Plant Prod. Sci. 13(2): 132—140 (2010)

Effects of Temperature on the Digestible Protein Content of Grains during Ripening in a Seed-protein Mutant Rice Cultivar LGCsoft

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Abstract: The effects of temperature during the ripening period on digestible protein contents of the rice grains of a seed-protein mutant rice cultivar LGCsoft were examined. The plants were grown under a natural condition until the booting stage, and then in temperature-controlled greenhouses set at 24.0°C, 28.0°C, and 30.6°C (mean temperature). The protein compositions and the protein contents of the rice grains were analyzed quantitatively. The protein compositions in the LGCsoft grains varied with the temperature condition. The ratio of the digestible to total protein was higher in high-temperature conditions, and that of difficult-to-digest proteins, especially 13 kDa prolamin was lower in high-temperature conditions. The protein compositions in a normal-type cultivar Nihonmasari, which was the original cultivar of LGCsoft also varied with the temperature. However, the effect of temperature on the ratio of the digestible to total protein was larger in LGCsoft than in Nihonmasari. The ratios of the digestible protein in the grains under 24.0°C and 30.6°C conditions were 74.3% and 81.3%, respectively, in Nihonmasari. On the other hand, they were 52.0% and 63.1%, respectively, in LGCsoft. In LGCsoft, the total protein content of grains was 70.6-72.5 mg g^{-1}, and it was affected only slightly by temperature during the ripening period. Therefore, the digestible protein content of grains under 24.0°C and 30.6°C conditions was 36.7 mg g^{-1} and 45.7 mg g^{-1}, respectively, in LGC soft. It was clarified that the digestible protein content was higher at elevated temperatures because of the increased ratio of digestible to total protein.

Key words: Digestible protein, LGCsoft, *Oryza sativa* L., Protein composition, Protein content, Seed-protein mutant cultivar, Temperature.

In Japan, seed-protein mutant cultivars of rice (*Oryza sativa* L.) with low ratios of digestible proteins such as glutelin to total protein have been developed in recent years (Nishimura, 2002; Uehara et al., 2002; Iida et al., 2004; Nishimura et al., 2005). Such rice cultivars are anticipated for production of dietary foods for patients with kidney diseases (Mochizuki and Hara, 2000; Muromoto and Shigeta, 2007). Moreover, they have been examined for brewing Japanese sake (Takahashi and Sakurai, 2000; Iwano et al., 2002; Furukawa et al., 2006), and indeed, rice cultivars for sake-brewing have been developed (Uehara et al., 2002; Iida et al., 2008). In seed-protein mutant cultivars, it is necessary to control the digestible protein contents of rice grain because higher contents of digestible protein degrade the quality of dietary foods and Japanese sake production. Consequently, it is important to elucidate the influence of environmental conditions on the protein composition and the total protein content of grains. The findings will help establish suitable cultivation techniques for these cultivars.

Several investigations using temperature-controlled greenhouses have demonstrated that the total protein content of rice grains is increased by exposure to high temperatures during the ripening period (Honjo, 1971; Yanatori, 1975; Seo and Chamura, 1980; Maeshige, 1981; Tamaki et al., 1989). However, few reports have described the effects of temperature on the protein composition in rice grains. Katsube-Tanaka et al. (2005) reported that the ratio of 13 kDa prolamin to total protein in rice grains ripened at 35°C was lower than that of those ripened at 25°C, but the ratio of 60–70 kDa protein fraction was higher in the grains ripened in high-temperature conditions. Yamakawa et al. (2007) reported that the content of 13 kDa prolamin was markedly lower in the grain ripened in a high-temperature condition (33°C/28°C) during 5–20 d after flowering compared with the grains ripened in the control condition (25°C/20°C). The 13 kDa prolamin has been reported to be the main

Received 16 December 2008. Accepted 25 August 2009. Corresponding author: Y. Ohdaira (yodaira@affrc.go.jp, fax+81-84-924-7893).

component of protein body type I which is resistant to pepsin digestion (Ogawa et al., 1987). Therefore, the lower ratio of 13 kDa prolamin in rice grains under high-temperature conditions mean higher ratio of digestible protein. The digestible protein contents of rice grains are higher under high-temperature conditions in normal cultivars. Perhaps a similar phenomenon might be observed in seed-protein mutant cultivars. However, there are no reports on the response of the total protein content and protein composition to temperature during the ripening period. Recently, in Japan, the decrease in the rice grain weight and appearance quality caused by the high temperature during the ripening period has become a serious problem (Morita, 2008). In consideration of ongoing global warming (IPCC, 2007), the influence of temperature during the ripening period on the total protein contents and protein composition of rice grains in seed-protein mutant cultivars should be elucidated in order to control the digestible protein content more strictly. Moreover, quantitative analyses are needed because, in seed-protein mutant cultivars, the digestible protein content of grain must be suppressed for use in dietary foods and for brewing Japanese sake. Only a few reports have presented qualitative data related to the electrophoretic profile of proteins (Katsube-Tanaka et al., 2005; Yamakawa et al., 2007).

This study was done to elucidate the effect of temperature during the ripening period on the digestible protein content of rice grains of a seed-protein mutant cultivar LGCsoft (Iida et al., 2004). Rice plants were grown under natural conditions until the booting stage, and then under different temperature conditions in temperature-controlled greenhouses. The protein composition and the total protein contents of grains were analyzed quantitatively. In previous reports, the accumulation patterns of nitrogen in grains during the ripening period differed with the position of the grains on a panicle (Arai and Kono, 1979) and the influence of environmental conditions on the total protein contents of grains varied with the position on a panicle (Matsue and Ogata, 1999). To elucidate the temperature effects in detail, we investigated the total protein contents and protein composition of grains on the primary and secondary rachis branches.

Materials and Methods

A seed-protein mutant LGCsoft and a normal-type cultivar Nihonmasari were used. The LGCsoft was bred from the progeny of a cross between a low-glutelin line, NM67 (later registered as LGC1), and a low-amylose line, NM391 (Iida et al., 2004). Both NM67 and NM391 were bred from Nihonmasari by mutation. LGCsoft has a low glutelin gene *Lgc1* (Iida et al., 1993a, 1993b).

Pre-germinated rice seeds were sown on 31 May 2005 in nursery boxes (Minoru pot 448, Minoru Industrial Co. Ltd., Japan), with 448 holes (16 mm in diameter; 25 mm in depth). Three seeds were sown per hole, and all holes had been filled with nursery soil for rice seedlings (Pete-baido, Minoru Industrial Co. Ltd., Japan). The seedlings were grown for 21 d, and then transplanted circularly into plastic pots of 1/2000 a. The pots contained paddy soil (gray lowland soil) for eight hills per pot. Compound fertilizer was incorporated into the soil at 1.88 g per pot ($N:P_2O_5:K_2O$=16:16:16%) as basal dressing. The soil in the pots was puddled for a few days before transplanting. As top-dressing, 1.43 g of ammonium sulfate (21%N) was applied at 25 d after transplanting. The same compound fertilizer was applied at 1.40 g per pot at 35 d after transplanting. Rice plants were grown until the booting stage in the open at the National Agricultural Research Center for Western Region in Fukuyama, Japan (34° 30′N, 133° 23′E). Each plant was restricted to the main culm through occasional removal of tillers. The pots were transferred to temperature-controlled natural light greenhouses, in which the temperatures were set at 25°C/23°C (mean temp. 24.0°C: L treatment), 30°C/26°C (mean temp. 28.0°C: M treatment), and 33°C/29°C (mean temp. 30.6°C: H treatment) with a day/night cycle (0630–1730/1830–0530, time for changing the temperature; 1730–1830 and 0530–0630) at the booting stage. Three pots per temperature treatment were used for each cultivar. Amounts of solar radiation during the ripening period were 15.4±5.3, 15.3±5.8, 15.6±5.9 MJ m^{-2} d^{-1} (mean value±SD) in L, M and H treatments, respectively. Rice plants in the pots were harvested when the cumulative temperature after the heading stage exceeded 1000°C d. Then they were air dried, and the panicles were hand-threshed. The spikelets were separated from the primary branch and the secondary branch. After counting the spikelets, the hulled grains were screened through 1.8 mm sieves. The weights of ripened grains and the 1000-grain weights were adjusted to 15% water content. The percentage of ripened grains was calculated as the ratio of the number of ripened grains to the number of spikelets.

Protein composition was analyzed and protein content measured as follows. About 6 g of the ripened grain from each experimental plot was ground into flour using a grinder (Cyclone Sample Mill, UDY Corp., USA). The seed protein was extracted from 20 mg of the flour using 700 μL of SDS-urea solution (4% SDS, 8M urea, 5% mercaptoethanol, 125 mM Tris-HCl (pH6.8), 20% glycerin). The stirred mixture of rice flour and SDS-urea solution in a tube with a 2 mL volume was laid in an incubator at 35°C for a day. Then, the tube was set upright in an incubator at 35°C for a day. Proteins in the supernatant were separated using polyacrylamide gel electrophoresis (SDS-PAGE). After electrophoresis, gels were stained with 0.08% Coomassie Brilliant Blue R-250 solution. SDS-PAGE was done according to the procedure

Table 1. Yield components and grain yield of normal rice cultivar Nihonmasari and seed-protein mutant rice cultivar LGCsoft.

Position of rice grain in panicle	Cultivar	Temperature treatment	Number of grains per panicle	1000-grain weight (g)	Percentage of ripened grains (%)	Ripened grain weight (g)
Primary branches (P)	Nihonmasari	L	45.8±0.5	25.1±0.6	89.0±1.8	1.02±0.04
		M	45.4±0.8	24.5±0.4	91.6±1.5	1.02±0.02
		H	46.2±1.5	24.5±0.4	92.0±0.8	1.04±0.04
	LGCsoft	L	43.6±1.1	22.7±0.2	79.0±4.7	0.78±0.07
		M	44.5±0.7	22.2±0.3	82.5±1.7	0.81±0.03
		H	43.9±0.6	21.9±0.3	76.9±2.7	0.74±0.04
	ANOVA	Temperature (A)	NS	*	NS	NS
		Cultivar (B)	NS	**	*	**
		A×B	NS	NS	NS	NS
Secondary branches (S)	Nihonmasari	L	21.9±1.2	21.3±0.5	85.7±3.3	0.40±0.01
		M	22.1±1.3	20.8±0.2	88.7±3.4	0.41±0.04
		H	22.7±2.7	20.7±0.4	83.6±0.8	0.39±0.05
	LGCsoft	L	26.9±1.5	19.0±0.1	80.9±3.6	0.41±0.02
		M	26.3±2.7	18.6±0.2	76.7±1.0	0.37±0.04
		H	26.3±1.0	18.3±0.2	64.2±3.1	0.31±0.03
	ANOVA	Temperature (A)	NS	*	*	NS
		Cultivar (B)	*	***	***	NS
		A×B	NS	NS	*	NS
	ANOVA	Temperature (A)	NS	*	*	NS
		Position (C)	***	***	**	***
		A×C	NS	NS	*	NS
P+S	Nihonmasari	L	67.7±1.2	23.9±0.6	88.0±1.7	1.42±0.05
		M	67.5±1.2	23.3±0.2	90.7±2.0	1.43±0.05
		H	68.9±1.2	23.3±0.4	89.5±0.4	1.44±0.03
	LGCsoft	L	70.4±0.8	21.3±0.2	79.6±4.2	1.19±0.08
		M	70.7±3.3	20.8±0.2	80.6±1.5	1.19±0.06
		H	70.2±1.2	20.5±0.3	72.7±2.5	1.05±0.06
	ANOVA	Temperature (A)	NS	*	NS	NS
		Cultivar (B)	**	***	**	**
		A×B	NS	NS	NS	NS

Values represent the mean values±standard deviation. Grain weight was adjusted to 15% water content. *, **, and ***, represent statistical significance at $P<0.05$, 0.01, and 0.001, respectively. NS, not significant at $P>0.05$.

described by Nishio (1996). Eight protein fractions—>57 kDa protein, 57 kDa protein, 37–39 kDa glutelin α, 26 kDa globulin, 22–23 kDa glutelin β, 16 kDa prolamin, 13 kDa prolamin, and 10 kDa prolamin—were quantitated using a densitometer (Phoretix 1D Advanced, Cosmo Bio Co. Ltd., Japan). The ratio of each protein fraction was calculated as the sum of these values being 100%. For this study, prolamin fractions were regarded as difficult-to-digest protein, and other fractions as digestible protein according to previous reports (Uehara et al., 2002; Iida et al., 2004; Nishimura et al., 2005; Ohdaira et al., 2009). The nitrogen content of the rice flour that had been dried for three or more days at 80ºC was measured by the Dumas combustion method (rapidN III, Elementar Analysensysteme GmbH, Germany). The total protein content was calculated by multiplying the nitrogen content by 5.95, which was the constant coefficient to convert nitrogen content to total protein contents of rice. The digestible protein contents were calculated by multiplying the total protein contents by the ratio of digestible proteins to total protein.

Effects of temperature, cultivar, position of grain within a panicle (primary or secondary branches) and their interactions on the yield components, grain yield, protein composition and contents of total and digestible protein in grains were analyzed by analysis of variance (ANOVA) as a

Table 2. Protein composition in ripened grain in normal rice cultivar Nihonmasari and seed-protein mutant rice cultivar LGCsoft.

Position of rice grain in panicle	Cultivar	Temperature treatment	Ratio of digestible protein (%)						Ratio of difficult-to-digest protein (%)			
			>57 kDa protein	57 kDa protein	37-39 kDa glutelin α	26 kDa globulin	22-23 kDa gultelin β	sum	16 kDa prolamin	13 kDa prolamin	10 kDa prolamin	sum
Primary branches (P)	Nihonmasari	L	10.8±0.8	7.9±0.1	28.0±0.4	6.0±0.2	20.8±0.4	73.5±0.5	7.1±0.6	17.5±0.9	1.9±0.4	26.5±0.5
		M	12.0±0.8	9.1±0.4	29.7±0.3	5.4±0.4	21.6±0.7	77.9±1.1	7.0±0.4	13.5±0.5	1.7±0.3	22.1±1.1
		H	11.8±0.2	9.9±0.5	31.4±0.5	4.4±0.1	22.9±0.1	80.3±0.7	6.3±0.2	11.3±0.6	2.1±0.1	19.7±0.7
	LGCsoft	L	11.6±0.2	6.8±0.2	12.5±0.3	9.9±0.2	8.7±0.3	49.6±0.3	8.8±0.5	39.2±0.9	2.4±0.3	50.4±0.3
		M	13.3±0.8	8.9±0.5	14.2±0.1	10.3±0.2	8.7±0.1	55.4±1.1	8.2±0.5	34.1±0.8	2.2±0.2	44.6±1.1
		H	14.4±0.4	10.4±0.2	16.3±0.7	10.0±0.2	9.3±0.4	60.5±1.4	7.9±0.4	29.1±1.3	2.5±0.3	39.5±1.4
	ANOVA	Temperature (A)	**	**	***	*	*	***	*	***	NS	***
		Cultivar (B)	***	NS	***	***	***	***	**	***	NS	***
		A×B	NS	*	NS	*	NS	NS	NS	NS	NS	NS
Secondary branches (S)	Nihonmasari	L	11.3±0.7	9.9±0.4	28.6±0.3	5.4±0.2	21.0±0.5	76.3±0.1	6.2±0.2	15.6±0.6	1.9±0.3	23.7±0.1
		M	12.7±0.8	11.7±0.7	30.5±0.3	4.6±0.2	21.4±0.3	80.9±0.9	5.6±0.3	11.5±0.5	1.9±0.4	19.1±0.9
		H	12.7±0.5	12.3±0.2	32.2±0.4	4.0±0.1	22.6±0.3	83.8±0.5	5.2±0.3	9.1±0.4	1.9±0.1	16.2±0.5
	LGCsoft	L	14.9±0.3	8.9±0.3	13.9±0.1	9.7±0.3	9.0±0.7	56.4±1.7	7.3±0.5	34.7±1.1	1.6±0.3	43.6±1.7
		M	15.4±0.6	11.1±0.4	14.6±0.1	9.9±0.3	8.7±0.2	59.6±0.9	7.4±0.2	30.8±0.8	2.1±0.1	40.4±0.9
		H	18.4±1.9	12.8±0.6	18.1±0.5	10.2±0.6	9.7±0.1	69.2±2.0	6.3±0.3	22.9±1.5	1.6±0.2	30.8±2.0
	ANOVA	Temperature (A)	*	***	**	NS	*	***	*	***	NS	***
		Cultivar (B)	*	NS	***	***	***	***	**	***	NS	***
		A×B	NS	NS	*	*	NS	*	*	*	NS	*
	ANOVA	Temperature (A)	**	***	***	*	*	***	*	***	NS	***
		Position (C)	***	**	**	*	NS	***	*	***	NS	***
		A×C	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P+S	Nihonmasari	L	10.9±0.7	8.5±0.1	28.2±0.2	5.8±0.2	20.9±0.4	74.3±0.4	6.8±0.4	16.9±0.8	1.9±0.2	25.7±0.4
		M	12.2±0.3	9.9±0.2	29.9±0.2	5.2±0.2	21.6±0.5	78.8±0.5	6.5±0.2	12.9±0.3	1.8±0.1	21.2±0.5
		H	12.1±0.2	10.5±0.3	31.6±0.4	4.3±0.1	22.8±0.1	81.3±0.5	6.0±0.1	10.7±0.5	2.0±0.1	18.7±0.5
	LGCsoft	L	12.8±0.0	7.5±0.2	13.0±0.6	9.9±0.1	8.8±0.4	52.0±0.8	8.3±0.5	37.6±0.6	2.1±0.3	48.0±0.8
		M	14.0±0.7	9.6±0.5	14.3±0.1	10.1±0.2	8.7±0.1	56.8±1.1	8.0±0.4	33.1±0.8	2.2±0.1	43.2±1.1
		H	15.6±0.7	11.1±0.3	16.8±0.6	10.1±0.1	9.4±0.3	63.1±1.4	7.4±0.2	27.3±1.2	2.2±0.3	36.9±1.4
	ANOVA	Temperature (A)	**	***	***	*	*	***	*	***	NS	***
		Cultivar (B)	**	NS	***	***	***	***	**	***	NS	***
		A×B	NS	*	NS	*	NS	*	NS	*	NS	*

Values represent the mean values±standard deviation. *, **, and ***, represent statistical significance at $P<0.05$, 0.01, and 0.001, respectively. NS, not significant at $P>0.05$.

split-block design. For the data of protein composition, ANOVA was conducted on transformed protein ratio by arcsine transformation. The significance of mean values of the total and the digestible protein contents among cultivars and positions of grains was analyzed using the least significant differences (LSD) test ($P<0.05$).

Results

1. Yield and yield components

The 1000-grain weight was lighter at higher temperatures in both LGCsoft and Nihonmasari and on both primary and secondary branches (Table 1). The difference in the 1000-grain weight between the L and H treatments was 0.7–0.8 g in LGCsoft, and 0.6 g in Nihonmasari. The 1000-grain weight on the primary branch was 3.6–3.8 g higher than that on the secondary branch in both cultivars. The 1000-grain weight of Nihonmasari was 2.2–2.6 g heavier than that of LGCsoft on both the primary and secondary branches.

Although there were no significant differences ($P>0.05$) among the three temperature conditions in the percentage of ripened grains including those on the

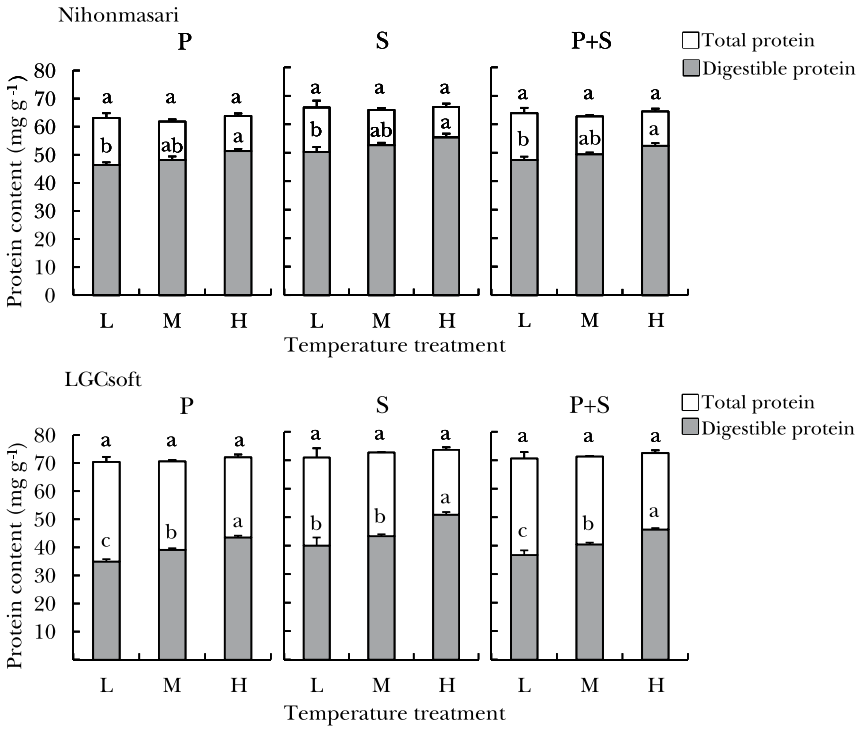

Fig. 1. Effects of temperature during the ripening period on the contents of total protein and digestible protein in grain of normal rice cultivar Nihonmasari and seed-protein mutant rice cultivar LGCsoft.
P, Primary branches; S, Secondary branches; L, mean temp. 24.0°C, M; mean temp. 28.0°C, H; mean temp. 30.6°C. Vertical bars represent standard deviations of three replicates. Means followed by the same letter in each protein fraction do not differ significantly (P>0.05, LSD).

primary and secondary branches, the percentage of ripened grains in the H treatment was 13–17% lower than that in the L and M treatments on the secondary branches in LGCsoft (Table 1). In both cultivars, the percentage of ripened grains on the secondary branches was lower than that on the primary branch's grain, and the tendency was remarkable in higher temperature conditions. The percentage of ripened grains in LGCsoft was lower than that in Nihonmasari. The varietal difference in the percentage of ripened grains was remarkable in H treatment.

There were no significant differences (P>0.05) among temperature treatments in the ripened grain weight (Table 1). Weights of the ripened grains per panicle were 1.42–1.44 g in Nihonmasari and 1.05–1.19 g for LGCsoft. The varietal difference in ripened grain weight was larger in the H treatment.

2. Effect of temperature on protein composition in rice grain

Ratios of >57 kDa protein, 57 kDa protein, 37–39 kDa glutelin α and 22–23 kDa glutelin β to the total protein in the grains on both the primary and secondary branches were higher under elevated temperature conditions in LGCsoft and Nihonmasari (Table 2). As a result, the ratio of digestible protein—the sum of the ratios of five digestible protein fractions to total protein—was higher at elevated temperatures in both cultivars. In all grains on the primary and secondary branches, the ratio of digestible to total protein in the L and H treatments was 52.0% and 63.1%, respectively, in LGCsoft. In Nihonmasari, the ratio in the L and H treatments was 74.3% and 81.3%, respectively. Although the ratio of digestible to total protein in grains was higher under elevated temperature conditions in both cultivars, the ratios were influenced by temperature more greatly in LGCsoft than in Nihonmasari. The difference in the ratio of digestible to total protein between H and L treatments was 11.1% in LGCsoft and 7.0% in Nihonmasari. There was a significant interaction (P<0.05) between cultivar and temperature for the ratio of digestible to total protein.

The ratios to total protein of 16 kDa prolamin and 13 kDa prolamin, which were components of difficult-to-digest protein, were lower at elevated temperatures (Table 2). The change of the ratio with the temperature was remarkable for 13 kDa prolamin. In all grains on the primary and secondary branches, the differences in the ratio of 13 kDa prolamin between the H\and L treatments

Table 3. Analysis of variance (ANOVA) of the effects of temperature on the contents of total protein and digestible protein in ripened grain in normal rice cultivar Nihonmasari and seed-protein mutant rice cultivar LGCsoft.

Position of rice grain in panicle	Source of variation	Total protein content	Digestible protein content
Primary branches (P)	Temperature (A)	NS	***
	Cultivar (B)	**	***
	A×B	NS	*
Secondary branches (S)	Temperature (A)	NS	***
	Cultivar (B)	**	**
	A×B	NS	*
	Temperature (A)	NS	**
	Position (C)	**	**
	A×C	NS	NS
P+S	Temperature (A)	NS	***
	Cultivar (B)	**	**
	A×B	NS	*

*, **, and ***, represent statistical significance at $P<0.05$, 0.01, and 0.001, respectively. NS, not significant at $P>0.05$.

were 10.3% in LGCsoft, and 6.2% in Nihonmasari. There was a significant interaction ($P<0.05$) between cultivar and temperature for the ratio of 13 kDa prolamin.

The protein compositions in the grains on the secondary branches were different from those on the primary branches (Table 2). The ratios of >57 kDa protein, 57 kDa protein and 37-39 kDa glutelin α fractions in the grains on the secondary branches were higher than those on the primary branches in both cultivars. The ratios of 26 kDa globulin and 22–23 kDa glutelin β in the grains on the secondary branches differed only slightly from those on the primary branches. Thus, the ratios of digestible protein were higher in the grains on the secondary branches than in those on the primary branches. In contrast, the ratios of 16 kDa prolamin and 13 kDa prolamin in the grains on the secondary branches were lower than those on the primary branches in both cultivars. The patterns of change in the protein compositions with the temperature during the ripening period were similar for the secondary and the primary branches. There was no significant interaction ($P>0.05$) between grain position and temperature for the ratios of all protein fractions.

3. Effect of temperature on the contents of total protein and digestible protein in rice grain

Total protein content of grains including those on the primary and secondary branches was 70.6–72.5 mg g^{-1} in LGCsoft, and 62.8–64.5 mg g^{-1} in Nihonmasari (Fig. 1). Although apparent varietal differences ($P<0.01$) existed in the total protein contents, the effect of temperature on the total protein content was not significant ($P>0.05$) (Fig. 1, Table 3). The effect of temperature was not significant ($P>0.05$) in the grains on the primary or secondary branches either. In both cultivars, the total protein content of the grains on the secondary branches was higher than that of those on the primary branches.

In all grains on the primary and secondary branches, digestible protein content was 36.7–45.7 mg g^{-1} in LGCsoft, and 47.5–52.4 mg g^{-1} in Nihonmasari (Fig. 1). The digestible protein contents were higher under the higher temperature condition in both cultivars. Differences in the digestible protein content between L and H treatments were 9.0 mg g^{-1} in LGCsoft and 4.9 mg g^{-1} in Nihonmasari. There was a significant interaction ($P<0.05$) between cultivar and temperature for the digestible protein content (Table 3). The increase in the digestible protein content under the high temperature condition was greater in LGCsoft than in Nihonmasari.

Discussion

The ratio of the digestible to total protein in rice grains of a seed-protein mutant cultivar LGCsoft was increased by exposing the plants to higher temperatures during the ripening period (Table 2). In contrast, the ratio of difficult-to-digest protein, such as 13 kDa prolamin, was decreased under the higher temperature conditions. A similar tendency was observed in a normal-type cultivar Nihonmasari. Reportedly, the ratio of 13 kDa prolamin to total protein in rice grains that had been ripened at 35°C was smaller than that of those ripened at 25°C in the experiment using a culture system of detached ears of a normal-type cultivar Yukara (Katsube-Tanaka et al., 2005). Moreover, the 13 kDa prolamin content of rice grains ripened at 33/28°C was markedly lower than that of rice

grains ripened at 25/20°C in normal-type cultivars (Yamakawa et al., 2007). Our results were in agreement with these two investigations. According to Yamakawa et al. (2007), the gene expression level of 13 kDa prolamin was suppressed extremely under high-temperature conditions during the ripening period, although high temperatures suppressed expression levels of various genes associated with seed-protein development. Therefore, the decreased biosynthesis of 13 kDa prolamin under the high-temperature condition in LGCsoft and Nihonmasari was considered to have resulted from suppression of gene expression in 13 kDa prolamin.

In this experiment, the ratio of digestible to total protein was higher in the rice grown at elevated temperatures. However, the increase varied with the fraction of digestible protein (Table 2). Although further study is necessary to elucidate the difference in the temperature dependence among the digestible protein fractions, the gene expression level of each protein fraction might be related to the temperature dependence, because the gene expression level under the high temperature condition varied with the fraction (Yamakawa et al., 2007).

The content of free amino acids in caryopsis is extremely low throughout the ripening period, and nitrogen translocated to caryopsis is considered to be used immediately for synthesis and accumulation of seed proteins (Juliano, 1985). In normal-type rice cultivars, the glutelin content of caryopsis increases rapidly and the globulin content increases gradually from 5 d after flowering (Tanaka et al., 1995). In contrast, the prolamin content increases only slightly until 5–10 d after flowering, and then increases reaching a maximum level at 30 d after flowering, similarly to glutelin. Such a time course of accumulation of seed proteins during the ripening period was confirmed not only in normal-type cultivars but also in a seed-protein mutant cultivar, LGC-1, which is a parent of LGCsoft (S. Iida, personal communication). Therefore, if biosynthesis of 13 kDa prolamin is suppressed by the reduction of 13 kDa prolamin gene expression under high temperature conditions, then perhaps nitrogen translocated to developing grain may be used for biosynthesis of digestible protein such as glutelin and 57 kDa protein instead of 13 kDa prolamin. Besides, it is supposed that a high temperature promotes the maturation of grains, reducing the accumulation of 13 kDa prolamin. Further study of the relation between the grain maturation and the protein accumulation in seed-protein mutant cultivars is necessary.

In the present study, the difference in the ratio of digestible to total protein between H and L treatments was larger in LGCsoft than in Nihonmasari (Table 2). It was assumed that the high temperature suppressed 13 kDa prolamin biosynthesis more strongly in LGCsoft than in Nihonmasari because the ratio of 13 kDa prolamin in LGCsoft was at least two times higher than that in Nihonmasari. The result suggests that for the seed-protein mutant cultivars, much attention must be paid to the temperature during the ripening period.

The ratios of >57 kDa protein, 57 kDa protein and 37–39 kDa glutelin α were higher in the grains on the secondary branches than in those on the primary branches in both LGCsoft and Nihonmasari (Table 2). In contrast, the ratio of 13 kDa prolamin was lower in the grains on the secondary branches. These results might be attributable to (1) the differences in the accumulation pattern in grains and (2) the differences in the filling period between the grains on the primary and the secondary branches. Tanaka et al. (1995) reported that the prolamin accumulation in a grain started later than the glutelin accumulation and increased gradually. Arai and Kono (1979) reported that the nitrogen accumulation in a superior spikelet preceded that of an inferior spikelet, and that the accumulation velocity of nitrogen in a grain was lower in the inferior spikelets than in the superior spikelets throughout the ripening period. Based on these reports, it is inferred that the grains on the secondary branches matured before completion of protein synthesis, especially 13 kDa prolamin synthesis, resulting in a much lower ratio of 13 kDa prolamin on the secondary branches than on the primary branches. Furthermore, the higher ratio of 57 kDa protein in the grains on the secondary branches suggests that the grains on the secondary branches matured before completion of protein synthesis, because the 57 kDa protein is considered to be a precursor of glutelin (Yamagata et al., 1982; Sarker et al., 1986).

Although the protein composition of grains on the secondary branches differed from those on the primary branches, the pattern of change with the temperature during the ripening period resembled that of the grains on the primary branches (Table 2). Additionally, no significant interactions ($P>0.05$) between grain position and temperature found in the ratios of all protein fractions. These results indicate that little difference exists in the effects of temperature on the protein synthesis in grains according to the grain position within a panicle.

Several investigations have shown that the total protein content of rice grains was increased by the exposure to a high temperature during the ripening period (Honjo, 1971; Yanatori, 1975; Seo and Chamura, 1980; Maeshige, 1981; Tamaki et al., 1989). Researchers attributed these findings to the insufficient accumulation of starch and the lighter 1000-grain weight (Yanatori, 1975; Seo and Chamura, 1980; Maeshige, 1981). In this study, the 1000-grain weight was indeed lower under the higher-temperature conditions on both the primary and secondary branches (Table 1). However, the total protein contents of grains was not influenced significantly ($P>0.05$) by temperature during the ripening period in

both LGCsoft and Nihonmasari (Fig. 1). Yanatori (1975) reported that the total protein content of some cultivars was rarely influenced by temperature. Matsue et al. (2003) also found no significant relation ($P>0.05$) between the total protein contents of rice grains and the temperature during the ripening period in some cultivars cultivated under different years and transplanted at different times. Concerning Matsue's report, Morita (2008) suggested that the total protein content might vary with the degree of grain weight decrease under high-temperature conditions. Therefore, further study on the mechanism of high-temperature resistance is needed to clarify the varietal difference in the change of total protein content of rice grains.

Generally, the total protein content of grains is influenced by the percentage of ripened grains. In our study, no significant relation ($P>0.05$) was found between the percentage of ripened grains and temperature during the ripening period (Table 1). However, the percentage of ripened grains tended to be lower and the total protein content higher in the H treatment than in the L treatment on the secondary branches of LGCsoft (Table 1, Fig. 1). These results indicate that LGCsoft should not be cultivated under extremely high temperature conditions during the ripening period.

In conclusion, cultivation techniques that avoid extremely high temperatures during the ripening period are needed in order to stabilize the digestible protein content of rice grain at a low level in the seed-protein mutant cultivar LGCsoft. Selection of cultivation site is important. In Japan, a high altitude and/or high latitude may be suitable for cultivating LGCsoft, although the growth period must be considered. Another way to avoid high temperatures during the ripening period is to delay the transplanting time. However, further study is needed to clarify whether this method can stabilize the digestible protein content of rice grain at a low level. Numerous reports show that the total protein content of rice grain increased by delayed transplanting, even when temperatures during the ripening period are low (Matsue et al., 1991; Otomo et al., 1992; Konno et al., 1994; Kawaguchi et al., 1995; Koide et al., 1995; Ueda et al., 1998; Usuzaka and Kimura, 2004). We cannot deny the possibility that the digestible protein content of rice grain is increased by increasing the total protein content by delayed transplanting even though the ratio of digestible to total protein was low under the low-temperature condition. The influence of the cropping season on the digestible protein contents of rice grains in seed-protein mutant cultivars will be reported in another paper.

Acknowledgements

We are grateful to Dr. Shuichi Iida, National Agricultural Research Center for Western Region, and Dr. Takehiro Masumura, Kyoto Prefecture University, for critically reviewing the manuscript. We also thank Ms. Kazuko Matsuura, National Agricultural Research Center for Western Region, for analyzing the protein composition. This study was supported by a grant for "Integrated Research for Providing Fresh and Delicious 'Brand Nippon' Agricultural-Products V Rice" from the Ministry of Agriculture, Forestry and Fisheries of Japan.

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* In Japanese.
** In Japanese with English abstract.
*** In Japanese with English summary.
****Translated from Japanese except book title by the present authors.