

Full Length Research Paper

Effects of nitrogen levels on growth, yield and nitrogen uptake of fiber-rich cultivar, Goami 2

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To investigate the effect of nitrogen (N) application level on fiber-rich cultivar, Goami 2, an experiment was carried out on a paddy soil at the rice experimental farm of the National Institute of Crop Science (NICS), Rural Development Administration (RDA) in Suwon, Korea. Rice growth, yield and its components for Goami 2 were investigated under different N application levels (0, 50, 70, 90, 110, 130 and 150 kg ha⁻¹). Goami 2 showed higher SPAD values and N content in plant than that of Daeam (reference cultivar) at 29 days after transplanting (DAT), but lower at 93 DAT. The ammonium-N (NH₄-N) increased in the Daeam cultivated soil, but decreased in the Goami 2 cultivated soil at 93 DAT. Among yield components, brown to rough rice ratio, liter (ℓ) weight, and 1,000-grain weight of Goami 2 were lower compared to Daeam as reference cultivar. Also, hulled rice yield decreased. The N application levels of Goami 2 from 70 to 150 kg ha⁻¹ did not show significant different in view of yield and N efficiency. We also found out that a number of small vascular bundles (SVB) in the stem of Goami 2 were significantly lower in the stem of Goami 2 than those of Daeam. Therefore, the optimum N application level of Goami 2 was at the 70 kg ha⁻¹, considering the yield of milled rice and N efficiency.

Key words: Rice, nitrogen, N efficiency, yield, SEM.

INTRODUCTION

Rice is the most important agricultural produce and the main staple food for many Asian countries. With growing concerns regarding national health and expanding markets of functional products worldwide, some specialty rice cultivars, including giant embryonic rice, black rice, and red rice are being developed in Korea (Li et al., 2008; Kong and Lee, 2010). Researches have been made to develop rice of high nutrition level since 1994. IR164 was developed, which has iron content of 25 mg kg⁻¹ (Hu, 2003). Also, the golden rice of high content of vitamin A was developed by transformation of β -carotene gene (Beyer et al., 2002). In Japan, twenty-four specialty rice with low amylose, high amylose, big grains, small grains, aroma, giant embryo, and color rice were developed in 1995 (Ning et al., 2008). It has been reported that 100 special rice such as color and aroma rice, has developed since China started developing cultivars breeding (Li et al., 2008). It was also reported that 45 specialty rice such as glutinous rice, medium glutinous rice, color rice, aroma rice and functional rice were developed in Korea (Kim et al., 2008). Among specialty rice cultivars, Goami 2 was

bred by mutation of methyl-N-nitrosourea (MNU) in japonica cultivar which is known to be the highest quality of japonica cultivar in Korea (Lee et al., 2006). Goami 2 has two times and five times higher non-digestibility compared to brown and milled rice, respectively, and the mineral contents, such as phosphorous, iron, zinc and magnesium have two to five times higher compared to the general japonica cultivar (Kim et al., 2007; Kang et al., 2003). It was reported that the weight and medial fat were decreased by the group of eating Goami 2 compared to general rice eating group. Although the diet, ingredient, and process of Goami 2 have been previously studied, basic information on N fertilization of the cultivar has not been reported yet. We assumed that Goami 2 may be different to N response due to different ingredient in grains. N is essential to rice production. The high input N had been accomplished to high yield rice cultivars such as short culm length, resistance to lodging and high N fertilization (Cho and Koh, 2007) because Korean government policy focused on high production up to 2001 (Jang, 2010). However, policies and researches were

caused to environmental pollution of soil, river and atmospheres as reported by researches (Rice et al., 2001; Cho and Koh, 2007). Also, N levels are different to maximum yield and N use efficiency is depended upon cultivars (Ducan et al., 1967; Fageria, 1992; Lee et al., 1985; Raun and Johnson, 1999).

This study was conducted to determine the N application amount considering N use efficiency and to clarify the characteristics of vascular bundle formation of Goami 2 cultivar.

MATERIALS AND METHODS

Soil and site

The experiments for this study were conducted in paddy soil (fine loamy, mixed, nonacid, mesic, family of Aeric Fluventic Haplaquepts) at the National Institute of Crop Science (NICS), Korea, in 2005. The soil characteristics in the 0 to 15 cm layer are as follows: pH 5.4 (water 1:5); 22.1 g kg⁻¹ organic matter; 98 mg kg⁻¹ available P₂O₅; 0.15 cmol kg⁻¹ exchangeable K; 3.25 cmol kg⁻¹ exchangeable Ca; 1.60 cmol kg⁻¹ exchangeable Mg. The soil texture was silt loam.

Cultivar and raising seedling

Two cultivars of rice (*Oryza sativa* cv. Goami 2 and Daean) were used for the experiment. Goami was developed as a mid-late maturity cultivar at the National Institute of Crop Science (NICS), Rural Development Administration, Korea, in 2002. This cultivar has high content of non-digestibility ingredient such as D-xylose and arabinose. Daean was used as reference cultivar. 30 days (cv. Daean) and 35 (Goami 2) days old seedlings were machine-transplanted in a space of 30 × 14 cm on May 24th, respectively. The seedlings of Goami 2 were raised for 35 days for root matting formation.

N treatments

A split design with N levels as main plots and cultivars as subplots was adopted. The N levels included no N application (N 0, 0 kg ha⁻¹), N 50, N 70, N 90, N 110, N 130, and N 150 kg ha⁻¹ with urea as the source (50% as basal fertilizer, and 20 and 30% as topdressing fertilizer for tillering and promoting panicle development, respectively). Phosphorus was applied to basal in all plots at 45 kg ha⁻¹ as fused phosphate (P₂O₅). Potassium was applied to 70% basal and 30% topdressing in all plots at 57 kg ha⁻¹ as potassium chloride.

Plant and soil analysis

The carbon and nitrogen content in rice plant and brown rice grains were analyzed using a CNS2000 combustion analyzer (Leco, USA). The ammonium N content in the soil was determined using a FIAstar 5000 analyzer (FOSS, Sweden) after extraction in 2 M potassium chloride solution (RDA, 1988). Agronomic N-use efficiency (ANUE) (Singh et al., 1998) was calculated as:

$$ANUE = \frac{GY_F - GY_0}{N_F}$$

Where, GY_F is the grain yield (14% moisture content) with fertilizer N application, GY₀ is the grain yield (14% moisture content) without N application and N_F is the fertilizer N applied.

Vascular bundle observation

The three hill of rice were selected from 0, 90 and 150 kg N ha⁻¹ plot at milking stage. These samples were average hill of each plot. Stem samples were from 1 cm below the panicle neck. The samples were fixed at Karnovsky's fixative at 4°C for 24 h, and then washed with 0.05 M cacodylate buffer for 10 min three times. Then, the samples were fixed with 1% osmium tetroxide (buffered in 0.05 M cacodylate buffer), and washed three times by pure water. The samples were stuck to the copper-base for spraying of gold in vacuum condition, and then vascular bundle of rice stem was observed by SEM (LEO440, Zeiss, Germany).

Rice growth and yield

The plant height, tiller and leaf color were measured at 29 and 59 DAT and heading stage. The leaf color was determined from the uppermost leaves using a chlorophyll meter (SPAD-502, Minolta, Japan). Grain yield was determined from 4 m² areas. Total grain dry weight was determined using seed moisture content (MC). Total straw weight was determined after drying at 70°C to a constant weight. Yield components such as panicle number per hill, number of grains per panicle, liter (ℓ) weight, and 1,000-grain weight were also determined (RDA, 2003).

Statistical analyses

Differences among treatments were estimated by one-way ANOVA using the SAS program (SAS institute, ver. 9.2, 2004) with Tukey's LSD. Differences were considered significant at p < 0.05.

RESULTS AND DISCUSSION

Table 1 shows the seedling quality of Goami 2 and Daean cultivars. Goami 2 was nursed at bed for 35 days for root mat formation. Goami 2 was delayed to early growth as compared to other japonica cultivars. Therefore, the seedling nursery of Goami 2 was raised for more than 30 days (Yang et al., 2005). The seedling height, leaf number, and root mat formation of Goami 2 were below those of Daean 2, even though it was nursed for 35 days. The dry weight/seedling height is criterion to measure seedling health degree. The above-ground dry weight and dry weight/seedling height of Goami 2 had good quality than Daean.

The SPAD values of Goami 2 decreased, but dry weight increased in all N levels treatments compared to Daean (Table 2). Among N levels of Goami 2, the SPAD values did not differ significantly at N 70 to N 130 in all of the treatments except N 0. However, dry weight almost had a similar trend, but the differences of N levels were bigger than SPAD values.

Ammonium-N (NH₄-N) in soil was shown by Goami 2 and Daean at 29, 64 and 93 DAT (Table 3). Rice mainly takes up NH₄-N in flooding soil condition (Nyle, 1990).

Table 1. Seedling quality of experimental cultivars.

Cultivar	Seedling height (cm)	Leaf number	Above-ground dry weight (mg plant ⁻¹)	Dry weight/ seedling height	Root mat formation
Goami 2	14.6 ^b	5.4 ^a	26.3 ^a	1.80 ^a	Good
Daeon	15.4 ^a	4.9 ^b	24.4 ^b	1.58 ^b	Excellent

Table 2. Changes of rice growth characteristics in Goami 2 and Daeon by different N levels and growing stages.

Cultivar	N level (kg ha ⁻¹)	29 DAT ¹		59 DAT		Heading ²	
		SPAD ³	Dry weight (kg ha ⁻¹)	SPAD	Dry weight (kg ha ⁻¹)	SPAD	Dry weight (kg ha)
Goami 2	0	36.4 ^c	389 ^c	33.7 ^b	2.660 ^c	33.2 ^b	6.749 ^f
	50	40.0 ^b	589 ^b	34.3 ^b	3.646 ^b	35.7 ^a	7.436 ^d
	70	41.1 ^b	581 ^b	36.5 ^a	3.521 ^b	37.7 ^a	9.188 ^{bc}
	90	41.9 ^b	718 ^a	37.7 ^a	4.343 ^a	35.9 ^a	8.802 ^c
	110	42.1 ^b	700 ^a	38.0 ^a	4.987 ^a	37.3 ^a	8.727 ^c
	130	42.0 ^b	687 ^a	39.7 ^a	4.861 ^a	38.5 ^a	9.641 ^b
	150	44.0 ^a	625 ^a	41.5 ^a	4.512 ^a	38.3 ^a	10.890 ^a
Daeon	0	35.8 ^c	550 ^d	33.9 ^c	3.617 ^d	38.8 ^b	5.986 ^d
	50	37.9 ^b	629 ^c	35.1 ^b	4.671 ^c	41.2 ^a	6.941 ^c
	70	39.5 ^a	670 ^c	35.8 ^b	4.846 ^{bc}	41.9 ^a	7.899 ^b
	90	39.9 ^a	778 ^b	36.7 ^b	5.101 ^b	40.7 ^a	7.989 ^b
	110	39.0 ^a	858 ^a	36.2 ^b	5.596 ^b	40.2 ^a	9.054 ^a
	130	40.2 ^a	814 ^a	36.7 ^b	5.340 ^b	42.2 ^a	8.802 ^a
	150	41.4 ^a	827 ^b	38.4 ^a	6.093 ^a	42.7 ^a	9.339 ^a

¹Days after transplanting; ²Goami 2 and Daeon were investigated at 93 and 85 DAT, respectively. ³SPAD, SPAD-502 (Minolta, Japan).

While the NH₄-N concentrations increased in proportion to N levels at 29 DAT, its concentrations decreased in all treatments with time. Therefore, its concentration in soil didn't show significantly in all treatments except for N 0 level of reference cultivar at 64 DAT. Compared with the reference cultivar, NH₄-N concentration of Goami 2 was lower than that of Daeon at 29 DAT; although it passed the stage where its concentrations were low. Therefore, the N absorb pattern of Goami 2 was low at the early stage, but increased a little bit at 93 DAT.

N content of rice plant was shown by Goami 2 and Daeon at 29 and 64 DAT and heading date (Table 4). N content was not significantly different at all N levels treatments. The changing pattern of N content was similar to that of SPAD values. The N content of Goami 2 was a little high at 29 DAT, no difference at 64 DAT, and a little low at heading stages as compared to Daeon. N content of Goami 2 in stem + leaf sheath panicle had no difference in Daeon, but the leaf N content was a little low at heading. Therefore, N location of Goami 2 estimated problem.

The yield components of Goami 2 were different from other japonica cultivars. We could not measure 1,000-grain weight because of low specific gravity of grains. So we weighed ℓ weight. The N application rates higher than 90 and 110 kg ha⁻¹ did not increase the panicle number m⁻² of Goami 2 (321.3 m⁻²) and Daeon (395.9 m⁻²), respectively (Table 5). The spikelets of Goami 2 were higher than those of Daeon. The brown/rough rice ratio, ℓ weight and 1,000-grain weight of Goami 2 were lower than those of Daeon. Different N levels revealed a little increase of these weights as N application increased. Previous research has shown that structure of Goami 2 starch was different from general japonica cultivars (Kang et al., 2003). The amylose of Goami 2 was known as an indigestible ingredient such as fiber (Kim et al., 2008). We inferred that the specific gravity of Goami 2 was reduced.

The N application rates higher than 70 and 90 kg ha⁻¹ did not increase milled rice yield of Goami 2 and Daeon, respectively (Table 6). The N application rates of Goami2 from 70 to 150 kg ha⁻¹ did not show significantly

Table 3. The change of NH₄-N in soil in Goami 2 and Daean by different N levels and growing stages.

Cultivar	N level (kg ha ⁻¹)	29 DAT	64 DAT	93 DAT
		mg kg ⁻¹		
Goami 2	0	35.4 ^c	4.0 ^a	0.58 ^a
	50	35.7 ^c	4.4 ^a	0.51 ^a
	70	45.7 ^b	4.0 ^a	0.96 ^a
	90	47.5 ^b	4.9 ^a	1.19 ^a
	110	48.4 ^b	4.5 ^a	1.09 ^a
	130	86.0 ^a	4.0 ^a	1.00 ^a
	150	83.0 ^a	4.2 ^a	1.58 ^a
Daean	0	22.0 ^c	3.6 ^b	0.85 ^a
	50	25.4 ^c	5.4 ^a	0.58 ^a
	70	34.2 ^{bc}	5.3 ^a	1.11 ^a
	90	45.4 ^b	5.7 ^a	1.38 ^a
	110	48.8 ^b	5.2 ^a	1.36 ^a
	130	69.8 ^a	5.5 ^a	1.40 ^a
	150	78.0 ^a	5.3 ^a	1.83 ^a

Table 4. N content of rice plant in Goami 2 and Daean by different N levels and growing stages.

Cultivar	N level (kg ha ⁻¹)	29 DAT (%)	64 DAT (%)	Heading (%)				Heading date
				Leaf	Stem + leaf sheath	Panicle	Mean	
Goami 2	0	2.61 ^a	1.28 ^a	2.07 ^a	0.50 ^a	1.00 ^a	1.19 ^a	August 24th
	50	2.64 ^a	1.25 ^a	2.31 ^a	0.55 ^a	1.12 ^a	1.33 ^a	August 24th
	70	2.66 ^a	1.40 ^a	2.27 ^a	0.52 ^a	1.06 ^a	1.28 ^a	August 24th
	90	2.74 ^a	1.41 ^a	2.70 ^a	0.68 ^a	1.11 ^a	1.49 ^a	August 24th
	110	2.61 ^a	1.43 ^a	2.74 ^a	0.72 ^a	1.18 ^a	1.50 ^a	August 24th
	130	2.84 ^a	1.53 ^a	2.71 ^a	0.82 ^a	1.29 ^a	1.61 ^a	August 25th
	150	3.12 ^a	1.85 ^a	2.46 ^a	0.68 ^a	1.20 ^a	1.45 ^a	August 25th
Daean	0	2.41 ^a	1.37 ^a	2.23 ^a	0.51 ^a	1.04 ^a	1.26 ^a	August 15th
	50	2.58 ^a	1.35 ^a	2.45 ^a	0.68 ^a	1.27 ^a	1.47 ^a	August 15th
	70	2.45 ^a	1.40 ^a	2.79 ^a	0.78 ^a	1.36 ^a	1.64 ^a	August 15th
	90	2.85 ^a	1.57 ^a	2.89 ^a	0.85 ^a	1.32 ^a	1.69 ^a	August 15th
	110	2.58 ^a	1.55 ^a	2.80 ^a	0.83 ^a	1.27 ^a	1.63 ^a	August 16th
	130	2.85 ^a	1.53 ^a	2.78 ^a	0.90 ^a	1.31 ^a	1.66 ^a	August 16th
	150	2.91 ^a	1.73 ^a	2.91 ^a	0.99 ^a	1.33 ^a	1.74 ^a	August 16th

different yield. The weight of rice straw increased as N levels increased. These trends were similar to milled rice yield. Increasing the N application rate from 50 to 70 kg ha⁻¹ significantly increased N efficiency of Goami 2 and Daean from 50 to 90 kg ha⁻¹.

In this study, the optimum N level of Goami 2 was at the 70 kg ha⁻¹, considering milled rice and straw, and N efficiency. Therefore, increasing level was not advantageous for N uptake in fiber-rich cultivar, Goami 2.

With SEM, the stem could be observed (Figure 1), and large and small vascular bundle of rice stems could be counted in Goami 2 and Daean (Table 7). The vascular bundle is an important tissue in plants due to the determination of the genotype's yield capacity and translocation ability of assimilates into grains (Park et al., 1999). The number of large vascular bundle (LVB) was higher in stem of Goami 2 than Daean but the number of small vascular bundle (SVB) was significantly lower in stem of

Table 5. Yield components in Goami 2 and Daean by different N levels.

Cultivar	N level (kg ha ⁻¹)	Panicle (Number m ⁻²)	Spikelets (number per panicle)	Brown/rough rice ratio (%)	ℓ weight (g)	1,000-grain weight (brown rice, g)
Goami 2	0	198.3 ^c	98.3 ^a	75.1 ^a	502 ^a	17.2 ^a
	50	253.5 ^b	102.6 ^a	74.4 ^a	497 ^a	16.8 ^a
	70	276.8 ^b	95.4 ^a	78.0 ^a	507 ^a	16.6 ^a
	90	321.3 ^a	94.4 ^a	77.3 ^a	497 ^a	16.5 ^a
	110	318.1 ^a	95.9 ^a	77.3 ^a	495 ^a	16.4 ^a
	130	322.0 ^a	95.2 ^a	77.7 ^a	488 ^b	16.1 ^{ab}
	150	317.4 ^a	106.3 ^a	74.3 ^a	487 ^b	15.8 ^b
Daean	0	288.1 ^c	79.3 ^b	83.0 ^a	610 ^a	23.1 ^a
	50	327.4 ^b	82.4 ^a	83.5 ^a	599 ^a	22.9 ^a
	70	342.6 ^b	86.0 ^a	83.6 ^a	606 ^a	22.5 ^a
	90	352.6 ^b	83.1 ^a	83.7 ^a	602 ^a	22.6 ^a
	110	395.9 ^a	87.4 ^a	83.9 ^a	605 ^a	23.0 ^a
	130	375.9 ^a	85.6 ^a	84.1 ^a	601 ^a	22.5 ^a
	150	358.0 ^b	89.4 ^a	83.9 ^a	590 ^b	22.6 ^a

Table 6. Yield and N efficiency in Goami 2 and Daean by different N levels.

Cultivars	N levels (kg ha ⁻¹)	Milled rice (kg ha ⁻¹)	Rice straw (kg ha ⁻¹)	N efficiency ¹ (%)
Goami 2	0	3.160 ^c	3.066 ^d	-
	50	3.778 ^b	4.657 ^c	18.9 ^b
	70	4.389 ^a	5.179 ^b	22.0 ^a
	90	4.373 ^a	5.407 ^b	17.5 ^b
	110	4.428 ^a	5.495 ^b	15.0 ^c
	130	4.525 ^a	6.125 ^a	13.5 ^c
	150	4.529 ^a	5.997 ^a	13.7 ^c
Daean	0	4.224 ^d	4.927 ^c	-
	50	5.000 ^c	6.170 ^b	19.5 ^a
	70	5.349 ^b	6.390 ^b	20.3 ^a
	90	5.719 ^a	6.913 ^{ab}	21.0 ^a
	110	5.818 ^a	7.140 ^a	18.3 ^{ab}
	130	5.697 ^a	7.325 ^a	14.0 ^b
	150	5.765 ^a	7.307 ^a	12.9 ^c

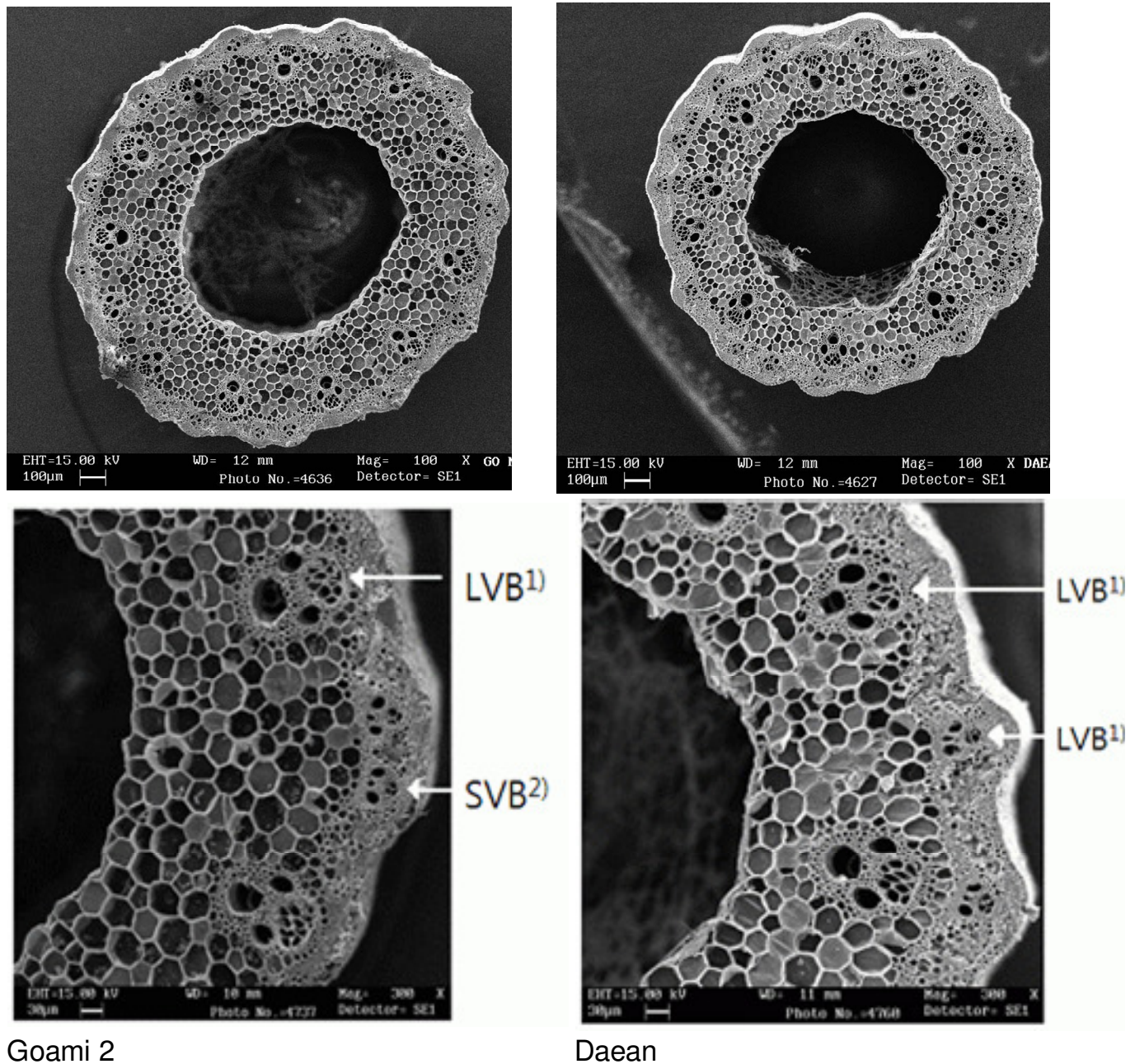
N efficiency: (Grain yield at target plot-Grain yield at no N plot)/applied N amount.

Goami 2 than Daean. We could not find the trend according to the N application levels. Based on the observation of SEM and the number of LVB and SVB, we suggested that vascular bundle of stems affect nutrient translocation of rice. However, more detailed studies are needed to find the structure of stem and nutrient translocation.

Conclusion

Fiber-rich, Goami 2 is a functional rice cultivar for diet. However, agronomic traits of this cultivar has not been

known. Therefore, we evaluated the growth, yield and the components of Goami 2 by different nitrogen application level in paddy. Also ammonium-N in soil and SPAD values of rice plant were measured during the rice growing season. These results show that the Goami 2 nitrogen take-up in soil and SPAD values of Goami 2 decreased as rice growth increased. At harvest season, brown to rough rice ratio, ℓ-weight, and 1,000-grain weight of Goami 2 were low compared to Daean as reference cultivar. So, hulled rice yield decreased. The N application rates of Goami 2 did not show significant difference to yield, and the N efficiency from 70 to 150 kg



Goami 2

Daean

Figure 1. SEM imagines of large and small vascular bundle in Goami 2 and Daean by application rate of 90 kg N ha⁻¹. ¹LVB, Large vascular bundle; ²SVB, small vascular bundle.

Table 7. The varietal difference of large and small vascular bundle of rice stems¹ grown under different N levels.

N level (kg ha ⁻¹)	Goami 2		Daean	
	LVB ²	SVB ³	LVB	SVB
0	11.0 ± 0.01	15.6 ± 0.79	10.0 ± 0.45	17.0 ± 0.45
90	12.0 ± 0.01	15.0 ± 0.73	10.0 ± 0.01	19.3 ± 0.49
150	11.7 ± 0.34	14.3 ± 0.34	10.7 ± 0.34	18.3 ± 0.69

¹Neck of panicle under 1 cm; ²LVB, large vascular bundle; ³SVB, small vascular bundle.

ha⁻¹ Goami 2 might be a kind of low fertilizing requiring cultivar. Also, we found out that Goami 2 had less numbers of small vascular bundle in the stem. Therefore, nitrogen application should be reduced for the cultivation of Goami 2.

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