Jpn. J. Crop Sci. 64(1) : 148—155 (1995)

Root System Morphology of Four Rice Cultivars : Response of Different Component Roots to Nitrogen Application

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Received July 29, 1994

Abstract : A crop root system morphology can be determined by the development of different component roots, such as nodal root axes and their concomitant lateral roots of two different types ; long, large in diameter, and branching (L type), and short, small in diameter, and non-branching (S type). In this respect, we attempted to examine the cultivar difference in root system morphology among rice cultivars and to evaluate phenotypic plasticity in the root system morphology when grown under different nitrogen (N) application regimes. Four rice cultivars that are known to differ in adaptability for heavy manuring (AHM) and ecotype (indica and japonica) were grown for 14 days under three N levels. Cultivar difference in root system morphology existed among the four cultivars. The differences were recognized in the ratio of lateral roots to the entire root system length, and more remarkably in the ratio of S type to L type lateral root number. The difference in the former parameter could be well related to that in the ecotype, while the latter to the AHM of the cultivar. As to the phenotypic plasticity, the three component roots differed in production response, while they were fairly similar in elongation response. The root system morphology of a cultivar with low AHM was considerably phenotypically plastic, while that of the other cultivars were relatively stable to the changes of N conditions in soil.
Key words : Adaptability, Ecotype, Heavy manuring, Lateral root, Nitrogen, Phenotypic plasticity, Rice, Root system.

根系構成根の窒素施肥に対する反応性からみたイネ 4 品種の根系形態 : 田中実秋・山内　章・河野恭廣（名古屋大学農学部）

要　旨 : 作物の根系を構成する根は，主軸根としての節根とそれらの根軸上に発生する側根に大別でき，さらにその側根には形態の異なる 2 つの型の側根が存在する．それらは，長く，太く，高次の側根を発生させるもの（L型側根）と，短く，細く，高次の側根を発生させないもの（S型側根）である．したがって，根系の形態はこれらの 3 種類の根の発達程度によって規定することができる．そこで，耐肥性の程度および生態型（インド型と日本型）の異なるイネ 4 品種を用い，こうした観点からみた，根系形態の品種間差異，および異なる窒素施用量条件下で生育させた時の根系形態の可塑性を評価しようとした．根系採取は播種後 14 日目に行なった．その結果，これら 4 品種間で明確な根系形態の差異が存在することを見いだした．そしてその差異はまず総側根長が総根長に占める割合において認められ，品種の生態型によって整理することができた．また，S型，L型側根の根数比においてさらに顕著な品種間差異を認め，これは品種の耐肥性によって整理できた．根系を構成する各根の異なる窒素施用量に対する反応性（可塑性）についてみると，発生数における反応は 3 者間で明確に異なっていたが，伸長反応では類似の傾向を示した．また，根系全体でみると，異なる窒素施用量に対する根系形態の可塑性は，耐肥性の小さな 1 品種でとくに大きかった．
キーワード : イネ，可塑性，根系，生態型，側根，耐肥性，窒素．

Cultivar differences of root system morphology in rice plant have not been fully investigated. However, it should be reasonable to suspect that root system morphology has been altered, together with shoot morphology, along with the development of high-yielding cultivars which are adapted for heavy manuring.

In many of crop species, evidence has been presented that lateral root is the major component in a root system. For example, Dittmer[2] showed that lateral roots of four-month-old winter rye accounted for more than 99% of the total root length. Yamauchi et al.[11] reported that lateral roots of 30-day-old rice and maize accounted for about 96% and 95% of the total root length, respectively. Kawashima[3] estimated that lateral root length was 99% of total root length in rice plants after heading. In 30-day-old soybean, Kono et al.[7] showed that lateral roots accounted for 99% of total root length.

Therefore, it should be very important to include lateral root development when a root system morphology is to be quantitatively characterized and compared with others. We previously reported that at heading stage, the rice cultivars with low adaptability for heavy manuring (AHM) showed a substantial increase in the total lateral root length with increasing nitrogen (N) application, whereas in cultivars with high AHM, the total lateral root length remained relatively unchanged under different N application regimes[8]. In addition, cultivar difference related to their ecotype was also observed. The total lateral root length of indica type cultivars were greater than that of japonica type cultivars under all different N application regimes. These facts indicate that the lateral root is the key organ also when the response of root system to a given environmental trigger like N application is to be studied. However, only very few studies have been conducted so far on the growth response of lateral roots to the different N application[4].

We have classified lateral roots of cereal plants into two types by their morphology[5,11]; one is L-type lateral root, which is relatively long, large in diameter and produces the higher order lateral roots, and the other is S-type lateral root, which is short, small in diameter and non-branching. Therefore, in the evaluation of root system morphology, we paid special attention to the development of three different component roots; nodal root axes (including one seminal root), L type, and S type lateral roots.

The aims of this study were to compare the root system morphology among four rice cultivars which differ in their AHM and ecotype (indica and japonica type), and to evaluate its phenotypic plasticity by examining the growth responses of root system to different N application amounts as the integrated production and elongation responses of each cmponent root.

Materials and Methods

The following four rice cultivars that differ in AHM were selected; Gin-nen (indica type, low AHM[1]), Tamanishiki (japonica type, low AHM[1]), Habataki (indica-japonica hybrid cultivar and regarded as indica type cultivar, developed at Hokuriku National Agricultural Experiment Station, Japan as a high yielding cultivar in 1989, thus expected to have high AHM), Norin 25 (japonica type, high AHM[1]).

Rice plants were grown in root boxes (25 cm × 2 cm × 40 cm) filled with 2.6 kg of loamy sand soil uner submerged conditions in a vinyl shed. Three different N level plots were prepared; 5N plot (equivalent to 5 kg N/1000 m^2), 10N plot (10 kg N/1000 m^2) and 30N plot (30 kg N/1000 m^2). Assuming that in paddy fields, the depth of cultivated soil layer where fertilizer is applied is 10 cm, the amount of N application to the root box soil was determined so that the whole soil profile in the box contained the equivalent rate of N as the paddy field. Compound fertilizer (12% N, 16% P_2O_5, 14% K_2O) was used for 5N plot as an N source and ammonium sulphate was additionally used for 10N and 30N plot.

Three root boxes were prepared for one plot of each cultivar. Three pregerminated seeds of each cultivar were sown in the root box on July 23, 1990 and thinned to one plant per root box four days after sowing. All plants then were sampled 14 days after sowing. At sampling, plant height, plant age in leaf number and number of tillers were recorded, then the shoots were separated from the root systems. The shoots were oven-dried at 80°C for two days and weighed. Then, the total nitrogen content of the shoots was determined by the semi-micro Kjeldahl method. Root systems were sampled by the revised root box method which facilitates the sampling with little damage and loss of fine roots[6] and then preserved in FAA (formalin, acetic acid, 70% alcohol (1 : 1 : 18)) for further study.

The preserved root systems were stained with 0.25% Coomassie Brilliant Blue R. This procedure is important to get good contrast of root image against background during scanning by the image scanner for root length measurement as described later. After separating all nodal roots from the node base, the number per plant was recorded and each nodal root axis length was measured visually. The number of L type and S type lateral roots on each nodal roots was counted. Subsequently, L type lateral roots, together with their concomitant 2nd order laterals, were separated from the nodal root axes in each sample, and then the rest of the root, i.e., nodal root axes with S type lateral roots were cut into about 2-cm segments. The length of these two root parts was measured by the image analysis method previously reported[9]. Consequently,

Table 1. Shoot growth and nitrogen (N) content of four rice cultivars as affectd by different amounts of N application.

Cultivar	N-level (kg/1000 m^2)	Numer of tillers (No./plant)	Plant height (cm)	Shoot dry weight (g/plant)	N content (% Dry weight basis)
Gin-nen	5	1.7 a	43.5 a	0.191 a	2.03a
	10	2.0 a	45.2 a	0.187 a	2.03a
	30	2.0 a	45.9 a	0.206 a	2.06a
Tamanishiki	5	2.3 a	33.9 a	0.157 ab	2.14a
	10	2.3 a	35.2 a	0.144 b	1.96a
	30	2.0 a	33.3 a	0.185 a	2.03a
Habataki	5	2.0 a	37.2 a	0.182 a	2.09a
	10	2.0 a	37.5 a	0.195 a	1.99a
	30	2.0 a	37.1 a	0.223 a	2.13a
Norin 25	5	1.7 a	32.3 a	0.149 a	2.19a
	10	2.0 a	31.1 a	0.126 a	2.00a
	30	2.0 a	32.6 a	0.181 a	2.12a

Data are means of 3 plants. Data followed by the same letter in each column are not significantly different by Duncan's multiple range test (P=0.05).

the length of L type lateral roots included those of their concomitant 2nd order laterals. The total length of S type lateral roots was estimated by subtracting the length of the nodal root axis from that of the nodal root axis with S type lateral roots. Root dry weight was determined as ash-free organic root matter[10].

Results

Overall plant responses to the three N application regimes expressed by shoot growth parameters and N content are summarized in Table 1. Number of tillers per plant, plant height and N content of a plant were not affected by the N application regimes in any of the cultivars, while shoot dry weight showed a tendency to increase with increasing N application. The manner of responses was not different among cultivars. Plant ages in leaf number were about 6 for all cultivars of all plots.

Root growth parameters are presented in Fig. 1 and Table 2. As indicated in Fig. 1, the total length of nodal root axes tended to decrease as the amount of applied N increased. This tendency was more evident for the two indica type cultivars, Gin-nen and Habataki.

For the total lateral root length, a similar trend with nodal root axes was recognized for the indica type cultivars, while for the japonica type cultivars, no consistent trend with N application regimes was noted. Since in the calculation of this parameter, the total number of laterals roots produced and their single average length are involved, the results of more detailed analysis on the lateral roots

Table 2. Number of nodal root axes and root dry weight of four rice cultivars as affected by different amounts of N application.

Cultivar	N-level (kg/1000 m^2)	Number of nodal root axes (No./plant)	Root dry weight (g/plant)
Gin-nen	5	30.3 a	0.028 a
	10	31.0 a	0.020 ab
	30	33.7 a	0.018 b
Tamanishiki	5	31.0 a	0.028 a
	10	30.3 a	0.022 a
	30	32.3 a	0.023 a
Habataki	5	35.0 a	0.028 a
	10	34.7 a	0.026 a
	30	34.7 a	0.020 b
Norin 25	5	33.3 a	0.027 a
	10	31.3 a	0.018 b
	30	35.0 a	0.022 ab

Data are means of 3 plants. Data followed by the same letter in each column are not significantly different by Duncan's multiple range test (P=0.05).

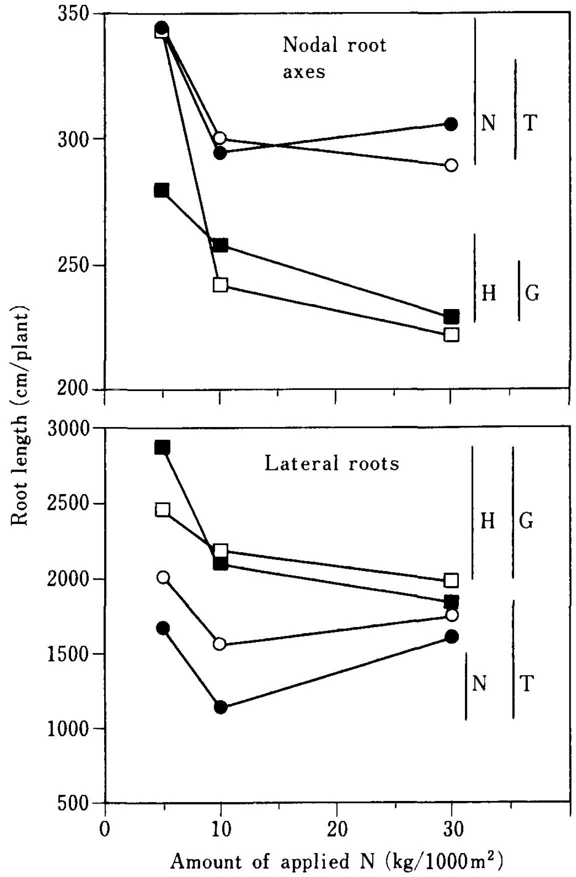

Fig. 1. Changes in total nodal root axis length and total lateral root length of Gin-nen (□), Tamanishiki (○), Habataki (■) and Norin 25 (●) as affected by different amounts of N application. Vertical bars with letters in the figure indicate LSD (p=0.05) of each cultivars. G, Gin-nen; T, Tamanishiki; H, Habataki; N, Norin 25.

response, which is the key response of the entire root system, will be presented later. In contrast to the ecotype, the AHM of cultivars could not be related to these root elongation response patterns for both nodal root axes and their concomitant lateral roots.

It is worthy to note that in the total nodal root axis length, the two japonica type cultivars were longer than the indica cultivars at all N levels, while an opposite trend was evident in the total lateral root length.

Production of nodal roots was not affected by different N levels in any cultivars, while total root dry weight showed a similar trend with total nodal root axis length (Table 2).

Now lateral roots responses to different N application regimes are explained by paying special attention to the two different types, i.e., L and S types. In this detailed analysis, lateral root response differences were found to correspond more consistently to the differences in the AHM of cultivars rather than those in the ecotypes.

Production of L type lateral roots per unit length of nodal root axis (density in Table 3) tended to decrease with an increasing amount of N application for Gin-nen, a cultivar with low AHM, and it did not respond at all for Tamanishiki, a cultivar with low AHM, while it tended to increase for the two cultivars with high AHM. For S type, all the cultivars showed a tendency to increase in this parameter, and were most pronounced in Gin-nen (Table 4).

Elongation responses (Fig. 2) were also different between the two types of lateral roots and the responses of S type lateral roots could be well related to the AHM of cultivars, but not those of the L type. The average length of single S type lateral root tended to decrease as the amount of applied N increased. This response occurred more sharply in the cultivars with high AHM than those with low AHM, which was the main factor leading to a drastic decrease in the total length from a 5N plot to a 10N plot (Table 4). L type lateral root responses were rather similar to those of nodal root axes described earlier except for Gin-nen (Fig. 1).

It is also interesting to note here that the cultivars with high AHM had a capacity to produce more L type lateral roots either per plant base or per unit nodal root axis length base (Table 3), but that no such difference was found in S type lateral root production among the four cultivars (Table 4).

Discussion

First, our present results clearly showed that a genetic difference in root system morphology existed in the four rice cultivars examined.

An entire root system length was accounted for by mainly lateral roots as many other studies have already proved[2,3,7,10]. As summarized in Table 5, the cultivar difference was primarily recognized in (1) the composition in length of nodal root axes and lateral roots to form the entire root system length; the lateral roots accounted for about 90% for Gin-nen and Habataki (indica type), about 85% for Tamanishiki, and a few percent lower for Norin 25, and (2) the lateral root length

Table 3. L type lateral root development of four rice cultivars as affected by different amounts of N application.

Cultivar	N-level ($kg/1000\ m^2$)	Total length (cm/plant)	Number (No./plant)	Density (No./cm nodal root axis)
Gin-nen	5	119.5 a	32.0 a	0.14 a
	10	99.5 a	22.7 ab	0.12 a
	30	40.8 b	11.7 b	0.07 a
Tamanishiki	5	205.2 a	62.0 a	0.31 a
	10	179.5 a	56.7 a	0.31 a
	30	132.3 a	57.7 a	0.31 a
Habataki	5	378.7 a	101.7 a	0.50 a
	10	383.4 a	114.3 a	0.61 a
	30	275.1 b	111.0 a	0.65 a
Norin 25	5	199.4 a	84.0 a	0.39 a
	10	121.5 a	67.7 a	0.37 a
	30	181.3 a	89.7 a	0.43 a

Data are means of 3 plants. Total length includes those of their 2nd order laterals. Data followed by the same letter in each column are not significantly different by Duncan's multiple range test ($P=0.05$).

Table 4. S type lateral root development of four rice cultivars as affected by different amounts of N application.

Cultivar	N-level ($kg/1000\ m^2$)	Total length (cm/plant)	Number (No./plant)	Density (No./cm nodal root axis)
Gin-nen	5	2238.9 a	2456.3 a	10.9 b
	10	2101.9 a	2108.3 a	11.7 ab
	30	1923.5 a	2414.7 a	14.3 a
Tamanishiki	5	1754.1 a	2211.3 a	10.3 a
	10	1410.3 a	1855.3 a	10.5 a
	30	1613.9 a	2434.3 a	13.0 a
Habataki	5	2493.3 a	2290.7 a	10.9 a
	10	1720.6 ab	2029.3 a	10.9 a
	30	1539.5 b	2320.7 a	13.5 a
Norin 25	5	1510.9 a	1903.0 a	9.2 a
	10	1024.9 b	1828.3 b	9.8 a
	30	1401.4 ab	2495.3 a	12.0 a

Data are means of 3 plants. Data followed by the same letter in each column are not significantly different by Duncan's multiple range test ($P=0.05$).

composition by L type and S type laterals ; e.g. at 5N plot, L type lateral roots accounted for about 9 to 10% for the two japonica type cultivars, while the percentage was almost half for Gin-nen and a few percent higher for Habataki as compared with the japonica type cultivars. Further, the cultivar difference was even more drastic in the ratio of the number of S type to L type lateral roots (Table 6). This ratio was found to be highest in Gin-nen, followed by Tamanishiki, and lowest, but almost equal, in Habataki and Norin 25.

Such characteristics of root system morphology could be related to either the ecotype or AHM of the cultivars. The composition in length could be strongly related to the ecotype difference, while that in number could in turn be strongly related to the AHM of the cultivar

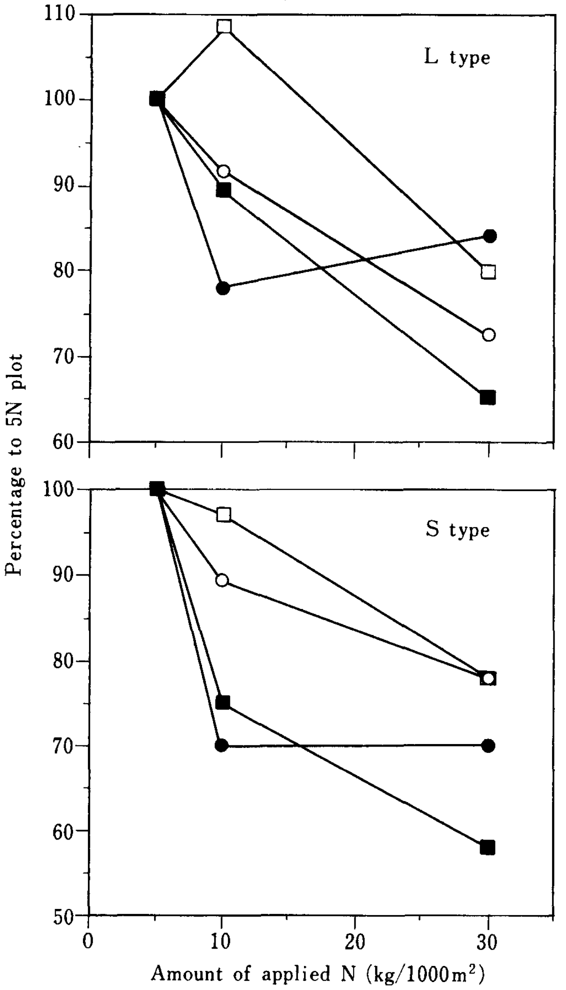

Fig. 2. Changes in average length of single L type and S type lateral root of Gin-nen (□), Tamanishiki (○), Habataki (■) and Norin 25 (●) as affected by different amounts of N aplication. Data are expressed in ratio of 10N and 30N plot to 5N plot.

i.e. the cultivars with low AHM produced substantially more S type lateral roots relative to L type lateral roots than the ones with high AHM (Table 6). In other words, the latter cultivars were superior in capacity to produce L type lateral roots as compared with the former cultivars.

Second, an attempt was made in this study to evaluate the growth response (phenotypic plasticity) to different N application levels of three component roots (nodal root axis, L type, and S type lateral root) in a root system of four rice cultivars, and for this purpose, their production (number) and elongation (length) were determined under three N application regimes. In production response, nodal root axes were scarcely affected by N application (Table 2). On the other hand, although the differences between different N regimes plots were not significant in many cases, an increase of S type lateral root production tended to be associated with that of the amount of N applied for all the cultivars, and most pronounced in Gin-nen (low AHM) (Table 4), while, by the same N treatments, L type lateral root production was significantly inhibited for Ginl-nen, not affected for Tamanishiki (low AHM), and showed a slight increase tendency for the cultivars with high AHM (Table 3).

In elongation response, effects of increased amount of N application were mostly inhibitory for S type lateral root elongation, and severe reduction was observed at lower N level (10N plot) for the cultivars with high AHM than those with low AHM (Fig. 2). L type lateral roots responded differently to N treatment but the effects were still inhibitory though no relationship of response patterns to the cultivars AHM was observed. As for nodal root axes, since the total length was decreased (Fig. 1), while their numbers remained almost unchanged by the increased N application (Table 2), the elongation of each nodal root axis was also inhibited by the treatment. Further, comparing the responses between lateral and nodal root axes in their total length, their response patterns were basically similar (Fig. 1).

These facts indicate that the phenotypic plasticities of the three component roots substantially differed in production response, while they were similar in elongation responses under different N concentrations in soil.

Finally, we attempted to understand the root system morphology response as a consequence of above-described developmental responses of component roots that are summarized in Tables 5 and 6. We found that the root system morphology was altered by the N treatments, but differently among the cultivars. The most pronounced difference was found in the ratio in number of the S type to L type lateral roots (Table 6). The ratio showed a tendency for sharp increase correspondingly with increasing N application for Gin-nen, slight increase for Tamanishiki, while the ratio seemed to be relatively unchanged for the cultivars with high AHM. On the other hand, when root system morphology changes

Table 5. Composition of a root system in length by nodal root axes, L type and S type lateral roots of four rice cultivars as affected by different amounts of N application. Data are expressed in percentage of each component root to the entire root system length.

Cultivar	N-level (kg/1000m²)	Nodal root axes (%)	Lateral roots (%)		
			Total	S-type	L-type
Gin-nen	5	12.3 a	87.7 a	83.5 a	4.2 a
	10	10.5 a	89.5 a	85.3 a	4.2 a
	30	10.2 a	89.9 a	88.0 a	1.8 b
Tamanishiki	5	15.6 a	84.4 a	75.4 a	9.0 a
	10	16.2 a	83.8 a	74.6 a	9.2 a
	30	14.3 a	85.6 a	79.1 a	6.5 a
Habataki	5	9.8 a	90.2 a	78.3 a	11.9 a
	10	11.1 a	88.9 a	71.7 a	17.2 a
	30	11.5 a	88.5 a	74.6 a	13.9 a
Norin 25	5	16.8 a	83.2 a	73.6 a	9.6 a
	10	20.6 a	79.4 b	71.0 a	8.4 a
	30	16.4 a	83.6 a	74.0 a	9.6 a

Data are means of 3 plants. Data followed by the same letter in each column are not significantly different by Duncan's multiple range test (P=0.05).

Table 6. Ratio of S type to L type lateral root number of four rice cultivars as affected by different amounts of N application.

Cultivar	N-level (kg/1000 m²)	ratio
Gin-nen	5	86.8 a
	10	97.5 a
	30	269.3 a
Tamanishiki	5	38.8 a
	10	37.9 a
	30	44.7 a
Habataki	5	23.6 a
	10	18.2 a
	30	21.1 a
Norin 25	5	24.8 a
	10	31.0 a
	30	28.2 a

Data are means of 3 plants. Data followed by the same letter in each column are not significantly different by Duncan's multiple range test (P=0.05).

are evaluated based on the composition in length of the three roots (Table 5), they were rather stable under the different N conditions, except for Gin-nen. Therefore, it can be concluded that root system morphology of Gin-nen, the cultivar with low AHM is phenotypically plastic, while that of the other cultivars are relatively stable to the changes of N conditions in the soil.

Acknowledgment

We are grateful to the Japanese Ministry of Education, Science and Culture for its support in carrying out this investigation under Grant No.05304012.

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* In Japanese with English summary.
** In Japanese with English abstract.
*** Translated from Japanese by the present authors.