Suitable Level of Nitrogen Fertilizer for Tea (*Camellia sinensis* L.) Plants in Relation to Growth, Photosynthesis, Nitrogen Uptake and Accumulation of Free Amino Acids*

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Abstract : The physiological responses of pot-grown tea plants to various levels of nitrogen fertilizer were investigated to determine the suitable level of nitrogen for tea plants. Defining an arbitrary unit of nitrogen application as 1 N plot (200 mgN pot⁻¹ year⁻¹, corresponding to 10 kgN 10a⁻¹ year⁻¹), experimental plots from 0 to 27 N were prepared using ammonium sulphate. The plants exhibited symptoms of nitrogen deficiency below 3 N plots. High photosynthetic activity and favorable growth were observed from 6 to 12 N plots. Tip-burn of mature leaves and inhibition of photosynthesis were first detected at the level of 15 N plot. At the level of more than 18 N plots, the falling of mature leaves, depression of root respiration and death of some plants occurred. Based on these results, the critical level for growth and yield was determined to be 6 N plot. Nitrogen uptake increased with the increase in the amount of nitrogen applied, while the capacity of uptake gradually saturated. The recovery rate of applied nitrogen declined linearly with the increase in nitrogen dressing. The concentration of free amino acids in the first flush shoots increased in an unlimited manner with the increase in nitrogen dressing. However, the absolute amounts in the new shoots were greatest in 9 to 15 N plots due to the inhibition of new shoot growth in heavily manured plots. Thus, with respect to the accumulation of free amino acids, the critical level for leaf quality is estimated to be around 12 N plot. The theanine content in the first flush shoots increased up to 9 N plot. Thereafter, a high content of arginine was detected, implying disorder in the nitrogen metabolism. These results demonstrate that the critical level of nitrogen for leaf quality is very close to the toxic level and is twofold greater than that for growth and yield.

Key words : Arginine, *Camellia sinensis*, Critical level, Excess nitrogen, Free amino acid, Green tea, Nitrogen uptake, Nutritional diagnosis.

生長、光合成、窒素吸収及び遊離アミノ酸蓄積からみた茶樹の適正窒素施用量：岡野邦夫・忠谷浩司***・松尾喜義（野菜・茶業試験場・***滋賀県茶業指導所）

要 旨：新芽品質向上に有効な窒素施用量の限界値や生理障害が発現する施用量を明らかにする目的で、様々な窒素施用条件下における幼茶樹の生理反応を調べた。ポット当たり年間 200 mg の窒素施用量を基準の 1 N 区（10 a 当たり年間 10 kgN に相当）とし、0 N～27 N 区までの処理区を硫酸を用いて設定した。3 N 区以下では窒素欠乏症状が見られたが、6 N 区～12 N 区にかけては光合成活性は高く、個体や新芽の生育は良好であった。葉の褐変や光合成活性の低下は 15 N 区以上で発現した。18 N 区以上では落葉、根の呼吸活性低下、一部個体の枯死等が観察された。これらより生長や収量からみた最適レベル（critical level）は 6 N 区と判断された。茶樹による窒素吸収量は施用量の増大につれて増加したが、吸収能力は次第に飽和した。施用窒素の吸収率は施用量の増大につれて直線的に低下した。一番茶新芽の遊離アミノ酸濃度は、窒素施用量の増大に伴って上昇を続けた。しかし窒素多肥区では新芽生育が阻害されたため、新芽中に蓄積した遊離アミノ酸量は 9 N 区～15 N 区で最高となった。これらより新芽品質からみた最適レベルは 12 N 区前後と判断された。新芽中のテアニン濃度は 9 N 区まで上昇したが、それ以上の多肥区では大量のアルギニンが集積し、窒素代謝の異常が推察された。以上より、新芽品質からみた窒素の最適レベルは樹体に生理障害が発現する直前のレベルであり、生長や収量からみた最適レベルの 2 倍近い値であると考えられる。

キーワード：アルギニン、栄養診断、過剰窒素、最適レベル、窒素吸収、チャ、遊離アミノ酸、緑茶。

In tea cultivation, a large amount of nitrogen is removed annually from the plant body

by the plucking of new shoots or skiffing and pruning. Therefore, mature tea trees need a good amount of nitrogen to maintain nutritional status at a desirable level. Annual input of nitrogen to the tea field was recommended to be 54 kg 10a⁻¹, determined by several data, such as the amount of nitrogen removed,

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recovery rate of applied nitrogen and growth response to applied nitrogen^{4,8)}. However, the amount of nitrogen applied to the tea field has gradually increased year by year, and has often exceeded 120 kg 10a⁻¹ in recent years³⁾. This is caused by the grower's intention to produce new shoots with a higher content of free amino acids, which are demonstrated to be a determinant of the quality of green tea¹⁶⁾. Meanwhile, the limit of nitrogen dressing effective for improving leaf quality is yet to be defined.

A large quantity of nitrogen dressing to the tea field has led to several environmental problems^{9,12,13)}. Furthermore, excess fertilizer under present conditions would cause a physiological disorder of tea plants, such as the death of feeder roots²³⁾. Although the tea plant is considered to be tolerant to heavy manuring, Maehara and Hakamata¹⁴⁾ stated that adaptability of tea plants to heavy manuring was not so high compared to other crops. Thus, tolerance of tea plant to excess nitrogen has not been fully understood. In a preliminary study¹⁹⁾, we examined the responses of young tea plants to relatively lower levels of nitrogen dressing, and suggested that the optimum level of nitrogen for the quality of new shoots might be greater than that for the growth of tea plants. In the experiment, however, any injury caused by excess nitrogen could not be detected.

In the present study, growth, photosynthesis, root respiration, nitrogen uptake and free amino acid content in new shoots were compared among young tea plants grown under a wider range of nitrogen levels than

used in the previous study¹⁹⁾. The main purpose is to know the limit of nitrogen dressing effective for the increase in leaf amino acids and the nitrogen level that is hazardous to tea plant.

Materials and Methods

1. Plant materials and nitrogen dressing

One-year-old cuttings of *Camellia sinensis* (L.) cv. Yabukita were transplanted from a nursery bed to 4 liter volume of unglazed pots containing field soils in March 1994. Two cuttings were planted in a pot. Fertilizer experiment was conducted from May 1994 to May 1995 in a glasshouse at NIVOT in Kanaya, Shizuoka prefecture, Japan. An arbitrary unit of nitrogen application (200 mgN pot⁻¹ year⁻¹, corresponded to 10 kgN 10a⁻¹ year⁻¹ on surface area basis) was defined as 1 N plot (Table 1). Ten experimental plots from 0 N to 27 N were prepared at 3 N intervals. As the nitrogen fertilizer was not applied in 0 N plot, the only nitrogen source in 0 N plot was soil nitrogen. The field soil used in the present experiment contained 0.3–0.4% of total nitrogen. The maximum level of 27 N was more than twofold greater than that of 12 N in the previous study¹⁹⁾. Each plot consisted of eight pots with 16 plants. Ammonium sulphate was used as a nitrogen source. Superphosphate of lime and potassium chloride were administered to all pots in equal amounts. According to the customary practice⁸⁾, fertilizer was applied with five splits, that is, summer dressing I, II, III, autumn dressing and spring dressing (Table 1). Watering was carried out carefully to lessen the leaching of nitrate from pot soils.

Table 1. Time of fertilizer application and amount of fertilizer applied to basal unit of 1 N*.

Time of application**	Amount of fertilizer (mg pot ⁻¹)		
	Ammonium sulphate	Superphosphate of lime	Potassium chloride
Summer I (May 26)	160	280	100
Summer II (Jun. 28)	160	0	0
Summer III (Jul. 29)	160	0	0
Autumn (Sep. 21)	240	280	100
Spring (Feb. 23)	240	280	100
Total	960***	840	300

* See Materials and Methods.

** 1994 ~1995.

*** Corresponds to 10 kgN 10a⁻¹ year⁻¹.

2. Harvest and nitrogen determination

Each four pots were harvested in early November when the growth of shoots ceased and at early May of the next year when the first flush shoots were plucked. The harvested plants were divided into new shoots, mature leaves, stems and roots. The roots were washed carefully to eliminate the soils. Separated plant parts were dried in an oven at 80°C for one week. The new shoots were killed by steaming before drying. After weighing, the samples were ground to a fine powder with a vibrating sample mill (Model TI-100, Heiko Manufacturer, Tokyo). Total nitrogen content of the samples was determined by the standard Kjeldahl method.

At the harvesting of the first flush shoots, percentage of *banjhi* shoots (shoots temporarily ceased to develop new leaves) to total sprouting shoots (P.B.S.), which was used as an indicator of shoot maturation¹⁾, were in the range from 50–70% in 0 N to 12 N plots. Meanwhile, the P.B.S. of the new shoots in the heavily-manured plots more than 15 N was less than 15% because of the diminished growth of the buds.

3. Measurement of photosynthesis

Eight mature leaves were selected from each plot before harvesting. Photosynthetic activity of the leaves was determined with an oxygen electrode (Rank brothers, Cambridge) as described previously¹⁸⁾. Two leaf slices (5 mm × 10 mm) were collected from both sides of the midrib of a leaf by a leaf punch, and floated on the distilled water under an incandescent lamp of 3 klx ($60 \mu\text{mole m}^{-2} \text{s}^{-1}$) for 60 min. Then, the leaf slices were cut into small pieces of 1.7 mm square by a razor, and put in a reaction vessel containing 1.9 ml of phosphate buffer (pH 7.0). Temperature in the reaction vessel was maintained at 25°C. After the incubation for 6 min under light conditions of 60 klx ($1,200 \mu\text{mole m}^{-2} \text{s}^{-1}$) which was prepared by a slide projector, 0.1 ml of 400 mM sodium bicarbonate was added to the vessel. The rate of oxygen evolution under the light was designated as the photosynthetic activity.

4. Measurement of root respiration

Root respiratory activity was measured by an O₂ uptake manometer (O₂ uptester Model 5-B, TAITEC, Koshigaya) as previously reported²⁰⁾. Approximately half of root system

was excised from the plant and introduced to a 500 ml volume of a reaction vessel, which contained 350 ml of phosphate buffer (50 mM, pH 7.0). The vessel was dipped in a water bath with constant temperature of 25°C. Oxygen consumption over four hours was measured by a manometer and consumption rate was expressed based on unit root dry weight.

5. Determination of free amino acids

Free amino acids in the first flush shoots were determined by high performance liquid chromatography (HPLC) coupled with pre-column derivatization of *o*-phthalaldehyde (OPA)⁶⁾. The powdered samples were extracted with hot water (80°C), then the extract was derivatized with OPA. The amino acid derivatives were separated by reverse-phase HPLC (Capcell Pak C₁₈ SG 120, Shiseido, Tokyo) in a linear gradient mode using 16% and 50% ethanols.

6. Measurement of soil pH and electric conductivity (EC)

Twenty-five ml of distilled water was added to 10 g of air-dried soil collected from the surface layers of the pots. After mixing well, pH was measured with a pH meter. Additional 25 ml of distilled water was added to the mixture, then EC was measured with a conductivity meter (Model CM-30ET, TOA Electronics, Tokyo).

Results

As experimental results were basically the same between the two harvesting times of November in 1994 and May in 1995, the results from the latter harvesting will be mainly shown in the following figures and tables.

1. Growth, photosynthesis and root respiration

Leaves attached to the plants in 0 N plot appeared to be yellowish, a typical symptom of nitrogen deficiency. Under the nitrogen application from 3 N to 12 N plot, the plants grew normally with no visible symptoms. Tip-burn of the mature leaves was first observed in 15 N plot. A portion of the mature leaves began to fall in 18 N plot. More than 21 N plot, a pronounced falling of mature leaves and the death of some plants occurred. A stunted growth of the first flush shoots was observed in heavily-manuring plot more than

Fig. 1. Dry weights in component parts of tea plants grown under various levels of nitrogen environment.

N: new shoots, M: mature leaves, S: stems and R: roots. Values are the mean of 8 plants. Vertical bars indicate the standard deviation of whole plant weight.

Table 2. Effect of various levels of nitrogen dressing on the rate of root respiration in young tea plants.

Nitrogen level	Oxygen consumption rate* ($\mu\text{l gDW}^{-1} \text{h}^{-1}$)	
	Nov. in 1994	May in 1995
0 N	328 $\pm$ 110**	193 $\pm$ 19
3 N	352 $\pm$ 42	269 $\pm$ 25
6 N	389 $\pm$ 80	272 $\pm$ 63
9 N	392 $\pm$ 101	250 $\pm$ 23
12 N	441 $\pm$ 77	280 $\pm$ 74
15 N	353 $\pm$ 58	218 $\pm$ 30
18 N	375 $\pm$ 57	186 $\pm$ 30
21 N	286 $\pm$ 89	150 $\pm$ 7
24 N	260 $\pm$ 40	181 $\pm$ 51
27 N	264 $\pm$ 79	180 $\pm$ 25

*Consumption rate was measured by an O_2 uptester.

**Values are the mean of 4 plants $\pm$ S.D.

18 N.

Fig. 1 shows dry weight of the plants grown under various levels of nitrogen fertilizer. As some plants had died beyond 21 N plot, the growth data were collected only from the surviving plants. The plants in 0 N plot showed a retarded growth due to the nitrogen deficiency. Among the wide range of nitrogen applications from 3 N to 12 N plot, the plants showed favorable growth. In heavily-manuring plots of more than 15 N, plant growth was suppressed in proportion to the amount of nitrogen fertilizer applied. Individ-

Fig. 2. Effects of various levels of nitrogen dressing on the photosynthetic activity of mature leaves.

The activity was measured with an oxygen electrode at November in 1994 (closed column) and at May in 1995 (open column). Values are the mean of 8 measurements with 8 leaves. Vertical bars indicate the standard deviation.

ual plant parts such as new shoots or roots exhibited a similar growth response to the nitrogen dressing, as in the case of whole plant. In heavily-manuring plots, a fraction of the mature leaves was markedly decreased by the extensive falling of the leaves. The growth of the first flush shoots, which was equivalent to the yield of the first crop, was vigorous in moderately-manuring plots from 6 N to 9 N, but was depressed both in the nitrogen-deficient plot below 3 N and nitrogen-excess plots of more than 15 N. In 24 N and 27 N plots, the unfolding of new leaves was completely inhibited and the buds remained as they were.

The effect of nitrogen level on photosynthetic activity of mature leaves is indicated in Fig. 2. Photosynthetic activity was relatively low in 0 N plot, possibly due to nitrogen deficiency. Thereafter, activity increased with the increase in nitrogen dressing up to 12 N plot. Maximum activity was observed from 6 N to 12 N plots. Photosynthetic activity started to be inhibited at the 15 N plot, where the tip-burn of the leaves was observed. Under the nitrogen-excess plots of more than 15 N, the activity gradually declined with the increase in nitrogen dressing. These trends were more obvious in the measurement in May 1995 than in November 1994.

Table 2 shows the influence of the nitrogen level on root respiration. Activity of root respi-

Fig. 3. Changes in the amount of fertilizer nitrogen absorbed by the plants and recovery rate of applied nitrogen with the increase in nitrogen dressing.

Fertilizer N = (N in plants of each plots) — (N in plants of 0 N plot, soil N). Values are the mean of 4 measurements with 8 plants.

Fig. 5. Free amino acid contents in the first flush shoots of tea plants grown under various levels of nitrogen environment.

Thea, theanine; Arg, arginine; Gln, glutamine; Asn, asparagine; and Other, other amino acids. Values are the mean of 3 measurements. Vertical bars indicate the standard deviation of total free amino acids.

ration was higher in November than in May of the next year. There were large variations in the activities of root respiration among the plants. No clear difference could be detected in plants grown under various levels of nitrogen from 0 N to 18 N plots. Decline of root respiration was first observed under heavily-manuring plots of more than 21 N where most of the leaves had fallen.

2. Nitrogen uptake

The amount of nitrogen fertilizer absorbed by the plants grown under various levels of nitrogen and recovery rate of applied nitrogen by the plants are indicated in Fig. 3. Nitrogen

Fig. 4. Effects of various levels of nitrogen dressing on total nitrogen contents of each plant parts.

Symbols are the same as in Fig. 1. Vertical bars indicate the standard deviation.

derived from the fertilizer was determined by subtracting the nitrogen in plant of 0 N plot (soil nitrogen) from that of the other plots (3 N to 27 N). Nitrogen uptake increased with the increase in the amount of nitrogen applied in the plots up to 15 N, but the uptake showed a saturation curve against the nitrogen dressing. As leaf fall occurred beyond the 18 N plot, net uptake of nitrogen could not be determined. Recovery rate of applied nitrogen by plants linearly declined in proportion to the increase in the amount of nitrogen applied. Approximately 72% of the applied nitrogen was absorbed by the plants of the 3 N plot, while only 37% of applied nitrogen was recovered in the 15 N plot where the largest amount of nitrogen was taken up.

Fig. 4 shows the effects of nitrogen level on total nitrogen contents of various plant parts. Nitrogen contents in all parts increased with the increase in nitrogen dressing, except for the roots. The value in the roots reached the uppermost limit at 18 N plot. Among the plant parts, new shoots exhibited the highest content, followed by the mature leaves, roots and stems. The value in the new shoots was 3.3% at 0 N plot, but exceeded 8.1% at the heaviest-manuring plot in 27 N.

3. Free amino acids in first flush shoots

The effects of various levels of nitrogen dressing on the contents of free amino acids in the first flush shoots are shown in Fig. 5. Content of total free amino acids was 3.2 mg gDW⁻¹ at 0 N plot, then gradually increased in proportion to the amount of nitrogen applied, and finally reached 168 mg gDW⁻¹ at

27 N plot. Theanine content increased with the increase in nitrogen application up to 9 N plot, but any significant increase could not be observed under the more heavily-manuring plots. In the 9 N plot, about 50% of total free amino acids was occupied by theanine. Beyond 9 N plot, a high content of arginine accumulated instead of theanine. Arginine accumulation further progressed in the more heavily-manuring plots, and accounted for 50 to 60% of total free amino acids in the new shoots. Under extremely heavy-manuring plots such as 24 N or 27 N, considerable amounts of glutamine and asparagine were detected in addition to arginine.

4. Soil pH and EC

Table 3 shows the changes in pH and EC values of the soils administered with various levels of nitrogen fertilizer. The pH value in 0 N plot exceeded 6.0, then gradually lowered with the increase in fertilizer dressing. It reached to the lowermost value of 4.4 at 12 N plot. Thereafter no changes were observed. Similar results were also observed on measurement in May, namely, even in the heavily-manuring plots, the lowermost value of pH remained at 4.3 to 4.4. EC value increased with the increase in fertilizer dressing from 0.2 mS cm⁻¹ at 0 N plot to nearly 2.0 mS cm⁻¹ at 27 N plot. The EC values were higher in May than in November of the preceding year. These results mean that the salt accumulation in the soil processed with the increase in fertilizer application.

Discussion

1. Critical level of nitrogen for growth and yield

The tea plants exhibited favorable growth under the nitrogen environment from 6 N to 12 N plots (Fig. 1 and Fig. 2). On the other hand, depressions of photosynthesis and new shoot growth were observed below the 3 N plot, and physiological disorders were detected beyond the 15 N plot. According to the concepts of nutritional diagnosis^{2,14)}, 0 N to 3 N plot in the present experiment may be regarded as deficiency zone, 6 N to 12 N plot as adequate or luxury zone, and more than 15 N plot as the toxic zone. A critical level of nitrogen, which is defined as the minimum nitrogen for obtaining the maximum growth or yield¹⁴⁾, is determined to be 6 N plot (Fig. 1 and Fig. 2). Surplus nitrogen in 9 N and 12 N plot was not necessarily needed for growth and yield of the plants. Assuming that the results obtained from the pot experiment can be simply converted into field conditions, critical levels of nitrogen in 6 N plot would be equivalent to 60 kgN 10a⁻¹ year⁻¹. Although the propriety of this assumption should be demonstrated in future studies, it is interesting that this figure is very similar to the recommended value⁸⁾ of 54 kgN 10a⁻¹.

Total nitrogen (T-N) content in mature leaves is often used as an indicator for nutritional diagnosis of tree crops. Maehara and Hakamata¹⁴⁾ reported on hydroponically-

Table 3. Changes in pH and EC values* of the soils administered with various levels of nitrogen fertilizer.

Nitrogen level	pH		EC (μS/cm)	
	1994	1995	1994	1995
	November	May	November	May
0 N	6.03±0.05	5.24±0.09	213±21	193± 13
3 N	5.53±0.07	4.49±0.02	254± 8	193± 2
6 N	5.07±0.04	4.49±0.04	143± 6	219± 8
9 N	4.85±0.05	4.33±0.12	230±19	283± 50
12 N	4.41±0.02	4.53±0.04	307±16	435± 27
15 N	4.39±0.01	4.44±0.04	384±25	552± 92
18 N	4.60±0.03	4.39±0.06	302±50	644± 71
21 N	4.40±0.01	4.26±0.03	493±55	934± 1
24 N	4.40±0.02	4.41±0.01	620±77	1,382±106
27 N	4.32±0.02	4.32±0.01	925±35	1,929±164

* Values are the mean of three measurements ± S.D.

grown tea plants that the T-N content of mature leaves was 4% under the critical nitrogen level and that the value was not significantly different from those under toxic levels. In the present study, the T-N content in the mature leaves was 2.38% at the critical level of 6 N plot and 3.77% at toxic level of 15 N plot (Fig. 4), which were different from one another and were considerably lower than those reported by Maehara and Hakamata¹⁴⁾. The reason for the discrepancy in critical levels between the two results is not clear, but part of this may be due to the difference in culture conditions, namely, soil culture *versus* water culture. In addition, the critical level would vary depending on plant age. Further investigation will be needed to determine the standard of nutritional diagnosis in tea plants.

2. Critical level of nitrogen for quality

Contents of total nitrogen or free amino acids are usually used as indicators of tea quality^{15,16)}. Tea growers have applied a large amount of nitrogen fertilizer to the tea fields to produce new shoots with higher contents of free amino acids. However, the upper limit of nitrogen dressing effective for increasing the content of amino acids has not been defined. In the present study, concentrations of the free amino acids in the new shoots increased without limit with the increase in the amount of nitrogen applied (Fig. 5). Thus, there seems to be no upper limit for nitrogen dressing to the extent we regard the concentrations of free

amino acids in new shoots as the sole determinant for tea quality. However, continuous rise in amino acid concentration in the new shoots was not due to the net accumulation of free amino acids but due to the depression of new shoot growth (Fig. 1). Absolute amount of free amino acids in the new shoots, which was determined by multiplying the concentration into the shoot weight, was greatest in the 9 N to 15 N plots and declined under the more-heavily manuring plots (Fig. 6). These results indicate that concentration of free amino acids as an indicator of leaf quality should be considered in relation to the growth of new shoots. In this context, the absolute amount of free amino acids shown in Fig. 6 seems to be a better indicator than the concentration to determine the suitable level of nitrogen for tea quality. From these considerations, the critical level of nitrogen with respect to leaf quality is estimated to be around 12 N, which is twofold greater than that for growth. Nitrogen level in the 12 N plot in the present study can be converted into 120 kgN 10a⁻¹ year⁻¹, which is not an uncommon value in recent tea cultivation³⁾. However, the critical level for leaf quality was very close to the toxic level to tea plants as suggested in the present study.

Excess dressing of nitrogen fertilizer must reduce the yield of new shoots through physiological disorders, even in mature tea trees. However, the influence of excess-nitrogen on the growth and yield of mature trees is difficult to detect because of the large buffer capacity of tea trees against environmental stresses. Moreover, the yield itself can not be defined strictly in tea cultivation because the new shoots are harvested during the rapidly-growing phase. All of these factors must bring about the unlimited increase of nitrogen input to tea fields in recent years. Appropriate techniques for growth diagnosis in mature tea trees should be urgently developed to detect excess-nitrogen stress.

3. Accumulation of free amino acids in new shoots

Ammonia taken up by tea roots is first synthesized to glutamine, then converted into glutamic acid or theanine^{5,24)}. Theanine, a peculiar amide in tea plant, is synthesized from glutamic acid and ethylamine in roots^{10,21)}. These amino acids are transported to shoots *via* xylem^{5,22)}. During the process,

Fig. 6. Absolute amount of total free amino acids accumulated in the first flush shoots of a plant grown under various levels of nitrogen environment.

Absolute amount of free amino acid = (Dry weight of new shoots, g) $\times$ (Concentration of free amino acids, %).

glutamine is rapidly metabolized to other amino acids, while theanine is relatively stable and concentrated in new shoots^{11,25)}. Consequently, theanine usually accounts for 40–70% of the free amino acids in the new shoots of tea plant¹⁵⁾.

In the present study, theanine increased with the increase in nitrogen dressing up to 9 N plot. Thereafter, no significant increase was observed (Fig. 5). Under a more heavily-manuring plot than 9 N, the high content of arginine began to accumulate. Although the synthetic pathway of arginine is not related to glutamine, both theanine and arginine are known to be increased by nitrogen dressing^{14,24)}. These compounds are generally considered to be storage or detoxification forms of ammonia^{7,26)}. As the content of arginine is usually high in high-grade tea¹⁵⁾, it is considered to be a good indicator for tea quality. The result in the present study, however, suggests that excess accumulation of arginine might be an indication of the disorder in nitrogen metabolism. Arginine content or theanine : arginine ratio in the new shoots may be used for diagnosis of nitrogen nutrition.

4. Effects of excess nitrogen on roots

Tea researchers have generally considered that injuries caused by excess-nitrogen might appear not on the aerial parts but on the roots. In fact, Tachibana et al.²³⁾ recently reported that the ratio of sound roots in the surface layer of inter-row soil were considerably reduced when the nitrogen dressing exceeded 140 kg 10a⁻¹ even in an apparently-healthy tea field. In the present study, however, a clear effect on root respiration was not evident, even in the 15 N plot, where the inhibition of photosynthesis in mature leaves was detected (Table 2). Depression of root respiration was first recognized in more heavily-manuring plots where many leaves had fallen. These results mean that the mechanism for excess-nitrogen injuries may be different between the pot experiment and the mature tea field. In the mature tea field, the fertilizer application was usually restricted to the inter-low spaces, resulting in extremely high concentrations of salts in the inter-low soils. Tea roots would not be able to survive under such a severe environment. On the other hand, aerial parts of the trees might be healthy because they were supported by the remaining intact roots just

below the canopy. If the fertilizers were sprayed to the entire surface area of the tea field and all roots were in contact with the applied nitrogen, the efficiency of nitrogen uptake by the tea trees would rise remarkably, and excess-nitrogen injuries would appear first, not on the roots but on the aerial parts.

5. Recovery rate of applied nitrogen

The results here indicate that tea plant grown under an excess-nitrogen environment continues to absorb nitrogen until the physiological disorders have appeared (Fig. 3). Meanwhile, the recovery rate of applied nitrogen linearly declined with the increase in nitrogen dressing. Although behavior of applied nitrogen could not be fully traced, most of the nitrogen not absorbed by the plants would remain in the soil with some leaching from the pots. Continuous rise in EC value of the pot soil with the increase in nitrogen dressing (Table 3) suggests that a large part of the nonabsorbed nitrogen might remain in the pot soil in inorganic form. Sulphate ion originated from the ammonium sulphate might also contribute to the rise of EC value, because pH of the soil decreased with the increase in nitrogen dressing.

Hoshina⁴⁾ indicated that the recovery rate of applied ¹⁵N-nitrogen was approximately 40% in a mature tea field under the fertilizer management of 60 kgN 10a⁻¹ year⁻¹. Ogawa¹⁷⁾ reported based on a lysimeter experiment that the recovery rate of ¹⁵N-nitrogen was 38% under the condition of 54 kgN 10a⁻¹, while the rate declined to 22% in the case of 108 kgN 10a⁻¹. He¹⁷⁾ also stated that if nitrogen dressing doubled from 54 kg to 108 kg, the leached nitrogen became fourfold and the soil-remaining one became threefold. The same situation must also exist in the tea fields. A large amount of nitrogen dressing reaching 120 kg 10a⁻¹ in recent years may pollute ground water with leached nitrate^{9,12)} and accelerate the strong acidification of the soils with sulphate ion^{13,23)}.

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* In Japanese with English summary.

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*** Translated from Japanese by the present authors.