Influence of Rooting Substrates on the Morphology of Papaya Root Formed in vitro

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Abstract Effect of rooting substrates on the morphological characters of papaya roots formed in vitro was observed microscopically. The root primordium appeared near the vascular bundles six days after cutting in both agar and vermiculite media.

In the agar medium, the root number and length were lower than those in the vermiculite medium, while the root diameter was much larger. In both media, root hairs were observed on the root surface. The root hairs observed in the agar medium were much shorter and thicker than those in the vermiculite medium. The anatomical structure of the roots in both media was normal at the early stage of development. After 4 weeks of rooting, however, hypertrophy of subepidermal cells and occurrence of cracking in the epidermis of root were observed in the agar medium. On the other hand, no structural abnormalities of roots were observed in vermiculite medium.

Key words Micropropagation, Morphology, Papaya, Root, Rooting substrate

Introduction

To increase the production efficiency in mass propagation through tissue culture, it is very important to improve the rate of plantlet survival during acclimation as well as to enhance shoot proliferation and rooting in vitro. Besides environmental conditions such as humidity and light intensity, the quality of roots which had been determined in vitro before transplanting is known to markedly affect the survival rate\(^6,7\).

Although microcuttings were usually rooted in agar-solidified medium in papaya, it was difficult to acclimate the plantlets because they tended to degenerate after being transferred outside\(^3\). On the other hand, we found that the acclimation of plantlets could be readily achieved with a higher survival rate of plantlets even under ex vitro conditions, when using vermiculite as rooting substrate in place of agar\(^3,4\).

These facts suggest that the rooting substrate may induce considerable changes in the root quality which in turn affects the plantlet survival during acclimation.

In this study, to determine the effect of rooting substrates on the morphological characters of papaya roots, microscopic observation on roots formed in agar and vermiculite media was carried out.

Materials and Methods

Preparation of microcuttings Proliferation culture was established using the shoot tip of Carica papaya L. (dioecious strain, female) by the methods previously reported\(^3\) on MS medium containing 1 \(\mu\)M 6-benzylaminopurine, 3% sucrose and 0.8% agar. Single shoot tips 3 mm long were excised from the proliferating shoot

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mass and transferred to the medium supplemented with 2 μM 6-(r, r-dimethylallylaminomethyl)purine (2 iP). The cultures were grown at 27 °C under a 16-hr photoperiod by cool white fluorescent lamps at 40 μ mol/s/m². After 4 weeks of culture, the shoots were collected and prepared as microcuttings. Basal leaves were removed and three terminal leaves were left.

**Rooting medium and auxin treatment**

Hormone-free half-strength MS basal medium supplemented with 2% sucrose was used for rooting. As substrates for the rooting medium, agar and vermiculite were tested. Twenty ml of medium solidified with 1% of agar was added to a test tube (25 mm × 150 mm). On the other hand, ten ml of liquid medium was put into a test tube containing 25 ml of vermiculite (diameter of grains, 2.4–5 mm).

The base of the microcuttings was dipped in 10 mM indole-3 butyric acid (IBA) in 50% ethanol for 5 sec. Then the microcuttings were inserted into these rooting media. The rate of rooting was monitored weekly and the number, length and fresh weight of roots were determined after 4 weeks of rooting.

**Observation of initial process of rooting**

Microcuttings were sampled at 0, 3 and 6 days after insertion. Transverse hand-sections of the basal parts of the microcuttings were made and the development of the root primordia and vascular connection was observed using a light microscope after staining with 0.5% safranine solution.

**Anatomical observation of roots**

The root formed in the respective rooting media were collected each week. Transverse hand-sections of the roots at about 10 mm from root tips were prepared and then the inner structure of the root was observed under a light microscope after staining with safranine.

**Observation of surface structure by cryo-scanning electron microscopy (SEM)**

Roots were collected from the microcuttings after 3 weeks of culture. Immediately after excision, root samples about 5 mm long were taken at approximately 5 mm from root tips and mounted on aluminum stubs using silver paste. The samples were frozen in liquid nitrogen and then examined with a SEM (Hitachi, S 800) equipped with a cryo-system.

**Results**

Immediately after the preparation of the microcuttings, no root primordia was found (Fig. 1 a), while a marked accumulation of starch grains was observed in the xylem of the basal parts (Fig. 1 b). The density of the starch grains considerably decreased three days after insertion, but no evidence of root initiation was detected in any of microcuttings in the agar and vermiculite media (data not shown). In the microcuttings collected six days after insertion, actively developing root primordia were observed in both media. The site of origin of the primordia was identical in both media; the root primordium arose near the vascular bundle. Vascular connections between these root primordia and the vascular bundles of the cutting normally developed in both media (Fig. 1 c, d). In the vermiculite medium, the development of primordium resulted in the protrusion of the cortex tissue and caused a swelling of the basal part of the microcuttings (Fig. 1 c). On the other hand, the cortex of the microcutting in the agar medium formed a considerable amount of callus (Fig. 2 d).

After 4 weeks of rooting period, although all the microcuttings in both media eventually rooted, the number of roots was much smaller in the agar than in vermiculite medium, whereas the length of individual roots was almost the same. The fresh weight of the root system in the agar medium was much larger than that in the vermiculite (Table 1). In appearance, the roots in the agar medium were thick and less branched. The fact that the roots in the agar medium were thick was indicated by the date of length and fresh weight of the root.

The observation using cryo-SEM showed the presence of root hairs on the surface of the roots even in the agar medium. The roots in the vermiculite medium exhibited much longer root hairs than in the agar, but the density of the root hairs was considerably higher in agar medium (Fig. 2 a–d). In vermiculite, the epidermal cells of the roots were arranged regularly and their surface was very smooth (Fig. 2 e). In the roots formed in agar medium, on the contrary, the
Fig. 1 Development of root primordium of papaya microcuttings in different rooting substrates.
(a) Transverse section of stem base immediately after preparation of the microcuttings.
(b) Accumulation of starch grains in xylem (stained with I₂KI solution).
(c) Basal section of microcutting in vermiculite medium 6 days after cutting. Arrow shows the vascular connection.
(d) Basal section of microcutting in agar medium 6 days after cutting. Arrow shows the vascular connection.
CA; callus, CO; cortex, EP; epidermis, RP; root primordium, S; starch grains, VB; vascular bundle, X; xylem, (Bars=0.5mm).
Fig. 2  Surface structure of papaya roots formed in different rooting substrates observed by scanning electron microscopy.

(a,c) Distribution and development of root hairs on the roots formed in vermiculite medium.
(b,d) Distribution and development of root hairs on the roots formed in agar medium. Arrow shows the crack of epidermis and the cavity in subepidermal layer.
(e) Epidermal structure of root formed in vermiculite medium.
(f) Epidermal structure of root formed in agar medium.
Bar = 1,000µm in (a, b), 500µm in (c, d) and 100µm in (e, f).
Table 1  Effect of rooting substrate on root growth of papaya in vitro (after 4 weeks of rooting).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Rooting (%)</th>
<th>No. of roots/Plant</th>
<th>Total root length (Plant cm)</th>
<th>Average root length (cm)</th>
<th>Fresh weight of roots/Plant (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
<td>100</td>
<td>5.1 (2.1)</td>
<td>6.8 (1.6)</td>
<td>1.3 (0.3)</td>
<td>1.66 (0.38)</td>
</tr>
<tr>
<td>Vermiculite</td>
<td>100</td>
<td>14.1 (1.3)</td>
<td>19.6 (3.5)</td>
<td>1.4 (0.3)</td>
<td>0.44 (0.07)</td>
</tr>
</tbody>
</table>

\(^{2}\) Means±(95% confidence interval) N = 25

As observed in other species\(^{6}\), the decrease in the total amount of roots in agar medium which was associated with the reduced elongation and branching of the roots is likely to limit the absorption of water and nutrients, which may be one of the major reasons for the failure in acclimation of papaya plantlets.

In several species, the lack or poor development of the root hair was observed in the roots formed in the agar medium\(^{6,8}\). In this experiment, it was found that the length of the root hairs in the agar medium became considerably shorter but that density was much higher than in the vermiculite medium. Through the anatomical observation of root sections, a severe abnormality of the inner structure of the root consisting of hypertrophy of subepidermal cells accompanied by the unusual expansion of intercellular spaces was revealed. This observation suggests that defective water absorption may result from the difficulty in the movement of water absorbed by the root hairs to the vascular bundles of roots rather than the reduction in water absorption by the root hairs.

Another important structure involved in water absorption is the vascular connection between the roots and stem of the microcuttings. When the roots derived from the callus are formed at the base of the microcuttings, the vascular connections are known to be weak\(^{9}\). In this experiment, however, most of the root primordia derived from the adjacent area of the vascular bundles in the stem of the microcuttings and vascular connection developed well even in agar medium.

The contamination with fungi or bacteria is also an important factor involved in the decline of papaya plantlets rooted in agar medium during acclimation. To avoid this
Fig. 3 Inner structure of papaya roots formed in different rooting substrates.
(a) Whole area of transverse section of root formed in vermiculite medium.
(b) Magnified transverse section of root formed in vermiculite medium.
(c) Whole area of transverse section of root formed in agar medium.
(d) Magnified transverse section of root formed in agar medium. Arrows show the hypertrophied subepidermal cell (I) and the cavity beneath the epidermis (II).
CO: cortex, EP: epidermis, RH: root hair, X: Xylem
Bar = 0.5mm in (a) and (c), = 0.2mm in (b) and (d)

Table 2 Anatomical characters of roots formed in different rooting substrates (after 4 weeks of rooting)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Area (mm$^2$)</th>
<th>No. of cortical cell layer</th>
<th>Diameter of subepidermal cell (μm)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vascular</td>
<td>Cortical</td>
<td>Total</td>
</tr>
<tr>
<td>Agar ratio</td>
<td>0.28 (0.08)$^y$</td>
<td>1.13 (0.21)</td>
<td>1.54 (0.33)</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>73</td>
<td>100</td>
</tr>
<tr>
<td>Vermiculite</td>
<td>0.12 (0.05)</td>
<td>0.56 (0.10)</td>
<td>0.78 (0.17)</td>
</tr>
<tr>
<td>ratio</td>
<td>15</td>
<td>72</td>
<td>100</td>
</tr>
</tbody>
</table>

$^a$ Average longitudinal diameter of subepidermal cells
$^y$ Means±(95% confidence interval) N = 25
problem, the medium adhering to the root surface must be washed off before transplanting outside of the culture. However it is very difficult to completely remove the medium which had infiltrated the cavities of the cortex through the cracks of the root epidermis in the agar medium. Even if most of the medium could be removed, the bare parenchymatous cells of the cortex themselves may be easily attacked by fungi or bacteria.

As was reported in several species, the hypertrophy of the cortical cells and expansion of intercellular spaces are typical morphological changes found in plants under anaerobic conditions. The low gas exchanges in the agar medium could be one of the major causes of the development of the abnormal structure of the papaya roots formed there. To improve the root quality and increase the production efficiency of plantlets, it is essential to use a more permeable material like vermiculite as a substrate for rooting medium.

References