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Carbon and Nitrogen Dynamics with Aging in Seminal Root System of Rice Seedling*

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Abstract: The allocation pattern of carbon (C) and nitrogen (N) with aging among several organs of rice seedling was investigated with particular attention to the difference in seminal root axis (SRA) and concomitant lateral roots (LR). Rice (*Oryza sativa* L. cv. Aichiasahi) was grown in root boxes under submerged conditions for 35 days after sowing (DAS). Overall C and N concentration of LR were higher than those of SRA, and the difference was more pronounced in N concentration. In the seminal root portion of the same age, C and N content decreased in both SRA and LR with progression of age, however a trend of declining N content in LR was alleviated by the rooting of higher (2nd order) LR. The ratios of root length to C content and dry weight in LR were 27 times that of SRA, and the ratio of root length to N content in LR was 12 times that of SRA after Day 21. These results suggest that the aging patterns of LR and SRA are different, and LR plays a more important role in controlling the metabolic activity of the whole root system than SRA. Moreover, increase in N content in SRA after Day 12 and the increase in the number of LR up to 35 DAS suggested that the seminal root of rice was alive and maintained its metabolic activity for at least 35 DAS.
Key words: Aging, Carbon, Lateral roots, Nitrogen, Partitioning, Rice, Seminal root axis.

水稲種子根のエイジングに伴う炭素および窒素の動態: 郭　康洙・飯嶋盛雄・山内　章・河野恭廣（名古屋大学農学部）

要　旨: 水稲芽生えの各器官における炭素および窒素の分布パターンを経時的に測定し，とくに種子根の主根軸と側根のエイジングに伴う変化を比較検討した．水稲品種・愛知旭の芽生えを，根箱を用いて湛水条件下で播種後35日間生育させた．炭素および窒素含有率は主根軸に比べて側根の方が高く，とくに窒素含有率で顕著であった．同じエイジの種子根部位において，炭素および窒素含有量は加齢するにつれて，両器官ともに減少する傾向にあったが，新たな高次（2次）の側根が発根することにより，側根における窒素含有量の減少パターンが緩和された．主根軸と側根における炭素および窒素含有量と乾物重から長さへの転形効率は，播種後21日目以降になると側根の方が主根軸に比べて，炭素含有量と乾物重では約27倍，窒素含有量では約12倍高かった．これらの結果は，主根軸と側根とではエイジングのパターンが異なること，また主根軸より側根の方が，全根系の代謝活性を制御する器官として重要な役割を果していることを示唆した．さらに，播種後12日目以降の主根軸における窒素含有量が増加することや，側根数が実験終了時まで増加したことは，水稲の種子根は少なくとも播種後35日目までは生存し，代謝活性を維持していることを示すものであった．
キーワード: エイジング，種子根軸，水稲，側根，炭素，窒素，分配．

Carbon (C) and nitrogen (N) needed for plant growth are translocated from absorbing parts to the other plant parts. Large amounts of carbohydrates translocated into roots are used for root respiration, and the energy obtained by root respiration is mainly utilized for root growth, nutrient uptake and root maintenance[1]. As the root system ages, root respiration activity generally decreases[5], and the proportion of respiration for root growth and nutrient uptake decreases rapidly, whereas that for root maintenance increases[12]. Considerable amounts of N taken up by roots are translocated to leaves, and large amounts of organic N needed for root growth are supplied through re-translocation from leaves[6,7]. As plant ages, the ability of reduction into organic N in both roots and leaves, and N uptake by roots generally decreases.

As mentioned above, C and N metabolism and their translocation patterns in roots are affected by ages. Therefore, it is of great interest to study the changes in these processes within the root system as plant age progresses. Tatsumi and Kono[9,10] demonstrated that old nodal roots and leaves of rice plants were the major supply source to the actively growing

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young nodal roots with nitrogenous compounds, and concluded that the roots with different ages have different N metabolism.

In addition to nodal roots, the cereal root system is composed of several other component roots, and the major part of a root system is accounted for by lateral roots (LR) emerged from seminal and nodal roots. It was reported[11] that root surface area of the LR reached up to 90% of the total root surface area of the entire root system and about 90% of water was taken up by the LR. Most probably, LR would also be greatly responsible for nutrient uptake. Therefore, high respiration rate in a root system is closely associated with proliferative growth of LR[2]. These observations indicate that LR would have an important role in controlling the translocation pattern and metabolic activities of C and N in a root system. Thus it is very important to estimate C and N allocation pattern in root axes and LR separately.

In the study concerning translocation of C and N in a root system, many researchers have employed hydroponics because of the convenience of the sampling of root systems[5,7,12]. Since hydroponically-grown roots are very much different from soil-grown roots in their development and function, it is seemed that they are also different in C and N metabolism. Therefore, in this study, we investigated the C and N dynamics in seminal root axis (SRA) and concomitant LR of the rice seedling grown in soil to evaluate the relationship of the activity of C and N metabolism and aging in SRA and LR.

Materials and Methods

The experiments were conducted in an environment-controlled phytotron, which was the natural irradiance type, and maintained at 30°C day/25°C night temperature and 80% relative humidity. A total of 90 root boxes (40 cm×25 cm×2 cm) were filled with loamy sand soil and submerged in water. Seeds of rice (*Oryza sativa* L. cv. Aichiasahi) were pre-germinated at 30°C for 2 days, and were sown in the root boxes on July 15, 1993. Three to ten plants were grown in each root box depending on the growth period without any fertilizer. At each sampling, plants from ten replicate root boxes were sampled. Seedlings were sampled at 2-day intervals from 4 to 14 days after sowing (DAS), and thereafter 7-day intervals until 35 DAS. To ensure intact sampling of the root system, the revised root box pin-board method was employed[4]. Using this method, the root system can be sampled with minimal disturbance and loss. In the present study, we conducted two experiments separately. The allocation pattern of C and N in relation to aging was compared among several organs in Exp. 1, and between SRA and LR in greater details in Exp. 2.

1. Experiment 1

Each sampled seedling was divided into the above-ground part (shoot), endosperm, nodal roots and seminal root. Moreover, all of the LR emerged on SRA was separated from SRA using fine forceps. Each divided organ was cut into pieces and mixed for homogeneity. These were divided into three masses for the three replicate measurements of C and N concentration, and dry weight. Each organ, except for SRA and LR, was milled using a vibrating sample mill (T1—100, Heiko Co. Ltd.).

2. Experiment 2

In this experiment, we focused on a given portion of seminal root, and attempted to evaluate the age effects on the C and N content and dry weight. For this purpose, only the same portion of seminal root, which elongated during the first 4 days with exception of 3 mm-root tip, i.e. a portion of 11.7 cm from the root base, was used at each sampling (Fig. 1). In the root portion, all of the LR emerged was separated from the SRA using fine forceps. Some of the seminal roots were fixed with FAA (Formalin, Acetic acid, 70% EtOH ; 1 : 1 : 18 parts by volume) for the measurement of the number and length of LR.

All of the organs sampled in Exps. 1 and 2 were dried for 3 days in a drying oven at 80°C, and preserved in desiccators to prevent moisture until analysis. The dry weights of each plant organ were measured using micro balance (MT-5, Mettler), and then C and N concentrations were determined using a CHN corder (MT-5, Yanaco Co. Ltd.).

Results

1. Experiment 1

The seminal root elongation pattern is shown in Fig. 1. Since the patterns were almost the same between Exps. 1 and 2, data only from Exp. 2 is shown for simplification.

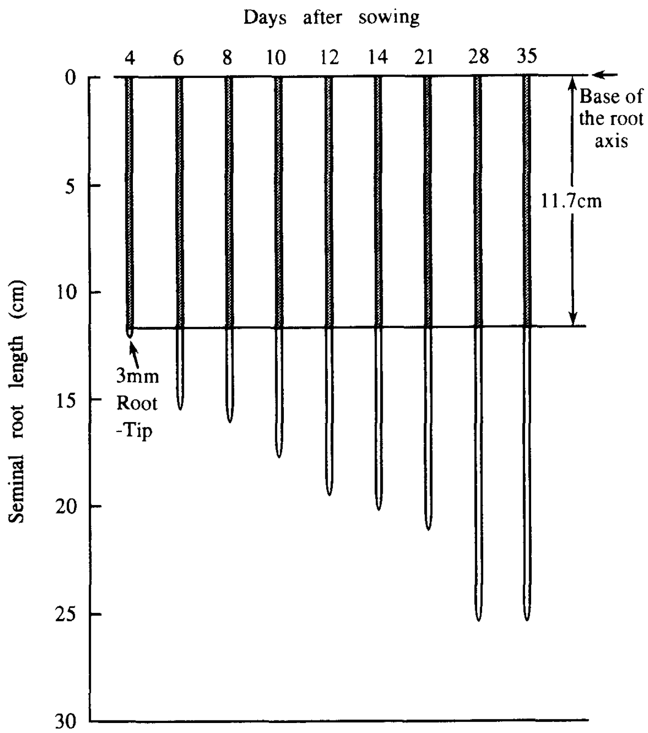

Fig. 1. Schematic diagram of the seminal root elongation pattern in Exp. 2. Shade parts show the seminal root portion which was used for analyses in Exp. 2. This portion elongated during the first four day after sowing and 11.7 cm in length.

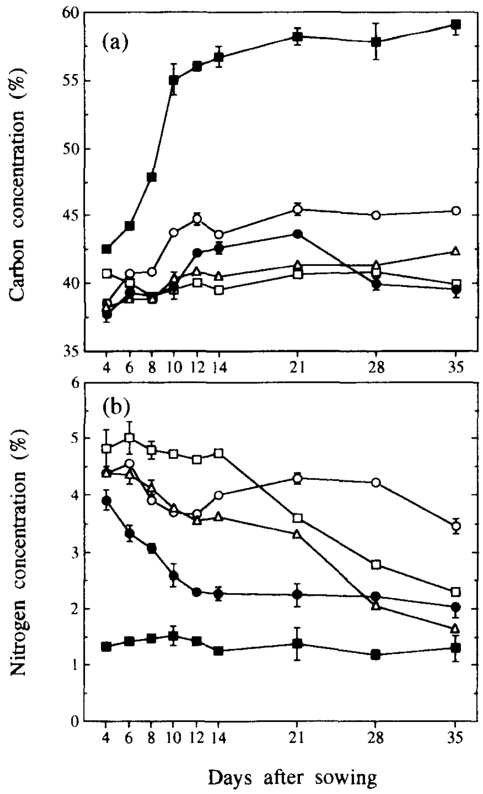

Fig. 2. Changes in carbon (a) and nitrogen (b) concentration with aging in several organs of rice seedling in Exp. 1. The values are dry weight bases, and represent 3 replicate measurements. Vertical error bars indicate standard deviation. shoot (□——□) ; endosperm (■——■) ; lateral roots (○——○) ; seminal root axis (●——●) ; nodal roots (△——△).

The concentration of C and N in individual plant organs, including the SRA and LR, are shown in Fig. 2. The pattern of changes of C concentration in shoot and endosperm were contrastingly different from that of N concentration. Namely, the concentration of C in shoot was almost constant during the experimental period, whereas that of endosperm increased drastically up to about 55% at 10 DAS, and thereafter maintained the high level. In contrast, the concentration of N in shoot was the highest level of all the organs examined until 14 DAS, but thereafter gradually decreased to about 2.5% at 35 DAS. However, that in endosperm was almost constant, and maintained the lowest level during the experimental period.

In the case of SRA and LR, although only slightly higher concentration was observed in LR, there were no marked differences in the value of C concentration between them until 21 DAS. However, thereafter the value of SRA decreased rapidly, whereas that of LR was constant. As a result, the C concentration in LR was 6% higher than that in SRA at the end of the experiment. In the concentration of N, the value of SRA was gradually decreased to about 2% at 14 DAS, and thereafter maintained at an almost constant level. Although the concentration was also decreased up to 12 DAS, there was a tendency for further increase in the concentration in LR from 14 to 21 DAS. Therefore, the N concentration in LR was about twice that of SRA from 21 to 28 DAS, and was the highest of all the organs examined after Day 21.

In nodal roots, in which root axes and their lateral roots were mixed for the analysis, the concentration of C exceeded that of SRA during 28 to 35 DAS (Fig. 2). However, the concentration was always lower than LR of seminal root during the experimental period. In the case of N concentration, nodal roots showed higher levels than SRA during 4 to 21 DAS, whereas the concentration showed higher levels during only 8 to 10 DAS than LR

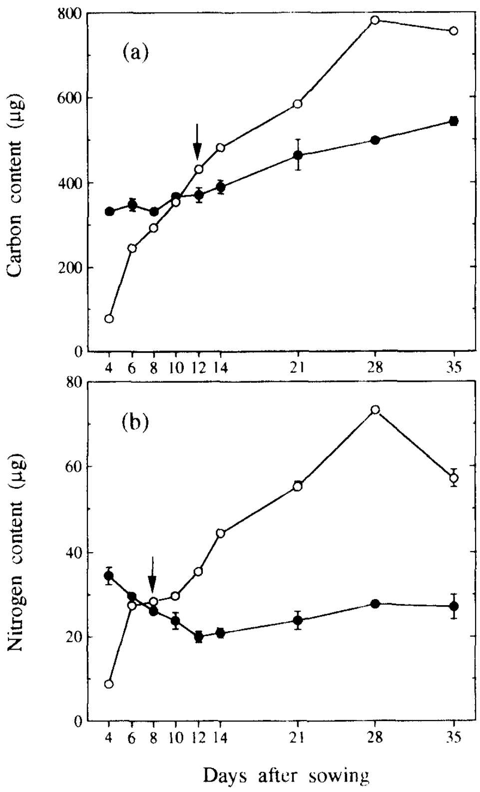

Fig. 3. Changes in the content of carbon (a) and nitrogen (b) in the whole SRA (●—●) and LR (○—○) with aging in Exp. 1. The values of LR represent the sum of all of the LR attached on the SRA. Arrows indicate the mean time when the contents in LR exceeded those in SRA.

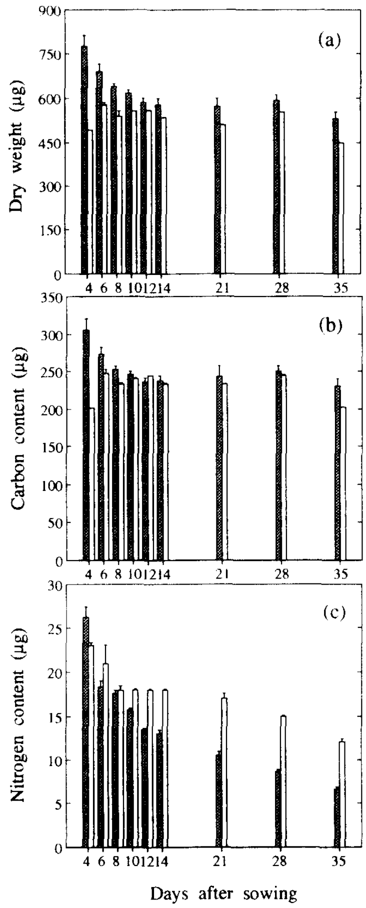

Fig. 4. Changes in dry weight (a), C (b) and N (C) content in SRA (■) and LR (□) in Exp. 2. Vertical error bars represent standard deviation from three replicate measurements.

of seminal root.

As for the content of C and N, there were also differences of C and N allocation patterns (Fig. 3). As the seminal root grew, LR emerged vigorously on the SRA, and soon the contents of C and N in LR came to exceed those in SRA. However, the point in time (DAS) when the contents in LR exceeded those in SRA was somewhat different between C and N. The time was 12 DAS for C content and 8 DAS for N content. The contents of C and N in LR gradually increased, except for the values at 35 DAS. In the case of SRA, the content of C constantly increased. However, that of N decreased till 12 DAS, and thereafter exhibited a pattern of increase.

2. Experiment 2

In the seminal root portion of the same age, i.e. 11.7 cm in the length from the root base, the content of C and dry weight in SRA and LR showed no marked differences during 8 to 28 DAS, whereas N content gradually decreased in both organs (Fig. 4). At first, N content in SRA was higher than in LR. However, thereafter the content gradually decreased to 25% of original content at the end of the experiment. In the case of LR, however, N content also showed a decreasing pattern at first, and then no significant change was observed from 8 to 14 DAS. Therefore, N content was always higher in LR compared to SRA after 8 DAS. In this period, higher (2nd order) LR emerged vigorously on the 1st order LR. The number of 2nd order LR increased 256% from 8 to 10 DAS, and 56% from 12 to 14 DAS (Fig. 5). The emergence of new LR had the effect of alleviating the decrease of N content in LR overall. As the age of LR proceeded from 28 to 35 DAS, the effect disappeared. However, the N content in LR was still about twice that in SRA at the end of the experimental period.

In the case of C/N ratio, LR was always

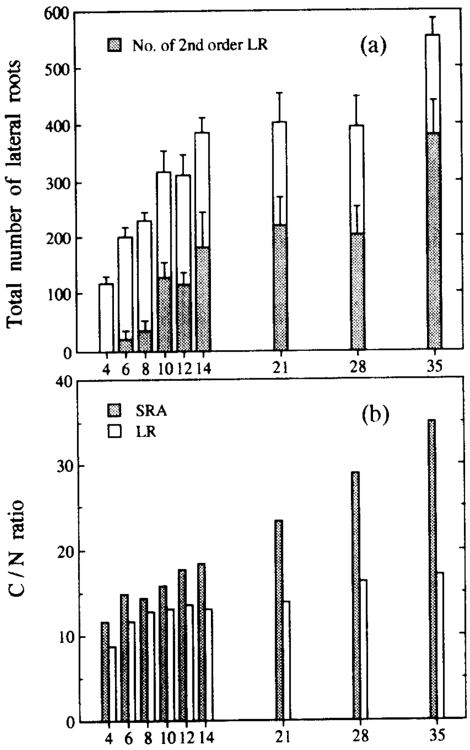

Fig. 5. Changes in the number of total and 2nd order LR (a) and C/N ratio in SRA and LR (b) in Exp. 2. Vertical error bars in the upper graph represent standard deviation from three to ten replicate measurements.

lower as compared with SRA, and only 44% of that of SRA at 35 DAS (Fig. 5). These results suggested that the activity of N metabolism was much higher in LR than in SRA.

Now, to determine how these resources (C, N and dry matter) are utilized for the root growth, we calculated the ratio of root length to C content (L/C), N content (L/N) and dry weight (L/D). We termed these ratios as conversion efficiency to length. When the conversion efficiency of root length was calculated, including both SRA and LR, the values at 4, 14 and 35 DAS were 2.032, 5.528 and 6.745 (mm/μg) in L/C; 21.917, 87.343 and 162.572 (mm/μg) in L/N; 0.812, 2.339 and 2.995 (mm/μg) in L/D, respectively. When efficiency was calculated separately for SRA and LR, however, considerable differences were noted between them (Table 1). The values of L/C, L/N and L/D in LR, and L/N in SRA showed a gradual increment during the experimental period. In contrast, the L/C and L/D in SRA was almost constant after Day 8 as the same pattern in its C content and dry weight shown in Fig. 4. Comparing the absolute values, the LR were about 8 to 12 times higher than SRA at the beginning of the experiment. Thereafter, the difference between the two organs gradually increased in the case of L/C and L/D, and at the end of the experimental period, the values in LR were about 28 times those in SRA. As for L/N, the values in LR were consistently about 12 to 15 times those in SRA.

Discussion

In this study, we focused on the C and N metabolisms of LR, whose physiology is scarcely known. When C and N concentrations were compared among several organs, N concentration in LR was the highest levels among several organs after Day 21 (Fig. 2). And when C and N metabolisms were compared between LR and SRA in a given portion of seminal root, N metabolism in LR was also greater than SRA, especially in the later growth period (Fig. 4). This fact strongly indicates that LR have an important role in controlling metabolism in a root system.

It is very difficult to estimate how long a root system is alive, and to evaluate its functional activity. In fact, such information is very limited. In this work, we found that even the elongation of SRA almost stopped after Day 28 (Fig. 1), the content of N after Day 12 showed an increase pattern up to the end of the experimental period (Fig. 3). In the case of LR grown on SRA, the concentration of C and N maintained high levels up to 35 DAS (Fig. 2), and the number of LR continued to increase even at 35 DAS (Fig. 5). We interpreted these results in such a way that the seminal root was alive and sustained metabolic activity until 35 DAS, although its activity seemed to decrease with aging.

In general, protein-nitrogen in plant tissues is easily decomposed with progression of age, and translocated to other active plant parts[8], whereas C, which is firmly bounded in cell walls, is hardly decomposed, even if the cells are dead. Based on this phenomenon, Helal[3] proposed that the protein content of soil-grown roots could be used as an index for estimating active root parts. Therefore, our results strongly indicate that LR are physiologically more active than SRA, though

Table 1. The ratio of root length to C and N content, and dry weight in SRA and LR (mm/μg) in Exp. 2. Values are means of three replications ± standard deviation, and the values in parentheses represent the ratio of LR to the SRA.

Days after sowing	Dry weight (μg)		Length/C (mm/μg)		Length/N (mm/μg)		Length/Dry weight (mm/μg)	
	SRA	LR	SRA	LR	SRA	LR	SRA	LR
4	778±36.6	491±4.4	0.38±0.017	4.5±0.04 (12.0)	4.8±0.20	40±0.35 (8.3)	0.15±0.007	1.8±0.01 (12.4)
6	685±18.6	578±12.0	0.43±0.017	8.2±0.24 (19.1)	7.3±0.25	95±2.06 (13.0)	0.17±0.005	3.5±0.07 (20.7)
8	490±11.6	541±14.6	0.50±0.008	8.7±0.23 (17.4)	8.3±0.12	110±3.31 (13.3)	0.20±0.003	3.7±0.10 (18.7)
10	610±13.1	558±1.7	0.48±0.008	9.8±0.02 (20.5)	8.7±0.12	128±0.56 (14.8)	0.19±0.004	4.2±0.01 (22.4)
12	581±12.3	559±1.3	0.50±0.010	10.1±0.01 (19.9)	9.5±0.18	135±0.10 (14.2)	0.20±0.004	4.3±0.01 (21.7)
14	585±29.9	536±6.3	0.48±0.017	10.7±0.09 (22.3)	9.7±0.30	140±0.94 (14.5)	0.20±0.011	4.7±0.05 (23.3)
21	568±23.7	510±6.0	0.48±0.026	11.5±0.03 (23.9)	12.8±0.60	159±5.37 (12.4)	0.20±0.010	5.2±0.06 (26.3)
28	588±17.4	554±1.8	0.47±0.014	12.4±0.04 (26.4)	15.0±0.10	201±0.79 (13.4)	0.20±0.006	5.5±0.02 (27.5)
35	529±23.1	449±7.5	0.50±0.022	13.8±0.01 (27.6)	19.6±0.79	238±8.13 (12.0)	0.22±0.010	6.2±0.11 (28.4)

we only measured the total content of N, not protein content directly.

In paddy rice, aerenchyma generally develops in axile roots, through which oxygen is supplied from shoot to roots for respiration. In this study, it was found that dry weight and content of C and N in SRA decreased gradually from 4 to 12 DAS (Fig. 4), and subsequently N concentration in LR slightly increased (Fig. 2) and 2nd order LR emerged vigorously on the 1st order LR (Fig. 5). These facts suggest that the decrease of dry weight and the content of C and N in SRA during this period would be mainly caused by collapse of the cortical cells and development of aerenchyma, and the released C and N from SRA would be utilized for the metabolism and development of the LR. In addition, the relatively low C/N ratio in LR indicates that the translocation rate from SRA to LR may be higher for N than for C. And from this, we speculate that the senescence of SRA took place earlier than the LR, and the LR maintained a higher metabolic activity throughout the experimental period.

In conclusion, we attempted to determine the aging pattern of the root system of rice seedling grown in soil, based on the C and N allocation pattern among several organs in general, and between SRA and LR in particular, and found that the aging pattern of LR was quite different from SRA, and LR play a key role in controlling the whole root metabolism in a root system of rice seedlings. Moreover, we observed that the seminal root was alive and functioning for at least 35 DAS.

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References

1. Gliński, J. and J. Lipiec 1990. Soil physical conditions and plant roots. CRC Press, Florida. 65—172.
2. Granato, T.C., C.D.J. Raper and G.G. Wilkerson 1989. Respiration rate in maize roots is related to concentration of reduced nitrogen and proliferation of lateral roots. Physiol. Plant. 76 : 419—424.
3. Helal, H.M. 1992. An index for estimating the living part of plant roots. In Kutschera, L., E. Hübl, E. Lichtenegger, H. Persson and M. Sobotik eds., Root ecology and its practical

application 2. Proceedings of the 3rd ISRR-symposium. Verein für Wurzelforschung A-9020 Klagenfurt, Austria. 739—742.

4. Kono, Y., A. Yamauchi, T. Nonoyama, J. Tatsumi and N. Kawamura 1987. A revised experimental system of root-soil interaction for laboratory work. Environ. Control in Biol. 25 : 141—151.
5. Lambers, H., F. Posthumus, I. Stulen, L. Lanting, V.D.S.J. Dijk and R. Hofstra 1981. Energy metabolism of plantago major ssp. major as dependent on the supply of mineral nutrients. Physiol. Plant. 51 : 245—252.
6. Larsson, C.M., M. Larsson, J.V. Purves and D.T. Clarkson 1991. Translocation and cycling through roots of recently absorbed nitrogen and sulphur in wheat (*Triticum aestivum*) during vegetative and generative growth. Physiol. Plant. 82 : 345—352.
7. Okano, K., J. Tatsumi, T. Yoneyama, Y. Kono and T. Totsuka 1983. Investigation on the carbon and nitrogen transfer from a terminal leaf to the root system of rice plant by a double tracer method with ^{13}C and ^{15}N. Jpn. J. Crop Sci. 52 : 331—341.
8. Peoples, M.B. and M.J. Dalling 1988. Senescence and aging in plants. Academic Press, New York. 181—217.
9. Tatsumi, J. and Y. Kono 1980. Nitrogen uptake and transport by the intact root system of rice plants : Comparison of the activity in roots from different nodes. Jpn. J. Crop Sci. 49 : 349—358.
10. ——— and ——— 1981. Translocation of foliar-applied nitrogen to rice roots. Jpn. J. Crop Sci. 50 : 302—310.
11. Varney, G.T. and M.J. Canny 1993. Rates of water uptake into the mature root system of maize plants. New Phytol. 123 : 775—786.
12. Werf, A.V.D., A. Kooijman, R. Welschen and H. Lambers 1988. Respiratory energy costs for the maintenance of biomass, for growth and for ion uptake in roots of *Carex diandra* and *Carex acutiformis*. Physiol. Plant. 72 : 483—491.