Influence of Nitrogen Enrichment during Reproductive Growth Stage on Leaf Nitrogen Accumulation and Seed Yield in Soybean

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Abstract: Nitrogen assimilation during seed filling limits the seed yield in soybean. Seed nitrogen dependence on either redistributed nitrogen or absorbed nitrogen from soil during seed filling shows varietal differences. The objective of this study was to investigate the timing of nitrogen enrichment for effective nitrogen assimilation. 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 containing 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 concentrations. The high nitrogen concentration from R1 to R5 delayed the decline in SPAD value and leaf nitrogen concentration and improved the seed yield performance in Sachiyutaka, whereas stimulated the decline in SPAD value and leaf nitrogen concentration and had 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 large seed yield increase by nitrogen enrichment during R5 to R7 in Tamahomare could be caused by both the high photosynthetic rate and vigorous nitrogen uptake during seed filling. These results suggested that the most effective timing of nitrogen enrichment during the reproductive growth period to increase seed yield varies with the cultivar due to the difference in the pattern of nitrogen assimilation.

Key words: Leaf nitrogen accumulation, Nitrogen enrichment, Seed yield, Soybean.

Nitrogen assimilation or redistribution during the seed filling period is 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). During seed filling period, a large amount of nitrogen is redistributed from vegetative organs, but 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.

However, many studies have shown that the redistributed nitrogen improves the seed yield. For example, Shiraiwa and Hashikawa (1995) reported that high yielding modern cultivars exhibited greater dry matter production and nitrogen accumulation during the seed filling period 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 determining the length of seed filling period, which has close relationship with yield performance in soybean. Shibles and Sundberg (1998) showed that the leaf nitrogen content at the beginning of seed filling correlated significantly with seed yield. On the other hand, some researchers insist that high ability of nitrogen assimilation during seed filling contribute to the high seed yielding rather than nitrogen redistribution (Vasilas et al., 1995; Kumudini et al., 2002). These inconsistent results indicate that the effective nitrogen utilization for the seed yield could be different genetically and conditionally.

In the previous research, the seed yield in some cultivars was positively correlated with the amount of redistributed nitrogen from leaves, while in the high seed yielding cultivar Tamahomare, the high seed yield was associated with rather lower nitrogen redistribution, implying the importance of the direct nitrogen uptake during seed filling for high seed yielding beside nitrogen redistribution.
(Zhao et al., 2014).

On the other hand, nitrogen topdressing could be a practice to satisfy the rapid nitrogen demand during seed filling. Nitrogen topdressing at flowering time has been shown to increase the nitrogen assimilation and seed yield (Hamdi, 1976; Nakano et al., 1989; Wood et al., 1993; Nishioka and Okumura, 2008). However, the effect of nitrogen topdressing varies with the genotype, soil fertility, and topdressing timing (Watanabe, 1982; Gan et al., 2003; Salvagiotti et al., 2008), and occasionally it has no effect on seed yield (Abe and Onuma, 1981). Thus, the relationship between nitrogen assimilation and seed production is still unclear.

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

Materials and Methods

1. Plant cultivation

Two soybean cultivars Sachiyutaka and Tamahomare were used to conduct a series pot experiments in 2010 and 2012. The two cultivars are grown widely in western Japan and belong to the same maturity group. According to the previous reports (Zhao et al., 2014), Sachiyutaka was more dependent on redistributed nitrogen than nitrogen assimilation in seed production, whereas the reverse was the case in Tamahomare.

The experiments were conducted in a side-opened plastic house in Coastal Bioenvironment Center, Saga University, Karatsu City of Japan (33º 27’ N and 129º 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 the seedlings were thinned to one plant pot$^{-1}$ at the stage when the first trifoliate leaf appeared. After emergence, all the plants were watered twice a week with a half concentration of basic nutrient solution contained 50

| Nutrient | Concentration (ppm) | Reagents |
|----------|---------------------|----------|
| N        | 5~800               | NH$_4$NO$_3$ |
| P        | 70                  | KH$_2$PO$_4$ |
| K        | 110                 | K$_2$SO$_4$, KH$_2$PO$_4$ |
| Mg       | 90                  | MgSO$_4$ |
| Ca       | 35                  | CaCl$_2$ |
| Fe       | 3.5                 | NaFeEDTA |
| Mn       | 0.3                 | MnSO$_4$ |
| B        | 0.06                | H$_2$BO$_3$ |
| Zn       | 0.009               | ZnSO$_4$ |
| Cu       | 0.009               | CuSO$_4$ |
| Mo       | 0.009               | MoO$_3$ |

Modified from Matsunaga et al. (1983).

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Fig. 1. Changes in leaf SPAD value in Sachiyutaka and Tamahomare treated with different concentrations of nitrogen during R1 – R5 stages. 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. 2. Changes in leaf nitrogen concentration in Sachiyutaka and Tamahomare treated with nitrogen at different concentrations during R1 – R5. 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. Changes in leaf SPAD value in Sachiyutaka and Tamahomare treated with different concentrations of nitrogen during R5 – R7 stages. 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.
ppm nitrogen, and then with full concentration of basic solution (100 ppm nitrogen) from four weeks after emergence to harvest (Table 1).

2. Treatment with different nitrogen concentrations
The nitrogen concentration in the basic nutrient solution was changed from the flowering stage to the beginning of seed filling stage (R1 – R5), or from R5 to the physiological maturity stage (R5 – R7). During the treatment period, the nitrogen concentrations were adjusted to 5, 25, 100 (control), 200 and 400 ppm in 2010, and 5, 100 (control), 200, 400 and 800 ppm in 2012. The nutrient solution for replacing the solution in the sand medium (500 to 800 mL per plant) was applied every 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 with a chlorophyll meter SPAD-502 (Konica Minolta, Inc., Osaka, Japan) once or twice a week on the second or third fully expanded leaf from the top during the R1 to R7 stage.

One leaflet on the third or fourth fully expanded leaf from the top was taken at R1, R5, R6 and R7 stages. 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 the 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 μmol m⁻² 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 31.2 to 34.6°C during the measurement. Air was introduced through a rubber hose from 10 m far from the plants outside the plastic house.

At the harvest maturity stage (R8), all the plants in each treatment were harvested for the determination of the seed yield and yield components. In 2012, the roots were collected from the plants in 5, 100 and 800 ppm
The high nitrogen concentration (400 ppm in 2010 and 800 ppm in 2012) enhanced the leaf nitrogen accumulation at the R5 stage clearly in Sachiyutaka, but only slightly in Tamahomare (Fig. 2). At the R7 stage, the nitrogen concentration in the leaves treated with 400 ppm (in 2010) and 800 ppm (in 2012) nitrogen solution was at a high level in Sachiyutaka, but at the lowest level in Tamahomare.

In the nitrogen treatment during the R5 to R7 stage, the leaf SPAD values in both cultivars declined after the R5 or R6 stage in all nitrogen treatment, but the decrease was delayed by nitrogen enrichment, even in Tamahomare, in which the SPAD value at the R7 stage was at very high level in 400 ppm (in 2010) and 800 ppm (in 2012) nitrogen treatments (Fig. 3).

The changes in leaf nitrogen concentration in the treatment during R5 to R7 stage were similar to those in SPAD value (Fig. 4). At the R7 stage, the effect of nitrogen concentration on the leaf nitrogen concentration was larger in Tamahomare than in Sachiyutaka. Especially in the treatments with 400 ppm nitrogen in 2010 and 800 ppm nitrogen in 2012, the leaf nitrogen concentration in Tamahomare at R7 stage did not differ greatly from that at the R5 stage, while in the treatments with the lowest nitrogen concentration (5 ppm), it was much lower than that at the R5 stage.

Fig. 5 shows the leaf photosynthetic rate at the time between R5 and R6 stages in 2010. The photosynthetic rate in R1 – R5 treatment was higher in Tamahomare than in Sachiyutaka irrespective of the nitrogen concentration. In both cultivars, the photosynthetic rate was not increased by nitrogen enrichment during R1 – R5, but in Tamahomare it was significantly increased by nitrogen enrichment during R5 – R7 though not in Sachiyutaka.

4. Statistical analysis
Least significant difference (LSD) analysis was used for the analysis of significance on the parameters of leaf photosynthetic rate, root dry weight and yield components among different treatments in each cultivar. Single-factor analysis of variance (ANOVA) method was used (Figures 1 to 4).

Results
1. Influence of nitrogen concentration on leaf SPAD value and leaf nitrogen content
In the treatment during R1 – R5, leaf SPAD value gradually increased until the R5 stage and then decreased rapidly in all treatments (Fig. 1). However, the response of leaf SPAD values to the nitrogen concentration in Sachiyutaka was different from that in Tamahomare in both 2010 and 2012. Before the R5 stage, with the increase in nitrogen concentration in nutrient solution, the leaf SPAD values increased in both cultivars though slightly. After the R5 stage, however, then a high nitrogen concentration delayed the decrease of SPAD value in Sachiyutaka but accelerated it in Tamahomare. The SPAD value in Tamahomare treated with 400 or 800 ppm nitrogen was very low at the R7 stage.

2. Influence of nitrogen concentration on seed yield and yield components
Nitrogen enrichment during R1 – R5 increased total pod number plant\(^{-1}\), fertile pod number plant\(^{-1}\) and seed number plant\(^{-1}\) in both cultivars in 2010 and 2012 (Table 2), however, it increased the seed weight plant\(^{-1}\) and 100 seed weight only in Sachiyutaka. In Tamahomare in 2012, nitrogen enrichment did not increase seed weight plant\(^{-1}\), and rather significantly decreased 100 seed weight.

Nitrogen enrichment during R5 – R7 treatment, greatly increased the fertile pod number plant\(^{-1}\), seed number plant\(^{-1}\), seed weight plant\(^{-1}\) and 100 seed weight in Tamahomare in both 2010 and 2012, but not so much in Sachiyutaka (Table 3). The highest nitrogen concentration (800 ppm) during R5 – R7 in 2012 increased the seed weight to 1.42 times of the control (100 ppm) in Tamahomare, but only to 1.26 times in Sachiyutaka. In the treatment with the lowest nitrogen concentration (5 ppm in 2012) during R5 – R7, the seed weight was 0.78 times of the control in Tamahomare, but 0.94 times of the control.
in Sachiyutaka. Thus, the nitrogen enrichment had a greater effect on yield performance in Tamahomare compared with Sachiyutaka.

There was a strong interaction between the effects of nitrogen enrichment and cultivar on seed weight and 100 seed weight in both R1 – R5 and R5 – R7 treatments (Table 2, 3), which indicated that the effect of nitrogen enrichment varied with the cultivar.

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

3. Influence of nitrogen enrichment on root dry weight

The root dry weight increased significantly with the increase in nitrogen concentration during R1 – R5 in Sachiyutaka, but not in Tamahomare (Fig. 6). However, 800 ppm nitrogen during R5 – R7 increased the root dry weight by 1.5 times compared with 100 ppm (control) nitrogen in Tamahomare, but only by 1.2 times in Sachiyutaka.

Discussion

Although soybean plants need to accumulate much nitrogen in the seeds during seed filling, the nitrogen topdressing is rarely conducted in the commercial soybean production. This might be because the nitrogen fertilizer

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Table 2. Seed yield and yield components in the plants treated with different concentrations of nitrogen during R1 – R5 stages.

|            | 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 5 ppm | 46 ± 4.1 d | 33 ± 4.3 c | 63 ± 4.2 c | 19.3 ± 1.5 c | 30.5 ± 0.7 c |
| Sachiyutaka, 2010 R1-R5 25 ppm | 47 ± 3.1 cd | 35 ± 3.7 c | 66 ± 4.0 c | 20.4 ± 1.9 c | 30.7 ± 1.1 c |
| Sachiyutaka, 2010 R1-R5 100 ppm | 52 ± 2.4 b | 41 ± 3.1 b | 74 ± 3.8 b | 23.2 ± 1.4 b | 31.4 ± 0.4 bc |
| Sachiyutaka, 2010 R1-R5 200 ppm | 53 ± 3.7 b | 45 ± 7.4 b | 77 ± 4.3 ab | 24.7 ± 1.4 b | 32.1 ± 0.3 b |
| Sachiyutaka, 2010 R1-R5 400 ppm | 59 ± 5.8 a | 51 ± 5.2 a | 82 ± 4.3 a | 27.5 ± 1.8 a | 33.3 ± 0.7 a |
| Tamahomare, 2010 R1-R5 5 ppm | 52 ± 3.1 c | 49 ± 4.3 bc | 88 ± 6.7 a | 24.7 ± 3.5 a | 28.1 ± 1.5 a |
| Tamahomare, 2010 R1-R5 25 ppm | 53 ± 4.1 c | 49 ± 4.2 c | 89 ± 5.9 a | 24.9 ± 1.5 a | 27.9 ± 2.0 a |
| Tamahomare, 2010 R1-R5 100 ppm | 67 ± 5.4 b | 53 ± 2.7 ab | 89 ± 8.5 a | 24.8 ± 2.2 a | 27.8 ± 0.5 a |
| Tamahomare, 2010 R1-R5 200 ppm | 69 ± 3.7 ab | 54 ± 5.0 a | 91 ± 7.5 a | 24.6 ± 1.1 a | 27.1 ± 1.6 a |
| Tamahomare, 2010 R1-R5 400 ppm | 73 ± 3.4 a | 57 ± 4.6 a | 94 ± 5.9 a | 24.9 ± 1.1 a | 26.5 ± 1.1 a |
| Sachiyutaka, 2012 R1-R5 5 ppm | 44 ± 3.6 c | 35 ± 3.3 d | 59 ± 9.5 c | 17.2 ± 1.8 d | 29.0 ± 0.7 c |
| Sachiyutaka, 2012 R1-R5 100 ppm | 49 ± 4.8 bc | 38 ± 5.9 cd | 69 ± 4.3 b | 21.3 ± 2.3 c | 30.8 ± 0.6 b |
| Sachiyutaka, 2012 R1-R5 200 ppm | 51 ± 2.7 b | 41 ± 4.8 bc | 71 ± 9.2 b | 22.4 ± 3.9 c | 31.5 ± 0.7 b |
| Sachiyutaka, 2012 R1-R5 400 ppm | 58 ± 5.4 a | 47 ± 3.5 b | 79 ± 6.1 a | 25.3 ± 3.1 b | 31.9 ± 0.7 ab |
| Sachiyutaka, 2012 R1-R5 800 ppm | 62 ± 5.0 a | 53 ± 5.4 a | 85 ± 10.3 a | 27.6 ± 2.6 a | 32.6 ± 0.6 a |
| Tamahomare, 2012 R1-R5 5 ppm | 53 ± 5.6 d | 45 ± 6.1 d | 80 ± 5.2 b | 22.4 ± 3.2 a | 28.0 ± 1.2 a |
| Tamahomare, 2012 R1-R5 100 ppm | 61 ± 4.7 c | 51 ± 5.1 c | 81 ± 4.2 b | 22.1 ± 2.5 a | 27.4 ± 1.4 ab |
| Tamahomare, 2012 R1-R5 200 ppm | 63 ± 7.6 bc | 53 ± 5.8 bc | 83 ± 4.2 ab | 21.8 ± 3.1 a | 26.3 ± 0.9 b |
| Tamahomare, 2012 R1-R5 400 ppm | 69 ± 6.3 ab | 57 ± 6.2 ab | 89 ± 7.9 a | 22.3 ± 4.3 a | 25.1 ± 0.6 b |
| Tamahomare, 2012 R1-R5 800 ppm | 74 ± 3.3 a | 62 ± 3.9 a | 89 ± 3.3 a | 22.1 ± 3.7 a | 24.9 ± 0.8 b |

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.
application could restrict the nitrogen fixation by nodules. Our results showed that 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 of raising the seed yield potential by nitrogen control.

Drastic nitrogen redistribution from leaf to seed during the seed filling stage triggers leaf senescence (Sinclair and de Wit, 1976), shortens the seed filling period (Loberg et al., 1984; Hortensteiner and Feller, 2002), and limits the seed yield. The present results showed that the increase in nitrogen concentration in the culture solution (nitrogen enrichment) during the R1 – R5 stage delayed the decline in the leaf SPAD value (Fig. 1), increased leaf nitrogen concentration at R5 (Fig. 2), and promoted the yield performance in Sachiyutaka (Table 2). However, in Tamahomare, the nitrogen enrichment during R1 – R5 did not delay the decline of leaf SPAD value (Fig. 1), even decreased the leaf nitrogen concentration greatly (Fig.2). Although the nitrogen enrichment promoted the total and fertile pod number plant$$^{-1}$$ in Tamahomare, it decreased the 100 seed weight (Table 2), resulting in no change in the seed yield (Table 2). Since the nitrogen accumulation in the leaves was slight in Tamahomare compared with Sachiyutaka (Zhao et al., 2014), the present results imply that the increased nitrogen source during R1 – R5 enhanced the pod set rather than nitrogen accumulation in the leaves, but the lack of nitrogen supply

| Table 3. Seed yield and yield components in the plants treated with different concentrations of nitrogen during R5 – R7 stages. |
|---------------------------------------------------------------|
| **Total Pod** | **Fertile Pod** | **Seed** | **Seed** | **100 seed** |
|               | (plant$$^{-1}$$) | (plant$$^{-1}$$) | No. | weight (g plant$$^{-1}$$) | weight (g) |
| Sachiyutaka, 2010 | | | | | |
| R5-R7 5 ppm | 52 ± 2.6 a | 34 ± 3.7 c | 71 ± 4.3 b | 21.9 ± 1.6 c | 30.8 ± 0.5 a |
| R5-R7 25 ppm | 51 ± 5.6 a | 32 ± 4.2 c | 72 ± 5.6 b | 22.7 ± 1.6 bc | 31.6 ± 2.1 a |
| R5-R7 100 ppm | 52 ± 6.8 a | 41 ± 2.7 b | 74 ± 3.3 b | 23.2 ± 1.1 bc | 31.4 ± 0.6 a |
| R5-R7 200 ppm | 53 ± 6.7 a | 48 ± 3.4 a | 84 ± 3.7 a | 23.1 ± 1.4 ab | 29.9 ± 1.2 a |
| R5-R7 400 ppm | 52 ± 5.8 a | 45 ± 4.3 a | 83 ± 3.3 a | 26.2 ± 1.2 a | 31.4 ± 2.1 a |
| Tamahomare, 2010 | | | | | |
| R5-R7 5 ppm | 66 ± 6.4 a | 42 ± 5.3 c | 85 ± 8.7 c | 18.4 ± 3.0 d | 21.6 ± 1.9 b |
| R5-R7 25 ppm | 65 ± 3.3 a | 44 ± 7.7 c | 93 ± 6.8 b | 20.8 ± 2.6 c | 22.3 ± 2.2 b |
| R5-R7 100 ppm | 67 ± 8.1 a | 53 ± 3.9 b | 89 ± 8.1 bc | 24.8 ± 3.8 b | 27.8 ± 0.9 a |
| R5-R7 200 ppm | 66 ± 3.6 a | 55 ± 4.6 b | 90 ± 7.3 bc | 25.3 ± 3.6 b | 28.1 ± 0.9 a |
| R5-R7 400 ppm | 67 ± 5.1 a | 62 ± 4.8 a | 103 ± 5.3 a | 30.1 ± 2.3 a | 29.3 ± 0.6 a |
| Sachiyutaka, 2012 | | | | | |
| R5-R7 5 ppm | 50 ± 2.1 a | 37 ± 5.7 b | 68 ± 8.6 c | 20.1 ± 3.1 d | 29.4 ± 0.9 a |
| R5-R7 100 ppm | 49 ± 1.9 a | 38 ± 4.4 b | 69 ± 5.9 c | 21.3 ± 3.0 cd | 30.8 ± 0.7 a |
| R5-R7 200 ppm | 49 ± 5.9 a | 42 ± 5.3 ab | 75 ± 6.7 bc | 22.8 ± 2.8 bc | 30.6 ± 1.1 a |
| R5-R7 400 ppm | 48 ± 3.5 a | 46 ± 2.9 a | 81 ± 4.4 ab | 24.9 ± 2.9 ab | 30.3 ± 0.5 a |
| R5-R7 800 ppm | 49 ± 4.2 a | 46 ± 2.4 a | 88 ± 7.1 a | 26.8 ± 1.8 a | 30.5 ± 0.6 a |
| Tamahomare, 2012 | | | | | |
| R5-R7 5 ppm | 62 ± 7.5 a | 47 ± 4.9 c | 79 ± 8.3 d | 17.2 ± 1.9 d | 21.9 ± 0.6 c |
| R5-R7 100 ppm | 61 ± 4.3 a | 51 ± 4.4 c | 81 ± 9.2 d | 22.1 ± 3.5 c | 27.4 ± 0.7 b |
| R5-R7 200 ppm | 60 ± 6.8 a | 53 ± 6.8 bc | 88 ± 7.4 c | 24.3 ± 4.0 c | 27.3 ± 1.5 b |
| R5-R7 400 ppm | 62 ± 6.3 a | 58 ± 6.2 ab | 96 ± 8.9 b | 27.8 ± 3.7 b | 28.9 ± 0.6 ab |
| R5-R7 800 ppm | 61 ± 6.0 a | 61 ± 5.2 a | 104 ± 5.4 a | 31.4 ± 3.6 a | 30.3 ± 1.1 a |
| 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.
after R5 induced a drastic leaf nitrogen redistribution and early senescence. In addition, although the treatment with a low nitrogen concentration (5 ppm and 25 ppm) during R1 – R5 aggravated the decrease in SPAD value and nitrogen concentration in leaves (Fig. 1, 2), and reduced the seed yield in Sachiyutaka (Table 2), this was not the case in Tamahomare.

On the other hand, the low and high nitrogen concentration during R5 – R7 affected the yield performance in Tamahomare. The lowest nitrogen concentration (5 ppm) reduced the seed yield (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) (Table 3). In the highest nitrogen concentration (800 ppm) treatment during R5 – R7 in 2012, the redistribution of nitrogen seemed to contribute a little to the increase in seed yield, since the leaf nitrogen concentration at R7 was slightly different from that at R5 (Fig. 4). These results imply that a higher soil nitrogen content and active root system during seed filling could increase the seed yield, regardless of the amount of redistributed nitrogen from vegetative organs. In fact, a heavier root dry weight was found in Tamahomare in 800 ppm nitrogen treatment during R5 – R7 (Fig. 6).

Generally, the soybean plants start senescence after R5, when nutrient assimilation is 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 in 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 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.

In the present study, the nitrogen enrichment during reproductive stage could enhance seed yield dramatically. However, the most effective timing of nitrogen enrichment was different with the cultivar due to the different pattern of nitrogen assimilation. This could be why the effects of nitrogen topdressing were not stable as reported previously. The results presented here will help in conducting adequate nitrogen topdressing on soybean seed production.

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**Fig. 6.** Root dry weight of the plants treated with different concentrations of nitrogen during R1 – R5 and R5 – R7 stages in 2012. Bars indicate standard deviations with six replications. Different letters in each figure indicate significant difference according to LSD analysis at $p < 0.05$ level.
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