Timecourse Changes in Two Different Topological Indices with Seminal Root System Development of Rice*

Yasuhiro IZUMI, Yasuhiro KONO, Akira YAMAUCHI and Morio IIJIMA

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

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Abstract : For the topological analysis of plant root systems, two sets of topological indices have been proposed ; the ratios of measured altitude (a) and total external pathlength (Pe) to the expected value of the respective parameter when lateral root branching is assumed to occur randomly $\{a/E(a), Pe/E(Pe)\}$ and to the maximum value of the respective parameter when all lateral roots emerge from the main root axis (herringbone type) $\{a/Max(a), Pe/Max(Pe)\}$. We traced the timecourse changes in these indices with root system development of two rice cultivars grown with the excised root tip culture method, and evaluated their adaptability for the actual complexity of the branching pattern. The $a/E(a)$ and $Pe/E(Pe)$ did not adequately express the progress of complication with root growth and the difference between cultivars in branching pattern because root system size substantially affected the values. On the other hand, changes in $a/Max(a)$ and $Pe/Max(Pe)$ showed close correlation with those in several root developmental characters such as the number and mean branching order of lateral roots (MBO). Thus, we conclude that $a/Max(a)$ and $Pe/Max(Pe)$ are better indices than $a/E(a)$ and $Pe/E(Pe)$ for the topological characterization of morphology in the developmental study of rice root systems.

Key words : Branching pattern, Herringbone type, *Oryza sativa* L., Rice, Root system, Topological index, Topology.

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要 旨 : 根の分枝パターンの評価に用いられるトポロジー指数を求める際に、アルチチュード (a , 最長の外部パス長) と外部パス長の総和 (Pe) について、根がランダムに分枝すると仮定したときの期待値 $\{E(a), E(Pe)\}$ に対するそれぞれの比 $\{a/E(a), Pe/E(Pe)\}$ と、最も単純な分枝パターンである魚骨型の場合の最大値 $\{Max(a), Max(Pe)\}$ に対するそれぞれの比 $\{a/Max(a), Pe/Max(Pe)\}$ とで表される指数が提唱されている。本研究では、経時的にサンプリングした水稻の培養種子根系を材料としてトポロジー解析を行い、根系発達に伴うこれらの指数の変化を追跡することによって、実際の分枝パターンの複雑さをどちらがより正しく表現しているかについての比較を行った。 $a/E(a)$ や $Pe/E(Pe)$ では、分枝パターンの複雑化による指数の減少を根系サイズの増大による影響が打ち消してしまうために、実際の複雑さの変化と一致した推移を示さなかった。それに対して、 $a/Max(a)$ や $Pe/Max(Pe)$ は、側根の発生数や平均分枝次元 (MBO) といった根系形質との間に高い相関を示しており、これらを指標とすることによって実際の分枝パターンの経時的な複雑化の過程や品種間差異を把握することが可能であった。これらのことから、 $a/Max(a)$ と $Pe/Max(Pe)$ はイネ根系発達に伴う分枝パターンの複雑化を解析する場合に用いる指標としては、 $a/E(a)$ や $Pe/E(Pe)$ よりも適していると結論した。

キーワード : イネ, 魚骨型, 根系, 水稻, トポロジー, トポロジー指数, 分枝パターン。

Recently, topological analysis has been applied to the characterization of plant root system morphology in addition to or instead of measurement of developmental growth parameters such as number and length of different component roots¹⁻⁵⁾. This method is useful to express the characteristics of root system morphology, especially when the complexity of branching pattern of a root system is of main concern.

Topologically, a root system is considered to

consist of links which are defined as portions from the root tip to the branching point, or between the two branching points. The former is called an “external (exterior) link” and the latter an “internal (interior) link”^{2,3,5)}. There are two extreme branching patterns in root systems. The simplest branching pattern is called the herringbone type, in which lateral root emergence (new external link production) is restricted on the main root axis and no branching root higher than second order (Fig. 1-A). In contrast, the most complex branching pattern is called the dichotomous type, in which lateral root emergence takes place on

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Fig. 1. Two extreme branching patterns of root system whose magnitude (μ), equivalent to total root number, is 16; herringbone type (A) and dichotomous type (B). The numbers near the root tips indicate the exterior pathlength to the base of root system. The altitude (a) which is defined as the longest exterior pathlength and the total exterior pathlength (Pe) are 16 and 151 in A, 5 and 80 in B, respectively.

any external link with equal probability so that branching order of lateral root is highly advanced (Fig. 1-B). The topological variables, total external pathlength (Pe , the sum of external pathlength, which is defined as the number of links from a root tip to the base) and the altitude (a , the longest external pathlength within a system), are at a maximum in the herringbone type, and at a minimum in the dichotomous type in a root system with a given magnitude (μ , total number of root tips, i.e., a main root + lateral roots in the case shown in Fig. 1)^{2,3,5}. Consequently, the a and Pe values of a root system are in the range shown by these two extreme root system types. Using such topological variables as a , Pe and μ , two different sets of topological indices have been largely proposed used for the quantification on how closely the branching pattern of a root system resembles either herringbone or dichotomous type. Fitter et al.² proposed the ratios of $a/E(a)$ and $Pe/E(Pe)$, where $E(a)$ and $E(Pe)$ is the expected value of a and Pe at given μ , respectively, when root systems show

random branching pattern. Since these ratios are one for the random branching pattern, any increases in the ratio from one show that the branching pattern of the root system is closer to the herringbone type, while any decreases indicate it is closer to the dichotomous type.

On the other hand, the ratio of a and Pe to their theoretical maximum values was designated as the *reduced diameter* (diameter is equivalent to altitude) and the *reduced external pathlength*, respectively, by Werner and Smart⁶. We use the term, $a/\text{Max}(a)$ and $Pe/\text{Max}(Pe)$, respectively, in this paper as once described by Fitter³. In a root system with a given μ , the complete herringbone type gives the maximum value for a and Pe . Therefore, $a/\text{Max}(a)$ and $Pe/\text{Max}(Pe)$ are one for the complete herringbone type. Any decrease in these ratios from the maximum value ($=1$) indicates that the branching pattern of a root system is closer to the dichotomous type.

In this report, we attempted to trace the timecourse changes in topological indices on these two different bases, namely ratios of a and Pe to either expected or maximum values, with root system development.

Materials and Methods

Each of the expected and maximum value of a and Pe on a given μ is calculated with the following equations proposed by Werner and Smart⁶;

$$E(a) = 1.4 \cdot (2\mu - 1)^{0.58} \quad (1)$$

$$E(Pe) = 2^{2(\mu-1)} / \left(\frac{1}{2\mu-1} \cdot {}_{2\mu-1}C_{\mu} \right) \quad (2)$$

$$\text{Max}(a) = \mu \quad (3)$$

$$\text{Max}(Pe) = (\mu^2 + 3\mu - 2) / 2 \quad (4)$$

Note that the first formula (Eq.1) was empirically induced from the regression of a to μ based on the result of a simulation model, and that ${}_{2\mu-1}C_{\mu}$ in the second equation (Eq.2) means the number of combinations in selection of μ from $2\mu - 1$. Using these topological variables, the topological indices on the two different bases as explained earlier, i.e., $a/E(a)$, $Pe/E(Pe)$, $a/\text{Max}(a)$ and $Pe/\text{Max}(Pe)$, were calculated, and their timecourse changes were examined with the progressive complications in branching pattern.

According to Fitter et al.², topological indices are scale-dependent because primary

topological parameters, i.e., a and Pe are complicated by μ . They reported that this problem can be avoided by the use of covariance analysis, with common logarithm of magnitude ($\log \mu$) as the covariate. Hence we also calculated the adjusted mean values of these indices in each cultivar and sampling period with the covariance analysis in two-way (cultivar $\times$ growth period) to reduce the effect of root system size.

We used the same root samples examined in our previous study⁵⁾, that is, the seminal root

systems of two rice cultivars (Taichung Native 1, indica type; Yukara, japonica type) grown with the excised root tip culture method, in which the root systems were sampled weekly for six weeks. From these sampled root systems, the relationship between development and topological indices were examined. Further, as the quantitative expression of the complexity of branching pattern on a developmental basis we calculated the mean branching order (MBO) of lateral roots in a root system as follows;

$$\text{MBO} = \frac{1 \times \text{Number of 1st order lateral roots} + 2 \times \text{Number of 2nd order lateral roots} + \dots}{\text{Total number of lateral roots}}$$

Results

Table 1 shows the timecourse changes in the magnitude (root system size) and the mean branching order (MBO) of root systems of two rice cultivars which were cultured for six weeks. Significant differences in the magnitude were observed from the first to the fourth week in TN-1, between the second and the third week, and between the third and the sixth week in Yukara. Because no root system sampled at the first week produced any second order lateral roots, MBO was 1.00 in both cultivars. Thereafter, MBO significantly increased from the second to third week in TN-1, and from the second to fourth week in Yukara. These results indicate that the branching pattern became more complex during such periods.

The timecourse changes in topological indices are shown in Table 2. At the first week, $a/\text{Max}(a)$ and $Pe/\text{Max}(Pe)$ was 1.00 (maximum value) in both cultivars. Thereafter, these ratios continued to decrease significant-

ly, indicating that the root branching pattern changed and departed away from the herringbone type as root systems developed with the emergence of higher branching order lateral roots (Table 1).

On the other hand, $a/E(a)$ and $Pe/E(Pe)$ increased from the first to the second week rapidly in TN-1 and gradually in Yukara (Table 2), which implies that the branching pattern becomes closer to herringbone type, though in fact the complexity of the branching pattern is scarcely changed in both cultivars during the period as shown in the almost constant MBO (Table 1). Further, the ratios did not change from the second to the third week in TN-1, and from the second to the fourth week in Yukara (Table 2), when significant complication in the branching pattern occurred in each cultivar as shown in MBO (Table 1). These facts show that the complication of branching pattern cannot be evaluated based on $a/E(a)$ and $Pe/E(Pe)$ because timecourse change in these indices tended to be offset by the effect of root system size.

Table 1. Timecourse changes in root system size (magnitude, μ) and the mean branching order (MBO) of lateral roots in a root system of two rice cultivars grows with excised root culture method.

Cultivars		Growth period in weeks					
		1	2	3	4	5	6
TN-1	μ	32a	241b	574c	849d	994d	950d
	MBO	1.00a	1.04a	1.27b	1.34b	1.38b	1.37b
Yukara	μ	71a	94a	132b	168bc	160bc	180c
	MBO	1.00a	1.01a	1.15b	1.27c	1.23c	1.24c

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

Table 2. Timecourse changes in topological indices of root systems of two rice cultivars grown with excised root culture method.

Cultivars		Growth period in weeks					
		1	2	3	4	5	6
TN-1	$a/E(a)$	2.02a	4.56b	4.78b	5.07b	5.02b	4.79b
	$Pe/E(Pe)$	1.74a	4.14b	4.53bc	5.19c	4.89bc	5.04c
	$a/Max(a)$	1.00a	0.96a	0.71b	0.64bc	0.59bc	0.56c
	$Pe/Max(Pe)$	1.00a	0.93a	0.68b	0.65b	0.57b	0.58b
Yukara	$a/E(a)$	2.87a	3.17ab	3.13ab	3.01ab	3.09ab	3.24b
	$Pe/E(Pe)$	2.49a	2.81b	2.90bc	2.92bc	2.97bc	3.09c
	$a/Max(a)$	1.00a	0.99a	0.87b	0.75c	0.78bc	0.78bc
	$Pe/Max(Pe)$	1.00a	0.99a	0.90a	0.80b	0.83b	0.82b

The same letters adjacent to each value mean no significant difference among growth periods at 5% level according to Duncan's multiple range test.

Table 3. Timecourse changes in topological indices of root systems of two rice cultivars grown with excised root culture method. The data were corrected for difference in root system size.

Cultivars		Growth period in weeks					
		1	2	3	4	5	6
TN-1	$a/E(a)$	4.09	4.48	3.77	3.65	3.43	3.25
	$Pe/E(Pe)$	3.86	4.05	3.50	3.73	3.26	3.46
	$a/Max(a)$	0.86	0.96	0.78	0.74	0.70	0.66
	$Pe/Max(Pe)$	0.82	0.94	0.77	0.77	0.71	0.72
Yukara	$a/E(a)$	4.08	4.09	3.68	3.30	3.44	3.47
	$Pe/E(Pe)$	3.73	3.75	3.46	3.22	3.33	3.32
	$a/Max(a)$	0.92	0.92	0.83	0.73	0.76	0.77
	$Pe/Max(Pe)$	0.89	0.91	0.85	0.78	0.80	0.80

Data are mean values adjusted by two way (growth period $\times$ cultivar) analysis of covariance using log magnitude as the covariate²⁾.

Moreover, the branching pattern of root system of Yukara was closer the herringbone type than that of TN-1 after the second week as shown in MBO (Table 1). However, these indices were higher in TN-1 than in Yukara as shown in Table 2 due to the larger root system size in the former cultivar (Table 1). Therefore, $a/E(a)$ and $Pe/E(Pe)$ did not correctly express the cultivar difference in branching pattern. In contrast, it is obvious that such topological indices as $a/Max(a)$ and $Pe/Max(Pe)$ correctly expressed the difference between the two cultivars (Table 2).

Next, the adjusted mean values of the topological indices by covariance analysis are shown in Table 3. Throughout the culture period, $a/E(a)$ and $Pe/E(Pe)$ continued to decrease, indicating that the complexity of

branching pattern increased. However, the indices as well as non-adjusted value increased in TN-1 from the first to the second week, when magnitude rapidly increased (Table 1). Further, these indices during a subsequent growth stage did not reflected the difference in branching pattern between the two cultivars. These results mean that adjustment by covariance analysis was incomplete.

In the adjusted $a/Max(a)$ and $Pe/Max(Pe)$ (Table 3), the range of timecourse changes was reduced as compared to that of non-adjusted value (Table 2). The increase of values from the first to the second week in TN-1 due to the increased magnitude was also found as $a/E(a)$ and $Pe/E(Pe)$, and the cultivar difference was less clear in adjusted values than in non-adjusted values (Table 2).

Table 4. Correlation coefficients between the topological indices and the number or mean branching order (MBO) of lateral roots of root system of two rice cultivars.

Cultivars		$a/E(a)$	$Pe/E(Pe)$	$a/Max(a)$	$Pe/Max(Pe)$
TN-1	1st-L No.	-0.935**	-0.877*	-0.992***	-0.990***
	2nd No.	-0.921**	-0.831*	-0.972**	-0.966**
	MBO	-0.908*	-0.838*	-0.997***	-0.996***
Yukara	1st-L No.	-0.984***	-0.981***	-0.994***	-0.993***
	2nd No.	-0.983***	-0.987***	-0.989***	-0.995***
	MBO	-0.996***	-0.997***	-0.999***	-0.999***

1st-L No., Number of L-type first order lateral roots.

2nd No., Number of second order lateral roots.

*, **, ***: 5, 1, 0.1% level of significance.

Correlation coefficients were calculated in adjusted mean values of $a/E(a)$ and $Pe/E(Pe)$ shown in Table 3, and non-adjusted values of $a/Max(a)$ and $Pe/Max(Pe)$ shown in Table 2.

These results indicate that covariance analysis on $a/Max(a)$ and $Pe/Max(Pe)$ was not effective in our study.

To evaluate the adaptability of topological indices, we calculated the correlation coefficients of adjusted $a/E(a)$ and $Pe/E(Pe)$ (Table 3), or non-adjusted $a/Max(a)$ and $Pe/Max(Pe)$ (Table 2), with MBO as well as the number of L-type first order and second order lateral roots (Table 4). Namely, we chose the values in either of two Table (Table 2 and 3) which seemed to show better fitness to root system development. The correlation coefficients with non-adjusted $a/E(a)$ and $Pe/E(Pe)$, or adjusted $a/Max(a)$ and $Pe/Max(Pe)$ showed respectively lower significance than the coefficients shown in Table 4 (data are not shown).

The L-type lateral root is the one which is long, thick and has the capacity to produce higher branching, and the growths of L-type lateral roots and concomitant higher branching order lateral roots (most of them were second order in the samples examined) greatly affect the branching pattern and root system development⁷⁾. Correlation coefficients between developmental root characters and topological indices were all significant (Table 4), especially the coefficients with $a/Max(a)$ as well as $Pe/Max(Pe)$. These were always higher than those with $a/E(a)$ and $Pe/E(Pe)$. This indicates that $a/Max(a)$ and $Pe/Max(Pe)$ better expressed the complexity of root branching pattern on a developmental basis, i.e., the botanical branching order system which we have conventionally used.

Discussion

Our results showed that $a/Max(a)$ and $Pe/Max(Pe)$ expressed the timecourse changes with rice root growth and the cultivar difference in branching pattern better than $a/E(a)$ and $Pe/E(Pe)$.

We assumed that $a/E(a)$ and $Pe/E(Pe)$ would be suitable for evaluation of root system morphology of similar size if the root systems show a nearly random branching pattern. However, in fact the rice root systems used in this study showed more herringbone-like rather than a random branching pattern. In such cases, adjustment by covariance analysis was indispensable because of strong scale-dependency (Table 2). Indeed, the fitness of these indices as the root growth parameter was improved to some extent, but the range of magnitude was too large to completely offset the effect of root system size (Table 3). Further, the modified means are not absolute but relative values because the adjustment is not identical. In other words, it depends on covariates ($\log \mu$) of other plots. Hence, it would be difficult to compare the branching pattern of root systems using these indices among different experiments.

During the course of our study, we found that the branching pattern of a rice root system is almost always closer to herringbone than a dichotomous type because a and Pe seldom show less than their expected values, and the branching pattern of any root system samples changed from complete herringbone type to random with the progress of root

growth. These facts clearly show that it is reasonable to use $a/\text{Max}(a)$ and $Pe/\text{Max}(Pe)$ for the developmental study of rice root system formation; and, additionally, that these indices can well describe the progress of the complication of branching pattern. Practically, it is convenient to use these indices in the comparison of branching patterns in other experiments because the maximum value, which was attained when root branching pattern is simplest, is constant ($=1$), as compared to the variable maximum value in $a/E(a)$ and $Pe/E(Pe)$.

We therefore conclude that the topological indices, $a/\text{Max}(a)$ and $Pe/\text{Max}(Pe)$ for the topological characterization of the developmental features of root system morphology in the case of rice. We assume that $a/\text{Max}(a)$ and $Pe/\text{Max}(Pe)$ can also be applied in topological analysis to a wide range of field crops, which is a subject for further study, where the branching pattern is relatively close to herringbone type.

However, if root systems show a more random branching pattern, these indices would be substantially affected by root system size. Hence, in such case, the avoidance of its effect should also be required on $a/\text{Max}(a)$ and $Pe/\text{Max}(Pe)$. In any case, however, it is unavoidable that the increase of root system size affects the topological indices which we used in this study because neither a nor Pe are independent of μ . Thus, the application of topological analysis to developmental study might be limited for short growth period during which the change of magnitude is

relatively small. A further study will be needed to develop some suitable parameter for topological analysis for longer term research.

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