Hormonal Regulation of Growth and Development of Tomato Embryos in Vitro

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Abstract. Indoleacetic acid (IAA), gibberellic acid (GA3), and kinetin were incorporated into tissue culture media to determine the effective concentrations for sustaining growth of excised immature tomato (Lycopersicon esculentum Mill.) embryos. GA3 (10^-7 to 10^-5 M) stimulated growth of 13- and 15-day-old embryos but resulted in morphological abnormalities. Kinetin (10^-6 or 10^-5 M) promoted embryonic development and expansion of cotyledons but inhibited subsequent growth. Combinations of kinetin and GA3, or kinetin and IAA were most beneficial for embryos excised 12 days after pollination, when embryonic development was at a globular to early heart-shaped stage.

Excised embryos can be grown successfully on artificial medium without hormones once they have reached an autotrophic stage, marked by the differentiation of cotyledons. Few investigators, however, have been able to culture undifferentiated embryos to maturity on synthetic media with or without the addition of hormones or complex substances such as coconut milk and casein hydrolysate. The endosperm surrounding the embryo in vivo provides a source of hormones as well as nutrients that direct the normal growth of the embryo until the embryo is capable of synthesizing these substances. An imbalance of growth substances in the endosperm or between the

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Table 1. Analysis of variance for germination rate and plant length in Expt. 1.4

<table>
<thead>
<tr>
<th></th>
<th>Germination rate</th>
<th>Length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>m.s.</td>
</tr>
<tr>
<td>13-day-old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatments</td>
<td>16</td>
<td>0.123*</td>
</tr>
<tr>
<td>Replications</td>
<td>2</td>
<td>0.213</td>
</tr>
<tr>
<td>Error</td>
<td>31</td>
<td>0.085</td>
</tr>
<tr>
<td>15-day-old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatments</td>
<td>14</td>
<td>0.308**</td>
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<tr>
<td>Replications</td>
<td>3</td>
<td>0.675**</td>
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<tr>
<td>Error</td>
<td>40</td>
<td>0.073</td>
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</tbody>
</table>

4Differences significant at 5% (*) or 1% (**) levels.
5Data transformed into radians.
6Measurements after 6 weeks for 13-day-old and after 4 weeks for 15-day-old embryos.

Table 2. Analysis of variance for germination rate and plant length in experiment 2 after 6 weeks in culture.6

<table>
<thead>
<tr>
<th>Source</th>
<th>Germination rate</th>
<th>Length (mm)</th>
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</thead>
<tbody>
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<td></td>
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<td>m.s.</td>
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<td>Treatments</td>
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<td>IAA</td>
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<td>Kinetin</td>
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<tr>
<td>GA3</td>
<td>2</td>
<td>0.121</td>
</tr>
<tr>
<td>IAA × Kinetin</td>
<td>4</td>
<td>0.058</td>
</tr>
<tr>
<td>IAA × GA3</td>
<td>4</td>
<td>0.072</td>
</tr>
<tr>
<td>Kinetin × GA3</td>
<td>4</td>
<td>0.089</td>
</tr>
<tr>
<td>Replications</td>
<td>2</td>
<td>1.204**</td>
</tr>
<tr>
<td>Error</td>
<td>35</td>
<td>0.083</td>
</tr>
</tbody>
</table>

6Differences significant at 5% (*) or 1% (**) levels.
7Data transformed into radians.

The objectives of the work reported here were to test the responses of excised embryos of tomato (*L. esculentum*) to IAA, GA3, kinetin and combinations of these incorporated in the basal medium. We thought one or more of these hormones might enable us to culture globular to early heart-shaped embryos, and eventually the results might be applied to culture systems for circumventing incompatibility in the cross *L. esculentum* × *L. peruvianum*.

**Materials and Methods**

**Expt. 1.** Single additions of IAA (10⁻¹⁰ to 10⁻⁴ M), GA3 (10⁻⁷ to 10⁻⁴ M), or kinetin (10⁻¹⁰ to 10⁻⁴ M) were incorporated into Murashige and Skoog’s (6) medium. IAA and GA3 were added to the autoclaved medium via Millipore filters, since these compounds are heat-labile.

Embryos (“Vendor”) were excised 13 and 15 days after pollination and transferred onto the artificial media. Sixteen embryos of each age were cultured for 12 weeks, 4 replications of 4 embryos per dish. Length measurements were made biweekly on the roots and shoots, and observations on morphological development were recorded.

**Expt. 2.** The HLH medium described by Neal and Topoleski (8) was adopted as the basal medium in this experiment. Two-way combinations of IAA (10⁻¹⁰ and 10⁻⁹ M), GA3 (10⁻⁸ and 10⁻⁷ M), and kinetin (10⁻⁸ and 10⁻⁷ M) were incorporated into the medium and a control was included without hormones. Globular or very early heart-shaped embryos were excised 12 days after pollination. Three replications of 4 embryos each were observed for abnormal morphological development and analyzed for differences in growth measured as plantlet length.

Other materials and methods for both experiments followed those of Neal and Topoleski (8). The term ‘germination’ refers to the differentiation and growth of a root or a stem bearing cotyledons or leaflets.

**Results**

**Expt. 1.** Overall germination rates were 60% for the 15-day-old embryos and 36% for the 13-day-old embryos. The high concentrations of IAA or GA3 inhibited embryo germination, accounting for the significance shown in Table 1.

Figures 1, 2, and 3 show the mean embryo or plantlet lengths after 4 or 6 weeks in culture for both age groups on media containing IAA, GA3, or kinetin. The relative patterns did not change subsequently. Both 13- and 15-day-old embryos were stimulated to elongate by the lower concentrations of IAA and GA3 (Table 1).

The GA3 treatments that induced the greatest growth response resulted in plantlets with severely elongated shoots, minimal leaf growth, and morphologically abnormal root growth (Fig. 4). Kinetin, in contrast, seemed to promote normal, compact growth at low concentrations but extended embryonic development at high concentrations, marked by an early expansion of the cotyledons and a lack of root development. Most embryos failed to germinate on the high kinetin media. As the opposite of precocious germination, the prolonged period of embryonic development seemed to be potentially beneficial if it could be balanced by the stimulative effects of IAA or GA3.

**Expt. 2.** GA3 at 10⁻⁷ M again appeared to stimulate growth once an embryo had germinated. The average plantlet size after
Fig. 2. Growth responses of embryos excised 13 and 15 days after pollination; mean embryo or plantlet length after 6 and 4 weeks, respectively, in culture on media containing GA3 (Expt. 1).

Fig. 3. Growth responses of embryos excised 13 and 15 days after pollination; mean embryo or plantlet length after 6 and 4 weeks, respectively, in culture on media containing kinetin (Expt. 1).

6 weeks in culture on $10^{-7}$ M GA3 was twice that of the control (Fig. 5). IAA or a reduced concentration of GA3 was not as effective. Combinations of IAA plus GA3 were antagonistic, resulting in growth poorer than the control (Fig. 6). None of these differences were statistically significant, however, due to a high degree of variability in plant response (Table 2).

We feel that the observations on morphological effects that
could be attributed to the hormone treatments are more important than growth measurements. Certain combinations of kinetin with either IAA or GA₃ appeared to promote a balance between pregerminal development and postgerminal growth. The combinations of 10⁻⁸ M kinetin with 10⁻⁹ M IAA or 10⁻⁸ M GA₃ resulted in no reduction in germination and a more normal type of growth than that often seen on IAA or GA₃ without kinetin. Figure 7 illustrates the types of development often associated with 10⁻⁸ M kinetin, and kinetin in combination with 10⁻⁹ M IAA or 10⁻⁸ M GA₃.

The combination of kinetin and GA₃ induced development and growth of a few extremely small, globular embryos. Growth of such small undeveloped embryos was not observed on any other medium; therefore, the combination of GA₃ and kinetin appears to be worth further evaluation for embryo culture research.

Discussion

The results of our experiments are in accordance with the types of responses reported in the literature. Auxin effects previously reported ranged from inhibition of root initiation and growth to stimulation of both root and shoot growth, depending on concentration and other factors such as the composition of the basal medium, species and age of the embryos, and light conditions (12, 13, 14, 17). Embryos also may show differential growth...
sensitivities in respect to concentration; root growth may have a lower requirement for auxin than does shoot growth (3, 4). Such a distinction was not obvious in these experiments; hypocotyl elongation preceded root growth at most auxin concentrations.

GA₃ has been reported to stimulate cell division and cell elongation in differentiated embryos of *Gossypium* (1) and *Capsella* (12, 15) but globular and heart-shaped embryos did not respond favorably to GA₃. Stimulation of growth by GA₃ generally is reported as a favorable response, but we observed that GA₃ induced undesirable morphological effects in tomato plantlets. Similar observations have been reported for orchid embryos, where an overall stimulation of growth by GA₃ was accompanied by extreme elongation and chlorosis (10).

Inhibition of root growth and necrosis of the root meristem were the primary effects of cytokinins on heart-shaped and older *Capsella* embryos in culture (9, 12, 16, 17). Low concentrations (5 × 10⁻¹¹ M to 5 × 10⁻¹⁰ M) also caused precocious expansion of the embryonic leaves, and high concentrations (5 × 10⁻⁹ M to 5 × 10⁻⁷ M) induced callus growth. Younger embryos were influenced favorably by kinetin, which increased the rate of survival at concentrations of 5 × 10⁻⁸ M to 5 × 10⁻¹² M. However, young heart-shaped and globular embryos grew abnormally large, since kinetin induced cell division without differentiation at the apical end. In our experiments, tomato embryos also were induced to develop embryonically and differentiate cotyledons in the presence of kinetin; further growth and germination was inhibited at high concentrations (10⁻⁷ M) but not at lower concentrations.

In Expt. 2, kinetin (10⁻⁷ or 10⁻⁸ M) in combination with GA₃ (10⁻⁷ or 10⁻⁸ M) or IAA (10⁻⁹ M) showed the greatest potential for inducing development and growth of embryos excised before differentiation has occurred. Raghavan and Torrey (11) reported a similar interaction among hormones employed in the culture of globular *Capsella* embryos, where a balanced combination of IAA, kinetin, and adenine sulphate induced normal growth and differentiation. The promotion of pregerminial development by kinetin appears to be critical to the normal morphological development of the embryo, whereas GA₃ or IAA apparently is required to stimulate germination and postgerminial growth. IAA is not as effective as GA₃ in this respect. The less differentiated the embryos at excision, the more important it is to include kinetin and GA₃ (or kinetin and IAA) in the medium and the more exacting is the requirement for the proper balance between them.

**Literature Cited**