AgriEngineering

Article

Validation of Relation between SPAD and Rice Grain Protein Content in Farmer Fields in the Coastal Area of Sendai, Japan

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Abstract: In present-day Japan, high quality is the first requirement of rice production. To maintain the quality of rice, the prejudgment technique has been proposed to control rice growth or to separately harvest rice depending on its quality. Since the quality of rice is generally indexed by grain protein content, which is strongly affected by nitrogen content of leaves, one of the major prejudgment techniques is based on leaf greenness evaluation (i.e., SPAD value). However, the technique is under research and not popular with the farmers because the reliability of prejudgment is inadequate. In this study, we investigated the leaf SPAD value at different growth stages of different cultivars and with cultivation methods in farmer fields over four years, and we validated the applicability of prejudgment by comparing with the grain protein content. The results showed that the grain protein content was positively correlated with leaf SPAD value at the maturity stage, but correlated weakly with those at the booting, heading, and milking stages. Since the regression coefficients significantly differed depending on the year, cultivar, and planting method, the acquisition of a regression equation for each target is recommended to predict grain protein content more accurately. The validation in this study suggests that the prejudgment of grain protein content just before harvest has generality for several targets and is useful for harvesting rice depending on the quality. The results in this study may contribute to the attempts to evaluate SPAD value and then rice quality by remote sensing.

Keywords: rice; prejudgment; planting method; growth stage; SPAD; grain protein content

Citation: Zhang, L.; Hashimoto, N.; Saito, Y.; Obara, K.; Ishibashi, T.; Ito, R.; Yamamoto, S.; Maki, M.; Homma, K. Validation of Relation between SPAD and Rice Grain Protein Content in Farmer Fields in the Coastal Area of Sendai, Japan. *AgriEngineering* **2023**, *5*, 369–379. https://doi.org/10.3390/agriengineering5010024

Academic Editor: Pankaj B. Pathare

Received: 2 January 2023
Revised: 2 February 2023
Accepted: 8 February 2023
Published: 13 February 2023

1. Introduction

Rice (*Oryza sativa* L.) a staple food crop, that is cultivated and consumed worldwide in different countries [1]. With the development of the economy and the improvement in people's living standards, higher requirements for rice quality have been recognized. Improving rice quality is now one of the major research goals of rice production. A large number of studies have shown that amylose and protein contents are important factors affecting the cooking and eating quality of rice [2–4]. In particular, grain protein content can explain 38.6% of the variation in the taste value of indica rice [5]. Shi et al. [6] also reported that protein can explain 66.8% of the food and taste value. Accordingly, grain protein content is the major index of grain quality to be controlled. The fact that the amylose content is generally controlled by genotypes and the effect of management is not clear [7] has also enhanced the priority of grain protein content to evaluate grain quality. The protein is mainly decomposed in the leaves and transported to be synthesized in the grain [8,9]. Accordingly, the grain protein content is mainly affected by the leaf protein content [10–12].

At present, Japan's agricultural employment population is sharply declining, resulting in the expansion of managed agricultural land per farm. However, this expansion together with the aging population, is a huge burden on farmers and agricultural businesses. To reduce this burden, the development of economic and labor-saving farmland management techniques is required. In particular, the control of yield and quality is required for rice-producing farmers in terms of the economic aspects. In this context, SPAD (an index of relative chlorophyll content; the name comes from the Soil and Plant Analyzer Development project [13])-based management has been proposed to control grain protein content [14]. This SPAD-based management approach helps farmers to optimize fertilizer application and to harvest rice separately depending on the quality. The quality separation is generally more profitable than ordinal mixed quality. For this purpose, the prejudgment technique is more important for farmers.

A SPAD meter (SPAD 502 plus, Konica Minolta, Tokyo, Japan) measures the absorbance of 650 nm and 940 nm light, which reflects the relative content of chlorophyll [15]. The measurement is non-destructive and rapid. Since chlorophyll is the main substance of plant photosynthesis, the content is used as an indicator of plant status in farmer fields, as well as in plant physiology research [16–18]. Since the chlorophyll content is related to plant nitrogen status, the SPAD value is also used in nitrogen diagnosis to optimize nitrogen application and to control pests, diseases, and yield [19–24]. The quality management of rice is based on the relation between grain protein content and SPAD via leaf nitrogen content.

The relation has been partly proven by field experiments [25] and farmer field investigations [26,27]. The grain protein content appeared to be strongly correlated with SPAD during the reproductive stage [25]. Fukuyama and Abe [28] and Wakamatsu et al. [29] reported positive correlations between grain protein content and SPAD from the full heading stage to the maturation stage. Wang et al. [30] also confirmed that the SPAD of the surviving leaf in indica rice after the heading stage was strongly correlated with the protein content of rice, especially at the yellow ripening stages. However, the SPAD-based management is presently under research. Few farmers use a SPAD meter to manage their fields. Previous studies have reported that the grain protein content varies according to many factors, such as the temperature during the ripening stage [31,32] and water management [33]. Even the timing of the transplantation and harvest sometimes affects the grain protein content [34,35]. These facts suggest that the reliability of SPAD-based management is inadequate for farmers. The validation is strongly recommended to be conducted in actual farmer fields, where several management approaches are applied and many environmental factors affect rice yield and quality. For this purpose, we investigated the grain protein content in relation to planting methods and cultivars in farmer fields in the coastal area of Sendai, Japan, for 4 years. We also measured the SPAD value in the booting stage, heading stage, milking stage, and maturity stage to validate the relation with the grain protein content. The statistical analysis was focused on the yearly variation and the effects of planting methods and cultivars on the relation between the SPAD value and the grain protein content. The study is expected to contribute the utilization of remote sensing in the future.

2. Materials and Methods

2.1. Research Fields

The research was conducted in rice fields managed by a large-scale agricultural producer cooperative in the coastal area of Sendai City, Miyagi Prefecture, Japan (Figure 1). The cooperative managed about 100 ha of fields and planted rice about 60 ha. We selected representative fields according to planting methods and cultivars from 2018 to 2021. Table 1 shows the number of investigation points and the cultivation details for each year. In 2018 and 2021, additional fertilizer was applied. The days of booting, heading, milking, and maturity stage varied among years due to the weather conditions. Weeds and insect pests were managed by the conventional practices of farmers. Table 2 shows the seasonal changes in the mean solar radiation, temperature, and precipitation during the rice-growing periods in 2018, 2019, 2020, and 2021.

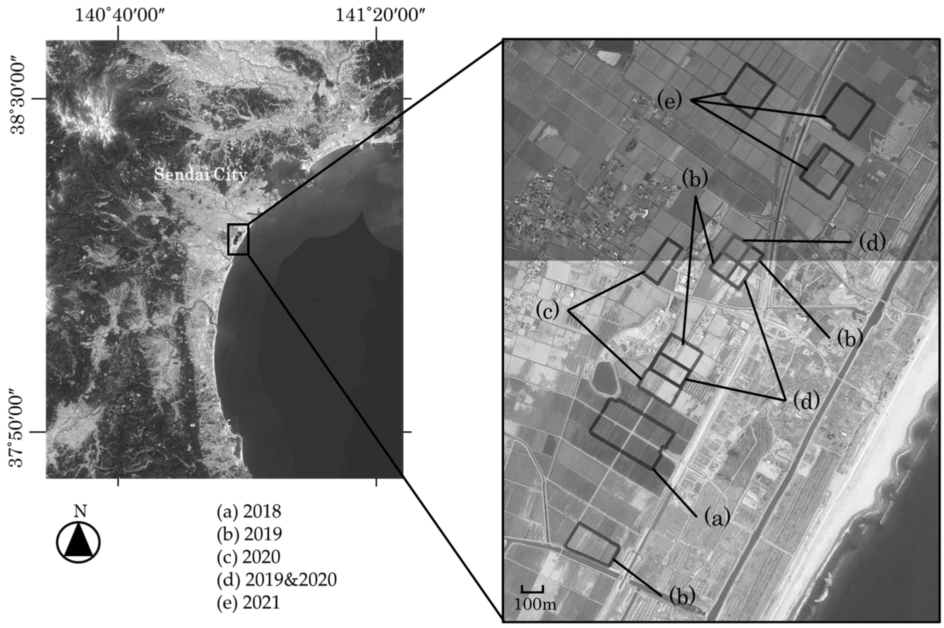

Figure 1. Location of research fields in this study. This figure was modified from aerial image taken by the Geospatial Information Authority of Japan (https://www.gsi.go.jp/top.html accessed on 18 November 2022). The black rectangles in the right figure are the fields investigated in 2018, 2019, 2020 and 2021.

Table 1. Number of investigation points and cultivation details from 2018 to 2021.

Year	Cultivar	Planting Method	Number of Points	Planting Density (Row × Column)	Fertilizer	
					Basal ($g\ m^{-2}$)	Additional ($g\ m^{-2}$)
2018	Hitomebore	Transplanting	40	0.3 m × 0.21 m	40 [a]	5 [b]
	Manamusume	Transplanting	20	0.3 m × 0.21 m	40 [a]	5 [b]
	Datemasayume	Transplanting	20	0.3 m × 0.21 m	40 [a]	5 [b]
	Hitomebore	Direct sowing [c] (flooded)	80	0.3 m × 0.21 m	40 [a]	-
2019	Hitomebore	Direct sowing [c] (flooded)	48	0.3 m × 0.2 m	40 [d]	-
	Hitomebore	Transplanting [e] (dense)	48	0.3 m × 0.2 m	40 [d]	-
	Hitomebore	Transplanting	48	0.3 m × 0.2 m	40 [d]	-
2020	Manamusume	Transplanting [e] (dense)	8	0.3 m × 0.18 m	40 [c]	-
	Manamusume	Direct sowing	8	0.3 m × 0.18 m	40 [d]	-
	Hitomebore	Transplanting	16	0.3 m × 0.18 m	40 [d]	-
2021	Manamusume	Direct sowing	48	0.3 m × 0.18 m	40 [d]	8 [b]
	Hitomebore	Transplanting	48	0.3 m × 0.2 m	40 [d]	8 [b]
	Manamusume	Transplanting [e] (dense)	48	0.3 m × 0.2 m	40 [d]	8 [b]

[a] Hitomebore senyouhiryou 2gouR (Central Chemical Co, Ltd., Tokyo, Japan). [b] Minakuchi NK (Central Chemical Co, Ltd.); [c] Miyagimai-ippatsu 204 (Katakura and Co-op Agri Corporation, Tokyo, Japan); [d] Direct sowing under flooded conditions; [e] Direct sowing under dry conditions; [e] Transplanted with densely seeded seedlings.

Table 2. Daily average (Avg.) solar radiation ($MJ\ m^{-2}$), temperature (°C) and precipitation (mm) during the growing periods in 2018, 2019, 2020, and 2021.

Month	Period	Solar Radiation ($MJ\ m^{-2}$)				Temperature (°C)				Precipitation (mm)			
		2018	2019	2020	2021	2018	2019	2020	2021	2018	2019	2020	2021
May	early	14.2	23.8	20.3	20.9	15.4	15.4	17.4	15.9	3.3	1.5	0.3	1.9
	late	20.2	23.5	15.8	16.0	18.5	19.3	16.3	18.0	3.3	3.7	6.3	2.8
June	early	18.4	19.4	22.4	20.4	19.0	18.5	21.8	20.5	5.0	5.3	1.2	1.8
	late	16.9	13.6	14.1	15.5	21.5	19.5	20.6	20.7	1.7	6.0	1.7	2.3
July	early	15.9	11.7	8.5	9.5	24.6	20.3	20.9	21.8	3.6	4.0	14.7	9.1
	late	18.6	15.0	10.7	21.3	26.4	24.5	21.5	26.2	0.3	3.2	11.1	3.3
August	early	15.9	17.1	18.0	13.8	25.8	27.4	26.3	24.6	7.5	0.3	2.5	7.3
	late	13.3	12.3	18.0	13.6	24.2	25.1	26.9	25.0	10.0	5.3	2.2	4.5
September	early	9.9	14.1	11.9	12.3	21.9	24.0	24.7	20.7	4.9	3.9	7.5	5.8
	late	11.6	13.1	10.6	13.9	19.7	20.9	20.2	21.0	7.6	1.0	5.3	2.4
Avg.		15.5	16.4	15.0	15.7	21.7	21.5	21.7	21.5	4.7	3.4	5.3	4.1

2.2. Measurements

The SPAD value was measured using SPAD502Plus (Konica Minolta Japan, Inc., Tokyo, Japan) following the standard method [36]. The measurement was conducted at the booting stage (19th July, 17th July, 20th July and 20th July), heading stage (10th Aug, 8th Aug, 7th Aug and 6th Aug), milking stage (25th Aug, 23rd Aug, 22nd Aug and 21st Aug), and maturity stage (5th Sep, 3rd Sep, 4th Sep and 7th Sep) in 2018, 2019, 2020, and 2021, respectively. The stages were followed to the standard guideline in Japan [37]. In the milking stage, the rice grain contains a white solution of starch and its leaves are yellowish. In the maturity stage, the color of the rice grain turns brown, and that of leaves turns yellow but remains green. A total of 5 plants were measured in every plot, and three SPAD values per leaf, including one value around the midpoint of the uppermost fully expanded leaf blade and 2 values 3 cm from the midpoint were averaged as the mean SPAD value of the leaf.

Rice was harvested at each investigation point when the grains had more than 95% ripe yellow color and then dried in the air for 2 weeks. Rice grain protein content (PC) was measured for 2 × 25 g samples for each investigation point one month after harvest using a rice taste analyzer (K-TA200, Kubota, Osaka, Japan). The grain moisture content was determined by drying at 80 °C for 72 h in an oven. The grain protein content was corrected on a 15% moisture content basis.

2.3. Statistical Analysis

Statistical analysis was performed with R (version 4.0.0) and RStudio software. The effects of year, cultivar, and planting method on SPAD value and grain protein content were tested for each growth stage with following equations:

$$\text{SPAD} = \text{Year} \times \text{Cultivar} \times \text{Planting method} \tag{1}$$

$$\text{Grain protein content} = \text{Year} \times \text{Cultivar} \times \text{Planting method} \tag{2}$$

The regression of grain protein content against SPAD value was obtained for all data including years, cultivars, and planting methods for each growth stage with following equation:

$$\text{Grain protein content} = \text{SPAD} \tag{3}$$

The effects of year, cultivar, and planting method on the regression of grain protein content against SPAD value were tested for milking stage and maturity stage with following equation:

$$\text{Grain protein content} = \text{Year} \times \text{SPAD} + \text{Cultivar} \times \text{SPAD} + \text{Planting method} \times \text{SPAD} \tag{4}$$

The above equations were referred to a textbook of general linear regression [38].

3. Results

3.1. Effect of Year, Cultivar and Planting Method on SPAD and Grain Protein Content

The SPAD value was the highest in the booting stage and decreased in the later growth stage (Table 3). The effects of the interactions of year, cultivar, and planting method on the SPAD value were significant, except for the effect of year × cultivar × planting method at the heading and milking stages. Although the interactions were significant, the SPAD value in 2020 tended to be relatively higher while that in 2019 was lower. The order of cultivars in the SPAD value was not consistent. Datemasayume was the highest at the booting stage while Manamusme was the highest at the maturity stage. Transplanting with densely seeded seedlings tended to have lower SPAD value, while direct sowing tended to have higher SPAD value especially at the milking stage and maturity stage.

Table 3. Averages and three-way ANOVA results of SPAD at booting stage, heading stage, milking stage, and maturity stage, as well as grain protein content (PC).

Cultivar	Planting Method	Year	Booting Stage	Heading Stage	Milking Stage	Maturity Stage	PC (%)
Hitomebore	Transplanting	2018	43.91	34.31	31.15	26.15	7.7
	Transplanting	2019	40.83	32.55	31.27	24.42	7.0
	Transplanting	2020	41.77	35.35	32.34	24.12	7.0
	Transplanting	2021	41.18	34.38	34.42	28.05	7.8
	Transplanting (dense)	2019	40.38	31.93	30.53	24.57	7.3
	Direct sowing (flooded)	2018	41.44	36.37	27.99	26.16	7.8
	Direct sowing (flooded)	2019	38.99	34.00	29.06	30.11	7.8
Manamusume	Transplanting	2018	46.97	34.35	30.05	26.76	7.8
	Transplanting (dense)	2020	42.98	39.89	37.75	34.47	8.8
	Transplanting (dense)	2021	39.88	35.86	34.58	28.90	8.6
	Direct sowing	2020	37.97	36.36	33.35	28.29	7.7
	Direct sowing	2021	35.89	31.04	32.80	30.90	8.2
Datemasayume	Transplanting	2018	44.05	34.09	31.94	28.37	8.2
Average							
Year	2018		43.13	34.92	30.36	26.89	7.9
	2019		40.07	32.83	30.29	26.37	7.4
	2020		40.91	37.20	34.48	28.96	7.8
	2021		38.98	33.76	33.93	29.28	8.2
Cultivar	Hitomebore		41.21	34.13	30.97	26.23	7.5
	Manamusume		40.74	35.50	33.71	29.86	8.2
	Datemasayume		44.05	34.09	31.94	28.37	8.2
Planting method	Transplanting		43.12	34.17	31.86	26.31	7.6
	Transplanting (dense)		40.38	31.93	30.53	24.57	7.3
	Direct sowing		36.93	33.70	33.08	29.60	8.0
	Direct sowing (flooded)		40.22	35.19	28.53	28.14	7.80
Overall			41.25	34.65	32.09	27.79	7.8
ANOVA results							
	Year	-	***	***	***	***	***
	Cultivar	-	***	***	***	***	***
	Planting method	-	***	***	***	***	***
	Year × Cultivar	-	***	***	***	***	***
	Year × Planting method	-	**	***	**	***	***
	Cultivar × Planting method	-	**	***	**	***	***
	Year × Cultivar × Planting method	-	*	ns	ns	**	ns

ns: non-significant at 0.05 probability level; *, **, *** significant at 0.05, 0.01, 0.001 probability level, respectively.

The rice grain protein content also varied among the cultivars and planting methods. The interactions of year, cultivar, and planting method were significant except for the effect of year x cultivar x planting method. Although the interactions were significant, the grain protein content in 2021 was the highest while that in 2019 was the lowest. Hitomebore tended to show lower grain protein content than Manamusume. Transplanting both custom and sense seedling showed relatively lower grain protein content than direct sowing both under dry and flooded conditions.

3.2. Estimation of Grain Protein Content Based on SPAD Value

The relation between the grain protein content and the SPAD value is shown in Figure 2. The regression, including all the years, cultivars, and planting methods, for each growth stage was not significant at the booting stage but significant at later growth stages. The SPAD value and the grain protein content rather formed clusters for each year, cultivar and planting method, but distributed along the regression line at latter growth stages. The coefficients of determination increased at later growth stages and reached 0.5 at the maturity stage.

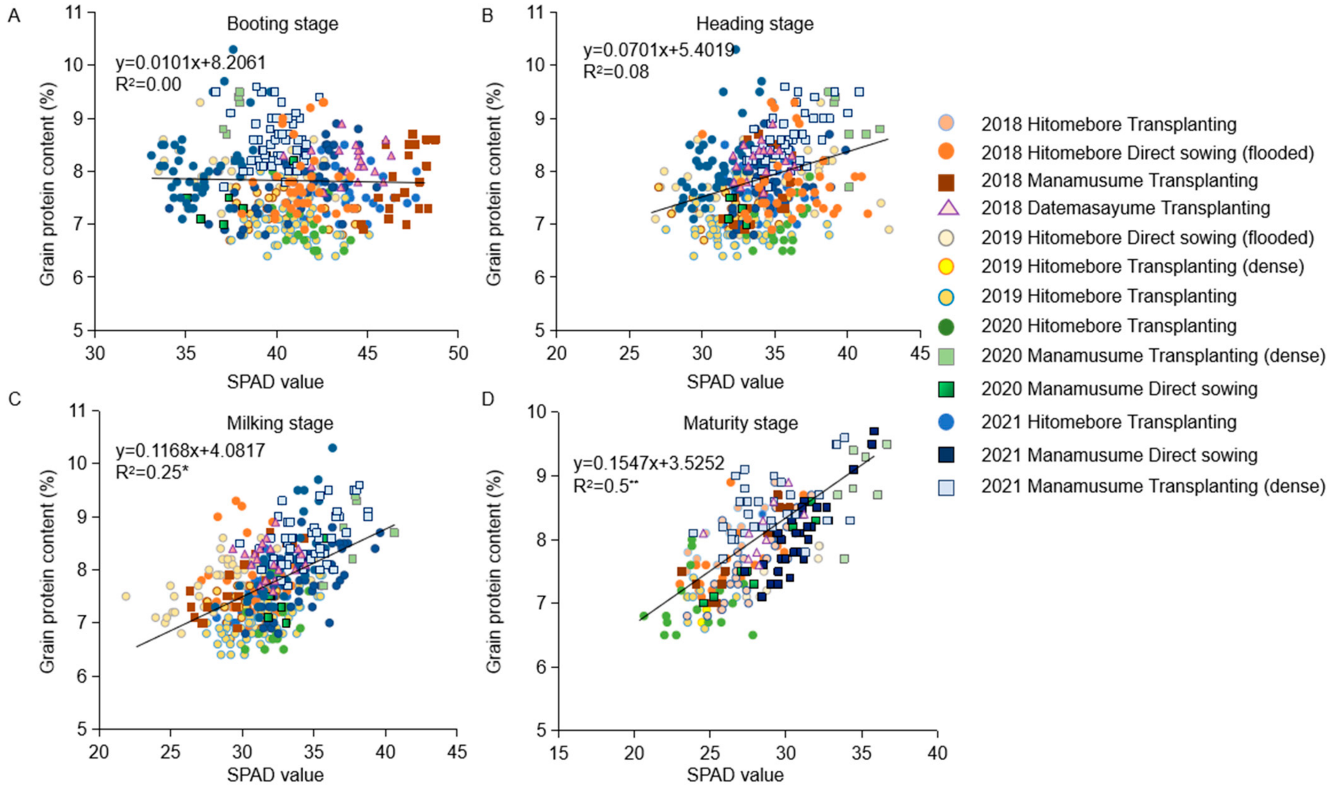

Figure 2. Relationships between grain protein content and SPAD value at different growth stages over four years. *, ** significant at 0.05, 0.01 probability level, respectively.

The effects of year, cultivar, and planting method on the regression were evaluated by Equation (4). The significant interactions were detected at the milking stage (Table 4), The regression coefficients significantly differed in 2019, for Datemasayume and for direct sowing. These different regression coefficients increased the coefficient of determination from 0.25 in Figure 2 to 0.66 in Table 4. However, the interactions were not significant at the maturity stage (Table 5). Only the planting method was significant in addition to the SPAD. The different regression coefficients increased the coefficient of determination from 0.50 in Figure 2 to 0.68 in Table 5.

Table 4. Regression coefficients and ANOVA results by Equation (2) for milking stage.

	Regression coefficients [a]				ANOVA results	
	Intercept		×SPAD			
Main effect	0.701		0.222	***	Intercept	ns
					SPAD	***
Year					Year	***
2018	0.000		0.000		Year × SPAD	***
2019	3.328	***	−0.125	***		
2020	−0.326		−0.017			
2021	1.767		−0.066			
Cultivar					Cultivar	**
Hitomebore	0.000		0.000		Cultivar × SPAD	**
Manamusume	0.118		0.012			
Datemasayume	8.243	**	−0.245	**		
Planting method					Planting method	**
Transplanting	0.000		0.000		Planting method × SPAD	**
Transplanting (dense)	1.068		−0.040			
Direct sowing (flooded)	1.564		−0.005			
Direct sowing	−3.661	*	0.113	*		
R^2	0.662	***				

[a] Coefficients can be obtained with sum of components. For example, protein content for direct sowed (flooded) Hitomebore in 2019 is expressed as (0.701 + 3.328 + 0.000 + 1.564) + (0.222 − 0.125 + 0.000 − 0.040) × SPAD. Probability of coefficient was obtained by t-test against 0.00 as null hypothesis. ns: non-significant at 0.05 probability level; *, **, *** significant at 0.05, 0.01, 0.001 probability level, respectively.

Table 5. Regression coefficients and ANOVA results by Equation (2) for maturity stage.

Regression Coefficients [a]					ANOVA Results	
	Intercept		x SPAD			
main effect	4.695	**	0.117	*	Intercept	**
					SPAD	*
Year					Year	ns
2018	0.000		0.000		Year × SPAD	ns
2019	−1.262		0.020			
2020	−0.774		0.016			
2021	−1.457		0.044			
Cultivar					Cultivar	ns
Hitomebore	0.000		0.000		Cultivar × SPAD	ns
Manamusume	−0.896		0.037			
Datemasayume	0.162		−0.001			
Planting method					Planting method	*
Transplanting	0.000		0.000		Planting method × SPAD	ns
Transplanting (dense)	2.368		−0.070			
Direct sowing (flooded)	−1.013		0.033			
Direct sowing	−1.311		0.032			
R^2	0.683	***				

[a] Coefficients can be obtained with sum of components. For example, protein content for direct sowed (flooded) Hitomebore in 2019 is expressed as (4.695 − 1.262 + 0.000 + 2.363) + (0.117 + 0.020 + 0.000 − 0.070) × SPAD. Probability of coefficient was obtained by t-test against 0.00 as null hypothesis. ns: non-significant at 0.05 probability level; *, **, *** significant at 0.05, 0.01, 0.001 probability level, respectively.

4. Discussion

Many previous studies have reported the relationship between leaf SPAD and grain protein content as mentioned in the introduction [28–30]. Based on the studies, many dissemination institutes recommend the management target of SPAD value. However, the studies were conducted under relatively uniform conditions, such as cultivar, fertilizer application, and planting methods. The applicability is unknown in actual farmer fields, where various conditions are mixed. Therefore, few farmers utilize SPAD meter to control rice grain quality. In this study, the relation between the leaf SPAD and the grain protein content of rice were validated in farmer fields which an agricultural producer cooperative managed in the coastal area of Sendai City, Miyagi Prefecture, Japan. The validation revealed that the regression coefficients converged at the ripening stage, but they significantly varied at the milking stage according to year, cultivar, and planting method.

The cultivar difference should be considered as the major factor. Wang et al. [39] and Ravier et al. [40] also insisted that cultivar differences should be considered when estimating rice grain protein content using SPAD. The regression coefficients of Datemasayuume were significantly higher for the intercept and significantly lower for the slope than those of Hitomebore at the milking stage (Table 4). Insufficient of data from Datemasayume may affected the statistical results. Otherwise, the low-amylose characteristic of Datemasayume [41] may have affected the relation. However, the difference was not significant at the maturity stage. Moreover, the difference between Hitomebore and Manamusume was not significant, even at the milking stage. These non-significances might be derived from the similarity in background of the genotypes. Hitomebore is the most popular cultivar in the prefecture. Manamusume was bred from the cross between Chiyonishiki and Hitomebore [42]. Datemasayume is one of the progenies of Hitomebore [41]. The major purposes of production are slightly different among cultivars: Hitomebore is produced for a general market with a relatively high price; Manamusume is produced for the restaurant industry or feed due to its higher productivity; and Datemasayume is produced for high-grade edible rice. Instead of the difference in production purpose, the characteristic of the relation between the SPAD and the grain protein content of Hitomebore may remain in Manamusume and Datemasayume. This fact recommends further investigations to evaluate the relation for other cultivars. However, the majority of present rice cultivars are progenies of Koshihikari in Japan and have quite similar genotypes [43]. Hitomebore is also a progeny of Koshihikari [44]. Accordingly, similar regression coefficients are expected for Japanese cultivars.

The effects of the planting method and the weather conditions on grain protein content were previously reported but without a focus on the relation between SPAD and grain protein content. Nakano et al. [25] reported the significant effect of N application and temperature on grain protein content. Hirai et al. [26] determined fertilizer application, meteorological environment, and growth conditions as the major factors affecting rice grain protein content and yield by analyzing farmer fields. The differences in grain protein content were explained in the SPAD, but the variation in the relation between these two components was unknown. The effect of the planting method on the regression coefficients, such as those shown in Tables 4 and 5, was not recognized. The difference in regression coefficients might partly be caused by the decreased rate of SPAD. For example in Hitomebore, the transplanting in 2018 was associated with the recording of the highest SPAD, 43.91, at the booting stage and decreased to 26.15 at the maturity stage, producing 7.7% of the grain protein content; while the direct sowing (flooded) in 2019 was associated with the recording of the lowest SPAD, 38.99, at the booting stage and decreased to 30.11 at the maturity stage, producing 7.8% of the grain protein content. Since the optimum SPAD varies, depending on the number of spikelets used to control the grain protein content [45], further analysis is necessary to quantify the effect of the decrease rate of the SPAD on the grain protein content based on the number of spikelets.

The validation in this study suggests that the prejudgment of the grain protein content does not have high accuracy and that regression coefficients are required for each year, cultivar, and planting method from the booting to the milking stage. The original concept of SPAD-based management is to control nitrogen status of rice to obtain higher yield and quality [25,26]. For this purpose, evaluation should be conducted before the heading stage, presumably the booting stage, to optimize nitrogen fertilizer application. However, the validation in this study suggests that simple and reliable guideline for famers seems difficult. Recently, higher air temperature which may be caused by global warming, has affected rice production in Japan [46]. The most significant effect is that the higher temperature during grain filling increased chalky grains [47,48]. The increase in chalky grain decreases palatability and downgrades rice products. Nitrogen fertilizer application is proposed as the counter measure because the increase in photosynthesis by the application increases carbohydrate accumulate in the grain which alleviates the occurrence of chalky grain [49,50]. However, the application often decreases the rice quality due to the increase in grain protein content. Accordingly, the guideline of counter measure generally includes agronomic traits such as numbers of spikelets and tillers in addition to SPAD value. These guidelines might also suggest that generalization of SPAD-based management for grain protein content requires the assessment of agronomic traits. For this purpose, the development of a rapid and easy assessment method is necessary.

Although the prejudgment of grain protein content before the milking stage has several problems, prejudgment at the maturity stage is high enough in accuracy with common regression coefficients. Even at the maturity stage, the prejudgment allows separate harvesting depending on the quality, resulting in higher profitability. Although further validations under various situations are required to provide reliability for farmers, this method is considered to be almost established. Recently, remote sensing technology has been widely used to evaluate crop growth in farmland [51–55]. Although LAI has been a major target of estimation by remote sensing for controlling rice yield [56,57], the estimation of SPAD has also been studied [58–60]. Although further improvement seems necessary to evaluate SPAD by remote sensing, the results of this study provide the possibility of utilizing remote sensing to evaluate the grain protein content just before harvesting.

Author Contributions: Conceptualization, K.H.; methodology, N.H. and K.H.; formal analysis, L.Z.; investigation, Y.S., K.O., T.I., R.I., M.M. and S.Y.; data curation, N.H. and L.Z.; writing—original draft preparation, L.Z.; writing—review and editing, K.H. and N.H.; funding acquisition, K.H. All authors have read and agreed to the published version of the manuscript.

Funding: This work was partly supported by JSPS KAKENHI, Japan, grant number 19H03069 and 22H02449 and by JICA-JST SATREPS, Japan, grant number JPMJSA1909.

Data Availability Statement: Not applicable.

Acknowledgments: The authors appreciate Sendai Arahama, the agricultural producer cooperative, for the permission and support in the study, and members of Crop Science Laboratory of the Graduate School of Agricultural Science, Tohoku University for their assistance in the measurement.

Conflicts of Interest: The authors declare no conflict of interest. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Maclean, J.; Hardy, B.; Hettel, G. *Rice Almanac: Source Book for One of the Most Important Economic Activities on Earth*; IRRI: Los Baños, Philippines, 2013.
2. Zhang, L.X.; Zhang, C.Q.; Yan, Y.; Hu, Z.J.; Wang, K.; Zhou, J.H.; Zhou, Y.; Cao, L.M.; Wu, S.J. Influence of starch fine structure and storage proteins on the eating quality of rice varieties with similar amylose contents. *J. Sci. Food Agric.* **2020**, *101*, 3811–3818. [CrossRef]
3. Martin, M.; Fitzgerald, M.A. Proteins in rice grains influence cooking properties. *J. Cereal Sci.* **2002**, *36*, 285–294. [CrossRef]
4. Lu, L.; Zhu, Z.W. Prediction model for eating property of Indica rice. *J. Food Qual.* **2014**, *37*, 274–280. [CrossRef]
5. Chen, H.; Chen, D.; He, L.H.; Wang, T.; Lu, H.; Yang, F.; Deng, F.; Chen, Y.; Tao, Y.F.; Li, M.; et al. Correlation of taste values with chemical compositions and Rapid Visco Analyser profiles of 36 indica rice (*Oryza sativa* L.) varieties. *Food Chem.* **2021**, *349*, 129176. [CrossRef]
6. Shi, S.J.; Wang, E.T.; Li, C.X.; Cai, M.L.; Cheng, B.; Cao, C.G.; Jiang, Y. Use of protein content, amylose content, and RVA parameters to evaluate the taste quality of rice. *Front. Nutr.* **2022**, *8*, 758547. [CrossRef]
7. Wang, G.Y.; Abe, T.; Sasahara, T. Concentrations of Kjeldahl-digested nitrogen, amylose, and amino acids in milled grains of rice (*Oryza sativa* L.) cultivated under organic and customary farming practices. *Jpn. J. Crop Sci.* **1998**, *67*, 307–311. [CrossRef]
8. Gaju, O.; Allard, V.; Martre, P.; Gouis, J.L.; Moreau, D.; Bogard, M. Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and grain nitrogen concentration in wheat cultivars. *Field Crops Res.* **2014**, *155*, 213–223. [CrossRef]
9. Wang, Z.J.; Wang, J.H.; Liu, L.Y.; Huang, W.J.; Zhao, C.J.; Wang, C.Z. Prediction of grain protein content in winter wheat (*Triticum aestivum* L.) using plant pigment ratio (PPR). *Field Crops Res.* **2004**, *90*, 311–321. [CrossRef]
10. Wang, H.; McCaig, T.; DePauw, R.; Clarke, J. Flag leaf physiological traits in two high-yielding Canada Western Red Spring wheat cultivars. *Can. J. Plant Sci.* **2008**, *88*, 35–42. [CrossRef]
11. Thomas, J.R.; Gausman, H.W. Leaf reflectance vs. Leaf Chlorophyll and Carotenoid Concentrations for Eight Crops1. *Agron. J.* **1997**, *69*, 799–802. [CrossRef]
12. Himelblau, E.; Amasino, R.M. Nutrients mobilized from leaves of Arabidopsis thaliana during leaf senescence. *J. Plant Physiol.* **2001**, *158*, 1317–1323. [CrossRef]
13. Uddling, J.; Gelang-Alfredsson, J.; Piikki, K.; Pleijel, H. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. *Photosynth. Res.* **2007**, *91*, 37–46. [CrossRef]
14. Song, X.; Gu, X.; Yang, G.; Chen, L.; Ma, Q.; Li, Z. Comparison of Leaf and Canopy Parameters for Estimating Wheat Nitrogen and Grain Protein Content. In Proceedings of the International Conference on Agro-Geoinformatics, Hangzhou, China, 6–9 August 2018; pp. 1–6.
15. Ling, Q.; Huang, W.; Jarvis, P. Use of a SPAD-502 meter to measure leaf chlorophyll concentration in Arabidopsis thaliana. *Photosynth. Res.* **2011**, *107*, 209–214. [CrossRef]
16. Palta, J.P. Leaf Chlorophyll Content. *Remote Sens. Rev.* **1990**, *5*, 207–213. [CrossRef]
17. Zhang, J.; Blackmer, A.M.; Ellsworth, J.W.; Koehler, K.J. Sensitivity of Chlorophyll Meters for Diagnosing Nitrogen Deficiencies of Corn in Production Agriculture. *Agron. J.* **2008**, *100*, 543–550. [CrossRef]
18. Eitel, J.U.H.; Long, D.S.; Gessler, P.E.; Hunt, E.R. Combined Spectral Index to Improve Ground-Based Estimates of Nitrogen Status in Dryland Wheat. *Agron. J.* **2008**, *100*, 1694–1702. [CrossRef]
19. Balasubramanian, A.C. Adaptation of the chlorophyll meter (SPAD) technology for real time N management in rice: A review. *Int. Rice Res. Notes* **2000**, *25*, 4–8.
20. Ghosh, M.; Swain, D.K.; Jha, M.K.; Tewari, V.K. Precision nitrogen management using chlorophyll meter for improving growth, productivity and N use efficiency of rice in subtropical climate. *J. Agric. Sci.* **2013**, *5*, 253–266. [CrossRef]
21. Yang, H.; Yang, J.; Lv, Y.; He, J. SPAD values and nitrogen nutrition index for the evaluation of rice nitrogen status. *Plant Prod. Sci.* **2014**, *17*, 81–92. [CrossRef]

22. Ata-Ul-Karim, S.T.; Cao, Q.; Zhu, Y. Non-destructive assessment of plant nitrogen parameters using leaf chlorophyll measurements in rice. *Front. Plant Sci.* **2016**, *7*, 1829. [CrossRef]
23. Giamerti, Y.; Hongo, C.; Saito, D.; Caasi, O.; Susilawati, P.N.; Shishido, M.; Sudiarta, I.P.; Wijaya, I.M.; Homma, K.K. Evaluating Multispectral Imaging for Assessing Bacterial Leaf Blight Damage in Indonesian Agricultural Insurance. In Proceedings of the International Conference on Agribusiness and Rural Development, IConARD, Yogyakarta, Indonesia, 13–14 October 2020; Volume 232, p. 03008.
24. Xue, L.H.; Cao, W.X.; Yang, L.Z. Predicting grain yield and protein content in winter wheat at different n supply levels using canopy reflectance spectra. *Pedosphere* **2007**, *17*, 646–653. [CrossRef]
25. Nakano, H.; Tanaka, R.; Guan, S.; Okami, M.; Wada, H.; Hakata, M.; Ohdan, H. Identification of growth-related indicators affecting the appearance and protein content of rice grains. *Agron. J.* **2022**, *114*, 565–581. [CrossRef]
26. Hirai, Y.; Keisuke, S.; Hamagami, K. Evaluation of an analytical method to identify determinants of rice yield components and protein content. *Comput. Electron. Agric.* **2012**, *83*, 77–84. [CrossRef]
27. Mori, S.; Yokoyama, K.; Fujii, H. Classification of brown rice with different protein content using the diagnosis of leaf color during the ripening period in Shonai area of Yamagata prefecture. *Jpn. J. Crop Sci.* **2010**, *79*, 113–119. [CrossRef]
28. Fukuyama, T.; Abe, N. Relationships between protein content of rice grain and leaf color in paddy field of Budokubo, Nagaoka city. *Bull. Fac. Agric. Niigata Univ.* **2011**, *63*, 55–62. (In Japanese, with English Summary)
29. Wakamatsu, K.; Sasaki, O.; Uezono, I.; Tanaka, A. Effect of the amount of nitrogen application on occurrence of white-back kernels during ripening of rice under high-temperature conditions. *Jpn. J. Crop Sci.* **2008**, *77*, 424–433. [CrossRef]
30. Wang, Z.L.; Cheng, Y.B.; Gong, R.; Zhou, S.C.; Wang, C.R.; Li, H.; Huang, D.Q.; Zhou, D.G.; Zhao, L.; Pan, Y.Y.; et al. Correlation Between SPAD Value of Flag Leaf and Rice Quality of High Quality indica Rice. *Chin. J. Rice Sci.* **2021**, *35*, 89–97. (In Chinese, with English Summary)
31. Matsue, Y. Studies on palatability of rice in northern Kyushu. VI. Effects of seedling characteristics under abnormal weather in 1993 on the palatability and physiochemical characteristics of rice. *Jpn. J. Crop Sci.* **1995**, *64*, 714–716. [CrossRef]
32. Wada, T.; Oosato, K.F.; Hamachi, Y. Effects of high air temperature and insufficient solar radiation during ripening period on the palatability and physicochemical properties of rice in 1999 in Kyushu regions. *Jpn. J. Crop Sci.* **2002**, *71*, 349–354. [CrossRef]
33. Chen, W.; Zhang, G.; Zhao, G.; Yao, H.; Xu, H. Variation in rice quality of different cultivars and grain positions as affected by water management. *Field Crops Res.* **2003**, *80*, 245–252. [CrossRef]
34. Ueda, K.; Kusutani, A.; Asanuma, K.; Ichii, M. Effect of transplanting timing on growth of rice cultivar kinuhikari in Kagawa Prefecture. *Jpn. J. Crop Sci.* **1998**, *67*, 289–296. [CrossRef]
35. Matsue, Y.; Mizuta, K.; Furuno, K.; Yoshida, T. Studies on palatability of rice in northern Kyushu. II. Effects of harvest time on palatability and physiochemical properties of milled rice. *Jpn. J. Crop Sci.* **1991**, *60*, 497–503. [CrossRef]
36. Saito, K.; Miyama, M.; Yamamoto, J.; Katsukita, H. Diagnosis of nitrogen nutrition of rice cv. koshihikari using chlorophyllmeter. *Bull. Chiba Agric. Exp. Stn.* **1992**, *33*, 27–35.
37. Crop Investigation Criteria Editing Committee. *Crop Investigation Criteria*; Kyushu Branch, Crop Science Society of Japan: Fukuoka, Japan, 2013. (In Japanese)
38. Causton, D.; Grafen, A.; Hails, R. *Modern Statistics for the Life Sciences*; Oxford University Press: London, UK, 2002.
39. Wang, G.Y.; Bronson, K.F.; Thorp, K.R. Multiple leaf measu rements improve effectiveness of chlorophyll meter for durum wheat nitrogen management. *Crop Sci.* **2014**, *54*, 817–826. [CrossRef]
40. Ravier, C.; Quemada, M.; Jeuffroy, M.H. Use of a chlorophyll meter to assess nitrogen nutrition index during the growth cycle in winter wheat. *Field Crops Res.* **2017**, *214*, 73–82. [CrossRef]
41. Endo, T.; Nagano, K.; Sasaki, K.; Chiba, B.; Wagatsuma, K.; Hayashi, H.; Saeki, K.; Sato, H.; Sakai, M.; Nakagomi, Y. New rice cultivar "Datemasayume". *Bull. Miyagi Pref. Furukawa Agric. Exp. Stn.* **2018**, *13*, 19–44.
42. Matsunaga, K.; Sasaki, T.; Nagano, K.; Okamoto, E.; Abe, S.; Uematsu, K.; Kano, A.; Takizawa, H.; Hayasaka, H.; Usuki, S.; et al. New rice cultivar "Manamusume". *Bull. Miyagi Pref. Furukawa Agric. Exp. Stn.* **2002**, *3*, 53–68.
43. Kobayashi, A.; Hori, K.; Yamamoto, T.; Yano, M. Koshihikari: A premium short-grain rice cultivar its expansion and breeding in Japan. *Rice* **2018**, *11*, 15. [CrossRef] [PubMed]
44. Sasaki, T.; Abe, S.; Matsunaga, K.; Okamoto, E.; Nagano, K.; Tanno, K.; Chiba, Y.; Kano, A.; Uematsu, K. A new rice cultivar "Hitomebore". *Bull. Miyagi Pref. Furukawa Agric. Exp. Stn.* **1993**, *2*, 1–17.
45. Ozaki, K.; Kobayashi, T.; Ohashi, Y.; Kawase, K. Establishment of predict method to regulate desirable spikelet numbers and protein contents by growth diagnosis production, and application to 'Koshihikari' cultivated in Tango area, Kyoto prefecture. *Bull. Agric. For. Tech. Dept. Kyoto Pref.* **2012**, *35*, 1–10.
46. Horie, T. Global warming and rice production in Asia: Modeling, impact prediction and adaptation. *Proc. Jpn. Acad. Ser. B* **2019**, *95*, 211–245. [CrossRef]
47. Shimoyanagi, R.; Abo, M.; Shiotsu, F. Higher temperatures during grain filling affect grain chalkiness and rice nutrient contents. *Agronomy* **2021**, *11*, 1360. [CrossRef]
48. Masutomi, Y.; Takimoto, T.; Shimamura, M.; Manabe, T.; Arakawa, M.; Shibota, N.; Ooto, A.; Azuma, S.; Imai, Y.; Tamura, M. Rice grain quality degradation and economic loss due to global warming in Japan. *Environ. Res. Commun.* **2019**, *19*, 121003. [CrossRef]
49. Tsukaguchi, T.; Iida, Y. Effects of assimilate supply and high temperature during grain-filling period on the occurrence of various types of chalky kernels in rice plants (Oryza sativa L.). *Plant Prod. Sci.* **2008**, *11*, 203–210. [CrossRef]

50. Yoshida, H.; Takehisa, K.; Kojima, T.; Ohno, H.; Sasaki, K.; Nakagawa, H. Modeling the effects of N application on growth, yield and plant properties associated with the occurrence of chalky grains of rice. *Plant Prod. Sci.* **2016**, *19*, 30–42. [CrossRef]
51. Zhang, J.; Chen, Y.; Zhang, Z. A remote sensing-based scheme to improve regional crop model calibration at sub-model component level. *Agric. Syst.* **2020**, *181*, 102814. [CrossRef]
52. Maki, M.; Sekiguchi, K.; Homma, K.; Hirooka, Y.; Oki, K. Estimation of rice yield by SIMRIW-RS, a model that integrates remote sensing data into a crop growth model. *J. Agric. Meteorol.* **2017**, *73*, 2–8. [CrossRef]
53. Ma, J.J.; Zheng, B.Y.; He, Y. Applications of a Hyperspectral Imaging System Used to Estimate Wheat Grain Protein: A Review. *Front. Plant Sci.* **2022**, *13*, 837200. [CrossRef] [PubMed]
54. Iwahashi, Y.; Sigit, G.; Utoyo, B.; Lubis, I.; Junaedi, A.; Trisasongko, B.H.; Wijaya, I.M.A.S.; Maki, M.; Hongo, C.; Homma, K. Drought damage assessment for crop insurance based on vegetation index by unmanned aerial vehicle (UAV) multispectral images of paddy fields in Indonesia. *Agriculture* **2023**, *13*, 113. [CrossRef]
55. Yamamoto, S.; Nomoto, S.; Hashimoto, N.; Maki, M.; Hongo, C.; Shiraiwa, T.; Homma, K. Monitoring spatial and time-series variations in red crown rot damage of soybean in farmer fields based on UAV remote sensing. *Plant Prod. Sci.* **2023**. [CrossRef]
56. Hirooka, Y.; Homma, K.; Shiraiwa, T. A leaf area-based non-destructive approach to predict productivity. *Agron. J.* **2021**, *113*, 3922–3934. [CrossRef]
57. Hashimoto, N.; Saito, Y.; Yamamoto, S.; Ishibashi, T.; Ito, R.; Maki, M.; Homma, K. Feasibility of yield estimation based on leaf area dynamicsmeasurements in rice paddy fields of farmers. *Field Crops Res.* **2022**, *286*, 108609. [CrossRef]
58. Botha, E.J.; Leblon, B.; Zebarth, B.J.; Watmough, J. Non-Destructive Estimation of Wheat Leaf Chlorophyll Content from Hyperspectral Measurements through Analytical Model Inversion. *Int. J. Remote Sens.* **2010**, *31*, 1679–1697. [CrossRef]
59. Zhang, R.; Yang, P.; Liu, S.; Wang, C.; Liu, J. Evaluation of the Methods for Estimating Leaf Chlorophyll Content with SPAD Chlorophyll Meters. *Remote Sens.* **2022**, *14*, 5144. [CrossRef]
60. Obara, K.; Homma, K.; Tajima, R.; Maki, M.; Saito, Y.; Hashimoto, N.; Yamamoto, S.; Hongo, C. Analysis of RGB images to estimate SPAD values in rice for UAV remote sensing. *Jpn. J. Crop Sci.* **2020**, *89*, 50–51. [CrossRef]