor. The B-6 and T-37 strains were also inoculated into non-genetic tumor forming N. glauca and N. langsdorffii in order to provide controls.

Materials and Methods

Seeds of Nicotiana glauca and N. langsdorffii were obtained from Dr. H. H. Smith of the Brookhaven National Laboratory, while those of the hybrid were obtained from Dr. G. L. Hagen at the Institute for Cancer Research in Philadelphia. The seeds were germinated in a high humidity greenhouse (R.H. 80%, temp 25°) on a 12-hr illumination cycle. The N. langsdorffii and hybrid seedlings were potted at the end of 6 weeks, while the N. glauca seedlings were potted after 8 weeks. Under these conditions the time of flowering of N. glauca, N. langsdorffii, and the hybrid was ca. 193, 133, and 141 days, respectively.

Cultures of Agrobacterium tumefaciens (Towne and Smith) strains B-6 and T-37 were obtained from Dr. Armin C. Braun of Rockefeller University, while a B-6-G2 (avirulent) strain was obtained from Dr. R. E. Beardsley of Manhattan College. These strains were subcultured on tryptone-glucose-yeast extract (4) and transferred on a biweekly schedule. One week after potting, the young tobacco plants (at the 5-6 leaf stage) were inoculated with 48-hr cultures of 1 of the 3 strains of Agrobacterium or with distilled water and all replicated 16 times. Kalanchoe plants (16 replicates of each treatment) were inoculated with B-6, T-37, and B-6-G2 strains of Agrobacterium tumefaciens, after the method of Braun and Stonier (3).

Results

Inoculations of Kalanchoe (2,7), confirmed the fact that the B-6-G2 strain of Agrobacterium tumefaciens is avirulent, the T-37 strain produces partially transformed cells, and the highly virulent B-6 strain produces transformed cells, (Figs. 1-3).

Extensive crown gall tumor formation occurred after 20 days in all 3 taxa inoculated. The tumors formed on N. glauca and N. langsdorffii were readily

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identified as crown gall tumors. In the hybrid the objective is to induce crown gall tumors not genetic tumors at the site of wounding. To succeed, this inoculation must be made at an early (5-6 leaf) stage of development. When the hybrid was inoculated with distilled water (or avirulent B-6-G2) wound callus formed (Fig. 2); when inoculated with the T-37 strain a small amorphous or organoid tumor resulted (Fig. 4); and, finally, a B-6 inoculation induced a large, amorphous tumor mass (Fig. 3). These results are expressed quantitatively in Table I.

Table I. Crown Gall Tumor Formation in Nicotiana glauca-langsdorffii

<table>
<thead>
<tr>
<th>Host response</th>
<th>Strains</th>
<th>B6-6</th>
<th>T-37</th>
<th>B6-G2</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Large tumor (amorphous)</td>
<td></td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Medium tumor (amorphous)</td>
<td></td>
<td>10</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Wound callus</td>
<td></td>
<td>3</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Teratomata</td>
<td></td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

At a much later stage of development a T-37 inoculated plant is somewhat stunted in stem growth and the tumor mass grows into a large and vigorously growing teratoma (Fig. 5, left). A B-6 inoculated plant is quite stunted and has succumbed at an early date and the tumor remains amorphous (Fig. 5, right). The hybrid tobacco plants used in this study respond in the same growth patterns with respect to each strain of Agrobacterium as did Kalanchoe.

A differential in stem elongation was observed among hybrid plants inoculated with different strains of Agrobacterium from right to left (Fig. 6). The B-6-G2 avirulent strain stimulated stem growth over the uninoculated control; the T-37 inoculated stem was reduced by nearly half; and the B-6 inoculated stem was quite stunted and soon succumbed. A quantitative expression of these growth effects may be seen in Table II.

To obtain tumors for comparison with the induced crown gall neoplasms, hybrid plants were allowed to grow to maturity in the high humidity greenhouse. Genetic tumors readily formed on such plants particularly at the nodes from which flowers or leaves have abscissed. Fig. 7 shows the tenth oldest node on a hybrid plant. The genetic tumor is a small, compact, largely amorphous mass of partially transformed cells. Fig. 8 and 9 show tumors, a mixture of amorphous and organoid growth centers, at the eighth and fifth oldest nodes, respectively. Morphologically, these genetic tumors are not distinguishable from their corresponding growth form in the in vivo crown gall tumor.

<table>
<thead>
<tr>
<th>Inoculation</th>
<th>Avg height of 16 plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated control</td>
<td>80.5</td>
</tr>
<tr>
<td>B6-6</td>
<td>10.9</td>
</tr>
<tr>
<td>T-37</td>
<td>40.4</td>
</tr>
<tr>
<td>B6-6-G2</td>
<td>102.0</td>
</tr>
<tr>
<td>Distilled water</td>
<td>80.7</td>
</tr>
</tbody>
</table>

Fig. 1. Kalanchoe plants inoculated with 3 strains of Agrobacterium tumefaciens: Right, the B6-G2 strain is avirulent so that the stem below the decapitated apex (arrow) produces only slight callus. Center, the T-37 strain induces partially transformed cells which proliferate to produce a large mass of tumor cells which later (at arrow) produce aberrant roots, stems, and leaves. Left, the highly virulent B-6 strain induces completely transformed cells since only amorphous tumor masses are formed. ×1/3.

Fig. 2. Nicotiana glauca-langsdorffii inoculated (at arrow) with distilled water. Only a hard, compact wound callus forms. ×1/4.

Fig. 3. N. glauca-langsdorffii, inoculated (at arrow) with the highly virulent B-6 strain, forms a large, loose tumor mass which must be composed of completely transformed cells since it never becomes organoid. ×1/3.

Fig. 4. N. glauca-langsdorffii inoculated (at arrow) with the T-37 strain quickly forms a large teratoma. ×1/4.

Fig. 5. AT-37 inoculated plant (left) at a later stage of development than that in Fig. 4. Overall stem growth is somewhat stunted and the tumor mass grows into clusters of aberrant stems, leaves, and buds. A B-6 inoculated plant (right), at a later stage of development than that shown in Fig. 3, becomes quite stunted; the growth of the tumor mass remains amorphous, and such plants soon succumb. ×1/3.

Fig. 6. Differentials in stem elongation of N. glauca-langsdorffii plants. Leit to right: Response to the avirulent B-6-G2 strain, a non-inoculated control, the T-37 strain, and the highly virulent B-6 strain.

Fig. 7-9. Spontaneous tumors in the amphidiploid hybrid, N. glauca-langsdorffii. All ×1/3. Fig. 7. Tenth node from the base on a hybrid plant showing a small amorphous tumor mass at the point of leaf abscission. Fig. 8. Partially transformed cells at the eighth oldest node forming a mixture of amorphous and organoid growth centers. Fig. 9. Highly organoid tumor mass at the fifth oldest node.
Discussion

Although previously it was not possible to demonstrate any essential differences between genetic and crown gall tumors (3) the data (contained in this report) conclusively show that the 2 tumor species can be established in a single taxon. The B-6 transformed tumors are recognizable on the basis of a rapid growth rate, an amorphous morphology, and a severe stunting effect on the host plant. The T-3/7 tumor is characterized by a slow growth rate, either amorphous or organoid morphology dependent on the fortuitous location of the tumor on the plant (2), and a moderate degree of stunting to the host plant. Genetic tumors form during senescence at the nodes of flower or leaf abscission while their morphology consists of a series of morphological states ranging from small amorphous tumors to large teratomata (5, 6).

Further studies, which will be reported separately, show that nutritional differences exist between these tumors in vitro. Characterization of these tumor types and normal callus tissues on nutritional and physiological grounds should contribute to our knowledge of normal and abnormal growth.

Literature Cited