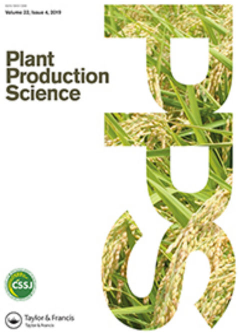

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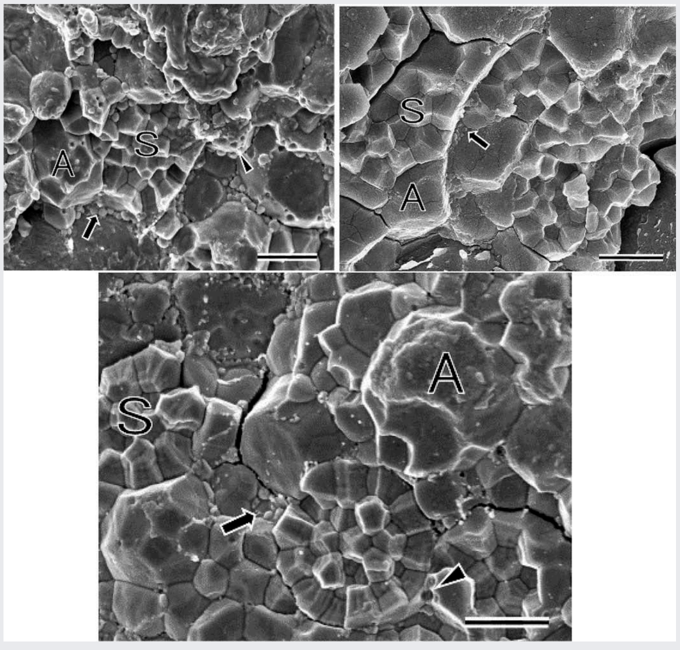
Morphological analysis on comparison of organic and chemical fertilizers on grain quality of rice at different planting densities

Kifayatullah Kakar[a,b], Youji Nitta[c], Naomi Asagi[d], Masakazu Komatsuzaki[d], Fumitaka Shiotsu[e], Toshiaki Kokubo[d] and Tran Dang Xuan[a]

[a]Graduate School for International Development and Cooperation, Hiroshima University, Hiroshima, Japan; [b]Faculty of Agriculture, Nangarhar University, Nangarhar, Afghanistan; [c]Faculty of Food and Agricultural Sciences, Fukushima University, Fukushima, Japan; [d]The College of Agriculture, Ibaraki University, Ibaraki, Japan; [e]Faculty of Agriculture, Meiji University, Kawasaki, Japan

ABSTRACT

Effects of organic (Italian ryegrass and Bokashi) and chemical fertilizer on growth, yield, and grain quality of rice (*Oryza sativa* L.) were compared under different planting densities in 2013/2014 and 2014/2015. Italian ryegrass was incorporated into the soil as green manure. Bokashi (a mixture of organic materials) was applied as basal dressing. To measure yield and its components, 30 hills were chosen for each treatment. Rice grains were harvested from each treatment to assess the grain quality and to evaluate accumulation structures using a scanning electron microscope. Bokashi treatment increased panicle number per hill, ripened grain percentage, panicle number per m^2, and grain yield compared to no fertilizer treatment at normal planting density. Chemical fertilizer treatment increased plant length at high planting density. Italian ryegrass and Bokashi treatments promoted the taste point (taste score as reference) by reduction of amylose and protein contents at normal planting density in contrast to chemical fertilizer. 1000-grain weight, panicle number per m^2, and grain yield were higher at high planting density than at normal planting density. However, high planting density decreased panicle number per hill and spikelet number per panicle. It also enhanced the amylose content of rice grain. Scanning electron microscopic observation revealed that chemical fertilizer treatment marked up protein bodies and their traces on amyloplasts. However, Bokashi treatment produced large amyloplasts, which included many starch granules. These results show that Italian ryegrass and Bokashi can offset reductions of chemical fertilizer and can lead to sufficient starch accumulation structures in rice grains.



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CONTACT Youji Nitta nittay@agri.fukushima-u.ac.jp

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Introduction

World pollution and food security problems have compelled people to return to eco-friendly farming systems to decrease threats to human health posed by inorganic farming systems (Mader et al., 2002). The adverse effects of chemical fertilizer on soil and environment limit its usage in sustainable agricultural systems (Peyvast, Kharazi, Tahernia, Nosratierad & Olfati, 2008). The continuous use of chemical fertilizers may also lead to the accumulation of heavy metals in plant tissues which compromises the nutrition value and fruit quality (Shimbo et al., 2001). Excessive use of chemical fertilizers and pesticides has caused pollution of several kinds, involving leaching of nutrients and destruction of soil physical characteristics (Agbede, 2010).

Chemical fertilizers take less time to decompose and can use directly, and this condition causes to release nutrients quickly and increase plant growth (Sharma & Chetani, 2017) as well as improve crop yield (Ojeniyi, 2002). It is reported that chemical fertilizers lead the way for imbalance in nutrient uptake, decrease in soil organic matter, increase in soil acidity (Ojeniyi, 1981), and production of non-secured foods and, consequently, harm human life (Sharma & Chetani, 2017). In addition, such fertilizers decreased rice grain quality through enhancing amylose and protein contents in rice grain endosperm (Sandhu, Mahal & Kaur, 2015).

Application of organic fertilizers can manage the application rate of chemical fertilizers. Green manures are concerning as an important soil management practice with the potential to improve soil quality (Haque, Kim, Kim & Kim, 2015), increase rice yield (Ali, 1993), and reduce the dependence of chemical fertilizers. The presence of extra nitrates in water is a challenge for public health and environmental protection; cultivation of green manure as catch crops traps the residual mineral nitrogen in the soil and is environmentally friendly (Jeon, Ryoo, Hahn, Walia & Nakamura, 2010).

Green manure and its incorporation into soil reduce dependence on chemical fertilizers and must be regarded as an influential soil management practice while maintaining soil organic matter contents (Elfstrand, Bath & Martensson, 2007). Green manures have become an extremely important strategy for low-cost and sustainable soil fertility. Moreover, they add organic matter to the soil, improving its structure as an important soil amendment (Bin, 1983), and constrain nitrates from leaching. In addition, green manures reduce soil erosion and protect fields from weeds.

In mono-rice paddy fields, the cultivation of green manures and their incorporation into soils are strongly recommended. Haque et al. (2015) mentioned that legume cover crops improve rice vegetative growth while decrease grain quality; however, non-legume cover crops decrease rice productivity. Besides, Bokashi (a mixture of rice bran and husks, soybean meal, sugar syrup, water, and *Aspergillus oryzae* with anaerobic fermentation method) is developed in Japan and applies as an organic fertilizer to enhance soil organic matter and crop yield. Dou, Komatsuzaki and Nakagawa (2012) have mentioned that the Bokashi application improved root system and yield of paddy rice.

Rice grain quality includes several elements, mostly related to cooking and eating quality. Actually, rice grain quality is the principal component of evaluation in Asian countries, most of which consume rice as a dietary staple food (Song, Choi, Sharma & Kang, 2012). Grain quality also includes complex characteristics such as accumulation structures of starch, protein, and lipids (Kakar et al., 2019). Among these characteristics, the main determinant factors for cooking and eating quality are amylose and protein contents of rice grains (Juliano, 1979).

Yadav, Singh, Kumar, Yadav and Singh (2013) described that cultivation of rice plants with farmyard manure, vermicompost, and poultry manure could be the key factor for achieving and maintaining the high level of grain quality. However, Sangeetha, Balakrishnan and Devasenapathy (2013) observed that rice grain eating quality was higher under the organic farming system than in the conventional farming system. Therefore, an adequate nutrient supply and planting density are necessary for consideration to manage the physicochemical properties of rice grains.

On the other hand, in rice plants, planting density plays important roles on growth, yield, and grain quality through the influence of tillers, panicles, and spikelet numbers (Wu, Wilson & McClung, 1998). Bozorgi et al. (2011) mentioned that high planting density increased plant height, leaf area index, yield, and its components compared to medium or low planting densities. Many reports have described that high planting density optimizes the rice grain yield, but information related to rice grain quality is less available.

Several studies elucidated the numerous effects of organic fertilizers and planting density on rice growth, yield, and grain quality. Nevertheless, little information has been reported for Italian ryegrass as green manure, Bokashi, and suitable planting densities with them. For this study, experiments were conducted on rice (*Oryza sativa* L.) to clarify the effects of

Italian ryegrass, Bokashi, and chemical fertilizer under different planting densities by consideration of physicochemical properties using morphological analysis.

Materials and methods

Experimental site and design

Identical experiments were conducted in 2013/2014 and 2014/2015 in The Field Science Center, The College of Agriculture, Ibaraki University, Japan. The experimental farm, located at 36°01′48.31″N latitude and 140°21′40.26″E longitude, has an average annual temperature and rainfall of 14.4°C and 1187.8 mm, respectively. Experiments were conducted in open fields with the following soil properties (0–20 cm depth): 0.8 g cm^{-3} bulk density, 50.8 g kg^{-1} total carbon, 5.0 g kg^{-1} total nitrogen, P_2O_5 47.2 mg kg^{-1} dry soil, pH 6.2, and 0.08 dS m^{-1} electroconductivity.

Experiments were conducted with a split-plot design in three replications and eight treatments. The main factor was green manure (Italian ryegrass), Bokashi, chemical fertilizer (as basal dressing, 6:6:6 g m^{-2}), and no fertilizer application. The sub-factor was rice normal planting density (30 cm × 15 cm) and high planting density (30 cm × 7.5 cm) with each plot size of 3 m × 2 m.

Italian ryegrass and Bokashi fertilizer application

Italian ryegrass (*Lolium multiflorum* Lam.) cv. Hanamiwase was selected as a winter green manure crop cultivated from 3 December 2013 through 14 April 2014 for the 2013/2014 experiment and from 18 November 2014 through 13 April 2015 for the 2014/2015 experiment at a seeding rate of 10 g m^{-2}. Italian ryegrass plants were then incorporated into the soil with a tractor on April 13 and 14 in 2014 and 2015, respectively. In addition, Bokashi was applied to the soil as a basal dressing with the application rate of 120 g m^{-2} at the time of Italian ryegrass incorporation.

Total nitrogen content of Italian ryegrass and Bokashi was measured using a CN coder machine (JM3000CN; JP-SCIENCE LAB Co, Ltd., Kyoto, Japan). To quantify the amounts of phosphorus (P_2O_5) and potassium (K_2O) in Italian ryegrass and Bokashi, dried and ground samples were digested with HNO_3 and $HClO_4$ and filtered. Phosphorus was measured based on the colorimetric method described by Murphy and Riley (1962) as well as potassium was analyzed by atomic absorption spectrometry (3100, Perkin-Elmer, Norwalk, USA). The amounts of N, P_2O_5, and K_2O for treatments using different organic and chemical fertilizers sources are shown in Table 1.

Table 1. Amount of N, P_2O_5, and K_2O (gm^{-2}) applied in treatments from different organic and chemical fertilizer sources.

Treatments	Chemical fertilizer	Italian ryegrass	Bokashi	Total
No fertilizer	0:0:0	0:0:0	0:0:0	0:0:0
Chemical fertilizer	6:6:6	0:0:0	0:0:0	6:6:6
Italian ryegrass	0:0:0	1.6:0.4:0.8	0:0:0	1.6:0.4:0.8
Bokashi	0:0:0	0:0:0	3.6:3.3:0.1	3.6:3.3:0.1

Aboveground dry matter was calculated for Italian ryegrass to estimate the amount of N, P_2O_5, and K_2O. Amounts of N, P_2O_5, and K_2O in Italian ryegrass and Bokashi were measured based on the dry weight.

Plant materials

Rice (*O. sativa* L.) cv. Koshihikari (japonica) was used as the main crop. Dry seeds were treated with Aphelenchoides pesticide to protect seedlings from disease and pest attacks. Then, 160 g seeds were sown per nursery box in the greenhouse of the Field Science Center, The College of Agriculture, Ibaraki University, on 11 April 2014 and 2015. The 33-day-old rice seedlings of 4.1 plant age in leaf number were transplanted to the paddy field on 13 May 2014 and 15 May 2015 with a transplanting machine. They were thinned to be three seedlings per hill by hand. Two planting densities were applied on crops: normal planting density and high planting density as mentioned above. Plants were harvested on 6 September 2014 and 14 September 2015.

Ten hills were chosen from each replication (90 plants per treatment) to evaluate grain yield and yield components and followed the technique described by Hoshikawa (1989). Grains were de-husked using a small impeller hulling machine (FC2K; Otake Co. Ltd., Japan). Three hundred grams of brown rice samples with three replications were used to measure grain quality such as amylose content, protein content, and taste point (score of quality) through applying a taste analyzer machine (RCTA11A; Satake Co. Ltd., Japan).

Preparation for scanning electron microscopic observation

Perfect brown rice grains were collected from each treatment of both planting densities (Italian ryegrass, Bokashi, chemical fertilizer, and no fertilizer) to observe the surface and internal structures of grains using a scanning electron microscope (JSM6360A; JEOL, Japan). Thirty perfect brown rice grains per treatment were freeze-dried with a freeze vacuum dryer (–60°C, 10^{-3} Pa, LFD-100NDPS1; Nihon Techno Service Co. Ltd., Japan). After drying, grains that had been cross-cut using a razor blade (for internal observation) and whole grains were placed on specimens. They were coated with platinum using the sputtering machine (JUC-5000; JEOL, Japan) and were observed using the scanning electron microscope.

Statistical analysis

All data were analyzed using SPSS statistical software (SPSS, 13.0, IBM Corporation, USA). Univariate analysis of variance (ANOVA) and interactions between treatments and planting densities were carried out. Pearson correlation was conducted to analyze the relation between quality traits. Then, all the percentage data were analyzed by Tukey's test after the cube root transformation.

Results

Almost identical results were obtained from growth parameters, yield, yield components, grain quality with taste analyzer, and scanning electron microscopic observations for 2013/2014 and 2014/2015 experiments. Therefore, we describe data of the 2013/2014 experiment as follows.

Growth, yield, and yield components

Growth, yield, and yield components were differed in fertilizer treatments and planting densities as exhibited in Table 2 . Panicle number per hill, the percentage of ripened grain, and panicle number per m^2 were higher in Bokashi treatment than in no fertilizer treatment at normal planting density. Consequently, grain yield in Bokashi treatment was higher than that in Italian ryegrass and no fertilizer treatments at normal planting density. However, there was no significant difference among no fertilizer, chemical fertilizer, and Italian ryegrass treatments. Therefore, these results mean that grain yield in Bokashi treatment was higher than the other treatments. Chemical fertilizer treatment increased plant length at high planting density. Spikelet number per panicle was not significantly different among treatments but was higher in chemical fertilizer treatment.

Panicle number per hill and spikelet number per panicle were greater under normal planting density and were ranged from 14.1 to 16.0 and 95.9 to 98.4, respectively. 1000-grain weight was significantly different between normal and high planting densities but not among treatments. Percentage of ripened grains was significantly different among treatments at normal planting density; however, it was higher in high planting density, but the difference was not significant between planting densities. In addition, grain yield was significantly higher under high planting density than under normal planting density.

Grain quality

Grain amylose and protein contents and the taste point (as a reference) were varied at both planting densities according to whether plants were grown in treatments with organic or chemical fertilizers (Table 3). Italian ryegrass and Bokashi treatments were associated with a reduction of amylose and protein contents in contrast to basal dressing chemical fertilizer treatment in both densities, which enhanced grain taste points. Amylose, protein, and taste point were ranged from 18.9 to 19.3, 6.6 to 7.1, and 73 to 78, respectively.

With high planting density, amylose and protein contents of rice grain were also increased, leading to a low grain taste point. Our observations revealed that amylose content was positively correlated with the planting density. Moreover, protein content and taste point, respectively, exhibited positive and negative correlation with planting density but not significant (Table 4). Amylose and protein contents had a negative correlation with

Table 2. Effect of Italian ryegrass, Bokashi, chemical fertilizer, and planting density on growth, yield, and yield components.

Treatments	Plant length (cm)	Panicle no. $hill^{-1}$	Spikelet no. $panicle^{-1}$	Ripened grains (%)	1000-grain weight (g)	Panicle no. m^{-2}	Grain yield (kg ha^{-1})
Normal density							
No fertilizer	107.4 a	14.1 b	89.2 a	75.1 b	20.4 a	318.9 b	4764.8 b
Chemical fert.	112.5 a	14.8 ab	98.4 a	76.2 ab	20.5 a	328.6 ab	5070.7 ab
Italian ryegrass	109.0 a	14.5 ab	95.9 a	76.9 a	20.7 a	323.0 ab	4899.1 b
Bokashi	109.7 a	16.0 a	96.7 a	76.7 a	20.4 a	355.2 a	5304.4 a
High density							
No fertilizer	105.6 b	8.2 a	87.7 a	76.3 a	20.8 a	384.1 a	5132.9 a
Chemical fert.	112.5 a	9.0 a	89.9 a	77.9 a	21.1 a	404.0 a	5818.1 a
Italian ryegrass	106.9 b	8.8 a	88.8 a	78.8 a	20.6 a	412.9 a	5834.5 a
Bokashi	107.3 b	9.3 a	88.2 a	79.6 a	20.9 a	413.8 a	5978.5 a
ANOVA							
Treatments (T)	*	ns	ns	*	ns	ns	ns
Density (D)	ns	***	**	ns	**	***	***
T × D	ns	ns	ns	*	ns	ns	ns

Univariate, SPSS statistical analysis was conducted for the variance. *, **, and ***, respectively, denote significant difference at 5%, 1%, and 0.1% probability levels. ns indicates not significant. The same letters in a column within planting density indicate no significant difference at the 5% probability level.

Table 3. Effect of Italian ryegrass, Bokashi, chemical fertilizer, and planting density on amylose content, protein content, and taste point.

Treatments	Amylose content (%)	Protein content (%)	Taste point (as reference)
Normal density			
No fertilizer	19.0 b	6.9 b	76 b
Chemical fert.	19.2 a	7.1 a	74 c
Italian ryegrass	18.9 b	6.7 c	78 a
Bokashi	18.9 b	6.8 bc	77 ab
High density			
No fertilizer	19.1 b	6.9 b	75 b
Chemical fert.	19.3 a	7.1 a	73 c
Italian ryegrass	19.2 bc	7.0 ab	74 bc
Bokashi	18.9 c	6.6 c	78 a
ANOVA			
Treatments (T)	***	**	***
Density (D)	*	ns	ns
T × D	***	**	***

Univariate, SPSS statistical analysis was conducted for the variance. *, **, and ***, respectively, denote significant difference at 5%, 1%, and 0.1% probability levels. ns indicates not significant. The same letters in a column within plating density indicate no significant difference at the 5% probability level.

Table 4. The correlation coefficient of planting density with amylose, protein, and taste point.

	Density	Amylose	Protein	Taste point
Density	1			
Amylose	0.367 ns	1		
Protein	0.067 ns	0.676 ***	1	
Taste point	−0.316 ns	−0.834 ***	−0.788 ***	1

*** denote significant difference at 0.1% level. ns indicates not significant.

taste point; however, amylose and protein contents had a positive correlation to each other.

Scanning electron microscopic observation

Scanning electron microscopic observation revealed no marked differences of grain surface structures among treatments in both high and low magnifications in terms of wrinkle formation. However, internal observation showed numerous differences in terms of amyloplast formation and amount of protein bodies. In the no fertilizer treatment, in addition to some protein bodies and their traces, small holes were detected on the surface of amyloplasts, and also, amyloplasts were small compared to other treatments (Figure 1(a–c)). The structure and size of the aleurone layer were similar among treatments, and several portions (central, dorsal, and ventral) within a single grain were not different in all treatments.

Observations of grains' internal structures revealed numerous protein bodies and their traces on amyloplasts of the chemical fertilizer treatment. Protein bodies were located around and on the surface of amyloplasts with spherical shapes and different sizes. Moreover, the amyloplast shapes were diverse and different from other treatments, and starch granules were gathered in two layers (circles) to make amyloplasts (Figure 1(d–f)).

Differences were observed among treatments in terms of amyloplasts and starch granule structures. The structures of amyloplasts and starch granules were round and polyhedral in Italian ryegrass (Figure 1(g–i)) and Bokashi treatments (Figure 1(j–l)). Additionally, Italian ryegrass and Bokashi treatments accumulated starch granules and amyloplasts without abnormality. Moreover, the Bokashi treatment grains had few protein bodies. Their traces were found between amyloplasts, but the amyloplasts were larger and the granules more numerous than in grains produced in other treatments (Figure 1(j–l)). Starch granules were placed as two to three layers in amyloplasts of Bokashi treatment, two layers with several starch granules and central layer with single starch granule, but in other treatments, they were placed as two layers.

Accumulation structures of grains in terms of starch granules, amyloplast, and protein bodies in high planting density also followed the same order as mentioned for treatments in normal planting density. However, high planting density marked up the presence of holes and cell walls around or within amyloplasts (Figure 2(a–l)). High planting density led the way for the diverse formation and different sizes of amyloplasts as well as increased protein bodies and its traces on the amyloplasts or around them.

Discussion

Rice growth and yield performance

Comparisons of results in hundreds of studies have revealed that green manures can increase rice yields (Ali, 1993). Among the nine green manure treatments (incorporation of white clover, Chinese milk vetch, hairy vetch, crimson clover, oat, rye, Italian ryegrass, white mustard, and lacy phacelia) in the Shikoku region of Japan, rice yields tend to be higher in white clover and hairy vetch treatments and lower in Italian ryegrass treatments (Asagi & Ueno, 2009). In our study, green manure (Italian ryegrass) treatment increased ripened grain percentage compared to no fertilizer treatment at normal planting density in the Kanto region of Japan. Therefore, the application effects of Italian ryegrass are apparently highly variable. They depend on the Italian ryegrass characteristics and on the soil system to which they are added.

The amount of nitrogen was higher in chemical fertilizer and Bokashi treatments, respectively; therefore, plant length, panicle number per hill, spikelet number per panicle, and panicle number per m^2 were

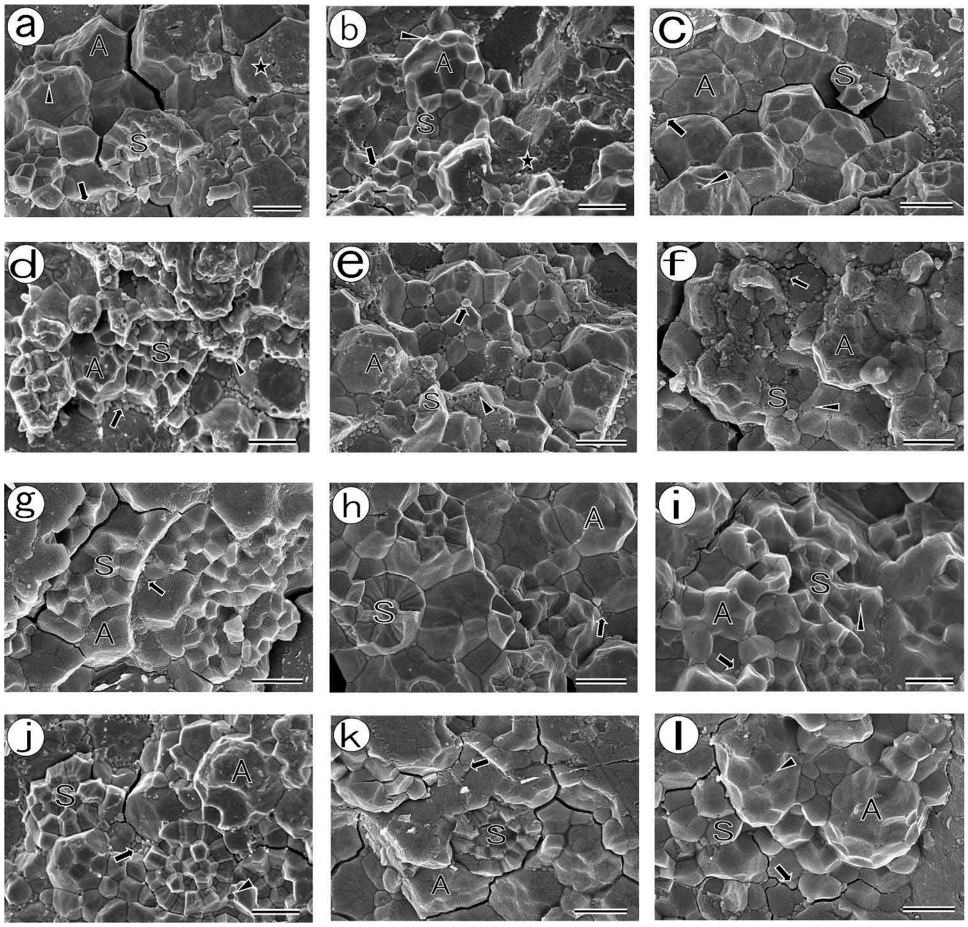

Figure 1. Accumulation structures of rice grain in normal planting density treatments. No fertilizer (a, b, & c), chemical fertilizer (d, e, & f), Italian ryegrass (g, h, & i), and Bokashi (j, k, & l). Central (a, d, g, & j), dorsal (b, e, h, & k), and ventral (c, f, i, & l) portions. A: amyloplast, S: starch granules, arrow: protein bodies, arrowhead: traces of protein bodies, star: holes. Bars: 10 μm.

increased which is reported by Fukushima, Shiratsuchi, Yamaguchi and Fukuda (2011), who noted that high nitrogen levels increased the yield components. Extremely high doses of nitrogen fertilizer or nitrogen unavailability increases spikelet sterility because excess nitrogen application decreases the number of engorged pollen grains in the anther and increases spikelet sterility (Gunawardena & Fukai, 2004). In this study, no fertilizer and chemical fertilizer treatments decreased the percentage of ripened grains at normal planting density.

In addition, Bokashi is apparently a candidate for use as a new organic fertilizer, especially in paddy rice cultivation and production (Dou et al., 2012). Because of its local production possibilities, it can be a key component of sustainable agriculture, a low-cost biomass resource, and a maintainer of soil productivity. Consequently, it can reduce demand for and the application rate of chemical fertilizers (Xiaohou, Min, Ping & Weiling, 2008). Bokashi treatment showed almost identical effects on growth parameters as Italian ryegrass treatment did.

High or low planting densities can increase rice yields, but high planting density might enhance rice yield more than low planting density. High planting density decreased the total leaf number of main culms and the spikelet number per panicle, which was also reported by Fukushima et al. (2011). Bozorgi et al. (2011) noted that plant height, yield, and yield components of rice were higher with high planting density than with either medium or low planting densities. Increased planting density raises the tiller number per unit of land area (Huang et al., 2013), which proves our results.

Rice tillering is well understood to be affected by nitrogen fertilizer input and planting density (Fadaga & De Datta, 1971). To obtain acceptable and high yields, planting density must be considered when rice plants are cultivated because it affects both aboveground and underground parts of rice plants (Baloch, Soomro, Javed & Ahmed, 2002). In this experiment, normal planting density increased

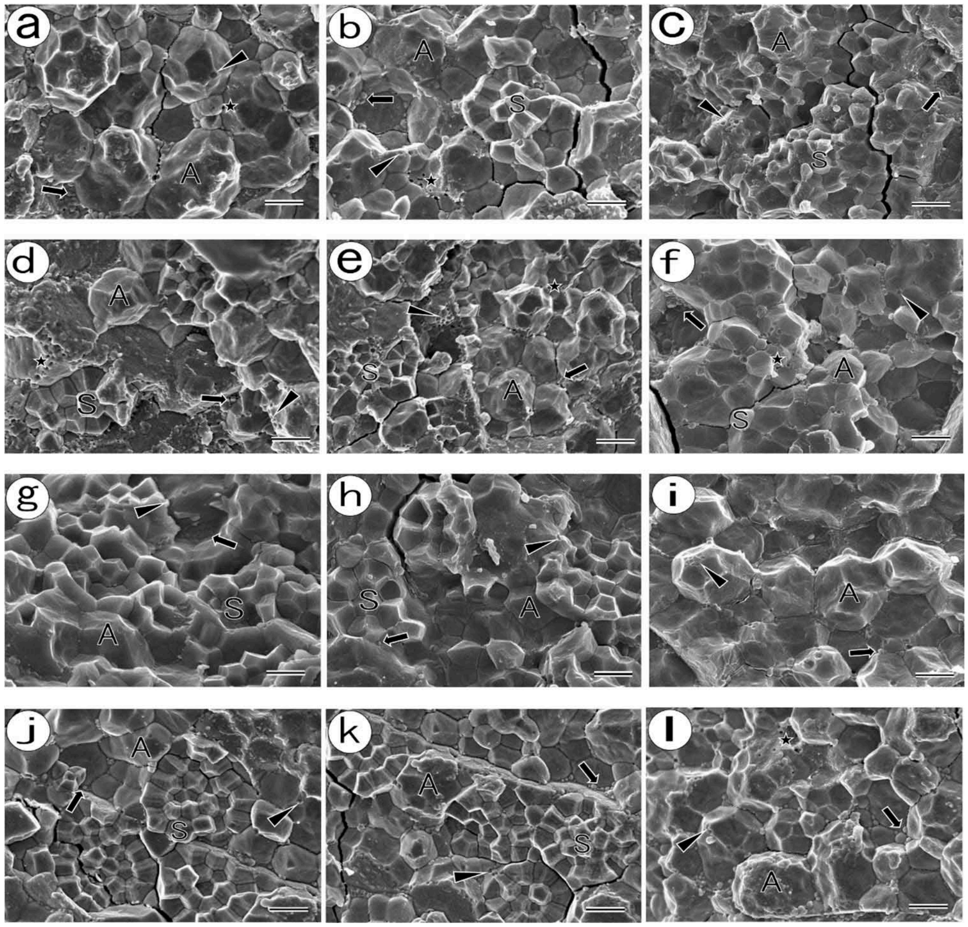

Figure 2. Accumulation structures of rice grain in high planting density treatments. No fertilizer (a, b, & c), chemical fertilizer (d, e, & f), Italian ryegrass (g, h, & i), and Bokashi (j, k, & l). Central (a, d, g, & j), dorsal (b, e, h, & k), and ventral (c, f, i, & l) portions. A: amyloplast, S: starch granules, arrow: protein bodies, arrowhead: traces of protein bodies, star: holes. Bars: 10 µm.

the panicle number per hill and spikelet number per panicle which was also observed by Baloch et al. (2002), which certified our results.

Grain quality and starch accumulation structure

Rice grain quality is evaluated by physical and chemical characteristics such as its appearance and the accumulation structures of starch, protein, and lipids. Chemical fertilizer application increased protein and amylose contents, resulting in a decreased taste point (Table 3). That is true because the level of nitrogen in this treatment was high. The high nitrogen level increases protein and amylose contents, as reported by Sandhu et al. (2015) and Singh, Pal, Mahajan, Singh and Shevkani (2011). In the Bokashi treatment, the nitrogen fertilizer amount was lower than in chemical fertilizer treatment. Therefore, it decreased amylose and protein contents of grains compared to chemical fertilizer treatment. Increase of protein content in no fertilizer treatment compared to Bokashi might be due to soil components including nitrogen and other minerals.

Increased planting density raised amylose and protein contents, which decrease the taste point (Table 3). However, Sandhu et al. (2015) reported opposite results to those of our observations. They explained that high protein content causes rice grains to be hard and to fracture during milling processes. In addition, Singh, Okadone, Toyoshima, Isobe and Ohtsubo (2000) described that high amylose contents engender rice grain hardness.

Cereal endosperm accumulated high levels of starches in amyloplasts, which occupy 76–78% of endosperm in rice grains (Shu, Sun & Wu, 2014). Starch granules consist of amylose and amylopectin accumulated in amyloplasts (Jeon et al., 2010). The starch granules are polyhedral, having a diameter of 3–9 µm. However, protein bodies are 1–4 µm in diameter and are located between amyloplasts (Harris & Juliano, 1997; Kakar et al., 2019).

Applications of primary elements (N, P_2O_5, and K_2O) were higher in the chemical fertilizer treatment than that in the other three treatments of normal planting density (Italian ryegrass, Bokashi, and no fertilizer) as listed in Table 1, which suggests that N uptake of rice at the late growth stage might be higher in the chemical fertilizer treatment. Therefore, the results of the internal structure of rice grains by scanning electron microscopic observation revealed many protein bodies and their traces in amyloplasts of chemical fertilizer treatment. Leesawatwong, Jamjod, Kuo, Dell and Rerkasem (2005) and Zakaria, Matsuda and Nitta (2000) reported that high nitrogen application can increase protein bodies in grain. Hayakawa, Seo and Igaue (1980) reported that traces (pits) formed by removal of protein bodies from starch granules and amyloplasts.

The Bokashi treatment had few protein bodies and their traces, but the amyloplasts were larger and the starch granules were more numerous than in other treatments. Bokashi might enlarge amyloplasts and increase the number of starch granules within amyloplasts. Blockage of the amyloplast distribution process can maintain enlargement of the amyloplast diameter. It might be related to pleomorphic amyloplasts having small starch granules (Yum & Kawagoe, 2009) or long starch granules (Jiang et al., 2010).

In our preliminary experiments, we tried to measure the number of protein granules per unit area and the size of amyloplast and starch granules. With regard to the number of protein granules per unit area, because protein granules were limitedly present among amyloplasts which occupy a large portion in the observation area under some magnifications, we could not measure it accurately. In addition, protein granules were locally present in a cell. For this reason, measurement of protein granules per unit area appeared to be difficult. On the other hand, for amyloplasts and starch granules, it was not clear where the observation surface correspond to any portion of them in three-dimensional view under scanning electron microscope observation. Therefore, we could not measure the major and/or minor axes of amyloplasts and starch granules accurately. From these reasons in our present experiments, we did not show the number of protein granules per unit area and the major and/or minor axes of amyloplasts and starch granules.

Applications of Italian ryegrass, Bokashi, and chemical fertilizers might change nitrogen and other nutrient mineralization patterns in soil, thereby affecting rice growth, yield, and grain quality. In this experiment, the Bokashi application had positive effects on rice yield and grain quality. To confirm the mechanisms of Bokashi application effects, it will be necessary to investigate nutrient release patterns in the soil to which Bokashi has been applied and then to compare those patterns to those produced by chemical fertilizers.

Our results presented above indicate that organic fertilizers can be a substitute for chemical fertilizer because they affect not only rice growth and yield but also improved grain quality and accumulation structures of starches in the endosperm. In addition, planting density affected rice growth, yield, and grain quality. Therefore, it must be administered based on soil fertility and crop characteristics.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Tran Dang Xuan http://orcid.org/0000-0001-7103-5698

References

Agbede, T. M. (2010). Tillage and fertilizer effects on some soil properties, leaf nutrient concentrations, growth and sweet potato yield on an Alfisol in southwestern Nigeria. *Soil and Tillage Research*, *110*(1), 25–32.

Ali, M. (1993). *An economic evaluation of green manure use in the rice based farming system*. (Terminal Report, Social Sciences Division. Los Baños, Laguna, Philippines: IRRI.

Asagi, N., & Ueno, H. (2009). Nitrogen dynamics in paddy soil applied with various N labeled green manures. *Plant and Soil*, *322*(1–2), 251–262.

Baloch, A. W., Soomro, M. A., Javed, M. A., & Ahmed, M. (2002). Optimum plant density for high yield in rice (*Oryza sativa* L.). *Asian Journal of Plant Science*, *1*(1), 25–27.

Bin, J. (1983). Utilization of green manure for raising soil fertility in China. *Soil Science*, *135*(1), 65.

Bozorgi, H. R., Faraji, A., Danesh, R. K., Keshavarz, A., Azarpour, E., & Tarighi, F. (2011). Effect of plant density on yield and yield components of rice. *World Applied Sciences Journal*, *12*, 2053–2057.

Dou, L., Komatsuzaki, M., & Nakagawa, M. (2012). Effects of Biochar, Mokusakueki and Bokashi application on soil nutrients, yields and qualities of sweet potato. *International Research Journal of Agricultural Science and Soil Science*, *2*(8), 318–327.

Elfstrand, S., Bath, B., & Martensson, A. (2007). Influence of various forms of green manure amendment on soil microbial community composition, enzyme activity and nutrient levels in leek. *Applied Soil Ecology*, *36*(1), 70–82.

Fadaga, S. O., & De Datta, S. K. (1971). Leaf area index, tillering capacity, and grain yield of tropical rice as affected by plant density and nitrogen level. *Agronomy Journal*, *63*(3), 503–506.

Fukushima, A., Shiratsuchi, H., Yamaguchi, H., & Fukuda, A. (2011). 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 Tohoko region of Japan. *Plant Production Science*, *14*(1), 56–63.
Gunawardena, T. A., & Fukai, S. (2004). The effects of nitrogen application and assimilate availability on engorged pollen production and spikelet sterility in rice. *Australian Agronomy Conference*, 12th AAC, Fourth ICSC, Australia.
Haque, M. M., Kim, S. Y., Kim, G. W., & Kim, P. J. (2015). Optimization of removal and recycling ratio of cover crop biomass using carbon balance to sustain soil organic carbon stocks in a mono-rice paddy system. *Agriculture, Ecosystems and Environment*, *207*, 119–125.
Harris, N., & Juliano, B. O. (1997). Ultrastructure of endosperm protein bodies in developing rice grain differing protein content. *Annals of Botany*, *41*(1), 1–5.
Hayakawa, T., Seo, S. W., & Igaue, I. (1980). Electron microscopic observation of rice grain. *Journal of the Japanese Society of Starch Science*, *27*(3), 173–179.
Hoshikawa, K. (1989). *The growing rice plant, an anatomical monograph: Panicle, flower and grain* (1 st ed., pp. 280–281). Tokyo, Japan: Nobunkyo.
Huang, M., Yang, C., Ji, Q., Jinang, L., Tan, J., & Li, Y. (2013). Tillering responses of rice to plant density and nitrogen rate in a subtropical environment of southern China. *Field Crops Research*, *149*, 187–192.
Jeon, J. S., Ryoo, N., Hahn, T. R., Walia, H., & Nakamura, Y. (2010). Starch biosynthesis in cereal endosperm. *Plant Physiology and Biochemistry*, *48*(6), 383–392.
Jiang, H., Horner, H. T., Pepper, T. M., Blanco, M., Campbell, M., & Jane, J. L. (2010). Formation of elongated starch granules in high-amylose maize. *Carbohydrate Polymers*, *80*(2), 533–538.
Juliano, B. O. (1979). Amylose analysis in rice, a review. In: Chemical aspects of rice grain quality. Proceedings of a workshop, International Rice Research Institute (IRRI), Los Banos, Laguna, Philippines. 251–260.
Kakar, K., Xuan, T. D., Abdiani, S., Wafa, I. K., Noori, Z., Attai, S., … Tran, H.-D. (2019). Morphological observation and correlation of growth and yield characteristics with grain quality and antioxidant activities in exotic rice varieties of Afghanistan. *Agriculture*, *9*, 167.
Leesawatwong, M., Jamjod, S., Kuo, J., Dell, B., & Rerkasem, B. (2005). Nitrogen fertilizer increases seed protein and milling quality of rice. *Cereal Chemistry*, *82*(5), 588–593.
Mader, P., Filessbach, A., Dubois, D., Gunst, L., Fried, P., & Niggli, U. (2002). Soil fertility and biodiversity in organic farming. *Science*, *296*(5573), 1694–1697.
Murphy, J. A. M. E. S., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, *27*, 31–36.
Ojeniyi, S. O. (1981). Effect of long term NPK application on secondary and micronutrient content of *Coffea canephora*. *Plant and Soil*, *60*(3), 477–480.
Ojeniyi, S. O. (2002). Soil management, national resources and environment. *Nigeria Journal of Soil Science, 16*, 131–135.
Peyvast, G., Kharazi, P. R., Tahernia, S., Nosratierad, Z., & Olfati, J. A. (2008). Municipal solid waste compost increased yield and decreased nitrate amount of broccoli. *The Society for Advancement of Horticulture*, *10*(2), 129–131.
Sandhu, S. S., Mahal, S. S., & Kaur, A. (2015). Physicochemical, cooking quality and productivity of rice as influenced by planting methods, planting density and nitrogen management. *International Journal of Food, Agriculture and Veterinary Sciences*, *5*, 33–40.
Sangeetha, S. P., Balakrishnan, A., & Devasenapathy, P. (2013). Influence of organic manures on yield and quality of rice (*Oryza sativa* L.) and black gram (*Vigna mungo* L.) in rice-black gram cropping sequence. *American Journal of Plant Sciences*, *4*(05), 1151.
Sharma, A., & Chetani, R. (2017). A review on the effect of organic and chemical fertilizers on plants. *International Journal for Research in Applied Science & Engineering Technology*, *5*(2), 677–680.
Shimbo, S., Zhang, Z. W., Watanabe, T., Nakatsuka, H., Matsuda-Inoguchi, N., Higashikawa, K., & Ikeda, M. (2001). Cadmium and lead contents in rice and other cereal products in Japan in 1998–2000. *Science of the Total Environment*, *281*(1–3), 165–175.
Shu, X., Sun, J., & Wu, D. (2014). Effects of grain development on formation of resistant starch in rice. *Food Chemistry*, *164*, 89–97.
Singh, N., Pal, N., Mahajan, G., Singh, S., & Shevkani, K. (2011). Rice grain and starch properties: Effects of nitrogen fertilizer application. *Carbohydrate Polymers*, *86*(1), 219–225.
Singh, V., Okadone, H., Toyoshima, H., Isobe, S., & Ohtsubo, K. I. (2000). Thermal and physicochemical properties of rice grain, flour and starch. *Journal of Agricultural and Food Chemistry*, *48*(7), 2639–2647.
Song, Y. J., Choi, I. Y., Sharma, P. K., & Kang, C. H. (2012). Effect of different nitrogen doses on the storage proteins and palatability of rice grains of primary and secondary rachis branches. *Plant Production Science*, *15*(4), 253–257.
Wu, G., Wilson, L. T., & McClung, A. M. (1998). Contribution of rice tillering to dry matter accumulation and yield. *Agronomy Journal*, *90*(3), 317–323.
Xiaohou, S., Min, T., Ping, J., & Weiling, C. (2008). Effect of EM Bokashi application on control of secondary soil salinization. *Water Science and Engineering*, *1*(4), 99–106.
Yadav, S. K., Singh, Y., Kumar, R. P., Yadav, M. K., & Singh, K. (2013). Effect of organic nitrogen sources on yield quality and nutrient uptake of rice (*Oryza sativa*) under different cropping system. *Vegetos-An International Journal of Plant Research*, *26*(1), 58–66.
Yum, M. S., & Kawagoe, Y. (2009). Amyloplast division progresses simultaneously at multiple sites in the endosperm of rice. *Plant and Cell Physiology*, *50*(9), 1617–1626.
Zakaria, S., Matsuda, T., & Nitta, Y. (2000). Effects of nitrogen application on the development and accumulation of protein bodies in developing rice seed. *Plant Production Science*, *3*(2), 84–93.