Promotion of Shoot and Root Formation in Asparagus in Vitro by Ancymidol

Chee-kok Chin
Department of Horticulture and Forestry, Rutgers University, New Brunswick, NJ 08903

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Abstract. Asparagus officinalis L. was propagated by culturing single-node spear segments on Murashige and Skoog's medium. Incorporation of ancymidol in culture medium accelerated the production of plantlets, promoted development of stronger roots and shoots, and suppressed undesirable proliferation of callus.

Yang and Clore (7) reported a simple and effective method to propagate asparagus in vitro using node segments on modified Murashige and Skoog's medium (5) containing naphthaleneacetic acid (NAA) and kinetin. At the end of 20 weeks, 25% of the cultures developed shoots and roots. One problem was that many cultures did not initiate roots. Production of complete plantlets could be increased if nodes, after developing shoots, were recultured in fresh medium (8). A relatively long period of time, normally more than 20 weeks, is needed for production of transplantable plants. This paper reports a modification of Yang and Clore's method that greatly reduces the time for production of transplantable plants.

Stock plants of clone 27, a pistillate selection, supplied by J. H. Ellison of Rutgers University, were explanted and maintained in culture as described by Yang and Clore (7). Shoots with well-developed cladophylls were excised and cut into single-node segments, each of which was then transferred to a 25 x 200-mm culture tube containing 20 ml of modified Murashige and Skoog's medium (5) with 1) 0.5 µM NAA and 0.5 µM kinetin, or 2) 0.5 µM NAA, 0.5 µM kinetin and 5 µM ancymidol. The cultures were maintained at 26°C under Cool White light at 1.2 klx. Each treatment involved 50 cultures and was replicated 4 times. Shoots developed in 4 weeks from 52% of the nodes in the medium without ancymidol, and from 90% of the nodes in the medium with ancymidol (Table 1).

At the end of the fourth week nodes with shoots were transferred to fresh media. In the medium without ancymidol no shoots rooted in 2 weeks, and only 26% rooted in 5 weeks (Fig. 1). In addition, the shoots and roots developed in the ancymidol medium were more vigorous than those developed in the medium without ancymidol (Fig. 2). Furthermore, callus often developed from explants in the medium without ancymidol, but not in the medium containing it (Fig. 2).

Six weeks after reculture, all the plantlets in the ancymidol medium possessed well-developed crowns with 4 to 7 spears and vigorous roots (Fig. 3). At this stage, plants were removed from the culture tube for transfer to a

Table 1. Effects of ancymidol on shoot development from cultured nodes of asparagus clone 27.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22</td>
<td>52</td>
</tr>
<tr>
<td>Ancymidol</td>
<td>45</td>
<td>90</td>
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</tbody>
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*Means were obtained from 4 experiments, each with 50 cultures.

Fig. 1. Effects of ancymidol on rooting of cultured 4-week-old nodal segments of asparagus (clone 27). Means were obtained from 4 experiments, each with 50 cultures.

Fig. 2. Five-week-old nodal segments from media with (right) and without (left) ancymidol.

Fig. 3. Six-week-old plantlets from media with (right) and without (left) ancymidol.
medium consisting of 1 vermiculite:1 sphagnum peatmoss, and kept under intermittent mist for 5 days. All plants survived establishment and grew into healthy plants. Twenty of these plants were randomly selected for chromosome counts and all were found to be diploid, 2n=20.

If the plantlets were not potted, but allowed to grow further, by week 8-10 they developed into relatively large crowns with 9–12 spears and 3–6 vigorous roots (Fig. 4). These were divided aseptically to smaller crowns, each with 3–5 spears and recultured in the medium containing ancymidol. In 1–3 weeks, all of these recultured crowns sent out more spears and roots.

Ancymidol greatly reduced the duration of time for production of transplantable plantlets, from about 20 weeks to 8 weeks. Time for production could be further shortened to about 3 weeks if in vitro crown division procedure was used. The incorporation of ancymidol also promoted stronger shoots and roots development and suppressed callus formation. Similar effects of ancymidol have been observed with 21 other clones, including pistillate (XX) and staminate (XY or YY) plants.

Ancymidol is an inhibitor of gibberellin (GA) synthesis and has been shown to block the oxidation of ent-kaurene, ent-kaurenol, and ent-kaurenal (2). GA, on the other hand, inhibits the formation of shoots and roots (1, 4, 6), and stimulates growth of callus in a number of plants (4, 6). It is possible that asparagus in culture possesses a quantity of GA that is partially inhibitory to shoot and root formation. Hence, the effect of ancymidol noted here may be due to inhibition of GA synthesis.

Literature Cited