Regeneration of Pea (*Pisum sativum* L.) Plants from Shoot Apical Meristems

K. K. Kartha, O. L. Gamborg and F. Constabel

With 3 figures

Received October 23, 1973

Summary

A procedure has been developed to obtain complete plants from meristems of three cultivars of *Pisum sativum* L., namely «Century», «Laxton’s Progress» and «Afghanistan». Benzyladenine (BA) alone or in combination with naphthaleneacetic acid (NAA) at molar concentrations of $5 \times 10^{-7}$ and $10^{-6}$, respectively, induced shoot differentiation in meristems cultured on B5 medium at 26° C, 60% relative humidity and exposed to fluorescent light (4000 lux, photoperiod 18 hrs). When applied alone, NAA induced complete plant formation. Root formation, on the shoots produced by culturing meristems, was induced by reculturing the shoots, 2 cm long, on half strength B5 medium supplemented with NAA at a concentration of $10^{-6}$ M.

Zusammenfassung


Introduction

In recent years plant tissue culture methods have gained importance as a means for vegetative propagation of plant species. From the point of view of pathology, apical meristem culture technique offers unparalleled advantages in the elimination of certain virus diseases (Morel and Martin, 1952; Nielsen, 1970; Quak, 1961; 1) Guest Research Worker supported by the International Development Research Centre, Ottawa, NRCC No. 13739.

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MORI, 1971; KARTHA et al., 1974). Organogenesis from the macerated shoot apical cells of *Pisum sativum* cv. Century has recently been demonstrated by GAMBORG et al. (1974). Since some of the legume viruses are transmitted through seeds of pea, it would be advantageous to be able to obtain virus-free plants by regeneration from apical shoot meristems. The present paper describes procedure for the regeneration of pea plants from shoot apical meristems.

**Material and Methods**

**Plant Material**

Three cultivars of *P. sativum*, Century, Laxton’s Progress and Afghanistan were used. Seeds were rinsed in 70% ethanol for 60 seconds, surface sterilized with 20% Javex (6% sodium hypochlorite) for 20 minutes, rinsed in sterile distilled water and germinated in the dark at 28°C on double layers of sterilized filter paper in a plastic petri dish.

**Culture Medium**

B5 medium (GAMBORG et al., 1968) containing 0.6% Difco agar and growth regulators (benzyladenine and naphthaleneacetic acid) at varying concentrations was employed to culture the apical meristems. The pH of the medium was adjusted to 5.7 with 0.1 N KOH. The medium (2.5 ml) was transferred into 10 × 1.2 cm pyrex tubes, plugged with absorbant cotton and autoclaved at 20 lbs p.s.i. for 20 minutes.

Four to five days after the germination of the seeds, apical meristematic domes, devoid of leaf primordia, measuring approximately 200–300 μ were dissected aseptically under a stereo microscope and transferred to the tubes. The meristems were incubated in a growth cabinet at 26°C at 60% relative humidity, under cool white fluorescent light of 4000 lux, using a light and dark cycle of 18/6 hrs.

**Results and Discussion**

The morphogenetic responses of pea (cv. Century) apical shoot meristems on B5 medium with different growth substances are described in Table 1. Similar results were obtained with the other two cultivars (Laxton’s Progress and Afghanistan).

Benzyladenine (5 × 10⁻⁷ M) alone or in combination with NAA (10⁻⁶ M) resulted only in shoot development (Fig. 1), whereas NAA alone (10⁻⁶ M) induced shoot and root formations from the meristematic cells (Fig. 2).

Since the combination of BA and NAA or BA alone induced only shoot formation, the conditions for inducing root formation were investigated. After one month’s growth the shoots were removed aseptically and cut into 2 cm length keeping the apical portion intact. They were recultured under the same environmental conditions in sterile plastic containers (Qualicum) containing 20 ml of the B5 medium supplemented with NAA at molar concentrations of 10⁻⁸, 10⁻⁷ and 10⁻⁶ respectively. Though the shoot explants remained green over a period of two months, no sign of root formation was observed. On the other hand, when the B5 medium was diluted (1:1) with glass distilled water before adding the auxin, more than 50% of the shoot explants showed signs of root development in 7 to 10 days at 10⁻⁶ M NAA. After two weeks the shoots produced well developed root systems (Fig. 3). NAA at

Table 1: Morphogenetic responses of pea (cv. Century) apical shoot meristems on B5 medium with different growth substances.

<table>
<thead>
<tr>
<th>Media</th>
<th>Rate of organogenesis</th>
<th>Morphogenetic responses After 10 days</th>
<th>Morphogenetic responses After 3 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>B5 + BA(5 × 10⁻⁷ M)</td>
<td>16/20</td>
<td>Swelling of meristem and slight callus formation from the cut ends. Initiation of shoot formation</td>
<td>Well developed shoots. No root formation. Considerable callus formed at the cut end</td>
</tr>
<tr>
<td>B5 + BA(5 × 10⁻⁷ M) + NAA(10⁻⁶ M)</td>
<td>15/20</td>
<td>Very good callus formation. Initiation of shoot formation</td>
<td>Well developed shoots. No root formation in two cases multiple shoot formation was observed</td>
</tr>
<tr>
<td>B5 + NAA(10⁻⁵ M)</td>
<td>8/20</td>
<td>Low incidence of callus formation. Initiation of shoot formation. Signs of root formation apparent</td>
<td>Complete plants with root. No multiple shoots produced. Callus formation ceased</td>
</tr>
<tr>
<td>B5 + NAA(10⁻⁷ M)</td>
<td>10/20</td>
<td>Low incidence of callus formation. Initiation of shoot development</td>
<td>Rooting occurred only on two shoots. Others only shoot development.</td>
</tr>
<tr>
<td>B5 + NAA(10⁻⁸ M)</td>
<td>8/20</td>
<td>Very slight callus formation. Initiation of shoot development</td>
<td>Only 3 meristems developed shoots. No rooting occurred. Others remained dormant without further development</td>
</tr>
<tr>
<td>B5 + no growth substances</td>
<td>0/20</td>
<td>No development</td>
<td>All explants died</td>
</tr>
</tbody>
</table>

1) Average of three experiments; results shown as number of differentiating meristems/number of meristems cultured.

10⁻⁷ M concentration promoted root development in less than 25% of the shoot explants while 10⁻⁸ M did not have any effect, although the shoots remained green.

The half strength B5 medium supplemented with NAA was tested for meristem culture. In these experiments, NAA was employed at molar concentrations of 10⁻⁶, 10⁻⁷ and 10⁻⁸ respectively and the meristems were cultured under the same conditions as those grown in undiluted B5 medium. Over a period of 4 to 6 weeks the organ differentiation was very low at 10⁻⁶ M, and no growth occurred at the other two levels of NAA. From 20 meristems, only one developed into a complete plant. These results indicate that the nutrient concentrations may be critical for plant regeneration from meristems.

A proper ratio of cytokinin and auxin is necessary for morphogenesis leading to the development of a complete plant. The findings reported in this communication

Figs. 1–3: Plant development from the shoot apical meristems of *Pisum sativum* cv. Century on B5 medium.

Fig. 1: Differentiation of shoot only when BA ($5 \times 10^{-7}$ M) and NAA ($10^{-6}$ M) were jointly employed (3 week old plant). Note the lack of root formation.

Fig. 2: Production of complete plants when NAA was employed at a molar concentration of $10^{-6}$ (after 3 weeks).

Fig. 3: Induction of roots on shoots when recultured on 1/2 strength B5 medium supplemented with $10^{-6}$ M NAA (2 week old plant from the time of reculturing).

illustrate an exogenous cytokinin-independent morphogenesis and the production of complete pea plants with an exogenous supply of NAA alone. Failure to obtain root initiation on shoots using BA alone indicates that an exogenous supply of an auxin is necessary for root formation. It seems likely that meristematic cells at the stage of culturing either contain sufficient amounts of cytokinins or are able to synthesize adequate amounts to maintain the proper cytokinin-auxin ratio needed for shoot and root formation. When BA is applied jointly with NAA, the exogenous BA apparently causes an imbalance in the auxin/cytokinin ratio which probably may account for the failure of root initiation in the differentiated shoots.

The induction of rooting on differentiated shoots, when cultured on 1/2 strength B5 containing the auxin, suggests that the mineral salt concentration may be inhibiting root initiation. However, the behaviour of meristematic cells and shoot explants appears to be very distinct from one another as the former did produce roots at full strength B5. Experiments conducted using 1/2 strength B5 medium supplemented with NAA for culturing apical meristems did not yield encouraging positive results. This further indicates that the nutritional requirements of the meristematic cells during the early stages of differentiation are far different from those later in the development of a plant.
Acknowledgments

The authors thank Dr. B. HOLL for the seeds of cv. Laxton's Progress and Afghanistan. The expert technical assistance by Mr. J. P. SHYLUK and Mr. D. HORN is gratefully acknowledged.

References


Dr. K. K. KARTHA, Dr. O. L. GAMBORG, and Dr. F. CONSTABEL, Prairie Regional Laboratory, National Research Council, Saskatoon, Sask. Canada S7N OW9.