

Study on the Role of Nitrogen Control on the Seed
Production in Soybean (*Glycine max* (L.) Merrill)
(ダイズ子実生産における窒素制御機構に関する研究)

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Chapter 1

General Introduction

Soybean (*Glycine max* (L.) Merrill) is a species of legume originated from East Asia. The seeds of soybean contain much valuable nutrient substance, such as 40% of protein, 20% of fat, 35% of carbohydrate and 5% of ash content approximately. The protein content in soybean is around 20% higher than the other legumes, and 3 to 5 times of cereals. Therefore, the soybean meal was very important raw material of animal feeds in the world and soybean oil occupies over 30% of the global vegetable oil market. Beyond that, the seeds of soybean contain significant amounts of phytic acid, alpha-linolenic acid, and isoflavones which have special health care effects for human body. Soybean also can be used for food production, such as bean curd, soy source and green soybean, etc. Nowadays, soybean has become one of the most important crops for oil, food and animal feeds production in the world.

From the late of 1990s, the world soybean production increased dramatically among main food crops in the world, compare with the cereal crops production, such as rice, maize and wheat. The main reason is because of an enlarged cultivation area in the north and south of America. Now, the main producers of soybean in the world are the United States (34.2%), Brazil (26.9%) and Argentina (19.9%) (FAO, 2012).

In contrary to the north and south of America, the soybean production in Asia only occupied a small part of the world, in which the two main producers China and India only occupied 5.7% and 4.8% of the world total

production, but the measure of consumption is huge which occupied over 60% of the total business volume of the world. Because it is difficult to increase total sown area largely, the enhancement of per unit area yield becomes to be the unique way to increase soybean production in Asia. Therefore, it is very important to understand the relationship between nutrient utilization and seed yield to increase the yield performance effectively and economically.

Generally, soybean plants require much nitrogen for seed production, because of the high protein content in the seeds compared with that in the seeds of other crops such as rice and maize (Osaki et al., 1991). The high nitrogen requirement appears not during the period of vegetative growth, but the seed filling period. To feed this high nitrogen requirement, the reinforcements of nitrogen absorption by roots, including nitrogen fixation by nodules and nitrogen redistribution from other vegetative organs during the seed-filling period are considered to be effective for improving the seed yield.

Seed filling period has been known as a limiting factor for seed production in soybean. The length of seed filling period has strong positive relationship with seed production (Smith and Nelson, 1986; Guffy et al. 1992; Egli, 2004). Before the start of seed filling, because the development of soybean plants is mainly in the vegetative organs, a part of the absorbed nitrogen is stored in vegetative tissues, such as leaves, stems, and petioles, and then redistributed to the seeds during the seed-filling period to satisfy the high nitrogen requirement for seed growth. Hanway and Weber (1971) reported that approximately half of the nitrogen in the matured seeds is redistributed from other plant parts, and the rest of the nitrogen is provided

by root absorption and nodules during the seed-filling period.

Many evidences support the redistributed nitrogen could improve the seed yield. For example, Shiraiwa and Hashikawa (1995) reported that high yielding modern cultivars exhibited greater dry matters and nitrogen accumulation during seed filling in comparison with old cultivars. Munier-Jolain et al. (1996) noted that the amount of available nitrogen in vegetative organs was a decisive factor for the length of seed filling period, which has close relationship with yield performance in soybean. Shibles and Sundberg (1998) showed the leaf nitrogen content at the beginning of seed filling correlated significantly with seed yield.

However, there are remains some disagreement about the relationships among redistributed nitrogen, the seed-filling period, and seed yield in soybean. For example, the large nitrogen redistribution process was also reported to induce the leaf senescence (Sanetra et al., 1998; Donnison et al., 2006), decline the photosynthetic activity (Buttery and Buzzell, 1988; Sinclair and Horie, 1989), shorten the duration of seed filling period (Sinclair and de Wit, 1976), and finally limit the seed yield.

Furthermore, some researchers even queried the existence of the relationship between redistributed nitrogen and seed yield in soybean. For example, Zeiher et al. (1982) showed no relationship between the contribution of redistributed nitrogen and seed yield in some commercial cultivars. Hayati et al. (1995) reported that leaf senescence did not occur because of the increase of seed nitrogen demand, and Egli and Bruening (2007b) pointed out that there is no relationship between increasing total

seed nitrogen at maturity and the amount of redistributed nitrogen or rapid leaf senescence.

On the other hand, the high ability of nitrogen assimilation during seed filling was reported to contribute to the high seed yielding rather than nitrogen redistribution (Vasilas et al., 1995). In addition, Kumudini et al. (2002) concluded that higher seed yielding was more associated with the nitrogen acquisition during seed filling rather than the nitrogen redistribution.

All of these inconstant evidences mentioned above indicate the role of nitrogen assimilation and redistribution on the seed yield is still unclear. With the consideration of seed protein content, maturity group and growth characteristics in different soybean cultivars, the effect of nitrogen utilization on the seed yield could be different genetically and conditionally. In addition, because the process of nitrogen redistribution during seed filling is also accompanied with nitrogen absorption simultaneously by root system, the only analysis of redistributed nitrogen was difficult to get credible results. Therefore, there is necessary to analyze the function of nitrogen utilization on seed yield synthetically and roundly.

In this study, at first to understand clearly the functions of nitrogen redistribution from vegetative organs on the seed yield production, the varietal difference in nitrogen redistribution and its contribution to the seed yield were investigated in Chapter 2 using various cultivars which are grown widely in Japan and other countries, in which the soybean cultivar Sachiyutaka and Tamahomare were found to have different patterns of

nitrogen utilization. In Chapter 3, the two soybean cultivars Sachiyutaka and Tamahomare were used to reveal the role of nitrogen assimilation and redistribution on the seed yield, by changing the intensity and timing of nitrogen supply during reproductive growth stage in soybean. In Chapter 4, the ability of nitrogen assimilation by root and nodule system during reproductive stage in Sachiyutaka and Tamahomare were evaluated by measuring seed yield, root dry weight, nodule number, and the nitrogen constitution in xylem sap including amino acid, nitrate, ureide and total nitrogen concentration at R1, R5 and R6 stage.

Finally, to find effective way to stimulate yield performance by nitrogen control, the effects of nitrogen topdressing at different timing on seed yield performance between Sachiyutaka and Tamahomare which have different nitrogen assimilation patterns were investigated in Chapter 5.

The achievements of this research are going to be published in the journal *Plant Production Science* (Zhao et al., 2014a, b).

Chapter 2

Varietal differences in nitrogen redistribution and its contribution to seed yield in soybean

I. SUMMARY

It is well known that large amount of nitrogen are redistributed from vegetative organs to the seeds during seed filling in soybean (*Glycine max* [L.] Merrill). However, the role of distributed nitrogen from leaves on the seed yield production is not clear. The varietal differences in nitrogen redistribution and its contribution to the seed yield were evaluated. Ten soybean cultivars were cultivated conventionally at the field in Saga, Japan. The plant samples were taken at various reproductive stages, and then the nitrogen contents in each part were determined. The redistributed nitrogen was estimated by the difference in the nitrogen contents between R5 and R7 stage. The nitrogen content in leaves began decreasing after R5 stage in all cultivars, indicating the start of nitrogen redistribution. About 13.8% to 37.9% of the total nitrogen in the seeds was estimated being redistributed from the leaf tissues among ten cultivars. The seed yield was correlated positively with the amount of redistributed nitrogen from leaves but with neither the nitrogen concentration in the leaves at R5 nor the proportion of redistributed nitrogen in the seeds. However, in high seed yielding year 2008 and 2009, the seed yield is not likely associated with nitrogen redistribution, even the lowest nitrogen redistribution was associated with relatively higher

seed yield in Tamahomare. The results indicated that large nitrogen redistribution does not always contribute to high seed yielding, implying the direct nitrogen uptake during seed filling could be more important factor for high seed yielding depend on the cultivars.

II. INTRODUCTION

Soybean plants require much nitrogen for seed production, because of the high protein content in the seeds compared with that in the seeds of other crops such as rice and maize (Osaki et al., 1991).

It is well known that large amount of nitrogen are redistributed from vegetative organs to the seeds during seed filling in soybean. Before the start of seed filling, because the development of soybean plants is mainly in the vegetative organs, a part of the absorbed nitrogen is stored in vegetative tissues, such as leaves, stems, and petioles, and then redistributed to the seeds during the seed-filling period. Hanway and Weber (1971) reported that approximately half of the nitrogen in the matured seeds is redistributed from other plant parts, and the rest of the nitrogen is provided by root absorption and fixation by nodules during the seed-filling period.

Many evidences support the redistributed nitrogen could improve the seed yield (Shiraiwa and Hashikawa, 1995; Munier-Jolain et al., 1996; Shibles and Sundberg, 1998). However, the redistribution of nitrogen from the vegetative to the reproductive parts of soybean plants is considered to be a compensation for the shortage of nitrogen supplied by the root system during seed filling. This redistribution may destroy the photosynthetic functions and cause leaf senescence and thus shorten the seed-filling period (Sinclair and de Wit, 1976; Egli et al., 1978; Shibles and Sundberg, 1998).

To understand clearly the functions of nitrogen redistribution from leaves on the seed yield production, the varietal difference in nitrogen

redistribution and its contribution to the seed yield were evaluated using various cultivars which are grown widely in Japan and other countries.

III. MATERIALS AND METHODS

1. Plant cultivation

Four soybean cultivars, Fukuyutaka, Akiyoshi, Sachiyutaka, and Tamahomare, all of which are widely cultivated in western Japan, were used to evaluate the nitrogen accumulation and redistribution in 2008 and 2009. Sachiyutaka and Tamahomare were the comparative cultivars with high and low protein contents, respectively, and slightly earlier maturing than the other two cultivars. Fukuyutaka is the most commercial cultivar and is grown widely in western Japan, and Akiyoshi was a leading cultivar before Fukuyutaka was bred. The seeds were sown on 10 July in 2008 and 9 July in 2009 in a loam field of the Coastal Bioenvironment Center, Saga University (33°27'N and 129°58'E). Four seeds were sown in each hole, with holes arranged at 15-cm intervals and 70 cm rows spacing. The split plot design was used for each cultivar consist of six rows with 12 m long in each row. Plants were thinned to allow only one plant to grow per hole (9.5 plants m⁻²) at the stage when the first trifoliolate leaf fully expanded. Chemical fertilizer was applied at a rate of 3:10:10 g m⁻² of N: P₂O₅: K₂O before sowing, and 100 g m⁻² of lime was also applied at the same time. The weeds were controlled by plowing or hands and the pesticides were sprayed when it was necessary. The growth stages were determined according to Fehr et al. (1971).

To further analyze the relationship between redistributed nitrogen and seed yield, ten and six cultivars were planted in a sandy field in 2010 and 2011, respectively. The cultivars used in 2010 were Fukuyutaka, Akiyoshi,

Sachiyutaka, Tamahomare, Akisengoku and Enrei (from Japan), Caviness and Stressland (from USA), Parana and IAS-5 (from Brazil), whereas in 2011 were Fukuyutaka, Sachiyutaka, Tamahomare, Akisengoku, Caviness and IAS-5. The plots were arranged in a random completely blocked design with three replications in each year-experiment. The seeds were sown on 20 July in 2010, and 15 July in 2011. Each plot's design consisted of four rows with 70 cm spacing and ten hills with 20 cm intervals (2.8 m × 2 m). The others were same as in 2008 and 2009.

2. Sampling and measurements

In 2008 and 2009, five plants of each cultivar were taken mainly at R1 (beginning flowering), R5 (beginning seed filling), R6 (full seed size), R7 (end of seed filling) and at some additional stage when is needed (Fehr et al., 1971). The sample plants were separated into stems, leaves, petioles, and pods, and then were weighed after drying at 80°C for 48 hours. Each part of the plant sample was ground into powder for the determination of total nitrogen content by the Kjeldahl method. The leaf SPAD value was measured by a chlorophyll meter SPAD-502 (Konica Minolta, Inc., Osaka, Japan) 1 or 2 times per week on the second or third fully expanded leaf from the top during R1 to R7. At the harvest stage (R8), eight medium-size plants of each cultivar were taken for the determination of the seed yield, yield components, and nitrogen content in the seeds. However in 2010 and 2011, the plant samples were taken only at R5 and R7, and the seed yield determination was conducted on 10 plants per plot with three replications.

The redistributed nitrogen was estimated as the difference in the nitrogen content in vegetative parts between R5 and R7. The nitrogen contents at R5 and R7 were calculated as multiplying the dry weight at R5 by the nitrogen concentration in each part at R5 and R7, respectively. This estimation method was according to Egli and Bruening (2007b). It is assumed that nitrogen lost from vegetative parts was redistributed to the seeds. The same estimation of nitrogen redistribution from vegetative parts was described also by Zeiher et al. (1982) and Kumudini et al. (2002).

3. Statistical analysis

Tukey-Kramer test was used for the significance analysis of parameters relating to redistributed nitrogen among cultivars. Pearson's correlation coefficient test was used to estimate the relationship between yield and nitrogen redistribution. The software Statcel 3 was used for the calculation of multiple comparison analysis.

IV. RESULTS

1. Nitrogen accumulation in vegetative organs and its redistribution

Fig. 2-1 shows a comparison of total nitrogen concentration among the vegetative organs of Fukuyutaka at R5 and R7 in 2009. The total nitrogen concentration in the leaf, pod wall, stem, and petiole was 50.3, 28.3, 16.5, and 11.2 mg g⁻¹ at R5, whereas it decreased to 11.9, 8.5, 3.7, and 5.1 mg g⁻¹ at R7, respectively.

Based on the hypothesis that the decrement of nitrogen content in the vegetative organs is because of redistribution to the seeds, the redistributed nitrogen from the leaves, stems, pod walls, and petioles occupied 28%, 11%, 6%, and 3% of total nitrogen in the seeds, respectively, showing that the leaves were the largest nitrogen source for the redistribution (Fig. 2-2). Approximately 48% of the nitrogen in the seeds was redistributed from vegetative organs, and the other 52% were considered to have been assimilated by the roots and nodules during seed filling.

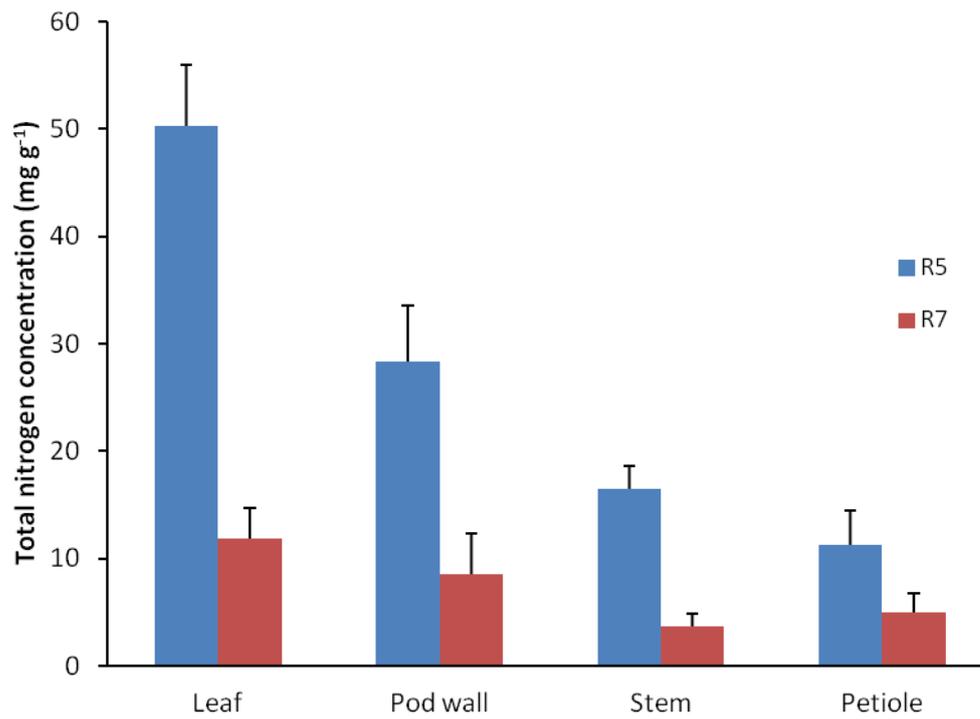


Fig.2-1. Nitrogen concentration in each vegetative organ at the R5 and R7 stages in Fukuyutaka in 2009. Bars indicate standard deviations with 5 replications.

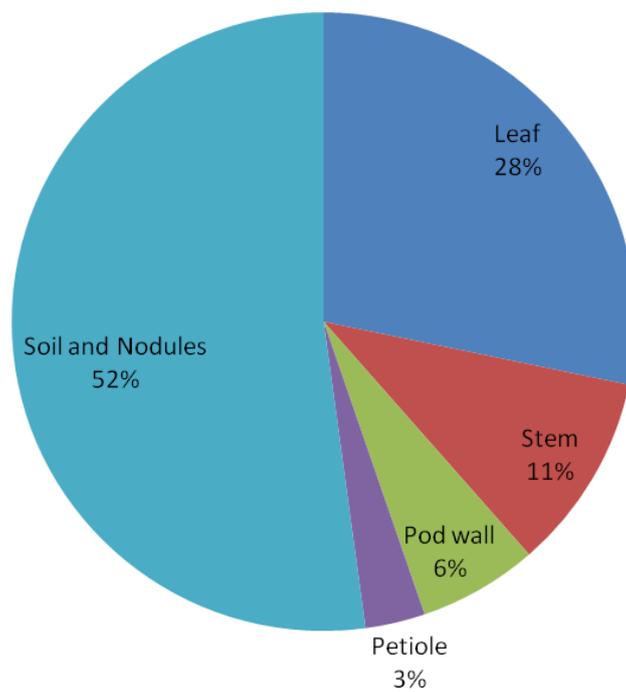


Fig. 2-2. Proportions of nitrogen sources in seeds during the seed-filling period in Fukuyutaka in 2009.

2. Varietal differences in nitrogen accumulation in the leaves and its redistribution to the seeds

Fig. 2-3 shows the changes in nitrogen concentration in the leaves in 4 commercial cultivars in 2008 and 2009. The nitrogen concentration in leaves remained at a high level for 40 to 60 days after sowing (DAS) in most of cultivars, and then it declined gradually from 60 DAS to 80 DAS and very sharply after 80 DAS. The nitrogen concentration decreased quickly in Sachiyutaka and Tamahomare, which were two early maturing cultivars, whereas it decreased slowly in Akiyoshi in 2009, especially after 80 DAS. However, the nitrogen concentration was constantly lower in Tamahomare in both years compared with the other 3 cultivars. The leaf SPAD value increased even after flowering, reached the maximum at 80 DAS, and then decreased sharply, synchronized with the changes in the nitrogen concentration (Fig. 2-4).

Table 2-1 shows the differences in nitrogen accumulation in the leaves and the redistribution to the seeds among 4 cultivars in 2008 and 2009. The nitrogen concentration in leaves at R5 was the highest in Fukuyutaka and the lowest in Tamahomare. However, the concentrations decreased to almost the same level at R7, except for Akiyoshi, in both 2008 and 2009.

The nitrogen redistributed from leaves to the seeds was 6.5 to 7.4 g m⁻² in Sachiyutaka, Fukuyutaka, and Akiyoshi, but significantly less in Tamahomare (4.6 to 5.0 g m⁻²) in both 2008 and 2009. Therefore, the two-year average proportion of nitrogen redistribution to seeds was 33.9% in Akiyoshi, 29.3% in Sachiyutaka, and 26.2% in Fukuyutaka, while it was only

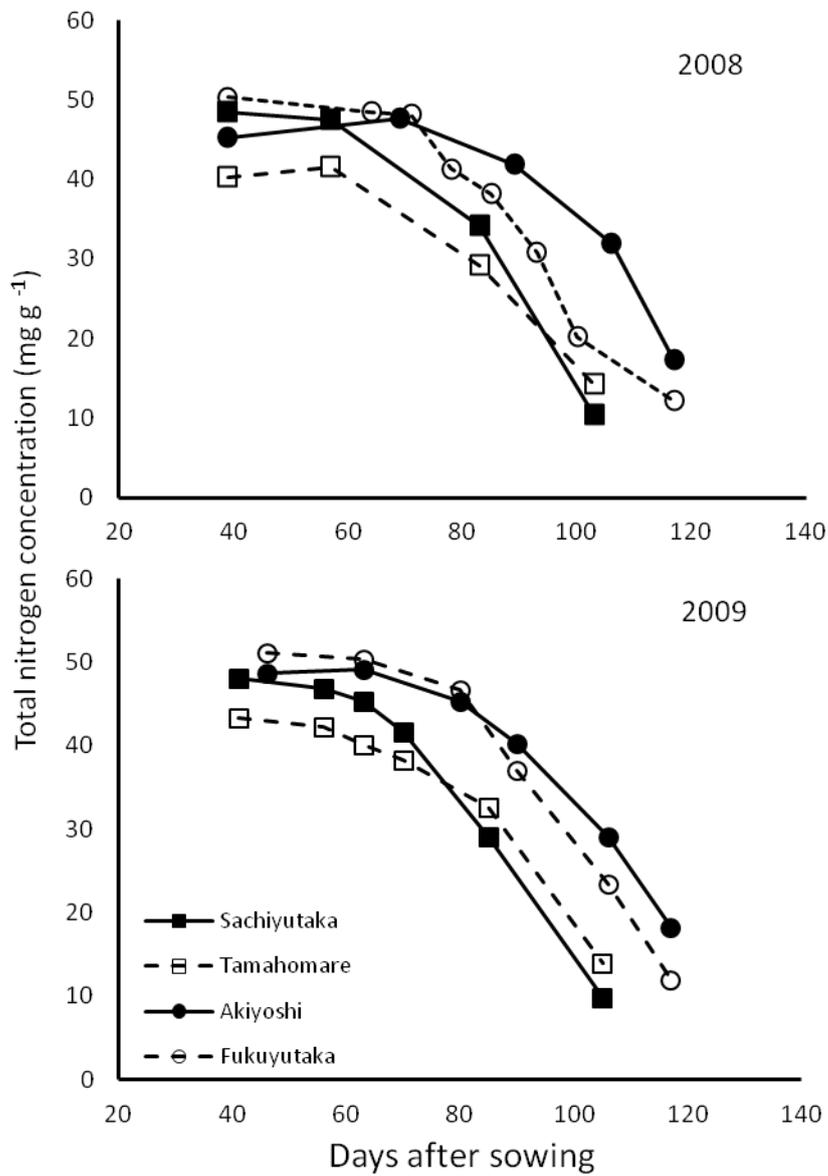


Fig. 2-3. Changes in nitrogen concentration in leaves during the reproductive stage.

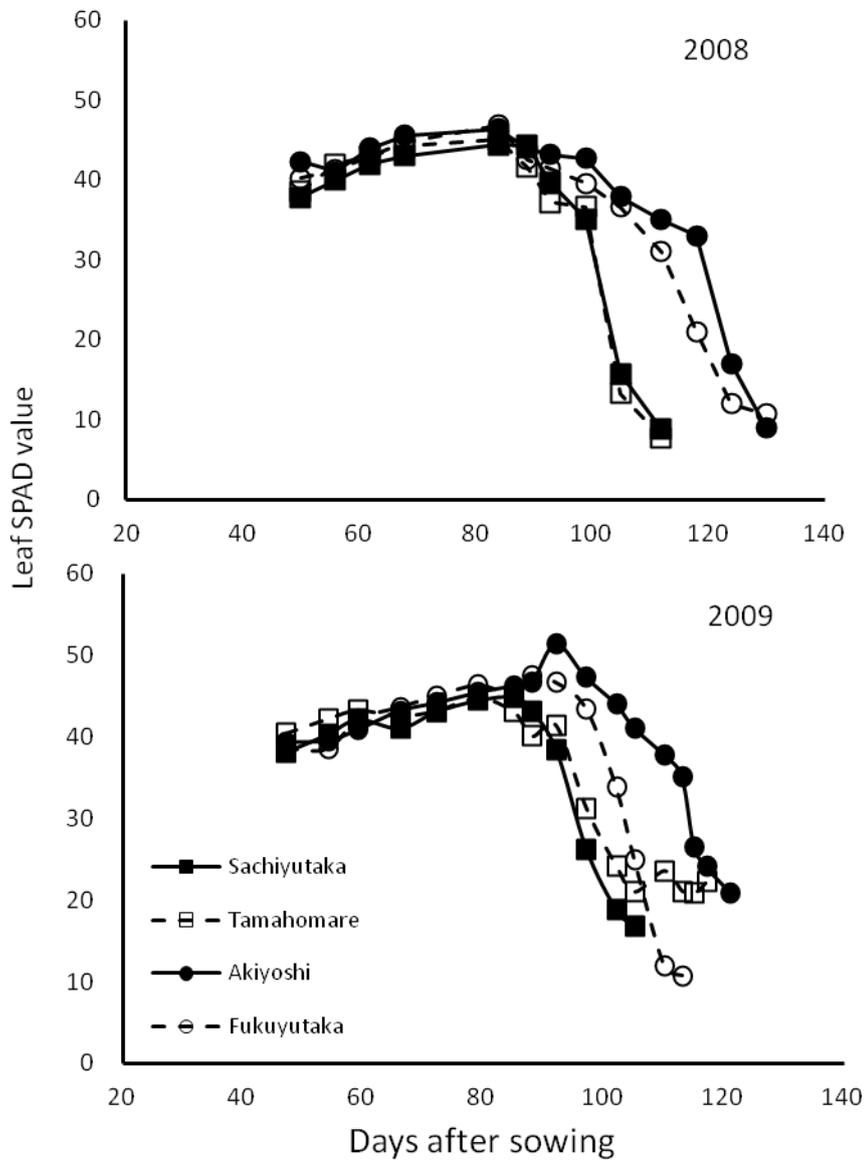


Fig. 2-4. Changes in SPAD value in leaves during the reproductive stage.

Table 2-1. Characteristics of nitrogen accumulation, redistribution and seed yield.

	Nitrogen concentration in leaves		Leaves weight	Nitrogen redistributed	Seed yield	Nitrogen content	Nitrogen concentration	Nitrogen redistribution
	R5	R7	R5	from leaves		in seeds	in seeds	to seeds ¹⁾
	(mg g ⁻¹)		(g plant ⁻¹)	(g m ⁻²)	(g m ⁻²)	(g m ⁻²)	(mg g ⁻¹)	(%)
2008								
Sachiyutaka	47.5±3.4 a	10.3±1.7 c	19.6±2.1 b	6.9±0.8 a	377±49 b	26.9±3.7 b	71.3±0.6 a	25.8±5.7 ab
Tamahomare	41.6±2.2 b	14.2±1.4 b	17.8±1.9 b	4.6±0.5 b	416±18 a	26.7±1.8 b	64.3±1.8 c	17.3±2.7 c
Akiyoshi	47.7±3.9 a	17.3±1.1 a	24.8±1.7 a	7.2±0.9 a	361±26 b	24.0±1.9 b	66.4±0.5 b	29.9±3.6 a
Fukuyutaka	48.5±2.1 a	12.2±1.6 bc	21.3±2.2 ab	7.3±0.5 a	452±26 a	30.5±2.2 a	67.5±0.7 b	24.1±2.1 b
2009								
Sachiyutaka	46.9±0.7 b	9.8±1.8 c	21.1±2.2 ab	7.4±0.3 a	322±23 b	22.8±1.7 ab	70.7±1.5 a	32.7±3.3 a
Tamahomare	42.3±2.2 c	13.9±1.3 b	18.5±1.5 b	5.0±0.4 c	334±33 b	20.1±2.7 b	60.1±2.1c	24.9±5.4 c
Akiyoshi	49.1±1.5 a	18.2±1.2 a	22.2±1.8 a	6.5±0.3 b	270±31 c	17.2±2.1 b	63.7±2.3 bc	37.9±6.8 a
Fukuyutaka	50.3±1.8 a	11.9±1.2 c	19.9±2.1 ab	7.3±0.7 a	382±42 a	25.7±2.8 a	67.2±2.5 ab	28.3±4.0 b

Data are expressed as mean values ± SD of five replications. Means followed by the same letter in the same column do not differ significantly at $p < 0.05$ level by Tukey-Kramer test.

¹⁾Nitrogen redistribution to seeds means the proportion of redistributed nitrogen in seed total nitrogen content.

21.1% in Tamahomare. Even though the redistributed nitrogen did not vary much from year to year, the proportion of redistributed nitrogen in the seeds was lower in 2008, which was a high seed yielding year, than in 2009. The proportion of nitrogen redistribution did not correlate with the seed yield. The nitrogen concentration in the seeds showed a significant difference among cultivars, that is, it was highest in Sachiyutaka (71.3 and 70.7 mg g⁻¹) and lowest in Tamahomare (64.3 and 60.1 mg g⁻¹) in both years.

3. Relationships between nitrogen redistribution from leaves and seed yield in different cultivars

Fig. 2-5 shows relationships between the seed yield with leaf nitrogen concentration, the proportion of redistributed nitrogen in the seeds and the amount of nitrogen redistributed from leaves to seeds over four years. No relationships were found between seed yield and nitrogen concentration in leaves at R5, or the proportion of redistributed nitrogen in the seeds (Fig. 2-5-A,B), however, the amount of redistributed nitrogen from leaves to seeds showed a significantly positive correlation ($r = 0.6447$, $p < 0.01$) with seed yield (Fig. 2-5-C). Therefore, compared with the nitrogen concentration in leaves at R5 and the proportion of redistributed nitrogen in the seeds, the amount of nitrogen redistributed from leaves to seeds was more suitable as the forecasting indicator for the seed yield.

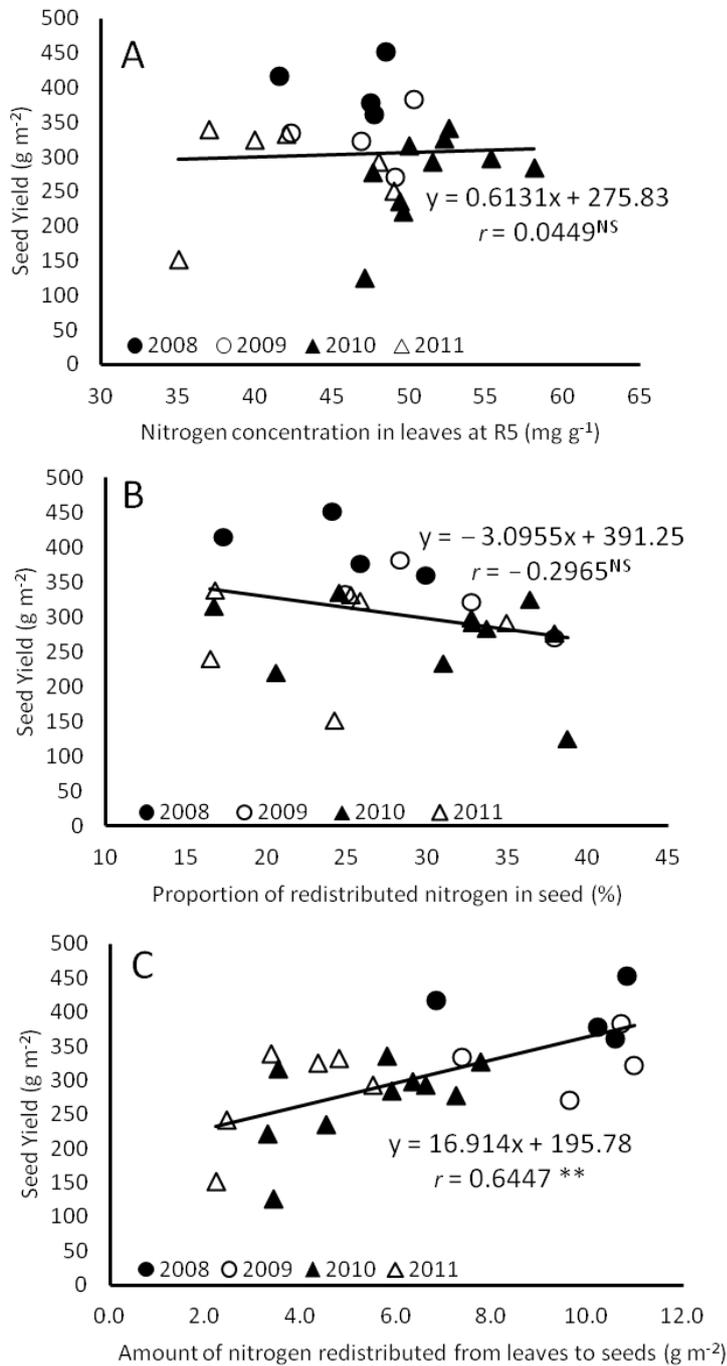


Fig.2-5. Relationships between the seed yield with leaf nitrogen concentration at R5 (A), proportion of redistributed nitrogen from leaves in seeds (B), and the amount of nitrogen redistributed from leaves to seeds (C).

** indicates significant difference at $p < 0.01$ level.

V. DISCUSSION

Most of the nitrogen assimilated by soybean plants is ultimately partitioned into the seeds. Before seed filling starts, except for the nitrogen used to support the development of vegetative tissues, a large amount of the assimilated nitrogen is considered to be stored in the vegetative organs (Egli et al., 1985; Warembourg and Fernandez, 1985; Hortensteiner and Feller, 2002). Therefore, the leaf nitrogen concentration and leaf SPAD value reached the maximum before seed filling started (Figs. 2-3, 2-4). However, the leaf nitrogen concentration decreased at around 70 DAS, after the seed filling started (R5), indicating that the nitrogen in the leaves was redistributed (Fig. 2-3). Hanway and Weber (1971) reported that almost half of the nitrogen, phosphorus, and potassium in the matured seeds were translocated from other plant parts in soybean. The results are consistent with this conclusion (Fig. 2-2). Moreover, the leaf tissue was the biggest nitrogen exporter, providing around 30% of the total nitrogen in the seeds.

Many researchers have discussed the importance of the nutrient assimilation in vegetative organs on the seed yield. For example, Munier-Jolain et al. (1996) analyzed the relationship between seed growth speed and the seed-filling period, noting that the amount of available nitrogen in vegetative organs was a decisive factor for the length of the seed-filling period. Wittenbach (1983) described how depodding can increase leaf dry weight and delay the loss of leaf chlorophyll and protein, thus proving the nutrient storage function of vegetative organs indirectly. Shibles

and Sundberg (1998) reported a positive linear relationship between nitrogen concentration in leaves at R5 and seed yield. The extensive investigation also found a significantly positive correlation between the seed yield and amount of redistributed nitrogen from leaves to seeds, but not with the nitrogen concentration in the leaves at R5 (Fig.2-5-A,C), showing inconsistent results with Shibles and Sundberg (1998). The results indicated that the amount of redistributed nitrogen from leaves was suitable as the forecasting indicator for seed yield.

In the high seed yielding year 2008, the highest yielding cultivar Fukuyutaka showed the highest amount of redistributed nitrogen, but a relatively lower proportion of redistributed nitrogen in the seeds (24.1%), while the highest proportion of redistributed nitrogen in the seeds (29.9%) was associated with the lowest seed yield in Akiyoshi (Table 2-1). On the contrary, in Tamahomare, the lowest nitrogen redistribution was associated with a relatively higher seed yield compared with Sachiyutaka, which belongs to the same maturity group with Tamahomare. Almost same trend was also observed in 2009. The results indicated that the high seed yield could be supported by not only large amount of redistributed nitrogen but also more nitrogen uptake (include nitrogen fixation) during the seed-filling period (R5-R7). Kumudini et al. (2002) pointed out that the new varieties with high seed yield showed a strong ability to take up nitrogen during seed filling. Even though we found significant correlation between the redistributed nitrogen and seed yield among ten cultivars over four years, high seed yielding is considered to be supported by both of nitrogen

remobilization and nitrogen uptake during seed filling (e.g. Fukuyutaka), even mostly by nitrogen uptake during seed filling (e.g. Tamahomare).

A large amount of nitrogen redistributed from leaves during seed filling could cause early leaf senescence (Sanetra et al., 1998; Guamet et al., 2002; Donnison et al., 2006), and retard photosynthesis (Boon-long et al., 1983; Buttery and Buzzell, 1988; Sinclair and Horie, 1989). Faster nitrogen partitioning and dry matter allocation into seeds are associated with shorter seed-filling duration, and lower seed yield in high seed protein genotypes (Salado-Navarro et al., 1985). In this experiment, although the nitrogen content in seeds of Sachiyutaka was higher than that in Tamahomare, the seed yield was lower in Sachiyutaka (Table 2-1). However, there was not a big difference in seed filling period (Fatichin et al., 2013) or leaf chlorophyll deterioration (Fig. 2-4) between Sachiyutaka and Tamahomare. Comparing with many evidences that prolonging the seed-filling period could increase the seed yield, Egli and Bruening (2007a, b) found a large nitrogen requirement of seeds was not associated with a short seed-filling period, and the stay-green genotype with a delayed onset of leaf senescence could not increase the seed yield (Luquez and Guamet, 2001). Therefore, the relationship between nitrogen assimilation and seed yield involves multiple physiological and environmental factors.

Chapter 3

Influence of nitrogen enrichment on leaf nitrogen accumulation and seed yield in soybean

I. SUMMARY

Seed nitrogen assimilation during seed filling limits the seed yield in soybean. In results of Chapter 2, significant varietal differences were found in seed nitrogen dependence on either redistributed nitrogen or absorbed nitrogen from soil during seed filling. In this chapter, the timing of nitrogen enrichment for effective nitrogen assimilation was investigated. Two soybean cultivars Sachiyutaka and Tamahomare were sown in the pots filled with well washed fine sand. The plants were well watered with nutrient solution contained 100 ppm nitrogen and other nutrient elements before and after the treatment. The treatments were conducted from reproductive stage R1 to R5 or from R5 to R7 by applying the nutrient solution with different nitrogen concentration. High nitrogen concentration during R1 to R5 delayed the decline in SPAD value and leaf nitrogen concentration and improved the yield performance in Sachiyutaka, whereas stimulated the decline in SPAD value and leaf nitrogen concentration and showed no effect on seed yield in Tamahomare. However, high nitrogen concentration during R5 to R7 delayed the decline in SPAD value and leaf nitrogen concentration and improved the yield performance more significantly in Tamahomare than in Sachiyutaka. The big seed yield increase by nitrogen enrichment during R5 to R7 in

Tamahomare could be caused by both of the high photosynthetic rate and vigorous nitrogen uptake during seed filling. These results suggested that the most effective timing of nitrogen enrichment on seed yield during reproductive growth period is different between the cultivars due to the pattern of nitrogen assimilation.

II. INTRODUCTION

In results of Chapter 2, the seed yield was positively correlated with the amount of redistributed nitrogen from leaves in Sachiyutaka, while in the case of another high seed yielding cultivar Tamahomare, the high seed yield was associated with even lower nitrogen redistribution, implying the nitrogen utilization patterns were different between Tamahomare and Sachiyutaka. On the other hand, these evidences also indicated the relationship between nitrogen assimilation and seed production is still unclear.

Seed filling period is known as a limiting factor for seed yield in soybean. The length of seed filling period has strong positive relationship with seed production (Smith and Nelson, 1986; Guffy et al., 1992; Egli, 2004). During seed filling period, a large amount of nitrogen is redistributed from vegetative organs to the seeds to satisfy the high nitrogen requirement for seed growth. However, this process could induce the leaf senescence (Sanetra et al., 1998; Donnison et al., 2006), decline the photosynthetic activity (Buttery and Buzzell, 1988; Sinclair and Horie, 1989), shorten the duration of seed filling period (Sinclair and de Wit, 1976), and finally limit the seed yield.

The objective in this Chapter was to reveal the role of nitrogen assimilation and redistribution on the seed yield between the cultivars with different nitrogen accumulation patterns, by changing the intensity and timing of nitrogen supply during reproductive growth stage in soybean.

III. MATERIALS AND METHODS

1. Plant cultivation

Two soybean cultivars Sachiyutaka and Tamahomare were used to conduct a series of pot experiments in 2010 and 2012. According to the results in Chapter 2, Sachiyutaka was more dependent of redistributed nitrogen in seed production, whereas Tamahomare was not.

The experiments were conducted in a side-opened plastic house in Coastal Bioenvironment Center, Saga University, Karatsu City, Japan (33° 27'N and 129° 58'E). Four seeds inoculated with *Bradyrhizobium* Spp. (Konryukin Mame-zo, Tokachi Nokyoren, Hokkaido, Japan) were sown in each pot (15 cm in diameter, 20 cm in depth) filled with well washed sand on 29 June in 2010 and on 23 July in 2012, then were thinned to only one plant per pot at the stage when the first trifoliolate leaf appeared. After emergence, all the plants were watered two times a week with the half concentration of basic nutrient solution contained 50 ppm nitrogen, and then with full concentration of basic solution (Table 3-1) from four weeks after emergence to harvest.

2. Treatments of nitrogen enrichment

The treatments were applied as changing only nitrogen concentration in the basic nutrient solution during flowering stage (R1) to the beginning of seed filling stage (R5), or from R5 to physiological maturity stage (R7). During treatment period, the nitrogen concentrations were adjusted to 5, 25,

Table 3-1. Components of nutrient solution.

Nutrient	Concentration (ppm)	Reagents
N	5 ~ 800	NH_4NO_3
P	70	KH_2PO_4
K	110	K_2SO_4 , KH_2PO_4
Mg	90	MgSO_4
Ca	35	CaCl_2
Fe	3.5	NaFeEDTA
Mn	0.3	MnSO_4
B	0.06	H_3BO_3
Zn	0.009	ZnSO_4
Cu	0.009	CuSO_4
Mo	0.009	MoO_3

Modified from Matsunaga et al. (1983).

100, 200 and 400 ppm in 2010, and 5, 100, 200, 400 and 800 ppm in 2012, and the nutrient solution were applied enough (500 to 800 mL per plant) for replacing the solution in the sand medium on each other day. Six plants were used in each treatment. The growth stages were determined according to Fehr et al. (1971).

3. Sampling and measurements

The leaf SPAD value was measured by a chlorophyll meter SPAD-502 (Konica Minolta, Inc., Osaka, Japan) 1 or 2 times per week on the second or third full-expanded leaf from the top during R1 to R7.

One leaflet on the third or fourth fully expanded leaf from the top was taken at R1, R5, R6 and R7 stage, respectively. After drying at 80°C for 48 hours, the leaves were ground into powder for the determination of total nitrogen content by Kjeldahl method.

The leaf apparent photosynthetic rate was measured on the second or third fully expanded leaf from top with a portable gas exchange system LI-6400 (LI-COR Bioscience, Lincoln NE, USA) from 1000 to 1200 on 17 September (Between R5 and R6) in 2010. Photosynthetic photon flux density (PPFD) was fixed at $1500 \mu \text{ mol m}^{-2}\text{s}^{-1}$ using a red blue LED light source, but the CO₂ concentration and temperature were not controlled. Consequently, the ambient CO₂ concentration was from 377.2 to 388.0 ppm and the leaf temperature was from 27.8 to 30.5 °C during the measurement. Air was induced through a rubber hose from 10 m far from the plants outside the plastic house.

At the harvest maturity stage (R8), all the plants of each treatment were taken for the determination of the seed yield and yield components. In 2012, the roots were collected in the plants in 5, 100 and 800 ppm treatments only after harvesting the shoots.

4. Statistical analysis

Least significant difference (LSD) analysis was used for the significance analysis on the parameters of leaf photosynthetic rate, root dry weight and yield components among different treatments in each cultivar. Single-factor ANOVA method was adapted for Figures 3-1 to 3-8.

IV. RESULTS

1. Influences of nitrogen enrichment on leaf SPAD value and nitrogen concentration

In the nitrogen enrichment during R1 to R5 stage, the response of leaf SPAD values to the nitrogen concentration was very different between Sachiyutaka (Fig. 3-1) and Tamahomare (Fig. 3-2) in both of 2010 and 2012. Before R5 stage, with the increase of nitrogen concentration in nutrient solution, the leaf SPAD values of both cultivars also increased. However, after R5 stage in high nitrogen concentration, the decrease in SPAD value in Sachiyutaka was slow, whereas in Tamahomare, it turned to be fast, and became to be the lowest at the last measurement at R7 stage.

The high nitrogen concentration (400 ppm in 2010 and 800 ppm in 2012) enhanced the leaf nitrogen accumulation apparently in Sachiyutaka (Fig. 3-5), while it did slightly in Tamahomare at R5 stage (Fig. 3-6). At R7 stage, the leaf nitrogen concentration which treated by 400 ppm (in 2010) and 800 ppm (in 2012) nitrogen solution was found at high level in Sachiyutaka, while in Tamahomare, it was the lowest.

In the nitrogen enrichment during R5 to R7 stage, even the leaf SPAD values in both of Sachiyutaka (Fig. 3-3) and Tamahomare (Fig. 3-4) declined in all nitrogen treatment after R5 stage, the decrease in leaf SPAD values was delayed by nitrogen enrichment, especially in Tamahomare, the SPAD value was found finally at very high level in 400 ppm (in 2010) and 800 ppm (in 2012) nitrogen treatments.

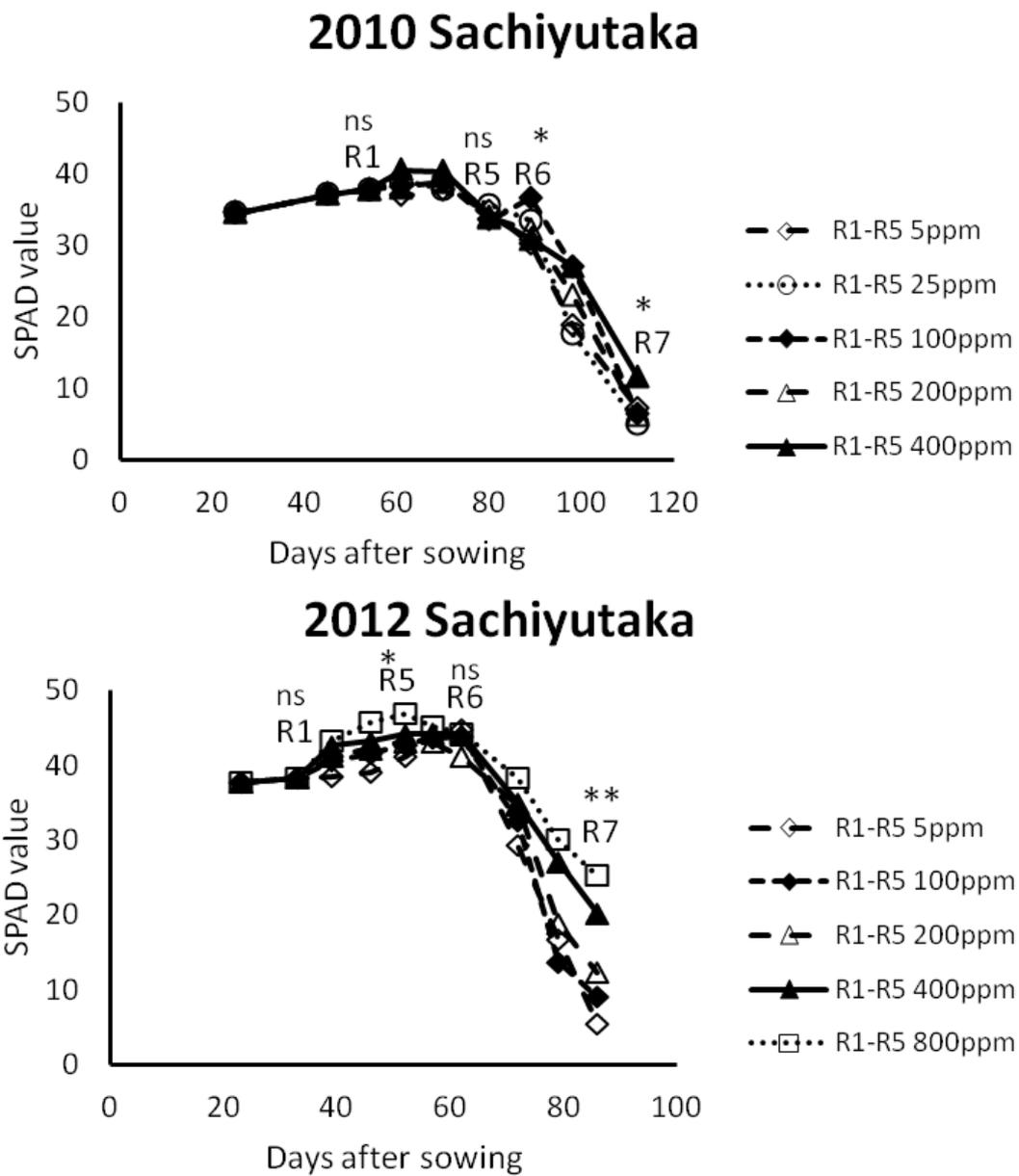


Fig. 3-1. Changes of leaf SPAD value in Sachiyutaka after sowing in R1-R5 treatment. R1, R5, R6 and R7 indicate the reproductive stages in control plants (100 ppm nitrogen), respectively.

* $p < 0.05$; ** $p < 0.01$; ns, not significant.

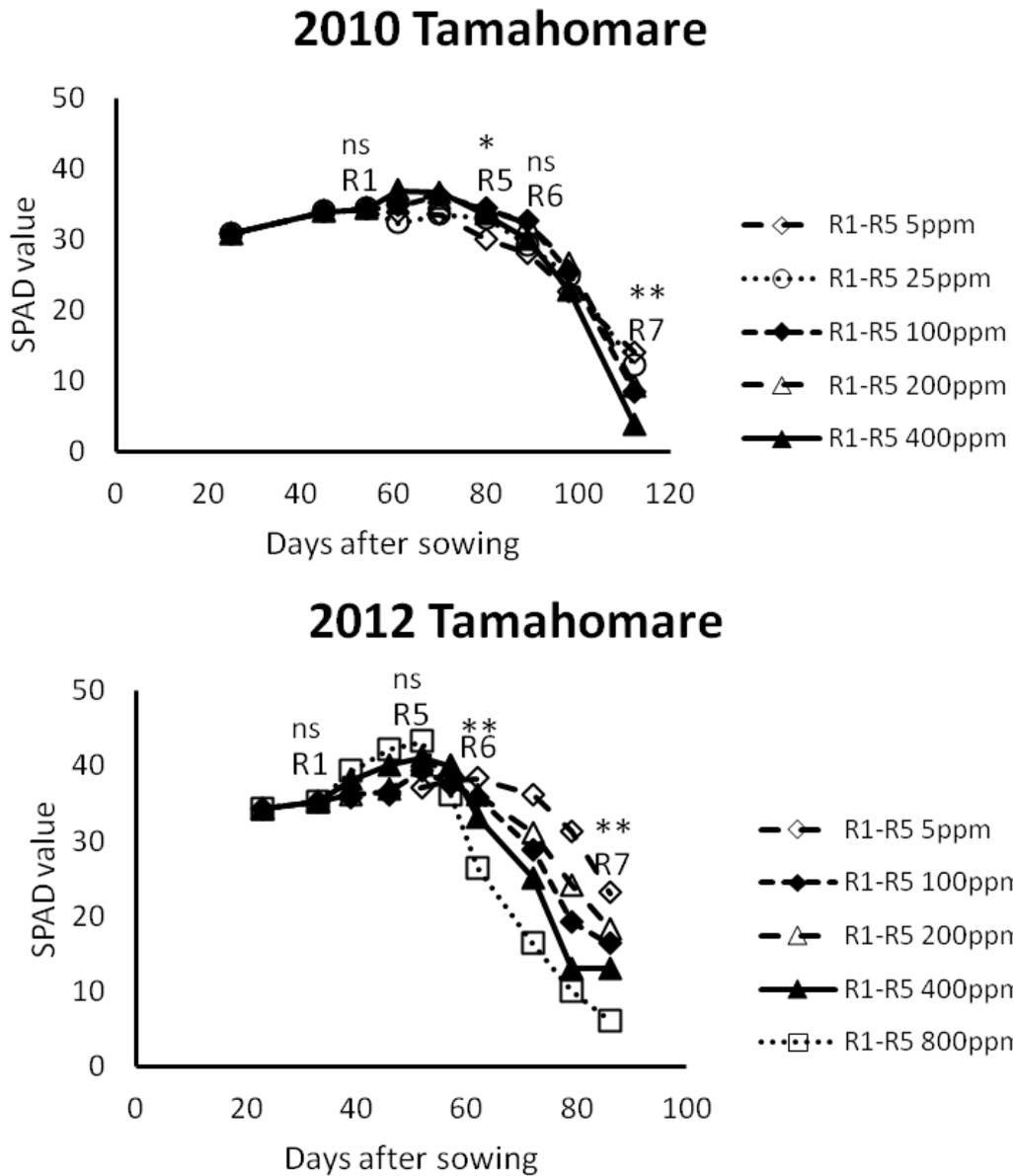


Fig. 3-2. Changes of leaf SPAD value in Tamahomare after sowing in R1-R5 treatment. R1, R5, R6 and R7 indicate the reproductive stages in control plants (100 ppm nitrogen), respectively.

* $p < 0.05$; ** $p < 0.01$; ns, not significant.

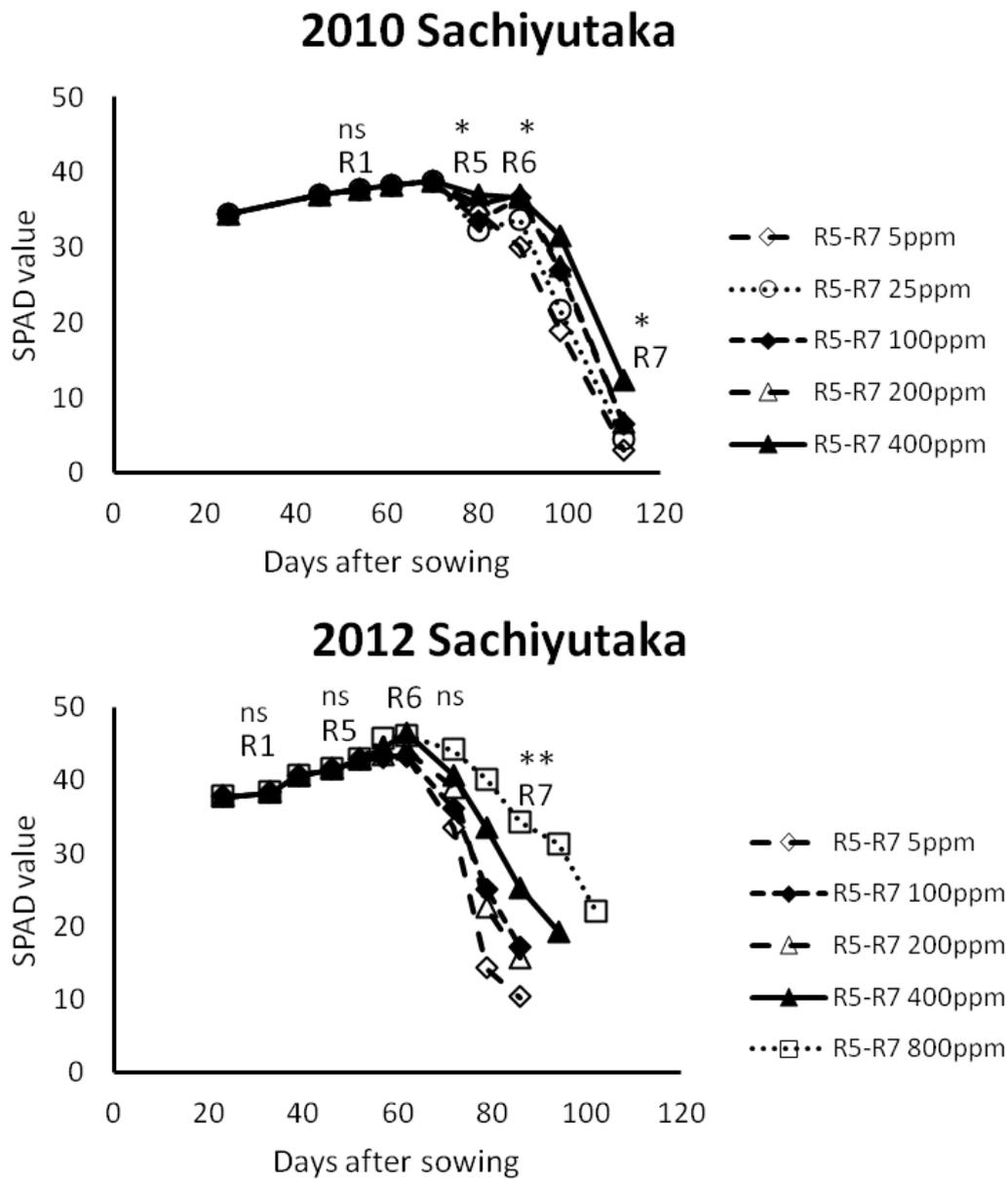


Fig. 3-3. Changes of leaf SPAD value in Sachiyutaka after sowing in R5-R7 treatment. R1, R5, R6 and R7 indicate the reproductive stages in control plants (100 ppm nitrogen), respectively.

* $p < 0.05$; ** $p < 0.01$; ns, not significant.

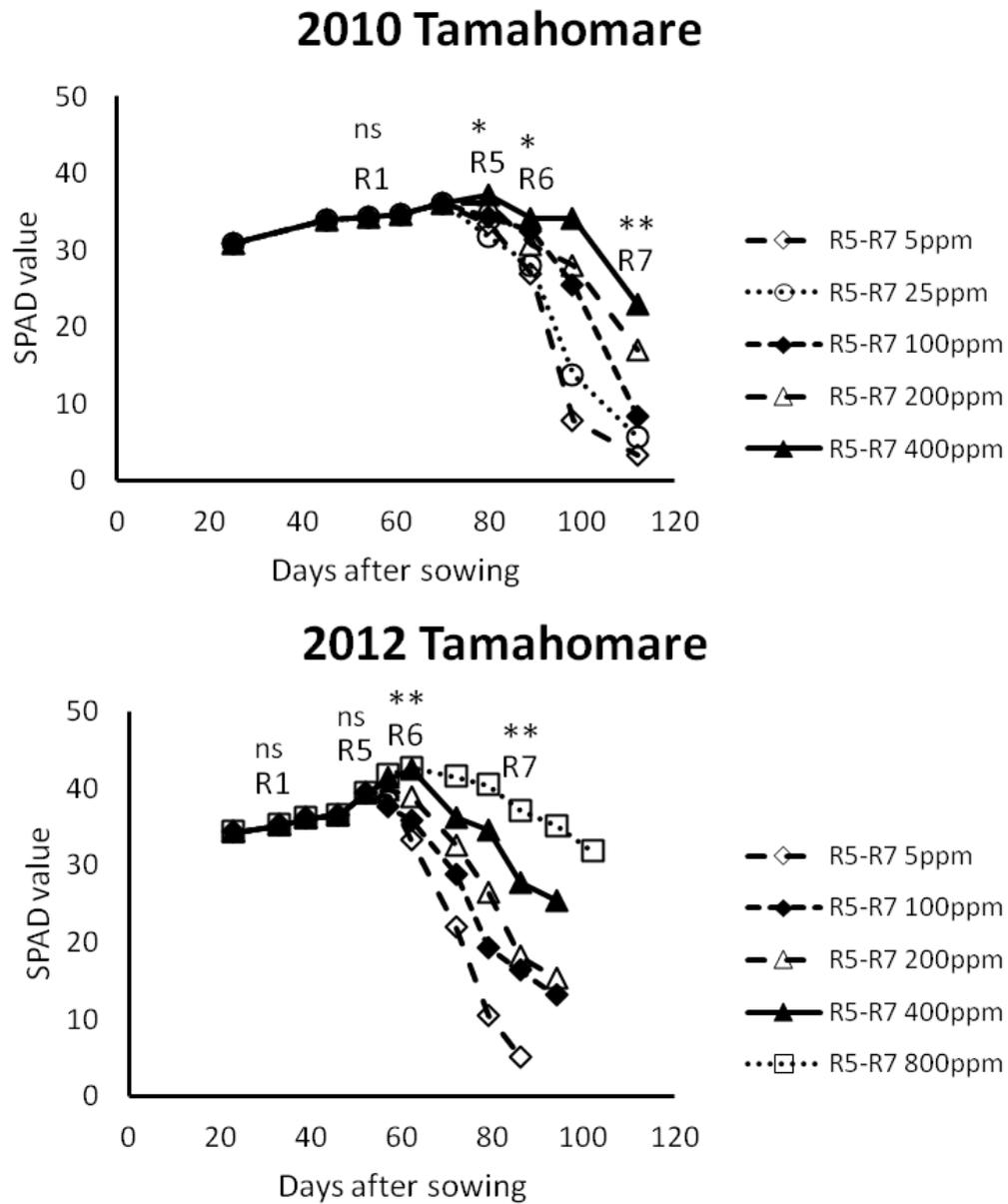


Fig. 3-4. Changes of leaf SPAD value in Tamahomare after sowing in R5-R7 treatment. R1, R5, R6 and R7 indicate the reproductive stages in control plants (100 ppm nitrogen), respectively.

* $p < 0.05$; ** $p < 0.01$; ns, not significant.

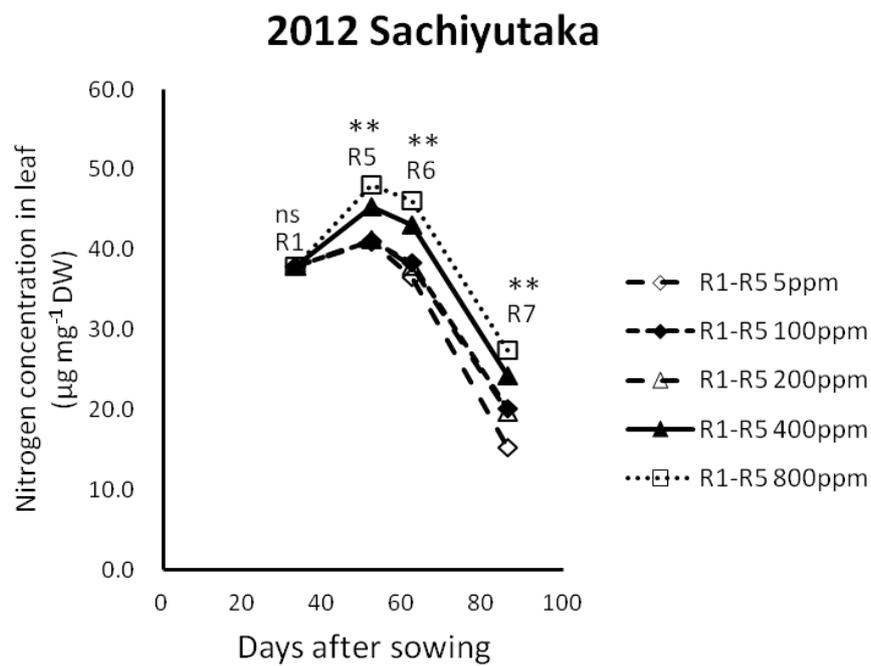
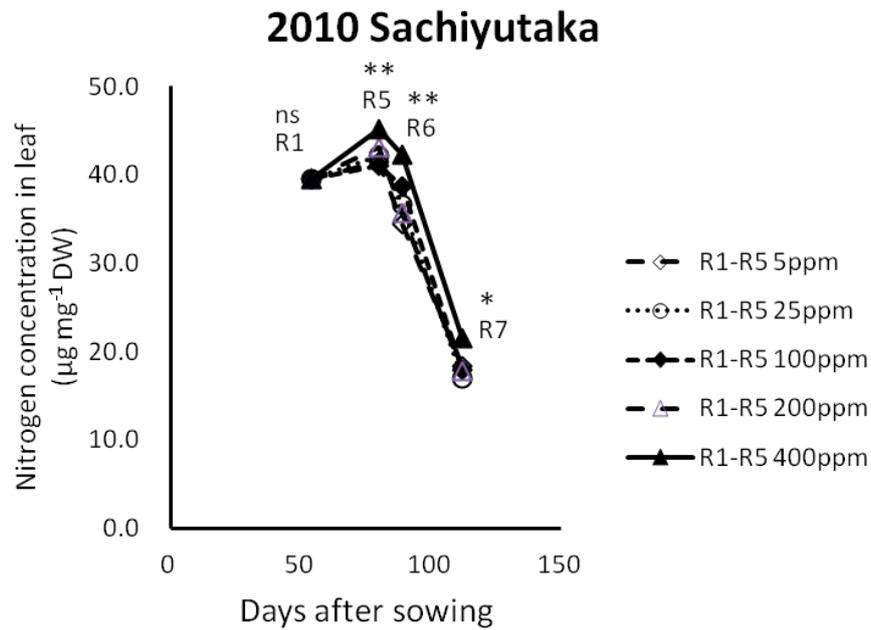


Fig. 3-5. Changes of leaf nitrogen concentration in Sachiutaka and Tamahomare after sowing in R1-R5 treatment. R1, R5, R6 and R7 indicate the reproductive stages in control plants (100 ppm nitrogen), respectively.

* $p < 0.05$; ** $p < 0.01$; ns, not significant.

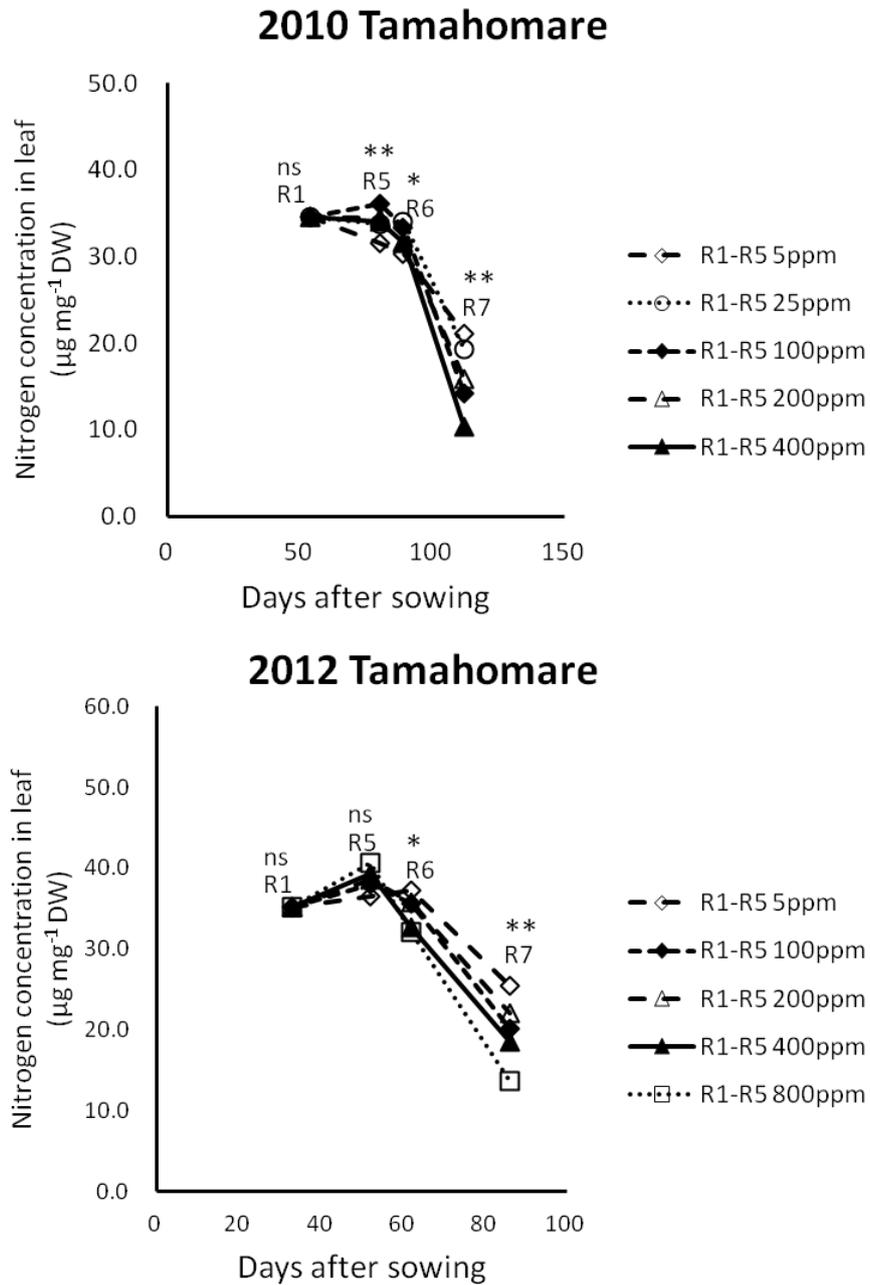


Fig. 3-6. Changes of leaf nitrogen concentration in Tamahomare after sowing in R1-R5 treatment. R1, R5, R6 and R7 indicate the reproductive stages in control plants (100 ppm nitrogen), respectively.

* $p < 0.05$; ** $p < 0.01$; ns, not significant.

The changes in leaf nitrogen concentration in the treatment during R5 to R7 stage were almost similar as that in SPAD value. At R7 stage, among the different concentrations of nitrogen enrichment, the leaf nitrogen concentration in Tamahomare (Fig. 3-8) showed much bigger difference than in Sachiyutaka (Fig. 3-7). Especially in the treatments of 400 ppm in 2010 and 800 ppm in 2012, the leaf nitrogen concentration in Tamahomare at R7 stage declined slightly compared with those at R5 stage, while in the treatments of the lowest nitrogen concentration (5 ppm), it declined greatly than Sachiyutaka.

Fig. 3-9 shows the leaf photosynthetic rate at between R5 and R6 stage in 2010. The photosynthetic rate was higher in Tamahomare than in Sachiyutaka. Since the measurement was only conducted after R5 stage, the photosynthetic rate was not increased by nitrogen enrichment during R1-R5 treatment. However, the significant increase in photosynthetic rate was observed accompanied with the increase of nitrogen concentrations in Tamahomare only during R5-R7 treatment.

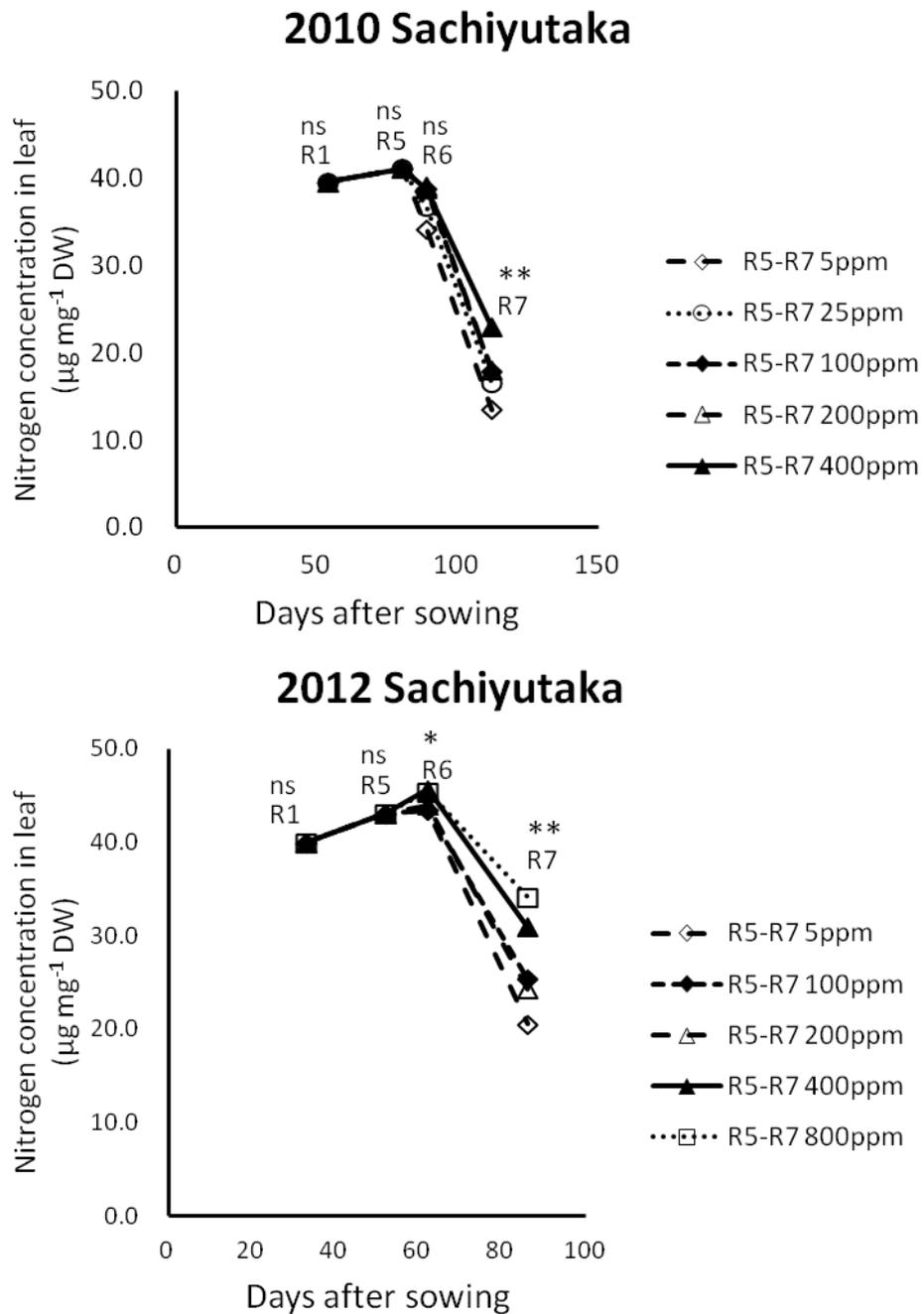


Fig. 3-7. Changes of leaf nitrogen concentration in Sachiyutaka after sowing in R5-R7 treatment. R1, R5, R6 and R7 indicate the reproductive stages in control plants (100 ppm nitrogen), respectively.

* $p < 0.05$; ** $p < 0.01$; ns, not significant.

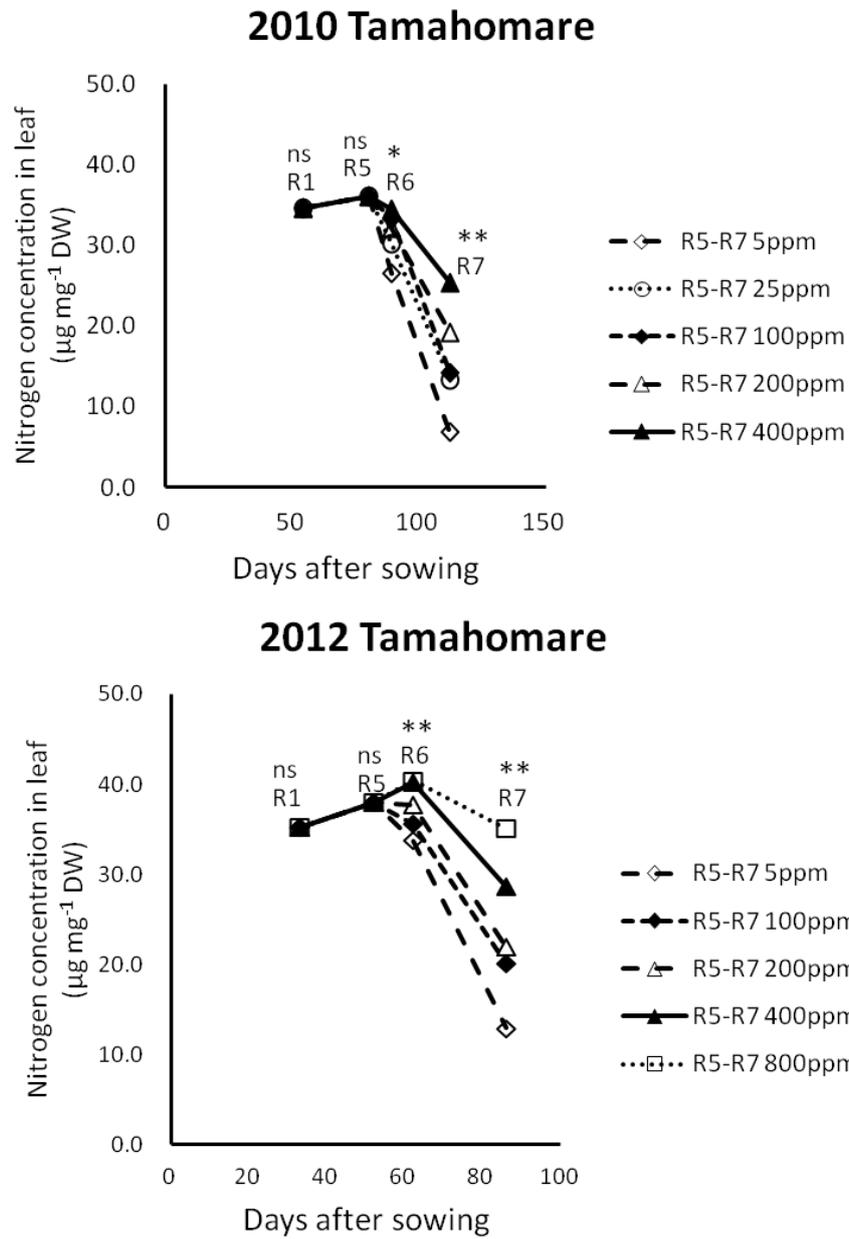


Fig. 3-8. Changes of leaf nitrogen concentration in Tamahomare after sowing in R5-R7 treatment. R1, R5, R6 and R7 indicate the reproductive stages in control plants (100 ppm nitrogen), respectively.

* $p < 0.05$; ** $p < 0.01$; ns, not significant.

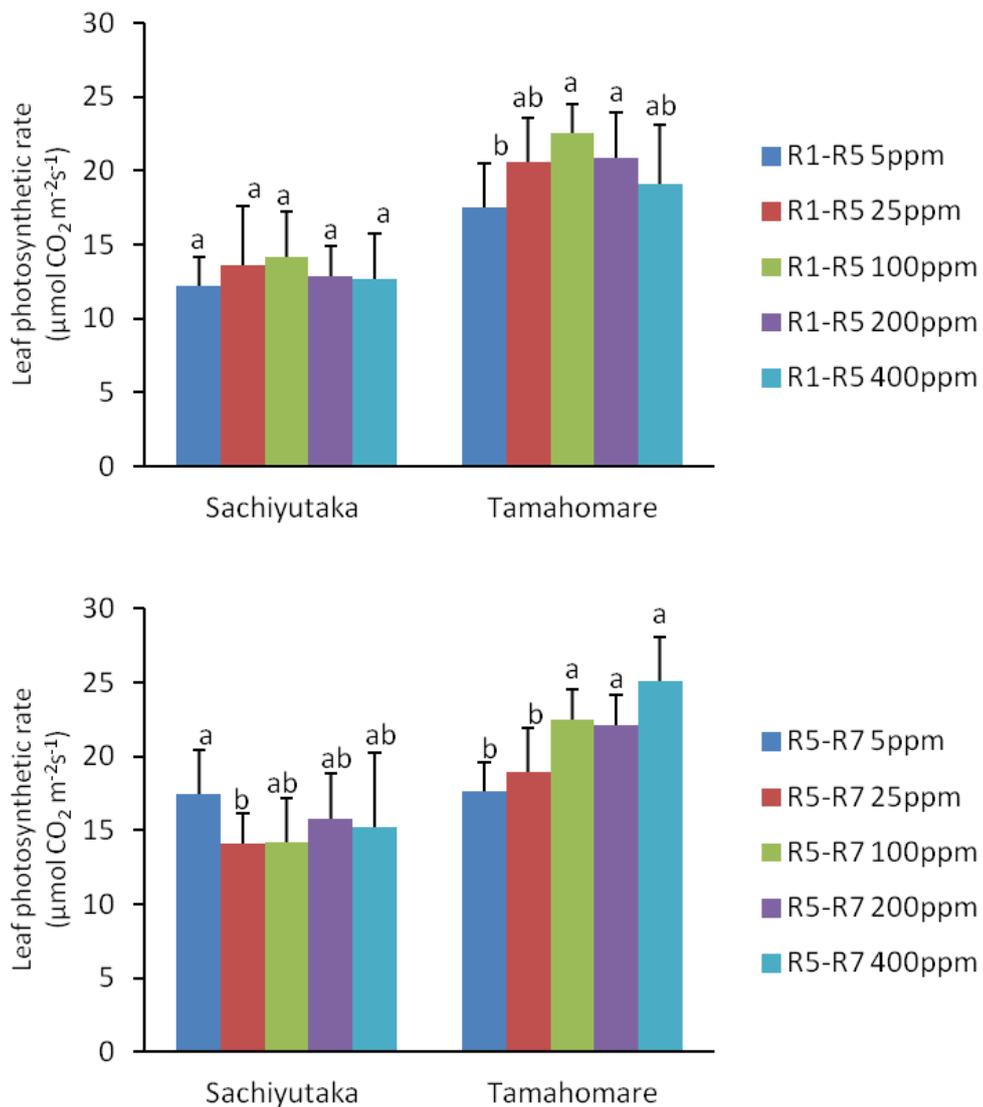


Fig. 3-9. Differences of photosynthetic rate in leaf in R1-R5 and R5-R7 treatments in 2010. The measurements were conducted on 17 September, 2010 (the stage between R5 and R6). Bars indicate standard deviations with five replications. In same cultivar, the different letters indicate significant difference according to LSD analysis at $p < 0.05$ level.

2. Influence of nitrogen enrichment on seed yield and yield components

In R1-R5 treatment, with the increase of nitrogen concentration, pod number per plant, fertile pod number per plant and seed number per plant increased in both cultivars in 2010 and 2012 (Table 3-2). However, the seed weight plant⁻¹ and 100 seed weight increased only in Sachiyutaka. There was no change in seed weight per plant, even the significant decrease in 100 seed weight in Tamahomare in 2010 and 2012.

In R5-R7 treatment, with the increase of nitrogen concentration, compared with Sachiyutaka, the dramatic increases in fertile pod number per plant, seed number per plant, seed weight per plant and 100 seed weight were observed in Tamahomare in 2010 and 2012 (Table 3-3). Especially in the highest 800 ppm mg nitrogen treatment in 2012, the seed weight in Tamahomare was 1.42 times of control (100ppm) comparing with the 1.26 times in Sachiyutaka, while in the lowest nitrogen concentration (5 ppm in 2012), it was 0.78 times of control in Tamahomare comparing with 0.94 times in Sachiyutaka. Therefore, the nitrogen enrichment caused much bigger effect on yield performance in Tamahomare compared with Sachiyutaka.

There were strong interaction between nitrogen enrichment and cultivar on seed weight and 100 seed weight in both R1-R5 and R5-R7 treatments (Table 3-2 and 3-3), which indicated that the effect of nitrogen enrichment varied in different cultivars.

Table 3-2. Differences in seed yield and yield components in R1-R5 treatment.

	Total Pod No. (plant ⁻¹)	Fertile pod No. (plant ⁻¹)	Seed No. (plant ⁻¹)	Seed weight (g plant ⁻¹)	100 seed weight (g)
Sachiyutaka, 2010					
R1-R5 5ppm	46±4.1d	33±4.3c	63±4.2c	19.3±1.5c	30.5±0.7c
R1-R5 25ppm	47±3.1cd	35±3.7c	66±4.0c	20.4±1.9c	30.7±1.1c
R1-R5 100ppm	52±2.4b	41±3.1b	74±3.8b	23.2±1.4b	31.4±0.4bc
R1-R5 200ppm	53±3.7b	45±7.4b	77±4.3ab	24.7±1.4b	32.1±0.3b
R1-R5 400ppm	59±5.8a	51±5.2a	82±4.3a	27.5±1.8a	33.3±0.7a
Tamahomare, 2010					
R1-R5 5ppm	52±3.1c	49±4.3bc	88±6.7a	24.7±3.5a	28.1±1.5a
R1-R5 25ppm	53±4.1c	49±4.2c	89±5.9a	24.9±1.5a	27.9±2.0a
R1-R5 100ppm	67±5.4b	53±2.7ab	89±8.5a	24.8±2.2a	27.8±0.5a
R1-R5 200ppm	69±3.7ab	54±5.0a	91±7.5a	24.6±1.1a	27.1±1.6a
R1-R5 400ppm	73±3.4a	57±4.6a	94±5.9a	24.9±1.1a	26.5±1.1a
Sachiyutaka, 2012					
R1-R5 5ppm	44±3.6c	35±3.3d	59±9.5c	17.2±1.8d	29.0±0.7c
R1-R5 100ppm	49±4.8bc	38±5.9cd	69±4.3b	21.3±2.3c	30.8±0.6b
R1-R5 200ppm	51±2.7b	41±4.8bc	71±9.2b	22.4±3.9c	31.5±0.7b
R1-R5 400ppm	58±5.4a	47±3.5b	79±6.1a	25.3±3.1b	31.9±0.7ab
R1-R5 800ppm	62±5.0a	53±5.4a	85±10.3a	27.6±2.6a	32.6±0.6a
Tamahomare, 2012					
R1-R5 5ppm	53±5.6d	45±6.1d	80±5.2b	22.4±3.2a	28.0±1.2a
R1-R5 100ppm	61±4.7c	51±5.1c	81±4.2b	22.1±2.5a	27.4±1.4ab
R1-R5 200ppm	63±7.6bc	53±5.8bc	83±4.2ab	21.8±3.1a	26.3±0.9b
R1-R5 400ppm	69±6.3ab	57±6.2ab	89±7.9a	22.3±4.3a	25.1±0.6b
R1-R5 800ppm	74±3.3a	62±3.9a	89±3.3a	22.1±3.7a	24.9±0.8b
ANOVA					
Nitrogen supply (N)	**	**	**	**	**
Cultivar (C)	**	**	**	ns	ns
N × C	*	ns	**	**	**

Data are expressed as mean values ±SD of six replications. Means followed by the same letter in the same column do not differ significantly at $p < 0.05$ level by LSD analysis.

* $p < 0.05$; ** $p < 0.01$; ns, not significant.

Table 3-3. Differences in seed yield and yield components in R5-R7 treatment.

	Total Pod No. (plant ⁻¹)	Fertile pod No. (plant ⁻¹)	Seed No. (plant ⁻¹)	Seed weight (g plant ⁻¹)	100 seed weight (g)
Sachiyutaka, 2010					
R5-R7 5ppm	52 ± 2.6a	34 ± 3.7c	71 ± 4.3b	21.9 ± 1.6c	30.8 ± 0.5a
R5-R7 25ppm	51 ± 5.6a	32 ± 4.2c	72 ± 5.6b	22.7 ± 1.6bc	31.6 ± 2.1a
R5-R7 100ppm	52 ± 6.8a	41 ± 2.7b	74 ± 3.3b	23.2 ± 1.1bc	31.4 ± 0.6a
R5-R7 200ppm	53 ± 6.7a	48 ± 3.4a	84 ± 3.7a	25.1 ± 1.4ab	29.9 ± 1.2a
R5-R7 400ppm	52 ± 5.8a	45 ± 4.3a	83 ± 3.3a	26.2 ± 1.2a	31.4 ± 2.1a
Tamahomare, 2010					
R5-R7 5ppm	66 ± 6.4a	42 ± 5.3c	85 ± 8.7c	18.4 ± 3.0d	21.6 ± 1.9b
R5-R7 25ppm	65 ± 3.3a	44 ± 7.7c	93 ± 6.8b	20.8 ± 2.6c	22.3 ± 2.2b
R5-R7 100ppm	67 ± 8.1a	53 ± 3.9b	89 ± 8.1bc	24.8 ± 3.8b	27.8 ± 0.9a
R5-R7 200ppm	66 ± 5.6a	55 ± 4.6b	90 ± 7.3bc	25.3 ± 3.6b	28.1 ± 0.9a
R5-R7 400ppm	67 ± 5.1a	62 ± 4.8a	103 ± 5.3a	30.1 ± 2.3a	29.3 ± 0.6a
Sachiyutaka, 2012					
R5-R7 5ppm	50 ± 2.1a	37 ± 5.7b	68 ± 8.6c	20.1 ± 3.1d	29.4 ± 0.9a
R5-R7 100ppm	49 ± 1.9a	38 ± 4.4b	69 ± 5.9c	21.3 ± 3.0cd	30.8 ± 0.7a
R5-R7 200ppm	49 ± 5.9a	42 ± 5.3ab	75 ± 6.7bc	22.8 ± 2.8bc	30.6 ± 1.1a
R5-R7 400ppm	48 ± 5.5a	46 ± 2.9a	81 ± 4.4ab	24.9 ± 2.9ab	30.3 ± 0.5a
R5-R7 800ppm	49 ± 4.2a	46 ± 2.4a	88 ± 7.1a	26.8 ± 1.8a	30.5 ± 0.6a
Tamahomare, 2012					
R5-R7 5ppm	62 ± 7.5a	47 ± 4.9c	79 ± 8.3d	17.2 ± 1.9d	21.9 ± 0.6c
R5-R7 100ppm	61 ± 4.3a	51 ± 4.4c	81 ± 9.2d	22.1 ± 3.5c	27.4 ± 0.7b
R5-R7 200ppm	60 ± 6.8a	53 ± 6.8bc	88 ± 7.4c	24.3 ± 4.0c	27.7 ± 1.5b
R5-R7 400ppm	62 ± 6.3a	58 ± 6.2ab	96 ± 8.9b	27.8 ± 3.7b	28.9 ± 0.6ab
R5-R7 800ppm	61 ± 6.0a	61 ± 5.2a	104 ± 5.4a	31.4 ± 3.6a	30.3 ± 1.1a
ANOVA					
Nitrogen (N)	**	**	**	**	**
Cultivar (C)	ns	**	**	ns	**
N × C	ns	ns	ns	**	**

Data are expressed as mean values ± SD of six replications. Means followed by the same letter in the same column do not differ significantly at $p < 0.05$ level by LSD analysis.

* $p < 0.05$; ** $p < 0.01$; ns, not significant.

Consequently, Sachiyutaka plants were more sensitive to the nitrogen enrichment during R1 to R5 rather than during R5 to R7, while, Tamahomare plants showed the dramatic response to the nitrogen enrichment during R5 to R7.

3. Influence of nitrogen enrichment on root dry weight

The root dry weight increased significantly with the increase in nitrogen concentration in Sachiyutaka, but not in Tamahomare in R1-R5 treatments (Fig. 3-10). However, in R5-R7 treatments, the root dry weight in 800 ppm nitrogen increased 1.5 times than in 100 ppm in Tamahomare, but only 1.2 times in Sachiyutaka.

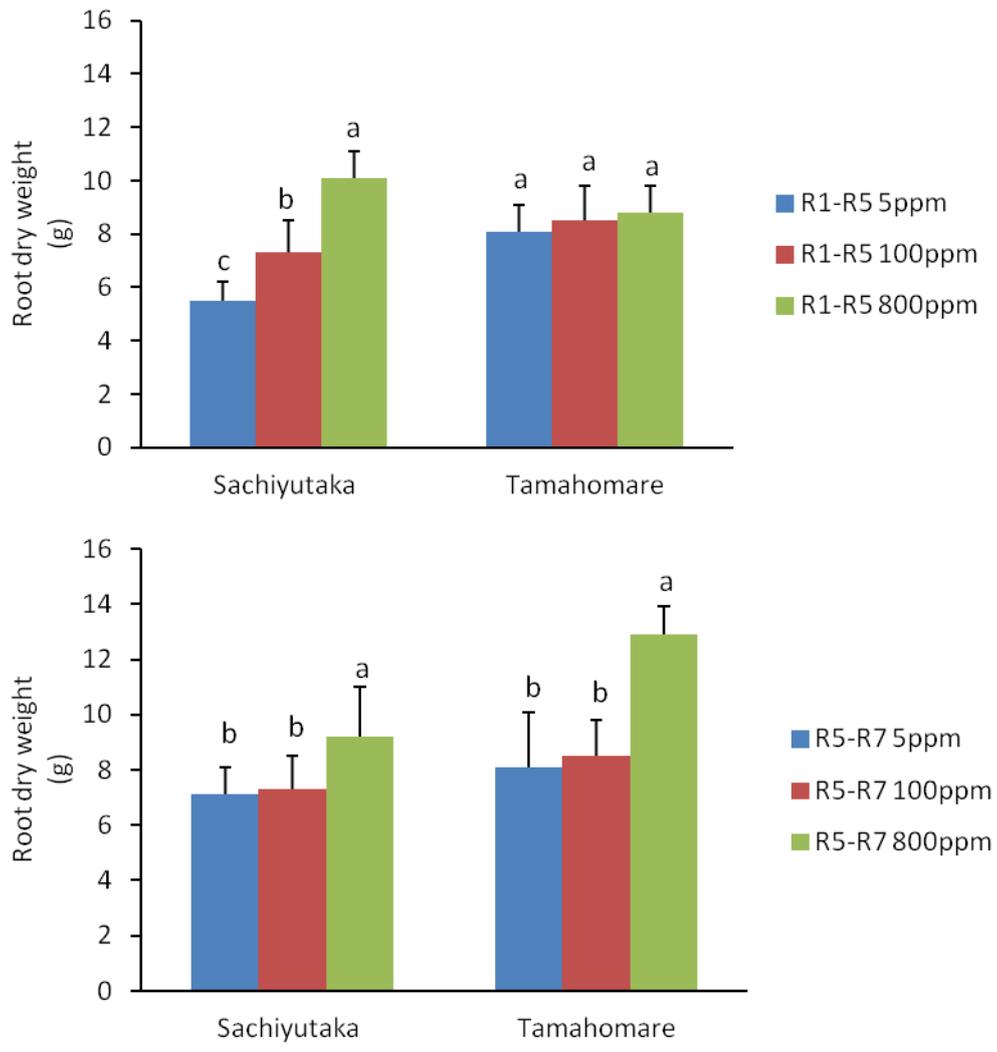


Fig. 3-10. Difference in root dry weight in both R1-R5 and R5-R7 treatments in 2012. Bars indicate standard deviations with six replications. In same cultivar, the different letters indicate significant difference according to LSD analysis at $p < 0.05$ level.

V. DISCUSSION

Although soybean plants need to accumulate much nitrogen in the seeds during seed filling, the practice of nitrogen topdressing in order to increase the seed yield is rarely done in the commercial soybean production. The reason might be due to that the nitrogen fertilizer application could stimulate the nitrogen uptake directly by root system but restrict the nitrogen fixation by nodules. The results showed the seed yield was very sensitive to the nitrogen enrichment during reproductive stage, even after the beginning of seed filling (R5 stage), indicating the possibility to raise the seed yield potential by nitrogen control.

It is known that the large amount of nitrogen redistribution from leaf to seed during seed filling stage triggers the leaf senescence (Sinclair and de Wit, 1976), shorten the seed filling period (Loberg et al., 1984; Hortensteiner and Feller, 2002), and limit the seed yield. The present results showed that the increase of nitrogen concentration during R1 to R5 actually relaxed the decline in the leaf SPAD value (Fig. 3-1), increased leaf nitrogen concentration at R5 (Fig. 3-5), and promoted the yield performance apparently in Sachiyutaka (Table 3-2). However, the exception is, in Tamahomare, the increased nitrogen concentration during R1 to R5 did not have effect on the delay of decline in leaf SPAD value (Fig. 3-2), nitrogen concentration (Fig. 3-6) and the improvement of seed yield (Table 3-2). Particularly, in high nitrogen concentration treatments (400 ppm and 800 ppm), the leaf SPAD value (Fig. 3-2) and nitrogen concentration (Fig.3-6) in

Tamahomare sharply decreased after R5 and reached to the lowest at R7. Although the nitrogen enrichment promoted the total and fertile pod number per plant in Tamahomare, the 100 seed weight even declined (Table 3-2), resulting in no change in the seed yield. Since the nitrogen accumulation in the leaves was little in Tamahomare compared with in Sachiyutaka (Zhao et al., 2014a), the present results implied that the rich nitrogen source before R5 enhanced the pod set rather than nitrogen accumulation in the leaves, but the short of nitrogen after R5 induced drastic leaf nitrogen redistribution and early senescence. In addition, although the treatment of the low nitrogen enrichment (5 ppm and 25 ppm) during R1 to R5 aggravated the declined trend in SPAD value (Fig. 3-1) and nitrogen concentration in leaf (Fig. 3-5), and reduced the seed yield finally in Sachiyutaka (Table 3-2), it did not show similar effects in Tamahomare (Fig. 3-2, 3-6).

On the other hand, in the treatment during R5 to R7, the low and high nitrogen concentration showed big effect on yield performance in Tamahomare. The lowest nitrogen concentration (5 ppm) reduced the yield apparently (11.5% in 2010 and 22.2% in 2012), while the high nitrogen concentration (400 ppm in 2010 and 800 ppm in 2012), increased the seed yield dramatically (21.4% in 2010 and 42.1% in 2012) compared with the control (constantly 100 ppm) in Tamahomare (Table 3-3). In the highest nitrogen concentration (800 ppm) treatment in Tamahomare during R5 to R7 in 2012, almost no contribution of redistributed nitrogen to the increase in seed yield was found, since the leaf nitrogen concentrations between R5 and R7 declined very little (Fig. 3-8). These results implied that more nitrogen

source in the soil and more active root system during seed filling could stimulate more increase in the seed yield, regardless of the amount of redistributed nitrogen from vegetative organs. In fact, Tamahomare showed heavier root dry weight in 800 ppm nitrogen treatment during R5 to R7 (Fig. 3-10).

When the soybean plants start the senescence generally after R5, the activity for nutrient assimilation is considered to be deteriorated. It could be the reason for big effect of nitrogen enrichment was found during R1 to R5, rather than during R5 to R7 in Sachiyutaka. On the other hand, some reports support the importance of nitrogen assimilation after R5. For example, Streeter (1978) reported that after R5, even short period of nitrogen stress can induce apparent decrease on the weight and nitrogen content in single seed. Jeppsen et al. (1978) reported that the increase of nitrogen supply after R5 can enhance the harvest nitrogen index at maturity, seed yield and seed nitrogen content. Wesley et al. (1998) claimed that nitrogen application at late-season (R3) can promote the yield of soybean significantly. In the present results of soybean cultivar Tamahomare, the big effect of nitrogen enrichment after R5 on seed yield is probably caused by its high ability of nitrogen absorption after R5.

In the commercial soybean production nowadays, nitrogen fertilizer is not applied much in basal dressing, because too much nitrogen in soil will restrain the growth of nodules and finally not satisfy the nitrogen requirement for seed production as well (Nakano et al. 1989). With the consideration of high nitrogen requirement in the seeds, the nitrogen

topdressing is often reported to improve the yield production but varied very much between the cultivars (Watanabe et al., 1983; Nakano et al., 1989; Nishioka and Okumura, 2008). However, the topdressing stage in the most cases was around flowering period (R1), which is before the seed filling. It would be the reason for less effect of nitrogen topdressing in some cultivars with less nitrogen storage functions, such as Tamahomare, therefore loss the benefit of topdressing.

The present results showed the nitrogen enrichment during reproductive stage could enhance seed yield performance dramatically. However, the most effective timing of nitrogen enrichment on seed yield during reproductive growth period is different between the cultivars due to the pattern of nitrogen assimilation. This result could be an explanation for why the effects of nitrogen topdressing were not constant as reported previously, and lead to be a better instruction for nitrogen topdressing on soybean yield.

Chapter 4

Varietal difference in the ability of nitrogen assimilation by root and nodule system during reproductive stage in soybean

I. SUMMARY

The soybean cultivar Sachiyutaka and Tamahomare were found to have different reactions to the nitrogen enrichment during seed filling period in Chapter 3, indicating the varietal difference in the ability of nitrogen assimilation by root and nodule system during seed filling period. To confirm this hypothesis, the two soybean cultivars were sown in the field of Saga University in 2011 and 2012. The seed yield, root dry weight, nodule number, and the nitrogen constitution in xylem sap including amino acid, nitrate, ureide and total nitrogen concentration at R1, R5 and R6 stage were measured. The results showed no apparent varietal differences in seed yield and total nitrogen concentration in the xylem sap, but the flowing speed of xylem sap was apparently faster in Tamahomare all the time which may associated with its heavier root dry weight. In addition, the nodule number and the proportion of ureide-nitrogen in xylem sap at R5 and R6 stages were apparently higher in Tamahomare compare with Sachiyutaka, indicating the higher activities of nodules in Tamahomare during late reproductive stage. On the other hand, Sachiyutaka showed higher proportion of amino-nitrogen in xylem sap from R1 to R6 stages indicating it supplied more redistributed nitrogen to seed growth. All of these results proved the varietal difference in nitrogen utilization patterns in soybean.

II. INTRODUCTION

In Chapter 3, the varietal difference in effect of nitrogen enrichment on seed yield between Sachiyutaka and Tamahomare indicated the ability of nitrogen absorption by root and nodule during seed filling period should be different. Therefore, it is necessary to investigate the difference in nitrogen assimilation by root and nodule system between Sachiyutaka and Tamahomare.

In soybean, generally the nitrogen assimilated by nodules is fixed as ammonia form firstly, after a series of chemical reactions, and then become to be ureide form. The ureides (allantoin and allantoate) are the final products of N_2 fixation in soybean (Kushizaki et al., 1964). McClure et al. (1980) suggested that the fraction of plant nitrogen derived from fixation could be estimated by analysis of the xylem sap. Van Berkum et al (1985) demonstrated that ureides content of either the whole soybean plant or young stem tissue was directly correlated to N_2 fixation under well-watered conditions. Therefore, the amount of ureides in xylem sap in soybean can be considered as an index for the activity of nodules.

On the other hand, the roots generally absorb the nitrate-nitrogen from soil directly (Latimore et al., 1977; David, 1982; Klotz and Horst, 1988). A part of the nitrate-nitrogen is moved through stem and petiole to leaf and reduced there. The other part of nitrate-nitrogen is reduced to ammonia in roots, and then via glutamine, glutamine acid, becomes to be asparagines, and moves to stem and leaf. Most of the nitrate-nitrogen is assimilated to

protein in leaf finally. After seed filling start, the protein in leaf will be resolved to amino acid, and then moved to seeds for protein synthesis. Therefore, the nitrate- and amino- nitrogen in xylem sap can be considered as indexes for roots activities and protein decomposition which associated with nitrogen redistribution after seed filling start.

The objective of this chapter was to compare the ability of nitrogen assimilation by root and nodule system between Sachiyutaka and Tamahomare by measuring the roots dry weight, nodules number, and the concentrations of ureide-, amino- and nitrate- nitrogen in xylem sap during reproductive stage.

III. MATERIALS AND METHODS

1. Plant cultivation

Two soybean cultivars, Sachiyutaka and Tamahomare, which showed different nitrogen utilization patterns in Chapter 3, were sown in 16 July in 2011 and 18 July in 2012 in a loam field of the Coastal Bioenvironment Center, Saga University (33° 27'N and 129° 58'E). Four seeds were sown in each hole, with holes arranged at 15 cm intervals and 70 cm row spacing. The split plot design was used for each cultivar consist of eight rows with 12 m long in each row. Plants were thinned to allow only one plant to grow per hole (9.5 plants m⁻²) at the stage when the first trifoliolate leaf fully extended. Chemical fertilizer was applied at a rate of 3: 10: 10 g m⁻² of N: P₂O₅: K₂O before sowing, and 100 g m⁻² of lime was also applied at the same time. The seeds were controlled by plowing or hands and the pesticides were spray when it was necessary. The growth stages were determined according to Fehr et al. (1971).

2. Sampling and measurements

In 2011 and 2012, when each cultivar nearly reached at reproductive stage R1 (beginning flowering), R5 (beginning seed filling) and R6 (full seed size), the field was irrigated well. On a sunshine morning of next days, five plants of each cultivar were chosen for xylem sap sampling. The plants were cut at 3 or 4 cm high from the ground, then the cut surface were covered immediately by the cotton packed in the plastic tube. After 30 to 60 minutes,

the cotton with the tube was take away, the weight of xylem sap soaked into the cotton was calculated by the difference in the cotton weight before and after set.

The xylem sap in the cotton of sampling tube was squeezed out by glass rod and moved to a saving tube by pipette, and then were used for determination of ureide (Un), nitrate (Nn) and amino nitrogen (An) by the Young-Conway, Cataldo and Moore-Stein method, respectively. The total nitrogen concentration (Tn) was the sum of Un, Nn and An.

At the harvest stage (R8), eight medium-size plants of each cultivar were taken for the determination of root dry weight, nodules number and seed yield.

IV. RESULTS

1, Xylem sap flow speed in different reproductive stages

Fig. 4-1 shows the speed of xylem sap flow from the fracture surface of stem. Both of Sachiyutaka and Tamahomare showed increased trends from R1 to R6. The flow speed of xylem sap in Sachiyutaka increased from 36.7 to 40.7 mg min⁻¹ in 2011 and 31.7 to 45.1 mg min⁻¹ in 2012, while in Tamahomare, the speed increased from 51.7 to 58.3 mg min⁻¹ in 2011 and 38.3 to 59.7 mg min⁻¹ in 2012. Compare with Sachiyutaka, Tamahomare showed higher flow speed of xylem sap all the time and the difference between two cultivars showed enlarged trend from R1 to R6 in both of 2011 and 2012.

2, Nitrogen content in xylem sap of soybean and its change along with reproductive stages

The total nitrogen concentration in xylem sap which defined as the sum of amino-, nitrate- and ureide- nitrogen concentration is showed in Fig. 4-2. Although total nitrogen concentration in both of Sachiyutaka and Tamahomare showed increased trend from R1 to R6, the varietal difference was not clear.

Fig. 4-3 shows the change of amino nitrogen concentration in xylem sap. Compare with R1 and R5 stage, amino nitrogen in both of Sachiyutaka and Tamahomare showed apparently increase at R6, and Sachiyutaka showed high concentration of amino- nitrogen than Tamahomare all the time.

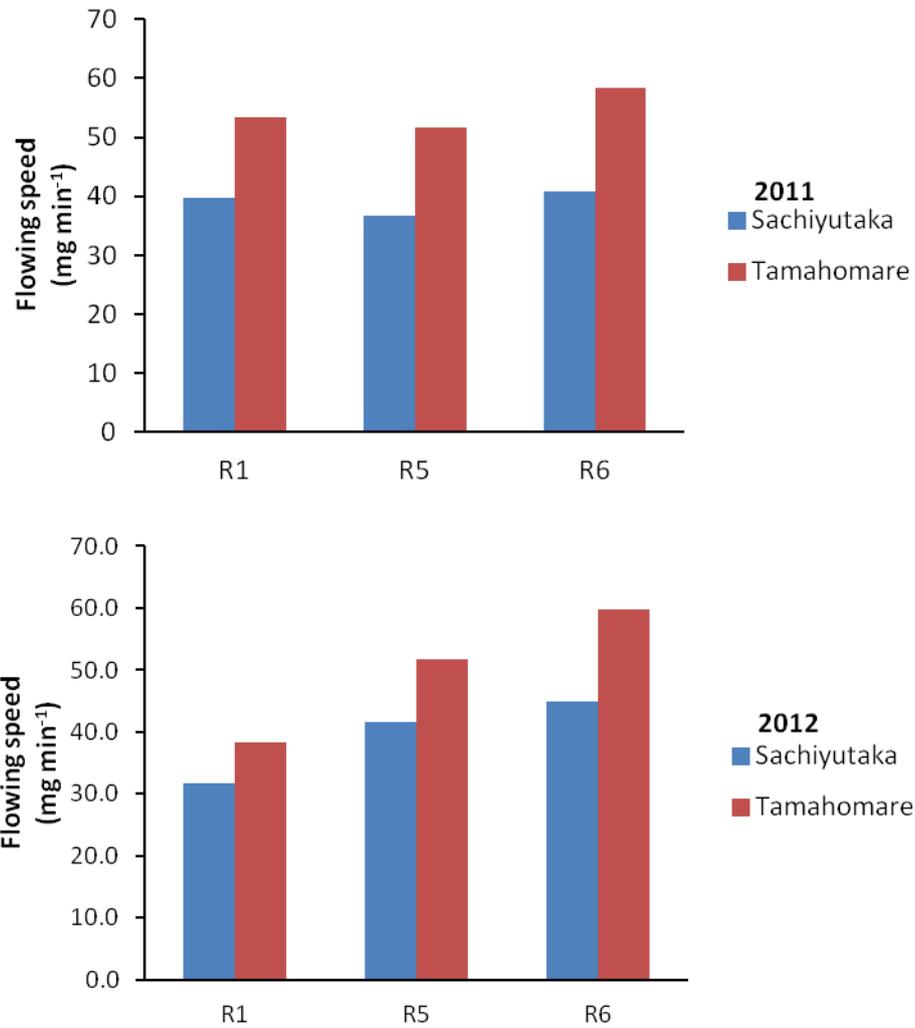


Fig. 4-1. Changes of flowing speed of xylem sap in Sachiyutaka and Tamahomare. R1, R5 and R6 indicate the reproductive stages of beginning flowering, beginning seed filling and full seed size, respectively.

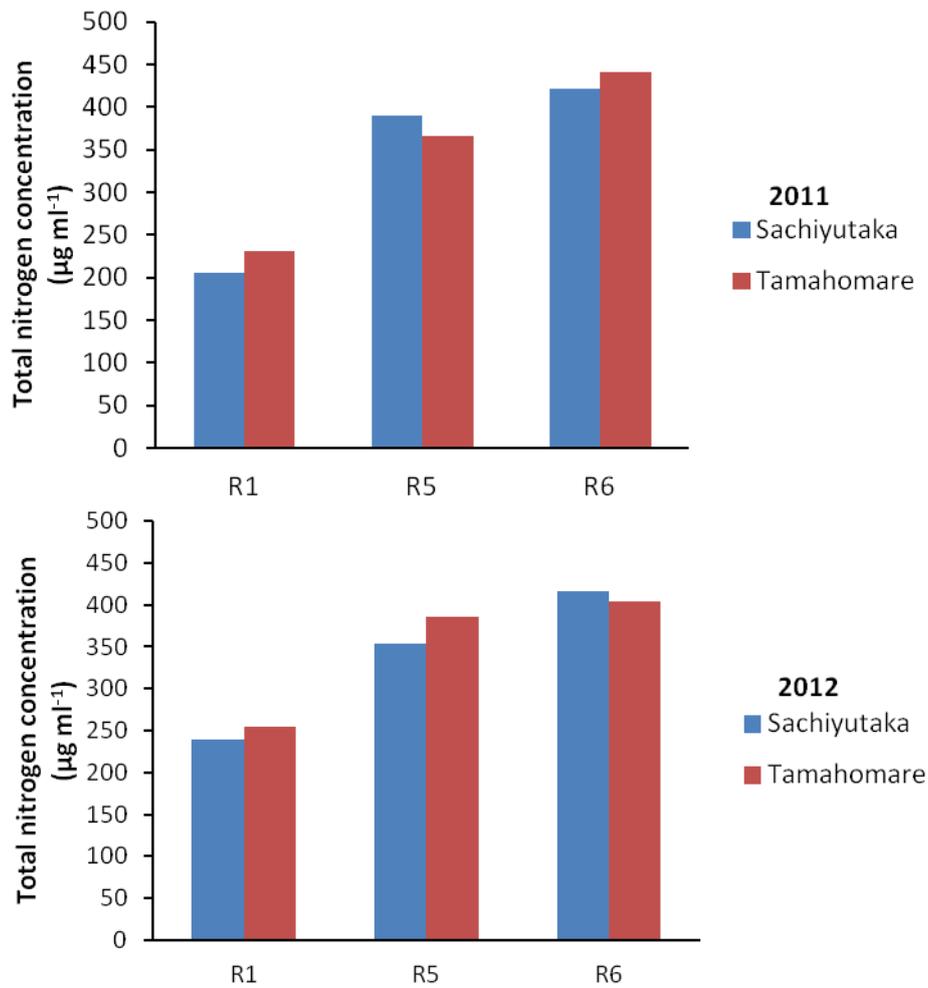


Fig. 4-2. Changes of total nitrogen concentration in xylem sap in Sachiyutaka and Tamahomare. R1, R5 and R6 indicate the reproductive stages of beginning flowering, beginning seed filling and full seed size, respectively.

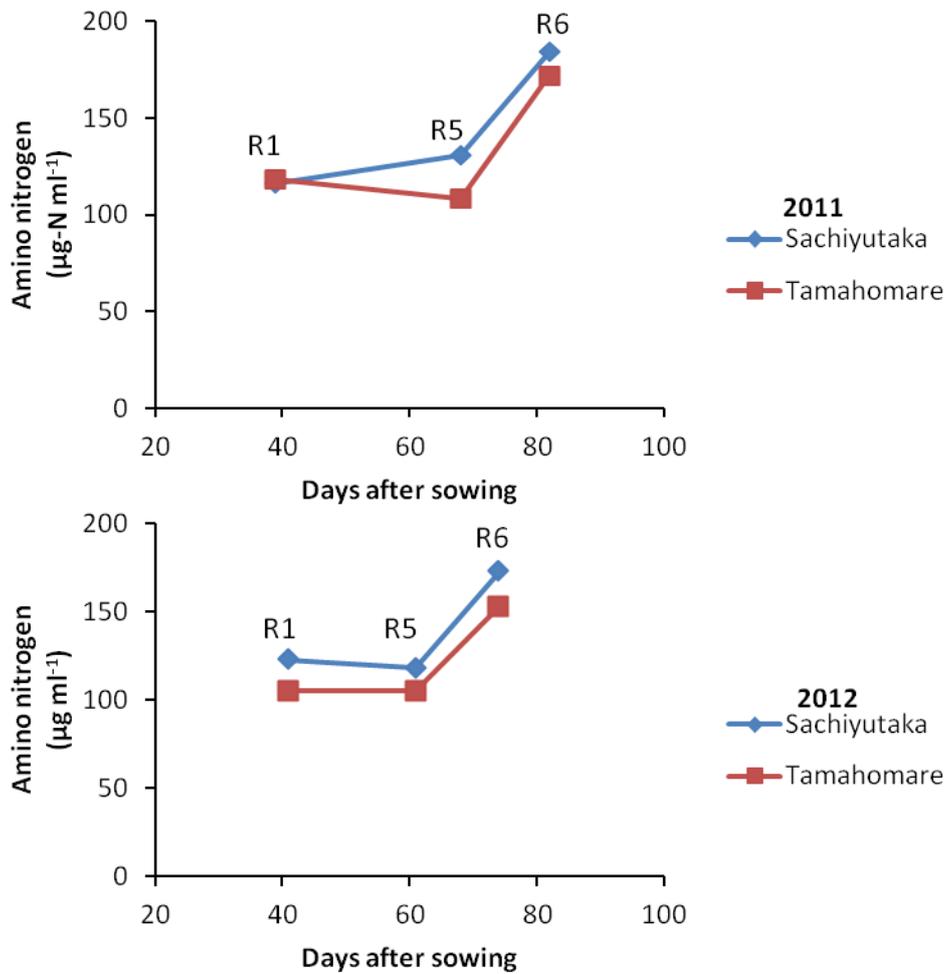


Fig. 4-3. Changes of amino nitrogen in xylem sap of Sachiyutaka and Tamahomare.

Fig. 4-4 shows the change of nitrate nitrogen concentration in xylem sap. Both of Sachiyutaka and Tamahomare showed big decrease in nitrate nitrogen from R1 to R6, but Sachiyutaka showed slower decrease than Tamahomare.

Fig. 4-5 shows the change of ureide nitrogen concentration in xylem sap. The ureide nitrogen concentration increased drastically from R1 to R5 in both of Sachiyutaka and Tamahomare, but the change was much smaller from R5 to R6, and Tamahomare showed more ureide nitrogen all the time.

3, Proportion of nitrogen constitute in xylem sap of soybean and its change along with reproductive stages

In the beginning of flowering (R1), the amino nitrogen concentration showed biggest proportion in both of Sachiyutaka and Tamahomare (Fig. 4-6), and the proportion in Sachiyutaka was 57% in 2011 and 48% in 2012, while in Tamahomare that was 51% in 2011 and 42% in 2012. Sachiyutaka showed bigger proportion of amino nitrogen than Tamahomare in both years.

However, in the beginning of seed filling (R5) stage, the biggest proportion became to ureide nitrogen in both of Sachiyutaka and Tamahomare (Fig. 4-7). In Sachiyutaka, the proportion of ureide nitrogen was 51% in 2011 and 49% in 2012, while in Tamahomare, that was changed to 67% in 2011 and 54% in 2012. Tamahomare showed apparently bigger proportion of ureide nitrogen than Sachiyutaka.

In the middle of seed filling (R6) stage, although the biggest proportion was still ureide nitrogen in both of Sachiyutaka and Tamahomare (Fig. 4-8),

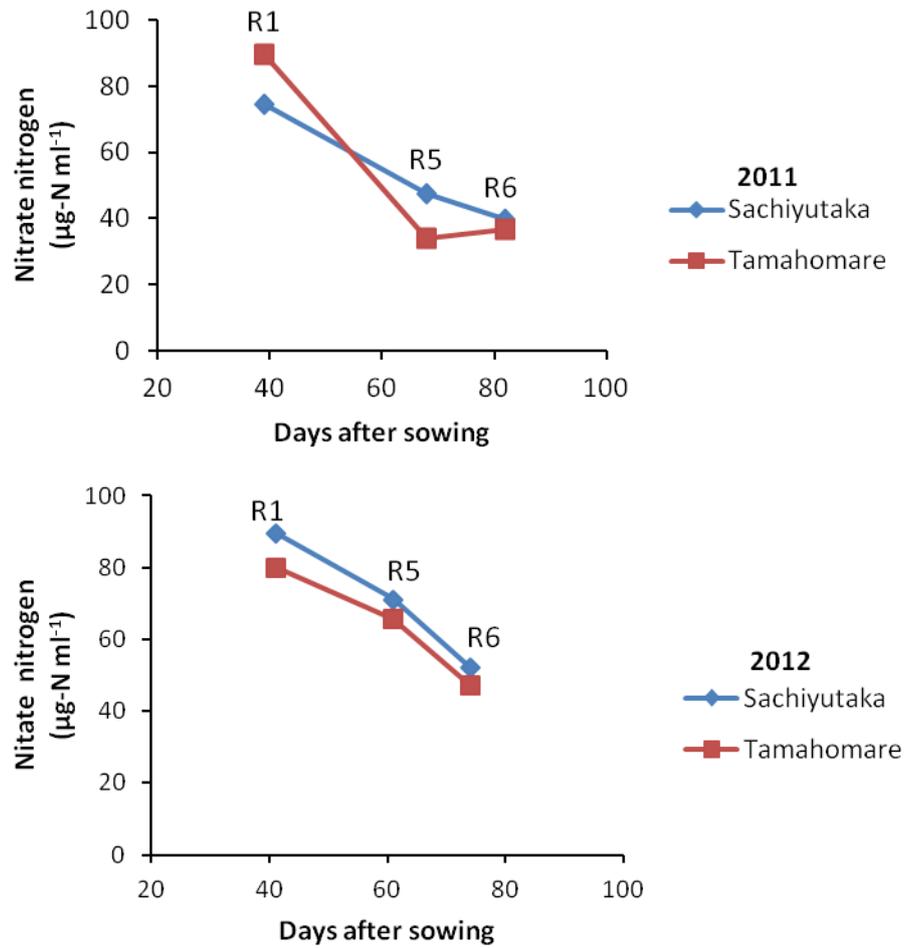


Fig. 4-4. Changes of nitric nitrogen in xylem sap of Sachiyutaka and Tamahomare.

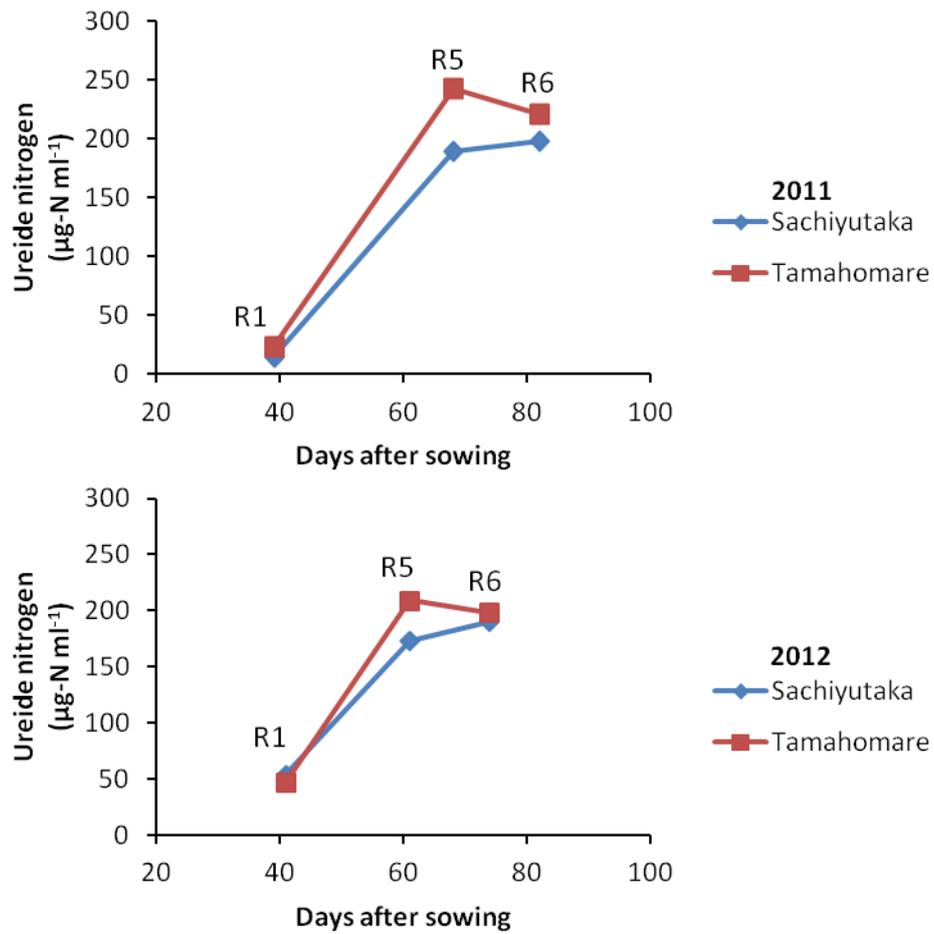


Fig. 4-5. Changes of ureide nitrogen in xylem sap of Sachiyutaka and Tamahomare.

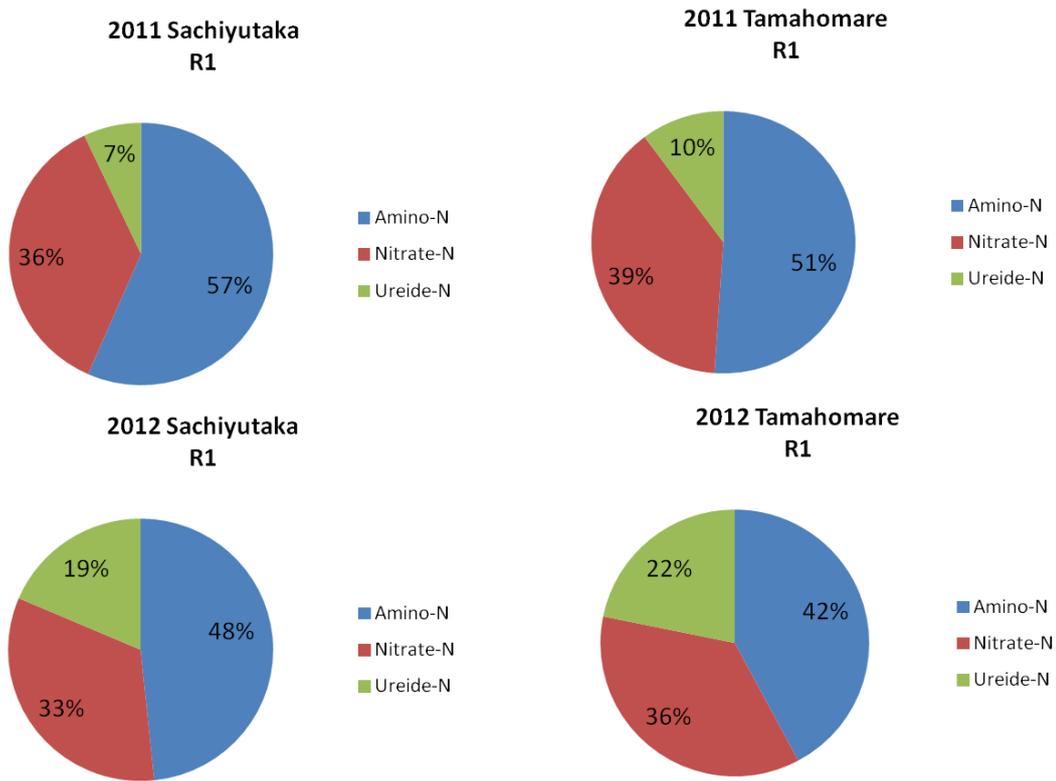


Fig. 4-6. Proportion of amino-, nitrate- and ureide- nitrogen in xylem sap of Sachiyutaka and Tamahomare at R1 stage.

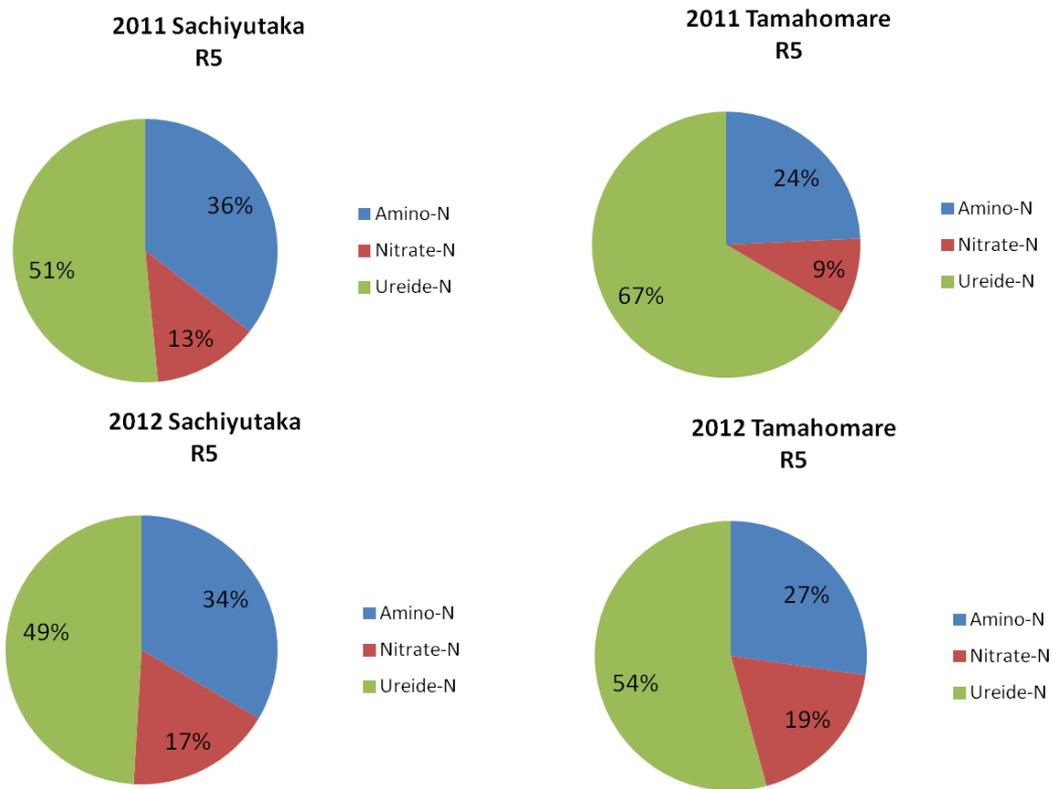


Fig. 4-7. Proportion of amino-, nitrate- and ureide- nitrogen in xylem sap of Sachiyutaka and Tamahomare at R5 stage.

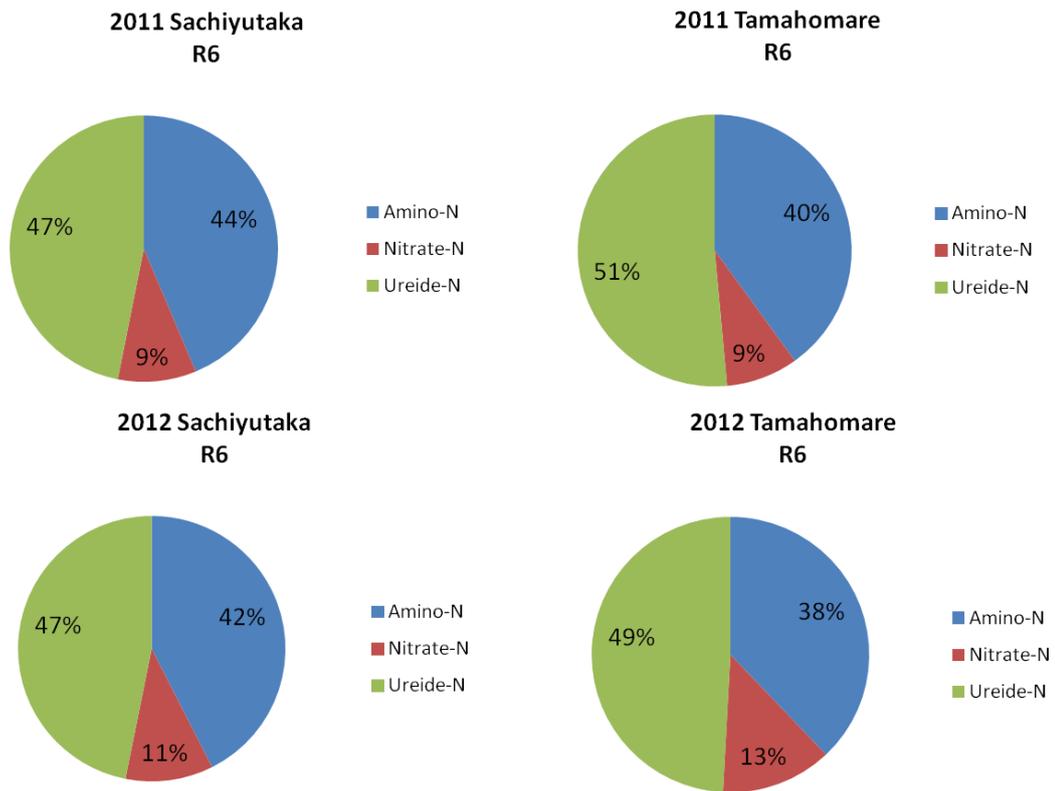


Fig. 4-8. Proportion of amino-, nitrate- and ureide- nitrogen in xylem sap of Sachiyutaka and Tamahomare at R6 stage.

the proportion of amino nitrogen increased dramatically again. In Sachiyutaka, the proportion of ureide and amino nitrogen was 47% and 44% in 2011, 47% and 42% in 2012, while in Tamahomare, the proportion of ureide and amino nitrogen was 51% and 40% in 2011, 49% and 38% in 2012. Compare with Sachiyutaka, Tamahomare showed bigger proportion of ureide nitrogen but smaller proportion of amino nitrogen from R1 to R6.

4, Varietal difference on root dry weight, nodules number, and seed yield

The root dry weight of Sachiyutaka and Tamahomare were 7.3 and 8.7 g plant⁻¹ in 2011 and 8.5 and 10.2 g plant⁻¹ in 2012 (Fig. 4-9), and the nodules number of Sachiyutaka and Tamahomare were 92 and 119 per plant in 2011 and 103 and 132 per plant in 2012 (Fig. 4-10), indicating both of root dry weight and nodules number in Tamahomare was more than Sachiyutaka. However, the seed yield of Tamahomare and Sachiyutaka (Fig. 4-11) were 293 and 332 g m⁻² in 2011 and 397 and 378 g m⁻² in 2012, which did not show apparently varietal difference.

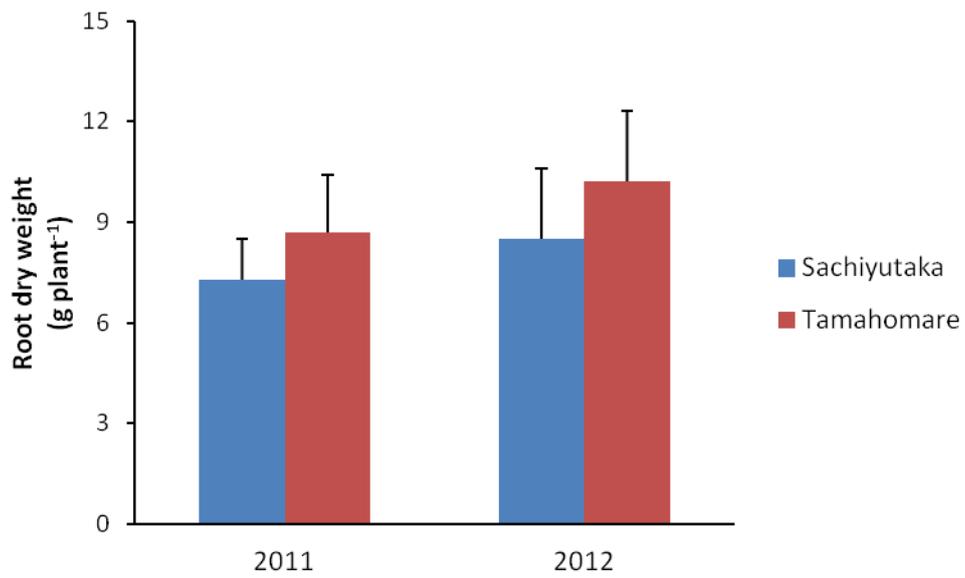


Fig. 4-9. Difference in root dry weight in Sachiyutaka and Tamahomare. Bars indicated standard deviations with eight replications.

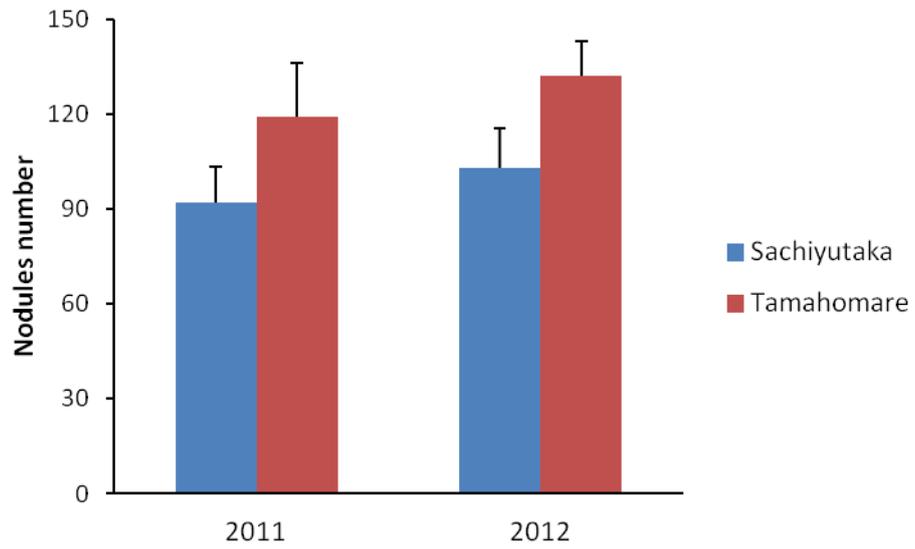


Fig. 4-10. Difference in nodule number in Sachiyutaka and Tamahomare. Bars indicated standard deviations with eight replications.

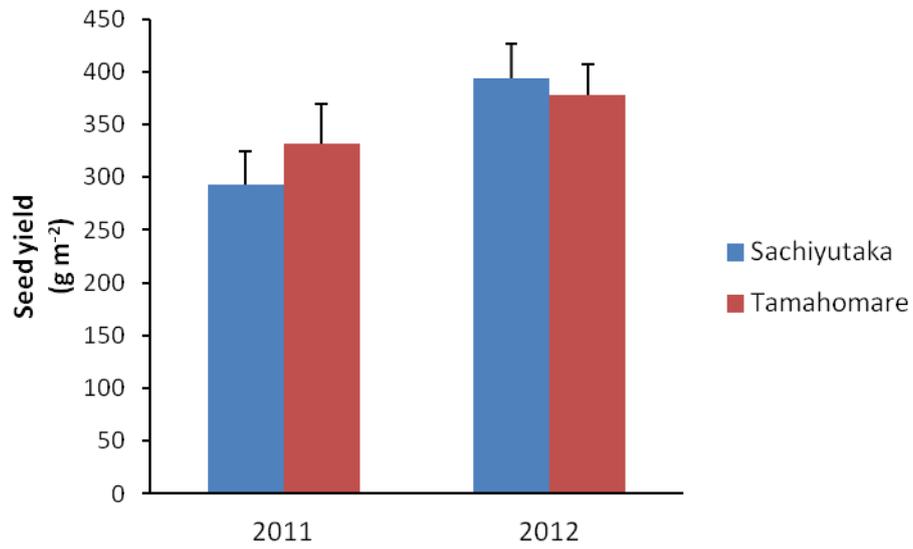


Fig. 4-11. Difference in seed yield in Sachiyutaka and Tamahomare. Bars indicated standard deviations with eight replications.

V. DISCUSSION

Many researchers mentioned the correlations between nitrogen forms and plant organs in soybean. For example, ureides (allantoin and allantoic acid) are reported as the predominant nitrogen transport compounds in N_2 fixing soybean (Streeter, 1979; Thomas and Schrader, 1981). Matsumoto et al. reported a correlation between the concentration of ureides in the soybean stem and nodules mass. McClure et al. (1980) found a correlation between the relative nitrogen input from N_2 fixation in greenhouse-grown soybeans and the relative ureide content of xylem. Therefore, ureides might serve as a useful indicator of N_2 fixation in the field. In this chapter, the amount of ureide nitrogen increased from R1 stage accompanied with the seed development in both cultivars (Fig. 4-5) and the ureide nitrogen also showed the biggest proportion (Fig. 4-7, 4-8) in R5 and R6 stage. However, Tamahomare showed bigger amount and proportion than Sachiyutaka all the time which may associate with its higher activities and number (Fig. 4-10) of nodules.

On the other hand, although nitrate-nitrogen is commonly absorbed by roots directly (Israel and Jackson, 1982), some-nitrate nitrogen taken up by soybeans is reduced in the root and transported as ureides in the xylem. Therefore, Herridge (1982) proposed a fraction of ureide nitrogen plus nitrate nitrogen to account for the contribution of nitrogen from root and nodules system. In this chapter, the amount and proportion of nitrate-nitrogen decreased from R1 to R6 all the time and did not show apparent

varietal difference (Fig. 4-4, Fig. 4-6 to 4-8) indicated either the lack of nitrogen source in soil or the activities of roots system.

It is known that the protein stored in leaves could be resolved to amino acid and moved to seeds after seed filling start for protein synthesis (David, 1982). Therefore, the increase of amino nitrogen in xylem sap can be considered as index for protein decomposition which associated with nitrogen redistribution after seed filling start. In this chapter, the amount of amino nitrogen showed apparently increase at R6 stage compare with R1 and R5 stage, and the amount and proportion of amino nitrogen was higher in Sachiyutaka all the time (Fig. 4-3, Fig. 4-6 to 4-7), indicated Sachiyutaka supplied more redistributed nitrogen to support the seed growth.

However, Sachiyutaka and Tamahomare did not show big difference in total nitrogen concentration in xylem sap (Fig. 4-2) and seed yield (Fig. 4-11), but the flow speed of xylem sap (Fig. 4-1) was faster in Tamahomare which may associated with its heavier root dry weight (Fig. 4-9). Therefore, compare with Sachiyutaka, Tamahomare had stronger roots system and more activated nodules during late reproductive stage to supply nitrogen for seed filling when the nitrogen source in the soil is rich.

Chapter 5

Enhancement of yield potential by nitrogen topdressing during reproductive stage in soybean

I. SUMMARY

The soybean cultivar Sachiyutaka and Tamahomare were found to have different nitrogen utilization patterns by nitrogen enrichment during seed filling period in Chapter 3, indicating the possibility of increasing seed yield by nitrogen control. To confirm this hypothesis at field condition, the two soybean cultivars were sown in the field of Saga University from 2011 to 2012 to investigate the effects of the timing of nitrogen topdressing on seed yield production. The nitrogen topdressing was applied at R1, R5 and R6 stage, respectively. The basal fertilizer was applied before sowing at the rate of 4 g m⁻² of nitrogen contained in the chemical fertilizer. When the plants in non-topdressing plot reached at R1, R5, and R6 stage, the urea was applied as topdressing by the rate of 6 g of nitrogen per m². Comparing with the no-topdressing treatment, the R1 topdressing treatment showed the biggest delayed effects on the decline of leaf SPAD value and nitrogen concentration after R5 stage, as well as the biggest enhancement on the seed yield and fertile pod number in Sachiyutaka, while in Tamahomare, the same effects were found in R5 topdressing treatment. These results indicated that the timing of nitrogen topdressing should be considered according to the pattern of nitrogen assimilation in the cultivar used, in order to get better economic benefits.

II. INTRODUCTION

In Chapter 3, the effects of nitrogen enrichment on seed yield were different between Sachiyutaka and Tamahomare due to the different patterns of nitrogen assimilation, in which the former was more dependent on nitrogen accumulated in vegetative organs, whereas the later was more dependent on its high ability of nitrogen absorption after the start of seed filling (R5). It indicated the possibility to raise the seed yield potential by nitrogen control according to the nitrogen assimilation pattern in different cultivars.

The nitrogen topdressing at flowering stage (R1) is often reported to improve the yield production. For example, Nakano et al. (1989) reported that nitrogen topdressing at flowering time could increase the nitrogen assimilation and seed yield in soybean. Wood et al. (1993) reported nitrogen topdressing was more efficient at the flowering stage than at the vegetative stages. Gan et al. (2003) compared the effect of nitrogen applications at various reproductive stages, and claimed that the best timing for nitrogen top-dressing during reproductive stage is at the flowering stage. On the other hand, very few evidences indicate the effect of nitrogen topdressing at the late reproductive stage with the consideration of big nitrogen requirement after the beginning of seed growth. For example, Jeppsen et al. (1978) reported that the increase of nitrogen supply after R5 can enhance the harvest nitrogen index at maturity, seed yield and seed nitrogen content. Wesley et al. (1998) claimed that nitrogen application at late-season (R3) can

promote the yield of soybean significantly. The above-mentioned reports indicated that the unsteady effects of nitrogen topdressing might be due to the neglect on the consideration of genotypic property for nitrogen assimilation.

To find an effective way to increase yield performance by nitrogen control, the effects of nitrogen topdressing timing on seed yield between two soybean cultivars Sachiyutaka and Tamahomare were investigated.

III. MATERIALS AND METHODS

1. Plant cultivation and treatments of nitrogen topdressing

Two soybean cultivars, Sachiyutaka and Tamahomare, both of them are widely cultivated in western Japan, were sown on 8 July in 2011 and 18 July in 2012 at the field of the Coastal Bioenvironment Center, Saga University (33°27'N and 129°58'E). According to the results in Chapter 3, the patterns of nitrogen assimilation were different between Tamahomare and Sachiyutaka in which the former was more dependent of its high ability of nitrogen absorption after R5, whereas the later was more dependent of nitrogen accumulated in vegetative organs.

Four seeds were sown in the holes arranged at 15-cm intervals and 70 cm rows spacing. Plants were thinned to allow two plants to grow in each hole (14 plants m⁻²) at the stage when the first trifoliolate leaf fully extended.

The split plot design was used between 4 treatments and 3 replications. Chemical fertilizer was applied at a rate of 4:15:20 g m⁻² of N: P₂O₅: K₂O before sowing, and 100 g m⁻² of lime was also applied at the same time. When the plants in no-topdressing treatment reached at flowering stage (R1), the beginning and the middle of seed filling (R5 and R6) stages, six g m⁻² of nitrogen by urea form (46% nitrogen contained) was applied to the field. The weeds were controlled by plowing or hands and the pesticides were sprayed when it was necessary. The growth stages were determined according to Fehr et al. (1971).

2. Sampling and measurements

The leaf SPAD value was measured by a chlorophyll meter SPAD-502 (Konica Minolta, Inc., Osaka, Japan) 1 or 2 times per week on the second or third full-expanded leaf from the top during R1 to R7.

One leaflet on the third or fourth fully expanded leaf from top was taken at R1, R5, R6 and R7 stage, respectively. After drying at 80°C for 48 hours, the leaves were ground into powder for the determination of total nitrogen content by Kjeldahl method.

At the harvest maturity stage (R8), eight to ten plants were taken from three replicated plots of each treatment for the determination of the seed yield and yield components.

3. Statistical analysis

Single-factor ANOVA was used in Fig. 5-1 to Fig. 5-4 to evaluate significant difference on leaf SPAD value and nitrogen concentration among treatments at R1, R5, R6 and R7 stage in each cultivar. Furthermore, Tukey-Kramer test was used in Table 5-2 for the significance analysis of parameters relating to vegetative and reproductive growth among different treatments in each cultivar.

IV. RESULTS

1. Influence of nitrogen topdressing on leaf SPAD value and nitrogen concentration

The response of leaf SPAD value to the timing of nitrogen topdressing was very different between Sachiyutaka and Tamahomare in both of 2011 and 2012. In R1-topdressing treatment, comparing with the control (no-topdressing), the decline of leaf SPAD value was delayed after R5 stage, but in R5 and R6 topdressing treatment, the SPAD value was not affected in Sachiyutaka (Fig. 5-1). While in Tamahomare, the decline in leaf SPAD value was delayed significantly by R5 and R6 topdressing treatments, but did not by R1-topdressing treatment compared with the control (Fig. 5-2).

The changes in leaf nitrogen concentration showed similar trends with the leaf SPAD value, in which only R1 topdressing treatment relaxed the nitrogen decline in Sachiyutaka (Fig. 5-3), whereas R5-topdressing treatment did same in Tamahomare (Fig. 5-4) after R5 stage. The other nitrogen topdressing treatments did not affect the leaf nitrogen concentration.

In addition, the timing of nitrogen topdressing also showed different effects on the seed filling period (the days from R5 to R7) between the cultivars (Table 5-1). Comparing with non-topdressing treatment, the period of seed filling was lengthened mostly in R1-topdressing treatment in Sachiyutaka (8 days in 2011 and 9 days in 2012), while in Tamahomare, the R5-topdressing treatment prolonged the seed filling period mostly (10 days in 2011 and 9 days in 2012).

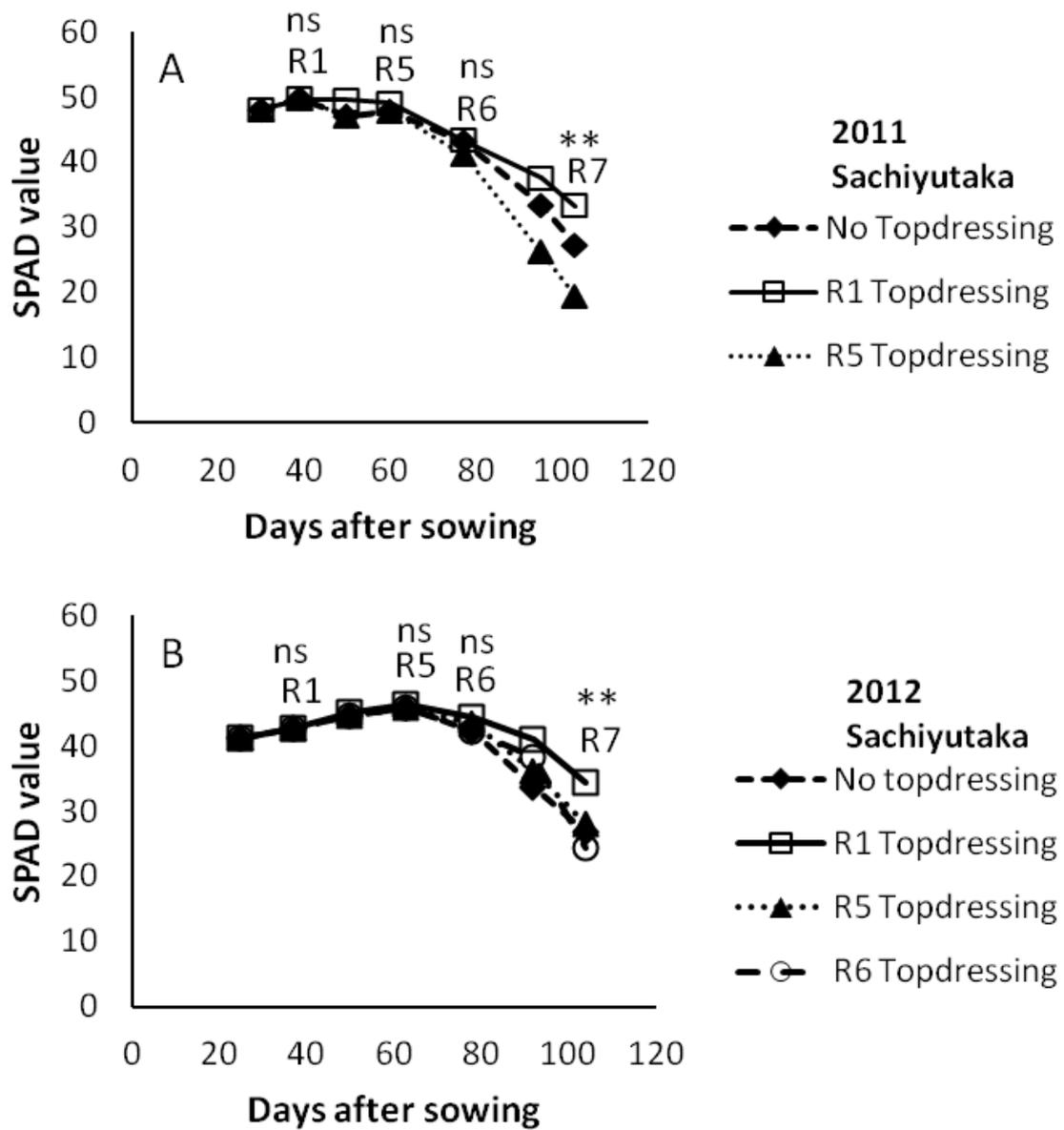


Fig.5-1. Changes of leaf SPAD value in Sachiutaka in 2011 and 2012. R1, R5, R6 and R7 indicate the reproductive stages in control plants (no-topdressing), respectively. ** $p < 0.01$; ns, not significant.

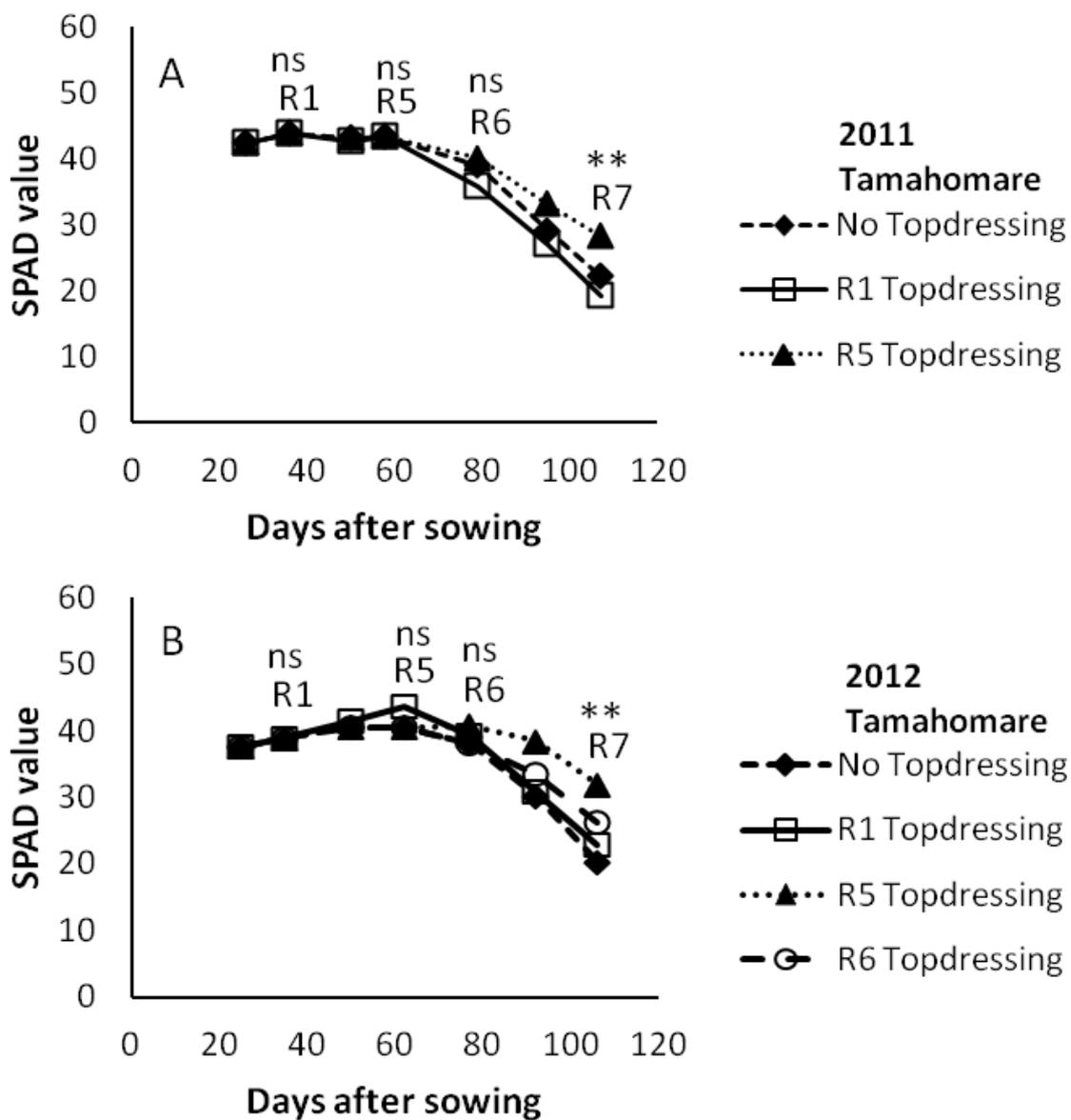


Fig.5-2. Changes of leaf SPAD value in Tamahomare in 2011 and 2012. R1, R5, R6 and R7 indicate the reproductive stages in control plants (no-topdressing), respectively. ** $p < 0.01$; ns, not significant.

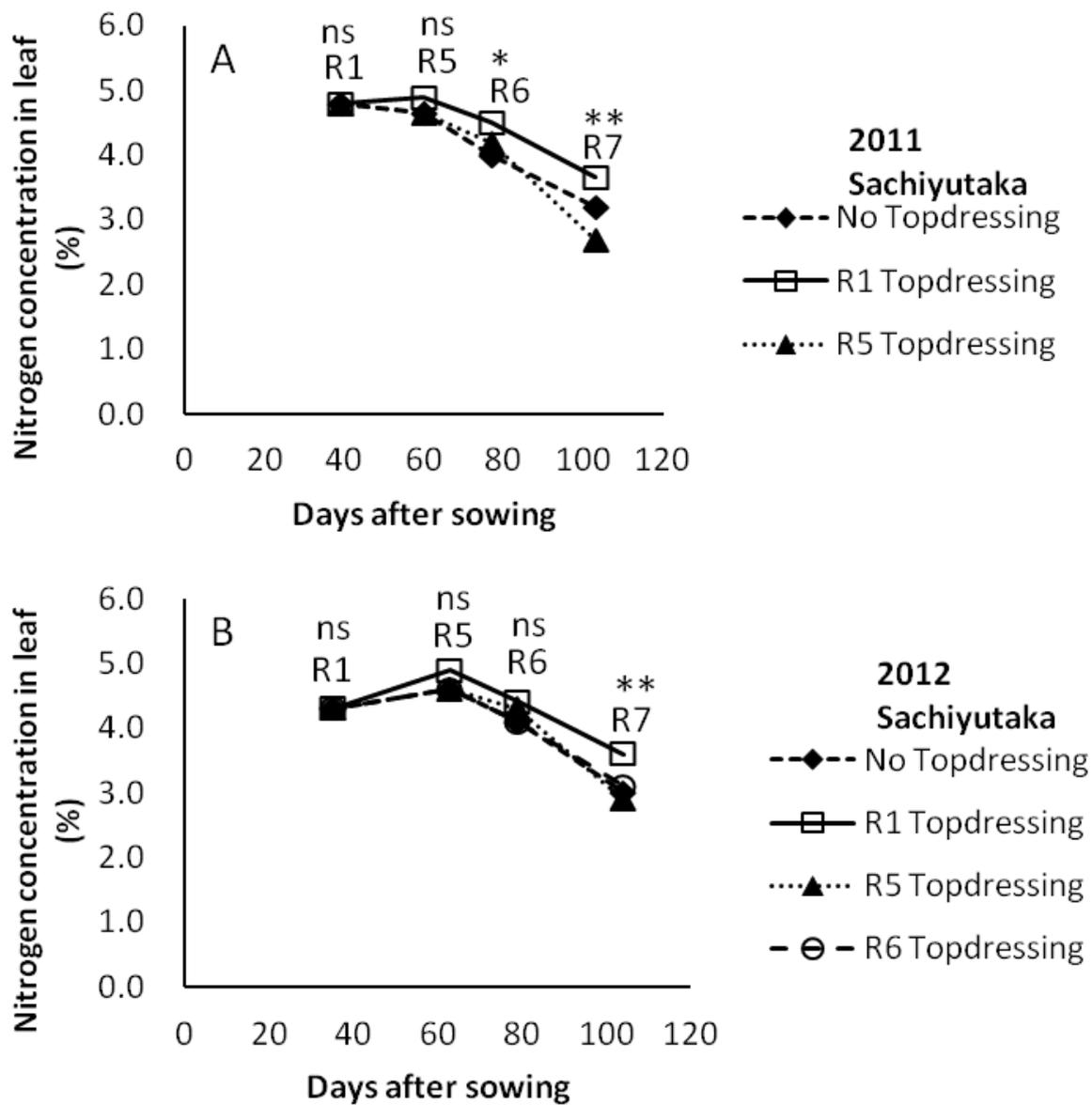


Fig.5-3. Changes of leaf nitrogen concentration in Sachiyutaka in 2011 and 2012. R1, R5, R6 and R7 indicate the reproductive stages in control plants (no-topdressing), respectively. * $p < 0.05$; ** $p < 0.01$; ns, not significant.

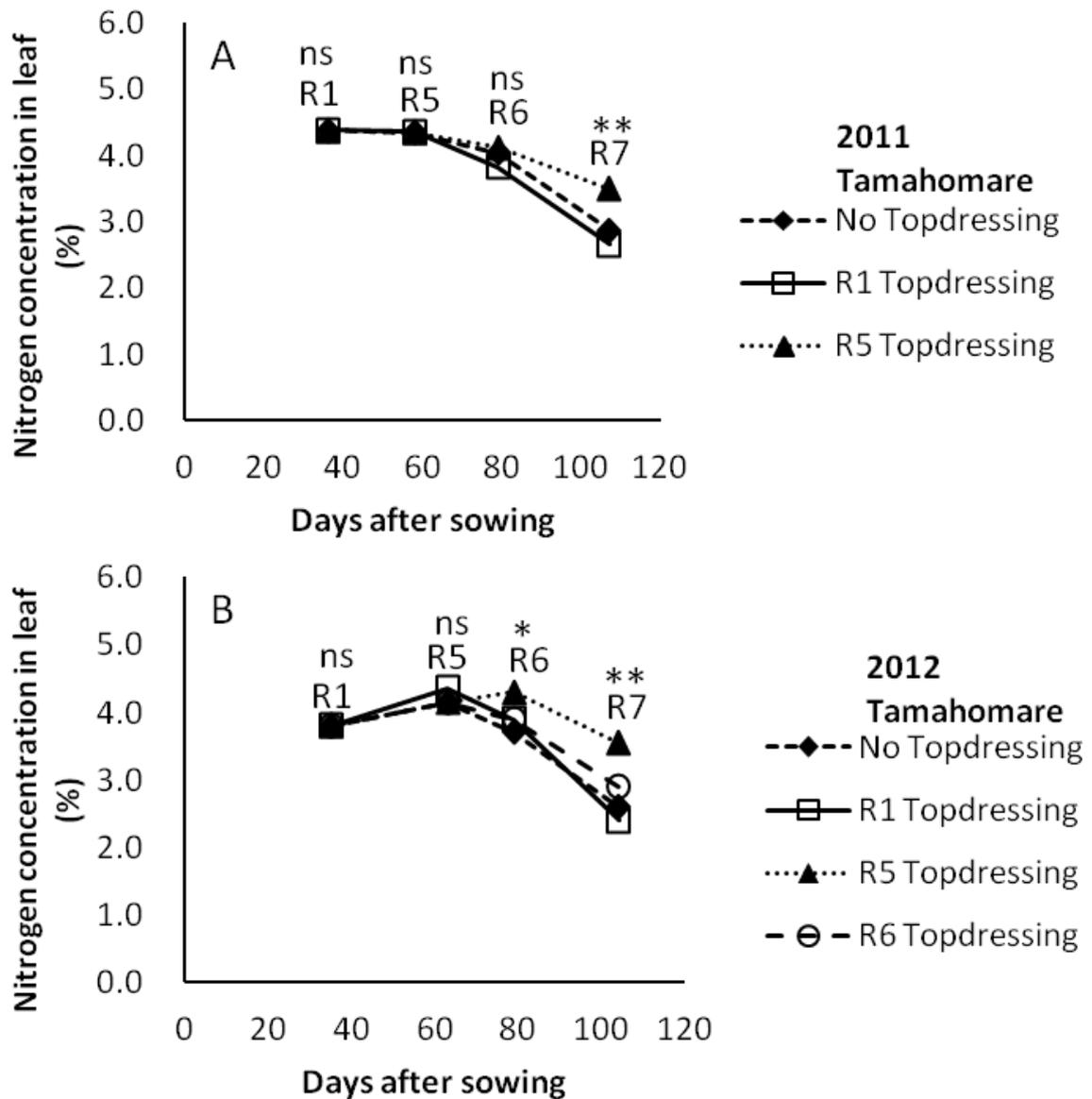


Fig.5-4. Changes of leaf nitrogen concentration in Tamahomare in 2011 and 2012. R1, R5, R6 and R7 indicate the reproductive stages in control plants (no-topdressing), respectively. * $p < 0.05$; ** $p < 0.01$; ns, not significant.

Table 5-1. The periods of each reproductive stage in Sachiyutaka and Tamahomare.

	Sowing-R1 (days)	Sowing-R5 (days)	Sowing-R6 (days)	Sowing-R7 (days)	R5-R7 (days)
Sachiyutaka, 2011					
No-topdressing	39	60	77	103	43
R1-topdressing	39	63	82	114	51
R5-topdressing	39	60	79	107	47
Tamahomare, 2011					
No-topdressing	36	58	79	107	49
R1-topdressing	36	60	79	107	47
R5-topdressing	36	58	82	117	59
Sachiyutaka, 2012					
No-topdressing	37	63	78	104	41
R1-topdressing	37	65	81	115	50
R5-topdressing	37	63	81	106	43
R6-topdressing	37	63	78	106	43
Tamahomare, 2012					
No-topdressing	35	62	77	106	44
R1-topdressing	35	63	77	106	43
R5-topdressing	35	62	81	115	53
R6-topdressing	35	62	77	110	48

2. Influence of nitrogen topdressing treatments on seed yield and yield components

The nitrogen topdressing showed big effects on seed yield performance in different timing between Sachiyutaka and Tamahomare (Fig. 5-5). In 2011, the seed yield of Sachiyutaka and Tamahomare in the no-topdressing treatment was 295.6 g m⁻² and 305.3 g m⁻², and increased 17.1% and 3.1% by R1-topdressing treatment, while 4.6% and 21.8% by R5-topdressing treatment. In 2012, the seed yield of Sachiyutaka and Tamahomare in the no-topdressing treatment was 282.8 g m⁻² and 291.7 g m⁻², and increased 15.2% and 5.1% by R1-topdressing treatment, while 5.7% and 17.3% by R5-topdressing treatment, whereas only 3.3% and 11.1% by R6-topdressing treatment.

The changes of seed size (100 seed weight) showed similar trend with seed yield (Fig.5-6). In 2011, the 100 seed weight in Sachiyutaka and Tamahomare was 31.4g and 23.6g in no-topdressing, and increased 11.8% and 6.4% by R1-topdressing treatment, as well as 3.5% and 20.8% by R5-topdressing treatment. In 2012, the 100 seed weight in Sachiyutaka and Tamahomare was 30.4g and 25.1g in non-topdressing, while it increased 13.2% and 3.2% by R1-topdressing treatment, 6.3% and 11.9% by R5-topdressing treatment, but only 2.3% and 3.9% by R6-topdressing treatment.

The effects of the timing of nitrogen topdressing to the total pod number were showed in Fig.4-7. Both of Sachiyutaka and Tamahomare showed the biggest increase in total pod number in R1-topdressing treatment, in which

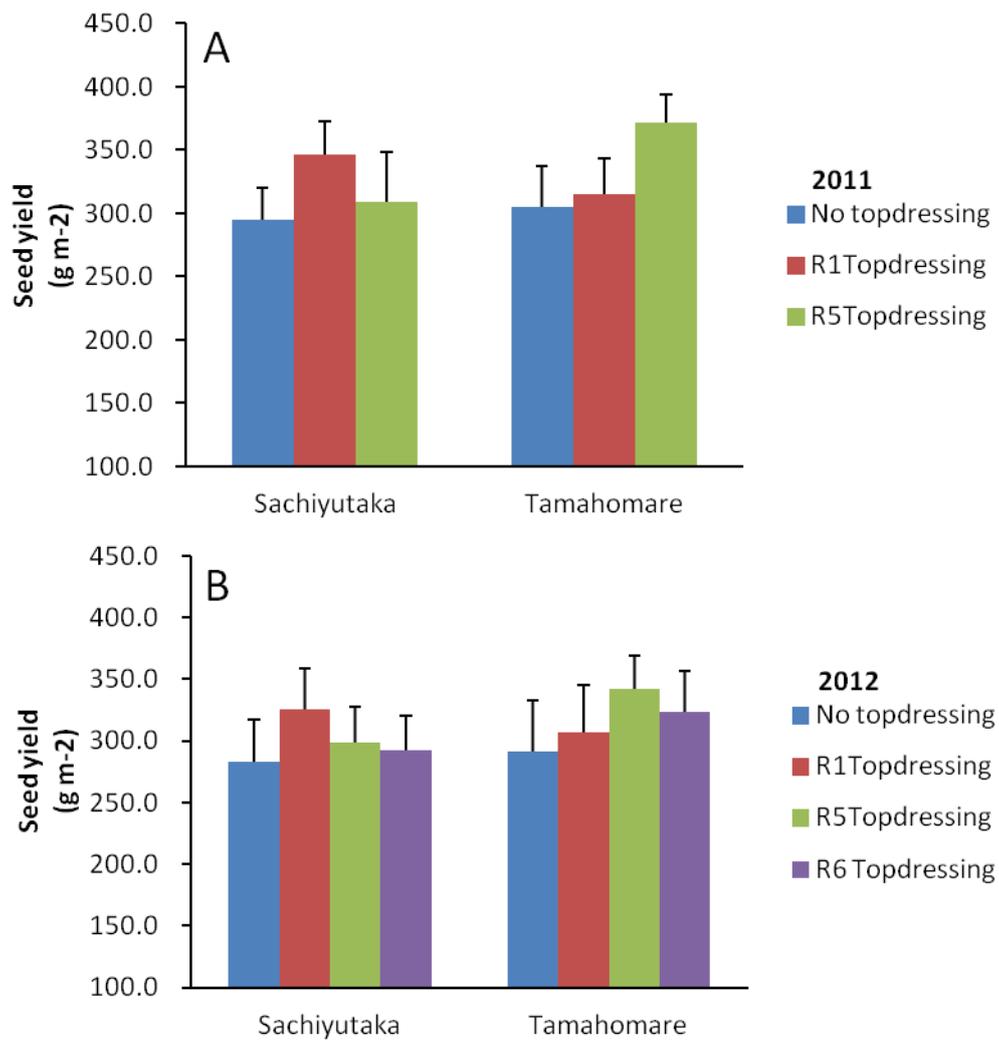


Fig.5-5. Seed yield in Sachiyutaka and Tamahomare among treatments in 2011 and 2012. Bars indicate standard deviations among replications.

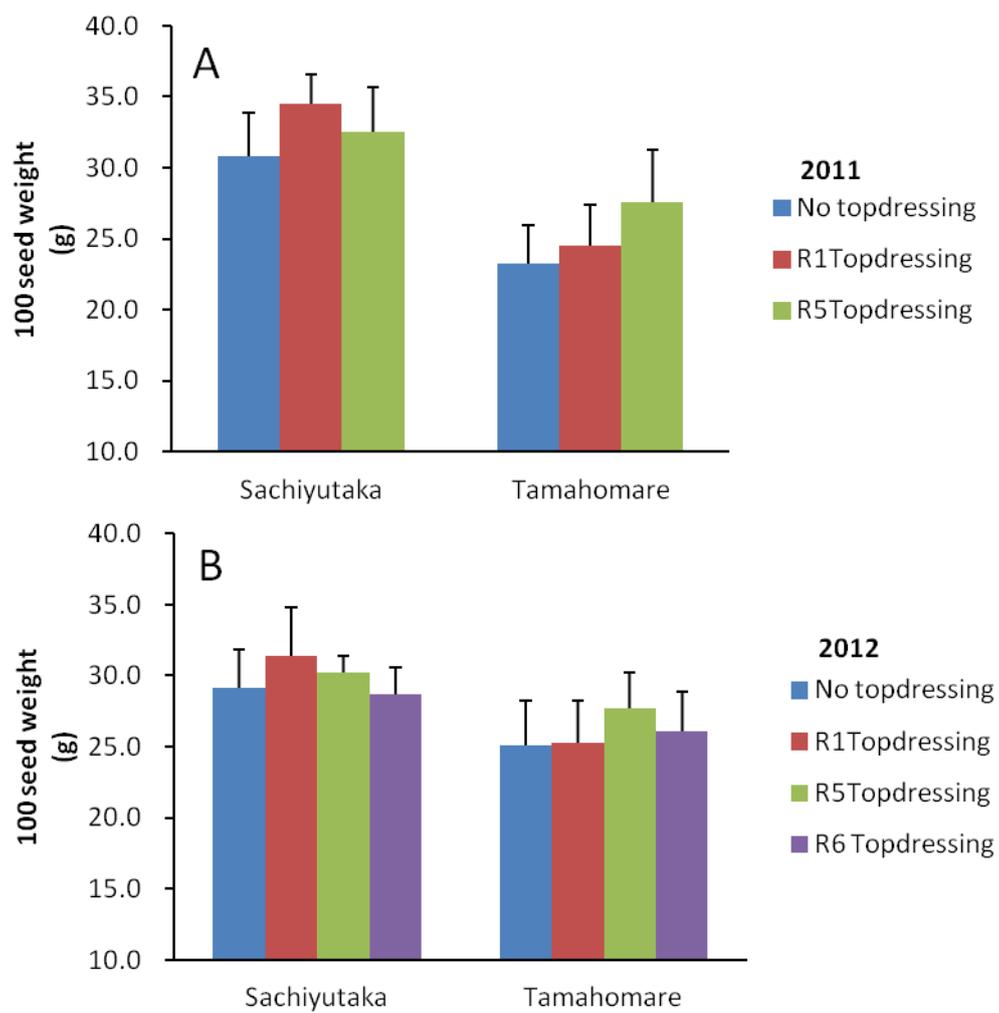


Fig. 5-6. The 100 seed weight in Sachiyutaka and Tamahomare among treatments in 2011 and 2012. Bars indicate standard deviations among replications.

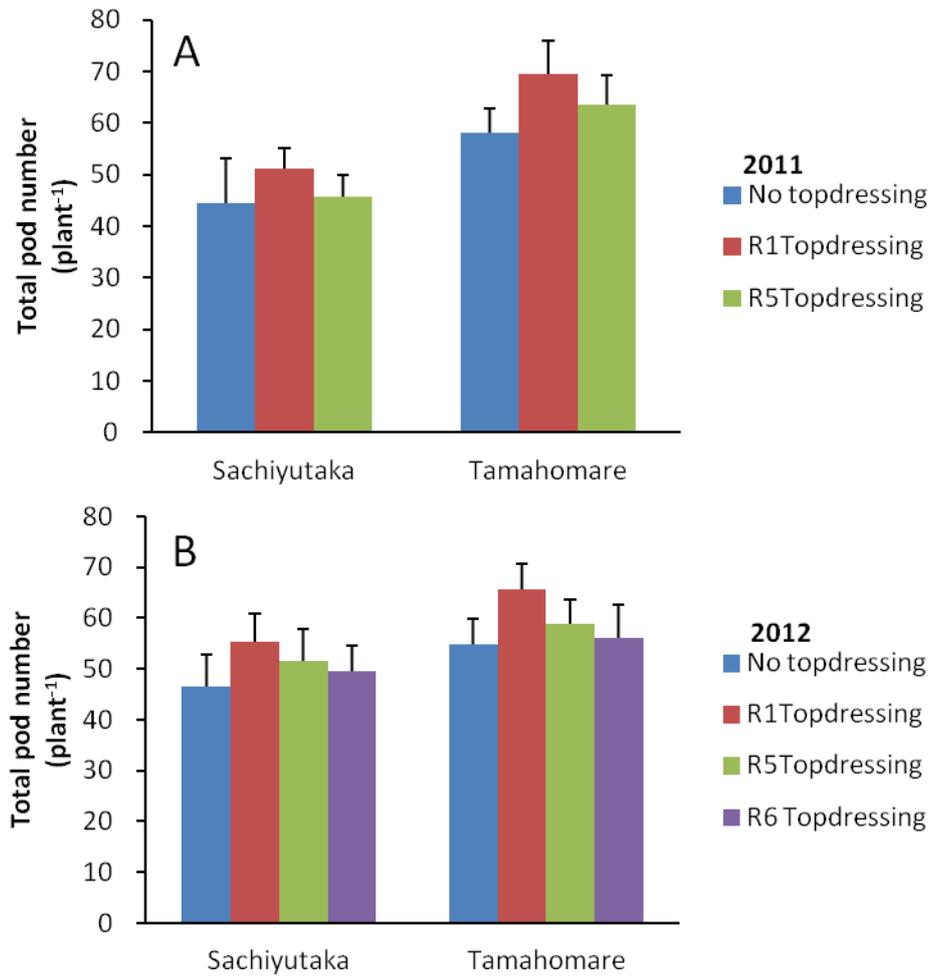


Fig.5-7. Total pod number in Sachiyutaka and Tamahomare among treatments in 2011 and 2012. Bars indicate standard deviations among replications.

it increased 14.8% and 19.6% in 2011, as well as 18.9% and 19.5% in 2012 compare with non-topdressing (45 and 58 plant⁻¹ in 2011 and 47 and 55 plant⁻¹ in 2012). However, the fertile pod number showed very different trends between two cultivars (Fig. 5-8), in which the biggest increase was found in R1-topdressing treatment in Sachiyutaka (13.2% in 2011 and 16.4% in 2012), while in R5-topdressing (12.9% in 2011 and 15.4% in 2012) in Tamahomare, compared with no-topdressing (40 and 53 in 2011 and 41 and 49 plant⁻¹ in 2012).

3. Influence of nitrogen topdressing treatments on vegetative growth components

The influence of nitrogen topdressing in different timing on the components of vegetative growth in 2011 and 2012 was showed in Table 5-2. The stem height, node number in main stem, branch number, total node number and stem weight were increased slightly only in R1-topdressing in both of Sachiyutaka and Tamahomare.

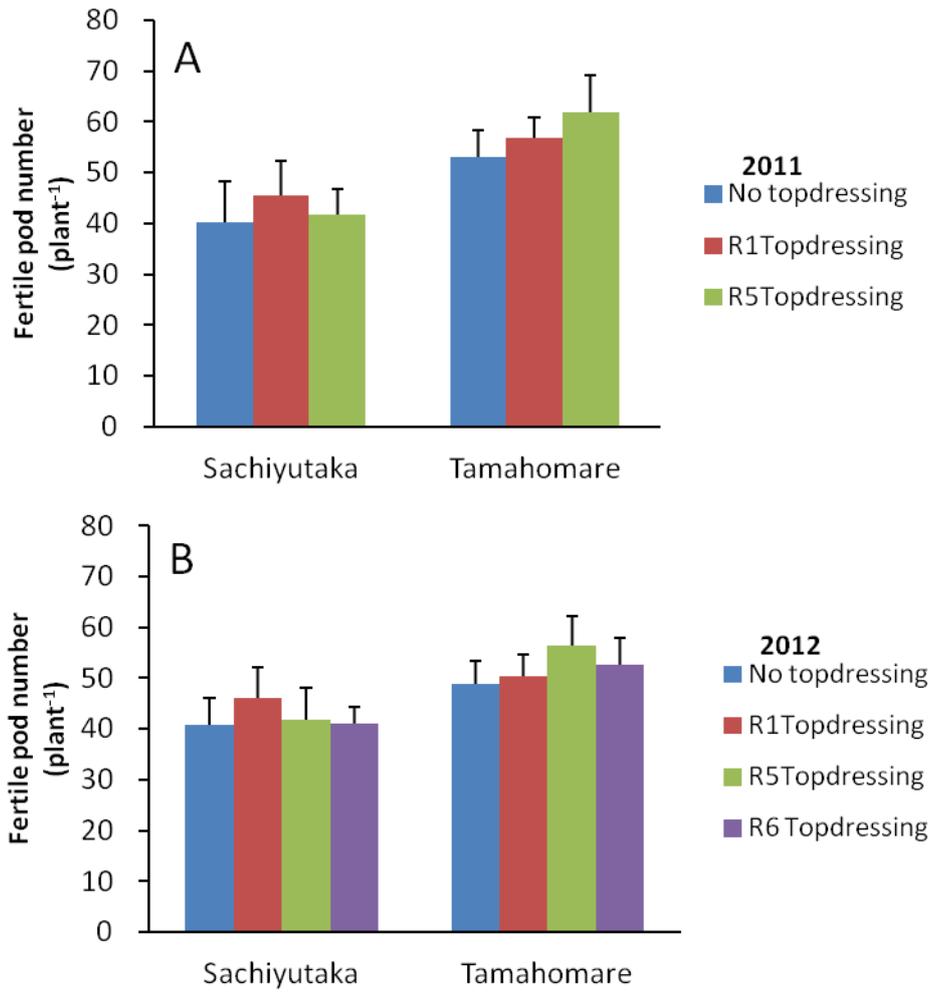


Fig.5-8 Fertile pod number in Sachiyutaka and Tamahomare among treatments in 2011 and 2012. Bars indicate standard deviations among replications.

Table 5-2. Components of vegetative growth in Sachiyutaka and Tamahomare.

	Stem height (cm)	Nodes No. in main stem	Braches No.	Total nodes No.	Stem weight (g)
2011					
Sachiyutaka					
No topdressing	39.1b	13.3b	7.1b	46.4b	9.7b
R1 topdressing	43.7a	14.1a	9.3a	51.8a	11.3a
R5 topdressing	37.8b	14.2a	7.2b	47.3b	9.8b
Tamahomare					
No topdressing	43.3b	14.2b	7.3a	47.7b	8.8b
R1 topdressing	53.1a	15.2a	7.2a	55.6a	11.1a
R5 topdressing	44.2b	14.3b	7.3a	49.2b	8.7b
2012					
Sachiyutaka					
No topdressing	36.6b	13.4b	7.4a	44.3b	9.1b
R1 topdressing	40.3a	14.3a	7.1a	50.2a	9.9a
R5 topdressing	37.3b	13.3b	7.3a	45.3b	8.8ab
R6 topdressing	36.5b	13.4b	7.3a	45.8b	9.2ab
Tamahomare					
No topdressing	40.1b	14.1b	6.2b	45.2b	8.2b
R1 topdressing	46.2a	15.1a	7.2a	50.7a	10.3a
R5 topdressing	41.2b	14.2b	7.4a	43.1b	9.1b
R6 topdressing	41.1b	14.4b	7.1a	47.3ab	8.8b

Data are expressed as mean values of eight replications. Means followed by the same letter in the same column do not differ significantly at $p < 0.05$ level by Tukey-Kramer test.

V. DISCUSSION

The large nitrogen redistribution from vegetative organs to seeds is considered to induce early leaf senescence (Sanetra et al., 1998; Donnison et al., 2006), decline the photosynthetic activity (Buttery and Buzzell, 1988; Sinclair and Horie, 1989) and shorten the duration of seed filling period (Sinclair and de Wit, 1976). In this research, the R1 nitrogen topdressing relaxed the decline in SPAD value (Fig.5-1) and nitrogen concentration (Fig. 5-3) after R5, lengthened the seed filling period (Table 5-1), therefore brought the biggest increase in seed yield (Fig. 5-5) in Sachiyutaka consequently. However, in another cultivar Tamahomare, the R1 topdressing did not show apparent effects on nitrogen redistribution and seed yield enhancement.

On the other hand, because the nitrogen requirement increased dramatically after the starts of seed filling in soybean, the effect of yield enhancement by nitrogen topdressing during late reproductive stage was also expected. Some reports mentioned the possibility of yield enhancement by nitrogen topdressing during late maturity stage (Hamdi, 1976; Wesley et al., 1998). In this research, comparing with the control (no-topdressing), the seed yield was enhanced in both two cultivars by R5 nitrogen topdressing treatment (Fig.5-5), especially in the cultivar Tamahomare, in which it showed the biggest increase of yield. However, the decline of SPAD value (Fig.5-2) and nitrogen concentration (Fig.5-4) in Tamahomare was apparently delayed after R5, indicating the nitrogen redistribution and leaf senescence were relaxed. These results confirmed the same conclusion as in

pot experiment in Chapter 3 again at field condition.

Furthermore, the R6 nitrogen topdressing even relaxed the decline of leaf SPAD (Fig.5-2) and nitrogen concentration (Fig.5-4), and enhanced seed yield (Fig.5-5) slightly in Tamahomare, but almost no effect in Sachiyutaka. These results showed R6 topdressing is too late for nitrogen assimilation by the plants.

In conclusion, the best timing of nitrogen topdressing was R1 for Sachiyutaka, but R5 for Tamahomare to get the best yield performance. It is supposed that R1 topdressing stimulated the seed yield in Sachiyutaka through the nitrogen accumulation in the vegetative organs and its redistribution after the start of seed filling, whereas R5 topdressing stimulated the seed yield in Tamahomare through its strong nitrogen absorption directly by the root system during seed filling. These results indicated that the timing of nitrogen topdressing should be considered according to the pattern of nitrogen assimilation in the cultivar used, in order to get better economic benefits.

Chapter 6

General discussion

Soybean is a crop with high protein and oil content in its seeds. Therefore, the nutrient acquirement of soybean, especially for nitrogen, is also much higher than cereal crops. The deficiency of nitrogen will reduce the seed yield apparently in soybean. Because the roots have the symbiosis system with nodule bacteria, soybean plants could also acquire nitrogen assimilated by nodules from air. Generally, soybean plants store nitrogen in the vegetative organs before seed development. However, after the start of seed filling, the nitrogen from roots and nodules cannot satisfy the big and rapid nitrogen requirement of seed (Harper, 1999; Unkovich et al. 2000; Salon et al. 2001). For the compensation of this nitrogen shortage, some parts of nitrogen in the vegetative organs are redistributed to the seeds. Therefore, the nitrogen supply by both redistribution and absorption should be very important for satisfying the requirement of nitrogen for seed growth.

In legume crops, the nitrogen redistributed from vegetative organs commonly occupy a big part in total nitrogen content in seeds. For example, in *Lens culinaris* the ratio of redistributed nitrogen in seeds was 43% to 90% (Kurdali et al., 1997), while in *vicia faba* the ratio was 80% (Dekhuijzen and Verkerke, 1984). The present research showed the nitrogen redistributed from vegetative organs provided around 48% of the total nitrogen in the seeds in soybean cultivar Fukuyutaka (Fig. 2-2). The significantly positive correlation between the seed yield and amount of redistributed nitrogen from

leaves to seeds was also found (Fig.2-5-C). These results indicated that the importance of redistributed nitrogen in soybean was similar with other legume crops reported previously.

Because the absorption of nitrogen from blooming to pod setting occupies was the most in the whole life of soybean, the nitrogen topdressing during blooming stage (R1) is often expected to increase the yield performance. For example, Wood et al. (1993) reported that the nitrogen topdressing during flowering stage could increase the seed yield effectively. Brevedan and Leggett (1978) reported that nitrogen topdressing during R1 stage could restrain pod dropping and increase seed yield. In this research, the soybean cultivar Sachiyutaka showed apparently bigger yield enhancement in nitrogen enrichment from R1 to R5 (Table 3-2) and the proportion of amino-nitrogen in xylem sap from R1 to R6 (Fig. 4-6 to 4-8) which indicated the seed growth was supported more by redistributed nitrogen in Sachiyutaka. While, the other high yielding soybean cultivar Tamahomare did not show apparent yield enhancement by the same treatment, because of its low nitrogen storage in leaf and high dependence on nitrogen absorption after the start of seed filling. The same results were also found at field condition, that is, the R1 nitrogen topdressing showed the biggest enhancement in Sachiyutaka in both of seed yield and seed size at last (Fig.5-4 and 5-5). However, in the cultivar Tamahomare, no apparent effect was found in seed yield and seed size.

On the other hand, the high seed yield was found not only supported by large amount of redistributed nitrogen but also more nitrogen uptake

(include nitrogen fixation) during the seed-filling period (R5-R7) in some soybean cultivars. For example, in the soybean cultivar Tamahomare, the much lower nitrogen redistribution was associated with a relatively higher seed yield compared with another high yielding cultivar Sachiyutaka (Table 2-1), which belongs to the same maturity group with Tamahomare. In nitrogen enrichment from R5 to R7 of this research, Tamahomare showed apparent yield enhancement (Table 3-3), which the trend of increase was much bigger than another cultivar Sachiyutaka. The same results were also confirmed at field condition (Fig. 5-4). Afza et al. (1987) indicated that because the roots still had relative high activity in the late period of reproductive stage (R5 to R7) in some soybean cultivars, the utilization efficiency of nitrogen fertilizer on yield production was very high. In this research, although the total nitrogen concentration in xylem sap showed no apparent varietal difference between Sachiyutaka and Tamahomare (Fig. 4-2), the flow speed of xylem sap (Fig. 4-1) as well as the proportion of ureide-nitrogen in xylem sap from R5 to R6 were much bigger in Tamahomare (Fig. 4-7 to 4-8), indicating the cultivar Tamahomare had more active nodules and stronger root system. Therefore, the nitrogen topdressing during late reproductive stage (after the start of seed filling) may be more effective to promote the seed production in some soybean cultivars with high ability of nitrogen absorption during late reproductive stage. In this research, the big root system (Fig. 3-10) and higher photosynthetic rate (Fig. 3-9) even during seed filling in Tamahomare might contribute the high seed yield.

All of the present results suggested the nitrogen assimilation and

utilization should be a main limiting factor for high seed yielding. The strategies of nitrogen acquisition for soybean plants are considered either accumulate much nitrogen in vegetative organs then redistribute to the seeds, such as the function found in the cultivar Sachiutaka, or create a strong root system (include nodulation) to facilitate the nitrogen assimilation during seed filling, such as in the cultivar Tamahomare. It could also be possible to install both functions in some cultivars which have high yielding potential. Therefore, according to the characteristics of nitrogen utilization in different soybean cultivars, the seed yield could be significantly increased by adjusting the timing of nitrogen application.

The seed yield in soybean is apparently lower than other main crops such as rice, wheat and maize in the world (Kokubun, 2001). Sinclair and de Wit (1976) suggested that the exchange rate from photosynthate to the seed content ingredient was around 80% in rice which mainly accumulates starch in seeds, while was only 50% in soybean which mainly accumulates fat and protein in seeds, therefore, it should be impossible to get higher seed yield in soybean than in rice. However, there were also some super high seed yield records on soybean. For example, Isoda et al. (2010) reported over 8000 kg ha⁻¹ yield from the soybean cultivar Toyokomachi grown in Xinjiang, China. The present study could encourage a new technology of high yielding soybean cultivation system, based on the better understanding on the mechanism of nitrogen assimilation and utilization.

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Summary

Soybean plants need to assimilate much nitrogen because of its high protein content in the seeds. The rapid nitrogen acquisition for the seed production during seed filling period often induces large amount of nitrogen redistribution from leaf to the seed, accelerates leaf senescence and shortens seed filling period, therefore limits the seed yield. In order to verify the mechanism of nitrogen assimilation and utilization, and find effective way to increase soybean seed yield by nitrogen control, in this research, the investigations of 1) evaluation of nitrogen redistribution from vegetative organs to the seeds, 2) varietal differences in nitrogen redistribution and its contribution to the seed yield, 3) varietal differences in the ability of nitrogen assimilation by root and nodule system during reproductive stage and 4) effects of nitrogen enrichment at various growth stage on leaf nitrogen accumulation and seed yield were conducted.

About 48% nitrogen in the matured seeds in field grow soybean was redistributed from vegetative organs, and more than 60% of redistributed nitrogen was from leaves, indicating the leaves are main nitrogen storing organs. The percentage of redistributed nitrogen in the matured seeds varied from 13.8 to 37.9%, and the seed yield was correlated positively with the amount of redistributed nitrogen from leaves between 10 cultivars over 4 years. However, in some high yielding cultivar such as Tamahomare, the high seed yield was not associated with a large redistributed nitrogen, implying the direct nitrogen uptake during seed filling could be more

important factor for high seed yielding depend on the cultivars.

In order to make clear the relations between redistributed nitrogen from leaves and absorbed nitrogen directly from the soil during seed filling, enriched nitrogen concentration in the soil before seed filling enhanced the nitrogen content in the leaves and seed yield in the cultivar Sachiyutaka which redistributed much nitrogen from leaves to the seeds whereas showed no effects on either nitrogen accumulation in the leaves or seed yield in cultivar Tamahomare which redistributed less nitrogen from leaves to the seeds. However, enriched nitrogen concentration in the soil after the start of seed filling increased seed yield dramatically in the cultivar Tamahomare, showing the absorbed nitrogen during seed filling was more effective on the seed yield rather than the redistributed nitrogen. These results indicated the varietal difference in the ability of nitrogen assimilation by root and nodule system during seed filling period.

To confirm this hypothesis, the seed yield, root dry weight, nodule number, and the nitrogen constitution in xylem sap were measured in Sachiyutaka and Tamahomare from 2011 to 2012. The results showed no apparent varietal differences in seed yield and total nitrogen concentration in the xylem sap, but the flowing speed of xylem sap was apparently faster in Tamahomare all the time which may be associated with its heavier root dry weight. In addition, the nodule number and the proportion of ureide-nitrogen in xylem sap at R5 and R6 stages were apparently higher in Tamahomare compare with Sachiyutaka, indicating the higher activities of nodules in Tamahomare during late reproductive stage. On the other hand,

Sachiyutaka showed higher proportion of amino-nitrogen in xylem sap from R1 to R6 stages, indicating more redistributed nitrogen was supplied to seed growth.

Therefore, the timing of nitrogen requirement by the soybean plants is different between the cultivars depending on the property of nitrogen assimilation. This probably is the reason of inconstant effect of nitrogen top dressing at flowering time in soybean. This function was also observed by nitrogen topdressing at field condition in both years of 2011 and 2012.

In conclusion, the differences in nitrogen assimilation and its effect on the seed yield between cultivars were revealed. The results indicate the possibility to raise the seed yield potential by control the nitrogen availability depending on the property of cultivar, and could guide the soybean producer for the nitrogen top dressing during growth season.

摘 要

(Summary in Japanese)

ダイズの子実は高タンパク質含量であるため、子実登熟するには大量の窒素を同化する必要がある。通常ダイズの子実肥大期間中における窒素の供給源としては土壌からの直接吸収分（固定窒素を含む）のみでなく、それまでに栄養器官に蓄積された窒素も転流され子実に再分配される。しかし、栄養器官からの窒素の流出は葉の老化および光合成機能の早期低下を誘発し、子実収量を制限する負の効果もある。したがって、ダイズの子実収量レベルを上げるためには如何に効率的に窒素を同化利用かことが極めて重要である。本論文では、ダイズの子実収量における窒素吸収及び再分配の役割を明確にし、窒素制御によるダイズ多収栽培の技術向上に貢献する目的で、①ダイズ栄養器官の貯蓄窒素の子実への再分配の評価、②貯蓄窒素が子実収量への影響の品種間比較、③根系及び根粒の窒素同化能力の品種間比較、④窒素供給制御による収量ポテンシャル向上の可能性について検討した。得られた結果は以下に要約される。

圃場栽培ダイズの成熟した子実に含まれる窒素のうち約 48%が葉、茎、葉柄などの諸栄養器官から転流されており、残りは子実肥大期間中に土壌から吸収されたと考えられる。また、転流窒素のうち約 60%が葉由来であり、葉が窒素貯蓄の重要な器官であることがわかった。さらに、国内外の品種を調査した結果、葉からの転流窒素は、成熟期の子実の全窒素含量の 13.8~37.9%を占めている。しかし、多収性品種であるサチユタカとタマホマレを比較してみると、転流窒素が多いサチユタカよりも、転流窒素が少ないタマホマレのほうで子実収量が高いことから、葉からの窒素再分配よりも、子実肥大期間中の窒素同化能力が高収量を得るのに大変重要であることが示唆された。

砂耕栽培したダイズについて開花後に培養液の窒素濃度を高めた場合、子実収量は、葉に貯蓄窒素が多い品種サチユタカでは開花直後の窒素給与に、貯蓄窒素が少ない品種タマホマレでは、開花約 1 ヶ月後の子実肥大期の窒素給与に強く反応した。即ち、窒素に対する要求性は前者が子実肥大前に強く、後者は子実肥大開始後に強いことが示唆された。

これらの結果は子実肥大期に根系及び根粒が窒素に対する同化能力の品種間差異を示唆している。この仮説を検証するため、2011 年と 2012 年に、圃場栽培のサチユタカとタマホマレについて、子実収量、根の乾物重、根粒数及び主茎液中の窒素成分を調査した。その結果、子実収量と主茎液中の総窒素濃度には品種間差が見られなかったが、主茎液の流出速度はタマホマレでは非常に速く、これはタマホマレのより重い根重に関連することを示唆している。さらに、根粒数及び R5 と R6 時の主茎液中のウレイド態窒素の割合もタマホマレで高く、タマホマレの根粒の活性が生育後期により高いことが示唆された。その一方、サチユタカは R1 から R6 まで主茎液中のアミノ酸態窒素の割合がつねに高く、より多い転流窒素を子実生長に供給していることが示唆された。品種によるこの興味深い結果を実証するために実際の圃場栽培ダイズに各生育時期に窒素追肥を行ったところ、子実収量が最も高いのは、サチユタカでは開花直後の追肥区、タマホマレでは子実肥大期の追肥区でみられた。これらの結果はこれまでのダイズ開花期追肥の効果が不安定な理由を窒素同化及び子実への取り込みの特性が品種によって異なることから説明できると推察された。

以上のように、本論文は、ダイズの窒素吸収蓄積、及び再転流パターンの品種特性を明らかにし、また品種の窒素同化特性に応じた窒素の制御による収量ポテンシャル向上の可能性を見出している。本論文で得た知見は、ダイズの多収栽培における窒素肥料管理に極めて有益な情報を呈示している。

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* In Japanese with English title.

** In Japanese with English summary.

*** In Japanese, the title was translated by author.