Levels of Nonstructural Carbohydrate in Leaves and Roots and Some Characteristics of Chloroplasts after Application of Different Amounts of Nitrogen Fertilizer to Peach Seedlings

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Summary

The effects of different amounts of nitrogen, applied in the soil, on the accumulation of nonstructural carbohydrate in leaves and roots were determined as well as on some characteristics of chloroplasts in one-year-old peach trees (*Prunus persica*) grown in 500-ml pots. The amounts of nitrogen, applied as NH_4NO_3 , were as follows: 0 mg/pot (N-0), 75 mg/pot (N-75), 150 mg/pot (N-150), 300 mg/pot (N-300), and 600 mg/pot (N-600). The weights of stems and leaves and shoot length were greatest in N-150 and N-300 trees, whereas root weights were heaviest in N-75 and N-150 trees. Shoot and root growth was significantly reduced in N-0 and N-600 trees. Leaf chlorophyll content ($\mu\text{g cm}^{-2}$), total soluble sugar (% dry weight), and nitrogen content (% dry weight) of leaves and roots increased with increased application of nitrogen, whereas starch content of leaves was inversely proportional to the amount of nitrogen administered. The amount of sorbitol (% fresh weight) in leaves was proportional to the growth rate of the trees. Chlorophyll was stained more heavily by 4', 6-diamidino-2-phenylindole in N-600 leaves than in N-0 leaves; the staining concentrated at the periphery of chloroplasts, indicating an accumulation of starch. The level of the enzyme, ribulose-bisphosphate carboxylase-oxygenase (RuBisCO), increased with increased applied nitrogen, reaching maximum at N-300 and N-600. Total soluble sugar in roots was high in vigorous trees, where root starch content was inversely proportional to root growth.

Key Words: *Prunus persica* L., peach seedlings, leaves and roots, sorbitol and starch, RuBisCO.

Introduction

Nitrogen is a very effective fertilizer for trees grown in most soils. In general, the volume and capacity of sink organs with vigorous vegetative growth increase with increases in the amount of nitrogen applied to fruit trees. Nitrogen enhances the export of soluble sugars from leaves so that the starch level in leaves decreases with an increased (De Jong et al., 1984; Nii et al., 1993, 1997a, b). Contrarily, with decreases in sink capacity due to limited availability of nitrogen, starch accumulates in the leaves of fruit trees, and both the chlorophyll content and the rate of photosynthesis (Pn) decrease (De Jong, 1982, 1983; De Jong et al., 1984; Nii et al., 1993, 1997a, b). The accumulation of starch in leaves of fruit trees can also be induced by ringing the shoot and removal of fruits (Nii, 1989, 1992, 1993, 1997). Thus, when starch accumulates at elevated levels in the leaves of fruit trees, leaf functions, principally Pn, tend to change (De Jong, 1982, 1983; De Jong et al., 1984; Nii,

1993). To determine why the accumulation of starch causes a decrease in Pn in fruit trees we studied the changes in chloroplast ultrastructure that occur after application of different amounts of nitrogen and found that the thickness of the thylakoidal layer becomes thinner with the accumulation of starch (Nii, 1989, 1992, 1993; Nii et al., 1993); other researchers have reported similar results (Schaffer et al., 1986; Gucci et al., 1991). With the decrease in thickness of the thylakoidal layer, accompanied by the accumulation of starch, the level of RuBisCO in peach leaves also decreases (Nii et al., 1995, 1997a).

The amount of sorbitol, a prominent translocatable carbohydrate in leaves, also changes when different amounts of nitrogen are applied (Nii et al., 1997a, b). Moreover, the amount of sorbitol in leaves of fruiting peach trees is higher than in leaves of non-fruiting trees (Nii, 1997). Layne and Flore (1993) reported that an increase in Pn in partially defoliated sour cherry trees was associated with a reduced level of starch in leaves and elevated levels of sucrose and sorbitol. These results suggest that the level of sorbitol in the leaves is high when the sink capacity of the tree is large. Although

some information is available about changes in levels of starch and sorbitol in leaves of rosaceous fruit trees supplied with various amount of nitrogen (Nii et al., 1997a, b), changes in levels of these carbohydrates when excess nitrogen is applied have not been reported. There is also little information about the characteristics of chloroplasts, root growth and levels of nonstructural carbohydrate in the roots after application of different amounts of nitrogen fertilizer. We need to consider sink organs in the context of the whole plant, as noted by Farrar (1996).

In this study, we monitored the growth of peach shoots and roots and levels of chlorophyll, total sugar, starch and sorbitol in leaves and roots after soil application of different amounts of nitrogen fertilizer. We also monitored the characteristics of chloroplasts and changes in levels of RuBisCO.

Materials and Methods

Plant materials

One-year-old peach seedlings [*Prunus persica* (L.) Batsch. cv. Ohatsumomo], grown in 10-cm diameter pots (volume, 500 ml) filled with sandy soil in a greenhouse. Nitrogen was supplied on May 12 and June 10, 1996, as NH_4NO_3 fertilizer as follows: zero nitrogen per pot (N-0), 75 mg/pot (N-75), 150 mg/pot (N-150), 300 mg/pot (N-300), 600 mg/pot (N-600), and 900 mg/pot (N-900). Other mineral salts applied to the trees were $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$ (1.0 g/pot), K_2SO_4 (1.0 g/pot), and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 g/pot). Twenty plants were used per treatment. During the experiment, all trees supplied with 900 mg of nitrogen per pot died.

The shoots ceased to elongate at the beginning of June and the trees were harvested on July 6. The weights of shoots, primary leaves, secondary leaves, and roots were determined separately. Mature leaves at middle positions on the main shoot were sampled for determinations of leaf area, levels of chlorophyll, and nonstructural carbohydrate.

Quantitation of chlorophyll, carbohydrate, and minerals

The chlorophyll content of leaves was measured after extraction of chlorophyll from five leaf discs (8 mm in diameter) per plant with 100% ethanol. Chlorophyll was quantified as described by Wintermans and De Mots (1965) and expressed on a per unit leaf area basis.

The levels of total soluble carbohydrates in leaves, shoot, and roots were determined from dried powders with the anthrone reagent after extraction with 80% ethanol. For determination of starch content, the ethanol-insoluble residue was boiled to hydrate the starch grains; after cooling, the mixture was incubated with 2% (w/v) glucoamylase at 55 °C for 1 hr. The mixture was then filtered and an aliquot of the filtrate was analyzed with the anthrone reagent. The sorbitol content was determined by HPLC as described elsewhere (Nii et al.,

1994).

Levels of minerals in dried leaves and roots were analyzed as follows: nitrogen was quantitated by the Kjeldahl method, phosphorous by reaction with ammonium molybdate, potassium by flame spectrophotometry, and calcium and magnesium by atomic absorption spectrophotometry.

Leaf anatomy

For anatomical observations of leaves, the central area of the lamina was excised and fixed overnight in 4% paraformaldehyde and 1% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) at 4 °C. Fixed specimens were dehydrated through a graded ethanol series and embedded in Technovit resin (Kulzer, Wehrheim, Germany) for examination under a light microscope after staining thin sections (1.5 μm) with methylene blue and/or I_2 -KI solution to follow the development of mesophyll tissues and the accumulation of starch.

Mesophyll cells of the mid-lamina were enzymatically separated and stained with 4',6-diamidino-2-phenylindole (DAPI) as described by Kuroiwa and Suzuki (1980).

Electrophoresis and immunoblotting for quantitation of RuBisCO

A leaf sample (0.5 g fresh weight) was macerated with 5 ml of 50 mM Tris-HCl buffer (pH 8.0) in a mortar and pestle that had been chilled with liquid nitrogen. The homogenate was centrifuged at $12,000 \times g$ for 5 min at 4 °C and the supernatant was subjected to SDS-PAGE (12.5% acrylamide), as described by Laemmli (1970). The proteins on the gel were then blotted electrophoretically onto a cellulose nitrate membrane (Schleicher and Schnell, Dassel, Germany). Antibodies against RuBisCO from spinach were prepared according to Nishimura and Akazawa (1974).

Results and Discussion

Figures 1, 2 A and Table 1 show details of the growth of the various trees at the final stage of treatment. The shoots of N-0 and N-600 trees were significantly shorter than those of N-75, N-150 and N-300 trees. There were no differences in shoot length between N-75 and N-150 trees. The dry weights of leaves and stems of N-150 and N-300 trees were greater than those of N-0 and N-600 trees. Root dry weight was greatest in N-75 and N-150 trees and lowest in N-600 trees. Although top growth was vigorous in N-300 trees, their roots were not proportionately heavier. Thus, the top/root (T/R) increased with increases in the application of nitrogen. These results also indicated that a supply of 600 mg of nitrogen per pot was excessive for the growth of one-year-old peach trees.

The nitrogen content of leaves increased with increases in the soil application of nitrogen (Table 2). The levels of other minerals in leaves also changed with

Fig. 1. Growth curves of peach shoots as a function of nitrogen level. The arrows in the Figure indicate the first and second applications of minerals to the soil (see text for details). $\diamond$: N-0, $\blacksquare$: N-75, $\blacktriangle$: N-150, $\bullet$: N-300, $\circ$: N-600.

changes in the amount of nitrogen applied. Levels of phosphorus and potassium in leaves were high in N-75, N-150, and N-300 trees but the level of phosphorus was clearly lower in N-600 trees. Levels of calcium and magnesium in leaves increased with increases in the application of nitrogen, as did the nitrogen content of roots. The levels of phosphorus, calcium, and magnesium in roots were generally higher when tree growth was vigorous. The potassium content of roots decreased with an increased application of nitrogen. Levels of phosphorus and potassium were markedly lower in roots of N-600 trees.

Total leaf area per tree or per leaf was smallest in N-0 trees, followed by N-600 trees (Table 3). Leaves from N-150 and N-300 trees were larger than those on N-0 and N-600 trees. When trees grew vigorously, their dry weight per unit leaf area was lower than that of trees which lacked vigor.

The chlorophyll content of leaves provided with sufficient nitrogen was significantly greater than that when little or no nitrogen was applied. The ratio of chlorophyll a to chlorophyll b was lowest in N-600 trees (Table 3).

The amount of total soluble sugar per unit dry weight of leaves increased with the application of increasing amounts of nitrogen (Table 4) but conversely, the amount of starch in leaves of nitrogen-deficient trees was significantly higher than that those on trees supplied with sufficient nitrogen. These results are similar to those obtained with kiwifruit (De Jong et al., 1984), peach (Nii et al., 1993, 1997a), and apple (Nii et al., 1997b) trees.

The sorbitol content of leaves was elevated in N-75, N-150, and N-300 trees, whereas it was lower in N-0 and N-600 trees (Table 4). The lower amount of sorbitol in the leaves of N-0 trees and N-600 trees might reflect a lower capacity for its translocation on

Fig. 2. (A) Effects of the application of different amounts of nitrogen on the growth of peach seedlings. Typical peach seedlings are shown, illustrating differences in shoot lengths and root volumes at harvest on July 6. (B, C) Fluorescence micrographs showing chloroplasts after staining with DAPI of mesophyll parenchyma cells from leaves of peach seedlings. B: N-0, C: N-600. Arrows point to central nuclei.

account of a weak sink activity. In previous studies of peach and apple trees, leaf sorbitol content increased with increases in the application of nitrogen (Nii et al., 1997a, b), especially on peach trees at the fruit maturation stage; it was higher than that of leaves of non-fruited trees. These results support our idea that a high level of sorbitol and a low level of starch reflect strong sink capacity. However, the sorbitol content was lower in N-600 leaves, even though the starch content of the leaves was the lowest and the chlorophyll content was highest among the various treatments. We attribute that

Table 1. Effects of the application of different amounts of nitrogen on shoot and root growth of peach seedlings.

Amount of nitrogen (mg per pot)	Leaf weight per tree (g) ^y		Stem weight per tree (g) ^y		Root weight per tree (g) ^y		Number of secondary shoots per tree	T/R ^z
	FW	DW	FW	DW	FW	DW		
N-0	2.05a	0.84a	1.36a	0.68a	2.81b	0.73b	0.5	1.6
N-75	6.89c	2.17c	3.43c	1.37c	4.76d	1.06c	6.7	2.6
N-150	8.85dc	2.61d	3.98d	1.46c	4.71d	1.01c	9.7	3.3
N-300	7.99c	2.56d	3.69d	1.45c	3.84c	0.89b	8.3	3.7
N-600	3.96b	1.40b	1.99b	0.87b	1.86a	0.49a	3.5	3.5

^z T: top, R: roots^y Means of results from six trees followed by the same letter are not significantly different ($p \leq 0.01$).**Table 2.** Effects of the application of different amounts of nitrogen on levels of minerals (% DW) in peach seedlings.

Amount of nitrogen (mg per pot)	N	P	K	Ca	Mg
Leaves ^z					
N-0	1.35	0.15	1.48	0.83	0.39
N-75	2.65	0.21	1.78	0.75	0.47
N-150	2.93	0.21	1.83	0.90	0.50
N-300	3.19	0.17	1.72	1.11	0.53
N-600	4.49	0.07	1.44	1.14	0.54
Roots ^z					
N-0	0.76	0.18	1.15	0.38	0.20
N-75	1.19	0.23	1.14	0.45	0.26
N-150	1.80	0.23	0.97	0.59	0.31
N-300	2.14	0.17	1.02	0.57	0.27
N-600	2.95	0.09	0.58	0.45	0.18

^z Values are means of results from duplicates in each case.**Table 3.** Effects of the application of different amounts of nitrogen on the characteristics of leaves from peach seedlings.

Amount of nitrogen (mg per pot)	Leaf weight (mg) ^z		Leaf area (cm ²) ^z	Dry weight per unit leaf area (mg cm ⁻²)	Chlorophyll ($\mu\text{g cm}^{-2}$) ^z	Chl a/Chl b ^z
	FW	DW				
N-0	62.3a	26.7a	4.5a	5.83b	24.6a	2.3b
N-75	157.0c	50.5c	10.3c	4.88a	48.8b	2.5b
N-150	168.7c	52.2c	11.6c	4.55a	56.8bc	2.5b
N-300	166.7c	53.0c	11.2c	5.13a	59.5cd	2.5b
N-600	118.8b	40.6b	7.7b	5.40b	67.6d	1.7a

^z Means of results from six trees followed by the same letter are not significantly different ($p \leq 0.01$).

to the tree growth which was inhibited. Thus, leaf functions in N-600 trees were impeded by the excessive application of nitrogen.

Figure 3 shows the anatomical features and the accumulation of starch in the mesophyll parenchyma of leaves. The lamina was thinner in nitrogen-deficient leaves, whereas the chloroplasts contained an abundance of starch grains, which accumulated within the palisade and spongy parenchyma cells. Several have reported that

the accumulation of starch within chloroplasts is accompanied by a reduction in the size of the chloroplast compartment and by damage to and disorganization of grana and thylakoids in fruit trees (Schaffer et al., 1986; Gucci et al., 1991). Our results from N-0 leaves are similar to those obtained in previous studies of peach and apple trees (Nii, 1989, 1992, 1993) in that the accumulation of starch induced a reduction in the volume of the chloroplast compartment that included

Table 4. Effects of the application of different amounts of nitrogen on the carbohydrate content of leaves, stems, and roots of peach seedlings.

Amount of nitrogen (mg per pot)	Leaves			Stems		Roots	
	Total soluble sugar (%DW) ^z	Starch (%DW) ^z	Sorbitol (%FW) ^y	Total soluble sugar (%DW) ^y	Starch (%DW) ^y	Total soluble sugar (%DW) ^z	Starch (%DW) ^z
N-0	6.1a	22.4a	2.6	2.8	10.8	4.2a	9.8c
N-75	7.8b	8.4b	4.1	2.8	6.4	4.8b	7.4b
N-150	8.3b	2.8c	4.0	2.8	5.5	5.5c	5.9a
N-300	8.2b	2.6c	4.1	2.9	4.9	5.1c	7.2b
N-600	9.0c	1.9d	3.0	2.9	6.0	4.8b	9.5c

^z Means of results from six trees followed by the same letter are not significantly different ($p \leq 0.01$).^y Values are means of results from duplicates in each case. **Fig. 3.** Effects of nitrogen application on the thickness of the mesophyll and the accumulation of starch in leaves of peach seedlings. A: N-0, B: N-600. Arrows point to starch grains.

thylakoidal layers (unpublished data).

The amount of total soluble sugar in roots was higher in N-150 and N-300 trees (Table 4) but the starch content of roots was lower in N-150, N-300, and N-75 trees and higher in N-600 and N-0 trees. Thus, the starch content of roots was inversely proportional to root growth. In deciduous fruit trees, the accumulation of starch, the principal reserve material in roots, generally increased in the autumn, before leaf senescence (Nii, 1993). In the present experiment, when shoots and roots continued to grow vigorously, less starch accumulated in roots.

Figures 2B and 2C show epifluorescence micrographs of leaf cells that had been stained with the fluorochrome DAPI. The intensity of DAPI fluorescence increased with increases in the chlorophyll content of leaves. Fluorescence was well distributed throughout the chloroplasts in nitrogen-sufficient leaves. By contrast, in

Fig. 4. Levels of RuBisCO after application of different amounts of nitrogen. Extracts from peach leaves were subjected to SDS-PAGE. Proteins were stained with Coomassie brilliant blue (A) or immunoblotted (B). An equal volume of sample was loaded in each lane. Numerals on the left indicate molecular weights (MW) of pre-stained protein standards that were run in parallel with samples. a: N-0, b: N-75, c: N-150, d: N-300, e: N-600. Upper arrow indicates large sub-unit, lower arrow indicates small sub-unit.

nitrogen-deficient leaves, fluorescence was observed only on the periphery of the chloroplasts which is a reflection of starch accumulation.

Figure 4A shows an electrophoretogram of leaf proteins after SDS-PAGE; Figure 4B shows the results of immunoblotting of RuBisCO from leaves. RuBisCO in extracts of peach leaves was found to cross-react with antibodies against the enzyme from spinach (Nii et al., 1997a). In this experiment, the immunoblot analysis revealed that nitrogen-deficient yellowish leaves contained only small amounts of RuBisCO and that the level of the protein increased as the chlorophyll became more abundant.

Changes in levels of RuBisCO in response to the application of nitrogen have contributed to our understanding of the physiological functions of leaves (Evans and Terashima, 1988; Terashima and Evans, 1988; Evans, 1989; Makino et al., 1992). Increases in levels of RuBisCO with increased levels of nitrogen in leaves depend on the plant species: levels of RuBisCO increase upon application of nitrogen in rice, spinach, bean, and pea but appear to be independent of applied nitrogen in wheat (Makino et al., 1992). In earlier studies of the effects of defruiting and of the application of limited nitrogen in peach trees, levels of both chlorophyll and RuBisCO decreased with the increased accumulation of starch in chloroplasts (Nii et al., 1995, 1997a). In this study, although the level of the RuBisCO increased in green leaves, there was no obvious difference between N-300 and N-600 leaves. On N-600 trees, shoot and root growth was inhibited but the levels of chlorophyll and RuBisCO remained high. Leaves of N-600 trees, which had an elevated level of nitrogen (4.5%), did not contribute to tree growth.

In conclusion, our results indicate that when the nitrogen content of leaves and roots are increased, vegetative growth increases but starch accumulation in these organs is affected inversely. However, when excess nitrogen fertilizer is applied to seedling peach trees, the leaves function significantly less efficiently in spite of higher chlorophyll and RuBisCO contents. When tree growth is vigorous, the sorbitol level is significantly elevated in the leaves. This study confirms that excess nitrogen is deleterious to peach trees; hence, the rate of nitrogen-fertilizer application should be regulated.

Acknowledgements

We thank Miss A. Jinno for her invaluable assistance. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture (Japan) and by a grant by Genetic Engineering of Environment Adaptable Plants from High-Tech Research Center in Meijo University.

Literature Cited

- De Jong, T. M. 1982. Leaf nitrogen content and CO₂ assimilation capacity in peach. *J. Amer. Soc. Hort. Sci.* 107: 955-956.
- De Jong, T. M. 1983. CO₂ assimilation characteristics of five *Prunus* tree fruit species. *J. Amer. Soc. Hort. Sci.* 108: 303-307.
- De Jong, T. M., A. Tombesi and K. Ryugo. 1984. Photosynthetic efficiency of kiwi (*Actinidia chinensis* Planch.) in response to nitrogen deficiency. *Photosynthetica* 18: 139-145.
- Evans, J. R. 1989. Photosynthesis and nitrogen relationships in leaves of C₃ plants. *Oecologia* 78: 9-19.
- Evans, J. R. and I. Terashima. 1988. Photosynthetic characteristics of spinach leaves grown with different nitrogen treatments. *Plant Cell Physiol.* 29: 157-165.
- Farrar, J. F. 1996. Sink-integral parts of a whole plant. *J. Exp. Bot.* 47: 1273-1279.
- Gucci, R., P. D. Petracek and J. A. Flore. 1991. The effect of fruit harvest on photosynthetic rate, starch content, and chloroplast ultrastructure in leaves of *Prunus avium* L. *Adv. Hort. Sci.* 5: 19-22.
- Kuroiwa, T. and T. Suzuki. 1980. An improved method for the demonstration of the *in situ* chloroplast nuclei in higher plants. *Cell Struct. Func.* 5: 195-197.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
- Layne, D. R. and J. A. Flore. 1993. Physiological responses of *Prunus cerasus* to whole-plant source manipulation. Leaf gas exchange, chlorophyll fluorescence, water relations and carbohydrate concentrations. *Physiol. Plant.* 88: 44-51.
- Makino, A., H. Sakashita, J. Hidema, T. Mae, K. Ojima and B. Osmond. 1992. Distinctive responses of ribulose-1,5-bisphosphate carboxylase and carbonic anhydrase in wheat leaves to nitrogen nutrition and their possible relationships to CO₂-transfer resistance. *Plant Physiol.* 100: 1737-1743.
- Nii, N. 1989. Fruiting effects on chloroplast structure and starch accumulation in apple leaves. *Sci. Rept., Fac. Agr., Meijo Univ.* 25: 35-39.
- Nii, N. 1992. Fruiting effects on chloroplast ultrastructure, starch accumulation, and localization of chloroplast nucleoids in peach leaves on the girdled shoots. *Sci. Rept., Fac. Agr., Meijo Univ.* 28: 41-48.
- Nii, N. 1993. Fruiting effects on leaf characteristics, photosynthesis, and root growth in peach trees. *J. Japan. Soc. Hort. Sci.* 62: 519-526.
- Nii, N. 1997. Changes of starch and sorbitol in leaves before and after removal of fruits from peach trees. *Ann. Bot.* 79: 139-144.
- Nii, N., K. Hase and H. Uchida. 1994. Anatomical features of the sieve elements and sorbitol content in various organs of Rosaceae fruit trees. *J. Japan. Soc. Hort. Sci.* 62: 739-747.

- Nii, N., M. Kato, Y. Hirano and T. Funaguma. 1993. Starch accumulation and photosynthesis in leaves of young peach trees grown under different levels of nitrogen application. *J. Japan. Soc. Hort. Sci.* 62: 547-554.
- Nii, N., K. Yamaguchi and M. Nishimura. 1995. Effects of fruiting on amylase activity and ribulose biphosphate carboxylase-oxygenase content in peach leaves. *J. Japan. Soc. Hort. Sci.* 64: 267-272.
- Nii, N., K. Yamaguchi and M. Nishimura. 1997a. Changes in carbohydrate and ribulose biphosphate carboxylase-oxygenase contents in peach leaves after applications of different amounts of nitrogen fertilizer. *J. Japan. Soc. Hort. Sci.* 66: 505-511.
- Nii, N., E. Yokotana and N. Shinkai. 1997b. Changes of nonstructural carbohydrate in leaves of apple trees grown after the application of different amounts of nitrogen. *Sci. Rept., Fac. Agr., Meijo Univ.* 33: 15-22.
- Nishimura, M. and T. Akazawa. 1974. Studies on spinach leaf ribulose biphosphate carboxylase. Carboxylase and oxygenase reactions examined by immunochemical methods. *Biochem.* 13: 2271-2281.
- Schaffer, A. A., K. C. Liu, E. E. Goldschmidt, C. D. Boyer and R. Goren. 1986. Citrus leaf chlorosis induced by sink removal: starch, nitrogen, and chloroplast ultra-structure. *J. Plant Physiol.* 124: 111-121.
- Terashima, I. and J. R. Evans. 1988. Effects of light and nitrogen nutrition on the organization of the photosynthetic apparatus in spinach. *Plant Cell Physiol.* 29: 143-155.
- Wintermans, J. F. G. M. and D. A. De Mots. 1965. Spectrophotometric characteristics of chlorophylls and their pheophytins in ethanol. *Biochim. Biophys. Acta* 109: 448-453.

窒素施肥量の相違がモモ実生樹の葉と根の炭水化物含量ならびに葉緑体の形質に及ぼす影響

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摘 要

本報告では、500 ml容のポットに定植したモモの1年生実生樹を用いて、窒素施肥量の相違が地上部と地下部の生長に及ぼす影響を調査するとともに葉と根の炭水化物含量を測定した。さらに葉の葉緑体に焦点を絞って、デンプン蓄積とRuBisCOタンパク量の変化を検討した。窒素施肥量は、1樹当たりNH₄NO₃を0 mg (N-0区), 75 mg (N-75区), 150 mg (N-150区), 300 mg (N-300区), 600 mg (N-600区), 900 mg (N-900区)施肥し、その他の肥料は各処理区とも同量とした。なお、N-900区は実験期間中枯死した。茎と葉の重量、枝長からみた地上部の生長は、N-150区とN-300区で最も大きかった。根はN-75区とN-150区で重かった。地上部と地下部ともにN-0区とN-600区で小さかった。葉と根の窒素含量(対乾物重%)ならびに葉面積当たりのクロロフィル含量($\mu\text{g}\cdot\text{cm}^{-2}$)は窒素施肥量の増加に伴い高くなっ

た。葉の全糖含量(対乾物重%)も窒素施肥量の増加に伴い高くなった。ソルビトール含量(対新鮮重%)は樹の生長が旺盛な処理区ほど高かった。葉内デンプン含量は窒素の施肥量とは逆の傾向がみられた。クロロフィル含量が最も高かったN-600区の葉緑体では、4',6-diamidino-2-phenylindole染色によってクロロフィルが濃い赤色に励起された。N-0区でのクロロフィルの励起は弱く、デンプン蓄積と関連して葉緑体の周辺部に集中していた。Ribulose biphosphate carboxylase-oxygenase (RuBisCO)は窒素施肥量に対応して増加したが、N-300区とN-600区の間には差がみられなかった。根の全糖含量は樹の生長が旺盛な処理区ほど高く、根のデンプン含量は根の生長量とは逆の傾向がみられ、N-0区とN-600区で高かった。