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出版情報：BIOTRONICS. 16, pp.13-23, 1987. Biotron Institute, Kyushu University

BIOTRONICS **16**, 13–23, 1987

QUANTITATIVE EVALUATION OF ROOT SYSTEM BY IMAGE ANALYSIS AS AFFECTED BY O_2 CONCENTRATION IN NUTRIENT SOLUTION OF WATER CULTURE

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(Received October 21, 1987; Accepted October 27, 1987)

YOSHIDA S. and EGUCHI H. *Quantitative evaluation of root system by image analysis as affected by O_2 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 O_2 levels in nutrient solution. The branching structure of root system was affected by O_2 level: Lower O_2 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 O_2 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 optimalized.

Key words: *Cucumis sativus* L.; cucumber plant; root system; root branching; water culture; O_2 concentration; image analysis; computed image.

INTRODUCTION

In root environment of water culture, O_2 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 O_2 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 O_2 level in the solution.

The present paper deals with computational image analyses of root structure of cucumber plants grown in different O_2 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 O_2 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

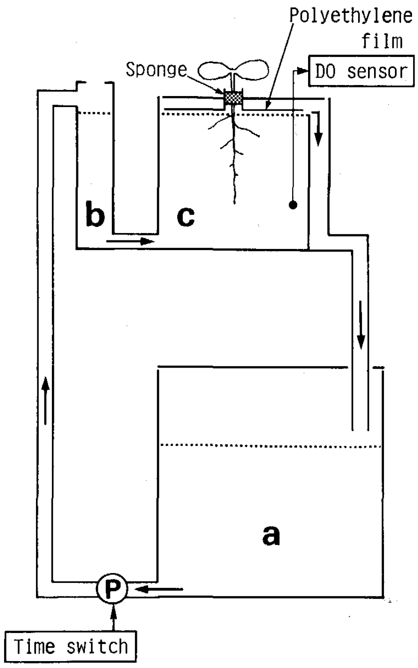

Fig. 1. Schematic diagram of the water culture system: P, a magnet pump; a, b and c, solution tanks.

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 O_2 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 KNO_3, 826 mg of $Ca(NO_3)_2 \cdot 4H_2O$, 114 mg of $NH_4H_2PO_4$, 492 mg of $MgSO_4 \cdot 7H_2O$, and suitable microelements in 1 litre of water. The plant was grown in the solution with different O_2 concentrations. For the high level of O_2 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 O_2 concentration, N_2 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 O_2 into the solution, and the solution was not circulated. The effect of O_2 concentration on roots was examined by using 7 plants at 4 leaves stage.

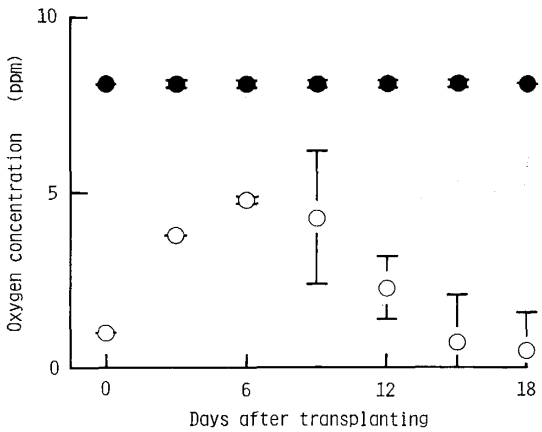

Fig. 2. O_2 concentration in aerated (●) and non-aerated (○) solutions during culturing cucumber plants: Means of the O_2 concentration measured three times are plotted with 95% confidence intervals.

Table 1. Leaf area, dry weight and *T-R* ratio of cucumber plants grown in the solutions of high and low O_2 levels: Respective 95% confidence limits are listed in parentheses.

O_2 level	Leaf area (cm²/plant)	Dry weight (g/plant)			*T-R* ratio (dry weight)
		Leaves	Top	Roots	
High	868.1	2.61	3.20	0.38	8.57
	(88.7)	(0.89)	(1.13)	(0.20)	(1.48)
Low	863.5	2.47	3.01	0.37	8.53
	(72.7)	(0.47)	(0.63)	(0.17)	(2.26)

Measurement of roots

Sampled roots were extended on a glass plate and were copied with a plain paper copier. The image of roots was digitized with a digitizer (KD4030A, Graphtec Corp.) and was transmitted to CPU through the interface. Data were analyzed about length and branching of roots.

RESULTS AND DISCUSSION

Oxygen concentration in the solution

Figure 2 shows O_2 concentrations in the tank c during culturing plants under aerated and non-aerated conditions. In the aerated solution, O_2 concentration was kept at the high level of 8.0 to 8.2 ppm. On the other hand, when N_2 gas was bubbled into the solution under the non-aerated condition, O_2 concentration decreased to about 1 ppm. Even in the non-aerated solution, the O_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_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.

a

b

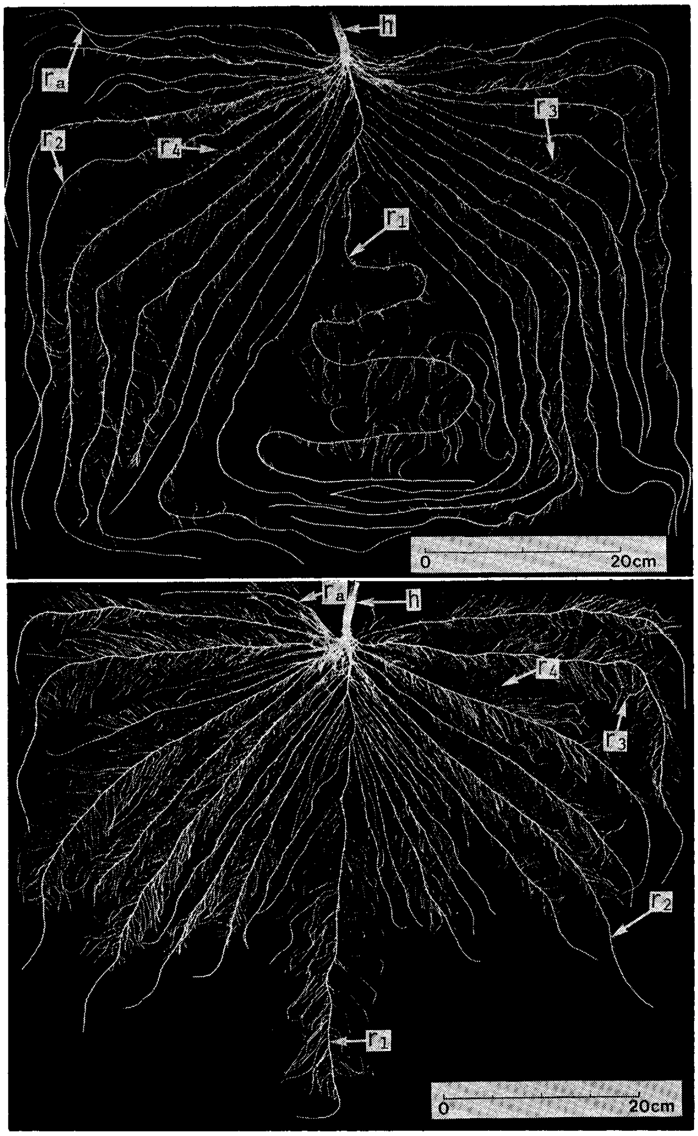

Fig. 3. Photographs of root systems of cucumber plants grown in the solutions of high (a) and low (b) O_2 levels: r_1, the first-order root; r_2, the second-order root; r_3, the third-order root; r_4, the fourth-order root; r_a, adventitious root; h, hypocotyl.

Feature of growth

When plants were 4 leaves stage (17 days old after transplanting), the plant growth was examined at high and low O_2 levels, as listed in Table 1. Leaf area, dry weight and T-R ratio were slightly larger at high O_2 level than those at low O_2 level. The difference, however, was not significant between two treatments at 5% level. Thus, influence of O_2 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 O_2 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 r_1, and the branching root developed

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.

O_2 level	Root	Number of roots	Total length (cm)	Mean length (cm)
High	r_1	1	101.1	—
	r_2	23	1143.5	49.7
Low	r_1	1	53.6	—
	r_2	21	784.5	37.4

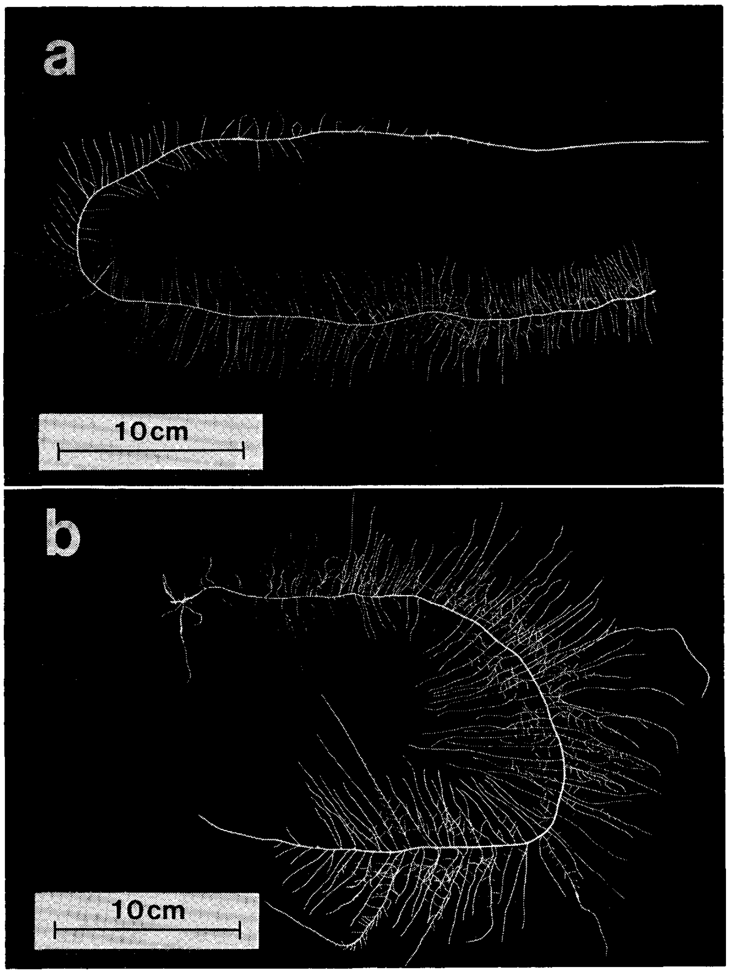

Fig. 4. Photographs of the longest second-order root (r_2) with branching roots (r_3 and r_4) in cucumber plants grown in the solutions of high (a) and low (b) O_2 levels.

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

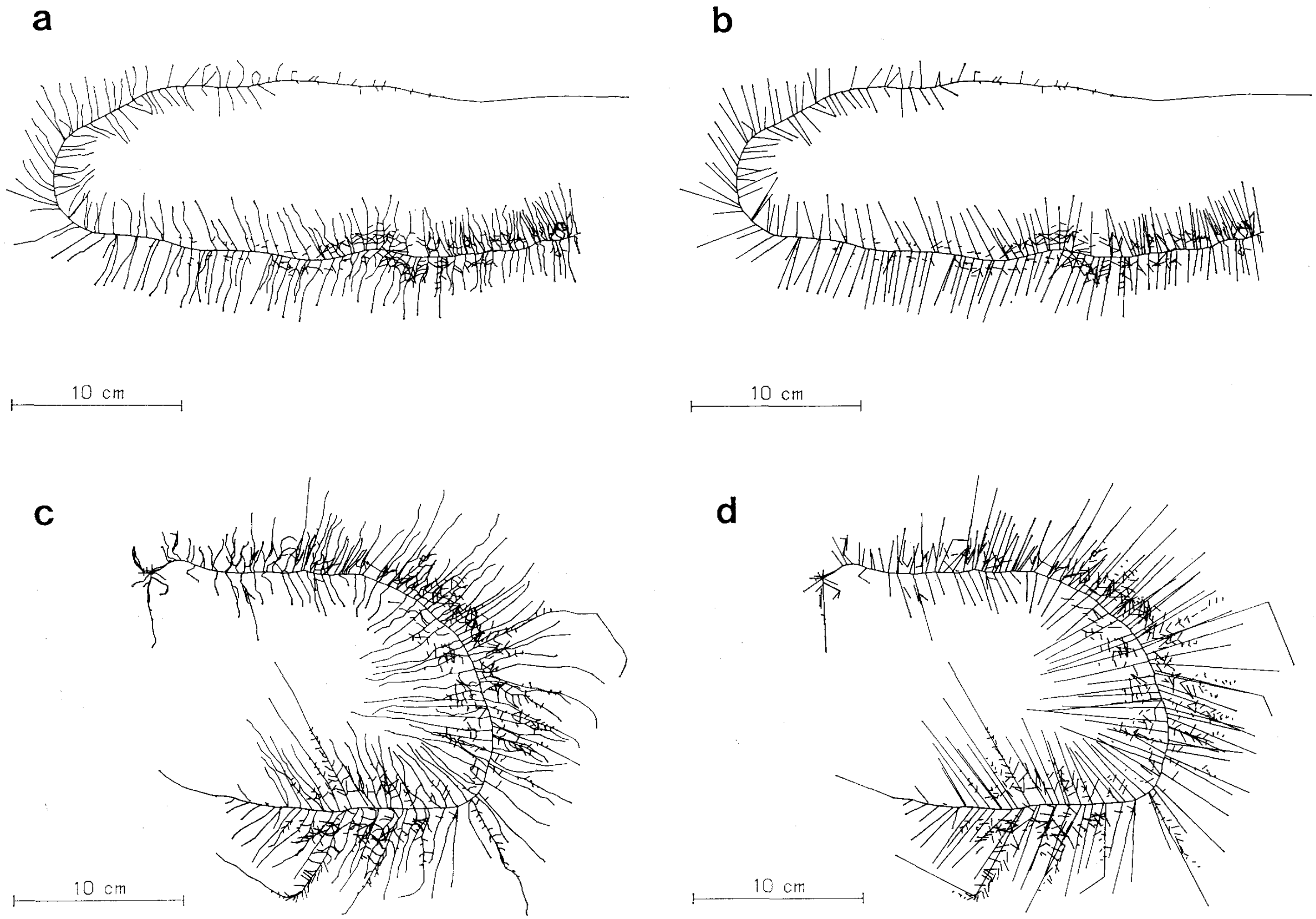

Fig. 5. Computed images of the longest second-order root (r_2 shown in Figs. 4a and b) displayed on X-Y plotter device: Images of roots grown at high O_2 level, which were sampled with fine digitization (a) and simplified digitization (b), and grown at low O_2 level, which were sampled with fine digitization (c) and simplified digitization (d).

Table 3. Root length measured by using different digitizing plots in the longest second-order root (r_2), which were obtained from the computed images (Figs. 5a, b, c and d): Lr_2, length of r_2; $\sum Lr_3$, total length of the third-order roots on r_2; $\sum Lr_4$, total length of the fourth-order roots on r_2

O_2 level			Fine digitization	Simplified digitization
High	Number of digitizing plots		2472	1291
	Lr_2	(cm)	70.06	70.79
	$\sum Lr_3$	(cm)	464.41	456.09
	$\sum Lr_4$	(cm)	93.76	90.66
Low	Number of digitizing plots		3821	1898
	Lr_2	(cm)	50.20	50.10
	$\sum Lr_3$	(cm)	708.94	681.33
	$\sum Lr_4$	(cm)	287.42	274.27

of r_1 and r_2 which developed r_3 and r_4. Lengths of r_1 and r_2 at high O_2 level were larger than those at low O_2 level. However, distinct difference in number of r_2 was not found between high and low O_2 levels. Thus, effect of O_2 level was clearly found in the structure of the root system, which was responsible for branching of r_3 and r_4 on the r_2.

Image processing of roots

For examination of branching roots, the longest r_2 with r_3 and r_4 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 r_2, 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 r_2 at high and low O_2 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 (Lr_2) of the longest second-order root and total length ($\sum Lr_3$) of r_3 and that ($\sum Lr_4$) of r_4 branching from the r_2. $\sum Lr_3$ at low O_2 level was slightly larger than that at high O_2 level, but the difference was not significant between two treatments at 5% level. On the other hand, Lr_2 at low O_2 level was smaller than that at high O_2 level, and there was significant difference between high and low O_2 levels at 5% level. However, $\sum Lr_4$ at low O_2 level was larger than that at high O_2 level, and the difference was significant between high and low O_2 levels

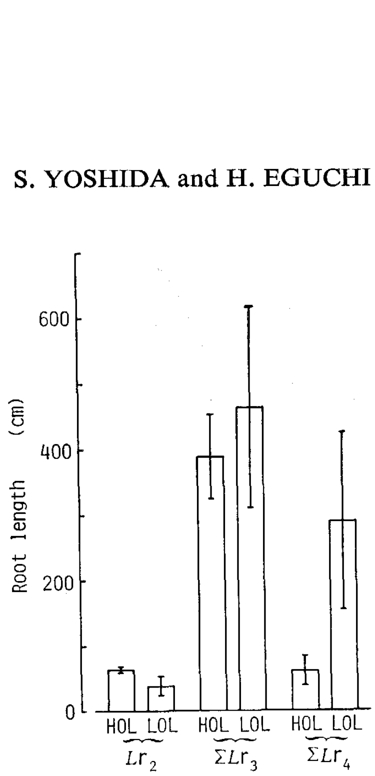

Fig. 6. Length of the longest second-order root (r_2) and total lengths of the third-order roots (r_3) and the fourth-order roots (r_4) in r_2 grown in the solutions of high and low O_2 levels: Lr_2, length of r_2; $\sum Lr_3$, total length of r_3; $\sum Lr_4$, total length of r_4; HOL, high O_2 level;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

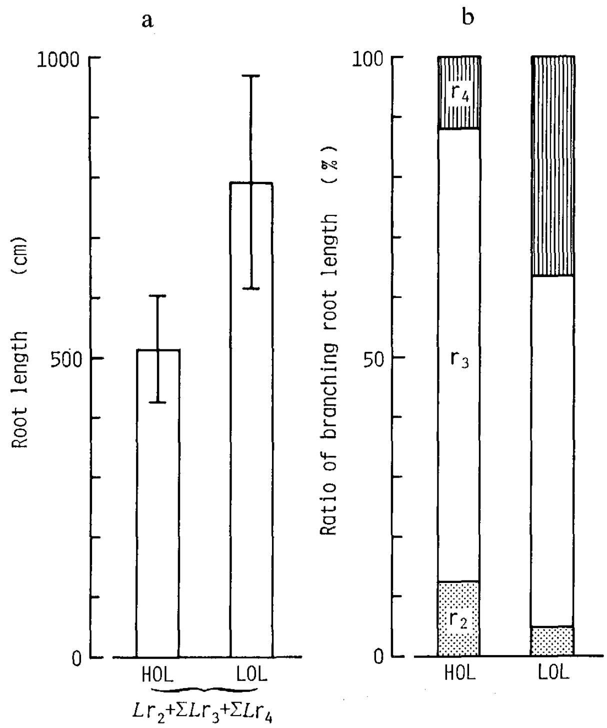

Fig. 7. Total root length (a) in the longest second-order root (r_2) and ratio (b) of the respective lengths of r_2, the third-order root (r_3) and the fourth-order root (r_4) to total root length in r_2 grown in the solutions of high (HOL) and low (LOL) O_2 levels: $Lr_2+\sum Lr_3+\sum Lr_4$, total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

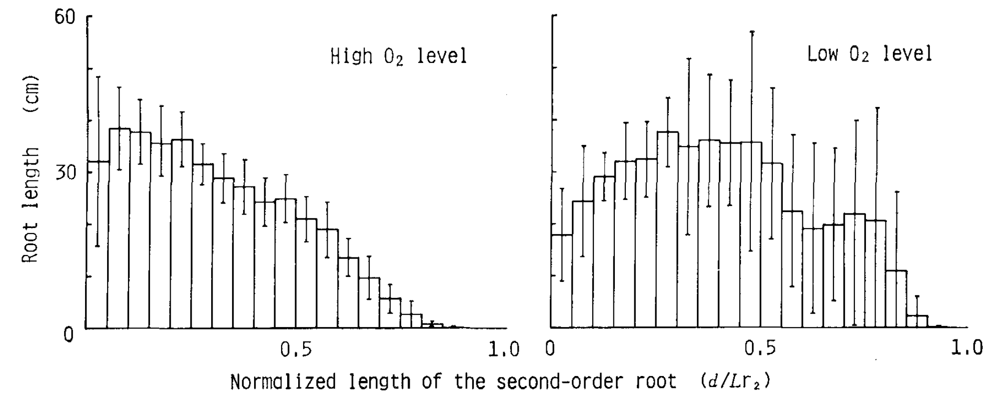

Fig. 8. Distributions of the third-order roots (r_3) on the longest second-order root (r_2) grown in the solutions of high and low O_2 levels, where distributions of r_3 are shown by mean values of total length of r_3 on respective twenty divisions of r_2, and means of 7 plants are plotted with 95% confidence intervals: Lr_2, length of r_2; d, distance from the base of r_2 to branching point of r_3.

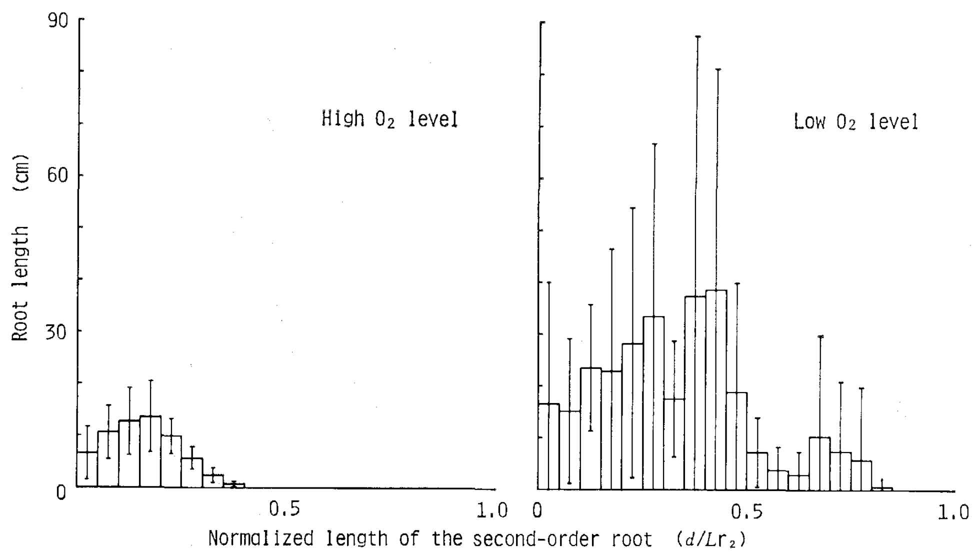

Fig. 9. Distributions of the fourth-order roots (r_4) on the longest second-order root (r_2) grown in the solutions of high and low O_2 levels, where distributions of r_4 are shown by mean values of total length of r_4 in respective twenty divisions of r_2, and means of 7 plants are plotted with 95% confidence intervals: Lr_2, length of r_2; d, distance from the base of r_2 to branching point of r_3 developing r_4.

at 1% level. Thus, at low O_2 level, inhibition of elongation of r_2 and promotion of branching of r_4 were observed.

Figure 7 shows total root length (a) in r_2 and ratio (b) of the respective lengths of r_2, r_3 and r_4 to the total root length. Total root length in r_2 at low O_2 level was larger than that at high O_2 level and the difference was significant between two treatments at 1% level. The ratio of length of r_4 at low O_2 level was higher than that at high O_2 level. Thus, development of r_4 resulted in remarkable increase in total root length at low O_2 level.

As mentioned above, significant difference in $\sum Lr_3$ 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 (Lr_3) of r_3 branching on respective twenty divisions of r_2. At high O_2 level, r_3 distributed in the pattern where Lr_3 decreased with longer distances from the base of r_2. At low O_2 level, r_3 distributed in broad pattern where Lr_3 were larger at the middle position of r_2 than those near the base and tip of r_2. On the other hand, $\sum Lr_4$ 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 d/Lr_2 in the region of 0 to 0.40. At low O_2 level, a lot of r_4 distributed in wider regions of d/Lr_2 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 optimalized.

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