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Genotypic Variation in the Development of Seminal Root System of Rice under Different Culture Conditions *in vitro**

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Abstract : To elucidate genotypic variations in the root system architectures of rice, which are determined by the emergence (branching) and elongation of lateral roots, we compared the development of seminal root systems (seminal root axis and lateral roots) among seven cultivars with different ecotypes using an *in vitro* culture method which can simplify growth conditions. Four different culture treatments were prepared by combining two factors (nitrogen source in medium and presence/absence of shoot) to evaluate genotypic variations as a whole. After three weeks of culture, sampled seminal root systems were developmentally, topologically and geometrically analyzed. First, we researched the responses of root system development to culture conditions. It was especially notable that the responses of L-type and S-type first order lateral roots were completely different, and that root system size was affected by both the composition of medium and the presence/absence of shoot, while branching pattern was mainly controlled by the former. Though it was difficult to find a general trend in cultivar variations throughout the treatments, we characterized and classified the seven cultivars mainly based on the root system size and responses to culture conditions. Instinctive genotypic variations were clearly recognized under culture conditions as compared to soil conditions. Thus, this study showed the possibility that selection of genotypes focused on architecture could be facilitated using an *in vitro* culture method.
Key words : Genotypic variation, *in vitro*, Lateral root, Link length, *Oryza sativa* L., Rice, Root system architecture, Topological index.

インビトロ培養条件下での水稲種子根系の発達における遺伝子型変異：泉　泰弘・河野恭廣・山内　章・飯嶋盛雄（名古屋大学農学部）

要　旨：水稲の根系構造（側根の発生と発達によって規定される形態）における遺伝子型変異を明らかにするため，生育環境の単純化が可能なインビトロ培養法を用い，種子根系（種子根軸と側根）の発達を生態型の異なる7品種間で比較した．遺伝子型変異を複数の生育条件下で総合的に評価するため，培地の窒素源・地上部の有無という2因子を組み合わせた4つの培養条件区を設けた．3週間培養した後に種子根系を採取し，発育学的解析（根数・根長など），トポロジー解析および幾何学的解析を行った．まず，各種培養条件に対する根系発達の反応を調査したが，とくに1次側根のL型とS型では反応が全く異なることや，根系サイズが培地の窒素源の違いと地上部の有無の両者によって影響されるのに対して，分枝パターンは主に前者によって支配され，後者の影響は比較的小さいことが注目された．続いて遺伝子型変異について考察したが，培養種子根系の発達は区間で非常に大きい多様性を示したため，品種間差異についても全ての区に共通する傾向を見い出すことは困難であった．しかし，根系サイズや応答反応の違いなどによって，各水稲品種の根系発達様式を生態型ごとに特徴づけ，分類することは可能であった．培養条件下での品種比較においては，土壌栽培条件下で比較を行った場合よりも明確な遺伝子型変異を検出することが可能であったことから，本研究は根系構造に注目して選抜を行う場合に，インビトロ培養法が有効である可能性を示したものといえる．
キーワード：遺伝子型変異，イネ，インビトロ，根系構造，水稲，側根，トポロジー指数，リンク長．

Since the pioneer work of Weaver[32] it has been reported that every plant species or variety has its own morphological characteristics in root systems as well as aboveground organs. In the case of rice (*Oryza sativa* L.), several comparisons have been made among different cultivars for spatial extension of main root axes (seminal and nodal root axes) which determine the "skeleton" of a root system[1,26,35]. Further, information on the genotypic variation in lateral root development which greatly contributes to the augmentation of root surface area[14,33] has also been accumulated in this decade[6,24,25,31] partly owing to the establishment of automatic root length measurement procedures. In most of these studies, growth of lateral roots was quantitatively evaluated based on root length or root length density.

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However, for absorption and transport of water and nutrients, the arrangement of lateral roots along the main axis is also important. Furthermore, the coexistence of two distinct types of lateral roots has long been known in rice root systems[13,15,20,33]. They do not only differ in length, diameter, and branching capacity but also in inner structure, which may imply a functional difference[34]. Therefore, it is not sufficient to understand lateral root development as a strategy for increase in length or surface area only. Moreover, the architecture of the root system as determined by the emergence (branching) and elongation of lateral roots should be paid attention as well. Nevertheless, the difference in architecture among rice cultivars has not been fully investigated.

It is known that the complicated effect derived from various soil environmental factors tends to mask genotypic variation in root growth under field conditions[29]. Thus, we attempted to compare the architecture of seminal root systems (seminal root axis and lateral roots) among rice cultivars grown with an *in vitro* root culture method because growth conditions can be simplified and easily controlled. Four different culture conditions were prepared by combining two factors following the view that any root growth in one way environment cannot reveal the extent of genetic variation among the entries[28]. First, we assessed the response of root system development to culture conditions, and next, we attempted to elucidate genotypic variations among several rice cultivars.

Materials and Methods

Seven rice cultivars on which we have reported elsewhere[12], i.e., Yukara (japonica cultivars), Peta (javanica cultivar), Tongil (japonica-indica hybrid cultivar), and four indica cultivars, Dee-Geo-Woo-Gen (DGWG), IR-8, Taichung Native 1 (TN-1) and Tsai-Yuang-Chung (TYC), which belong to the breeding line of Tongil, were used. The methods of germination and sterilization, and growth conditions (incubation) were the same as we previously reported[9]. Husked and surface-sterilized seeds were aseptically germinated on agar in Petri-dish. Two days after the seed bedding, the material for root culture was sampled. In addition to the conventional excised root culture method (excised culture)[9,19,22], germinated embryo was separated from endosperm and cultured so that shoot was also grown with root system in the same culture system (intact culture). By comparing excised and intact culture, the root growths, with or without shoots, could be evaluated. Two 1-cm root tip segments (excised culture) or one seminal root axis with scutellum and plumule (intact culture) were transferred into culture medium per flask. The following two culture media were used for each of excised and intact cultures to find the effect of nitrogen source in medium; Lai & Lee's[22] R_2 medium, which solely contains nitrate ions (45.5 ppm-N) as a nitrogen source, and revised R_2 medium (R_{2C} medium) which contains additional 0.1% Difco-certified casamino acid (casein acid hydrolyzes) and thus 82.6 ppm-N mainly as organic form (amino acid) other than nitrate ions. As a result, the following four treatments were prepared: Ex-R_2, excised culture in R_2 medium; Ex-R_{2C}, excised culture in R_{2C} medium; In-R_2, intact culture in R_2 medium; In-R_{2C}, intact culture in R_{2C} medium. Ten cultured root systems were sampled from each treatment at three weeks after transfer.

First, the root systems were developmentally analyzed. The seminal root axis length, and number and length of all the lateral roots were measured. Following to the previous studies[9,10,12,33,34], lateral roots were classified into two categories, i.e., L-type lateral root which is long, thick and produces higher branching order lateral roots, and S-type lateral root, which is short, slender and has no branching capacity[20,21]. Based on the data, we calculated the total root number and length, and the branching density (roots/per cm of mother root axis) of lateral roots. Further, we conducted topological and geometrical analyses as we have described elsewhere[12]. These analyses regard a root system to be a mathematical tree constituted by links which are defined as linear portions from the root tip to the branching point, or between the two branching points[2,3,4,10,11]. The mathematical parameters obtained are quite useful to indicate the characteristics of root system architecture concisely.

We used two types of topological indices, a/Max (a) and Pe/Max (Pe), i.e., ratio of altitude (a) and total external pathlength (Pe) to its maximum value, because they are good indices to evaluate the branching pattern of rice root system[11]. Both indices are maximum (=1) when the branching pattern is complete herringbone type (the simplest pattern in which the emergence of lateral roots is restricted within the first order), and the indices decrease as the branching pattern complicates. The geometrical variables, average lengths of external and internal links, were also calculated using the equations reported elsewhere[12]. The external and internal link length is greatly reflected by the average length and branching density of lateral roots, respectively. The root systems grown in the media with casamino acid were too fragile to spread two-dimensionally without destruction. Hence, fractal analysis was not conducted.

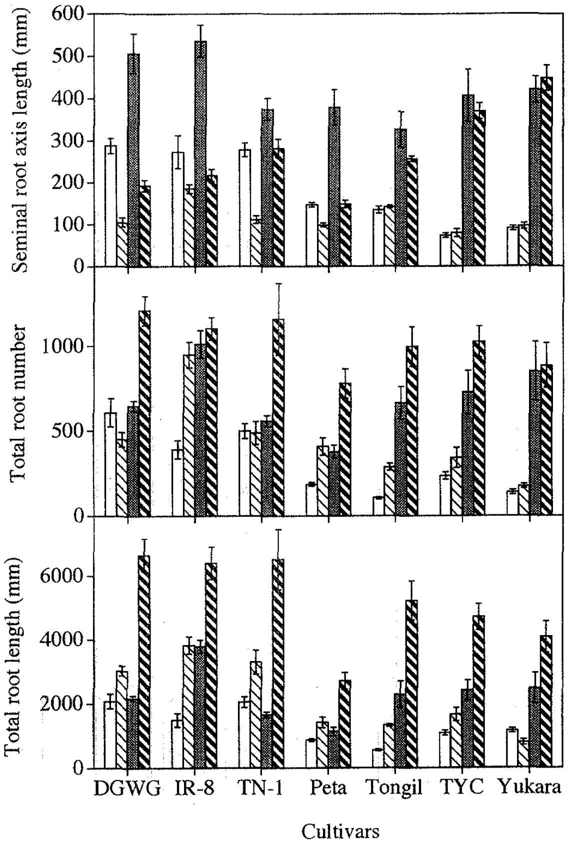

Fig. 1. Comparison of seminal root axis length (upper), total root number (middle) and total root length (lower) of seminal root systems among seven rice cultivars grown in four different culture treatments. Vertical bars show standard errors.

□ Ex-R2: Excised culture in R2 medium, ▧ Ex-R2C: Excised culture in R2C medium.
▩ In-R2: Intact culture in R2 medium, ▧ In-R2C: Intact culture in R2C medium.

Results

The seminal root axis length, total root number and length of cultured root system of seven rice cultivars are shown in Fig. 1. The seminal root axis length was shorter in Ex-R_{2C} than Ex-R_2 treatment and shorter in In-R_{2C} than In-R_2 treatment in DGWG, IR-8, TN-1 and Peta (upper). However, such a trend was not observed in the other cultivars. The axis length was longer in intact culture than in excised culture in most of the cultivars, and this trend was especially pronounced in TYC and Yukara.

The total root length was markedly promoted when the roots were cultured with either intact shoot or casamino acid in medium, hence the length was greatest in In-R_{2C} treatment for all the cultivars (lower). The total root number was also greatest in In-R_{2C} treatment for all the cultivars (middle), but responses to culture conditions were more varied among cultivars than those in length. On the whole, DGWG, IR-8 and TN-1 were larger in root system size than the others,

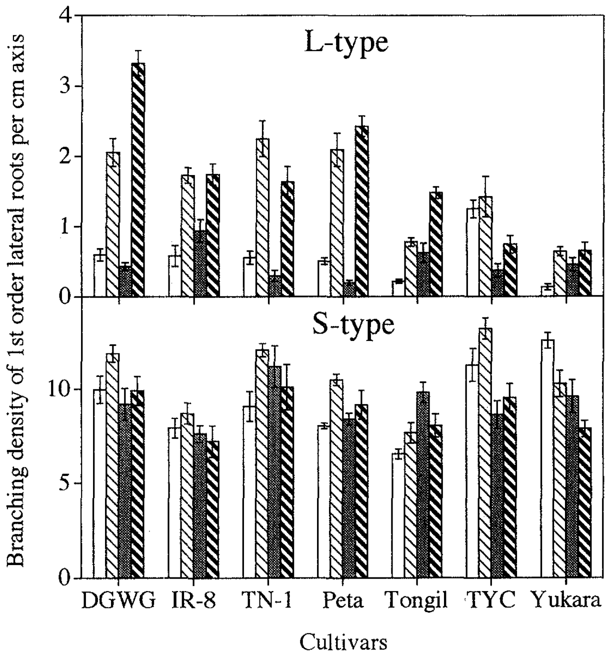

Fig. 2. Comparison of branching density of L-type (upper) and S-type (lower) first order lateral roots of seminal root systems among seven rice cultivars grown in four different culture treatments. Vertical bars show standard errors. Column of the bars are the same as those in Fig. 1.

□: Ex-R2, ▧: Ex-R2C, ▩: In-R2, ▧: In-R2C.

except In-R_2 treatment.

The branching density of L- and S-type first order lateral roots (per cm seminal root axis) are shown in Fig. 2. The density of L-type was greatly influenced by the culture conditions (upper), and increased by the addition of casamino acid in both excised and intact culture, especially in DGWG, IR-8, TN-1 and Peta. On the contrary, the density of S-type (lower) was less influenced by culture conditions, and the trends of cultivar variation were relatively consistent compared with those in L-type, i.e. IR-8, Peta and Tongil had lower branching density than DGWG and TN-1.

The topological index, a/Max (a) of seminal root systems was lower in R_{2C} treatments than R_2 treatments in both excised and intact culture irrespective of the cultivars (Fig. 3-upper). In other words, the branching pattern of root system was complicated, or less-similar to herringbone type when grown in R_{2C} medium. In comparing the same medium treatments, the indices in Ex-R_{2C} treatment were higher than those in In-R_{2C} treatment, or relatively similar between two treatments, whereas no consistent trend between Ex-R_2 and In-R_2 treatments was noted across the cultivars. All these trends shown above were almost the same in the other topological index, Pe/Max (Pe) because the values were relatively similar with a/Max (a) for any cultivars and treatments (lower).

The average length of external and internal link are shown in Fig. 4. In the intact culture, the external link length was longer in In-R_{2C} than In-R_2 treatment in all the cultivars (upper). In contrast, no remarkable trend across the cultivars was found in the excised culture. The length was considerably increased in DGWG and TN-1, and decreased in Yukara by casamino acid, whereas such clear effects were not observed in the other cultivars. On the other hand, the external link length grown in excised culture tended to be longer than seen in intact culture when they were compared with the same culture medium treatment with a few exceptions as seen in IR-8 and Tongil. The internal link length was longer in In-R_{2C} treatment than the other three treatments in all the cultivars but for

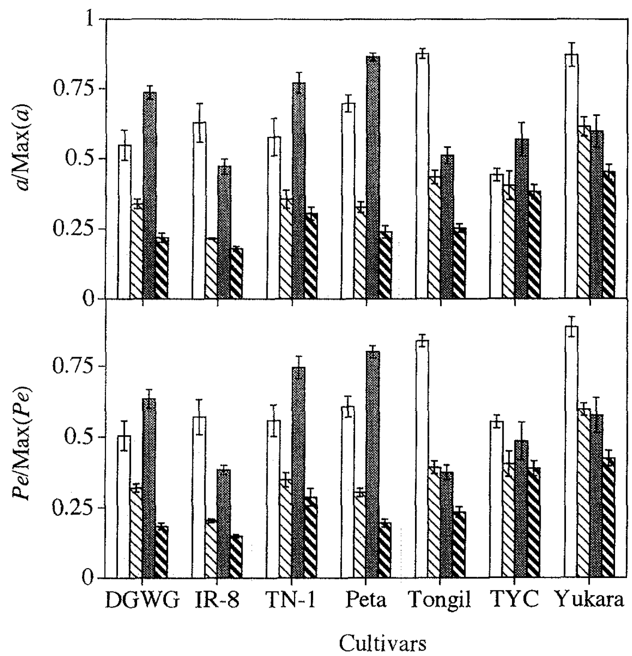

Fig. 3. Comparison of topological indices, a/Max (a) (upper) and Pe/Max (Pe) (lower) of seminal root systems among seven rice cultivars grown in four different culture treatments. Vertical bars show standard errors. Column of the bars are the same as those in Fig. 1.

□: Ex-R_2, ▧: Ex-R_{2C}, ▩: In-R_2, ▧: In-R_{2C}.

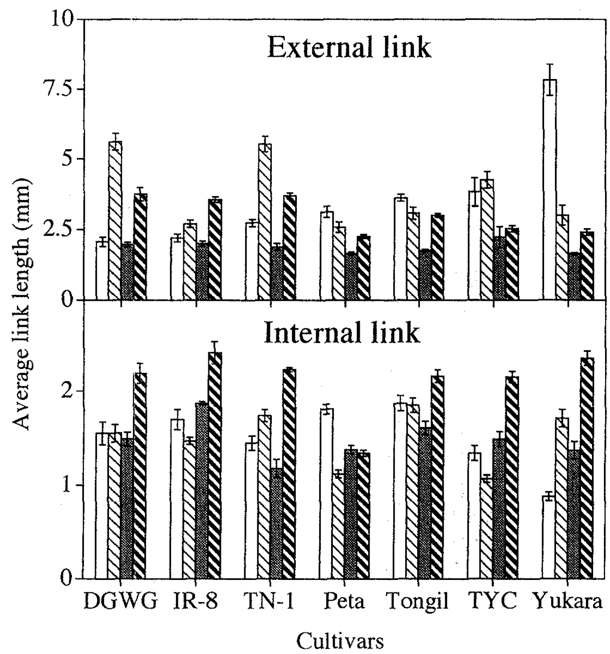

Fig. 4. Comparison of external (upper) and internal (lower) link length of seminal root systems among seven rice cultivars grown in four different culture treatments. Vertical bars show standard errors. Column of the bars are the same as those in Fig. 1.

□: Ex-R_2, ▧: Ex-R_{2C}, ▩: In-R_2, ▧: In-R_{2C}.

Table 1. Main effects of medium (M), shoot (S), cultivar (C) and their interactions on the developmental root characters, topological indices and average link lengths shown by F-ratio from three-way analysis of variance.

	Medium	Shoot	Cultivar	M×S	M×C	S×C	M×S×C
SRAL	143.8 ***	537.6 ***	11.7 ***	22.6 ***	18.4 ***	15.6 ***	5.8 ***
TRN	59.9 ***	284.6 ***	16.5 ***	12.7 ***	2.1	4.8 ***	6.6 ***
TRL	226.7 ***	289.8 ***	30.6 ***	79.1 ***	8.1 ***	5.0 ***	4.0 **
LBD	399.5 ***	0.4	25.8 ***	3.8	24.0 ***	10.9 ***	4.8 ***
SBD	6.1 *	15.4 ***	15.9 ***	11.6 ***	4.4 ***	6.7 ***	1.3
a / Max (a)	268.9 ***	8.3 **	11.7 ***	4.0 *	5.9 ***	7.8 ***	4.2 ***
Pe / Max (Pe)	252.0 ***	22.6 ***	14.6 ***	0.5	3.6 **	8.7 ***	6.2 ***
ELL	20.3 ***	106.5 ***	12.1 ***	18.8 ***	29.5 ***	12.5 ***	14.2 ***
ILL	42.1 ***	63.3 ***	11.2 ***	61.6 ***	14.2 ***	6.8 ***	1.2

*, **, ***: 5, 1, 0.1% level of significance.
SRAL, seminal root axis length; TRN and TRL, total root number and length;
LBD and SBD, branching density of L- and S-type first order lateral roots;
ELL and ILL, external and internal link length.

Peta (lower), which indicates that internal link length increased when the roots were cultured with both casamino acid in the medium and intact shoot.

The main effects of medium, shoot and cultivars, and their interactions on developmental root characters, topological indices and average link lengths were examined by three-way analysis of variance (Table 1). As already shown, the root growths considerably altered in response to culture conditions, so the significant main effects of medium and shoot were observed, except for that of shoot on the branching density of L-type first order lateral roots. Moreover, all the characters showed the significant main effect of cultivar. However, the interactions between cultivar and culture conditions were also significant in most cases, which indicated that the trends of cultivar variation were not consistent with the treatments or responses to the culture conditions.

Discussion

1. Responses to culture conditions

In excised root culture of rice, Oritani et al.[27] reported that the addition of 0.1% casamino acid markedly decreased seminal root axis length while total growth was improved. It was also reported for rice that the growth of cultured root with scutellum was vigorous as compared with excised root culture[16,23]. Thus, the inhibitive effect for seminal axis length and promotive effect for total number and length by casamino acid in our results (Fig. 1) are basically in good agreement with the previous studies. We suppose that the promotive effect of casamino acid to total growth was derived from the improvement of nutrition level, and that of shoot was due to that the shoot or scutellum supplied some materials for seminal root growth. Moreover, large interaction of medium and shoot in total root length (Table 1) implied that these two factors showed synergistic effect (Fig. 1-lower).

Kawata et al.[17] found that the concentration of sugar in medium altered the density of "branching" secondary roots, which is equivalent to our L-type first order lateral roots, in rice root culture. Our results showed that nitrogen level or form also affected the branching density of such roots, but presence/absence of shoot had little effect on it (Fig. 2-upper and Table 1). In contrast, on the branching density of S-type, the effect of shoot was significant while that of medium was relatively small (Fig. 2-lower and Table 1). Kono et al.[21] researched the morphological change in soybean root systems grown in root boxes with several soil moisture conditions, and found that the responses against moisture between L-type and S-type lateral roots were entirely different. We especially noticed their

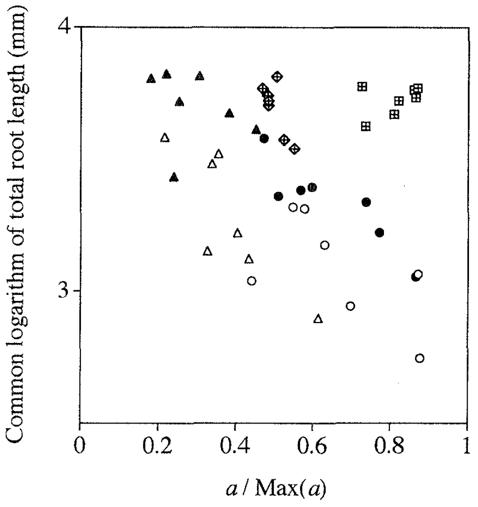

Fig. 5. Relationship between topological index and total root length of seminal root systems of seven rice cultivars in four culture treatments and two soil treatments. Column of the plots in culture treatments are the same as those in Fig. 1. Data of upland and submerged treatments were collected from seminal root systems of rice plants grown in root boxes for three weeks. Data and details on the soil treatments are reported elsewhere[12].

○ : Ex-R2, △ : Ex-R2C, ● : In-R2, ▲ : In-R2C, ⊕ : Upland, ⊞ : Submerged.

and our results because these suggest that genetic controls on the development of two types of lateral roots are different.

The responses of complexity of branching pattern to nutrient levels were reported before[2,3,4]. Generally, the branching pattern became more complex, i.e., changed from herringbone to dichotomous type as nutrient level increased. Influence of shoot, however, has not yet been documented. In Fig. 5, common logarithm of the total root length is plotted against a/Max (a). Both the white and black triangle symbols are situated at upper-left ward of the circle symbols with the same color indicating the impact of nitrogen source on both root parameters. On the other hand, comparing the distribution of the symbols with different colors, the black symbols are just above the white symbols with same shape, which meant that the presence of shoot promoted total root length but a/Max (a) remained within almost the same range in each medium treatment. This is because the main effect of medium on the topological indices showed higher significant level than that of shoot (Fig. 3 and Table 1), and is due to the similar response in the branching density of L-type first order lateral roots (Fig. 2-upper and Table 1). From these results, we assume that root system size is affected by both the composition of medium and presence/absence of shoot, while branching pattern is mainly affected by the former.

Fitter et al.[2,3,4] examined the effect of nitrogen on external link length for several plant species, but did not obtain explicit results. In our study for rice, the responses were considerably different among cultivars in excised culture (Fig. 4-upper). The decrease of external link length in some cultivars by casamino acid was due to the emergence of very short second order lateral roots. This means that external link length does not always increase with root system size. Though Fitter et al.[2,3,4] pointed out that nitrogen level did not affect or even diminish internal link length, both the significant effects of casamino acid and shoot were observed and mostly promoted the internal link length in our study (Fig. 4-lower and Table 1). However, the responses were not consistent for the cultivars shown in significant interactions as in the case of external link length (Table 1). Therefore, difference among cultivars in the responses of average link lengths should be further examined.

2. Genotypic variation in the root system development

Sadhu and Bhaduri[30] reported that the variability which depends on the nature of the media in which the roots grow influences the phenotype and masks the genotype in wheat. In our study, too, we faced difficulty in detecting genotypic variation in root system development because a common feature of each cultivar throughout all four treatments was rarely found, whereas the cultivar variation was markedly noticeable in any single treatment. However, we noticed that the trends of cultivar variation were analogous in several characters such as total root number and length whose main effect of cultivar was relatively large as compared with the interactions (Table 1). Thus, we characterized the seven cultivars mainly based on the root system size, and the response to culture conditions or remarkable traits shown in a few specific treatments.

It has been generally recognized that in excised root culture method the root system development in indica cultivars is more vigorous than in japonica cultivars[7,19,23]. We obtained the same result for two excised culture treatments and one intact culture (In-R_{2C}) treatment. Namely, three indica cultivars, DGWG, IR-8 and TN-1 had a relatively large root system size (Fig. 1). Further, these cultivars showed similar responses, not only in developmental characters, but also in other characters, such as topological indices (Fig. 3) and average link lengths (Fig. 4). Javanica cultivar, Peta showed small root system size in all treatments (Fig. 1). Peta was also characterized by relatively high branching density of L-type first order lateral roots as indica cultivars described above in R_{2C} treatments (Fig. 2-upper), which reflected complex branching pattern (Fig. 3).

In the other three cultivars, Tongil, TYC and Yukara, which showed intermittent root growth (Fig. 1), the most remarkable trait was noted as follows: In japonica-indica hybrid cultivar, Tongil, the promotive effect of not only medium but shoot on the branching density of L-type first order lateral roots and topological indices was recognized (Fig. 2-upper and Fig. 3). TYC, which is a native indica cultivar in Taiwan[18] (despite round grain shape as japonica type) showed rather similar responses to Tongil or Yukara in root system size compared to the other three indica cultivars, but was apparently distinguished by greater branching density of L-type first order lateral roots in excised culture treatments (Fig. 2-upper). Japonica cultivar, Yukara had the simplest branching pattern apart from In-R_2 treatment (Fig. 3) derived from low branching density of L-type first order lateral roots (Fig. 2-upper). Yukara was also characterized by very long external link in Ex-R_2 treatment (Fig. 4-upper).

In the previous papers[8,9,10], we discussed that organic nitrogen was indispensable for the emergence and elongation of L-type lateral roots, which greatly affects both root system size and branching pattern, in excised root culture of rice. From this view, the growth feature of Yukara seminal root system in Ex-R_2 treatment such as small root system size, low branching density of L-type first order lateral root or simple branching pattern, would be due to its low nitrate assimilation capacity. However, in the other treatments, the root growth was affected by more complicated factors, namely, casamino acid and/or the shoot. Hence, the cause of cultivar variation in seminal root growth should be explained by further studies on genotypic variations in allocation pattern of growth material in a whole plant and utilization of organic nitrogen in medium.

As shown above, the seven rice cultivars were classified according to the difference in seminal root system development. The classification almost agreed with the ecotype of each cultivar except for TYC. In addition to the partial similarity in cultivar variations between excised and intact culture, we further noticed that the cultivar variations shown *in vitro* also coincided with those grown in soil to some extent[12]. From these observations, we assume that cultivar variations expressed in intact seminal root system largely depend on the genotypic variation in genomes which only control root growth and are independent of shoot growth[36]. On the other hand, it was reported that rice root elongation was strongly affected by shoot organs because the trends of cultivar variations in excised-cultured and intact seminal root axis length were considerably different[5]. Therefore, we infer that the influence of shoot on lateral root growth is smaller than on main axes.

We expected that an instinctive genotypic variation would be more clearly recognized under culture conditions than field conditions. In fact, for example, distribution in the mean values of seven cultivars on both root system size and complexity of branching pattern were more scattered in four culture treatments than in two soil treatments (Fig. 5). Further, the main effect of cultivar on the topological indices was significant in all the culture treatments (Table. 1), whereas no significant cultivar variation was found when the same cultivars were grown in soil[12]. These showed the possibility that selection of genotypes focused on root system architecture could be facilitated by using *in vitro* culture method. Notably, the excised culture method allows comparison without shoot-root interaction and the effects of soil environment. To consider that we used only a limited number of cultivars and that a cultivar as exceptional as Peta showed quite different ranking in root system size between culture and soil conditions is grounds for more detailed study to confirm the possibility.

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* In Japanese.
** In Japanese with English summary.
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