CULTIVATIONAL STUDIES ON THE IMPROVEMENT OF NITROGEN USE EFFICIENCY IN RICE CULTIVARS

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Chapter 1

General introduction

1.1. Overview about rice production and nitrogen fertilizer

Rice is the most important staple food in Asia. More than 90% of the world's rice is grown and consumed in Asia, where around 60% of the world population lives. Rice accounts for between 35-60% of the caloric intake of three billion Asians (Guyer et al., 1998). Over 150 million hectares of rice are planted annually, covering about 10% of the world's arable land. In 1999-2000, this amounted to some 600 million tons of rice seed, equal to 386 million tons of milled rice. With the world population estimated to increase from 6.2 billion in the year 2000 to about 8.2 billion in the year 2030, the global rice demand will rise to about 765 million tons, or 533 million tons of milled rice (FAO, 2002).

To increase food production, one can either farm more land, or increase the yield from each unit of land. However, land resources are limited and cannot be expanding more in the future, it is still necessary to improve rice yield in order to satisfy people's food demand. There are many ways to improve rice yield such as using Nerica rice and high-yielding rice cultivars, applying fertilizer, installing irrigation systems, minimizing losses by enhancing post-harvest technology, etc. Among recent attempts to overcome rice yield limitation by improving yield are breeding programs and developing hybrid rice varieties which are being applied in some countries like China and Japan. Hybrid rice has been developed in China since 1974 and now is planted in almost 40% of Chinese rice fields (Li et al., 2009). Hybrid rice in China has a yield potential of 15 t ha^{-1} and these rice cultivars had some characters such as short-culmed, effect, thick and V–shape leaves, moderate tillers, medium plant height, heavy panicles (Peng et al., 2004). In Japan, the breeding for high-yielding cultivars began in 1981 with the target of developing new cultivars whose grain yield were higher than the older cultivars., Cultivar named "Takanari" was released this time, achieved a grain yield of 10 t ha^{-1}, was consistently higher than standard japonica varieties (San-oh et al., 2004). Besides breeding programs, understanding about types of fertilizers and methods, especially nitrogen fertilizer ones is crucial to enhance rice grain yield.

Nitrogen represents 67% of fertilizer applications to rice in a world basis (Eagle et al., 2011), and N fertilizer costs contain an important fraction of total production costs (Tirol-Padre et al., 1996). According to Hossain (2004), it was estimated that world rice production until 2030 will have to increase in 200 million tons of rough rice to supply the food demand. At current levels of N use efficiency (NUE), this would require to double the 10 million tons of N fertilizer currently used each year for rice production (Ladha et al., 2005). However, it has been observed a decrease in crop NUE with increasing N fertilization (Borrell et al., 1998; Fageria and Baligar, 2001; Jiang et al., 2004; Peng et al., 2006), and therefore increasing loss in N applied. In addition, recovery efficiency of applied N is relatively low in irrigated rice, because of rapid N loss from the soil-floodwater system (Singh et al., 1998; Peng et al., 2006). The highest losses are due to ammonia volatilization and, to a lesser extent, by denitrification and nitrate lixiviation (Belder et al., 2005; Peng et al., 2006). The development of high yielding and NUE rice cultivars is also required. It has been observed that rice presents a genetic variability for NUE (Singh et al., 1998). However, an improvement of this character through genetic selection has not yet been done (Koutroubas and Ntanos, 2003; Mae et al., 2006).

Nitrogen use efficiency (NUE) has been defined in several ways and NUE is also influenced by the form of nitrogen fertilizer (Raun and Schepers, 2008). However, these definitions generally take into account quantity of N accumulated in the plant, known as uptake efficiency and quantity of N utilized in grain production known as utilization efficiency. In soil with limited available N, utilization efficiency has been found to be more important than uptake efficiency in contributing to genotypic differences in grain production (Moll et al., 1982; Borrell et al., 1998; Koutroubas and Ntanos, 2003; Peng et al., 2006; Zhao et al., 2007). Utilization efficiency coupled with economic yield is a desired characteristic in crop plants if minimum depletion of soil N is a goal. Moll et al. (1982) recommended the development of genotypes with both high uptake and utilization efficiency.

In order to improve rice yield, many researchers have applied many methods, especially ones involved in nitrogen fertilizer doses, application times, and different types of fertilizers and methods at different conditions (Okumura et al., 1982; Seito et al., 1992; Mohammad et al., 2002; Ye et al., 2007; Artacho et al., 2009; Fukushima et al., 2011a). In the previous century, deep layer application methods were used to improving rice yield (Okumura et al., 1982; Wang et al., 1991). This method was a good way to increase rice yield than conventional method because N fertilizer concentrate near the root system with made it easy for rice plants uptake and use efficiency. Beside deep layer application methods, using slow-release fertilizer might be suitable in modern agriculture cultivation because of decreased the cost of labor (De Datta, 1986; Ohnishi et al., 1999; Taylaran et al., 2009). However, the understandings about nitrogen uptake and accumulation among fertilizer methods and grain yield differences among cultivars were still limited although this method was effective in supplying nutrients in timely manner and reduces nitrogen losses.

Recently, many researches attended to planting time and planting density on morphological traits, dry matter production and yield of rice cultivars (Nakano and Morita, 2009; Fukushima et al., 2011a) and research on effects of nitrogen fertilizer on nitrogen use efficiency under different soil conditions (Ye et al., 2007). However, just a few studies focus on nitrogen use efficiency, dry matter production at the panicle initiation stage and the effects of slow-release fertilizer on dry mater production, yield of rice at different cultivars.

1. 2. Objectives of this study

As stated above, the aims of study are as follows:

- To investigate effects of fertilizer types and methods on dry matter production, grain yield and nitrogen use efficiency of rice cultivars which probably useful information and guidelines for rice cultivation.

- To clarify the cultivar differences in nitrogen use efficiency at different levels of nitrogen fertilizer. Slow-release fertilizer and high-yielding cultivars were used in these experiments.

- To examine the dry matter production, yield and nitrogen use efficiency in varietal genotype in without nitrogen fertilizer application.

Chapter 2

Effects of different types of fertilizers and methods on dry matter production, yield and nitrogen use efficiency of rice cultivars under field conditions

2.1. Introduction

Rice is the staple food of more than a half of population in the world (Jackson et al., 1996; IRRI, 2002). Rice production accounts for 27% of total cereal production in the world, and 90% of rice production takes place in Asian countries (Mae et al., 2006; Artacho et al., 2009).

Nitrogen (N) is necessary for rice growth and development and it is the most yield-limiting nutrients in lowland rice production all around the world (Fageria and Barbosa Filho, 2001; Ye et al., 2007). Nitrogen uptake is affected by various factors such as type of fertilizers, soil and weather conditions (Ye et al., 2007), etc. Nitrogen is involved in all the metabolic processes in plants, and about 75% of leaf N is associated with chloroplast, which are essential for dry matter production during photosynthesis (Mae, 1997). Nitrogen requirement in rice is higher than that of other nutrients. High nitrogen availability to the plant has been associated with increase in plant height, high chlorophyll content, and leads to increased productivity (Arima, 1995). Moreover, nitrogen plays an important role in increasing leaf area and photosynthetic rate which consequently affects dry matter production and yields of rice plants.

In the efforts to improve rice yields, researchers have applied many methods such as using high yielding varieties, applying more nitrogen fertilizers and timely scheduling of topdressing (Nakano and Morita, 2009; Fukushima et al., 2011a; Hayashi et al., 2012).

However, heavy nitrogen application not only increases labor and costs of production but also pollutes the air and water quality (Hasegawa and Horie, 1996; Taylaran et al., 2009). Therefore, cultivation techniques have been applied such as fertilizer applications that help rice plant absorb nutrients at different growth stages and produce higher dry matter production, grain yield is still needing to improve.

Deep layer fertilizer application, known as deep fertilizer application was used in the previous century. Wada (1969) and Wang (1991) reported that deep layer application increased the number of spikelets m^{-2}, thus increasing rice grain yield (10－ 13%) to compared with conventional fertilizer method. However, deep layer fertilizer application increased the costs of labor than conventional method. Recently, use of slow-release fertilizer has been adopted before transplanting. This method is effective in supplying nutrients in a timely manner, and reduces nitrogen losses. Slow-release fertilizer application can be advantageous in rice production since plants efficiently absorb the available nitrogen leading to high dry matter production and grain yield. However, the amount of nitrogen uptake and accumulation by rice plants is not clearly understood, especially in some newly developed high-yielding cultivars. The objective of this study is to classify the uptake and use efficiency of nitrogen fertilizer based on dry matter production, grain yield, nitrogen accumulation and use efficiency with different fertilizer application methods.

2.2. Materials and methods

2.2.1. Plant materials

In this study, we selected two rice cultivars, "Nipponbare", widely grown in Japan, and "Takanari", one of the high yielding rice cultivars. The two rice cultivars were grown in a submerged paddy field.

The experiment was conducted in the experimental farm at the Field Science Center, Faculty of Agriculture, Okayama University, Japan (34^{0}41'N, 133^{0}55'N) in 2009. Two rice cultivars and five fertilizer methods were arranged in a randomized complete block design. Three fertilizer methods were used, namely; Control (0N); Conventional method; Deep fertilizer method for "Nipponbare" and "Takanari", and another two methods, Standard fertilizer method and High fertilizer method for "Nipponbare". The timing and amount of nitrogen fertilizer application are presented in Table 1.

Table 1. Timing and amount of nitrogen fertilizer application in rice cultivars.

Methods	Basal dressing (N g m^{-2})	Top dressing				Total dressing (g m^{-2})
		45 DBH	30 DBH	20 DBH	5 DAH	
0N	0	0	0	0	0	0
Conventional method	4	2	0	2	2	10
Deep fertilizer method	4	0	6	0	0	10
Standard fertilizer method	8	0	0	0	0	8
High fertilizer method	16	0	0	0	0	16

DBH: Days before heading; DAH: Days after heading

In the conventional method, chemical fertilizer (Rin-ka-an 44, N : P_2O_5 : K_2O = 14 : 17 : 13) was mixed and incorporated into the soil at 4 g m^{-2} as basal dressing, every 2 gN were applied at 45 DBH (days before heading), 20 DBH and 5 DAH (days after heading), respectively. In the deep fertilizer method, the same amount of fertilizer was applied with conventional method as a basal dressing and 6 gm^{-2} of paste fertilizer (Neo paste 1, N : P_2O_5 : K_2O = 12 : 12 : 12) was injected in the middle of 4 hills at the depth of 15 cm by soil injector at 30 DBH. In the standard and high fertilizer methods using slow release fertilizer (100D – 80, N : P_2O_5 : K_2O = 14 : 14 : 14) was applied as basal dressing at the rate of 8 and16 gN m^{-2}, respectively.

Seeds of rice were sown on 11 May and transplanted on 10 June, 2009 at a density of 22.2 hills m^{-2} (30 x 15 cm), with three plants per hill. Pests and diseases were intensively controlled to avoid yield loss.

2.2.2. Growth characteristics

In each plot, we selected 3 hills to measure the plant height, leaf numbers, stem numbers and SPAD values (leaf color) every week after transplanting

2.2.3. Dry weight and LAI

The plants were sampled every two weeks after transplanting 20 days to determine dry weight and leaf area. Sixteen hills were sampled and eight hills with average numbers of stem were selected at each stage. Plants were separated into leaves, stems and panicles. Leaf area was measured with leaf area meter (AAM-9; Hayashi Denko Co., Tokyo, Japan) after separation. All plant samples were dried in a ventilated oven at 80^0C until constant weight. The crop growth rates (CGR), net assimilation rates (NAR) and mean leaf area index (mean LAI) were calculated.

2.2.4. Growth analysis

From the plant dry weight and LAI measured as described above, the crop growth rate (CGR), net assimilation rate (NAR) and leaf area index (LAI) were also calculated by following formulas:

1) $\text{CGR (g m}^{-2}\text{ d}^{-1}) = \dfrac{W_2 - W_1}{t_2 - t_1}$

2) $\text{NAR (g m}^{-2}\text{ d}^{-1}) = \dfrac{(W_2 - W_1)(\ln A_2 - \ln A_1)}{(t_2 - t_1)(A_2 - A_1)}$

3) $\text{Mean LAI} = \dfrac{A_2 - A_1}{\ln A_2 - \ln A_1}$

Where, W_2, W_1 and A_2, A_1 indicate the dry weight and LAI at the corresponding date t_1 and t_2, respectively.

2.2.5. Yield and yield components

At the maturity stage, 20 hills for each replication were harvested manually for yield determinations. Yield was determined by brown rice weight. Fully ripened grains were selected by sieving through 1.8 mm and 1.6 mm mesh for Nipponbare and Takanari, respectively. Thousand grain weights was measured by using brown grains at 14.5% moisture.

2.2.6. Nitrogen accumulation and some relative parameters

At each stage, the plant parts from two hills were ground into powder by vibrating mill (Heiko, Co. Ltd) and the nitrogen concentration from each plant parts was analyzed by CN-Corder (MT-700, Yanaco Industry). Total nitrogen accumulation was calculated by multiplying the above-ground dry weight by nitrogen concentration.

On the basis of these measurements, nitrogen use efficiency (NUE) indices were calculated by the following formulas;

N use efficiency for biomass (BE_N) = BY /AN

Recovery efficiency of applied N (RE_N) = ($AN - AN_0$) / APN

Nitrogen use efficiency for grain yield (YE_N) = GY / AN

Partial factor productivity of applied N (PFP_N) = GY / APN

Where BY; Biomass yield (g m^{-2}), GY; Grain yield (g m^{-2}), AN; Accumulated N (g m^{-2}), AN_0; Accumulated N without fertilizer (g m^{-2}), APN; Applied N (g m^{-2})

2.2.7. Statistical analysis

The analysis of variance was performed to detect the different among treatments by ANOVA.

2.3. Results

2.3.1. Growth characteristics

Plant height

The plant height increased with increasing the amount of N fertilizer application during the growth period (Fig. 1). Plant height increased rapidly and got the highest at 71 days after transplanting (DAT). With deep fertilizer application method, plant height got the highest in both rice cultivars, followed by higher fertilizer method, standard fertilizer method and conventional method. Plant height of Nipponbare tended to be higher than Takanari with the same fertilizer methods.

Number of stems

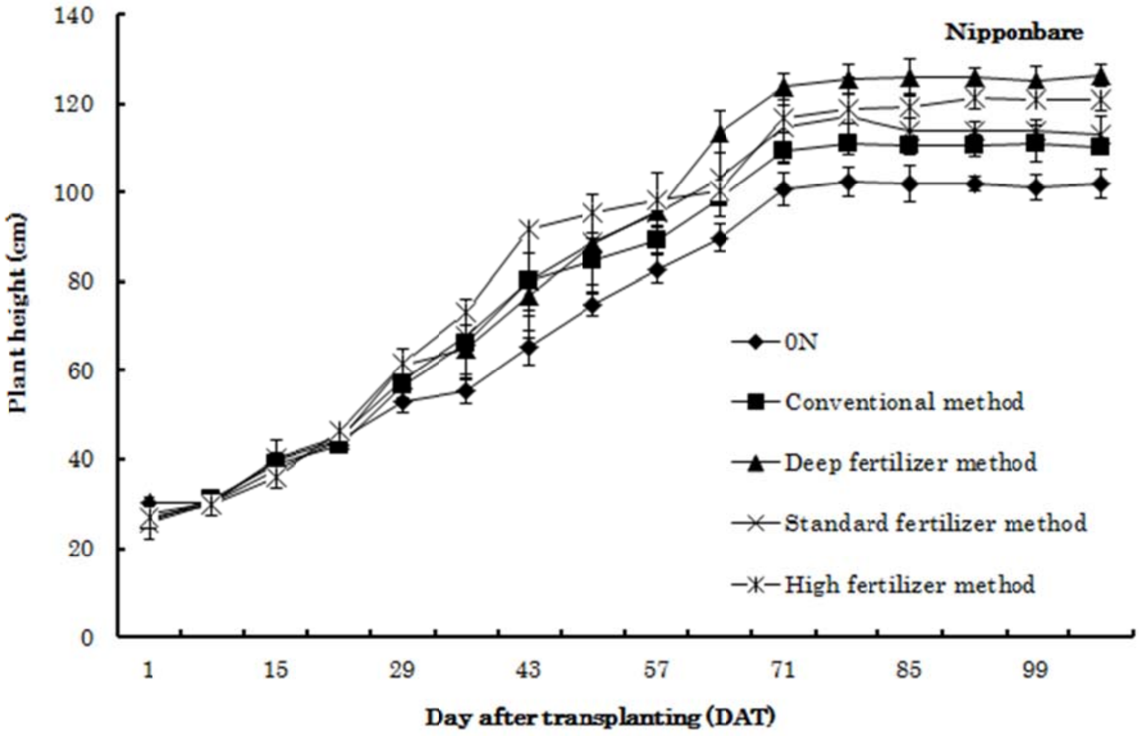
The number of stems were varied among fertilizer types and methods (Fig. 2). The number of stem increased dramatically from 15 DAT to 29 DAT. After that, number of stem slightly decreased and reached plateau after heading. In Takanari, number of stems at conventional and deep fertilizer method was not significant different and it was higher than that of control. In contrast, using slow released fertilizer the larger amount of nitrogen could increases the number of stem than other types and fertilization methods. Number of stem reached 25.4 (plant^{-1}), significantly higher than others.

Number of leaves

The number of leaves also higher in both cultivars with more N fertilizer applied (Fig. 3). However, the differences in leaves number among types and fertilizer methods were not significantly in both cultivars. In general, Takanari and Nipponbare developed the same leaf number during growth period.

SPAD meter readings

The SPAD values were observed from 15 DAT. The SPAD values reduced from 29 DAT and 22 DAT in Takanari and Nipponbare, respectively, then increased gradually and got peaked at heading in both rice cultivars. Deep fertilizer application methods had highest SPAD values than other type and fertilizer methods (Fig. 4).



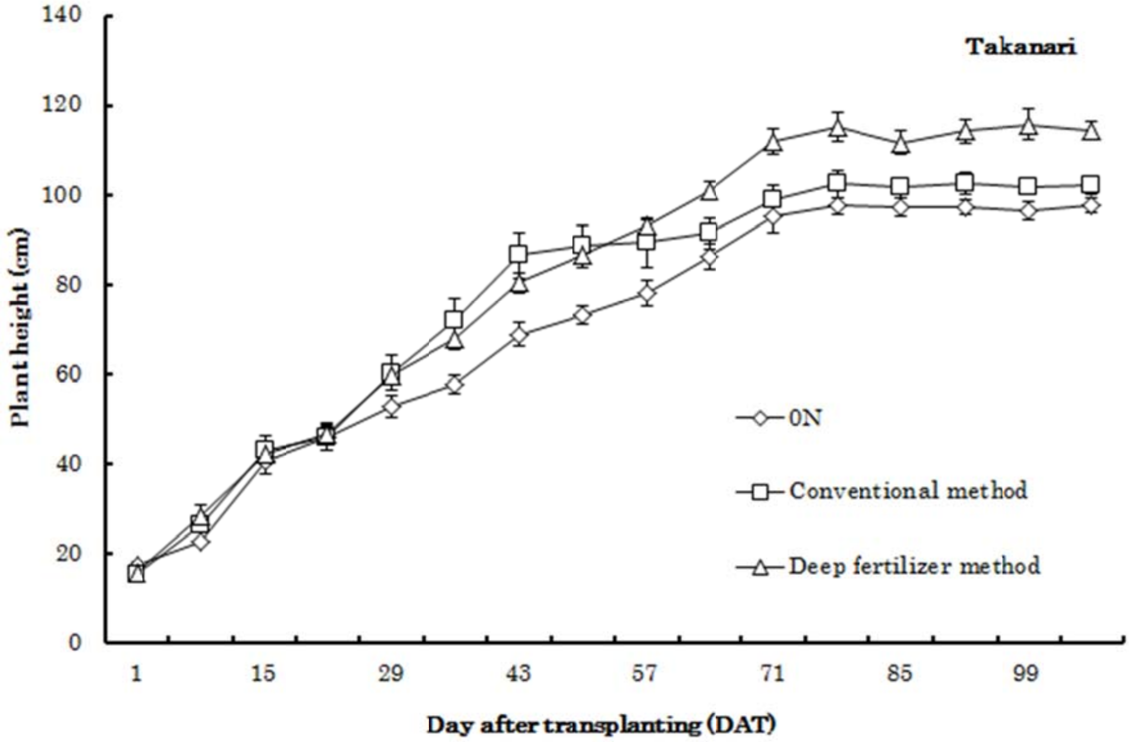


Fig. 1. Plant height of Nipponbare and Takanari at different types of fertilizers and methods.

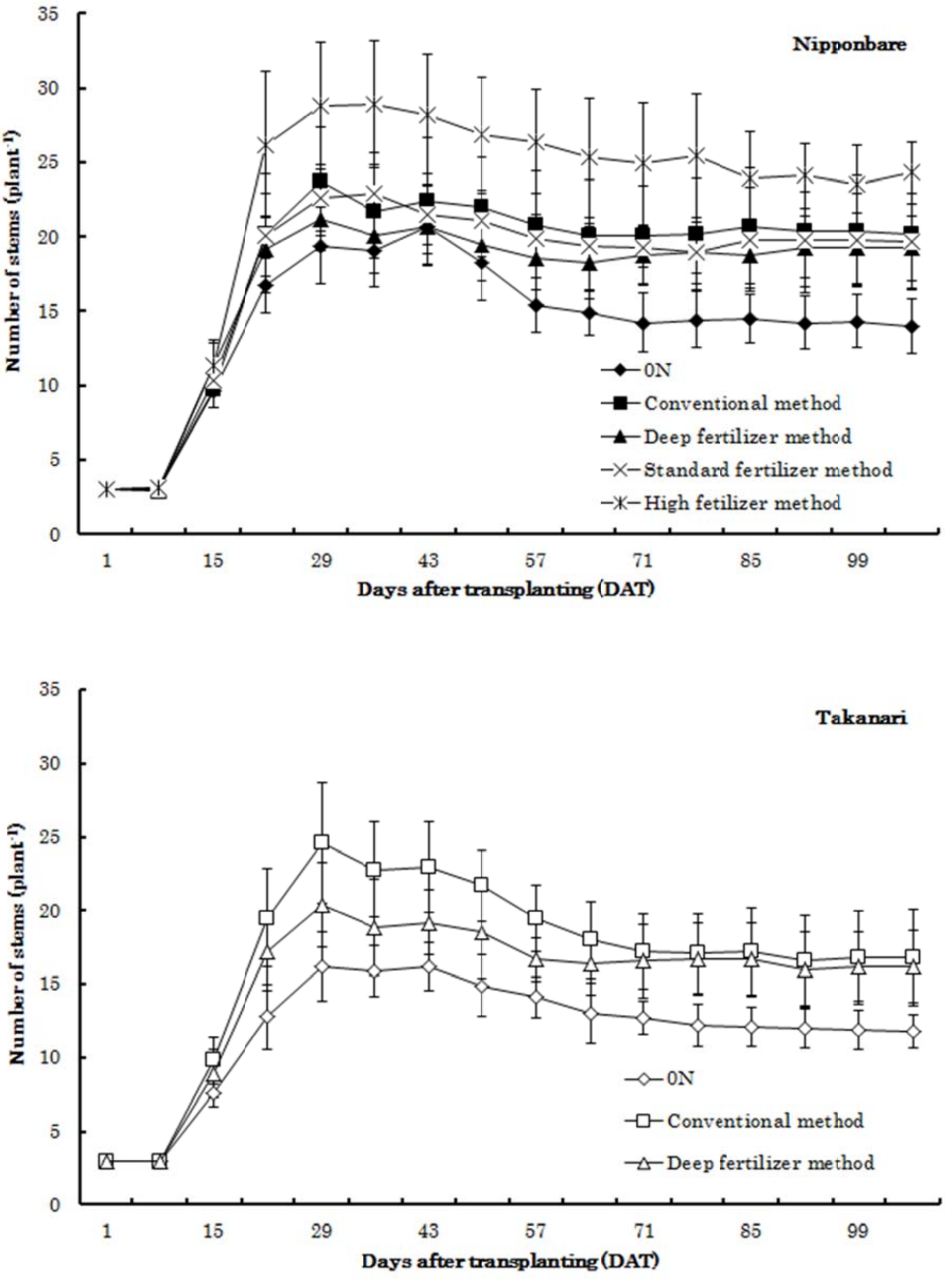


Fig. 2. Number of stems of Nipponbare and Takanari at different types of fertilizers and methods.

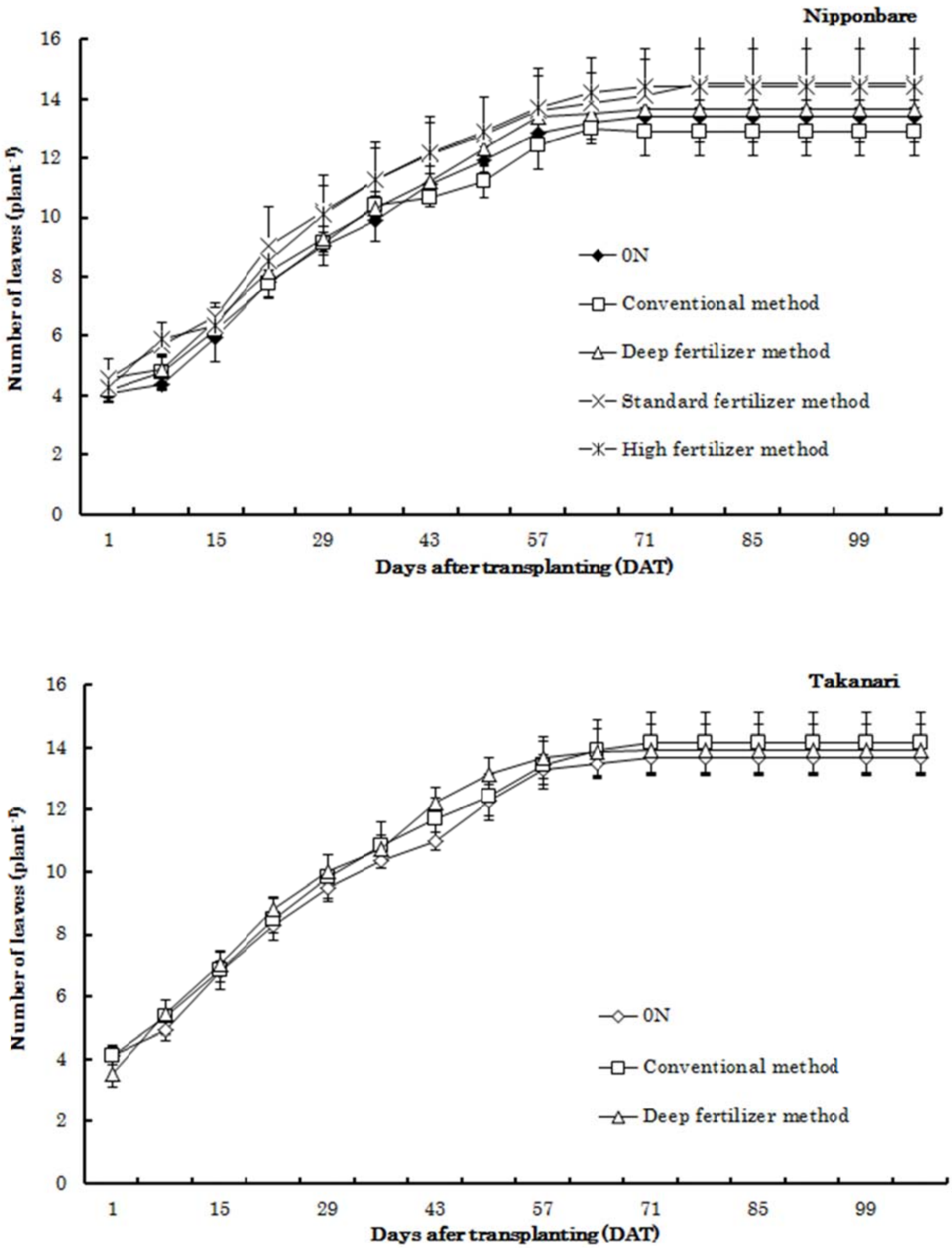


Fig. 3. Number of leaves of Nipponbare and Takanari at different types of fertilizers and methods.

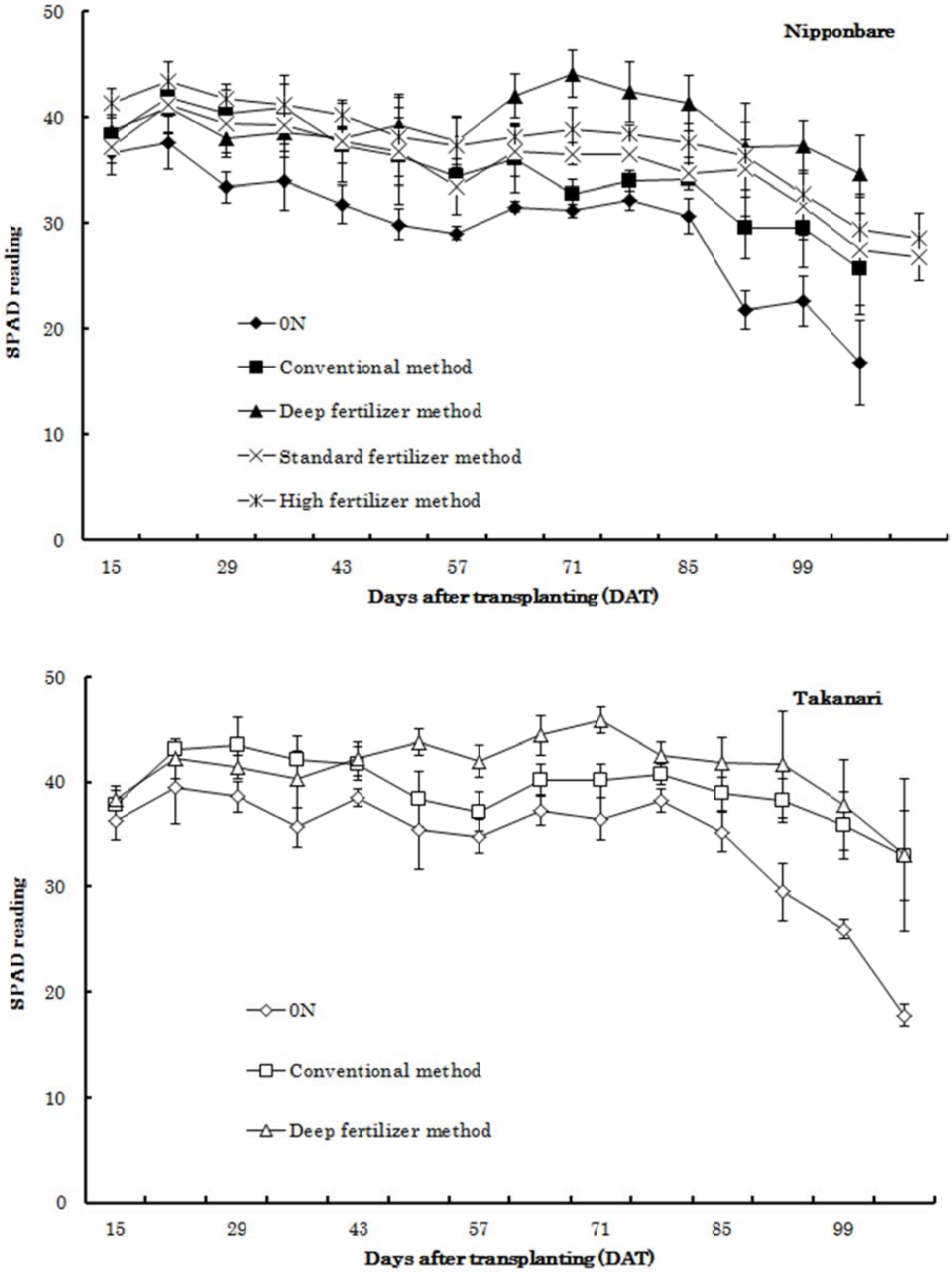


Fig. 4. SPAD reading values of Nipponbare and Takanari at different types of fertilizers and methods.

2.3.2. Dry matter production at different fertilizer application.

The effects of different nitrogen application methods on dry weight were significant for all growth and development stages, except for the young seedling in Nipponbare and Takanari (Table 2). Nitrogen fertilizer increased the number of tillers and top dry weight at full heading stage (76 DAT). At full heading stage, Takanari produced a significantly larger amount of dry matter at deep fertilizer method (1359 g m^{-2}) than that of the other methods. However, with more nitrogen fertilizer application in Nipponbare, plant dry weight also increased but there was no significant difference among fertilizer methods.

At harvesting time, plant dry weight with deep fertilizer method showed the largest amount of dry weight in Takanari (1891 g m^{-2}), and consistently higher than other methods, followed by standard fertilizer method in Nipponbare (1750 g m^{-2}). Without nitrogen application, Nipponbare produced the least biomass (1244 g m^{-2}), which is lower than Takanari (1411 g m^{-2}).

Table 2. Dry matter production at different fertilizer application methods in rice cultivars.

Cultivars	Methods	Days after transplanting (DAT)							
		0	20	34	48	62	76 *	90	104 **
Nipponbare	0N	4.2 a	58 b	172 c	437 e	704 d	984 b	1162 d	1244 c
	Conventional method	4.2 a	64 ab	297 b	539 d	805 c	1109 a	1380 c	1486 b
	Deep fertilizer method	4.2 a	64 ab	275 b	504 c	860 bc	1168 a	1482 b	1640 a
	Standard fertilizer method	4.2 a	68 a	287 b	605 b	923 ab	1169 a	1539 a	1750 a
	High fertilizer method	4.2 a	72 a	349 a	692 a	959 ab	1158 a	1414 c	1669 a
Takanari	0N	3.7 a	59 b	218 b	445 c	692 bc	991 c	1217 c	1411 b
	Conventional method	3.7 a	60 a	335 a	629 a	905 a	1166 b	1458 b	1635 ab
	Deep fertilizer method	3.7 a	60 a	306 a	566 b	942 a	1359 a	1703 a	1891 a

Means followed by the same letters are not significantly different at the 0.05 level by Tukey's test.

Unit: g m^{-2}. *, **: heading and maturity stages, respectively.

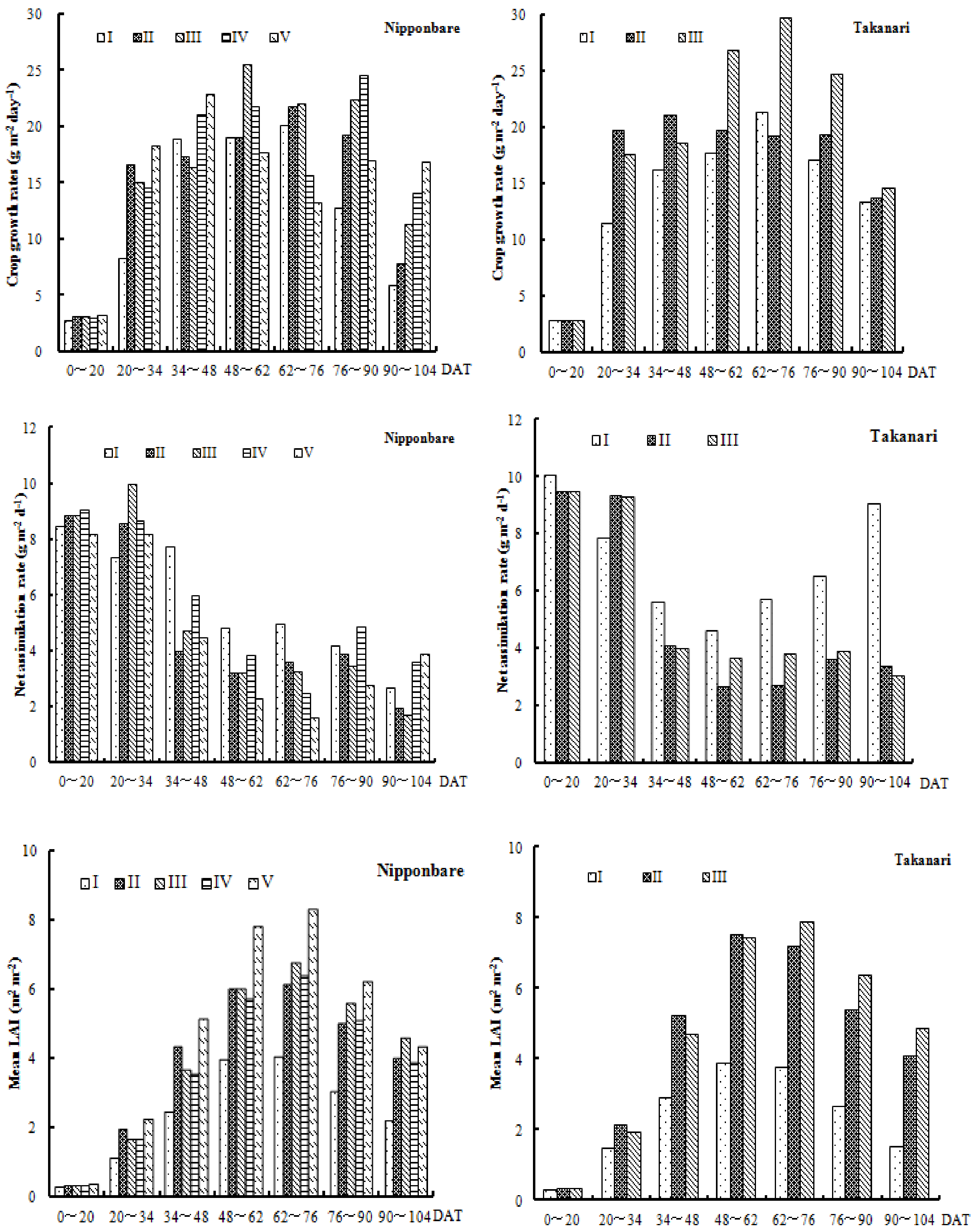


Fig. 5. Changes in CGR, NAR and mean LAI from transplanting to harvesting at different fertilizer methods in two rice cultivars. (I; 0N, II; Conventional method, III; Deep fertilizer method, IV; Standard fertilizer method, V; High fertilizer method).

To identify the factors affecting the differences in dry matter production, we compared CGR, NAR and mean LAI among fertilizer methods (Fig. 5). CGR increased after 20 DAT (days after transplanting) and it differed according to fertilizer method. In Takanari, CGR tended to be higher in deep fertilizer, especially 62 - 76 DAT and 76 - 90 DAT. During these periods, CGR was 26.8 and 29.7 g m^{-2} day^{-1}, respectively. The same result was observed inNipponbare. After heading, CGR decreased in both cultivars and fertilizer methods. The CGR after heading was consistently higher in standard and high fertilizer methods, especially at late ripening stage. Higher CGR after heading were responsible for the larger dry matter production.

NAR tended to be lower after transplanting and it differed by fertilizer method. NAR decreased with applied nitrogen fertilizer. However, it tended to be higher in standard and high fertilizer methods at the late ripening period.

Higher nitrogen application increased the number of stems with larger leaf area in both rice cultivars. The mean LAI increased after transplanting and it was largest at 76 DAT in both cultivars. In Takanari, deep fertilizer application method indicated the largest mean LAI (7.87 m^2 m^{-2}), whereas high fertilizer application (using slow release fertilizer) showed the largest mean LAI (8.29 m^2 m^{-2}) in Nipponbare. After heading, mean LAI decreased in all fertilizer application methods. At late ripening stage, deep fertilizer application method also showed the highest mean LAI values among the fertilizer applications in both cultivars.

2.3.3. Yield and yield components

Nitrogen fertilizer application methods had significant effects on grain yield, but the differences by fertilizer method were not significant (Table 3).

Grain yields without nitrogen application were 451 g m^{-2} and 706 g m^{-2} in Nipponbare and Takanari, respectively. The more nitrogen fertilizer is applied to rice, the more the grain yield increases. The grain yield of Takanari with deep fertilizer application method exceeded 863 g m^{-2} with the highest sink capacity (945 g m^{-2}), followed by conventional fertilizer application method (with grain yield 800 g m^{-2} and sink 854 g m^{-2}, respectively). The same tendency was observed in Nipponbare. Grain yield increased with more nitrogen fertilizer applied. Grain yield in high nitrogen fertilizer application (using slow-release fertilizer) exceeded 656 g m^{-2}, a little higher than deep fertilizer application method (644 g m^{-2}), due to the increased number of panicles. Higher grain yield and sink capacity in deep fertilizer method resulted from the larger number of spikelets per panicle and total spikelets m^{-2} in both rice cultivars.

Table 3. Yield and yield components of rice cultivars under different fertilizer application methods.

Cultivars	Methods	No. of panicles (panicle m^{-2})	No. of spikelets ($panicle^{-1}$)	No. of spikelets (10^3 m^{-2})	% ripened grains (%)	1000 grains weight (g)	Sink capacity (g m^{-2})	Grain yield (g m^{-2})
Nipponbare	0N	257 b	85 a	21.9 b	92.4 a	22.3 a	488 b	451 b
	Conventional method	368 a	82 a	30.2 a	93.2 a	22.4 a	676 a	630 a
	Deep fertilizer method	347 a	93 a	32.1 a	91.1 a	22.0 ab	707 a	644 a
	Standard fertilizer method	337 a	86 a	29.1 a	91.5 a	21.9 ab	638 a	590 a
	High fertilizer method	388 a	84 a	32.6 a	91.0 a	21.4 b	699 a	656 a
Takanari	0N	249 a	149 a	37.2 b	92.5 ab	20.5 b	763 b	706 b
	Conventional method	283 a	146 a	40.9 ab	93.7 a	20.9 ab	854 ab	800 a
	Deep fertilizer method	299 a	151 a	45.0 a	91.4 b	21.0 a	946 a	863 a

Means followed by the same letters are not significantly different at the 0.05 level by Tukey's test.

2.3.4. Nitrogen accumulation

Table 4 shows the accumulated nitrogen in rice cultivars with different fertilizer methods. The accumulated nitrogen varied among rice cultivars and fertilizer methods. Before heading stage, the amount of accumulated nitrogen tended to be larger in Takanari than Nipponbare. Deep fertilizer application method showed the largest amount of accumulated nitrogen in Takanari 16.4 g m^{-2}, followed by high fertilizer application method using slow release fertilizer in Nipponbare (15.8 g m^{-2}). In Nipponbare, the amount of accumulated nitrogen was significantly larger with deep fertilizer application than the other fertilizer methods, followed by high fertilizer application method (18.7 g m^{-2}). In Takanari, deep fertilizer application method also accumulated the larger amount of nitrogen (18.2 g m^{-2}) than conventional method (14.9 g m^{-2}), but the difference was not significant. Without nitrogen fertilizer application, accumulated nitrogen was higher in Takanari (10.8 g m^{-2}) than that in Nipponbare (8.0 g m^{-2}).

Table 4. Nitrogen accumulation in rice cultivars under different nitrogen fertilizer application methods

Cultivars	Methods	Days after transplanting (DAT)							
		0	20	34	48	62	76 *	90	104 **
Nipponbare	0N	0.14 a	1.6 c	2.6 d	5.8 e	6.6 d	7.4 d	7.5 e	8.0 d
	Conventional method	0.14 a	2.1 b	6.8 b	8.3 d	11.2 c	10.5 c	12.9 d	16.1 c
	Deep fertilizer method	0.14 a	2.1 b	5.7 c	9.2 c	12.6 b	16.0 a	19.2 a	24.0 a
	Standard fertilizer method	0.14 a	2.2 b	6.2 bc	9.9 b	11.5 bc	11.7 b	15.2 c	15.8 c
	High fertilizer method	0.14 a	2.7 a	9.4 a	14.0 a	15.8 a	15.4 a	17.3 b	18.7 b
Takanari	0N	0.08 a	1.6 b	3.6 b	5.0 b	6.1 c	7.1 c	8.0 c	10.8 b
	Conventional method	0.08 a	1.9 a	6.8 a	10.6 a	11.8 b	12.0 b	14.2 b	14.9 a
	Deep fertilizer method	0.08 a	1.9 a	6.5 a	10.7 a	16.4 a	18.6 a	19.5 a	18.2 a

Means followed by the same letters are not significantly different at the 0.05 level by Tukey's test.

Unit: g m^{-2}. *, **: heading and maturity stages, respectively.

2.3.5. Nitrogen use efficiency and some relative parameters

Nitrogen use efficiency and some relative parameters with different fertilizer application methods are presented in Table 5.

BE_N was varied among fertilizer and application methods and it tended to be lower with more nitrogen fertilizer application. BE_N varied from 104.0 to 134.9 g g^{-1} and 84.0 to 155.1 g g^{-1} in Takanari and Nipponbare, respectively. Standard fertilizer application method using slow-release fertilizer had higher biomass nitrogen use efficiency (108.9 g g^{-1}) than other fertilizer methods in Nipponbare.

RE_N reflected the capability of nitrogen accumulation from fertilizer application. In both rice cultivars, deep fertilizer application method showed the higher values than in other fertilizer methods, 159.5% and 75.7% in Nipponbare and Takanari, respectively. RE_N also decreased in high fertilizer method compared to standard fertilizer method using slow-release fertilizer (97.3%).

YE_N varied among fertilizer methods. In both rice cultivars, YE_N were highest with no nitrogen application, followed by the conventional method, and lowest in deep fertilizer method. YE_N was higher in Takanari than in Nipponbare at each fertilizer application method.

PFP_N was defined as the ratio of grain yield with nitrogen application, and it reflected the marginal effect of nitrogen absorbed by rice plant from N fertilizer. In both cultivars, PFP_N was higher in standard fertilizer application method (73.7 g g^{-1}) than the other methods in Nipponbare. Meanwhile, PFP_N was highest in standard fertilizer method than the other fertilizer methods in Nipponbare.

Table 5. Nitrogen use efficiency and some relative parameters

Cultivars	Methods	Grain yield ($g\ m^{-2}$)	Accumulated N ($g\ m^{-2}$)	BE_N ($g\ g^{-1}$)	RE_N (%)	YE_N ($g\ g^{-1}$)	PFP_N ($g\ g^{-1}$)
Nipponbare	0N	451	8.0	155.1	-	56.3	-
	Conventional method	630	16.1	92.2	81.0	39.1	63.0
	Deep fertilizer method	644	24.0	71.1	159.5	26.9	64.4
	Standard fertilizer method	590	15.8	108.9	97.3	37.3	73.7
	High fertilizer method	656	18.7	84.0	66.4	35.2	41.0
Takanari	0N	706	10.8	134.9	-	65.6	-
	Conventional method	800	14.9	109.9	41.2	53.7	80.0
	Deep fertilizer method	863	18.2	104.0	75.7	47.1	86.3

BE_N; N use efficiency for biomass, RE_N; Recovery efficiency of applied N,

YE_N; Nitrogen use efficiency for grain yield, PFP_N; Partial factor productivity of applied N.

2.3.6. Relationship between sink capacity, grain yield and accumulated N

Sink capacity had a significant relationship with grain yield (Fig. 6a). Higher sink capacity contributed to higher grain yield in Nipponbare and Takanari. In both cultivars, deep fertilizer application method had larger sink capacity than the other fertilizer methods. Close relation was found between accumulated N at heading and sink capacity but the sink capacity with the same level of accumulated N in Takanari was larger than that in Nipponbare (Fig. 6b). Sink capacity of Takanari was larger than that in Nipponbare with each fertilizer method. The more nitrogen accumulated, the higher the sink capacity produced. Especially with deep fertilizer application method in Takanari, the largest nitrogen accumulation contributed to the higher sink capacity.

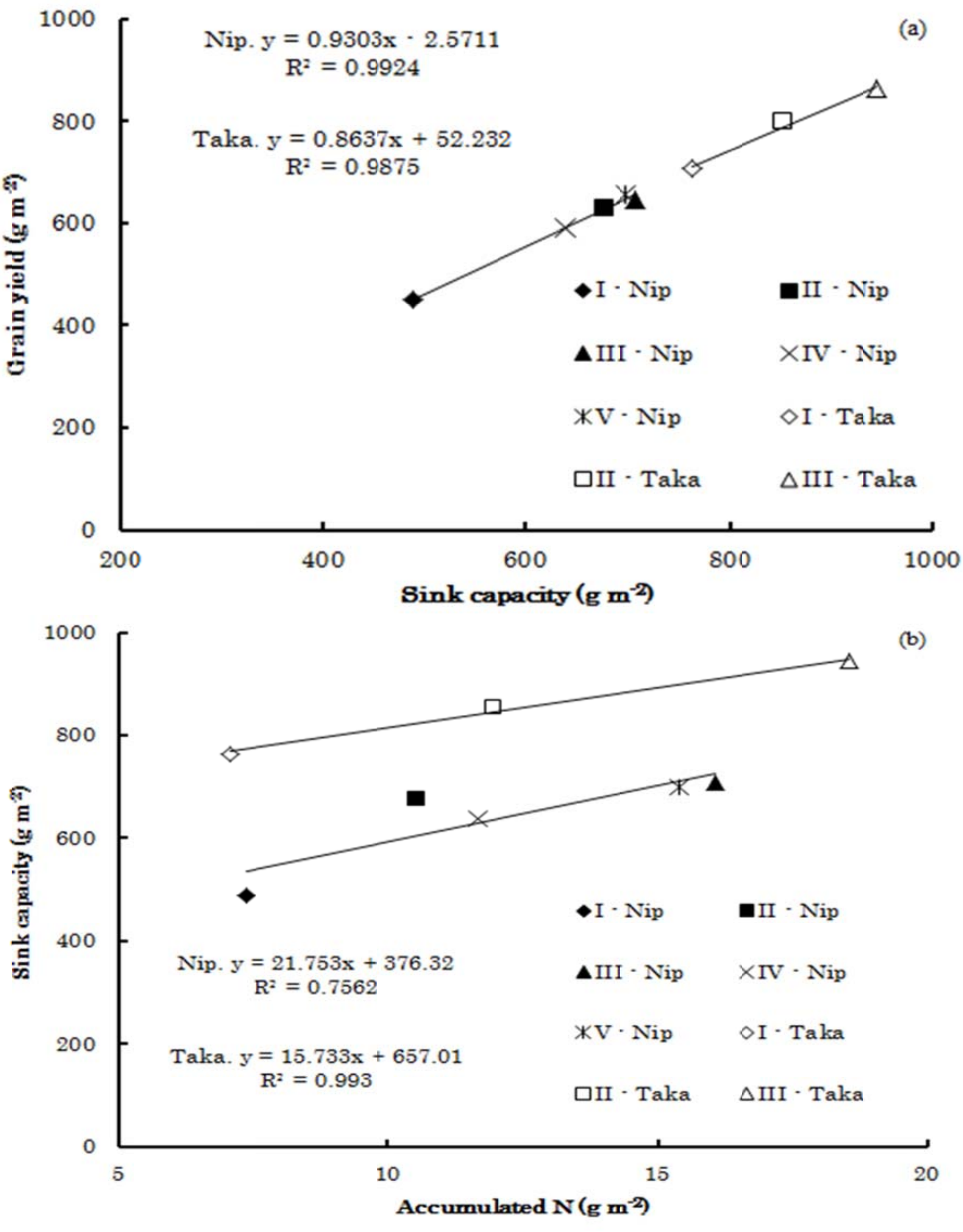


Fig. 6. Relationship between sink capacity and grain yield (a), accumulated N at heading and sink capacity (b) in Nipponbare and Takanari with different fertilizer methods.. (I; 0N, II; Conventional method, III; Deep fertilizer method, IV; Standard fertilizer method, V; High fertilizer method).

2.4. Discussion

In recent years, rice grain yield has increased markedly due to the improvement of rice cultivars and cultivation methods. In this study, we compared the effects of different fertilizer application methods on dry matter production, N accumulation and yield in rice cultivars. With more nitrogen fertilizer application, the rice plant can absorb and produce more dry matter, resulting in higher grain yield (Ju et al., 2009; Lin et al., 2009; Fukushima et al., 2011a). Previously, it was reported that nitrogen fertilizer plays an important role in increases dry mater and rice yield (Mohammad et al., 2002; Nakano and Morita, 2009; Taylaran et al., 2009), because it increases the photosynthetic rate and thus dry matter especially at the heading and ripening stages (Mohammad et al., 2002; Hayashi et al., 2012). In this study, plant dry weight increased with different nitrogen fertilizer applications (Table 2). Plant dry weight showed the higher values in deep fertilizer, standard fertilizer and high fertilizer application methods than both conventional method and control (0N). Takanari produced the highest dry matter production (1891 g m^{-2}) among rice cultivars and fertilizer methods. However, in Nipponbare, the highest plant dry weight was obtained with standard fertilizer method, followed by deep fertilizer method (Table 2). Thus, deep fertilizer application method is effective to obtain a high dry matter production in rice cultivars, and methods using slow-release fertilizer might have the potential to get higher dry matter production in rice cultivars.

With differing nitrogen fertilizer dose and timing of application, CGR and mean LAI tended to be higher with high nitrogen fertilizer applied (Lin et al., 2009; Taylaran et al., 2009). In the present study, CGR and mean LAI showed the highest values during 62 –

76 DAT. Deep fertilizer method had a higher CGR at this stage, 29.7 g m^{-2} d^{-1} and 25.5 g m^{-2} d^{-1} in Takanari and Nipponbare, respectively (Fig. 5). Similarly, the mean LAI in all fertilizer methods tended to be significantly higher than control, especially with deep fertilizer method and high fertilizer method (slow-release fertilizer), and CGR and mean LAI showed the maximum at the heading and flowering stages. Plants with higher CGR and mean LAI during this stage might be related with higher grain yield at harvest.

Rice plant can absorb and accumulate more nitrogen under high nitrogen applied condition. Accumulated N was remarkably affected by nitrogen fertilizer application and cultivation method (Okumura et al., 1982; Lin et al., 2009; Fukushima et al., 2011a). The present study showed that nitrogen uptake and accumulation increased consistently from transplanting to harvesting time. At the same time, nitrogen accumulated was lowest in control (0N) and higher in the other methods, especially with deep fertilizer methods (24.0 g m^{-2}) in Nipponbare. This means that plants can uptake and use more nitrogen when the fertilizer is applied deep and near in the root systems. This method is easy for rice plant uptake nitrogen fertilizer and produces higher sink capacity (Fig. 10b). Using slow-release fertilizer, plants can uptake and accumulate more nitrogen compared with other methods, especially in Nipponbare (Table 4).

Grain yield of rice is the product of different yield components. The panicle density is the most important component in determining grain yield (Fageria and Barbosa Filho, 2001; Artacho et al., 2009). Okumura reported that deep fertilizer application increased the number of spikelets m^{-2} and number of spikelets per panicle which leads to increasing yield. Wada and Wang also concluded with the same result, but confirmed that grain yield increased 10% to 13% compared with conventional methods. In this study, the number of panicles increased with different fertilizer application methods. The number of panicles ranged from 249 to 388 which was significantly higher than in the control (0N). High fertilizer application method produced higher number of panicles than other method in Nipponbare. On the other hand, the number of spikelets m^{-2} significantly increased with fertilizer application method. Deep fertilizer application showed the highest number of spikelets (45,000) in Takanari, and high fertilizer application method produced the highest number of spikelets (32,000) in Nipponbare. Higher yield components contributed to higher sink capacity and higher grain yield is determined by higher sink capacity (Fig. 6a). It indicated that larger sink capacity is one of the factors that determine grain yield.

YE_N decreased more among the other fertilizer application methods than in the control (0N). These results are similar with the other studies reported for japonica cultivars (Peng et al., 2006; Artacho et al., 2009). YE_N tended to be higher in Takanari than in Nipponbare. This was due to the higher amount of accumulated N resulting in larger sink capacity relative to accumulated N, and then increased the yield in Takanari. Both deep fertilizer application and basal application of slow release fertilizer increased the recovery efficiency and partial factor productivity of applied N. Using slow release fertilizer is recommended in terms of labor saving and lower cost.

Conclusion

Nitrogen fertilizer application methods were able to significantly increase dry matter production, panicle m^{-2}, total spikelet m^{-2}, grain yield, and nitrogen accumulation at harvesting time. Deep fertilizer application is a good way for rice cultivar uptake of more nitrogen fertilizer. However, using slow-release fertilizer might be suitable for modern rice cultivation because of labor saving and lower cost. Higher grain yield can be explained by higher dry matter production, higher nitrogen accumulated at harvesting and larger sink capacity.

Summary

To examine the effects of different types of fertilizer and application method on dry matter production, yield, nitrogen accumulation and use efficiency in rice cultivars, we used two rice cultivars (Nipponbare and Takanari) and five fertilizer methods, i.e. Control (0N), Conventional method, Deep fertilizer method, Standard fertilizer method and High fertilizer method in 2009. Dry matter production was more markedly increased with nitrogen fertilizer application than in control, and it was higher with deep fertilizer application in Takanari and standard fertilizer application in Nipponbare, respectively. The differences in dry matter production resulted from CGR and mean LAI in rice cultivars. Greater dry matter production was accompanied with the nitrogen accumulation at harvesting. Rice cultivars accumulated the largest amount of nitrogen at deep fertilizer application. Higher fertilizer application increased the number of panicle and total spikelets m^{-2}. The higher grain yield in Takanari resulted from the larger sink capacity. The grain yield of rice cultivars tended to be higher with deep fertilizer application due to the increase in sink capacity. Both deep fertilizer application and slow-release fertilizer increased the recovery efficiency and partial factor productivity of applied N, however, using slow-release fertilizer is recommended in term of labor saving and lower cost.

Chapter 3.

Cultivar differences in nitrogen use efficiency of field grown rice plant at different levels of nitrogen fertilizer.

3.1. Introduction

Rice is one of the most important cereal crops, widely grown in many localities throughout the world with favorable climatic conditions throughout the world (Yoshida, 1981; James, 1983). Rice is the staple food for more than a half of the world's population (Jackson et al., 1996; IRRI, 2002). Rice production occupies 27% of total cereal production in the world and 90% of total was produced in Asian countries (Mae et al., 2006; Artacho et al., 2009).

Nitrogen (N) plays an important role in irrigated rice production around the world (Cassman et al., 1998; Fageria and Barbosa Filho, 2001; Samonte et al., 2006). Nitrogen uptake is affected by various factors, such as soil types, weather conditions, type of fertilizers and application methods (Mae et al., 2006; Artacho et al., 2009). Rice plants require N during vegetative stage to raise growth and tiller numbers, which determines potential number of panicles. Nitrogen not only contributes to spikelets production during early formation stage but also contributes to sink size during the late panicle stage (Mae, 1997). Nitrogen also affects on increasing leaf area index and photosynthetic rate which consequently increases the dry mater production and grain yields (Arima, 1995).

In the past, to satisfy the human's food demand, farmers applied more fertilizer especially nitrogen fertilizers in the efforts to enhance rice yield (Matsushima, 1980).

However, heavy N application increases not only the costs of production and labors but also affects the environments (Hasegawa and Horie, 1996; Rivers et al., 1996). Therefore, we need to develop rice cultivation method that would be harmony between productions and environmental protection. One solution for this problem is using high yielding rice cultivars and slow release fertilizer.

In some studies, N use efficiency (NUE) is defined as agronomic N use efficiency (ANUE) (Peng et al., 2006), and also called internal NUE (Koutroubas and Ntanos, 2003), which was calculated by grain yield increasing with N application in comparison with no N application in relation to total N uptake. Recently, NUE has tended to be lower at different ecosystems because of an excessive amount of N fertilizer application to satisfy the food demands (Peng et al., 2006). Improving NUE in rice cultivation to get a stable and high yield, therefore, is necessary.

Many studies have attempted to evaluate the effects of slow-release fertilizer on N fertilization methods, planting time and densities on dry matter production at heading and after heading (Mohammad et al., 2002; Taylaran et al., 2009; Fukushima et al., 2011a; Fukushima et al., 2011b) and effects of N fertilizer on NUE under different soil conditions (Ye et al., 2007). Although the studies focused on NUE in rice cultivars, there is still little understanding about N use efficiency in high yielding rice cultivars and their relatives to dry matter production and yield components.

Thus, this study has been undertaken in order to evaluate the dry matter production, N accumulation, NUE and grain yield of high-yielding rice cultivars under different N fertilization levels.

3.2. Materials and methods

3.2.1. Plant materials and cultivation

Six high yielding rice cultivars (Koshihikari, Nipponbare, Takanari, Momiroman, Hinohikari and Akimasari) and the other two (Hokuriku 193 and Mizuhochikara) were used in 2011 and 2012, respectively to investigated the effects of different N fertilization levels on dry matter production, yield and nitrogen use efficiency. Heading and maturing time were got earlier in the order of Koshihikari, Nipponbare, Takanari, Hokuriku 193, Hinohikari, Momiroman, Mizuhochikara and Akimasari (Table 6).

Table 6. Dates of heading time in rice cultivars with different N levels in 2011 and 2012.

Cultivars	Notation	2011			2012		
		0N	1N	2N	0N	1N	2N
Koshihikari	Koshi	Aug. 10	Aug. 11	Aug. 13	Aug. 8	Aug. 8	Aug. 10
Nipponbare	Nippon	Aug. 20	Aug. 20	Aug. 20	Aug. 18	Aug. 18	Aug. 18
Takanari	Taka	Aug. 19	Aug. 19	Aug. 20	Aug. 19	Aug. 19	Aug. 27
Momiroman	Momi	Aug. 29	Aug. 29	Aug. 29	Aug. 31	Aug. 31	Sep. 2
Hinohikari	Hino	Aug. 27	Aug. 27	Aug. 27	Aug. 26	Aug. 26	Aug. 26
Akimasari	Aki	Sep. 5	Sep. 5	Sep. 7	Sep. 3	Sep. 3	Sep. 6
Hokuriku 193	Hoku	-	-	-	Aug. 26	Aug. 26	Aug. 28
Mizuhochikara	Mizuho	-	-	-	Aug. 31	Aug. 31	Sep. 3

Rice plants were grown in the experimental fields of Field Science Center, Okayama University in 2011 and 2012. The experiment was arranged in the split-plot design with three N levels (0N: 0 gN m^{-2}; 1N: 8 gN m^{-2}; 2N: 16 gN m^{-2}) in the main-plots and rice cultivars in the sub-plots. In main-plots, slow release fertilizer (100D-80 and 140E-80; N : P_2O_5 : K_2O = 14 : 14 : 14) were used and incorporated into the soil at a rates of 8 gN m^{-2} and 16 gN m^{-2} at 1N and 2N plots as a basal fertilizer, respectively. At 0N plot, phosphate (8 gP_2O_5 m^{-2}) and potassium chloride (8 gK_2O m^{-2}) were applied at the same time with N application.

Seeds of rice cultivars were sown on 16 May in both years and the rice seedlings with 5 leaf expansion were transplanted to the field at a hill spacing of 15 cm and a row spacing of 30 cm (22.2 hill m^{-2}, three plants per hill) on 14 June and 12 June, in 2011 and 2012, respectively. During two years, pests and diseases were controlled for optimum growth.

3.2.2. Growth analysis and nitrogen use efficiency

Eight hills for each replication were sampled at the panicle initiation (20 days before heading), heading and harvesting time (45 days after heading), and four hills with an average numbers of stems were selected to measure dry weight of whole plant. Plant samples were separated into leaves, stems, panicles and dead leaves. After separation, leaf area was measured by automatic area meter (AAM-9; Hayashi Denko Co., Tokyo, Japan). After that, all plant samples were dried in a ventilated oven at 80^0C until constant weight.

At the harvesting time, about one square meter sample (20 hills) for each replication was harvested to determine the yield and yield components. Yield was identified by brown rice weight. Fully ripened grains were selected by sieving through 1.8 mm and 1.6 mm mesh (Hokuriku 193 and Takanari). Thousand grain weights were measured by using brown grain and adjusted to a moisture content of 14.5%.

At each stage, eight hills with an average number of stems were selected in which two hills were used for the determination of accumulated N in a whole plant. All plant parts were ground into powder by vibrating mill machine (Heiko, Co. Ltd) and nitrogen concentration in each plant parts were analyzed by CN-Corder (MT-700, Yanaco Industry). Total accumulated N was calculated by multiplying the above-ground dry weight by N concentration.

On the basis of these measurements, NUE indices were calculated (Artacho et al., 2009; Ju et al., 2009).

Harvest Index (HI) (%) = Grain yield X 0.865 / dry matter production x 100%.

N use efficiency for biomass (BE_N) = BY / AN.

Recovery efficiency of applied N (RE_N) = ($AN – AN_0$) / APN.

Nitrogen use efficiency for grain yield (YE_N) = GY / AN.

Partial factor productivity of applied N (PFP_N) = GY / APN.

Where BY; Biomass yield (g m^{-2}), GY; Grain yield (g m^{-2}), AN; Accumulated N (g m^{-2}), AN_0; Accumulated N without fertilizer (g m^{-2}), APN; Applied N (g m^{-2}).

Factorial analyses of variances were performed to detect the difference among treatment by ANOVA. The significance levels of mean values were analyzed using Tukey's test.

3.3. Results

3.3.1. Dry matter production and leaf area index

Dry matter production of rice cultivars was significantly different in cultivars and N levels (Fig. 7). The dry matter production of rice cultivars was greater in 1N and 2N levels than 0N level in both years. At the panicle initiation stage (PI), dry matter production of Koshihikari is significantly smaller than the other rice cultivars in both years, while, Akimasari constantly produced higher dry matter than the other cultivars in 1N and 2N levels in accordance with the longer growth duration. At harvesting time, there were no differences between 1N and 2N levels in all cultivars. With N application from 0N to 1N levels, dry matter production sharply increased. However, the difference between 1N and 2N levels was not significant in 2011. Increasing the amount of N application from 8 g m^{-2} to 16 g m^{-2} did not mean increasing too much the dry matter in rice cultivars. Dry matter production of Takanari and Akimasari became higher than the other cultivars, which were 1874 and 1851 gm^{-2} at the harvesting, respectively.

The same trend was observed in 2012, the plant dry matter production in 1N and 2N was higher than 0N level. Koshihikari consistently produced the smallest amount of dry matter in comparison the other cultivars. While, Hokuriku 193 consistently produced a larger amount of dry matter production at 2N level, followed by Mizuhochikara and Akimasari.

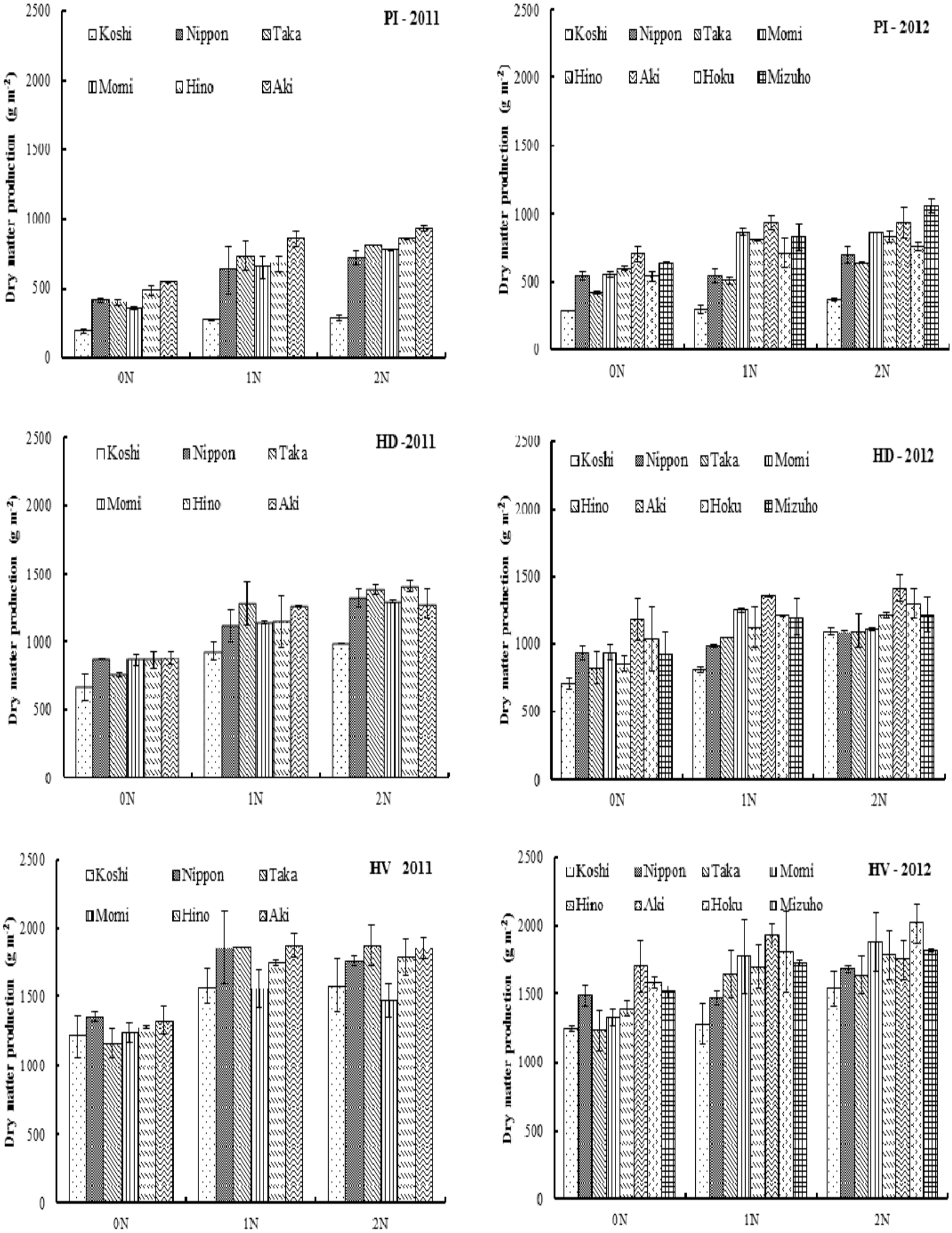
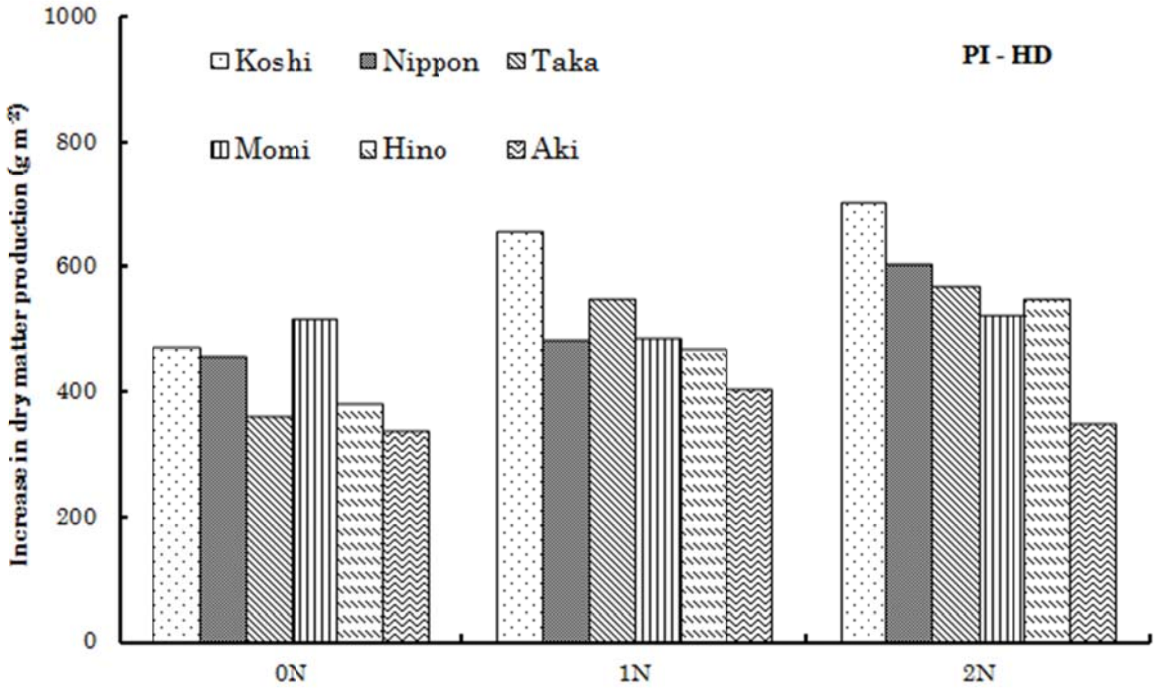


Fig. 7. Dry matter production in rice cultivars at panicle initiation (PI), heading (HD) and harvesting (HV) stages with different N levels in 2011 and 2012. The bars indicate the SE.



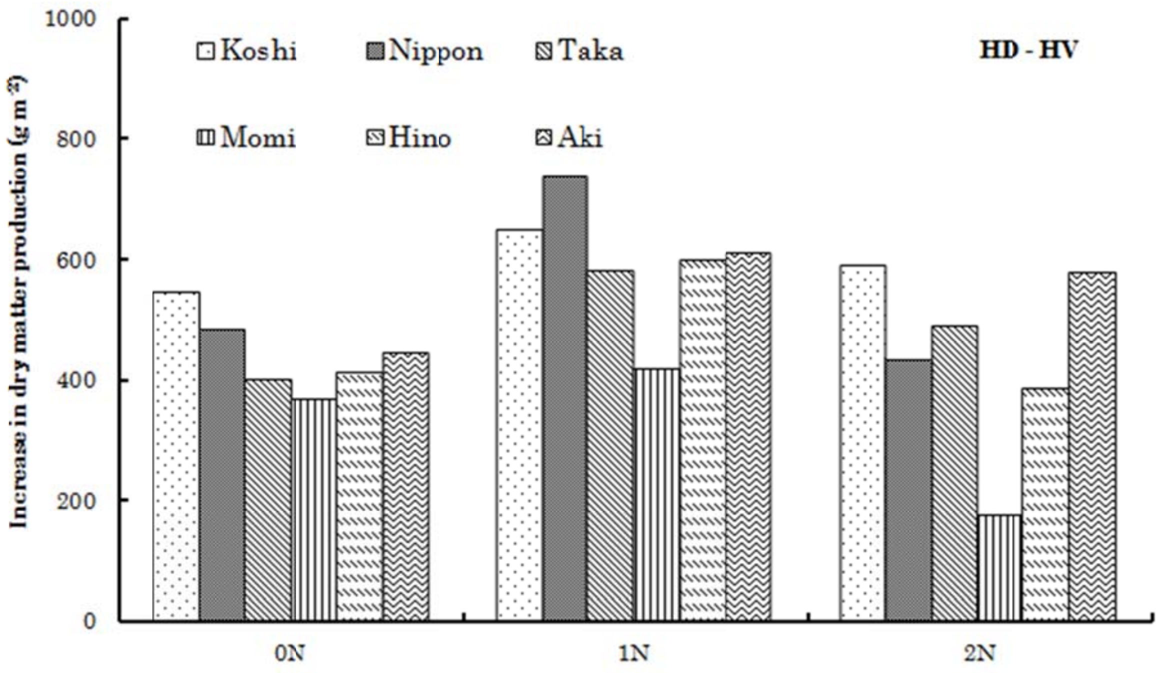
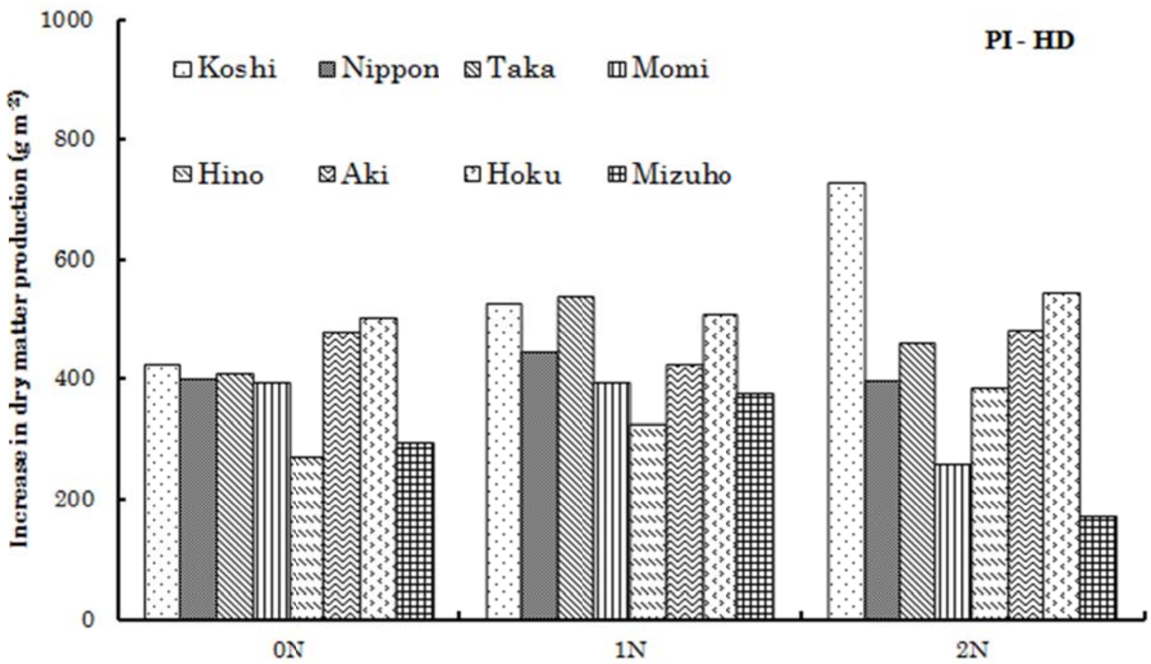


Fig. 8. Increase in dry matter production from PI to HD, and from HD to HV in rice cultivars in 2011.



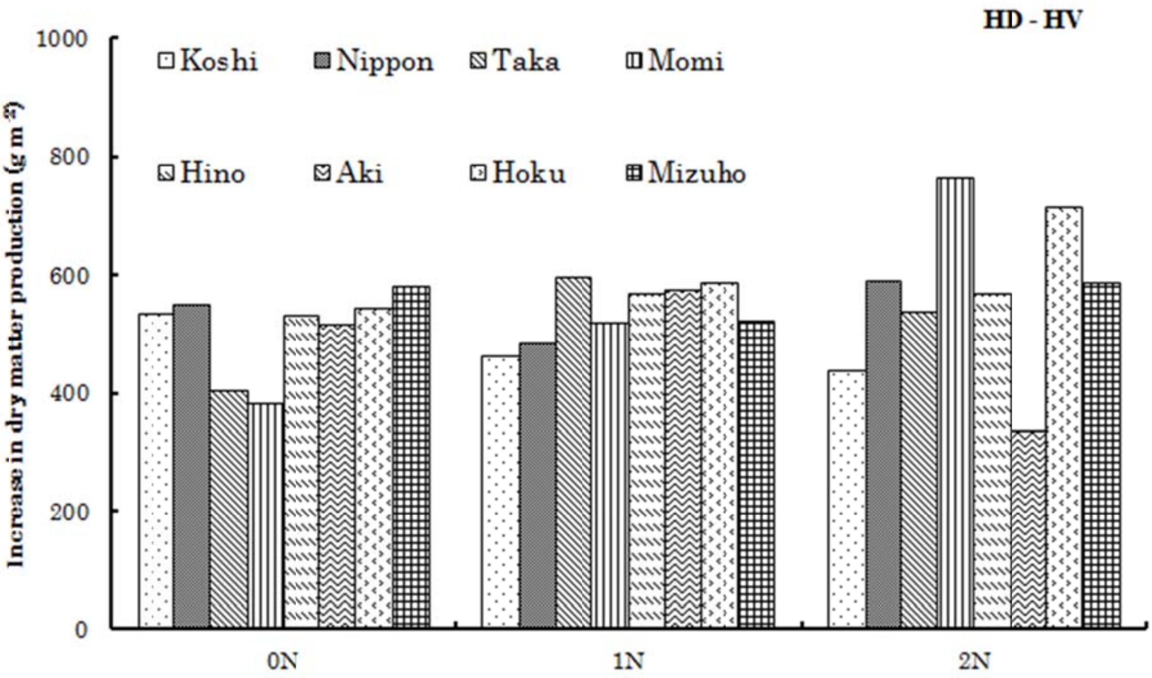
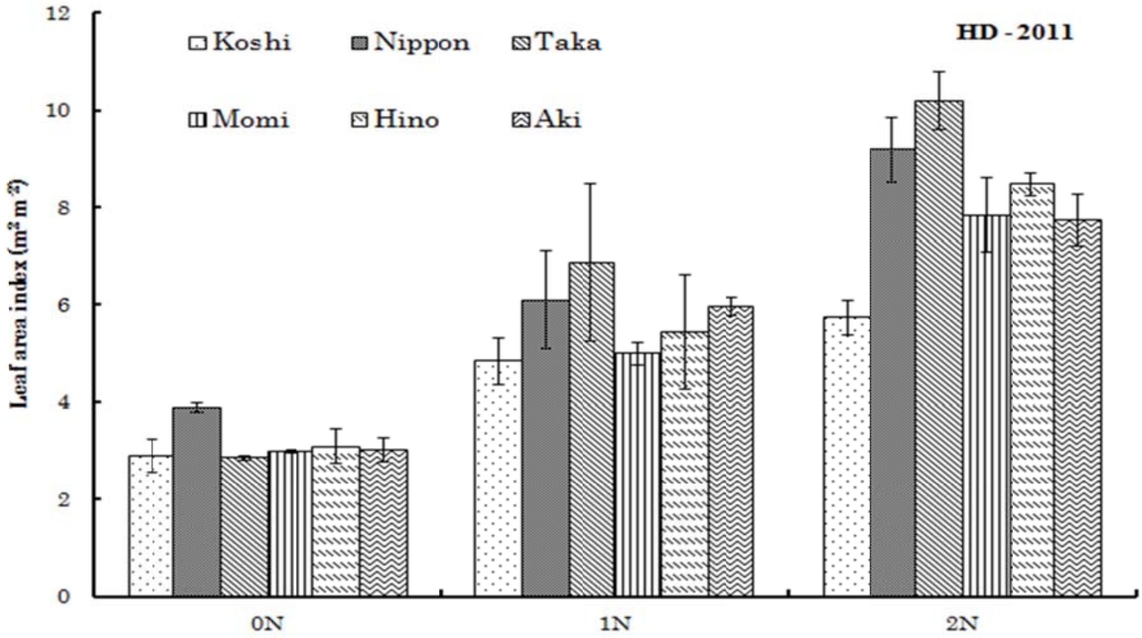


Fig. 91. Increase in dry matter production from PI to HD, and from HD to HV in rice cultivars in 2012.



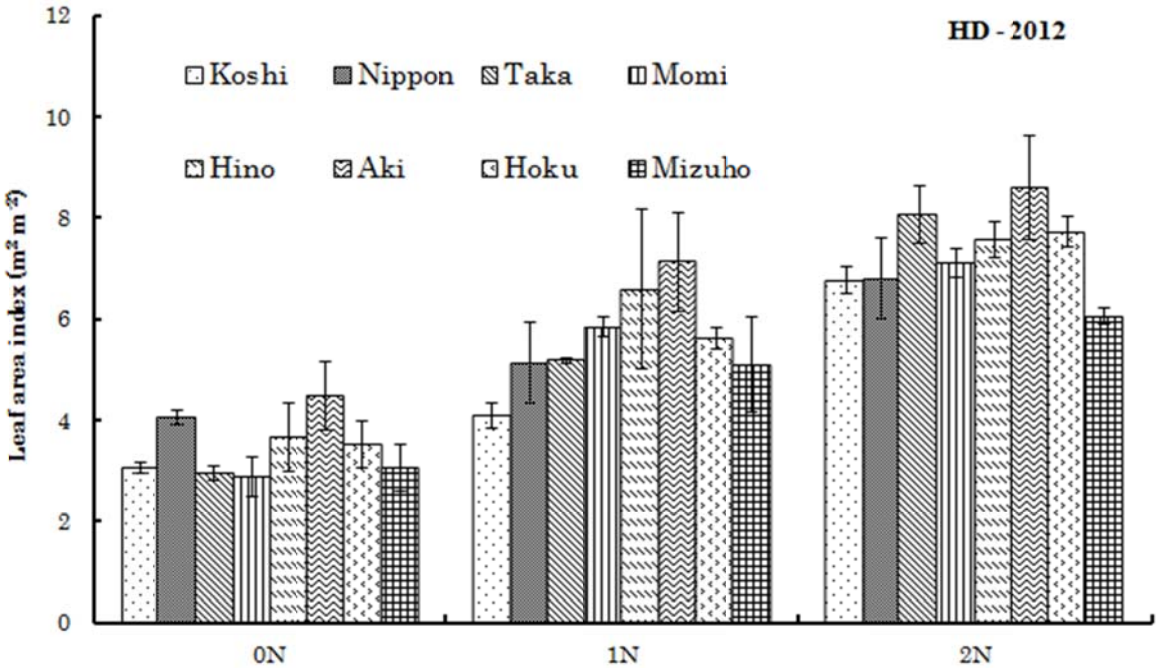


Fig. 10. Leaf area index (LAI) in rice cultivars at heading under different N applications in 2011 and 2012. The bars indicate the SE.

The increases in dry matter production from PI to HD and from HD to HV during two year experiments are clearly shown in Fig. 8 and Fig. 9. In 2011, the increase in dry matter production from PI to HD was higher in Koshihikari than the other cultivars. However, from HD to HV, the increase in dry matter production of Momiroman was lowest especially at 2N level, which resulted in lowest dry matter production at the harvesting than other rice cultivars (Fig. 8). At this stage, Takanari consistently showed a larger increase in dry matter production than other rice cultivars at 1N level.

In 2012, Koshihikari also showed the higher in increase dry matter production from PI to HD. However, from HD to HV, Momiroman and Hokuriku 193 at 2N level showed a larger increase in dry matter production than other rice cultivars.

To identify the factors affected the differences in dry matter production, leaf area index (LAI) among rice cultivars under different N levels was compared (Fig. 10). Higher nitrogen fertilizer application increased the number of tillers with larger leaf area in rice cultivars. LAI of rice cultivars were the largest at 2N, followed by 1N and 0N. LAI also increased after transplanting and reached maximum at heading. Takanari showed the largest LAI (10.2 m^2 m^{-2}) in 2011, followed by Nipponbare (9.2 m^2 m^{-2}), Hinohikari (8.5 m^2 m^{-2}). In 2012, Akimasari showed the largest LAI (8.6 m^2 m^{-2}), followed by Takanari (8.1 m^2 m^{-2}) and Hokuriku 193. In 2011, the LAI of Koshihikari (5.7 m^2 m^{-2}) was significantly smaller than other cultivars. However, Mizuhochikara showed the smallest LAI (6.1 m^2 m^{-2}) at 2N in 2012 than other cultivars. Plant with higher dry matter production and LAI might have the potential for higher grain yield.

3.3.2. Grain yield and yield components

Yield and yield components in rice cultivars in both years are listed in Table 7 and Table 8. N fertilization had the significant impacts on grain yield components, such as number of panicles m^{-2} and percentage of ripened grain.

Grain yield of rice cultivars at 1N level was 21.6 – 23.6% in 2011 and 8.0 – 15.7% in 2012 higher than 2N and 0N levels, respectively. During two years experiments, the number of panicles m^{-2} increased with N fertilizer application, which was in the order of 2N > 1N > 0N. The number of panicles m^{-2} in Takanari, Momiroman, Hokuriku 193 and Mizuhochikara was significantly lower than the other rice cultivars in both years. The highest number of panicles m^{-2} in Takanari and Momiroman was 274 and 229 panicles, in 2011, respectively, and those in Takanari, Momiroman, Hokuriku 193 and Mizuhochikara was 248, 274, 257 and 267 in 2012, respectively. The number of panicles in these cultivars was significantly lower than the other cultivars. However, the number of spikelets per panicle in four rice cultivars "panicle weight-type cultivars", was considerably higher and resulted in a significantly great number of spikelets m^{-2}. The number of spikelets m^{-2} in Takanari exceeded by 48,900 at 1N in 2011, enormously higher than the other cultivars in both years. In 2012, Takanari also showed the highest number of spikelets (46,100) at 1N, significantly higher than the other cultivars.

The rice grain yields in 1N level were higher than those in 2N and 0N levels during two years mainly due to the increase in lodging score. The grain yield of Takanari was highest (750 and 731 g m^{-2} in 1N) during 2 years, followed by Hokuriku 193 (669 g m^{-2} in 2012), Akimasari (597 g m^{-2} in 2011) and Hinohikari (594 g m^{-2} in 2012). However, the grain yield of Momiroman in 0N level was higher than 1N and 2N levels. Although this cultivars had a higher number of spikelets per square meter (45,200 in 2011), high N fertilizer application increased the lodging score, and decreased the percentage of ripened grains causing the lower grain yield especially in Momiroman.

The grain yield of Takanari and Hokuriku 193 exceeded by 560 g m^{-2}, significantly higher than the other rice cultivars without N fertilization in 2012. Grain yield at 0N level in Takanari and Hokuriku 193 was also higher due to the higher number of spikelets m^{-2} than the other rice cultivars.

Table 7. Grain yield and yield components of rice cultivars under different N levels in 2011.

Cultivars	N levels	No. of panicles (m^{-2})	No. of spikelets/panicle	No. of spikelets (x 10^3 m^{-2})	Percentage of ripened (%)	1000 grain weight (g)	Grain yield (g m^{-2})	Sink capacity (g m^{-2})	Lodging score
Koshihikari	0N	225 b	104 d	23.4 b	85.7 a	21.8 c	437 abc	511 bc	0.3
Nipponbare		243 a	92 e	22.4 bc	80.2 b	22.7 b	408 bc	509 bc	0.0
Takanari		199 c	152 b	30.2 a	81.7 b	18.2 d	449 abc	550 b	0.0
Momiroman		153 d	186 a	28.5 a	67.5 c	22.6 b	434 abc	643 a	0.0
Hinohikari		236 ab	86 e	20.2 c	85.3 a	23.3 a	402 c	471 c	0.0
Akimasari		203 c	113 c	23.0 b	85.4 a	23.1 a	454 a	532 b	0.0
Average		210	122	24.6	81.0	22.0	431	536	-
$LSD_{0.05}$		7.3	3.2	1.2	1.0	0.1	18.7	25.0	-
Koshihikari	1N	314 ab	110 c	34.6 c	59.3 c	21.8 b	448 d	754 bc	2.7
Nipponbare		336 a	100 d	33.5 c	69.7 b	22.0 b	511 cd	737 bc	1.3
Takanari		271 c	181 b	48.9 a	78.1 ab	19.6 c	750 a	960 a	0.0
Momiroman		220 d	192 a	42.2 b	37.1 d	21.2 b	324 e	895 a	0.3
Hinohikari		329 a	90 e	29.5 d	82.2 a	23.7 a	566 bc	689 c	0.0
Akimasari		300 b	115 c	35.6 c	75.4 ab	22.3 ab	597 b	793 b	0.3
Average		295	131	37.4	67.0	21.8	533	805	-
$LSD_{0.05}$		10.6	4.2	1.6	4.3	0.6	34.8	41.5	-
Koshihikari	2N	335 b	88 d	29.4 d	54.7 b	21.3 b	343 c	628 d	4.6
Nipponbare		365 ab	101 d	36.8 bc	59.4 b	21.3 b	466 b	785 bc	4.0
Takanari		274 c	176 b	48.2 a	71.7 a	20.0 c	690 a	963 a	0.3
Momiroman		229 d	198 a	45.2 a	21.9 c	21.5 b	211 d	974 a	1.0
Hinohikari		380 a	90 d	34.2 c	55.5 b	22.1 a	417 bc	754 c	0.3
Akimasari		343 ab	125 c	39.9 b	58.4 b	21.6 b	501 b	861 b	1.0
Average		321	130	39.0	53.6	21.3	438	827	-
$LSD_{0.05}$		18.7	7.8	1.7	4.5	0.2	40.6	36.9	-
N levels (A)		**	**	**	**	**	**	**	-
Cultivars (B)		**	**	**	**	**	**	**	-
A x B		*	**	**	**	**	**	**	-

Values within a column for each N levels followed by the same letter are not significantly different at the 0.05 probability level by Turkey's test (n =3). * and **, significant at the 0.05 and 0.01 probability level.
The lodging score was visually evaluated on a scale of none (0), very little (1), little (2), medium (3), much (4), and too much (5)

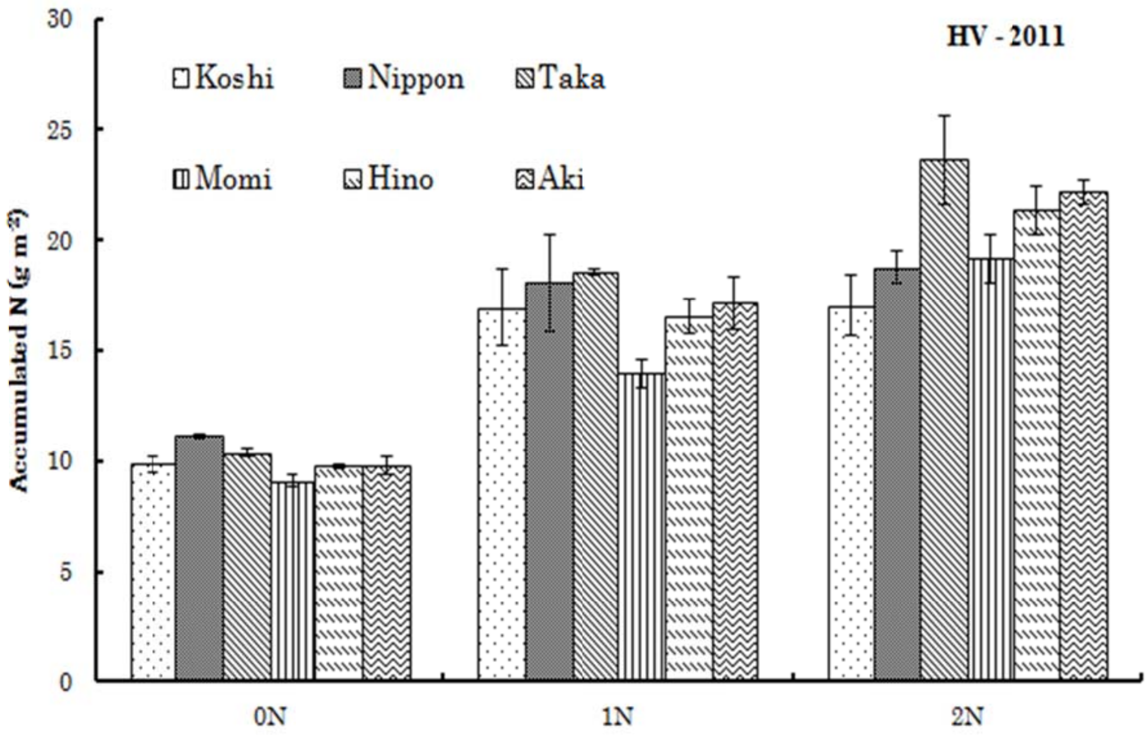
Table 8. Grain yield and yield components of rice cultivars under different N levels in 2012.

Cultivars	N levels	No. of panicles (m^{-2})	No. of spikelets/panicle	No. of spikelets (x 10^3 m^{-2})	Percentage of ripened (%)	1000 grain weight (g)	Grain yield ($g\ m^{-2}$)	Sink capacity ($g\ m^{-2}$)	Lodging score
Koshihikari	0N	284 a	93 e	26.4 c	79.4 de	20.3 b	427 def	538 bc	2.0
Nipponbare		282 a	90 e	25.5 c	89.6 abc	22.2 a	507 bc	567 ab	0.7
Takanari		221 c	157 b	34.5 a	94.2 a	17.3 c	562 ab	597 ab	1.0
Momiroman		177 d	154 b	27.1 c	77.8 e	21.9 a	463 cde	594 ab	0.0
Hinohikari		248 b	86 e	21.3 d	88.1 bc	22.4 a	420 ef	477 cd	1.0
Akimasari		235 bc	113 d	26.5 c	84.4 cd	21.9 a	491 cd	582 ab	0.3
Hokuriku 193		181 d	171 a	31.0 b	93.6 ab	20.3 b	589 a	630 a	1.0
Mizuhochikara		183 d	135 c	24.8 c	79.1 de	18.9 b	371 f	468 d	1.0
Average		226	125	27.1	85.8	20.7	479	556	-
$LSD_{0.05}$		8.0	6.7	1.3	2.7	0.7	31.8	32.0	-
Koshihikari	1N	367 a	94 f	34.5 bc	64.2 d	20.8 c	459 c	719 bc	3.7
Nipponbare		346 a	88 f	30.5 de	82.2 b	22.1 a	554 b	674 c	2.7
Takanari		238 cd	195 a	46.1 a	86.2 b	18.4 f	731 a	848 a	1.7
Momiroman		256 c	142 c	36.3 b	57.9 d	21.0 c	442 c	763 b	1.0
Hinohikari		354 a	87 f	30.9 cde	86.5 b	22.2 a	594 b	688 bc	3.7
Akimasari		310 b	110 e	34.0 bcd	73.8 c	21.6 b	542 b	735 bc	1.7
Hokuriku 193		209 d	171 b	35.8 b	94.0 a	19.9 d	669 a	713 bc	3.3
Mizuhochikara		230 cd	125 d	28.8 e	79.6 bc	19.3 e	442 c	555 d	0.0
Average		289	127	34.6	78.1	20.7	554	712	-
$LSD_{0.05}$		16.7	4.7	1.8	3.2	0.2	29.4	36.2	-
Koshihikari	2N	406 a	100 d	40.9 a	64.2 b	20.7 b	541 ab	844 a	4.7
Nipponbare		377 a	95 d	35.6 ab	74.7 ab	21.8 a	580 ab	778 ab	4.0
Takanari		248 c	160 a	39.6 ab	61.7 bc	18.4 d	452 bc	728 ab	2.0
Momiroman		274 c	125 c	34.5 b	47.5 c	21.2 ab	339 c	732 ab	2.7
Hinohikari		395 a	90 d	35.5 ab	71.6 b	21.4 ab	542 ab	760 ab	2.7
Akimasari		321 b	127 c	40.6 ab	62.0 bc	19.7 c	490 ab	795 ab	4.3
Hokuriku 193		257 c	143 b	36.7 ab	88.1 a	19.0 cd	614 a	696 b	3.3
Mizuhochikara		267 c	140 b	37.5 ab	75.9 ab	19.0 cd	545 ab	714 ab	1.3
Average		318	123	37.6	68.2	20.2	513	756	-
$LSD_{0.05}$		20.8	6.0	3.0	7.0	0.5	60.7	60.3	-
N levels (A)		**	ns	**	**	**	**	**	-
Cultivars (B)		**	**	**	**	**	**	**	-
A x B		**	**	**	**	**	**	**	-

Values within a column for each N levels followed by the same letter are not significantly different at the 0.05 probability level by Turkey's test (n =3). * and **, significant at the 0.05 and 0.01 probability level.
The lodging score was visually evaluated on a scale of none (0), very little (1), little (2), medium (3), much (4), and too much (5)

3.3.3. Nitrogen accumulation

N application levels significantly affected the uptake and accumulation of nitrogen in each rice cultivar (Fig. 11). In 2N level, rice plants could take up and accumulate a larger amount of N than 0N and 1N levels in both years. The amount of accumulated N increased from PI and was the largest at the harvesting. In 2011, Takanari consistently accumulated the largest amount of N (23.6 g m^{-2}) in 2N, while Koshihikari showed the smallest value (17.0 g m^{-2}). Hinohikari accumulated the largest amount of N (25.7 g m^{-2}), followed by Akimasari (24.8 g m^{-2}) in 2012.



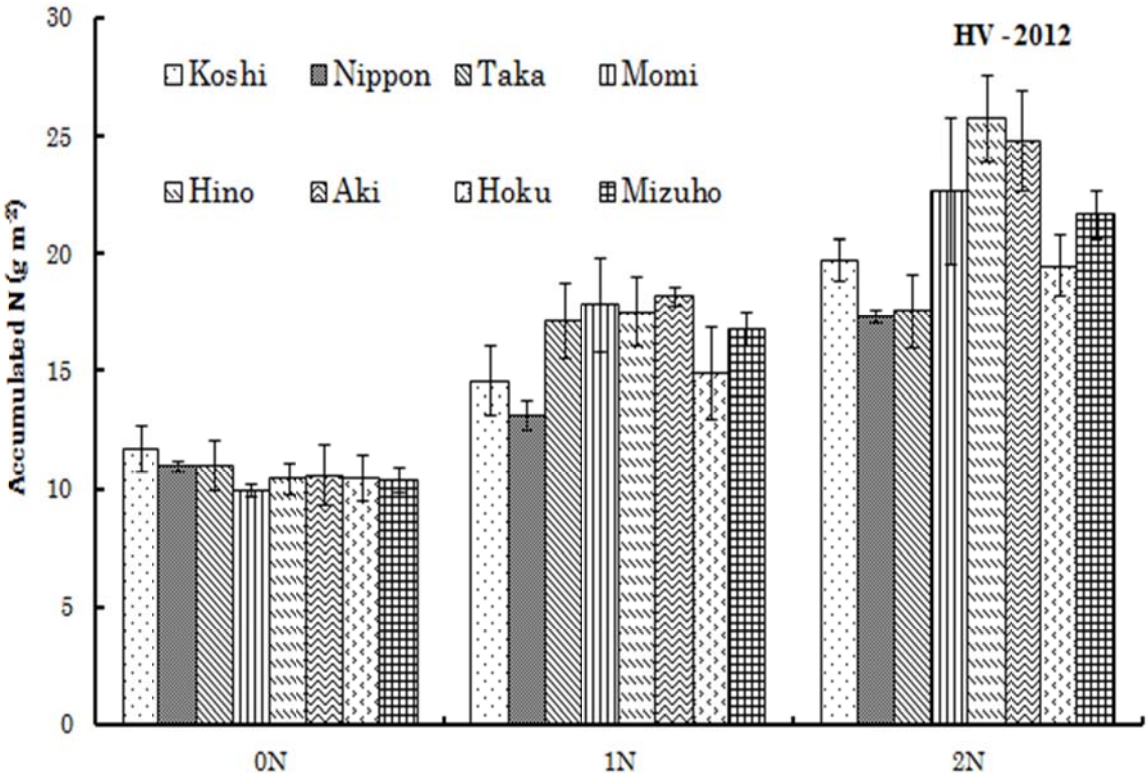


Fig. 11. Accumulated N in cultivars at harvesting time in 2011 and 2012. The bars indicate the SE.

3.3. 4. Nitrogen use efficiency and some relative parameters

N use efficiency and some relative parameters with different N fertilization levels were presented in Table 9 and Table 10. Nitrogen fertilization and cultivars affected significantly to BE_N, RE_N, YE_N and PFP_N. Interaction between N levels and cultivars were significant for all indices except for RE_N.

Harvest index (HI) of rice cultivars decreased with the increasing of N levels. HI in Takanari was significantly higher than the other rice cultivars in all N levels in both years except for 2N level in 2012. Higher HI contributed to higher grain yield in Takanari than the other rice cultivars.

N use efficiency for biomass (BE_N) tended to be lower with increasing in N levels. In 2011, BE_N were significantly higher in Momiroman at 1N, Nipponbare and Koshihikari at 2N level, respectively. However, in 2012, BE_N was higher in Hokuriku 193 than the other cultivars.

Recovery efficiency of N (RE_N) reflected the capability of N accumulation in rice cultivars. RE_N ranged from 45.0 – 101.8% and 27.4 – 98.2% in 2011 and 2012, respectively. RE_N in rice cultivars were significantly different in both years except 1N level in 2011.

N use efficiency for grain yield (YE_N) decreased with higher application of the N fertilizer and tended to be higher in 1N than 2N levels in both years. YE_N of Takanari and Hokuriku 193 at 0N (51.5 and 57.0 g g^{-1}) and 1N (43.0 and 45.4 g g^{-1}) were significantly higher than the other cultivars in 2012. These two cultivars showed the higher grain yield than the others.

Partial factor productivity for applied nitrogen (PFP_N) was defined as the ratio of grain yield with nitrogen application, which reflected the marginal effect of nitrogen absorbed by rice plant from N fertilizer. PFP_N was significantly higher in Takanari and Hokuriku 193 in both N levels and years than the other rice cultivars.

Table 9. Nitrogen use efficiency and some relative parameters at different N levels in rice cultivars in 2011.

Cultivars	N levels	HI (%)	BE_N ($g\ g^{-1}$)	RE_N (%)	YE_N ($g\ g^{-1}$)	PFP_N ($g\ g^{-1}$)
Koshihikari	0N	44.7 b	122.4 b	-	44.4 abc	-
Nipponbare		39.1 d	121.8 b	-	36.7 d	-
Takanari		46.8 a	112.1 c	-	43.3 bc	-
Momiroman		46.2 a	135.6 a	-	47.8 a	-
Hinohikari		37.0 e	131.2 a	-	41.2 c	-
Akimasari		40.5 c	136.0 a	-	46.4 ab	-
Average		42.4	126.5		43.3	
$LSD_{0.05}$		0.5	3.5		1.6	
Koshihikari	1N	41.4 b	94.5 f	88.6 ab	26.9 c	56.0 d
Nipponbare		38.8 cd	103.6 d	86.6 ab	28.6 bc	63.8 cd
Takanari		47.8 a	100.7 e	101.8 a	40.5 a	93.8 a
Momiroman		40.3 bc	111.5 a	61.2 b	23.2 c	40.5 e
Hinohikari		37.9 de	105.8 c	84.7 ab	34.2 ab	70.7 bc
Akimasari		36.9 e	108.6 b	91.8 ab	35.0 ab	74.6 b
Average		40.5	104.1	85.8	31.4	66.6
$LSD_{0.05}$		0.8	0.4	14.66	3.1	4.34
Koshihikari	2N	40.6 b	92.9 a	45.0 c	20.1 c	21.4 c
Nipponbare		37.6 c	93.5 a	47.6 c	24.9 b	29.1 b
Takanari		43.9 a	79.3 c	82.9 a	29.3 a	43.1 a
Momiroman		32.5 e	76.5 d	63.1 b	11.0 d	13.2 d
Hinohikari		36.1 cd	83.8 b	72.4 ab	19.5 c	26.1 bc
Akimasari		35.4 d	83.5 b	77.4 a	22.7 bc	31.3 b
Average		37.7	84.9	64.7	21.3	27.4
$LSD_{0.05}$		0.9	0.6	6.39	1.6	2.53
N levels (A)		**	**	**	**	**
Cultivars (B)		**	**	**	**	**
A x B		**	**	ns	**	**

Values within a column for each N levels followed by the same letter are not significantly different at the 0.05 probability level by Turkey's test (n =3). * and **, significant at the 0.05 and 0.01 level; ns, not significant by ANOVA.
HI: Harvest Index, BE_N: N use efficiency for biomass, RE_N: Recovery efficiency of applied N, YE_N: Nitrogen use efficiency for grain yield, PFP_N: Partial factor productivity of applied N.

Table 10. Nitrogen use efficiency and some relative parameters at different N levels in rice cultivars in 2012.

Cultivars	N levels	HI (%)	BE_N (g g^{-1})	RE_N (%)	YE_N (g g^{-1})	PFP_N (g g^{-1})
Koshihikari	0N	38.5 cd	104.9 f	-	36.4 de	-
Nipponbare		43.1 b	135.7 d	-	46.2 bcd	-
Takanari		49.0 a	112.0 e	-	51.5 ab	-
Momiroman		39.0 c	133.7 d	-	46.5 bcd	-
Hinohikari		35.4 e	134.4 d	-	40.4 cde	-
Akimasari		36.4 de	160.1 a	-	47.2 abc	-
Hokuriku 193		44.8 b	149.7 b	-	57.0 a	-
Mizuhochikara		36.0 de	145.2 c	-	35.9 e	-
Average		40.3	134.5		45.1	
$LSD_{0.05}$		1.2	1.3		4.8	
Koshihikari	1N	37.1 c	88.3 g	35.7 c	31.5 bc	57.3 cd
Nipponbare		42.0 b	112.3 b	27.4 c	42.2 a	63.4 bc
Takanari		45.0 a	95.3 f	76.7 ab	43.0 a	70.2 ab
Momiroman		35.5 cd	99.6 e	98.2 a	24.8 d	57.9 cd
Hinohikari		37.6 c	96.2 f	88.9 ab	34.1 b	52.5 de
Akimasari		38.2 c	106.3 c	95.2 a	29.8 bcd	61.3 c
Hokuriku 193		41.2 b	122.4 a	55.8 bc	45.4 a	73.7 a
Mizuhochikara		34.1 d	103.4 d	80.1 ab	26.3 cd	46.4 e
Average		38.8	103.0	69.8	34.6	60.3
$LSD_{0.05}$		1.3	0.6	16.98	3.0	60.3
Koshihikari	2N	39.6 ab	78.1 f	50.1 de	27.6 ab	33.8 cd
Nipponbare		42.9 a	97.1 b	39.9 e	33.4 a	34.6 c
Takanari		32.0 d	94.0 c	41.2 e	26.2 ab	45.7 a
Momiroman		31.2 d	82.1 e	79.3 abc	15.1 c	27.6 d
Hinohikari		35.0 cd	69.5 g	95.7 a	21.2 bc	37.1 bc
Akimasari		36.2 bc	70.2 g	88.7 ab	20.0 bc	33.9 cd
Hokuriku 193		34.7 cd	103.3 a	56.4 cde	31.8 a	41.8 ab
Mizuhochikara		33.9 cd	83.9 d	70.4 bcd	25.3 ab	27.6 d
Average		35.7	84.8	65.2	25.1	35.3
$LSD_{0.05}$		1.9	0.8	10.94	4.0	3
N levels (A)		**	**	ns	**	**
Cultivars (B)		**	**	**	**	**
A x B		**	**	ns	**	*

Values within a column for each N levels followed by the same letter are not significantly different at the 0.05 probability level by Turkey's test (n =3). * and **, significant at the 0.05 and 0.01 level; ns, not significant by ANOVA.

HI; Harvest Index, BE_N; N use efficiency for biomass, RE_N; Recovery efficiency of applied N, YE_N; Nitrogen use efficiency for grain yield, PFP_N; Partial factor productivity of applied N.

3.4. Discussion

Nitrogen is one of the most yield-limiting nutrients in lowland rice production and its uptake is affected by the varietal characteristics, kind of fertilizers, soils, weather conditions and cultivars (Ye et al., 2007). However, understanding the relation among LAI, dry matter production, nitrogen accumulation, grain yield and nitrogen use efficiency in some high-yielding rice cultivars is still limited. In this study, the dry matter production, nitrogen accumulation, nitrogen use efficiency among high-yielding rice cultivars under different N levels were compared with the results that indicated that the dry matter production in rice cultivars increased significantly associating with higher N levels in both years. However, increasing the amount of N application from 8 g m^{-2} to 16 g m^{-2} did not mean increasing too much the dry matter in rice cultivars (Fig. 7).

Akimasari consistently produced a larger dry matter production among rice cultivars, while Koshihikari showed a smaller value in dry matter production (Fig. 7), which corresponded to the late and early maturing cultivars in Okayama prefecture, respectively. The differences in dry matter production might result in not only dependence on nitrogen fertilizer but also growth duration. However, Takanari, Akimasari and Hokuriku 193 had lager dry matter production at the harvesting time. It implied that the differences among rice cultivars were caused by the increasing of dry matter production from HD to HV (Fig. 8).

Accumulated N in rice cultivars was remarkably affected by N levels (Fig. 11). It increased with the increasing in N levels, and was the highest in 2N, followed by 1N and 0N levels. Tananari and Hinohikari accumulated the largest amount of N. While, Koshihikari and Nipponbare accumulated the smallest amount of N compare with the other rice cultivars. Rice plants uptake and accumulate larger amount of nitrogen at 2N. However, increasing the amount of accumulated N from 1N to 2N level did not increase the grain yield (Table 7, 8). Moreover, the grain yield of Takanari, Momiroman, Hinohikari and Hokuriku 193 tended to decrease.

Rice grain yield is the product of different yield components, such as panicle m^{-2}, number of spikelets per panicle, thousand grain weight and ripened percentage (Yoshida, 1981; Fageria and Barbosa Filho, 2001; Koutroubas and Ntanos, 2003). Panicle number m^{-2} is the most important factor determining grain yields (Fageria and Barbosa Filho, 2001; Koutroubas and Ntanos, 2003; Artacho et al., 2009). In this study, panicle numbers significantly increased with higher N fertilization (Table 7, 8). Koshihikari, Nipponbare, Hinohikari had higher number of panicles than the others, while Takanari, Momiroman, Hokuriku 193 and Mizuhochikara had higher number of spikelets per panicle. The number of spikelets m^{-2} and sink capacity was larger in Takanari, Momiroman and Hokuriku 193 due to the greater number of spikelets per panicle. Differences in the number of panicles might be caused by the cultivars and N levels, especially N has the larger effects at the tillering stage (Zhong et al., 2003).

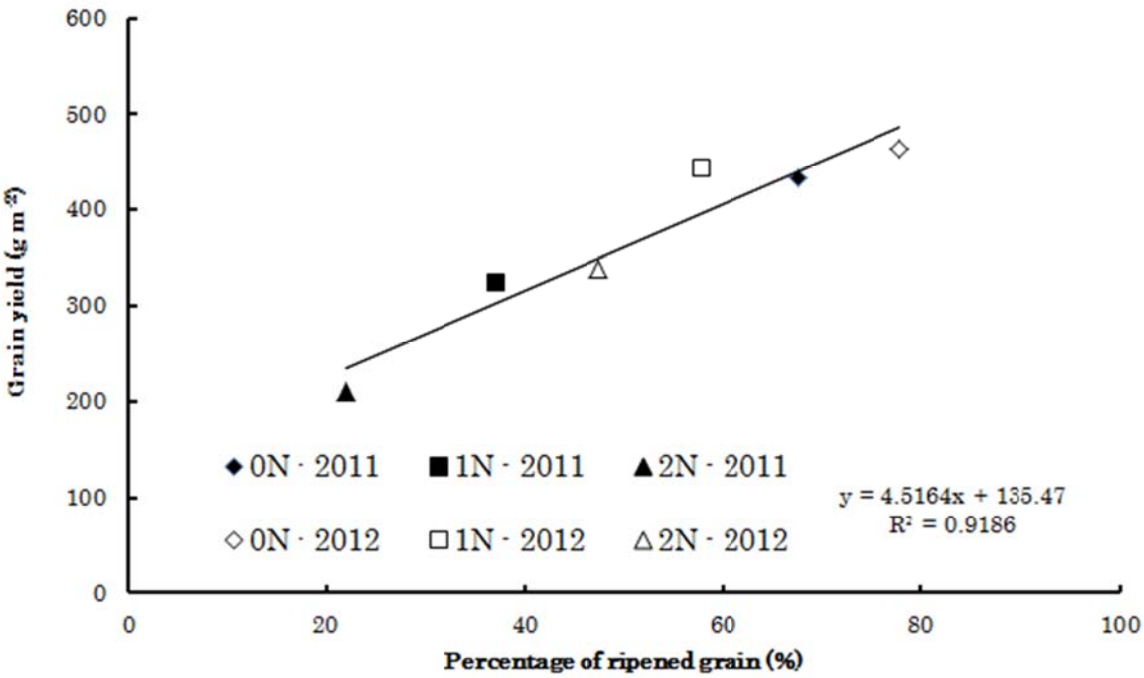


Fig. 12. Relationship between grain yield and percentage of ripened grain in Momiroman during 2 year experiments.

The differences in rice grain yields were affected by percentage of ripened grain. On one hand, the percentage of ripened grain decreased with more N application in rice cultivars. Especially in Momiroman, the higher the N application, the lower the grain yield is (Fig. 12). Although Momiroman showed the high sink capacity (974 g m^{-2} in 2011), lower percentage of ripened grain caused to decrease grain yield. Mukoyama et al., (2014) also observed the lower ripening ability in Momiroman which was necessary to clarify the lower ripening characters in Momiroman.

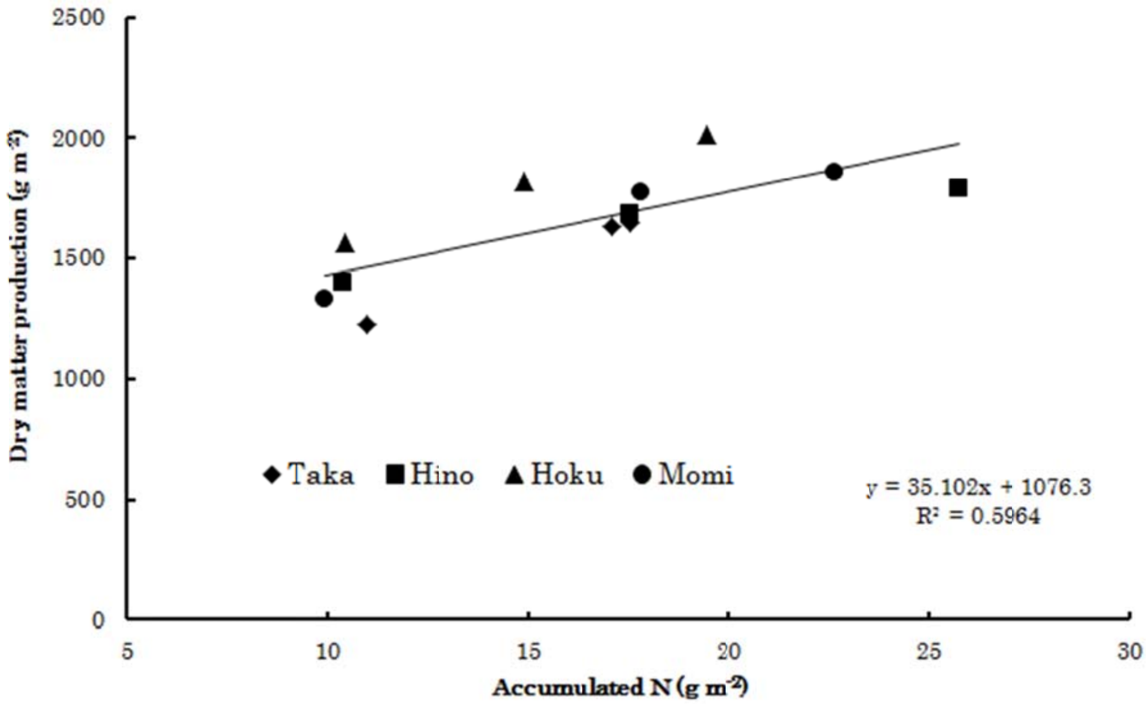


Fig. 13. Relationship between dry matter production and accumulated N at harvesting in some rice cultivars in 2012.

On the other hand, improving rice yield was attributed to increasing of harvest index rather than the total dry matter production (Ju et al., 2009). Total dry matter production is determined by total nitrogen uptake and nitrogen use efficiency for dry matter production (Akita, 1989; Amano et al., 1993). Besides, (Peng et al., 2000) reported that the increasing in rice grain yield should be based on the increasing in dry matter accumulation. In this study, dry matter production had a positive relation with accumulated N at harvesting significantly (Fig. 13). Larger amount of accumulated N contributed to a higher dry matter production, and Hokuriku 193 showed a higher dry matter production at the same levels of accumulated N with higher BE_N. The higher BE_N thought to be the higher partitioning of N to the leaves at the same levels of accumulated N. Further studies are necessarily conducted to clarify the factor affecting the higher BE_N in Hokuriku 193.

Besides, dry matter production of rice cultivars increased with higher N application (Fig. 7) as well as had a close relationship with N levels (Fig 13). It is means that rice cultivars which had higher dry matter production at heading might have a potential for higher grain yield. Furthermore, some rice cultivars had both higher dry matter productions at harvesting and high harvest index (Fig. 7; Table 9, 10), especially in Takanari and Hokuriku 193. These two rice cultivars had higher harvest index (47.8% and 41.2% at 1N level, respectively) than the others, although some cultivars were significantly higher in total dry matter production than these two cultivars. It can be concluded that the increasing in dry matter production at harvest combining with high HI in an important factor in order to improve rice grain yields.

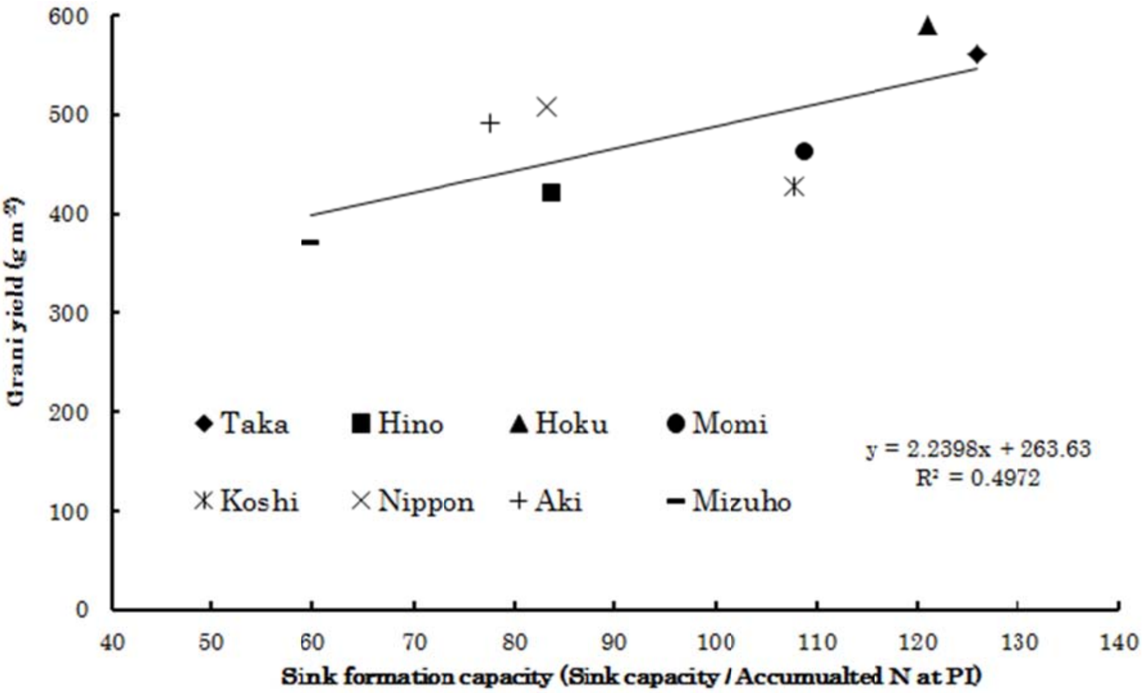


Fig. 14. Relation between sink formation capacity and grain yield without N fertilization in 2012.

Wada (1969) found out that the close linear relationship between accumulated N and spikelets number m^{-2} until late PI. The higher number of spikelets m^{-2} is an important factor that affects sink size. This means that sink size was determined by the N accumulated until PI. On the other hand, sink capacity which is divided by the accumulated N until PI, defined as sink formation capacity, indicates the efficiency of sink formation. Sink formation capacity in Takanari and Hokuriku 193 is almost higher than the other rice cultivars without N fertilization (Fig. 114). Higher sink formation capacity contributed to higher grain yield in Takanari and Hokuriku 193 than the other rice cultivars.

Dry matter production and N uptake were increased with the increasing of N application, in the order of 2N > 1N > 0N. However, the highest grain yield was attained at 1N levels. N use efficiency and some relative parameters showed a decreasing response to N levels in both years (Table 9, 10). YE_N decreased with N application and it was higher in 1N than 2N levels, because the increase in accumulated N was higher than the increasing in grain yield. Higher YE_N observed in Takanari and Hokuriku 193 in 2011 and 2012, respectively, which could be attribute to the higher HI. The same result was reported by Samonte et al., (2006).

In conclusion, dry matter production, grain yield, nitrogen accumulation increased corresponding with a higher N application. However, with the highest application of N level, dry matter production did not mean increasing too much and grain yield of rice cultivars tended to decrease because of lower the percentage of ripened grains. Grain yield of Takanari, Hokuriku 193, Akimasari and Hinohikari were the highest at 1N levels because of higher dry matter production, HI and YE_N. High HI and YE_N, therefore, considered to be important factors to evaluate high yielding cultivars.

Summary

We evaluated the cultivar differences in dry matter production, yield and yield components, nitrogen accumulation and use efficiency in selected high yielding rice cultivars. Experiments were conducted in the experimental field of Field Science Center, Okayama University during two years 2011 and 2012. Six rice cultivars was used in 2011 and more two cultivars in 2012, respectively. All cultivars were grown at three levels of nitrogen fertilization (0N: 0 gN m^{-2}; 1N: 8 gN m^{-2}; 2N: 16 gN m^{-2}). Dry matter production became larger in all cultivars with higher nitrogen application during two years. Dry matter production at harvest was highest in Takanari and Hokuriku 193. Takanari consistently accumulated the largest amount of N in 2N. The grain yield of Takanari was highest during 2 years (750 g m^{-2} and 731 g m^{-2} at 1N in 2011 and 2012, respectively) followed by Hokuriku 193 (669 g m^{-2} at 1N in 2012). Nitrogen use efficiency (YE_N) decreased with the raising of N levels and it was higher in Hokuriku 193 and Takanari. Both cultivars showed the highest yield among 0N plots (589 and 562 g m^{-2}) due to the highest YE_N and sink formation capacity (sink capacity / accumulated N at panicle initiation). This study clearly suggests that the higher yield is determined by the larger sink capacity, higher dry matter production and YE_N due to the lager sink formation capacity at the panicle initiation.

Chapter 4

Genotypic variation in dry matter production, grain yield and nitrogen use efficiency in selected rice cultivars under field conditions

4.1. Introduction

In rice production, nitrogen (N) fertilizer plays an important role in increasing dry matter production and grain yield (Nakano and Morita, 2009; Taylaran et al., 2009). Cultivars with higher rates of nitrogen accumulation and higher nitrogen use efficiency are vital to limit both production costs and environmental pollution while, at the same time, maximizing grain yield. Recently, modern agricultural production require a larger amount of N fertilizer, it is easy for rice plant uptake and develop the plant height and tiller numbers. A taller rice plant is more susceptible to lodging and responds less well to nitrogen (Yoshida, 1978). As the use of nitrogen fertilizer increases, breeding efforts focused on developing shorter-culmed and higher lodging resistant in rice is necessary. However, older rice cultivars which were widely grown in Japan might be have the potential adapt with lower levels of nitrogen fertilizer (Osada, 1995).

Nitrogen use efficiency for grain yield (YE_N) has been defined in various ways, but these definitions generally take into account quantity of N accumulated in the plant, known as uptake efficiency and quantity of N utilized in grain production known as utilization efficiency. In chapter 2, the result showed that YE_N decreased with increasing N doses and it was different among fertilizer methods. Without N fertilizer application, YE_N of Takanari and Nipponbare also showed the highest values compared with the other fertilization methods. The same result was observed in chapter 3, YE_N decreased with increase N levels. Besides, the grain yields of Takanari and Hokuriku 193 also higher than other cultivars and some japonica types at 0N level. These cultivars may adapt with low levels of nitrogen fertilizer. Moreover, there is a little understanding about rice cultivars grown without N fertilizer, especially cultivars originated from tropical area. Therefore, this study has been carried out to evaluate the varietal differences in dry matter production, grain yield and N accumulation of rice cultivars in field conditions without N fertilizer application.

4.2. Materials and methods

4.2.1. Plant materials

In the experiment, we used rice cultivars from NIAS Gene bank (25 accessions from NIAS World Global Rice Core Collection (WRC), 15 accessions from NIAS Rice Core Collection of Japanese Landraces (JRC). In addition, we included 9 high yielding (HY) cultivars, namely: Koshihikari, Nipponbare, Hokuriku 193, Takanari, Momiroman, Hinohikari, Mizuhochikara, Akimasari and SL 519, which were widely grown in Japan. Lists of rice cultivars, sites of origin and heading dates are presented in Table 11.

Table 11. Rice cultivars used in experiment.

ID	Name	Sites of Origin	Heading
W 1	Nipponbare	Japan	Aug. 18
W 2	Kasalath	India	Aug. 8
W 3	Bei Khei	Cambodia	Sep. 7
W 4	Jena 035	Nepal	Aug. 10
W 5	Naba	India	Sep. 5
W 6	Puluik Arang	Indonesia	Aug. 31
W 7	Davao 1	Philippines	Aug. 21
W10	Shuusoushu	China	Jul. 27
W 14	IR 58	Philippines	Aug. 9
W 15	CO 13	India	Aug. 17
W 16	Vary Futsi	Madagascar	Aug. 18
W 18	Qingyu (Seiyu)	Taiwan	Aug. 10
W 20	Takukan	Philippines	Sep. 5
W 21	Shwe Nang Gyi	Myanmar (Burma)	Aug. 7
W 30	Anjana Dhan	Nepal	Aug. 22
W 31	Shoni	Bangladesh	Aug. 1
W 32	Tupa 121-3	Bangladesh	Aug. 4
W 34	ARC 7291	India	Aug. 5
W 36	Ratul	India	Aug. 5
W 37	ARC 7047	India	July. 30
W 49	Padi Perack	Indonesia	Jul. 31
W 51	Urasan 1	Japan	Aug. 2
W 52	Khau Tan Chiem	Vietnam	Aug. 26
W 53	Tima	Bhutan	Sep. 11
W 55	Tupa 729	Bangladesh	Aug. 4
J 3	Hinode	Kinki	Aug. 9
J 5	Kaneko	Kantou Touzan	Aug. 7
J 10	Hirayama	Tokyo	Jul. 27
J 17	Akage	Akita	Aug. 15
J 20	Hosogara	Aomori	Jul. 29
J 22	Mansaku	Nagano	Aug. 8
J 23	Ishijiro	Toyama	Aug. 7
J 24	Joushuu	Yamagata	Aug. 8
J 26	Aikoku	Fukui	Aug. 13
J 30	Moritawase	Yamagata	Jul. 30
J 36	Sekiyama	Aomori	Jul. 27
J 37	Shinyamadaho 2	Hyougo	Aug. 16
J 41	Akamai	Tokushima	Aug. 20
J 45	Hiyadachitou	Yamagata	Jul. 30
J 46	Fukoku	Hokkaidou	Aug. 17
Koshi	Koshihikari		Aug. 10
Nippon	Nipponbare		Aug. 17
Hoku	Hokuriku 193		Aug. 26
Taka	Takanari		Aug. 19
Momi	Momiroman		Aug. 31
Hino	Hinohikari		Aug. 27
Mizuho	Mizuhochikara		Sep. 03
Aki	Akimasari		Sep. 04
SL 519	SL 519		Sep. 06

4.2.2. Cultivation

All WRC, JRC and HY cultivars were grown in the experimental field of Field Science Center, Okayama University in 2013. The field experiment was carried with no replication and the plot area for each cultivar was approximately 4.5 m^2 (50 x 90 cm). Seeds of rice cultivars were sown on 14 May and transplanted to the field on 12 June. All rice cultivars were transplanted manually at a density of 22.2 hill m^{-2} (30 x 15 cm) with 2 plants per hill. Calcium superphosphate (P_2O_5) and potassium chloride (K_2O) were incorporated into the soil at a rate of 8 g before transplanting.

4.2.3. Dry matter production, accumulated nitrogen and nitrogen use efficiency.

In each cultivar, eight hills were sampled at the harvesting time, and four hills with an average number of stems were selected to measure dry weight of whole plant. Plant samples were separated into leaves, stems and dead leaves. After separation, all plant samples were dried in a ventilated oven 80^{0}C until constant weight.

At the harvesting time, eight hills for each cultivar were harvested for the determination of grain yield and yield components. The plant samples were dried in a vinyl house and the total above dry weight was recorded. After counting the number of panicles, threshing and husking, the grains yield and yield components of brown rice were identified. Fully ripened grains were selected by sieving through 1.8 mm and 1.6 mm mesh. Thousand grain weights and grain yield were measured by using brown rice with 14.5% moisture.

The two of four hills, which were selected for the measurements of dry weight, were used for determination of accumulated nitrogen in a whole plant. All plant parts were ground into powder by vibrating mill machine (Heiko, Co. Ltd) and nitrogen concentration of the plant is determined by CN-Corder (MT-700, Yanaco Industry). Total accumulated nitrogen was calculated by multiplying the above-ground dry weight by nitrogen concentration.

Nitrogen use efficiency and some indices were calculated by the following formulas;

Harvest Index (HI) (%) = Grain yield x 0.865 / dry matter production x 100%.

N use efficiency for biomass (BY_N) = BY /AN.

Nitrogen use efficiency for grain yield (YE_N) = GY / AN.

Where BY; Biomass yield (g m^{-2}), GY; Grain yield (g m^{-2}), AN; Accumulated N (g m^{-2}).

4.3. Results

4.3.1. Dry matter production of rice cultivars.

Table 12. Dry matter production in rice cultivars

Unit: g m^{-2}

ID	Cultivars	Stem	Leaf	Panicle	Total
W 1	Nipponbare	393	165	419	977
W 2	Kasalath	475	184	552	1211
W 3	Bei Khe	560	293	420	1273
W 4	Jena 035	509	298	358	1165
W 5	Naba	465	297	514	1276
W 6	Puluik Arang	363	226	407	996
W 7	Davao 1	540	252	344	1136
W 10	Shuusoushu	514	163	580	1256
W 14	IR 58	353	209	288	850
W 15	CO 13	543	206	311	1059
W 16	Vary Futsi	826	377	371	1573
W 18	Qingyu (Seiyu)	444	235	297	977
W 20	Tadukan	534	178	442	1154
W 21	Shwe Nang Gyi	453	179	404	1035
W 30	Anjana Dhan	683	199	423	1305
W 31	Shoni	386	182	440	1009
W 32	Tupa 121-3	468	209	472	1149
W 34	ARC 7291	301	179	371	851
W 36	Ratul	448	238	482	1168
W 37	ARC 7047	448	162	460	1070
W 49	Padi Perack	389	160	366	915
W 51	Urasan 1	335	130	331	796
W 52	Khau Tan Chiem	542	210	332	1084
W 53	Tima	892	297	412	1601
W 55	Tupa 729	500	173	257	929
J 3	Hinode	375	120	411	907
J 5	Kaneko	381	101	327	809
J 10	Hirayama	402	144	534	1079
J 17	Akage	176	43	268	487
J 20	Hosogara	187	61	227	475
J 22	Mansaku	342	115	428	885
J 23	Ishijiro	451	148	405	1004
J 24	Joushuu	308	157	313	778
J 26	Aikoku	375	117	358	850
J 30	Moritawase	227	76	372	675
J 36	Sekiyama	291	72	444	807
J 37	Shinyamadaho 2	373	129	407	909
J 41	Akamai	452	162	561	1175
J 45	Hiyadachitou	225	70	313	607
J 46	Fukoku	199	48	184	432
Koshi	Koshihikari	412	126	455	993
Nippon	Nipponbare	482	174	426	1082
Hoku	Hokuriku 193	483	206	760	1449
Taka	Takanari	367	111	493	971
Momi	Momiroman	246	124	283	653
Hino	Hinohikari	412	126	455	993
Mizuho	Mizuhochikara	570	172	893	1635
Aki	Akimasari	640	214	600	1453
SL 519	SL 519	408	171	532	1111

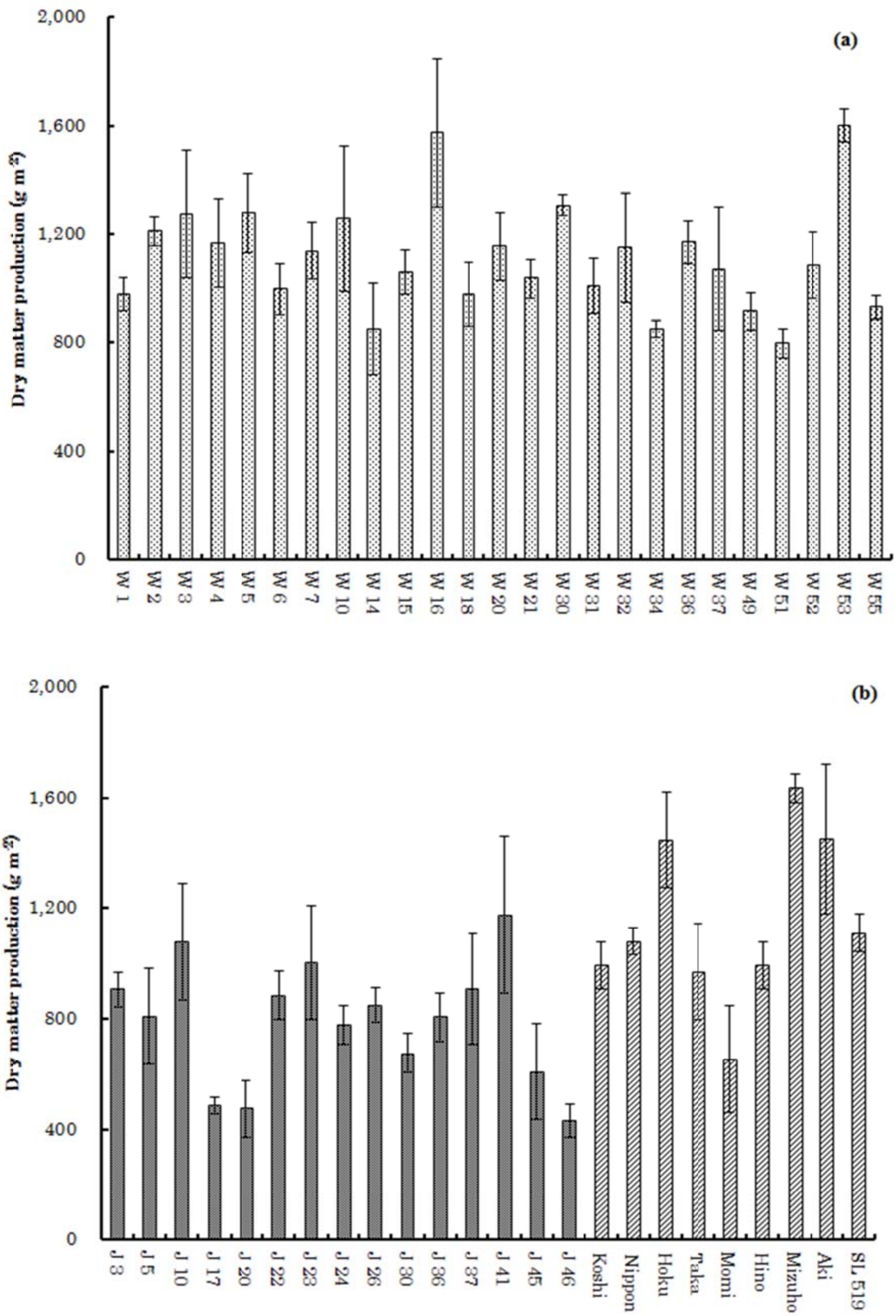


Fig. 15. Total dry matter production in WRC (a), JRC and HY (b) rice cultivars at harvesting time.

Table 12 and Fig. 15 showed the differences in dry matter production of rice cultivars and accessions cultivated without N fertilizer.

The stem dry weight was varied among rice cultivars and tended to be higher in WRC cultivars than JRC and HY cultivars. The stem dry matter production was ranged from 33 to 892, 176 to 452 and 246 to 640 in WRC, JRC and HY cultivars, respectively. The stem dry weight was higher in WRC than JRC and HY cultivars. W 53 (Tima) and W 16 (Vary Futsi) showed the highest values of stem dry weight.

Panicle dry weight is considered to be nearly equal to grain yield. HY cultivars produced the highest panicles dry weight than WRC and JRC cultivars. Mizuhochikara showed the heaviest panicle dry weight (893 g m^{-2}), followed by Hokuriku 193 (760 g m^{-2}). The panicle dry weight of WRC was ranging from 275 to 552 g m^{-2} in W 55 (Tupa 729) and W 2 (Kasalath), respectively. And also in JRC, the panicle dry weight was 184 to 561 g m^{-2} in J 46 (Fukoku) and J41 (Akamai), respectively.

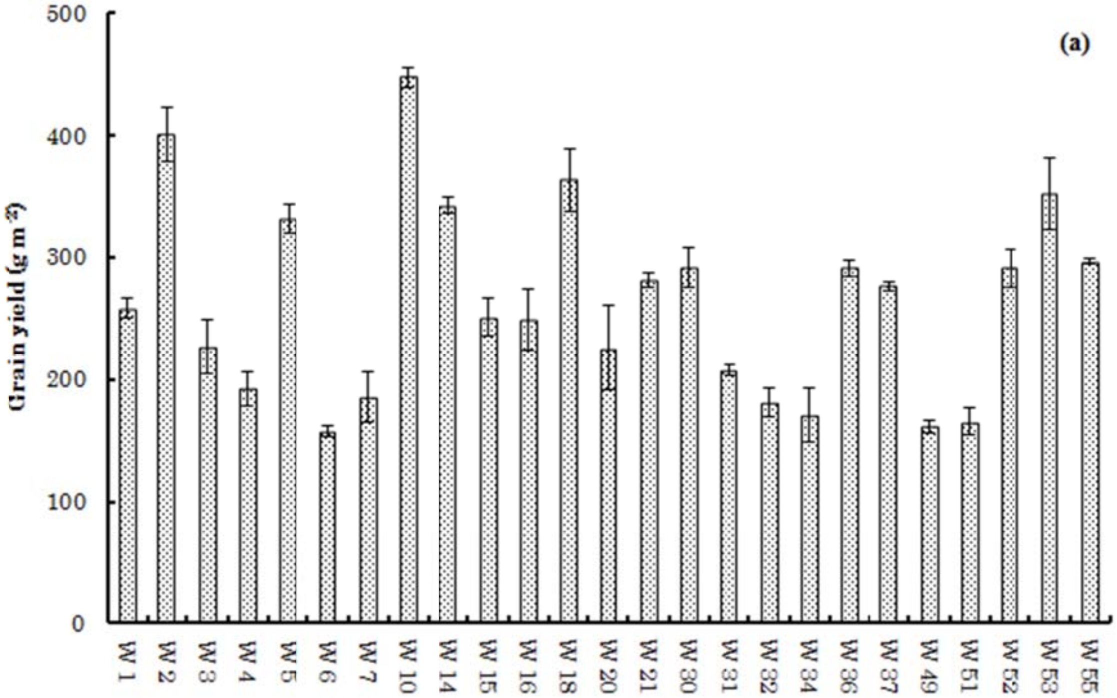
In WRC cultivars, 17 cultivars showed the total dry matter production more than 1000 g m^{-2}. W 53 (Tima), produced the highest dry matter production (1601 g m^{-2}) among the WRC cultivars, followed by W 16 (Vary Futsi – 1573 g m^{-2}), W 30 (Anjana Dhan – 1305 g m^{-2}), W 5 (Naba - 1276 g m^{-2}) and W3 (Bei Khe - 1273 g m^{-2}). W51 (Urasan 1 – 796 g m^{-2}) and W 14 (IR 58 – 850 g m^{-2}) showed the smallest dry matter production.

In JRC cultivars, J 41 (Akamai), J 10 (Hirayama) and J 23 (Ishijiro) had higher dry matter production. The total dry matter productions of these cultivars were 1175, 1079 and 1004 g m^{-2}. J 46 (Fukoku), J 20 (Hosogara) and J 17 (Akage) showed the smallest values among the JRC cultivars.

4.3.2. Yield and yield components

Table 13. Yield and yield components in rice cultivars

ID	Cultivars	No. of panicles (m^{-2})	No. of spikelets/panicle ($panicle^{-1}$)	No. of spikelets (10^3 m^{-2})	% of ripended grains (%)	1000 grains weight (g)	Sink capacity ($g\ m^{-2}$)	Grain yield ($g\ m^{-2}$)
W 1	Nipponbare	241	88	21.35	58.4	20.7	442.2	258.3
W 2	Kasalath	355	84	29.92	77.2	17.4	519.4	400.8
W 3	Bei Khe	294	78	23.07	57.3	17.2	396.1	226.9
W 4	Jena 035	275	79	21.62	53.2	16.7	361.9	192.5
W 5	Naba	283	84	23.75	71.2	19.6	465.8	331.6
W 6	Puluik Arang	269	56	15.15	51.3	20.2	306.6	157.3
W 7	Davao 1	236	87	20.46	50.4	18.0	367.9	185.2
W 10	Shuusoushu	294	87	25.54	87.3	20.1	512.1	447.0
W 14	IR 58	316	70	22.28	76.5	20.1	447.6	342.3
W 15	CO 13	400	58	23.26	55.4	19.5	452.7	250.7
W 16	Vary Futsi	264	99	26.21	47.6	20.0	523.2	248.9
W 18	Qingyu (Seiyu)	294	94	27.77	59.5	22.0	610.7	363.2
W 20	Tadukan	389	64	24.98	52.8	17.1	427.3	225.6
W 21	Shwe Nang Gyi	214	103	22.11	62.0	20.5	453.3	281.2
W 30	Anjana Dhan	211	131	27.60	60.3	17.5	483.2	291.4
W 31	Shoni	222	74	16.34	69.9	18.2	297.2	207.7
W 32	Tupa 121-3	155	97	15.08	67.4	17.8	268.2	180.9
W 34	ARC 7291	266	93	24.82	42.2	16.3	404.2	170.6
W 36	Ratul	444	60	26.68	63.8	17.1	456.4	291.1
W 37	ARC 7047	233	84	19.51	83.1	17.0	332.3	276.3
W 49	Padi Perack	150	123	18.50	48.8	17.9	330.6	161.4
W 51	Urasan 1	205	116	23.76	45.2	15.4	365.8	165.5
W 52	Khau Tan Chiem	255	111	28.23	57.5	18.0	506.7	291.1
W 53	Tima	175	143	24.96	77.9	18.1	452.9	352.6
W 55	Tupa 729	400	63	25.16	58.5	20.1	506.7	296.6
J 3	Hinode	194	56	10.87	59.7	17.1	186.0	110.0
J 5	Kaneko	219	64	14.13	45.9	19.9	280.5	128.8
J 10	Hirayama	225	101	22.59	71.4	21.4	482.4	344.4
J 17	Akage	295	43	12.80	62.8	22.0	281.7	176.8
J 20	Hosogara	130	100	13.11	35.8	17.8	233.3	82.0
J 22	Mansaku	269	65	17.59	40.1	17.6	310.6	124.3
J 23	Ishijiro	219	70	15.29	86.3	22.3	340.3	293.6
J 24	Joushuu	300	45	13.72	51.4	14.3	195.7	100.5
J 26	Aikoku	289	74	21.42	74.6	19.4	416.0	309.5
J 30	Moritawase	305	85	25.94	44.9	19.3	500.0	224.1
J 36	Sekiyama	194	145	28.23	57.6	21.0	591.7	340.8
J 37	Shinyamadaho 2	250	72	17.99	69.0	19.7	353.8	243.9
J 41	Akamai	150	62	9.26	57.9	22.3	206.0	117.8
J 45	Hiyadachitou	214	109	23.36	55.4	19.8	462.0	255.1
J 46	Fukoku	311	42	12.95	53.2	20.9	219.4	114.5
Koshi	Koshihikari	254	76	19.4	79.0	20.0	387.7	306.0
Nippon	Nipponbare	297	67	20.0	70.9	21.7	433.1	306.3
Hoku	Hokuriku 193	233	131	30.6	73.8	21.3	650.4	478.4
Taka	Takanari	203	137	28.0	88.5	17.9	501.8	443.9
Momi	Momiroman	155	142	22.1	55.6	21.4	472.4	260.2
Hino	Hinohikari	302	72	21.8	59.6	21.2	463.2	275.9
Mizuho	Mizuhochikara	230	133	30.6	63.3	20.6	632.4	400.3
Aki	Akimasari	311	94	29.3	55.1	21.6	631.3	347.9
SL 519	SL 519	272	93	25.4	57.6	20.6	523.6	301.6



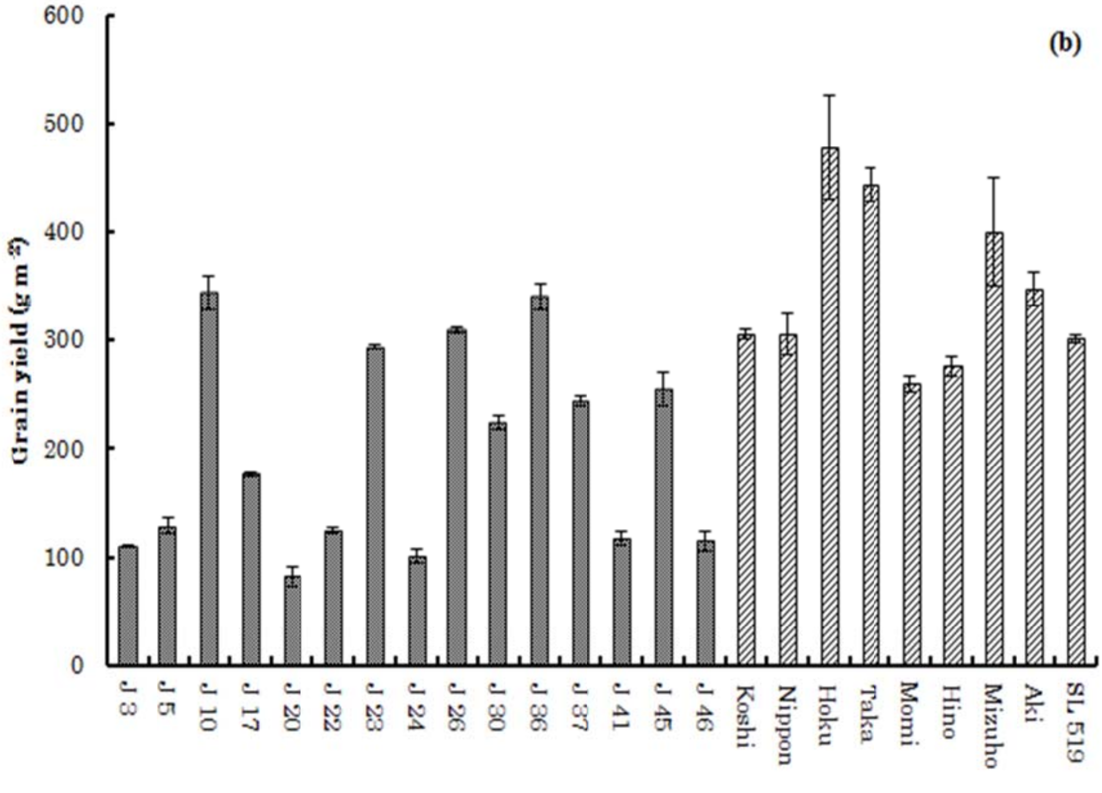


Fig. 162. Grain yields in WRC (a), JRC and HY (b) rice cultivars.

Yield and yield components in rice cultivars are listed in Table 13 and Fig. 16. In this experiment, WRC cultivars had higher number of panicles than JRC and HY cultivars. The number of panicles varied from 159 to 444 m^{-2}. W 36 (Ratul) had the highest number (444 m^{-2}) than the other rice cultivars, while W 49 (Padi Perack), W 32 (Tupa 121 - 3), J 20 (Hosogara), J 41 (Akamai), Momiroman and Takanari had the lower number of panicles m^{-2}, in the order of 150, 155, 130, 150, 155 and 214 panicle m^{-2}, respectively.

The number of spikelets per panicle were different among rice cultivars and was lower in WRC and JRC, especially in J 46 (Fukoku, 42), J 17 (Akage, 43), J 24 (Joushuu, 45), W 6 (Puluik Arang, 56), W 55 (Tupa 419, 63) and W 20 (Takukan, 64). HY cultivars had the higher number of spikelets than other rice cultivars such as Momiroman, Takanari, Hokuriku and Mizuhochikara. Such cultivars as J 36 (Sekiyama), J 45 (Hiyadachitou), J 20 (Hosogara), J 10 (Hirayama), W 52 (Khau Tam Chiem), W 51 (Urasan) and W 21 (Shwe Nang Gyi) also showed the higher spikelets numbers than the other cultivars.

The number of panicles m^{-2} combined with the number of spikelets per panicle could increase the total number of spikelets m^{-2}. Hokuriku 193, Mizuhochikara, Akimasari, Takanari, J 36 (Sekiyama), W 52 (Khau Tan Chiem), W 30 (Anjana Dhan) and W 2 (Kasalath) also showed the higher number of spikelets m^{-2} than other cultivars.

Grain yield (g m^{-2}) was higher in Hokuriku 193 (478), W 10 (Shuusoushuu, 447), Takanari (444), Mizuhochikara (400) than the other rice cultivars. These cultivars had higher number of spikelets per panicle, total num ber of spikekelts m^{-2} combined with higher percentage of ripened grains, therefore the higher grain yield. Some cultivars had a larger sink capacity but the lower percentage of ripened grain, caused to the grain yield decrease.

4.3.3. Nitrogen accumulation

Table 14. Accumulated N in rice cultivars

Unit: g m^{-2}

ID	Cultivars	Stem	Leaf	Panicle	Total
W 1	Nipponbare	2.8	1.5	6.5	10.8
W 2	Kasalath	3.1	1.1	8.2	12.4
W 3	Bei Khe	4.3	2.7	6.0	12.9
W 4	Jena 035	4.0	2.6	5.4	11.9
W 5	Naba	3.4	2.6	7.0	13.0
W 6	Puluik Arang	2.9	2.1	6.3	11.2
W 7	Davao 1	4.1	2.6	5.3	11.9
W 10	Shuusoushu	3.1	1.9	7.8	12.8
W 14	IR 58	3.1	2.5	2.9	8.6
W 15	CO 13	3.3	2.3	4.1	9.6
W 16	Vary Futsi	5.4	3.2	3.7	12.3
W 18	Qingyu (Seiyu)	3.2	2.1	3.2	8.6
W 20	Tadukan	3.0	1.5	7.2	11.6
W 21	Shwe Nang Gyi	2.9	2.3	6.3	11.6
W 30	Anjana Dhan	3.4	1.2	6.0	10.6
W 31	Shoni	2.2	1.4	6.7	10.3
W 32	Tupa 121-3	3.6	1.9	7.8	13.3
W 34	ARC 7291	2.8	2.0	5.7	10.5
W 36	Ratul	3.6	2.0	7.3	13.0
W 37	ARC 7047	3.1	1.3	6.6	11.0
W 49	Padi Perack	2.0	1.6	5.6	9.2
W 51	Urasan 1	2.3	1.4	4.7	8.4
W 52	Khau Tan Chiem	5.3	2.2	5.2	12.7
W 53	Tima	5.1	2.3	6.0	13.3
W 55	Tupa 729	4.8	2.2	3.2	10.2
J 3	Hinode	3.2	1.6	7.9	12.6
J 5	Kaneko	2.1	0.8	5.1	8.0
J 10	Hirayama	1.8	1.3	8.0	11.2
J 17	Akage	1.5	0.5	4.0	6.1
J 20	Hosogara	1.2	0.6	4.4	6.2
J 22	Mansaku	2.7	1.0	6.9	10.6
J 23	Ishijiro	3.7	1.6	6.6	11.9
J 24	Joushuu	1.8	1.5	4.6	7.9
J 26	Aikoku	2.0	1.2	5.8	9.0
J 30	Moritawase	1.6	0.8	6.8	9.2
J 36	Sekiyama	1.6	0.7	8.1	10.4
J 37	Shinyamadaho 2	3.8	1.7	8.2	13.7
J 41	Akamai	4.4	1.9	9.9	16.2
J 45	Hiyadachitou	1.5	0.6	5.7	7.8
J 46	Fukoku	2.9	1.1	3.0	6.9
Koshi	Koshihikari	3.1	1.3	6.4	10.8
Nippon	Nipponbare	3.1	1.9	4.5	9.5
Hoku	Hokuriku 193	2.4	1.0	6.1	9.6
Taka	Takanari	3.5	2.0	10.7	16.3
Momi	Momiroman	1.3	1.0	2.7	5.0
Hino	Hinohikari	2.3	1.0	5.0	8.3
Mizuho	Mizuhochikara	3.6	1.5	11.5	16.6
Aki	Akimasari	4.3	1.9	7.6	13.8
SL 519	SL 519	2.2	1.3	4.4	7.9

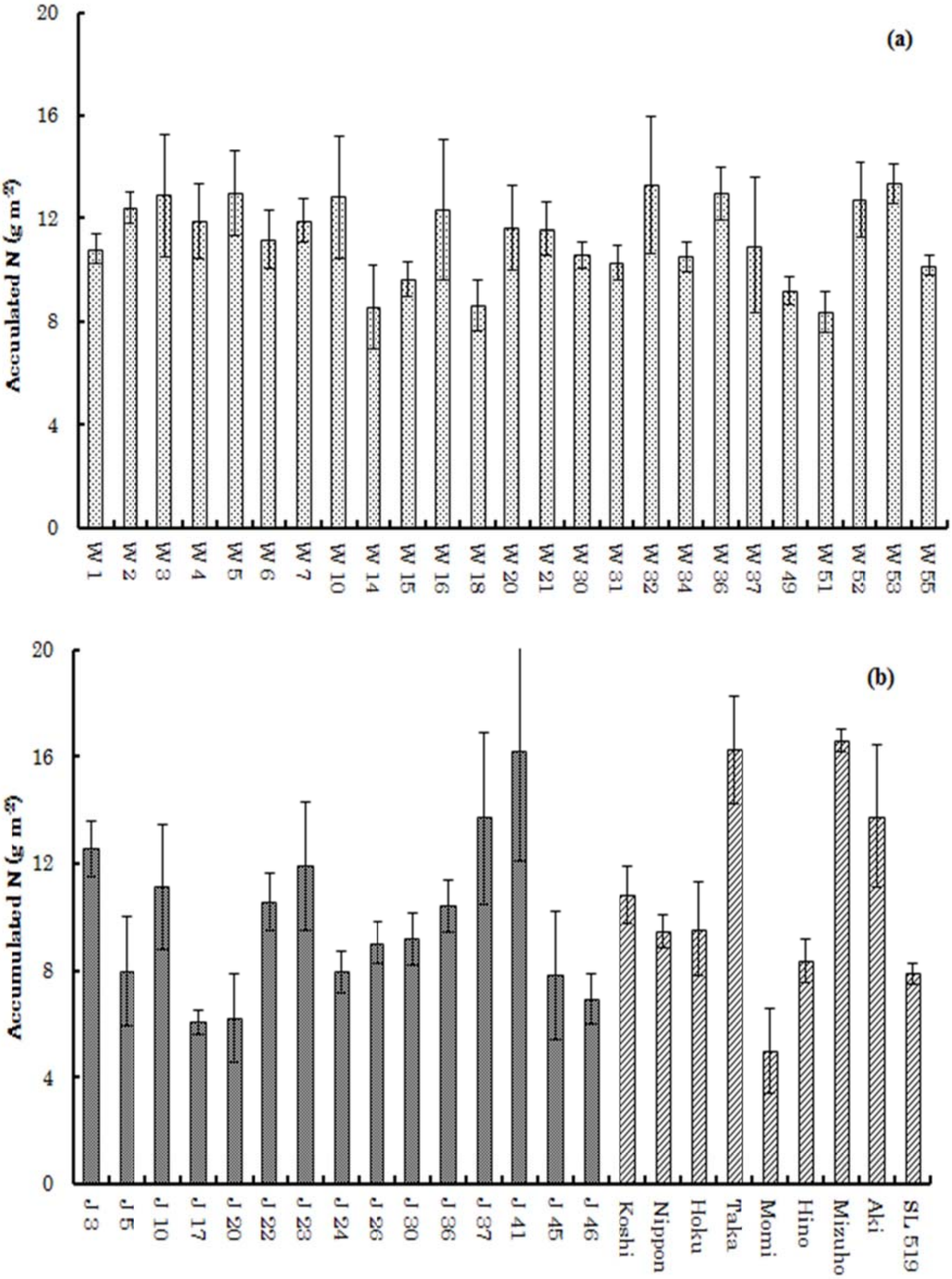


Fig. 173. Accumulated N in WRC (a), JRC and HY (b) rice cultivars

Accumulated N in rice cultivars are presented in Table 14 and Fig. 17. The amount of accumulated N in the HY and JRC cultivars tended to be larger than WRC cultivars. The amount of accumulated N were ranged between 8.4 – 13.3, 5.0 – 16.6 and 6.1 – 16.2 g m^{-2} in WRC, JRC and HYV cultivars, respectively. Mizuhochikara accumulated the largest amount of N (g m^{-2}) among rice cultivars (16.6), followed by Takanari (16.3), J 41 (Akamai, 16.2), Akimasari (13.8), W 53 (Tima, 13.3), J 37 (Shinyamadaho 2, 13.7). Momiroman, W 51 (Urasan 1), W 14 (IR 58), J 17 (Akage) and J 20 (Hosogara) accumulated the smallest amount of N than the other cultivars. The amount of accumulated N reflected the uptake ability of rice cultivars in the field conditions.

4.3.4. Harvest index and nitrogen use efficiency.

Table 15. Harvest index and nitrogen use efficiency in rice cultivars.

ID	Cutivars	Biomass yield (g m^{-2})	HI (%)	BE_N	YE_N
W 1	Nipponbare	293.4	39.8	90.3	23.9
W 2	Kasalath	383.5	36.1	97.6	32.3
W 3	Bei Khei	361.0	29.1	98.6	17.6
W 4	Jena 035	417.0	20.8	97.9	16.2
W 5	Naba	495.4	25.8	98.2	25.5
W 6	Puluik Arang	319.1	27.2	88.8	14.0
W 7	Davao 1	387.3	22.6	95.3	15.5
W 10	Shuusoushu	376.2	46.2	97.9	34.8
W 14	IR 58	306.7	50.0	99.2	40.0
W 15	CO 13	443.5	23.4	109.9	26.0
W 16	Vary Futsi	376.4	22.4	127.5	20.2
W 18	Qingyu (Seiyu)	222.1	40.2	113.3	42.1
W 20	Takukan	313.1	28.9	99.1	19.4
W 21	Shwe Nang Gyi	338.9	29.4	89.3	24.3
W 30	Anjana Dhan	515.9	27.2	123.4	27.5
W 31	Shoni	280.8	34.6	98.1	20.2
W 32	Tupa 121-3	221.5	37.4	86.3	13.6
W 34	ARC 7291	215.7	33.6	80.9	16.2
W 36	Ratul	406.5	33.8	90.2	22.5
W 37	ARC 7047	337.2	32.2	97.6	25.2
W 49	Padi Perack	246.6	35.2	99.6	17.6
W 51	Urasan 1	246.9	38.3	94.8	19.7
W 52	Khau Tan Chiem	369.1	26.6	85.2	22.9
W 53	Tima	390.1	23.9	120.0	26.4
W 55	Tupa 729	317.3	24.3	91.2	29.1
J 3	Hinode	207.8	30.9	72.2	8.8
J 5	Kaneko	272.8	27.6	101.5	16.1
J 10	Hirayama	390.7	37.9	96.8	30.9
J 17	Akage	231.4	37.3	80.4	29.2
J 20	Hosogara	133.7	39.4	76.5	13.2
J 22	Mansaku	357.9	22.0	83.7	11.8
J 23	Ishijiro	277.6	41.0	84.3	24.7
J 24	Joushuu	266.1	18.3	98.0	12.7
J 26	Aikoku	341.1	36.2	94.2	34.3
J 30	Moritawase	318.8	43.0	73.5	24.4
J 36	Sekiyama	292.9	51.5	77.5	32.7
J 37	Shinyamadaho 2	310.4	35.9	66.3	17.8
J 41	Akamai	327.3	16.1	72.4	7.3
J 45	Hiyadachitou	286.2	42.7	77.6	32.6
J 46	Fukoku	189.5	29.9	62.2	16.5
Koshi	Koshihikari	346.1	36.6	91.8	28.3
Nippon	Nipponbare	359.8	32.3	114.0	32.3
Hoku	Hokuriku 193	459.5	28.5	151.6	50.1
Taka	Takanari	341.2	43.7	59.7	27.3
Momi	Momiroman	284.6	38.9	130.6	52.1
Hino	Hinohikari	425.2	32.6	119.1	33.1
Mizuho	Mizuhochikara	349.4	43.5	98.4	24.1
Aki	Akimasari	456.5	40.7	105.4	25.2
SL 519	SL 519	457.0	36.6	140.9	38.2

Harvest index (HI), Nitrogen use efficiency for biomass (BE_N) and Nitrogen use efficiency for grain yield (YE_N) are listed in Table 15.

HI was varied among the rice cultivars. In WRC cultivars, HI was ranged from 20.8 to 50%. W 14 (IR58) showed the highest HI than the other JRC cultivars, followed by W 18 (Qingyu, 40.2%), W 10 (Shuusoushu, 46.2%) and W 1 (Nipponbare, 39.8%). J 36 (Sekiyama) showed the highest HI (51.5%) among JRC cultivars, and the lowest HI was observed in J 41 (Akamai, 16.1%). Next to J 36 (Sekiyama), HI of J 45 (Hiyadachitou, 42.7%) and J 30 (Moritawase, 43%) were higher than the other cultivars in the group.

In HY cultivars, HI was ranged from 32.2 to 46.7% and Takanari and Mizuhochikara had higher HI than the other cultivars.

BE_N was varied among rice cultivars and it tended to be higher in HY cultivars than WRC and JRC cultivars. Hokuriku 193 showed the highest BE_N (151.6) than the other cultivars, followed by SL 519 (130.8) and Momiroman (130.8). In WRC and JRC cultivars, BE_N in W 16 (Vary Futsi) and J 5 (Kaneko) were 127.5 and 101.5, respectively, and it was higher than the other cultivars.

YE_N was different among rice cultivars. YE_N was ranged 13.6 – 42.1, 7.3 – 34.3 and 24.1 – 52.1 in WRC, JRC and HY cultivars, respectively. Momiroman showed the highest in YE_N than other rice cultivars, followed by Hokuriku 193 (50.1), W 18 (Qingyu, 42.1), W 14 (IR 58, 40.0), SL 519 (38.2) and J 26 (Aikoku, 34.3).

4.4. Discussion

Recently, rice grain yield has increased markedly in order to coping with overpopulation and satisfy the food human's demand. Improvements of cultivation techniques such as fertilizer application, using high yielding rice cultivars, plays an important role in enhancing rice yield. More fertilizer used in rice cultivation coping with any negative problems, such as soils and water contaminations. Ishihara (1997) reported that some cultivars were grown in the previous time have some character might be adapt with lower levels of N fertilizer and accumulate larger amount of N. And in chapter 3 showed that, some high yielding cultivars could attain higher grain yield without N fertilizer. However, understandings about dry matter production, N accumulation, yield and nitrogen use efficiency for grain of varietal genotypes with no N fertilizer application is still limited. In the present study, we evaluated the dry matter production, grain yield, accumulated N and Nitrogen use efficiency for grain yield among WRC, JRC and HY cultivars without N fertilizer application.

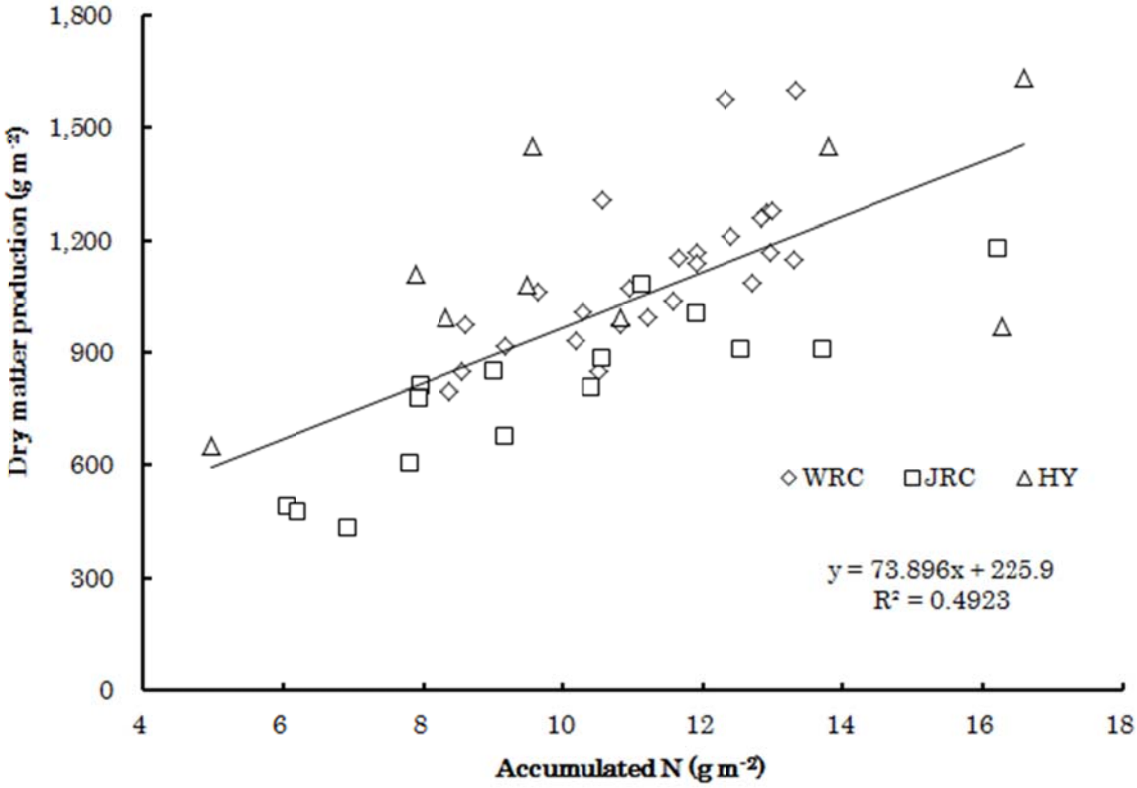


Fig. 18. Relationship between dry matter production and accumulated N at harvesting time in rice cultivars.

Close relation was found between accumulated N and dry matter production of all cultivars used (Fig. 18). The larger the accumulated N, the larger the dry matter production. JRC cultivars tended to produce smaller dry matter than WRC and HHY cultivars. Takanari, J 41 (Akamai) and w 53 (Tima) consistently accumulated the largest amount of N and produce higher dry matter than other rice cultivars (Fig. 15, 17). Table 12 and Fig. 18 also showed the correlation between amount of accumulated N and the dry matter production. The result notice that, some rice cultivars in WRC, JRC and HYV accumulated the larger amount of N and produced heavier dry matter production during ripening period.

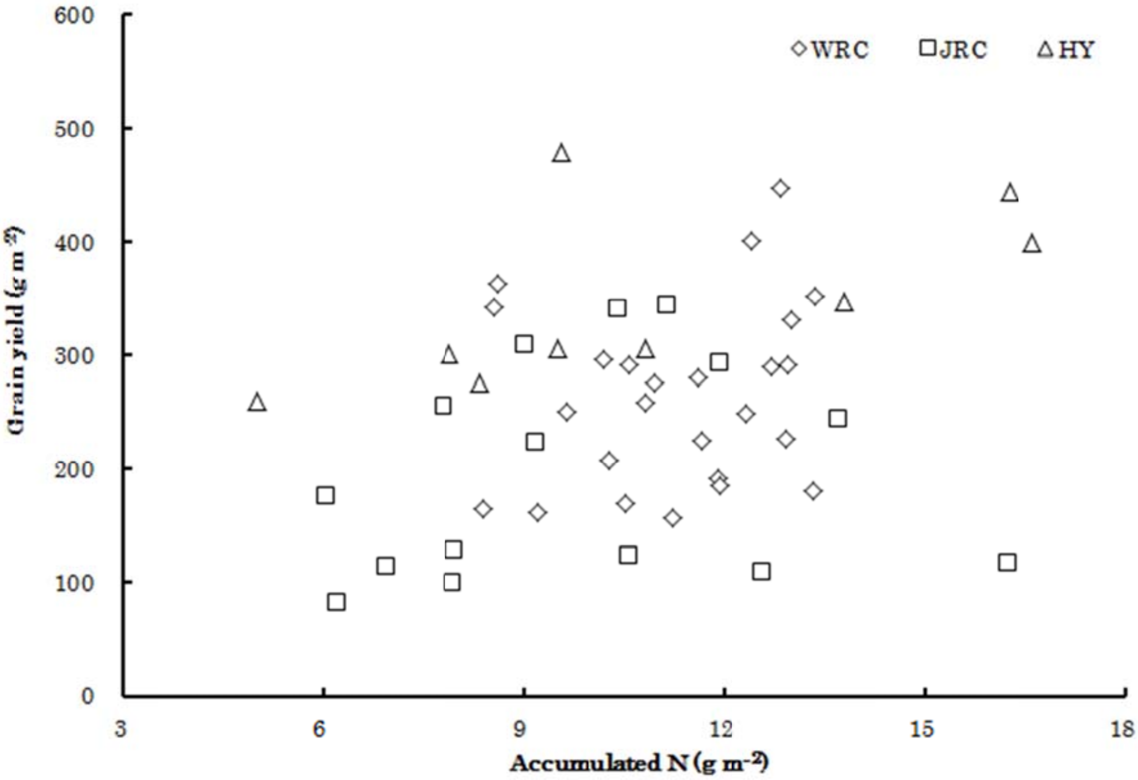


Fig. 19. Relationship between grain yield and accumulated N at harvesting time in rice cultivars.

Fig. 19 showed the relationship between grain yield and accumulated N at harvesting in rice cultivars. HY cultivars tended to be showed a higher grain yield than WRC and JRC cultivars. JRC cultivars could uptake more N to produce higher dry matter production, however, the higher the accumulated N, the higher the grain yields was not obtained. This was due to the lower HI in JRC cultivars (Table 15). Hokuriku 193, Takanari, Mizuhochikara, W 2 (Kasalath) and W 10 (Shuusoushu) showed higher grain yield and larger amount of accumulate N than the other rice cultivars.

On the other hand, improvement of rice cultivars was attributed to increase in harvest index (HI) rather than total dry matter production (Ju et at., 2009). In this study, HI of rice cultivars was varied among rice cultivars (Table 15). Rice cultivars having higher HI can attain the higher grain yield. W 10 (Shuusousuu), W 2 (Kasalath), Takanari and Mizuhochikara achieved both higher HI and grain yield than the other rice cultivars. Some cultivars in WRC and JRC showed higher grain yield with lower HI which was attributed to another factors such as dry matter production or yield components.

The field experiment was conducted using many cultivars without N fertilizer application, nitrogen used efficiency for grain yield (YE_N) was calculated (Table 15). YE_N was higher in HY cultivars than WRC and JRC cultivars. In WRC, W2 (Kasalath) and W 14 (IR 58) showed higher YE_N than the other rice cultivars.

In conclusion, without N fertilizer application, the higher dry matter production, grain yield and HI in HY cultivars and such cultivars as W 2 (Kasalath), W 10 (Shuusoushu), W 30 (Anjana Dhan), J10 (Hirayama), J 23 (Ishijiro) J 36 (Sekiyama), W 18 (Qingyu) and J 26 (Aikoku). These cultivars could accumulate the larger amount of nitrogen probably from the soil than the other rice cultivars. These cultivars can adapted with low levels of N fertilizer. Further study was needed to understand the mechanism responsible for the efficient accumulation of N with and without N fertilizer.

Summary

The nitrogen use efficiency for grain (YE_N) tended to decrease with the increasing N doses in chapter 2, 3. Besides, the grain yields of Takanari and Hokuriku 193 were also higher than other rice cultivars in 0N level. Therefore, in chapter 4, we investigated the cultivars differences in dry matter production, grain yield and nitrogen use efficiency in some selected rice cultivars.

The results showed that Mizuhochikara consistently produced the largest dry matter production to compare with WRC and JRC cultivars. Dry matter tended to be higher in WRC than JRC cultivars. Accumulated N were ranged from 8.4 – 13.3, 8.1 – 16.2 and 5.0 – 16.6 g m^{-2} in WRC, JRC and HY cultivars, respectively. YE_N was ranged from 13.6 – 42.1; 7.3 – 34.3 and 24.1 – 52.1 in WRC, JRC and HY cultivars, respectively. Momiroman showed the highest in YE_N among rice cultivars, followed by Hokuriku 193, W 16 (Qingyu), W 14 (IR 58), SL 519 and J 26 (Aikoku). Grain yield of HY cultivars and W 2 (Kasalath), W 10 (Shuusoushu), W 30 (Anjana Dhan), J10 (Hirayama), J 23 (Ishijiro) J 36 (Sekiyama), W 18 (Qingyu) and J 26 (Aikoku) tended to be higher because of higher dry matter production and harvest index. These cultivars could not only accumulate a larger amount of nitrogen from the soil than others but also are well-adapted to low levels of N fertilizer.

Chapter 5

General discussion

This study intended to investigate the effects of different types of fertilizers and methods on dry matter production, yield and nitrogen use efficiency of rice cultivars. Besides, the cultivar differences in nitrogen use efficiency at different levels of nitrogen fertilizer also were conducted in the experimental farm at the Field Science Center, Okayama University during 2 years 2011 and 2012.

First, the differences in dry matter production, grain yield, nitrogen accumulation and nitrogen use efficiency were characterized (Chapter 2). Then, the suitable fertilizer types and methods for rice cultivars were recommended to clarify nitrogen use efficiency of rice cultivars under different levels of nitrogen fertilizer (chapter 3). In chapter 3, grain yield of Takanari and Hokuriku 193 tended to be higher in 0N level than 2N level, and even higher than other japonica rice cultivars cultivated with conventional methods. Moreover, nitrogen use efficiency decreased with the increasing of N levels and it even was higher in 0N than 1N and 2N levels. In chapter 4, an experiment was conducted to evaluate genotypic differences in dry matter production, grain yield and nitrogen use efficiency in selected rice cultivars without N fertilizer.

The results of the experiment presented in the preceding chapter have been discussed and elucidated in this chapter. For more illustration factors and possible reasons of variation obtained due to treatment differences have been discussed in this chapter according to the objectives of the present investigation.

In term of improving rice yield to deal with the problem of overpopulation in the near future, researchers have focused on rice breeding programs and cultivation methods. The breeding programs also released new varieties which had a potential to higher grain yields than standard japonica varieties (San-oh et al., 2004; Mae et al., 2006).

The amount of absorbed N during panicle initiation makes the most effective contribution to spikelet production as well as grain filling. A larger amount absorbed N increases specific leaf weight and N content in leaves, which leads to increasing photosynthesis and promotion of carbohydrate accumulation in culms, leaves and transfer to grain after heading (Mae, 1997). Therefore, heavy nitrogen fertilization during panicle development, has been popular to improve population dynamics, make fertilizer use more efficient and raise grain yield (Jiang et al., 2004). However, increasing fertilizer N efficiency will be a major challenge for rice researchers and farmers. Greater fertilizer N efficiency may be achieved through improved timing and application methods and particularly through better incorporation of basal fertilizer N. Other promising alternative practices are use of N-efficient rice varieties, hand or machine deep placement of urea super granules, and use of slow-release N fertilizers

In chapter 2, nitrogen fertilizer was supplied at the 45 and 30 days before heading (DBH) in conventional and deep fertilizer methods as topdressing in order to provide nutrient for rice uptake to the contribution to dry matter and spikelets production. The dry matter production increased with fertilizer methods and was at the highest in Takanari with deep fertilizer method which was likely to be effective to obtain high dry matter production and improve the efficiency of nitrogen use. Roger et al., (1980), De Datta, (1986) and (Okumura et al., (1982) also reported the same results with this study. However, slow-release fertilizer application can be an advantage in rice production since plant can absorb nitrogen efficiently, leading to high dry matter production and grain yield (Ohnishi et al., 1999; Taylaran et al., 2009). In chapter 2, the method using slow-release fertilizer produced higher dry matter production than conventional methods which shows that slow-release fertilizer method can get higher dry matter production, and has the potential to higher grain yield.

Wu (2008) showed that, the rising LAI enhanced the CGR. The results indicate that the high yield of rice mostly comes from the products of photosynthesis after heading, which shown by the increased CGR at middle and later stages. Taylaran et al., (2009) also reported that CGR after heading of Takanari was larger than the other cultivars. This matter explained the greatest in dry matter production and grain yield. In this study, differences in timing and application methods also affected to CGR and mean LAI. CGR and mean LAI showed the maximum at the heading stage with deep fertilizer method ad high fertilizer method. After heading, the CGR were also higher in deep fertilizer application and fertilizer methods using slow-release fertilizer (Fig. 3). It is suggested that CGR might be used to explain the higher grain yield at harvest.

The importance of sink size in enhancing grain yield has been recognized by many researchers (Amano et al., 1993; Kropff et al., 1994). Rice yield can improve by increase sink size. Yoshida and Parap, (1976) reported that sink size accounted for 81% of the seasonal variations in rice yield. Grain yield is the product of different yield components, such as number of panicles m^{-2}, number of spikelets panicle, and thousand grain weight. Okumura, Wada and Wang also reported that deep fertilizer increased the number of spikelets m^{-2} and grain yield raised 10 to 13% compared with conventional method. In chapter 2, N fertilizer doses and methods increased number of spikelets m^{-2}, resulting in a higher sink capacity. Deep fertilizer method also showed higher grain yield in both cultivars. Takanari consistently produced the highest number of spikelets m^{-2} (45,000) while, high fertilizer method (using slow-release fertilizer) produced the highest number of spikelets (32,600) compared with other fertilizer methods in Nipponbare. Sink capacity had a significant relationship with grain yield (Fig. 6a). High sink capacity contributed to higher grain yield in rice cultivars. Deep fertilizer method had larger sink capacity than the other fertilizer methods. However, the more nitrogen accumulate, the higher sink capacity produced (Fig. 6b). Accumulated N was affected by nitrogen fertilizer application and cultivation method (Lin et al., 2009; Fukushima et al., 2011a). In chapter 2, Nitrogen accumulation increased with nitrogen fertilizer doses and it were highest with deep fertilizer method in both cultivars. Therefore, higher rice grain yield could be explained by larger amount of accumulated N.

YE_N decreased more among the other fertilizer application methods than in the control (0N). YE_N tended to be higher in Takanari than in Nipponbare. These results are similar with the other studies reported for japonica cultivars (Peng et al., 2006; Artacho et al., 2009).

Yoshida (1983) reported that LAI of a rice crop increases as growth advances and reaches maximum at around heading, when the rice plant has its five largest leaves. After heading, LAI declines as the lower leaves die. In chapter 3, LAI increased with more N fertilizer application and it was in the order of 2N > 1N > 0N. The maximum LAI values were 10.2 m^2 m^{-2} in Takanari, consistently largest in rice cultivars and N levels. High LAI contributed to higher CGR, leads to higher dry matter production in rice cultivars. Besides, the dry matter production in rice cultivars increased significantly associating with higher N levels in both years. However, increasing the amount of N application from 8 g m^{-2} to 16 g m^{-2} did not mean that increasing too much the dry matter in rice cultivars. Akimasari consistently produced a larger dry matter production among rice cultivars, while Koshihikari showed a smaller value in dry matter production (Fig. 7), which corresponded to the late and early maturing cultivars in Okayama prefecture, respectively. Yoshida, (1983) and Ying et al., (1998) also concluded biomass production can be increased by increasing growth duration or crop growth rate or both. These results were the same in this study. The differences in dry matter production might result in not only dependence on N fertilizer but also growth duration. However, Takanari, Akimasari and Hokuriku 193 had lager dry matter production at the harvesting time. It implied that the differences among rice cultivars were caused by the increasing of dry matter production from HD to HV (Fig. 8, 9).

Higher number of panicles m^{-2} and total number of spikelets m^{-2} also increased sink capacity in rice (Kropff et al., 1994). With high nitrogen fertilizer, number stem m^{-2} increased significantly with N levels and it was in the order of 2N, 1N and 0N. Number of stem is the most important components yield. Hinohikari, Koshihikari, Nipponbare and Akimasari also produced higher number of stems, resulted in higher number of panicles m^{-2}. Besides, the number of spikelets per panicle of Takanari, Hokuriku 193, Mizuhochikara and Momiroman was significantly higher than other cultivars, resulted in significantly great number of spikelets m^{-2}. Plants with higher sink capacity have a potential for higher grain yield. However, grain yields of some rice cultivars decreased with more N fertilizer application and the grain yield at 2N levels significantly lower to compare with 0N and 1N levels. Moreover, the accumulated N increased and showed the largest at harvesting. In 2011, Takanari consistently accumulated the largest amount of N (23.6 g m^{-2}) in 2N, while Koshihikari showed the smallest value (17.0 g m^{-2}). Hinohikari accumulated the largest amount of N (25.7 g m^{-2}), followed by Akimasari (24.8 g m^{-2}) in 2012. High accumulated N produced higher sink capacity and grain yield (Chapter 2). However, in chapter 3, rice grain yield tended to be decrease gradually, especially in Momiroman. These results indicate that increased sink capacity is not sufficient to increase rice yield. Otherwise, lodging also affected to grain yield. High N levels increased plant height and caused to lodging occurs in some cultivars. Grain yields in 1N level were higher than 2N and 0N levels during 2 years mainly due to the increase in lodging score in 2N levels. Besides, in 2012, we found that grain yield of Takanari and Hokuriku 193 exceeded 560 g m^{-2}. The grain yield in Takanari and Hokuriku in 0N levels was higher due to high sink formation capacity (Fig. 14). Wada (1969) also reported the same result.

Ripened percentage also affected to grain yield. Plants with lower ripened percentage had lower grain yield than other cultivars. During 2 year experiments, the grain yield of Momiroman showed the lowest values among cultivars and N levels. Although Momiroman showed the high sink capacity (974 g m^{-2} in 2011) but lower ripened percentage caused the decreasing of grain yield (Table 7, 8 and Fig. 12). Mukoyama et al. (2014) also observed the lower ripening ability in Momiroman which was necessary to clarify the lower ripening characters in Momiroman.

Grain yield can be improved by increasing total dry matter production (Ju et al., 2009). Total dry matter production is determined by total N uptake and N use efficiency for dry matter production (Akita, 1989). Besides (Peng et al., 2000) reported that the increasing in rice grain yield should be based on the increasing in dry matter accumulation. In this study, dry matter production had a positive relation with accumulated N at harvesting significantly (Fig. 13). A larger amount of accumulated N contributed to a higher dry matter production, and Hokuriku 193 showed a higher dry matter production at the same levels of accumulated N with higher BE_N. The higher BE_N was thought to be the higher partitioning of N to the leaves at the same levels of accumulated N. Further studies are necessarily conducted to clarify the factor affecting the higher BE_N in Hokuriku 193.

YE_N decreased with N application and it was higher in 1N than 2N levels, because the increase in accumulated N was higher than the increasing in grain yield. YE_N of Takanari at 1N were 40.5 and 43.0 in 2011 and 2012, relative with highest HI. Hokuriku 193 also showed higher YE_N (45.4) and HI (41.2) at 1N in 2012 (Table 9, 10). Higher YE_N observed in Takanari and Hokuriku 193 during 2 years which could be attribute to the higher HI. The same result was reported by Samonte et al., (2006).

YE_N tended to decrease with increasing in N doses in chapter 2 and 3. In addition, the grain yields of Takanari and Hokuriku 193 also higher than other rice cultivars in 0N. Therefore, in chapter 4, we investigated the genotypic differences in dry matter production, grain yield and nitrogen use efficiency in several selected rice cultivars in field conditions without N fertilizer application. In WRC cultivars, 17 cultivars showed the total dry matter production of more than 1000 g m^{-2}. W 53 (Tima), which produced the highest dry matter production (1601 g m^{-2}) among the WRC cultivars, followed by W 16 (Vary Futsi – 1573 g m^{-2}), W 30 (Anjana Dhan – 1305 g m^{-2}), W 5 (Naba - 1276 g m^{-2}) and W3 (Bei Khe - 1273 g m^{-2}). Among JRC cultivars, J 41 (Akamai), J 10 (Hirayama) and J 23 (Ishijiro) had higher dry matter production with the total of 1175, 1079 and 1004 g m^{-2}, respectively. J 46 (Fukoku), J 20 (Hosogara) and J 17 (Akage) showed the smallest values among the JRC cultivars. Close relation was found between accumulated N and dry matter production of all cultivars used (Fig. 18). The larger the accumulated N, the larger the dry matter produced. The result notice that, some rice cultivars in WRC, JRC and HYV accumulated the larger amount of N and produced heavier dry matter production during ripening period.

Fig. 19 showed the relationship between grain yield and accumulated N at harvesting in rice cultivars. HY cultivars tended to be showed a higher grain yield than WRC and JRC cultivars. JRC cultivars could uptake more N to produce higher dry matter production, however, the higher the accumulated N, the higher the grain yields was not obtained. This was due to the lower HI in JRC cultivars (Table 15). Hokuriku 193, Takanari, Mizuhochikara, W 2 (Kasalath) and W 10 (Shuusoushu) showed higher grain yield and larger amount of accumulate N than the other rice cultivars. (Kyaw et al., 2005) also reported the same result with this study.

Without N fertilizer application, the higher dry matter production, grain yield and HI in HY cultivars and such cultivars as W 2 (Kasalath), W 10 (Shuusoushu), W 30 (Anjana Dhan), J10 (Hirayama), J 23 (Ishijiro) J 36 (Sekiyama), W 18 (Qingyu) and J 26 (Aikoku). These cultivars could accumulate the larger amount of nitrogen probably from the soil than the other rice cultivars and adapt with low levels of N fertilizer. Further study was needed to understand the mechanism responsible for the efficient accumulation of N with and without N fertilizer.

References

Akita, S. 1989. Improving yield potential in tropical rice. *In* Progress in Irrigated Rice Research. p. 41-73. IRRI, Los Banos.

Amano, T., Zhu, Q., Wang, Y., Inoue, N. and Tanaka, H. 1993. Case studies on high yields of paddy rice in Jiangsu province, China. I. Characteristics of grain production. *Jpn. J. Crop. Sci*. 62: 267-274.

Arima, Y. 1995. Uptake and accumulation of nitrogen. *In* Science of the Rice Plant, Vol. 2. Physiology (Matsuo, T. et al. eds), pp. 327-343, Food and Agrciculture Policy Research Center, Tokyo.

Artacho, P., Bonomelli, C. and Meza, F. 2009. Nitrogen application in irrigated rice grown in Mediterranean conditions: Effects on grain yield, dry matter production, nitrogen uptake, and nitrogen use efficiency. *J. Plant Nutr*. 32: 1574-1593.

Belder, P., Bouman, B.A.M., Spiertz, J.H.J., Peng, S., Castaeda, A.R. and Visperas, R.M. 2005. Crop performance, nitrogen and water use in flooded and aerobic rice. *Plant Soil*. 273: 167-182.

Borrell, A.K., Garside, A.L., Fukai, S. and Reid, D.J. 1998. Season, nitrogen rate, and plant type affect nitrogen uptake and nitrogen use efficiency in rice. *Aust. J. Agr. Res*. 49: 829-844.

Cassman, K.G., Peng, S., Olk, D.C., Ladha, J.K., Reichardt, W., Dobermann, A. and Singh, U. 1998. Opportunities for increased nitrogen-use efficiency from improved resource management in irrigated rice systems. *Field Crops Res*. 56: 7-39.

De Datta, S.K. 1986. Improving nitrogen fertilizer efficiency in lowland rice in tropical *Asia. Fert. Res.* 9: 171-186.

Eagle, A.J., Bird, J.A., Hill, J.A., Horwath, W.R. and van Kessel, C. 2011. Nitrogen dynamics and fertilizer use efficiency in rice following straw incorporation and winter flooding. *Agron. J.* 93: 1346-1354.

Fageria, N.K. and Baligar, V.C. 2001. Lowland rice response to nitrogen fertilization. *Commun. Soil Sci. Plant Anal.* 32: 1405-1429.

Fageria, N.K. and Barbosa Filho, M.P. 2001. Nitrogen use efficiency in lowland rice genotypes. *Commun. Soil Sci. Plant Anal.* 32: 2079-2089.

FAO, 2002. Concern about rice production practices (http://www.fao.org/english/newsroom/news/2002/7538-en.html).

Fukushima, A., Shiratsuchi, H., Yamaguchi, H. and Fukuda, A. 2011a. Effects of nitrogen application and planting density on morphological traits, dry matter production and yield of large grain type rice variety Bekoaoba and strategies for super high-yielding rice in the Tohoku region of Japan. *Plant Prod. Sci.* 14: 56-63.

Fukushima, A., Shiratsuchi, H., Yamaguchi, H. and Fukuda, A. 2011b. Varietal differences in morphological traits, dry matter production and yield of high-yielding rice in the Tohoku region of Japan. *Plant Prod. Sci.* 14: 47-55.

Guyer, D., Tuttle, A., Rouse, S., Volrath, S., Johnson, M., Potter, S., Görlach, J., Goff, S., Crossland, L. and Ward, E. 1998. Activation of latent transgenes in Arabidopsis using a hybrid transcription factor. *Genetics.* 149: 633-639.

Hasegawa, T. and Horie, T. 1996. Leaf nitrogen, plant age and crop dry matter production in rice. *Field Crops Res.* 47: 107-116.

Hayashi, S., Ohshita, Y., Kimiwada, K., Tsuji, H., Ushiki, J., Miyaura, S. and Shibuya, Y. 2012. Yielding performance of "Kita-aoba", high-yielding rice variety for Hokkaido region, Northern Japan. *Plant Prod. Sci.* 15: 209-215.

Hossain, M. and Narciso, J., 2004. Global rice economy: Long-term perspectives. *In* Long-term prospects for the global rice economy. Hossain, M. et. al. eds, p. 2-19 FAO, Rome, Italy.

IRRI, 2002. Rice almanac. Los Baños, Laguna, IRRI, CIAT and WARDA. (http://books.irri.org/0851996361_content.pdf)

Jackson, P., Robertson, M., Cooper, M. and Hammer, G. 1996. The role of physiological understanding in plant breeding; from a breeding perspective. *Field Crops Res.* 49: 11-37.

James, A.D. 1983. Handbook of Energy Crops. (https://www.hort.purdue.edu/newcrop/duke_energy/Oryza_sativa.html)

Jiang, L., Dai, T., Jiang, D., Cao, W., Gan, X. and Wei, S. 2004. Characterizing physiological N-use efficiency as influenced by nitrogen management in three rice cultivars. *Field Crops Res*. 88: 239-250.

Ju, J., Yamamoto, Y., Wang, Y., Shan, Y., Dong, G., Miyazaki, A. and Yoshida, T. 2009. Genotypic differences in dry matter accumulation, nitrogen use efficiency and harvest index in recombinant inbred lines of rice under hydroponic culture. *Plant Prod. Sci.*12: 208-216.

Koutroubas, S.D. and Ntanos, D.A. 2003. Genotypic differences for grain yield and nitrogen utilization in Indica and Japonica rice under Mediterranean conditions. *Field Crops Res.* 83: 251-260.

Kropff, M.J., Cassan, K.G., Peng, S., Mattews, R.B. and Setter, T.L. 1994. Quantitative understanding of yield potential. *In* Breaking the yield barrier. Cassan, K.G. et. al. eds, p. 21-38. International Rice Research Institute, Los Banos, Philippines.

Kyaw, K., Toyota, K., Okazaki, M., Motobayashi, T. and Tanaka, H. 2005. Nitrogen balance in a paddy field planted with whole crop rice (*Oryza sativa* cv. Kusahonami) during two rice-growing seasons. *Biol.Fert. Soils.* 42: 72-82.

Ladha, J.K., Pathak, H., J. Krupnik, T., Six, J., van Kessel, C. and Donald, L.S. 2005. Efficiency of fertilizer nitrogen in cereal production: Retrospects and Prospects. *Adv. Agron.* 87: 85-156.

Li, J., Xin, Y. and Yuan, L. 2009. Hybrid rice technology development - Ensuring China's food security. International Food Policy Research Institute, Washington, USA. (http://www.ifpri.org/publication/hybrid-rice-technology-development)

Lin, X.-q., Zhu, D.-f., Chen, H.-z. and Zhang, Y.-p. 2009. Effects of plant density and nitrogen application rate on grain yield and nitrogen uptake of super hybrid rice. *Rice Science.* 16: 138-142.

Mae, T. 1997. Physiological nitrogen efficiency in rice Nitrogen utilization, photosynthesis, and yield potential. *Plant Soil.* 196: 201-210.

Mae, T., Inaba, A., Kaneta, Y., Masaki, S., Sasaki, M., Aizawa, M., Okawa, S., Hasegawa, S. and Makino, A. 2006. A large-grain rice cultivar, Akita 63, exhibits high yields with high physiological N-use efficiency. *Field Crops Res.* 97: 227-237.

Matsushima, 1980. Rice cultivation for the million. Japan Scientific Societies Press, Tokyo, Japan, pp.1-380.

Mohammad, A.Z.S., Murayama, S., Akamine, H. and Nakamura, I. 2002. Effect of nitrogen fertilization on photosynthetic characters and dry matter production in F1 hybrids of rice (*Oryza sativa* L.). *Plant Prod. Sci.* 5: 131-138.

Moll, R.H., Kamprath, E.J. and Jackson, W.A. 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. *Agron. J.* 74:562–564.

Mukoyama, T., Arai-sanoh, Y., Takai, T., Nakano, H. and Kondo, M. 2014. Effect of limiting sink capacity on ripening ability and NSC translocation of Momiroman, high yielding rice variety. *Jpn. J. Crop. Sci. 83 (Extra I)*: 196-197 (In Japanese)

Nakano, H. and Morita, S. 2009. Effects of planting time and nitrogen application on dry matter yield of the forage rice cultivar Tachiaoba in southwestern Japan. *Plant Prod. Sci.* 12: 351-358.

Ohnishi, M., Horie, T., Homma, K., Supapoj, N., Takano, H. and Yamamoto, S. 1999. Nitrogen management and cultivar effects on rice yield and nitrogen use efficiency in Northeast Thailand. *Field Crops Res.* 64: 109-120.

Okumura, T., Takeuchi, S. and Hasegawa, H. 1982. Study on the deeper layer application of additional nitrogen fertilizer for rice plant. II. Relationship between the depth of application of top dressing and growth and yield of rice plants. *Jpn. J. Crop Sci.* 51, 58-64. (In Japanese with English abstract)

Osada. A. 1995. Photosynthesis and respiration in relation to nitrogen responsiveness. *In* Science of the Rice Plant, Vol. 2. Physiology (Matsuo, T. et al. eds), p. 696-703 . Food and Agriculture Policy Research Center, Tokyo.

Peng, S., Buresh, R.J., Huang, J., Yang, J., Zou, Y., Zhong, X., Wang, G. and Zhang, F. 2006. Strategies for overcoming low agronomic nitrogen use efficiency in irrigated rice systems in China. *Field Crops Res*. 96: 37-47.

Peng, S., Laza, R.C., Visperas, R.M., khush, G.S., Virk, P. and Zhu, D. 2004. Rice: Progress in Breaking the Yield Ceiling. *In* Fischer T et. al (2004)., Brisbane, Australia, 26 September – 1 October 2004., Brisbane, Australia.

Peng, S., Laza, R.C., Visperas, R.M., Sanico, A.L., Cassan, K.G. and Khush, G.S. 2000. Grain yield of rice cultivars and lines developed in the Philippines since 1966. *Crop Science.* 40: 307-314

Raun, W.R. and Schepers, J.S. 2008. Nitrogen management to protect water resources. *In* Nitrogen in Agricultural Systems. (W.R. Raun. W.R. et. al. eds.) p.911-946. Agronomy Monograph 49. ASA, CSSA, and SSSA Madison, WI.

Rivers, C., Barrett, M., Hiscock, K., Dennis, P., Feast, N. and Lerner, D. 1996. Use of nitrogen isotopes to identify nitrogen contamination of the sherwood sandstone aquifer beneath the city of Nottingham, United Kingdom. *Hydrogeol. J.* 4: 90-102.

Roger, P.A., Kulasooriya, S.A., Tirol, A.C. and Craswell, E.T. 1980. Deep placement: A method of nitrogen fertilizer application compatible with algal nitrogen fixation in wetland rice soils. *Plant Soil.* 57: 137-142.

Samonte, S.O.P., Wilson, L.T., Medley, J.C., Pinson, S.R.M., McClung, A.M. and Lales, J.S. 2006. Nitrogen utilization efficiency: Relationships with grain yield, grain protein, and yield-related traits in rice. *Agron. J.* 98: 168-176.

San-oh, Y., Mano, Y., Ookawa, T. and Hirasawa, T. 2004. Comparison of dry matter production and associated characteristics between direct-sown and transplanted rice plants in a submerged paddy field and relationships to planting patterns. *Field Crops Res.* 87: 43-58.

Seito, F., Nakahori, T., Takagi, T. and Oyamada, Z. 1992. The relation between protein content in milled rice and eating quality, and effects of fertilizer application method. *Tohoku Agric. Res.* 45: 31-32.

Singh, U., Ladha, J.K., Castillo, E.G., Punzalan, G., Tirol-Padre, A. and Duqueza, M. 1998. Genotypic variation in nitrogen use efficiency in medium and long-duration rice. *Field Crops Res.* 58: 35-53.

Taylaran, R.D., Ozawa, S., Miyamoto, N., Ookawa, T., Motobayashi, T. and Hirasawa, T. 2009. Performance of a high-yielding modern rice cultivar Takanari and several old and new cultivars grown with and without chemical fertilizer in a submerged paddy field. *Plant Prod. Sci.* 12: 365-380.

Tirol-Padre, A., Ladha, J.K., Singh, U., Laureles, E., Punzalan, G. and Akita, S. 1996. Grain yield performance of rice genotypes at suboptimal levels of soil N as affected by N uptake and utilization efficiency. *Field Crops Res.* 46: 127-143.

Wada, G. 1969. The effect of nitrogenous nutrition on the yield determining process of rice plant. *Bull. Natl. Isnt. Agric. Sci.* A16:27-167 (In Japanese with English summary).

Wang, W.J., Katayama, K. and Takeda, T. 1991. Study on applicability of nitrogen-deep placement culture from the view point of productivity of various rice cultivars in warmer area. *Jpn. J. Crop Sci.* 60: 57-64.

Wu, W.-g., Zhang, H.-c., Qian, Y.-f., Cheng, Y., Wu, G.-c., Zhai, C.-q. and Dai, Q.-g. 2008. Analysis on dry matter production characteristics of super hybrid rice. *Rice Science.* 15: 110-118.

Ye, Q., Zhang, H., Wei, H., Zhang, Y., wang, B., Xia, K., Huo, Z., Dai, Q. and Xu, K. 2007. Effects of nitrogen fertilizer on nitrogen use efficiency and yield of rice under different soil conditions. *Front. Agric. China.* 1: 30-36.

Ying, J., Peng, S., He, Q., Yang, H., Yang, C., Visperas, R.M. and Cassman, K.G. 1998. Comparison of high-yield rice in tropical and subtropical environments: I. Determinants of grain and dry matter yields. *Field Crops Res.* 57: 71-84.

Yoshida, S. 1981. Fundamentals of rice crop science. International Rice Research Institute, Los Banos, Philippines.

Yoshida, S. 1983. Rice. *In* Potential Productivity of Field Crops under Different Environments (Smith, W. H. et. al. eds), p. 103-127. International Rice Research Institute, Los Baños, Philippines.

Yoshida, S. and Parap, F.T. 1976. Climatic influence on yield and yield components of lowland rice in the tropics. *In* Climate and Rice, p. 471-494. International Rice Research Institute Los Baños, Philippines.

Yoshida, S. 1978. Tropical climate and its influence on rice. Other. International Rice Research Institute, Los Banos, Laguna, Philippines. (http://eprints.icrisat.ac.in/8613/1/RP_01036_Tropical_climate_and_its........pdf)

Zhao, S.P., Zhao, X.Q., Li, S.M. and Shi, W.M. 2007. N-efficiency character of high-yield rice is not affected by soil nitrogen supply level. Acta Agriculturae Scandinavica, Section B. *Soil Plant Sci.* 57: 97-104.

Zhong, X., Peng, S., A. L. Sanico, A.L. and Liu, H. 2003. Quantifying the interactive effect of leaf nitrogen and leaf area on tillering of rice. *J. Plant Nutr.* 26: 1203-1222.

Appendix

List of symbols and abbreviation used in the thesis

Symbol/ Abbreviation	Description	Unit
0N	Treatments with total N fertilization of 0 g m^{-2}	
1N	Treatments with total N fertilization of 8 g m^{-2}	
2N	Treatments with total N fertilization of 16 g m^{-2}	
A_1	Leaf area index at corresponding time t_1	m^{-2} m^{-2}
A_2	Leaf area index at corresponding time t_2	m^{-2} m^{-2}
AN	Accumulated nitrogen	g m^{-2}
AN_0	Accumulated nitrogen without fertilizer	g m^{-2}
APN	Applied nitrogen	g m^{-2}
ANUE	Agronomic nitrogen use efficiency	g g^{-1}
BE_N	Nitrogen use efficiency for biomass	g g^{-1}
BY	Biomass yield	g m^{-2}
CGR	Crop growth rate	g m^{-2} day^{-1}
DAH	Day after heading	
DAT	Day after transplanting	
DBH	Day before heading	
FAO	Food Agriculture Organization	
GY	Grain yield	g m^{-2}
HD	Heading	

HI	Harvest index	%
HY	High yielding	
HV	Harvesting	
IRRI	International Rice Research Institute	
JRC	Rice core collection of Japanese Landraces	
LAI	Leaf area index	m^{-2} m^{-2}
N	Nitrogen	
NAR	Net assimilation rate	g m^{-2} day^{-1}
NUE	Nitrogen use efficiency	g g^{-1}
PFP_N	Partial factor productivity of applied nitrogen	g g^{-1}
PI	Panicle initiation	
RE_N	Recovery efficiency of applied nitrogen	%
W1	Dry weight at corresponding time t_1	
W2	Dry weight at corresponding time t_2	
WRC	World global rice core collection	
YE_N	Nitrogen use efficiency for grain	

Abstract

Rice is one of the most important cereal crops, widely grown in many localities with favorable climatic conditions throughout the world. Rice is the staple food of more than half of population. Rice production accounts for 27% of total cereal production in the world, and 90% of rice production takes place in Asian countries.

Nitrogen (N) is not only necessary for rice growth but also the most important nutrient in lowland rice production all over the world. N plays an important role in increasing dry matter production and grain yield. In order to improve rice yield to satisfy the food demands, farmers use high-yielding cultivars and excessively apply N fertilizer. However, heavy fertilizer application increases the cost of productions, decreases nitrogen use efficiency and pollutes the environment. Therefore, it is imperative to develop rice cultivation methods that would increase the absorption of nitrogen of rice plants, while still maintaining high and reliable dry matter production and grain yield.

To clarify the relationship between dry matter production, grain yield and nitrogen use efficiency, we conducted the field experiments at the Field Science Center, Okayama University. 1) effects of different types of fertilizers and methods on dry matter production, grain yield and nitrogen use efficiency of rice cultivars, 2) cultivar differences in nitrogen use efficiency at different levels of nitrogen fertilizer, and 3) genotypic differences in dry matter production, yield and nitrogen use efficiency in selected rice cultivars without nitrogen fertilizer, were examined.

In chapter 2, we used 2 rice cultivars (Nipponbare and Takanari) and five fertilizer methods, i.e. Control (0 gN m^{-2}), Conventional method (10 gN m^{-2}), Deep fertilizer method (10 gN m^{-2}), Standard fertilizer application method (8 gN m^{-2}) and High fertilizer

application method (16 gN m^{-2}). In this study, standard and high fertilizer methods use slow-release fertilizer. Dry matter production was increased more markedly with nitrogen fertilizer application than that in control, and it was higher with deep fertilizer application in Takanari and standard fertilizer application in Nipponbare, respectively. With differing nitrogen application doses and timing of application, crop growth rate (CGR) and mean leaf area index (mean LAI) tended to be increased and showed the maximum at heading and flowering stages. Plants with higher CGR and mean LAI during this stage might be resulted in higher grain yield at harvest. N accumulation increased consistently from transplanting to harvesting time and it showed the highest value with deep fertilizer method (24.0 g m^{-2}) in Nipponbare. Higher N accumulation contributed to larger sink capacity. Higher fertilizer application increased the number of panicle and total spikelets m^{-2}. The higher grain yield in Takanari resulted from the larger sink capacity. The grain yield of rice cultivars tended to be higher with deep fertilizer application due to the increase in sink capacity. Both deep fertilizer application and slow-release fertilizer increased the recovery efficiency and partial factor productivity of applied N. However, the uses of slow-release fertilizer is recommended in terms of labor saving and lower cost.

In chapter 3, experiments were conducted during two years, 2011 and 2012. Six rice cultivars (Koshihikari, Nipponbare, Takanari, Momiroman, Hinohikari and Akimasari) were used in 2011 and two more cultivars (Hokuriku 193 and Mizuhochikara) in 2012, respectively. All cultivars were grown at three levels of nitrogen fertilization (0N: 0 gN m^{-2}; 1N: 8 gN m^{-2}; 2N: 16 gN m^{-2}). Dry matter production became higher in all cultivars with higher the nitrogen application during two years. Dry matter production at harvest was highest in Takanari and Hokuriku 193. Takanari consistently accumulated the largest amount of N at 2N. The grain yield of Takanari was highest during the 2 years (750 g m^{-2} and 731 g m^{-2} at 1N in 2011 and 2012, respectively) followed by Hokuriku 193 (669 g m^{-2} at 1N in 2012). Nitrogen use efficiency for grain (YE_N) decreased with the raising of N levels and it was higher in Hokuriku 193 and Takanari. Both cultivars showed the highest yield among 0N plots (589 and 562 g m^{-2}) due to the highest YE_N and sink formation capacity (sink capacity / accumulated N at panicle initiation).

YE_N tended to decrease with increasing N doses in chapter 2 and 3. Besides, the grain yields of Takanari and Hokuriku 193 also higher than other rice cultivars in 0N. Therefore, in chapter 4, we investigated the genotypic differences in dry matter production, grain yield and nitrogen use efficiency in some selected rice cultivars. In this experiment, 49 rice cultivars (which were selected from NIAS Global Rice Core Collection, Rice Core Collection of Japanese Landraces and recently developed high-yielding cultivars) were grown in the field without N fertilizer. The results showed that high-yielding (HY) cultivars and such cultivars as W 2 (Kasalath), W 10 (Shuusoushu), W 30 (Anjana Dhan), J10 (Hirayama), J 23 (Ishijiro) J 36 (Sekiyama), W 18 (Qingyu) and J 26 (Aikoku) produced the higher dry matter production, grain yield and harvest index (HI) to compared with the other cultivars. These cultivars could accumulate a larger amount of nitrogen probably from the soil than the other rice cultivars and adapt to low levels of N fertilizer with high YE_N.

The overall results demonstrate that deep fertilizer application is a good way for rice cultivar to uptake more nitrogen fertilizer. However, using slow-release fertilizer might be suitable for modern rice cultivation because of labor saving and lower cost. Dry matter production, grain yield, N accumulation increased corresponding to higher N application. However, the highest application of N level did not mean that dry matter production was increased considerably and grain yield of rice cultivars tended to decrease because of the lower percentage of ripened grains. Gain yields of Takanari, Hokuriku 193, Akimasari and Hinohikari were the highest at 1N levels because of higher dry matter production, HI and YE_N. High HI and YE_N, therefore, are considered to be important factors to evaluate the high-yielding ability of rice cultivars. Some rice cultivars could accumulate a larger amount of N and produce higher grain yield. These cultivars can adapt with low levels of N fertilizer. High-yielding cultivars showed the higher grain yield and YE_N not only under standard fertilization, but also under without N fertilization.