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Efficacy of Irrigation Interval after Anthesis on Grain Quality, Alkali Digestion, and Gel Consistency of Rice

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Abstract: The management of amylose and protein contents and cooking quality are the main challenges in rice macronutrients and quality improvement. This experiment was conducted to examine the rice grain quality, alkali digestion, and gel consistency responses to irrigation interval after anthesis. Three rice varieties (K1, K3, and K4) were subjected to different irrigation intervals (1, 2, and 3 d) after anthesis. The findings of this study showed that the protein content was markedly increased from 6.53–6.63% to 9.93–10.16%, whilst the amylose content was decreased significantly from 22.00–22.43% to 16.33–17.56% under stressed treatments at irrigation intervals, whilst the quantity of fatty acids was not affected. The 3-d irrigation interval recorded the highest protein content but the lowest amylose value. In addition, this treatment shows lower gelatinization temperature, but it is negatively associated with hard gel consistency under irrigation interval. This study highlights that the water management following a 3-d irrigation interval from anthesis is a useful and simple treatment to improve rice nutrients and grain cooking quality.

Keywords: irrigation interval; protein; amylose; alkali digestion; gel consistency

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1. Introduction

Rice (*Oryza sativa* L.) is counted as the staple food in Asia, where approximately 90% of the world's rice is produced, plays a leading figure to provide food for billion people worldwide [1,2]. About 50% of the world population are dependent on rice for their initial food calories [3,4]. Rice grain as the main source of its nutritional part contains almost all the essential and beneficial elements for health. The grain quality is a key factor for market value and helps farmers to have a better economic return [5].

The rice grain quality contents are determined by genetic interaction and environmental conditions [6]. Based on previous studies, rice grain quality is mostly influenced by drought stress during the reproductive stage [7]. However, the breeders could increase the crop yield by genetic interaction and improving drought-tolerant varieties, but this process takes a long time and there is some misunderstanding for trait characteristics. The reproductive stage is a very critical period for rice crops and is a very susceptible stage to water deficit which generally results in the reduction of spikelet fertility during flowering [8].

The rice yield did not differ significantly at both intermittent and continuous flooding, while water use revenue was enhanced around 20–60% via intermittent flooding compared to the continuous flooding, the 2-d irrigation interval also saved the water supply remarkably; meanwhile, a decline of nitrogen uptake in continuous flooding irrigation was reported due to nitrate leaching [9]. Cai et al. [10] and Fofana et al. [11] found that the traits that determined rice quality, including head rice grain ratio, total milling rate, and protein content, were promoted by drought occurrence at the ripening stage.

The grain traits which meet consumer's preferences are part of rice grain quality [12]. The physical traits of rice grain encompass the grain shape, appearance, and milling recovery, while the physicochemical characteristics include alkali digestion, gel consistency, and amylose content besides physical attributes [13–15]. The rice grain physiochemical properties are diverse in different rice genotypes and influence the grain quality traits [16]. Gel consistency indicates the cold dough viscidity of cooked ground rice flour. It is used as an index for differentiating cooked rice contexture of amylose contents. Gel consistency extends from soft conditions to hard [17]. The weak and rigid gels are associated with starch polymers in the aqueous phase. The rice grains with soft gel consistency are preferred by the consumers [18]. On the other hand, the gene determines alkali digestion, is settled on chromosome six, and is controlled by Quantative Trait Locus QTL) in the *ALK* locus [19]. The values of alkali digestions are influenced by the nature of amylopectin which is a polysaccharide that exists in the endosperm of rice starch along with amylose.

Our previous study showed that water deficit after anthesis improved rice phytochemicals, antioxidant activities, and proline contents [20]. However, the rice nutrients and grain cooking qualities subjected to water stress have not been studied yet. Thus, this study was carried out to appraise the efficacy of irrigation intervals on rice grain quality, alkali digestion, and gel consistency after anthesis.

2. Materials and Methods

2.1. Materials and Experiment Design

Three rice varieties were selected for this study. K1 and K3 (Japonica subtype) are the cultivars that were developed from the Koshihikari variety grown in Higashi-Hiroshima, Hiroshima Prefecture, Japan. K4 is the Indica subtype (Khang Dan cultivar), originated from North Vietnam [20]. In the beginning, the sterilized seeds were soaked at 25 °C for 48 h. They were then washed with distilled water. Wagner pots with 30 cm height and 20 cm diameter and containing 4 kg commercial soil (JA-Zenchu, Hiroshima, Japan) were used for transplanting 20 d old rice seedlings. Three seedlings were transplanted in each pot and were thinned for two seedlings per pot one week later. The available ammonium nitrate was 0.16 g kg^{-1}, phosphorous 0.2 g kg^{-1} (P), and potassium (K) 0.26 g kg^{-1} in the original soil. The experiment was comprised of 3 levels of water and 3 varieties and laid out based on randomized complete block design (RCBD). Each block (replication) was replicated three times, making a total of 81 pots. The temperature in the greenhouse was (39/20 °C max/min). Urea fertilizer (N) was applied three times with the rate of 0.5 g N in each pot one day before transplanting as basal fertilizer. 0.5 g N as top dressing at 30 days after transplanting (DAT), and 0.2 g at 65 DAT. Furthermore, (P) and (K) were applied 2 g each, one day before transplanting.

From transplanting to anthesis, the pots were watered to sustain 85% soil moisture. The moisture in the pot soil was adjusted using the moisture meter (TDR-341F, Fujiwara Co., Ltd., Osaka, Japan). Later on, 3 levels of irrigation frequencies including continuous flooding in which the water level was kept at 5 cm above the soil as control (W1), intermittent flooding followed by a 2-d interval (W2), and intermittent flooding followed by a 3-d interval (W3) were applied until the plants were harvested. The collected panicles after harvest were dried at 50 °C for 72 h. Rice grains were then separated from rachises and adjusted to 12–14% moisture content using the oven method before further measurements.

2.2. Grain Quality Measurement

To obtain the brown rice, a rice husker machine (TR-250, Tokyo, Japan) was used to de-hull the grains. The quality contents including protein, amylose, and fatty acid were determined through a quality tester (PGC Shizuoka Seiki PS-500, Shizuoka Seiki Co., Ltd., Shizuoka, Japan).

2.3. Chemicals

Thymol blue, potassium hydroxide (KOH), and ethanol were used in this experiment. They were acquired from Kanto Chemical Co., Inc., Tokyo, Japan, and were analytical grade.

2.4. Determination of Gelatinization Temperature (GT)

Steps for the determination of GT were followed by the method reported by Chemutai et al. [21]. Briefly, 10 polished rice kernels were set in a Petri dish containing 10 mL of potassium hydroxide (KOH) 1.7% (w/v) which was prepared freshly. In order to have better spreading, enough space between the seeds was provided. Then, the dishes were covered and kept in an incubator at 30 °C for 24 h. The test was conducted in triplicate to ensure its validity and accuracy. The grains starch degradation of each sample was rated visually as illustrated in Figure 1 following on the 7-point numerical spreading scale described in Table 1 through the report of Graham [22].

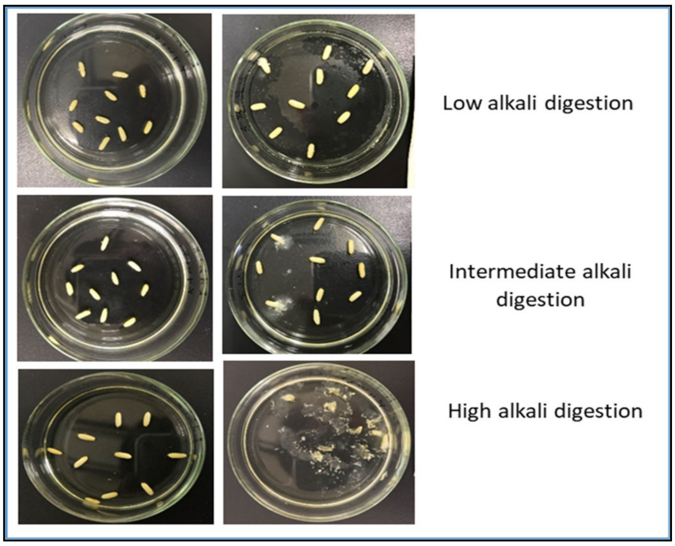

Figure 1. Degree of alkali digestion in rice seeds.

Table 1. Numerical scales for scoring gelatinization temperature.

Values	Degree of Dispersion	Alkali Digestion	Gelatinization Temperature
1	Grain not affected	Low	High
2	Swollen grain	Low	High
3	Inflated grain with imperfect and narrow collar	Low or intermediate	High-intermediate
4	Inflated grain with perfect and wide collar	Intermediate	Intermediate
5	Fragmented grain, perfect or wide collar	Intermediate	Intermediate
6	Dispersed grain, merging with collar	High	Low
7	Grain completely dispersed and intermingled	High	Low

2.5. Determination of Gel Consistency

The gel consistency of the rice grains was determined according to the method illustrated by Chemutai et al. [21] with fewer modifications. Briefly, test tubes sized 13 mm × 100 mm were filled with 0.5 g rice flour of the samples in triplicate. The samples were mixed with 0.026 mL of ethanol (95%) containing thymol blue (0.025%), then, the rice flour was prevented from clumping. The mixture was vortexed slowly, and 2 mL of potassium hydroxide (KOH) (0.2 N) was added and mixed again. To prevent the steam and reflow of samples, the tubes were covered with glass marbles. The samples were cooked in a boiling water bath at 92 °C for 6 min. Then, they were removed from the water bath and retained at room temperature for 5 min. Subsequently, they were put into an ice bath for 15 min. The tubes were positioned horizontally on a laboratory table marked with lines in millimeter graph paper (Figure 2). The length of gel was determined from the lowest part of the tube to the end of the gel. The classification of gel consistency was followed by the method reported previously by Graham [22]. The test differentiates rice with high amylose content into three classifications:

-> Very shelly rice with hard gel consistency (gel length, <40 mm)
-> Shelly rice with medium gel consistency (gel length, 41–60 mm)
-> Soft rice with soft gel consistency (gel length, >60 mm)

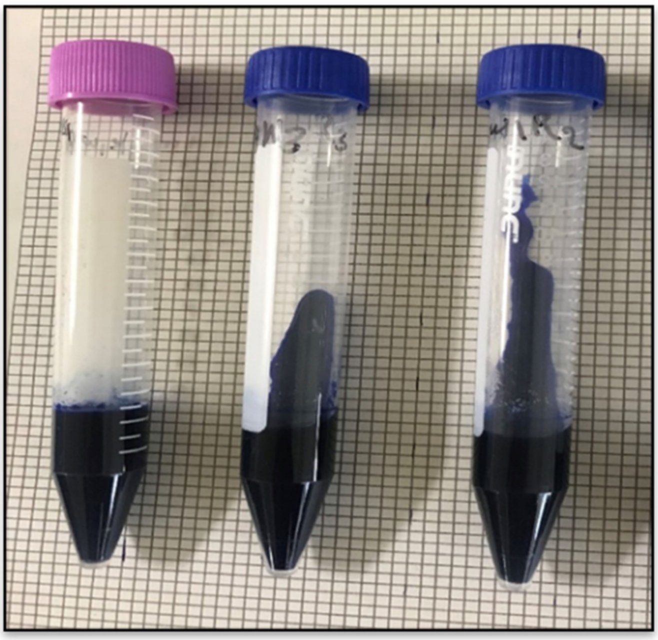

Figure 2. Gel consistency of the samples.

2.6. Data Analysis

All the data were analyzed using Minitab 16.0 (Minitab Inc., State College, PA, USA). Results are expressed as means ± standard deviation (SD). Significant differences among variety types and treatments were determined via ANOVA method, followed by Tukey's multiple comparison tests. The significant differences were defined at the 5% ($p < 0.05$).

3. Results

3.1. Grain Quality Contents

The grain quality parameters including protein, amylose, and fatty acids are shown in Table 2. In general, the discontinuity of water supply increased the protein content more than the control, of which W3 showed greater protein content (9.70–10.16%) than W2 (8.30–9.46%). In contrast, the amylose percentage was reduced remarkably at 2 d (19.10–21.50%) and 3 d intervals (16.33–18.20%), of which the 3 d interval caused a greater decline of amylose content. In the case of fatty acids, it was not influenced by the irrigation interval. Generally, only protein and amylose were affected by the imposition of water deficit. To contrast between the two subtypes Indica and Japonica rice varieties, K1 and K3 revealed higher protein percentage compared to K4 under W3, whereas the amylose content of the Indica (K4) was less affected than the Japonica (K1 and K3). In statistical analysis, significance was observed in protein and amylose contents among treatments. Additionally, non-significant difference was observed among varieties in protein and amylose contents (Table 2).

Table 2. Quality parameters of rice grain in response to irrigation interval.

Variety	Treatments	Protein (%)	Amylose (%)	Fatty Acid (%)
K1	W1	6.83 ± 0.78 [b]	22.43 ± 0.44 [a]	7.67 ± 3.18 [a]
	W2	8.63 ± 0.80 [ab]	19.14 ± 0.57 [b]	5.00 ± 0.58 [a]
	W3	9.70 ± 0.28 [a]	17.56 ± 0.60 [b]	9.67 ± 2.73 [a]
K3	W1	6.53 ± 0.46 [b]	22.30 ± 1.00 [a]	8.33 ± 3.84 [a]
	W2	8.30 ± 0.60 [ab]	19.10 ± 1.10 [ab]	9.00 ± 0.57 [a]
	W3	10.16 ± 0.60 [a]	16.33 ± 0.88 [b]	10.00 ± 1.00 [a]
K4	W1	6.63 ± 0.68 [b]	22.00 ± 0.72 [a]	4.33 ± 0.88 [a]
	W2	9.46 ± 0.48 [a]	21.50 ± 1.50 [a]	4.00 ± 0.00 [a]
	W3	9.93 ± 0.60 [a]	18.20 ± 1.10 [a]	3.66 ± 0.33 [a]
ANOVA				
Variety		NS	NS	*
Treatment		*	*	NS
Variety × Treatment		NS	NS	NS

W1, continuous flooding (control); W2, 2-d irrigation interval; W3, 3-d irrigation interval. Values with similar letters within watering treatments showed no significant difference at the 5% level. ∗: significant differences at (p <0.05); NS: not significant.

3.2. Gelatinization Temperature (GT)

The results of the impact of irrigation interval on rice grain GT are shown in Table 3. Based on the findings of the degree of alkali digestion, there was a considerable variation among the treatments for all GT of the varieties and it ranged from low to high level. The findings indicate that 3-d irrigation interval caused a greater reduction of GT. High GT was observed under continuous flooding for all varieties. Whilst the stressed seeds tended to have intermediate and low GT. Among the varieties, only K4 expressed low GT at 3-d which indicates that irrigation interval has made the variety soft which results in a short cooking-time.

Table 3. Gelatinization temperature of rice seeds based on 7-scale point of alkali digestion under irrigation intervals.

Varieties	Treatments	Alkali Digestion Value	Alkali Digestion	Gelatinization Temperature
K1	W1	2	Low	High
	W2	3	Intermediate	Intermediate
	W3	4	Intermediate	Intermediate
K3	W1	2	Low	High
	W2	4	High-intermediate	High-intermediate
	W3	3	Intermediate	Intermediate
K4	W1	2	Low	High
	W2	4	Intermediate	Intermediate
	W3	6	High	Low

W1, continuous flooding; W2, 2-d irrigation interval; W3, 3-d irrigation interval.

3.3. Gel Consistency (GC)

Figure 3 shows the GC of all rice varieties and treatments. The results revealed that GC decreased significantly in all varieties under irrigation interval treatments. GC was observed longer under continuous flooding rather than 2-d and 3-d irrigation intervals. K4 expressed the longest GC (74.3 mm) at continuous flooding and tended to decrease at 2-d and 3-d treatments. There were significant differences in GC among the treatments for K1. On the other hand, K1 and K3 had the lowest GC under 3-d.

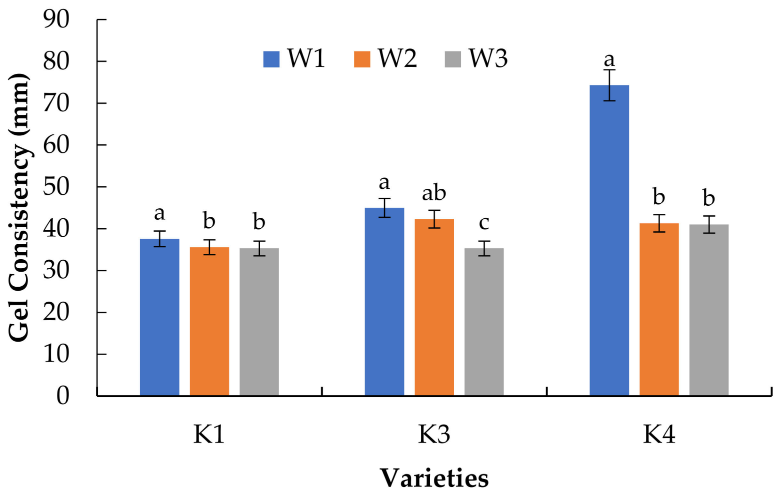

Figure 3. Rice gel consistency under water deficit. W1, continuous flooding; W2, 2-day irrigation interval; W3, 3-day irrigation interval. Values with similar letters are not significantly different ($p < 0.05$).

4. Discussion

Rice grain is counted as a vital food source for its valuable pharmaceutical properties and beneficial health values [23]. It is cultivated widely in the world, and its quality is affected by water stress deficit. In the current study, the protein percentage increased significantly at 2-d and especially at 3-d irrigation interval, while, the amylose content tended to decrease, meanwhile there was no change for the fatty acid content. These findings are consistent with the previous studies of Renmin and Yuanshu [24] and Rayee et al. [20] to report that when rice was subjected to water stress, the protein content was raised, while the amylose content was decreased. Upland and low land cultivars respond to water stress differentially, on the other hand, the significant changes in rice quality can be seen when rice is subjected to water stress at the grain filling period [25]. It was observed that proline and malondialdehyde accumulated significantly in rice leaves, and chlorophyll content tended to decrease with extended duration of irrigation interval [26]. The increase in rice protein will improve the nutritional condition of the human diet, but it negatively correlates to rice taste [27,28].

Determination of rice grain quality parameters through the evaluation of grain physio-chemical properties makes it easy to evaluate the cooking-eating properties of each variety under different treatments application. Alkali digestion is defined as a key character of cooking–eating quality of rice grain [21]. Based on the previous studies, there is a non-linear correlation between alkali digestion value and gelatinization temperature; the varieties with low alkali digestion have high gelatinization temperature [29,30]. Starch gelatinization depends on the type of starch, i.e., the proportion of amylose and amylopectin present, and availability of water. The swelling of the starch granules during the heating process causes cellular disruption, which together with starch gelatinization, give a softening in texture and increased palatability of the grain [30]. When rice is cooked, starch granules tend to absorb water and swell. As the temperature rises the starch continues to absorb more water till they reach their maximum volume, called gelatinization temperature [3]. Gelatinization temperature of rice grain refers to the cooking temperature and the starch granules swell irreversibly in hot water. The gelatinization temperature of rice grain can be classified as low (55–60 °C), intermediate (70–74 °C), and high (>74 °C) [22]. In the current study, the gelatinization temperature of rice grains ranged from high under continuous flooding to low and intermediate under stressed treatments of W2 and W2 irrigation intervals (Table 3). These findings revealed that the stressed rice grains at 3-d irrigation intervals have lower gelatinization temperature which results in shortened cooking time and saves energy during the cooking. This finding is consistent, as Cheng et al. [31] reported that alkali digestion and cooking quality significantly had been improved under intermittent irrigation compared to continuous flooding. Since grains with high gelatinization temperature require more water and time for cooking, thus, irrigation interval can be a desirable medium for decreasing gelatinization temperature and save time and energy for cooking.

Gel consistency was described as an indirect approach to screen cooked rice for its hardness [32]. This method is used in rice improvement programs to determine whether the genotype has a soft or hard tissue when cooked [33]. There are differences among the varieties for having gel consistency. The gel consistency of varieties with low amylose contents is soft and flaky. In this study, the gel consistency ranged from 35.3 mm to 74.3 mm among the varieties and treatments. The result reveals that K4 has a soft gel consistency, but it showed hard to medium gel consistency in response to drought conditions (Figure 3). K3 and K1 expressed hard gel consistency based on the gel consistency values reported by Graham [22]. However, different level of a waxy gene and the variation in the waxy gene locus is involved in the gel consistency classification. In this research, irrigation interval might have changed the interaction of genes and caused the varieties to have a hard gel consistency. Based on Graham's classification, soft rice mostly has a gel length of more than 61 mm [22]. In this study, the irrigation interval negatively affected the grain gel consistency. The highest gel consistency was observed in K4 (77 mm) at continuous flooding, whereas it was decreased to 41.3 mm at W2 treatment. This is the first time that rice grain physiochemical properties were analyzed in response to drought after anthesis stages. The current research illustrates the importance of irrigation management on rice grain quality and cooking properties and will assist farmers to increase their income by producing quality rice grain.

5. Conclusions

This study indicates that the application of water deficit at 3-d irrigation interval increases nutrients and cooking quality in rice grains. The treatment increases grain protein content significantly whilst the amylose quantity is decreased. The highest protein and the lowest amylose were associated with K3 variety. The result shows that rice grain gelatinization temperature level is reduced to the low (K4) and intermediate levels (K1 and K3) under 3-d irrigation interval, and it could be considered as benefits of water deficit, where as it negatively impacted rice gel consistency in all three treated rice cultivars. Considering the grain quality responses, K3 is suitable for cultivation under drought conditions. The simple treatment using water deficit interval is beneficial and economical for rice producers to improve rice quality and cooking values of rice grain. This research provides useful information on the changes in physicochemical properties in the rice grain under irrigation intervals.

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