Remobilization of Nitrogen from Vegetative Parts to Sprouting Shoots of Young Tea (*Camellia sinensis* L.) Plants*

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Abstract : Uptake, distribution and redistribution of ^{15}N -nitrogen in young tea plants were examined during the first flush season in order to know the seasonal changes in nitrogen uptake and the origin of nitrogen in sprouting shoots. Rate of nitrogen uptake was kept at relatively lower levels before bud break, while it rose 1.5-fold after the bud break. Most nitrogen absorbed before bud break was distributed to stem and roots, and after the bud break they retranslocated to the sprouting shoots. More than 75% of nitrogen absorbed after the bud break was partitioned to the sprouting shoots. Both the nitrogen balance and isotope dilution methods indicated that approximately 30% of nitrogen accumulated in sprouting shoots was newly absorbed and remaining 70% was retranslocated from the vegetative parts. The retranslocated nitrogen would play an important role for the growth of sprouting shoots both in quantity and in quality.

Key words : Isotope dilution, ^{15}N , Nitrogen, Nitrogen balance, Origin of nitrogen, Remobilization, Sprouting shoot, tea

茶樹体内から一番茶新芽への窒素の再移動 : 岡野邦夫・小牧晋哉・松尾喜義 (野菜・茶業試験場)

要 旨 : 窒素吸収能力の季節変化あるいは新芽窒素の起源を明らかにする目的で、一番茶生長期の幼茶樹における ^{15}N 標識窒素の吸収、分配および再分配を調べた。個体当り窒素吸収量は萌芽前の2月と3月は比較的少なかったが、新芽の萌芽・伸長が始まった4月以降は約50%増加した。萌芽前に吸収された窒素の多くは一度根や茎に分布し、萌芽後新芽に再転流した。一方、萌芽後に吸収された窒素の75%以上は新芽に集中した。窒素収支法および同位体希釈法で推定した結果、萌芽後に新たに吸収された窒素は新芽窒素の約30%にすぎず、残りの約70%は樹体内からの再転流窒素に由来することが判明した。この再転流窒素は、一番茶新芽の生長に対して量的あるいは質的に重要な役割を果たしていると思われる。

キーワード : ^{15}N , 再移動, 新芽, 窒素, 窒素収支, 窒素の由来, チャ, 同位体希釈。

Tea cultivation in Japan is characterized by heavy usage of nitrogenous fertilizer. More than $1,000 \text{ kg ha}^{-1}$ of nitrogen has often been applied to tea fields annually with the intention of producing new shoots with higher content of amino acids which are demonstrated to be a determinant of the quality of green tea¹⁰⁾. Excess dressing of fertilizer, however, has brought about many problems, such as strong acidification of the soils⁵⁾, pollution of ground water with leached nitrate⁶⁾ and rise of production cost of tea leaves.

Thus, it is matter of some urgency to reduce the nitrogen application to the tea fields without a decline of leaf nitrogen content. Several trials have been conducted to reduce the nitrogen dressing, for example, the continuous monitoring of nutrient concentration in the soil by an EC sensor³⁾ or the use of controlled-release fertilizer. One of the promising

approaches would be the improvement of fertilization efficiency. From these aspects, a few workers^{1,7)} have investigated the seasonal changes in nitrogen uptake by tea plants and the origin of nitrogen accumulated in sprouting shoots. Sufficient data are, however, still lacking, so that it is difficult to determine the adequate time of application and amounts of nitrogen to the tea fields. Tea is an evergreen woody plant. Therefore, it is necessary to elucidate the uptake, distribution and redistribution of nitrogen in the plant throughout the year for the purpose.

In the present study, changes in the capacity of nitrogen uptake by young tea plants and origin of nitrogen accumulated in sprouting shoots were examined during the first flush season using two different methods, the nitrogen balance and the isotope dilution.

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Materials and Methods

Nitrogen balance method

In June 1990, cuttings of cv. Yabukita were planted in a cylindrical pot (11 cm in diameter $\times$ 20 cm tall) containing field soils. In September after the root emergence, a small amount of compound fertilizer (containing 0.6 kg N a^{-1}) was applied. After one-year cultivation in a nursery bed, 10 plants each were harvested every 10 days from February to June in 1991. A few fallings of over-wintered leaves were observed during April to May. The harvested plants were separated into mature leaves, trunk, stalk, roots and sprouting shoots. The roots were washed carefully to eliminate the soils. Separated plant parts were dried in an oven at 80°C for at least seven days. After weighing, the samples were ground to fine powder with a vibrating sample mill (Model TI-100, Heiko Manufactory, Tokyo). Total nitrogen content was determined by the distillation method after standard Kjeldahl digestion since tea plants contained little nitrate nitrogen.

Isotope dilution method

In September 1991, one-year-old cuttings of cv. Yabukita were transplanted to a water culture container (30 liter in volume) filled with modified Yanase's tea culture solution¹⁸⁾, where 30 ppm of aluminum sulphate was added and adjusted to pH 4.5 by 1N HCL and 1N KOH. The solution was continuously aerated by an air-pump. Renewal of the solution was conducted twice a month. The plants were grown in a glasshouse.

From February to May in 1992, every four plants were fed with ^{15}N -labelled ammonium

sulphate (50 ppm N, 7.80 atom % excess ^{15}N) for each month (Fig. 1). Two plants were harvested at the termination of ^{15}N feeding and the remaining two plants were sampled at the plucking time of first flush (May 1). The bud break of the first flush was observed around March 20. The harvested plants were separated into mature leaves, stem (trunk + stalk), roots and sprouting shoots. Separated plant parts were dried, weighed and ground as described above. After determining the total nitrogen content, ^{15}N content was measured by the emission spectrographic method⁹⁾ with a ^{15}N analyzer (Model N-150, Japan Spectroscopic). A portion of the nutrient solution was dried up by a rotary evaporator. Total nitrogen and ^{15}N contents of the dried sample was determined after the Kjeldahl digestion where salicylic acid was used so that the nitrate nitrogen would be measured.

Contribution of nitrogen absorbed during each month to that accumulated in the sprouting shoots was calculated by the following equation based on the technique of isotope dilution^{12,13)}.

$$\text{Contribution (\%)} = \frac{{}^{15}N \text{ abundance in sprouting shoots}}{{}^{15}N \text{ abundance in nutrient solution}} \times 100$$

Results

Nitrogen balance method

Growth of the cuttings virtually ceased in early November and the plants were in dormancy during the winter. On the next spring, first, new roots emerged at the middle of February. Bud break was observed at the beginning of April (around April 5). After the bud break, new shoots continued to grow

Fig. 1. Time of ^{15}N application and harvesting schedule.

S1 : harvest at the termination of ^{15}N feeding.

S2 : harvest at the plucking time of first flush (May 1).

steadily.

Fig. 2 shows the changes in total nitrogen contents of each plant parts from February to June. During the period, plant dry weight increased from 4 gram to 6 gram. Nitrogen in mature leaves, stem and roots began to increase in early spring, and reached to peak values at the bud break stage, then gradually decreased. On the contrary, that of sprouting shoots increased rapidly after the bud break. This implies that remobilization of nitrogen might occur from the vegetative organs to the sprouting shoots after the bud break.

Nitrogen balance of each plant part between the bud break stage and the final harvest (May 29) was calculated assuming that all of newly absorbed nitrogen after the bud break was partitioned to the sprouting shoots (Table 1). During the period examined, 27.4 mg and 15.3 mg of nitrogen was lost from mature leaves and roots, respectively. Nitrogen contents in trunk and stalk also decreased slightly. In contrast, sprouting shoots gained a large quantity of nitrogen amounting to 67.3 mg. In whole plant, 18.3 mg of nitrogen increased during the period. This is originated from newly absorbed nitrogen from the soil. Contribution of various sources to the nitrogen accumulated in the sprouting shoots was calculated using these data. The result indicated that, of the nitrogen in sprouting shoots, only 27% came from the newly absorbed one and the remaining 73% was derived from the vegetative organs mainly from mature leaves (41%) and roots (23%).

Isotope dilution method

Fig. 3 shows the total amount of ^{15}N

absorbed during each month and the ^{15}N abundance in the whole plant. Although the values of ^{15}N abundance were relatively uniform, the amount of ^{15}N taken up by a plant varied largely among the plants. This might be due to a large difference ($\text{cv}=34.4\%$) in the size of the individual plants. Dry weight varied from 7 gram to 11 gram among the plants, and total amount of ^{15}N in a plant depended on the dry weight of the plant ($r=0.868^{**}$). Therefore, the amount of ^{15}N absorbed by a plant was corrected by the plant size. During February to March, the amount of nitrogen taken up by a plant was kept at relatively lower levels though the emergence of new roots was observed at the middle of February. The amount, however, rose 1.5 times greater than the previous months at April when the

Fig. 2. Changes in total nitrogen contents of each parts of young tea plants during February to June.

Values are the mean of 10 plants. Arrow in figure indicates the time of bud break.

Table 1 Nitrogen balance of each plant part between the bud break and the plucking of first flush.

Plant part	Total nitrogen (mg plant ⁻¹)			Contribution to sprouting shoots (%)
	Bud break	Plucking	Difference	
Sprouting shoots	8.4	75.7	+ 67.3	100
Mature leaves	49.8	22.4	- 27.4	41
Trunk	11.8	9.7	- 2.1	3
Stalk	13.2	9.0	- 4.2	6
Roots	30.3	15.0	- 15.3	23
Whole plant	113.5	131.8	+ 18.3*	27

* Amount of newly absorbed nitrogen during the period.

new shoots began to grow rapidly.

Fig. 4 indicates the distribution and redistribution of absorbed ^{15}N among the plant parts. In February and March, 70 to 80% of ^{15}N absorbed was distributed to the stem and roots by equal proportion, and less to the mature leaves. After the bud break, they retranslocated to the sprouting shoots, and 50 to 60% of them were in the sprouting shoots at the plucking time. On the contrary, in April, more than 75% of nitrogen absorbed after the bud break was directly partitioned to the actively developing shoots. These results clearly demonstrated that remobilization of nitrogen did occur from the vegetative parts to the sprouting shoots.

Table 2 shows the ^{15}N abundance in the sprouting shoots and the contribution of nitrogen absorbed at each month to that in the sprouting shoots. ^{15}N abundance in the sprouting shoots was relatively low when the labelled nitrogen was applied in February or March. While in the April application, the value increased up to 2.70 atom % excess. The contribution was calculated by the equation referred to in Materials and Methods. The results indicated that nitrogen absorbed during February, March and April accounted for 12.9%, 14.1% and 34.6% of that accumulated in the sprouting shoots, respectively. In this experiment, nitrogen absorbed during April was practically equivalent to the newly absorbed nitrogen, that is, nitrogen absorbed

after the bud break. Thus, it would be concluded that approximately 35% of nitrogen accumulated in the sprouting shoots was derived from the newly absorbed nitrogen, and the remaining 65% from the retranslocated one.

Discussion

Seasonal changes in uptake and partitioning of nitrogen

Tea plant is in a dormant phase from November to March in most areas of Japan. Photosynthesis of the leaves is, however, kept at a considerable level even in winter¹⁴⁾. The roots would be also active during the period. In the present study, nitrogen applied during February, when the temperature is lowest in the year, was obviously taken up by the plants (Fig. 3). Most of nitrogen absorbed stayed in the roots or stem, and a lesser portion was transported to the leaves. Konishi et al.⁷⁾ and Hoshina and Kozai²⁾ also reported that ^{15}N -nitrogen administered in the middle of winter was absorbed by tea plants, and a large part of them were partitioned to the roots, stem and aged leaves. After the bud break of first flush, the temporary reserved nitrogen was retranslocated to the developing shoots (Fig. 4).

In April, the rate of uptake of nitrogen by the plants clearly increased compared to the preceding months (Fig. 3). Part of this phenomenon might be explained by the emer-

Fig. 3. Amount of ^{15}N absorbed by a plant during each month and ^{15}N abundance in whole plant.

Left column (closed) indicates the amount of ^{15}N and right column (open) shows the ^{15}N abundance.

Values are the mean of four plants and vertical bars indicates the standard deviation.

Fig. 4. Distribution and redistribution of ^{15}N absorbed during each month.

n ; sprouting shoots, m ; mature leaves, s : stem and r ; roots.

Table 2 Contribution of nitrogen absorbed at each month to that in sprouting shoots

Time of ^{15}N application	^{15}N abundance (atom % excess)	Contribution (%)
February	1.01	12.9
March	1.10	14.1
April	2.70	34.6

^{15}N abundance in the nutrient solution was 7.80 atom % excess.

gence of dormancy accompanying the rise of air temperature. However, the more direct cause seems to be the presence of sprouting shoots. Most of nitrogen absorbed after the bud break was concentrated in the developing shoots (Fig. 4). Strong sink demand for nitrogen caused by the developing shoots might affect the nutrient uptake by the roots. The fact that the uptake rate of nitrogen did not rise in March when a considerable number of new roots had emerged may support this assumption. Hoshina and Kozai²⁾ also observed in the first flush season that nitrogen uptake was active when the new shoots were rapidly growing. In the present study, seasonal changes in nitrogen uptake by the tea plants were measured only on three months in early spring. If the same kind of measurements are conducted throughout the year, we can know when the tea plants require the nitrogen.

Origin of nitrogen in sprouting shoots

Origin of nitrogen accumulated in the sprouting shoots of first flush was estimated by the two different methods. In the nitrogen balance method, the calculation was conducted on the assumption that all of the nitrogen newly absorbed after the bud break was partitioned to the sprouting shoots. The result in Fig. 4 suggests that the assumption was virtually correct. However, a part of newly absorbed nitrogen was distributed to the organs other than the sprouting shoots. Therefore, contribution of newly absorbed nitrogen to the nitrogen in sprouting shoots might be overestimated by this method. Furthermore, the leaf falling during the experimental period would directly affect the results. Thus, this method seems to be more convenient but less accurate as compared to the isotope dilution method. In the present study, however, both methods gave similar results, that is, 27% to

35% of nitrogen accumulated in the sprouting shoots came from the newly absorbed one, and remaining 65% to 73% was derived from the retranslocated one from the vegetative organs. These values may vary depending on the plant size or nutritional conditions.

Watanabe^{16,17)} demonstrated the remobilization of protein nitrogen from old leaves to the sprouting shoots. Hoshina et al.¹⁾ reported on the second flush of young tea plants that ^{15}N -nitrogen applied after the bud break accounted for 23% of nitrogen in the new shoots. Oishi¹¹⁾ stated on mature tea trees that more than 80% of nitrogen in the plucked shoots of the first flush was originated from the nitrogen absorbed at the year before. All of these results clearly indicated that 70% to 80% of nitrogen in the sprouting shoots was derived from that accumulated in the vegetative parts, such as the mature leaves, stem and roots. It has been well known in herbaceous crops that the nitrogen retranslocated from the vegetative parts would play an important role for the development of new leaves and roots both in quantity and in quality^{4,8,15)}.

Since the nitrogen applied at early spring was effectively transported to the developing shoots of the first flush^{2,7)}, it would be necessary to apply fertilizer just before the bud break of first flush. In addition, the manuring practice before that time would also be important for the production of new shoots with high amino acid content.

References

1. Hoshina, T., S. Kozai and K. Ishigaki 1978. Effects of manuring time and nitrogen source on absorption and metabolism of summer fertilized nitrogen by tea plant. *Study Tea* 54: 28–32*.
2. Hoshina, T. and S. Kozai 1979. Absorption of nitrogen fertilized in winter season by tea plant.

- Tea Res. J. 50 : 84—86*.
3. Iwahashi, M., N. Suzuki and A. Morita 1992. Manuring of tea field with the aid of computer. Tea Res. J. 76 (Extra) : 56—57**.
 4. Kato, Y. and S. Kitada 1979. Studies on nitrogen metabolism of soybean plants. IV. The dynamic aspect of leaf nitrogen and its relation to protein turnover. Jpn. J. Crop Sci. : 229—242.
 5. Kosuge, N. 1992. Some aspects on pH in tea soil. Study Tea 62 : 1—7*.
 6. Kosuge, N., K. Ishigaki, M. Nakashimada, I. Watanabe and T. Hoshina 1987. Reduction of the nitrogen and phosphorous losses from tea fields. Bull. Natl. Res. Inst. Veg. Ornam. Plants Tea, B1 : 23—44*.
 7. Konishi, S., M. Ota and F. Iwase 1978. Studies on absorption and utilization of nitrogen in tea plants. I. Contribution of nitrogen applied at various stages of growth to that in new shoots. Jpn. J. Soil Sci. Plant Nutr. 49 : 221—225**.
 8. Mae, T. and K. Ohira 1981. The remobilization of nitrogen related to leaf growth and senescence in rice plants (*Oryza sativa* L.). Plant Cell Physiol. 22 : 1067—1074.
 9. Muhammad, S. and K. Kumazawa 1974. Assimilation and transport of nitrogen in rice. I. ¹⁵N-labelled ammonium nitrogen. Plant Cell Physiol. 15 : 747—758.
 10. Nakagawa, M., T. Anan and N. Ishima 1981. The relation of green tea taste with its chemical make-up. Bull. Natl. Res. Inst. Tea 17 : 69—123*.
 11. Oishi, S. 1985. Soil management and manuring. In Encyclopedia of Tea Cultivation. Nobunkyo, Tokyo. 98—127**.
 12. Okano, K. and A. Furukawa 1990. NO₂-nitrogen accumulated in plants as an indicator of NO_x pollution in the atmosphere. I. Fumigation experiment. J. Jpn. Soc. Air Pollut. 25 : 371—377*.
 13. Rennie, R.J., D.A. Rennie and M. Fried 1978. Concepts of ¹⁵N usage in dinitrogen fixation studies. In Isotope in Biological Dinitrogen Fixation. IAEA, Vienna. 107—133.
 14. Sakai, S. 1987. Studies on photosynthesis and matter production. Bull. Natl. Res. Inst. Tea 22 : 19—273*.
 15. Tatsumi, J. and Y. Kono 1981. Translocation of foliar-applied nitrogen to rice roots. Japan. Jour. Crop Sci. 50 : 302—310.
 16. Watanabe, I. and K. Ishigaki 1983. Senescence and mobilization of nitrogen in old leaves of tea plant in the second flush season. Soil Sci. Plant Nutr. 29 : 7—13.
 17. Watanabe, I. 1983. Effects of nitrogen application on the spring mobilization of protein nitrogen in old leaves of tea plant. Study Tea 65 : 31—36*.
 18. Yanase, Y. 1974. The observation of growing cycle on the part of upper and under ground of the tea plant using the mist box culture. Tea Res. J. 41 : 12—15*.
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- * In Japanese with English summary.
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