

Analysis of Timecourse Changes in Root System Morphology of Rice in Excised Root Culture

Yasuhiro IZUMI, Yasuhiro KONO, Akira YAMAUCHI
and Morio IJIMA

(School of Agriculture, Nagoya University, Chikusa, Nagoya 464-01, Japan)

Received January 31, 1995

Abstract : The development of cultured seminal root system of rice was analyzed to determine the most appropriate culture period for the characterization of root system morphology. Excised seminal root tips of Taichung Native 1 (TN-1, indica type) and Yukara (japonica type), were cultured for six weeks with the conventional tissue culture method, and their growth was evaluated with developmental, topological and fractal analyses. Developmental root growth parameters, such as the number and length of each root system component, reached almost maximum values at the fourth week in TN-1, and the third week in Yukara. On the other hand, it was possible to characterize the root system morphology by the branching densities of L- and S-type first order lateral roots and the average length of S-type first order lateral roots at the third week. In the timecourse changes of a topological parameter ($\log a/\log \mu$), which indicates the branching pattern, the difference between cultivars became clear at the third week. The fractal dimension (D), which indicates the complexity of root morphology, was higher in Yukara than in TN-1 throughout the experimental period. This suggested the possibility for characterizing the root system morphology of each cultivar by the D value. Therefore, we concluded that the most appropriate sampling time for the morphological research of cultured seminal root system was three weeks when the conventional excised root culture method is used.

Key words : Excised root culture, Fractal, *Oryza sativa* L., Rice, Root system development, Timecourse change, Topology.

根端培養系における水稻根系の経時的形態変化の解析 : 泉 泰弘・河野恭廣・山内 章・飯嶋盛雄 (名古屋大学農学部)

要 旨 : 本研究では、従来からの定法に従って水稻の種子根端を培養する場合における根系形態の特徴を把握するのに最適な培養期間を決定することを目的とした。培養にはインド型水稻品種、台中在来1号 (TN-1) と日本型水稻品種、ユーカラを供試し、根系の定量的解析とともにトポロジーおよびフラクタル解析によって根系発達を6週間にわたって追跡した。量的形質のうち根数・根長は TN-1 で4週目、ユーカラで3週目ではほぼ一定の値に達した。一方、側根の発生・発達に関する形質のうち、L型・S型1次側根の発根密度およびS型1次側根の平均長を用いることによって、3週目に各品種の根系が持つ形態的特徴を把握できることが明らかとなった。さらに根系の分枝パターンを示す指標となるトポロジー指数のうち、 $\log a/\log \mu$ 比においては3週目以降に両品種の差異が明確に認識された。根系形態の複雑さの指標であるフラクタル次元 (D) は、全培養期間を通じて常にユーカラの方が TN-1 よりも大きくなっており、この値によっても根系構造の特徴を把握できる可能性が示唆された。これらの結果を総合して、本研究で採用した培養条件下では、3週目に採取した水稻の培養種子根系において形態的特徴を調査するのが妥当であると結論した。またフラクタル解析・トポロジー解析がそのために有効な手法であることも明らかとなった。

キーワード : 経時変化, 根系発達, 根端培養法, 水稻, トポロジー, フラクタル。

The excised root culture method is useful for evaluating characteristics of root system structure and comparing it among different plant genotypes because complicated shoot-root interactions are eliminated and growing conditions of the roots can be simplified^{2,8)}.

Since the excised root culture method for rice was established by Kawata *et al.*¹⁰⁾, several studies on the development of seminal root system, which is defined as the seminal root axis and lateral roots, have been made by using the same method, or a modified

one^{10,11,12)}. In those studies, root growth was evaluated based only on seminal root axis length and root dry weight. However, to comprehend the morphological characteristics of the rice root system, it is crucial to take into account the development of the lateral roots because they constitute the major part of the total root surface area^{9,16)}. The nutrient acquisition capacity of plants should also be understood in terms of the quantitative growth and spatial extension of the roots¹⁾. However, the development of seminal root system including

emergence and elongation of lateral roots, and their duration of development *in vitro* have not been investigated so far.

Thus, we used two rice cultivars and monitored the development of all root system components over six weeks. The culture conditions were the same with those described in previous works^{10,11,12)} except that the culture period was longer. In addition to developmental analysis such as number and length of lateral roots, the changes of fractal dimension and topological parameters were also traced for the same periods. Based on these results, we also attempted to determine the appropriate sampling period to elucidate the characteristics of root morphology in a cultured seminal root system of respective rice cultivar for further researches.

Materials and Methods

In this experiment, we used two rice cultivars, Taichung Native 1 (TN-1, indica type) and Yukara (japonica type). Two-day-old seminal root tips which were grown on agar were excised to 1-cm-length. Then these root tips were transferred to the culture medium and cultured as described elsewhere⁸⁾. Thirty replicate flasks, each of which contained two root samples, (60 root samples) were used. Subsequently, ten seminal root systems were sampled weekly until six weeks after transfer to the culture medium. The pH and nitrate concentration of the medium were determined with pH meter (HORIBA, F-14) and ion meter (HORIBA, C-141), respectively at each sampling.

1. Developmental analysis

In all the root system components, length was measured with ruler and a sketch of the root system was drawn. Upon the measurement of the sampled roots, lateral roots were classified into L-type and S-type⁸⁾. From the measured values, total root number and length were determined. Furthermore, branching density (roots/cm) of first order lateral roots on the seminal root axis were also calculated.

2. Topological analysis

The basic idea for this analysis is to characterize the plant root system structure in the aspect of branching pattern⁴⁾. In the topological analysis, it is considered there are two extreme branching patterns, namely, the

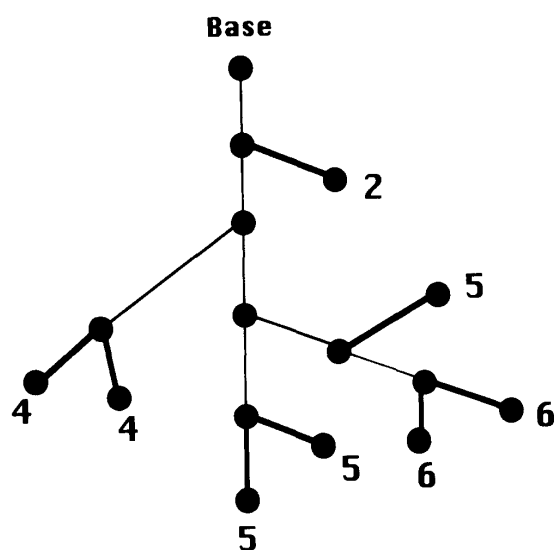


Fig. 1. Schematic diagram of the topological structure of a root system. Link is defined as the linear portion between two black circles. The thick one is called the external link and the fine one is called the internal link. The magnitude (μ) is defined as the number of external links. Number adjacent to root tip shows the pathlength which is the number of links from each external link to the base. The total pathlength (Pe) is calculated as the sum of pathlength from all the external links. The altitude (a) is the longest pathlength within a system. In this root system, μ , Pe and a is 8, 37 and 6, respectively.

herringbone type in which all lateral roots are emerged primarily from the main axes and dichotomous type in which the branch initiations occur with equal probability on all external links which are explained later. Every plant root system has a root structure between these two types, and the characterization is based on quantification of how the branching pattern of a root system is closer to either of them.

We followed the method for topological analyses described by Fitter^{4,5)}. He considered a root system to be a mathematical tree and constituted by links, which are defined as linear portions from the root tip to the branching point, or between the two branching points (Fig. 1). The former is called an "external link" and the latter an "internal link". Magnitude (μ) of root system is defined as the total number of external links. This is identical to the total root number, which have often been used for root system characterization. Total

pathlength (Pe) is the sum of the pathlength (the number of links from the root tip to base) from all external links. The altitude (a) is the longest pathlength within a system. The following topological parameters are calculated using these values.

Fitter^{4,5)} characterized the topology of the root system based on the slope of regression line between a or Pe in relation to μ on log scale, and reported that the larger the slope, the closer a root system is to a herringbone type. Instead, we calculated $\log a/\log \mu$ and $\log Pe/\log \mu$ ratios and used the mean values of these ratios at each sampling as topological parameters because significant correlations were not obtained in most cases. Nevertheless, the value was found to be useful to express the root branching pattern. The $\log a/\log \mu$ ratio is affected by the degree of higher order branching. When the branching pattern of a root system shows a complete herringbone type, the ratio is maximum ($=1$), and as the ratio decreases, the branching pattern becomes more complicated and closed to dichotomous type. The $\log Pe/\log \mu$ ratio depends on the position of the emergence of higher order branching. The increase of the ratio means that higher order branching lateral roots mainly emerge near the root tips of main axis.

3. Fractal analysis

Fractal analysis is also a mathematical method to evaluate the complexity of the shape of object, and it is recently used for the characterization of root system^{3,6,13,14,15)}. Fractal structure means that a shape has self-similarity. Fractal dimension (D) is a parameter of morphological complexity on the

root system analysis. In the two-dimensionally-expanded root system, the D value ranges between one and two. The D value of two means that the morphology has the most complex shape¹⁴⁾.

In this study, we followed the procedure of Tatsumi¹⁵⁾ for the fractal analysis. Stained seminal root systems were two-dimensionally spread in A4 size in the way that lateral roots did not overlap each other, and they were converted to digitized image with 256 gray levels and 100 DPI resolution using image scanner (Epson GT-6000). Image analyses were done with Image ver. 1.44 (NIH) with a MacintoshTM computer. No skeletonizing was held. Root systems were fractal when pixel size was in the range from 0.254 mm to 16.256 mm. D value was obtained based on the slope of regression line of the counted pixel number in relation to the scale of pixel when they were plotted on log scale.

Results

Seminal root axis length of TN-1 significantly increased from the first to the fourth week, but no significant differences were noted afterwards (Table 1). The same trend was found in the number and length of whole root system. On the other hand, the growth of Yukara's excised root tips was inferior to that of TN-1; the mean values in all three root characters were much lower in Yukara than in TN-1. However, the trends of increase in seminal root axis length and total root number in Yukara were similar to those in TN-1. The mean values of these root growth parameters were significantly different between the third and the sixth week. This means that there was

Table 1. Timecourse changes in seminal root axis length (SRAL), total root number (TRN) and total root length (TRL) of cultured root system of two rice cultivars.

Cultivars	Developmental characters	Culture period					
		1	2	3	4	5	6
TN-1	SRAL(mm)	54 a	218 b	340 c	445 d	506 d	494 d
	TRN	32 a	241 b	574 c	849 d	994 d	950 d
	TRL(mm)	236 a	836 b	1968 c	2727 d	3145 d	3118 d
Yukara	SRAL(mm)	47 a	58 ab	74 bc	80 cd	88 cd	93 d
	TRN	71 a	94 a	132 b	168 bc	160 bc	180 c
	TRL(mm)	311 a	771 b	1119 c	1247 c	1241 c	1228 c

Same letters adjacent to each value mean no significant differences among sampling periods at 5% level according to Duncan's multiple range test.

an increase during this period. The total root length, however, did not show an increase of this kind after the third week.

The number and length of lateral roots are shown in Table 2. The L-type first order lateral roots in TN-1 rapidly increased in number and length from the second to the third week, but afterwards their number and length did not significantly increase. The S-type lateral root growth showed the same pattern of increase as that of the seminal root axis length shown in Table 1. In contrast, the seminal root system of Yukara produced only a few L-type first order lateral roots. Their number and length reached constant values at the fourth week. The S-type lateral roots ceased to increase in number and length at the third week. The growth pattern of second order lateral roots was almost similar to that of their mother root, i.e., L-type first order lateral

roots.

Tables 3 and 4 show the average length and branching densities of L and S-type lateral roots per cm on the seminal root axis. After the second week, no significant change in the branching density of L-type first order lateral roots was observed in both cultivars. The branching density of S-type showed an almost constant value except between the first and the second week in TN-1 (Table 3). The average length of S-type first order lateral roots changed from the first to the second week, then, it did not change significantly until the end of the experiment in both cultivars (Table 4). Not all samples produced of L-type first order lateral roots at the second week in TN-1, and at the second and the third week in Yukara. Their average length did not change after the first week in TN-1, but increased until the sixth week in Yukara.

Table 2. Timecourse changes in lateral root number and length (mm) of cultured root system of two rice cultivars.

Cultivars	Type	Developmental characters	Culture period					
			1	2	3	4	5	6
TN-1	1st-L	Number	0 a	1 a	16 b	20 bc	27 c	26 c
		Length	0 a	30 a	464 b	655 bc	915 c	881 c
	1st-S	Number	31 a	229 b	374 c	497 d	546 d	546 d
		Length	181 a	580 b	1008 c	1297 d	1363 d	1381 d
	2nd	Number	0 a	10 a	183 b	331 c	420 c	377 c
		Length	0 a	8 a	156 b	330 c	361 c	361 c
Yukara	1st-L	Number	0 a	0 a	2 b	3 c	3 c	3 c
		Length	0 a	5 a	38 b	83 bc	77 bc	95 c
	1st-S	Number	70 a	91 b	104 c	115 c	117 c	127 c
		Length	264 a	707 b	957 c	999 c	1011 c	962 c
	2nd	Number	0 a	2 a	26 ab	49 b	39 b	49 b
		Length	0 a	1 a	50 ab	85 b	66 b	78 b

1st-L, L-type first order lateral root ; 1st-S, S-type first order lateral root ; 2nd, second order lateral root. Alphabet letters are the same as those in Table 1.

Table 3. Timecourse changes in branching densities (roots/cm) of first order lateral roots of cultured root system of two rice cultivars.

Cultivars	Type	Culture period					
		1	2	3	4	5	6
TN-1	1st-L	0.0 a	0.1 a	0.5 b	0.5 b	0.5 b	0.6 b
	1st-S	5.7 a	10.4 b	11.1 b	11.2 b	10.8 b	10.8 b
Yukara	1st-L	0.0 a	0.1 ab	0.2 bc	0.3 b	0.3 c	0.3 c
	1st-S	14.9 a	15.7 a	14.1 a	14.5 a	13.5 a	14.3 a

1st-L, L-type first order lateral root ; 1st-S, S-type first order lateral root. Alphabet letters are the same as those in Table 1.

Table 4. Timecourse changes in average length (mm) of first order lateral roots of cultured root system of two rice cultivars.

Cultivars	Type	Culture period					
		1	2	3	4	5	6
TN-1	1st-L	N.E.	29.9 *	29.3	32.4	33.7	33.5
	1st-S	6.4 b	2.5 a	2.7 a	2.7 a	2.5 a	2.6 a
Yukara	1st-L	N.E.	12.3 *	25.5 *	31.7	31.8	39.4
	1st-S	3.8 a	7.8 b	9.6 b	9.2 b	9.0 b	8.0 b

1st-L, L-type first order lateral root; 1st-S, S-type first order lateral root; N.E., Not emerged. Letters with asterisk (*) show that some root systems with no L-type first order lateral roots were excluded for calculation of mean values, hence the multiple range test was not applied. Alphabet letters are the same as those in Table 1.

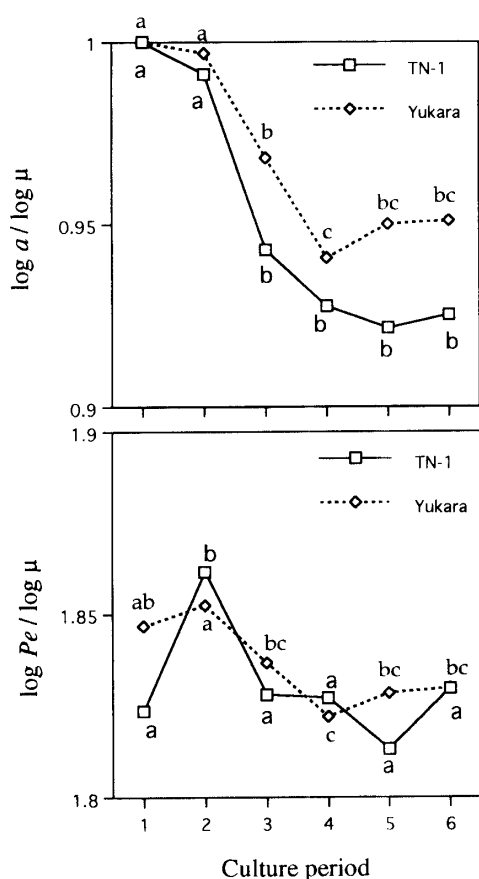


Fig. 2. Timecourse changes in topological parameters, $\log a / \log \mu$ (upper), $\log Pe / \log \mu$ (lower) of cultured root systems of two rice cultivars. Alphabet letters in figures are the same as those in Table 1.

The timecourse changes in topological parameters and fractal dimension are shown in Figs. 2 and 3. The topological parameter, $\log a / \log \mu$ ratio, significantly decreased from the second to the third week in TN-1, and from the second to the fourth week in Yukara

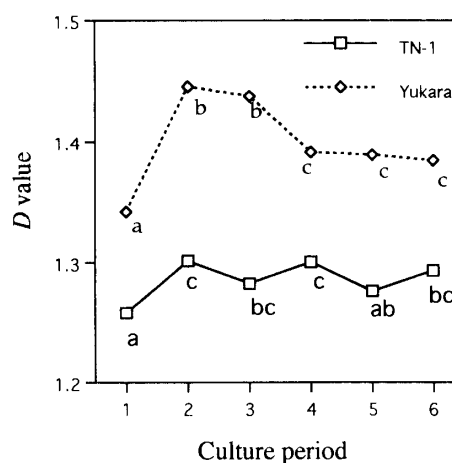


Fig. 3. Timecourse changes in fractal dimension (D) of cultured root systems of two rice cultivars. Alphabet letters in figure are the same as those in Table 1.

(Fig. 2-upper). This ratio was mostly higher in Yukara than in TN-1, except for the first week when all root systems were herringbone type (no second order lateral roots). The $\log Pe / \log \mu$ ratio did not show any significant changes and was relatively similar for both cultivars after the third week (Fig. 2-lower). Fractal dimension (D) was always higher in Yukara compared to TN-1 throughout the experiment (Fig. 3). The D value was significantly elevated from the first to the second week in both cultivars. Thereafter, the D value significantly decreased from the third to the fourth week in Yukara, while relatively small fluctuation of the D value was found in TN-1.

The pH values and nitrate concentration of growth medium are shown in Fig. 4. The pH drastically increased in the initial two weeks,

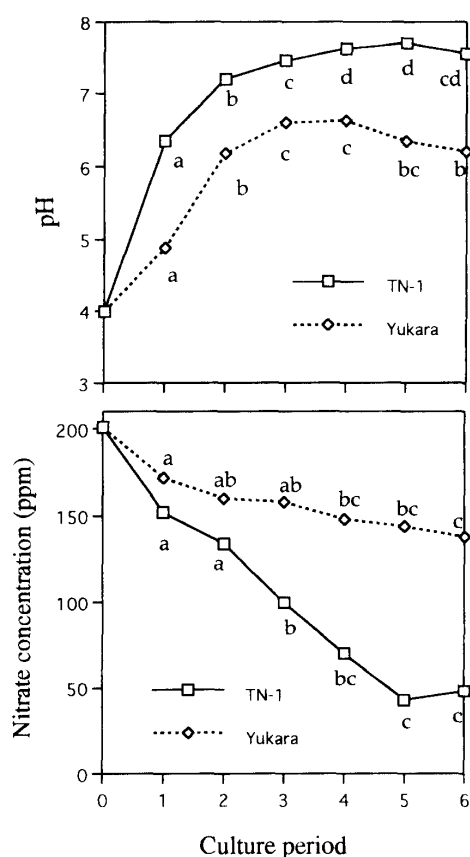


Fig. 4. Timecourse changes in pH (upper) and nitrate concentration (lower) of the culture medium with growth of cultured root of two rice cultivars. Alphabet letters in figures are the same as those in Table 1.

and then a moderate increase followed (Fig. 4-upper). The increase would be attributed mainly to the absorption of nitrate ions, which is a sole nitrogen source in the culture medium. A rapid absorption of nitrate ions by root tips of TN-1 took place earlier than Yukara, and the total consumption was also greater in the former cultivar (Fig. 4-lower).

Discussion

In this study, we analyzed the morphology of excised-cultured seminal root system of rice focusing particularly on the lateral root development with three different analytical approaches, i.e., developmental, topological and fractal analyses.

First, the root growth was evaluated by the length and number of different root system components, such as seminal root axis, and L and S-type lateral roots. Based on these parameters, the quantitative development of the seminal root system was found to be stabil-

ized at the fourth week in TN-1 and the third week in Yukara (Tables 1 and 2). The factors that mainly affected the root development, however, would presumably not be the same between the two cultivars. In the case of TN-1, it is assumed that root growth was limited by either space or nutrients because the flask was filled with the seminal root systems during the later stage of the growth. In contrast, root growth of Yukara was probably limited by relatively low capacity to utilize nitrate ions. Some researchers pointed out that nitrogen metabolism of excised-cultured root system differed among several rice cultivars especially between indica and japonica types^{7,11,12}). We also reported previously that the growth of excised root of Yukara depended on organic nitrogen originally contained in the excised root segment more than in TN-1⁸). In this aspect, it is assumed that Yukara's roots were not able to absorb or assimilate nitrate ions from the medium to sustain growth during later growth stages. The change of pH value and concentration of nitrate ions supported this assumption (Fig. 4).

Yamauchi *et al.*^{16,17}) found that the lateral root development of different branching orders and different types (L and S-type) is one of the major factors to characterize root system morphology. In this study, the branching density of the first order lateral roots almost reached stable at the third week in both cultivars (Table 3). Thereafter, the density of TN-1 was higher in L-type and lower in S-type than that of Yukara. The difference between cultivars in average length of S-type first order lateral root was also observed when the values became stable (Table 4). Hence we concluded that it is possible to evaluate the morphology of seminal root system with these branching density and average length of lateral roots. However, it is difficult to evaluate the average length of L-type first order lateral root because the time of emergence varied among root samples.

Second, in the topological analysis, after the $\log a/\log \mu$ ratio significantly decreased up to the third and fourth week, no significant changes were found in both cultivars (Fig. 2-upper). This means that the branching pattern of each cultivar did not change during later sampling stages. It is notable that the difference of $\log a/\log \mu$ ratio between two

cultivars became clear after the third week as seen in the difference of branching density of L-type first order lateral roots (Table 3). Hence, it was assumed that these two characters were closely related, while the density of S-type first order lateral roots, whose difference between cultivars was already recognized in earlier time, showed only a weak relationship with the $\log a/\log \mu$ ratio.

The $\log a/\log \mu$ ratio coincided well with quantitative root development expressed by the number and length of root system components (Tables 1 and 2). Thus, this ratio is useful as an indicator of developmental process in the culture system. In other words, when cultured roots were almost fully developed, these ratios also reached stable values.

However, the trend in quantitative analysis did not follow that in $\log Pe/\log \mu$ ratio (Fig. 2-lower). The reason for this is that Pe is strongly dependent on μ (total root number), especially in low root magnitude⁵⁾, so that the change in branching pattern was masked by size effect in the case of $\log Pe/\log \mu$ ratio. Thus, this value was considered not to be suitable for an indicator of either root system development or branching pattern in this study.

Finally, the fractal analysis was applied to evaluate the characteristics of seminal root system. Fractal dimension (D) is much less time-consuming to obtain than topological indices when the procedure proposed by Tatsumi¹⁵⁾ is used. The D value in Fig. 3 imply that the morphology of seminal root system in Yukara was always more complicated compared to TN-1 at all sampling periods. This trend coincided with those of the branching density and average length of S-type first order lateral roots after the second week (Tables 3 and 4). Tatsumi¹⁵⁾ reported that D value reflected the complexity of root system which was not determined by the length or area of root system but the branching order or density of lateral roots. In this respect, we also found the possibility to characterize the complexity of root system morphology by fractal dimension in relation to the emergence and elongation of S-type first order lateral roots which occupied the major part of total number and length of root systems in this experiment.

On the other hand, D value did not reflect quantitative root growth very well. Fitter *et*

*al.*⁶⁾ also observed the fluctuation of D value with plant growth. We suppose that a root system is a complex of different types and branching orders of lateral roots which have different effects on the D value. Further study is needed on this aspect to apply fractal dimension as an indicator of the degree of root system development.

In summary, we evaluated the appropriate sampling period for which the difference in characteristics of root system morphology became clear between rice cultivars. We have already shown the possibility for characterizing each cultivar with the branching density of L-type first order lateral roots, or the average length and branching density of S-type first order lateral roots. These characters showed similar timecourse trends with either the topological parameter ($\log a/\log \mu$ ratio) or fractal dimension (D value). The difference between cultivars was clearly recognized at the third week in $\log a/\log \mu$ ratio and the first week in D value, and these trend continued until the end of experiment (Tables 3 and 4).

Therefore, we concluded that three weeks at which the difference in branching pattern was clearly recognized are needed for the full characterization of morphology of seminal root system when the conventional excised root culture method is used, and that the topological and fractal analyses are useful techniques for this research purpose.

Acknowledgments

We are grateful to Dr. A.H. Fitter at York University in the UK for his useful suggestions for topological analysis. We also thank Dr. J.R. Pardales Jr. at Visayas State College of Agriculture in the Philippines for reviewing the manuscript. This work was supported by a grant from the Ministry of Education, Science and Culture in Japan (Grant No. 63560011).

References

1. Bray, R.H. 1954. A nutrient mobility concept of soil-plant relationship. *Soil Sci.* 78 : 9—22.
2. Butcher, D.N. and H.E. Street 1964. Excised root culture. *Bot. Rev.* 30 : 513—586.
3. Eghball, B., J.R. Settimi, J.W. Maranville and A. M. Parkhurst 1993. Fractal analysis for morphological description of corn roots under nitrogen stress. *Agron. J.* 85 : 287—289.
4. Fitter, A.H. 1985. Functional significance of root

- morphology and root system architecture. In Fitter, A.H. et al. eds., *Ecological Interaction in Soil*. Blackwell, Oxford. 87—106.
5. ——— 1987. An architectural approach to the comparative ecology of plant root systems. *New Phytol.* 106 : 61—77.
6. ——— and T.R. Stickland 1992. Fractal characterization of root system architecture. *Funct. Ecol.* 6 : 632—635.
7. Hou, C.R. and K.L. Lai 1983. Root physiology of japonica and indica rices (*Oryza sativa* L.). 2. Nitrogen uptake and the enzyme activities of nitrogen metabolism. *J. Agric. Assoc. China* 124 : 10—18.
8. Izumi, Y., Y. Kono, T. Aoshima, A. Yamauchi and M. Iijima 1995. Effects of physiological and morphological characteristics of root tips from rice seminal roots on subsequent growth under tissue culture condition. *Jpn. J. Crop Sci.* 64 : 622—628.
9. Kawashima, C. 1988. Root system formation in rice plant. III. Quantitative studies. *Jpn. J. Crop Sci.* 57 : 26—36***.
10. Kawata, S., A. Ishihara and S. Tsunoda 1967. On the composition of the medium for the culture of excised seminal roots of rice. *Proc. Crop Sci. Soc. Japan* 36 : 68—73**.
11. Kim, J.H., T. Oritani and J. Inoue 1986. Comparison of the growth of rice seminal roots between japonica and japonica-indica hybrids by the use of root-tip culture method. *Jpn. J. Crop Sci.* 55 : 217—222**.
12. Lai, K.L. and C.R. Hou 1983. Root physiology of japonica and indica rice (*Oryza sativa* L.). 1. Growth feature of excised root and embryo culture. *J. Agric. Assoc. China* 124 : 1—9.
13. Takagai, K. and J. Tatsumi 1994. Topological and fractal architecture of the root system of kidney bean (*Phaseolus vulgaris* L.) seedlings. *Jpn. J. Crop Sci.* 63 (Extra 1) : 198—199*.
14. Tatsumi, J., A. Yamauchi and Y. Kono 1989. Fractal analysis of plant root system. *Ann. Bot.* 64 : 499—503.
15. ——— 1995. Fractal geometry in root systems : quantitative evaluation of distribution pattern. *Jpn. J. Crop Sci.* 64 : 50—57***.
16. Yamauchi, A., Y. Kono and J. Tatsumi 1987. Quantitative analysis on root system structures of upland rice and maize. *Jpn. J. Crop Sci.* 56 : 608—617.
17. ———, ——— and ——— 1987. Comparison of root system structures of 13 species of cereals. *Jpn. J. Crop Sci.* 56 : 618—631.

* In Japanese.

** In Japanese with English summary.

*** In Japanese with English abstract.