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Comparison of Physicochemical Properties, Grain Quality, and Ultrastructure of Rice Cultivars

Zubair Noori[1], Abdul Ghani Qarluq[1,2], Kifayatullah Kakar[3]*, Saidajan Abdiani[3], Noor Agha Nawakht[3]

[1]Kunduz Directorate of Agriculture, Irrigation, and Livestock, Kunduz, Afghanistan
[2]Tsukuba Training Center, Japan International Cooperation Agency, Tsukuba, Japan
[3]Faculty of Agriculture, Nangarhar University, Nangarhar, Afghanistan

*Corresponding author: Kifayatullah Kakar (*kifayatullahkakar@gmail.com*)

Abstract

Genetic background, environmental conditions, and agronomical practices could influence yield components, grain quality, and physicochemical properties of rice. The study aim was to investigate the variations in yield potential, quality traits, and morphological observation among Akitakomachi (japonica), IR-28 (indica), and NERICA-4 (hybrid) varieties under the same environmental and agronomical conditions. For this, the cultivars were compared in a randomized complete block design with three replications at the paddy field of Tsukuba International Center, JICA, Tsukuba, Japan in 2017. All cultivars were transplanted at a spacing of 15×30 cm using three seedlings per hill. The results exhibited that Akitakomachi had a higher number of panicles per m2 (344.3), and the ripening ratio (94.3%) across all cultivars. In addition, IR-28 and Akitakomachi achieved significantly higher rough rice yield (6.9 t/h & 6.1 t/h), respectively, compared to NERICA-4 (4.9 t/h). However, NERICA-4 was associated with the significantly higher amylose and protein contents, which resulted in the reduction of the taste point than other cultivars. Furthermore, the lowest grain transparent (34.2%) and highest chalky grain (47.7%) were obtained in NERICA-4, which leads to lower grain quality. IR-28 and NERICA-4 were classified as long and slender grain types, whereas, Akitakomachi as a short and medium grain, respectively. The micrographs observation displayed that regular starch granules with polyhedral shape were arranged without air gaps in transparent grains of all cultivars. In contrast, the endosperm of chalky grains in NERICA-4 and IR-28 revealed that irregular starch granules with round shape were loosely packed. Thus, each cultivar should be considered based on growing regions and the cultivated aims.

Keywords: Yield components, Physicochemical properties, Grain quality, Rice cultivars.

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Transparency: The authors confirm that the manuscript is an honest, accurate, and transparent account of the study was reported; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained.
Ethical: This study follows all ethical practices during writing.

1. Introduction

Rice is one of the world's well-known cereal crops for human consumption and companion animals, supplying main food for over half of the world's population [1, 2]. Nevertheless, grain quality is a composite of physical and chemical specifications needed for interest by a specific customer class [3-5]. Though preferences for some of the quality characteristics differ over countries and regions and the priority for some of the specifications is widely shared [6]. Consumers prefer rice with invariable shape and translucent endosperm [7]. Although in Japan, sticky rice is more favorable due to its low amylose and protein contents which influence rice eating and cooking quality negatively [8].

Amylose is a crucial ingredient of rice starch and its content controlled mostly by granule-bound starch synthase consequently influences the rice grain texture [3]. Amylose content highly influenced by the environmental conditions, agronomical practices, grain dimension (including grain length, width, and the ratio of length to width), and genetic backgrounds [9]. Additionally, chalkiness in rice grains appertains to the opaque section and it is a consequential quality trait that decides rice price [10]. Chalkiness generally results in poorer rice milling quality, undesirable grain appearance, and crumbles eating quality due to the loose endosperm structure[11]. However, the starch granules provide energy for the plant during dark periods and for germination of seeds and tubers[12]. The scanning electron microscopic observation of a chalky portion of high temperature-ripened grains revealed those loosely arranged starch granules that create air gaps to reflect light randomly[13].

Shimono, et al. [14] reported that Akitakomachi is the predominant cultivar for the Tohoku region in the northern part of Honshu island, Japan (32.1% of a total cultivated area of 0.40 million ha in the Tohoku region in 2005), while NERICA-4 and IR-28 are the cultivars that researchers are practicing now in Japan. Many scholars have studied to differentiate these cultivars on yield and yield components as well as physicochemical properties and ultrastructure of starch accumulations of rice grains. Till date, information related to the quality traits of these cultivars is not available. Therefore, it is quite necessary to study the physicochemical properties, grain quality, and the chalky grain's ultrastructure of these rice cultivars using a scanning electron microscope.

2. Materials and Methods

2.1. Experimental Design and Plant Management

A field experiment was performed at the paddy field of Tsukuba International Center, JICA (36°32' N, 140°25' E), Japan. In this study, the seeds for each cultivar were procured from JICA Tsukuba International Center. Employing three different rice cultivars, Akitakomachi (japonica), IR-28 (indica), and NERICA-4 (derive of crossing the African rice (O. glaberrima Steud.) and the Asian rice (O. sativa L.)). The research was replicated thrice in a randomized complete block design. The rice cultivars were randomly arranged in plots (6.3 m × 2.7 m for each cultivar), separated by 1m distance between blocks and 50 cm between plots within a block. The field was puddled with puddling machine (Cyber Harrow, Kobashi Industries CO., LTD, Japan) twice and leveled manually. The fertilizers (N.P.K; 14-10-13; JA-ZEN CHU Co., Hiroshima, Japan) as a basal dressing were applied in the field by the broadcasting method at the rate of (N, P2O5, K2O: 60, 100, 80 kg ha^{-1}) and top dressing at (20, 0, 20 kg ha^{-1}) during the panicle initiation stage. Twenty-day-old seedlings were transplanted manually on May 15, 2017, using 3 seedlings per hill at 15 cm × 30 cm spacing. All plots were maintained under the flooded condition from transplanting to maturity stage to avoid water stress.

2.2. Data Measurements

Fifty hills were harvested from each plot to measure different parameters. Evaluation of grain yield and yield components were followed by the method delineated by Hoshikawa [15]. Rough rice grains were de-husked using a small impeller hulling machine (FC2K; Ohtake Co. Ltd., Japan). Grain quality and grain dimension were measured using a taste analyzer (RCTA11A; Satake Co. Ltd., Japan) and a mini vernier caliper (Niigata, SK-M100, Japan), respectively. To evaluate chalky and transparent grains, the brown rice was observed by putting the samples on a white light box (Fuji lightbox 5000 inverters, Japan) to make the grain transparent so the chalky portion (the white part inside the brown rice) could be viewed. Comparison of grain shape as well as appearance of transparent and chalky rice grains of all rice cultivars was conducted by a light microscopy (Keyence-VH500, Japan) see Figure 1.

2.3. Preparation for Scanning Electron Microscopy

The analysis and observation of a scanning electron microscope was conducted based on the method described by Zakaria, et al. [16]. To observe the endosperm of the transparent and chalky grains, brown rice grains were collected at the physiological maturity for each cultivar. Briefly, the milled rice grains for each of the three cultivars were selected and rapidly were submerged into slush nitrogen (solid and liquid, -210 °C). After vacuum freeze-dried with a freeze vacuum dryer (-60 °C, 10-3 Pa, LFD-100NDPS1; Nihon Techno Service). The grains were carefully divided into halves by using a razor blade. The separated halves were attached on a specimen, coated with platinum (JUC-5000; JEOL, Japan), and were observed by using scanning electron microscope (JSM6360A; JEOL, Japan). The micrographs of various magnifications were compared among the cultivars. All the above-mentioned data were analyzed in the Laboratory of Crop Science, The College of Agriculture, Ibaraki University, Ami-machi, Ibaraki, Japan.

2.4. Statistical Analysis

Data obtained for yield and its components, grain quality, and grain dimension were analyzed by one-way analysis of variance (ANOVA) using 13.0 statistical package for the social sciences (SPSS, Prentice-Hall, New Jersey, NJ, United State) software. The significant differences were defined at the $p < 0.05$ probability level.

3. Results and Discussion

3.1. Yield and its Components

In the current study, Akitakomachi was the prominent cultivar in panicle production, followed by IR-28. Both (Akitakomachi and IR-28) cultivars were significantly identical with each other but significantly disparate from NERICA-4 (Table 1). Variation in grain characteristics and yielding behavior was found in different rice cultivars in our previous study [17]. The minimum number of panicles per m2 was found in the NERICA-4 (211.1), because of poor tillers production in this cultivar. IR-28 revealed (101.8) the higher spikelets number per panicle across all cultivars, while, Akitakomachi produced the lowest number of spikelets per panicle (66.9); however,Wei, et al. [18] found significantly higher variation for spikelets number per panicle and the spikelets per m^2 across different genotypes. Shaibu, et al. [19] reported no significant differences in the total number of grains per panicle across irrigation regimes for NERICA-4. There were significant variations between some other genotypes, and the spikelets number per panicle displayed the extremely accordant and closest relationship with grain yield.

In addition, no significant variations in terms of panicle length were observed between Akitakomachi and IR-28, respectively. While, significantly lower panicle length was achieved in Akitakomachi (18.3 cm) than other cultivars. Khatun, et al. [20] stated no significant differences between panicle lengths of the genotypes. The maximum panicle weight was recorded in NERICA-4 (4.6 mg) while the lowest one was found in IR-28 (2.8 mg) followed by Akitakomachi (2.4 mg), respectively. However, Laza, et al. [21] reported that 1000-grain weight differed across cultivars. In the current study, IR-28 achieved the highest 1000-grain weight but it was at par with NERICA-4 (28.9 g) and significantly higher than Akitakomachi (28.0 g) cultivar. The ripened grains percentage for Akitakomachi and IR-28 were 94.3% and 85.0, respectively. While NERICA-4 achieved the lowest percentage of ripened grains compared to the remained cultivars; it is probably due to the environmental conditions at the ripening period Table 1. The percentage of ripened grains, which dynamically correlated with grain yield, displayed clear seasonal effects that varied significantly across the cultivars[22] .

Increasing rice grain yields has been a major objective of breeders to meet the needs of increasing population, but the predicted climate change threatens to reduce the advantage gained by drastically reducing grain yield [23]. In the current study, IR-28 revealed the highest rough rice yield (6.9 t/h) but it was identical with Akitakomachi (6.1 t/h) and significantly higher than the one in NERICA-4 (4.9 t/h). Peng, et al. [24] found significant higher variations in grain yield for different cultivars. Furthermore, temperature impacts rice yield by directly influencing the physiological activities involved in grain development [25].

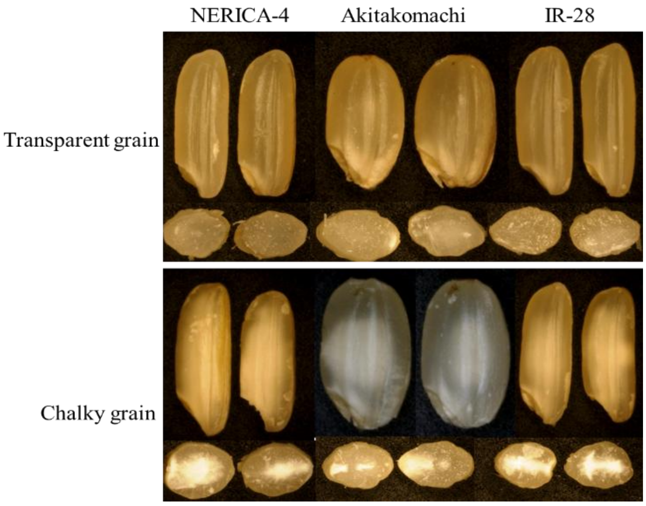

Figure-1.
Comparison of grain shape and appearance of transparent rice grains (T) and chalky rice grains (C) of NERICA-4, Akitakomachi, and IR-28. Photographs of brown rice were taken by a microscope (Keyence-VH500, Japan).

3.2. Physicochemical properties and grain quality

The physicochemical properties are important traits for rice researchers to improve grain quality based on consumer's preference. In this study, amylose, protein, and lipid contents, and the taste point (as a reference) of grain significantly differed across all cultivars Table 1. Besides increasing yields, improving rice grain quality is one of the most important goals of rice breeders to meet market demands [26]. The physicochemical properties and fine structure of starch greatly affect grain quality [9]. NERICA-4 was associated with the significantly higher amylose and protein contents than other cultivars, which finally declined the taste point. IR-28 revealed the lowest grain amylose and protein contents of 19.5% and 7.2%, followed by Akitakomachi 20.0% and 7.2%, respectively, which amplified the grain taste point. Average amylose content differed from 8% to 16% between places and from 5% to 22% among cultivars [27], the difference in amylose content is mostly described by cultivar (68%) and less by the environment (25%).Tsukaguchi, et al. [28] found highly significant variations in grain protein content across different genotypes. The protein content in rice grain was increased by applying nitrogen as topdressing [29].

Table-1.
Comparison of the cultivars based on yield and its components and physicochemical properties.

No	Traits	Cultivar		
		Akitakomachi	IR-28	NERICA-4
	Yield and its components			
1	Panicle No. m²	344.3 a	274.1 ab	211.1 b
2	Spikelet No. panicle^{-1}	66.9 c	101.8 a	89.8 b
3	Panicle length (cm)	18.3 b	24.2 a	25.4 a
4	Panicle weight (mg)	2.4 b	2.8 b	4.6 a
5	1,000-grain weight (g)	28.0 b	29.4 a	28.9 a
6	Ripening ratio (%)	94.3 a	85.0 ab	83.8 b
7	Rough rice yield (t/ha)	6.1 ab	6.9 a	4.9 b
	Physicochemical properties			
1	Amylose content (%)	20.0 b	19.5 c	21.2 a
2	Protein content (%)	7.2 b	7.2 b	9.2 a
3	Fat (%)	5.1 c	6.5 b	9.5 a
4	Moisture (%)	19.3 b	13.9 a	13.8 a
5	Taste point (as reference)	74.8 a	69.9 b	59.9 c
	Grain physical properties			
1	Grain length (mm)	5.1 b	6.6 a	6.6 a
2	Grain width (mm)	2.9 a	2.2 b	2.1 b
3	Grain thickness (mm)	2.0 b	2.0 b	2.1 a
4	Length/width ratio	1.8 b	3.1 a	3.2 a
5	Transparent grain (%)	84.5 a	52.1 b	34.2 c
6	Broken grain (%)	10.6 c	32.0 b	47.7 a
7	Chalky grain (%)	4.9 c	15.5 b	17.7 a

Same letters within a column denote no significant difference at the $p < 0.05$ probability level.

In addition, the finding of the current study also revealed that the grain lipid and moisture contents were significantly lower in Akitakomachi (Table 1). Rayee., et al. [30] reported that physicochemical properties of rice grain varied based on genetic background and cultivation method. The grain length in NERICA-4 (6.6 mm) and IR-28 (6.6 mm) were significantly higher compared to Akitakomachi (5.1 mm). Both (NERICA-4 and IR-28) cultivars revealed statistically identical results in terms of grain width with each other. Whereas, the highest grain width was measured in Akitakomachi (2.9 mm), across all cultivars. Zhang, et al. [31] reported that there were variations in grain width and the ratio of grain length to width in both cultivars in different seasons. There were identical and non-significant differences in grain thickness and the ratio of grain length to width in NERICA-4 and IR-28, both cultivars showed significantly higher grain thickness and the ratio of grain length to width compared to Akitakomachi Table 1. The length to width (L/W) ratio is used in the categorization of grain shape, a great value demonstrating slender shapes and an inferior value demonstrating medium intermediate, bold, or round shapes [32].

Highly significant variations in transparent and chalky grain percentages were observed across all cultivars Table 1. Additionally, Akitakomachi achieved the highest grain transparent percentage (84.5%), but significant the lowest chalky and broken grains percentages were achieved by Akitakomachi than other cultivars. While the lowest and highest transparent and chalky grain percentages were achieved in NERICA-4 (34.2% & 47.7%), followed by IR-28 (52.2% & 32.0%), respectively. Grain appearance is a substantial criterion in judging the quality of rice grain, and the chalky grains have a negative influence on the quality [33]. Researchers revealed that the percentage of chalky grains (reached 15%) declined eating quality of rice grain.Ishimaru, et al. [34] reported that the appearance of chalky grains reduces rice value in global markets and high-temperature stress during grain development stage assists the formation of chalky grains [35]. However, grain size and shape are between the first rice quality indicator that breeders account when progressing new cultivars for release and marketable production [36]. Preferences for grain dimension (size and shape) differ from one class of consumers to other [36]. Although grain size and grain shape were not significantly varied between Korean and Yunnan rice genotypes [37]. In the current study, according to the grain size and shape results, NERICA-4 and IR-28 were classified as long and Table 1. Additionally, Akitakomachi Table 1.

3.3. Scanning Electron Microscope Observations

To observe the variations between transparent and chalky grains on the development of the endosperm, cross-sections of the central portion of the grains were observed under a scanning electron microscope Figure 2. The endosperm of transparent grains revealed that there were no obvious variations in the endosperm structures across all cultivars. Additionally, the endosperm of all rice cultivars was almost identical and the starch granules with polyhedral shape had developed and regularly packed without air gaps among starch granules Figure 2, T1-T3. Particularly, the endosperm of transparent grains revealed that the grain development process in all cultivars was more advanced compared to chalky rains.

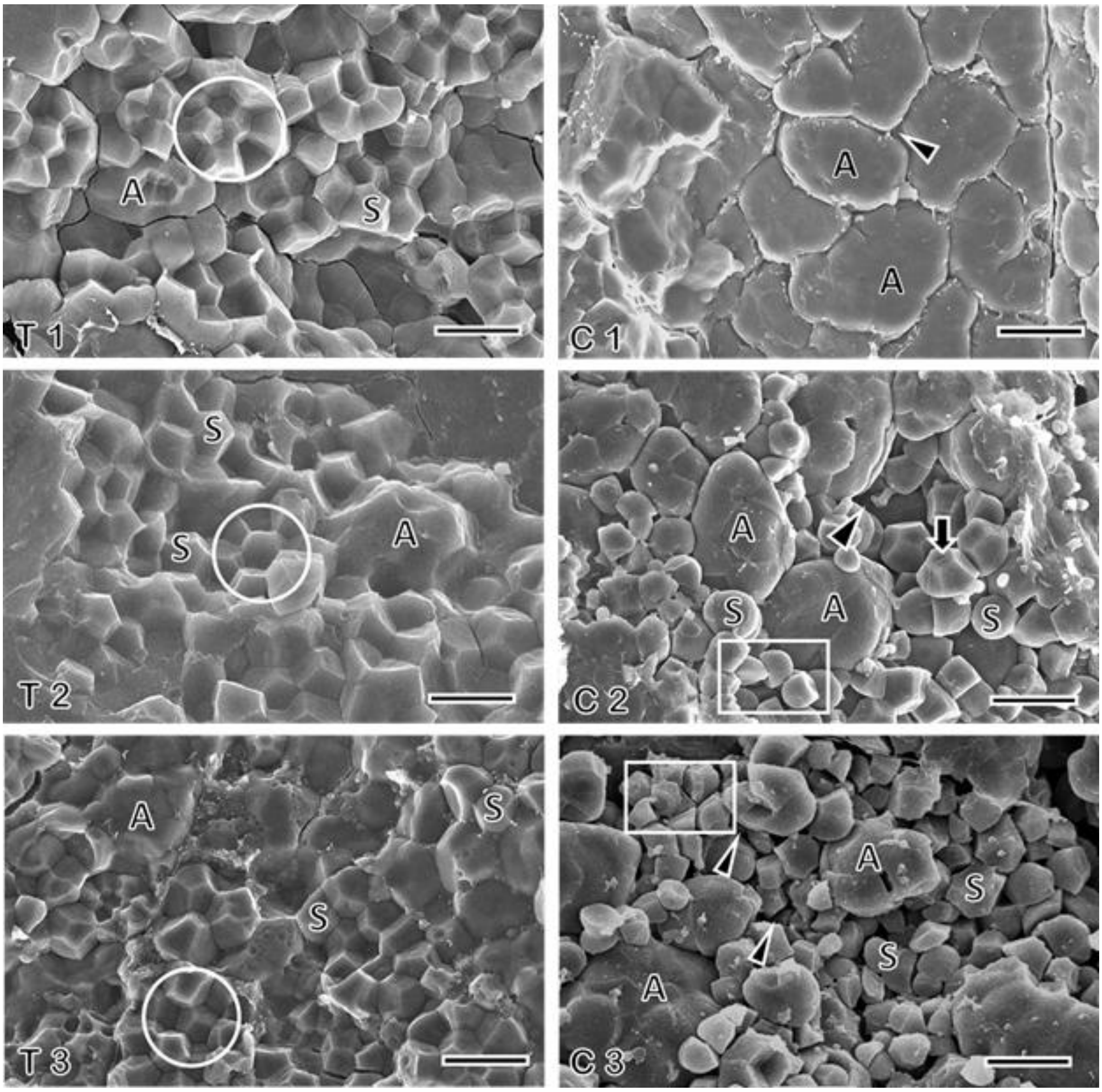

Figure-2.
Accumulation structure of brown rice grains of Akitakomachi (T1, C1), IR-28 (T2, C2), and NERICA-4 (T3, C3) under scanning electron microscopy. T: transparent grain, C: chalky grain. A: amyloplasts, S: starch granules, arrow: dent portions and arrowhead: air gaps. Scale bar: 10 µm. White circles display the polyhedral shape of starch granules grouping into amyloplasts without air gaps. White rectangles display loosely developed starch granules together with the single round shape and multiple size and shape of starch granules with numerous air gaps.

Liu, et al. [38] and Chen, et al. [12] observed that in the endosperm of the transparent grain, the starch granules with polygonal shape were regularly built up without air gaps between them. In this study, the scanning electron microscopy images illustrated that a few alterations of starch granules in the chalky grains were observed in the Akitakomachi endosperm. Furthermore, the high density of large amyloplasts with the round shape were packed with air gaps between amyloplasts were observed (Figure 2, C1). The endosperm of chalky grain which influenced by high temperature during the ripening period, the small starch granules with round shape were abnormally filled, and numerous air gaps appeared among amyloplasts [38, 39]. The endosperm of chalky grain was altered compared to the transparent grain in IR-28, and multiple irregulars and individual starch granules with round shape were poorly arranged. Numerous starch granules with dent portions on their surfaces were scattered and large airspaces among them were also observed (Figure 2, C2).

Our results agree with the findings of previous studies [12, 35, 39] who observed that the loose arrangement of starch granules, which caused by the high-temperature condition, facilitates the reduction of lower grain weight and quality. In contrast, single and high frequency of small starch granules in the endosperm of chalky grains of NERICA-4 were loosely built-up (round shape and small size), and numerous free air gaps were also observed among abnormal starch granules (Figure 2, C3). Hence, the poorly developed endosperm facilitates in lower grain weight and quality and might be influenced by high temperature during grain filling for this cultivar.. Kakar, et al. [40] stated that protein bodies in the rice endosperm may be varied based on genetic backgrounds and agronomical practices.

4. Conclusion

Rice cultivars demonstrated to respond dissimilarly under the field condition in their yield characteristics, physicochemical properties, and grain quality as well as on morphological structure. The endosperm of chalky grains in

NERICA-4 and IR-28 showed that individual starch granules with round shape were poorly arranged while Akitakomachi was accumulated starch granules without abnormality. The abnormal starch granules with dent portions on their surface were scattered and large air gaps among starch granules were observed due to high temperature during grain filling stage for these cultivars. Such poor development of endosperm declines both the weight and quality of grains. Akitakomachi is likely for the people who prefer sticky rice grain while NERICA-4 is more acceptable for non sticky rice grain consumers. The findings of this study may help rice producers to consider these cultivars based on cultivated region and production aimed.

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