QUANTITATIVE EVALUATION OF ROOT SYSTEM BY IMAGE ANALYSIS AS AFFECTED BY O₂ CONCENTRATION IN NUTRIENT SOLUTION OF WATER CULTURE

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QUANTITATIVE EVALUATION OF ROOT SYSTEM BY IMAGE ANALYSIS AS AFFECTED BY O₂ CONCENTRATION IN NUTRIENT SOLUTION OF WATER CULTURE

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YOSHIDA S. and EGUCHI H. Quantitative evaluation of root system by image analysis as affected by O₂ concentration in nutrient solution of water culture. BIOTRONICS 16: 13–23, 1987. Root images of cucumber plants were digitized for computation. The computed image was used for analysis of elongation and branching of the roots grown in different O₂ levels in nutrient solution. The branching structure of root system was affected by O₂ level: Lower O₂ concentration resulted in inhibition of elongation of the second-order roots and in development of many branching of the third- and the fourth-order roots. However, the effect of O₂ level in the solution on top growth was scarcely found. From the results, it could be conceivable that root system develops with increase in branching roots to maintain physiological function in plants for adaptation to the root environment which is not optimized.

Key words: Cucumis sativus L.; cucumber plant; root system; root branching; water culture; O₂ concentration; image analysis; computed image.

INTRODUCTION

In root environment of water culture, O₂ concentration in nutrient solution is responsible for plant growth (1, 5, 9, 10, 13, 14), as well as root temperature (2, 3) and nutrition (4, 11, 12). Effect of O₂ concentration has been found in elongation and branching of the roots, as reported by Erickson (6) and Geisler (8). It seems that root system varies with O₂ level in the solution.

The present paper deals with computational image analyses of root structure of cucumber plants grown in different O₂ levels in the solution of water culture for better understanding of morphological feature of root system.

MATERIAL AND METHODS

Water culture system

A water culture system was designed and constructed to control O₂ concentration in nutrient solution. Figure 1 shows the water culture system: The culture system was composed of three solution tanks (a, b and c) and a magnet pump. A
Fig. 1. Schematic diagram of the water culture system: P, a magnet pump; a, b and c, solution tanks.

Plant was cultured in the tank c. Nutrient solution pumped up was led to the tank b for buffering the movement of the solution. The solution was aerated when it was circulated from the tank a to the tank b and from the tank b to the tank c. The pump was manipulated by on-off action of a time switch. A dissolved oxygen meter (UC-12, Central Kagaku Co., Ltd.) was used for measurement of the O₂ concentration in the solution.

Plant material

Cucumber plants (Cucumis sativus L. "Chojitsu-Ochiai") were used in the experiments. The seeds were sown in Vermiculite moistened with tap water. Plants were grown at air temperature of 23°C, relative humidity of 70%, and light intensity of 25 nE cm⁻² s⁻¹ (PPFD, metal halide lamps; Yoko lamp, DR400, Toshiba Corporation) in photoperiod of 12 h. A cotyledonary plant (8 days old) was transplanted to the culture system. Nutrient solution was composed of 606 mg of KN0₃, 826 mg of Ca(NO₃)₂·4H₂O, 114 mg of NH₄H₂P0₄, 492 mg of MgSO₄·7H₂O, and suitable microelements in 1 litre of water. The plant was grown in the solution with different O₂ concentrations. For the high level of O₂ concentration, the solution was circulated for 5 min at an interval of 5 min where circulation rate was about 60 litre h⁻¹. For the low level of O₂ concentration, N₂ gas was bubbled into the solution to reduce oxygen concentration before transplanting of plants where solution surface in the tank c was sealed off with a polyethylene film to prevent diffusion of O₂ into the solution, and the solution was not circulated. The effect of O₂ concentration on roots was examined by using 7 plants at 4 leaves stage.
RESULTS AND DISCUSSION

Oxygen concentration in the solution

Figure 2 shows O\textsubscript{2} concentrations in the tank c during culturing plants under aerated and non-aerated conditions. In the aerated solution, O\textsubscript{2} concentration was kept at the high level of 8.0 to 8.2 ppm. On the other hand, when N\textsubscript{2} gas was bubbled into the solution under the non-aerated condition, O\textsubscript{2} concentration decreased to about 1 ppm. Even in the non-aerated solution, the O\textsubscript{2} concentration became 4.8 ppm 6 days after transplanting, and thereafter decreased in course of time; it became finally 0.7 ppm 15 days after transplanting. Thus, in the non-aerated culture, O\textsubscript{2} concentration in the solution was kept at the low level of 0.7 to 4.8 ppm. The solution temperature was 23±0.5°C in the growth chamber.
Feature of growth

When plants were 4 leaves stage (17 days old after transplanting), the plant growth was examined at high and low O₂ levels, as listed in Table 1. Leaf area, dry weight and T-R ratio were slightly larger at high O₂ level than those at low O₂ level. The difference, however, was not significant between two treatments at 5% level. Thus, influence of O₂ level on growth of top and dry weight of roots was not found at the growing stage.

Root systems grown in the solution of high and low O₂ levels were placed on a plate as shown in Fig. 3. Root systems developed to the fourth-order roots at 4 leaves stage. So, main root was designated as r₁, and the branching root developed
ROOTS AFFECTED BY SOLUBLE $O_2$

Table 2. Number and length of the first-order root ($r_1$) and the second-order roots ($r_2$) grown in the solution of high and low $O_2$ levels:

$r_2$, the second-order roots with branching roots.

<table>
<thead>
<tr>
<th>$O_2$ level</th>
<th>Root</th>
<th>Number of roots</th>
<th>Total length (cm)</th>
<th>Mean length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>$r_1$</td>
<td>1</td>
<td>101.1</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>$r_2$</td>
<td>23</td>
<td>1143.5</td>
<td>49.7</td>
</tr>
<tr>
<td>Low</td>
<td>$r_1$</td>
<td>1</td>
<td>53.6</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>$r_2$</td>
<td>21</td>
<td>784.5</td>
<td>37.4</td>
</tr>
</tbody>
</table>

Fig. 4. Photographs of the longest second-order root ($r_2$) with branching roots ($r_3$ and $r_4$) in cucumber plants grown in the solutions of high (a) and low (b) $O_2$ levels.

from $r_1$ was designated as $r_2$. Thus, $r_3$ and $r_4$ were designated in the same way. Adventitious roots which appeared at upper part of the root system were designated as $r_a$. At low $O_2$ level, respective elongations of $r_1$ and $r_2$ were found to be inhibited, but remarkable developments of $r_3$ and $r_4$ were observed. Root hairs in $r_3$ and $r_4$ appeared much more than those in $r_1$ and $r_2$. Table 2 shows numbers and lengths
Fig. 5. Computed images of the longest second-order root \( r_2 \) shown in Figs. 4a and b) displayed on X-Y plotter device: Images of roots grown at high \( O_2 \) level, which were sampled with fine digitization (a) and simplified digitization (b), and grown at low \( O_2 \) level, which were sampled with fine digitization (c) and simplified digitization (d).
ROOTS AFFECTED BY SOLUBLE O₂

Table 3. Root length measured by using different digitizing plots in the longest second-order root (r₂), which were obtained from the computed images (Figs. 5a, b, c and d): Lr₂, length of r₂; ΣLr₃, total length of the third-order roots on r₂; ΣLr₄, total length of the fourth-order roots on r₂

<table>
<thead>
<tr>
<th>O₂ level</th>
<th>Number of digitizing plots</th>
<th>Fine digitization</th>
<th>Simplified digitization</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lr₂ (cm)</td>
<td>70.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ΣLr₃ (cm)</td>
<td>464.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ΣLr₄ (cm)</td>
<td>93.76</td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lr₂ (cm)</td>
<td>50.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ΣLr₃ (cm)</td>
<td>708.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ΣLr₄ (cm)</td>
<td>287.42</td>
</tr>
</tbody>
</table>

of r₁ and r₂ which developed r₃ and r₄. Lengths of r₁ and r₂ at high O₂ level were larger than those at low O₂ level. However, distinct difference in number of r₂ was not found between high and low O₂ levels. Thus, effect of O₂ level was clearly found in the structure of the root system, which was responsible for branching of r₃ and r₄ on the r₂.

Image processing of roots

For examination of branching roots, the longest r₂ with r₃ and r₄ was sampled, which was copied as shown in Fig. 4a and b. The copied image was illustrated by compositions with curved lines. The curved lines were digitized as mentioned above, and the data were transmitted to CPU through the interface. Figure 5 shows computed images of the r₃, which were displayed on X-Y plotter device. The computed image was composed of straight lines, and there were some differences in pattern between digitizing ways (number of digitizing plots). Table 3 shows root lengths obtained from the computed images shown in Fig. 5. The digitizing plots in the simplified digitization were about a half of those in the fine digitization. Even in the images which were sampled with the simplified digitization, morphological feature was sufficiently illustrated in both of the r₂ at high and low O₂ levels. The difference between measured values which were obtained by the two ways of fine and simplified digitizations was less than 5% of measured values in fine digitization. So, it was estimated that the simplified digitization was enough for image processing to measure the roots.

Branching structure

Figure 6 shows length (Lr₂) of the longest second-order root and total length (ΣLr₃) of r₂ and that (ΣLr₄) of r₄ branching from the r₂. ΣLr₃ at low O₂ level was slightly larger than that at high O₂ level, but the difference was not significant between two treatments at 5% level. On the other hand, Lr₂ at low O₂ level was smaller than that at high O₂ level, and there was significant difference between high and low O₂ levels at 5% level. However, ΣLr₄ at low O₂ level was larger than that at high O₂ level, and the difference was significant between high and low O₂ levels.

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Fig. 6. Length of the longest second-order root ($r_2$) and total lengths of the third-order roots ($r_3$) and the fourth-order roots ($r_4$) in $r_2$ grown in the solutions of high and low $O_2$ levels: $Lr_2$, length of $r_2$; $\Sigma Lr_3$, total length of $r_3$; $\Sigma Lr_4$, total length of $r_4$; HOL, high $O_2$ level; LOL, low $O_2$ level. Means calculated by using 7 plants in each treatment are plotted with 95% confidence intervals.

Fig. 7. Total root length (a) in the longest second-order root ($r_2$) and ratio (b) of the respective lengths of $r_2$, the third-order root ($r_3$) and the fourth-order root ($r_4$) to total root length in $r_2$ grown in the solutions of high (HOL) and low (LOL) $O_2$ levels: $Lr_2 + \Sigma Lr_3 + \Sigma Lr_4$, total root length in $r_2$; Means calculated from the total root length in 7 plants are plotted with 95% confidence intervals.
ROOTS AFFECTED BY SOLUBLE O₂

High O₂ level
Low O₂ level

Fig. 8. Distributions of the third-order roots (r₃) on the longest second-order root (r₂) grown in the solutions of high and low O₂ levels, where distributions of r₃ are shown by mean values of total length of r₃ on respective twenty divisions of r₂, and means of 7 plants are plotted with 95% confidence intervals: Lr₂, length of r₂; d, distance from the base of r₂ to branching point of r₃.

Fig. 9. Distributions of the fourth-order roots (r₄) on the longest second-order root (r₂) grown in the solutions of high and low O₂ levels, where distributions of r₄ are shown by mean values of total length of r₄ in respective twenty divisions of r₂, and means of 7 plants are plotted with 95% confidence intervals: Lr₂, length of r₂; d, distance from the base of r₂ to branching point of r₃ developing r₄.

at 1% level. Thus, at low O₂ level, inhibition of elongation of r₂ and promotion of branching of r₄ were observed.

Figure 7 shows total root length (a) in r₂ and ratio (b) of the respective lengths of r₂, r₃ and r₄ to the total root length. Total root length in r₂ at low O₂ level was larger than that at high O₂ level and the difference was significant between two treatments at 1% level. The ratio of length of r₄ at low O₂ level was higher than that at high O₂ level. Thus, development of r₄ resulted in remarkable increase in

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total root length at low $O_2$ level.

As mentioned above, significant difference in $\sum L_{r3}$ was not found between low and high $O_2$ levels. However, feature of branching of $r_3$ at low $O_2$ level appeared different from that at high $O_2$ level. In particular, the distribution of $r_3$ on $r_2$ was found to be clearly affected by $O_2$ level. Figure 8 shows distribution of $r_3$ on $r_2$, where the distribution was illustrated with sum of the length ($L_{r3}$) of $r_3$ branching on respective twenty divisions of $r_2$. At high $O_2$ level, $r_3$ distributed in the pattern where $L_{r3}$ decreased with longer distances from the base of $r_3$. At low $O_2$ level, $r_3$ distributed in broad pattern where $L_{r3}$ were larger at the middle position of $r_2$ than those near the base and tip of $r_2$. On the other hand, $\sum L_{r4}$ at low $O_2$ level was remarkably larger than that at high $O_2$ level as mentioned above. Furthermore, the difference in distribution of $r_4$ on the $r_2$ was found between different $O_2$ levels, as shown in Fig. 9. At high $O_2$ level, $r_4$ distributed at the basal position of $djL_{r2}$ in the region of 0 to 0.40. At low $O_2$ level, a lot of $r_4$ distributed in wider regions of $djL_{r2}$ from 0 to 0.85.

Thus, effect of low $O_2$ level was found in inhibition of $r_2$ and in development of many branching roots of $r_3$ and $r_4$.

From the results in this experiment, it was suggested that cucumber plants can grow even under low $O_2$ level in the range of 0.7 to 4.8 ppm, where the root structure develops to be adaptable for deficient condition of $O_2$. Fitter (7) has reported that there are large differences in the branching process depending upon soil moisture level, and the root systems subjected to water deficits have more branching roots as compared with those adequately supplied with water. In these viewpoints, it could be conceivable that root system develops with increase in branching roots in order to maintain enough physiological function in plants even if root environment is not optimized.

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