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Varietal Difference of Polishing Characteristics and Suitability for Sake Brewing in "Hattan-Type Varieties" of Rice Suitable for Brewing Original Hiroshima Sake

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Abstract : By successive crossing using Hattan-type varieties originating from "Hattanso" as a parent, "Hattan-type varieties" of rice suitable for brewing the original Hiroshima sake have been bred. The varieties were improved inheriting the flavor of Hattan-type sake, and Hattan-nishiki No.1 and No.2 were bred in 1984. However, neither variety was suitable for brewing high-grade sake such as Ginjoshu and Daiginjoshu, which require high-degree polishing of rice grains. Therefore, their parent cultivar, Hattan No. 35, is attracting attention for the production of high-grade sake. We have been trying to breed a new variety, which retains the sake-brewing suitability of Hattan-type varieties but can endure high-degree polishing. In this study, to establish a guideline for further breeding, we examined the polishing characteristics and suitability for sake brewing of six Hattan-type varieties derived from Hattanso. In the process of breeding of "Hattan-type varieties" of rice, grain size, white-core size and % of white-core grains increased resulting in an increased suitability for sake brewing, such as water absorptivity and digestibility, in Hattan No.35, Hattan-nishiki No.1 and Hattan-nishiki No.2. However, Hattan-nishiki No.1 and No.2 had many ellipsoidal-white-cores with large white tissue, which cause the grains to be easily broken during high-degree polishing. On the other hand, Hattan No.35, having grains with relatively many lined-white-cores with small white tissue, was superior for high-degree polishing. In the future, breeding of a new variety, which has the superior cultivation characteristics of Hattan-nishiki No.1 and No.2, but with improved white-core characteristics, is expected.

Key words : Brewer's rice, Hattan-type varieties of rice, Polishing, Suitability for sake brewing, White-core grain.

Rice grain suitable for sake brewing has a white opaque endosperm, called "white-core" at the center of the grain. The endosperm at the white-core has less dense starch accumulation compared with other parts of the endosperm and has many gaps, which reflect the light randomly and make the appearance opaque (Nagato and Ebata, 1958; Del Rosario et al., 1968; Ando and Ichikawa, 1974; Yanagiuchi et al., 1996; Takahashi et al., 1999; Yoshii, 2000). The rice grain with a white-core easily absorbs water due to its rough structure, and is convenient for preparing malted rice (Koji) since Koji yeast can easily invade into the grain. In addition, unrefined sake (shubo and moromi) prepared with such grains is easily solubilized and saccharized. Therefore, this character has been considered to be indispensable for sake brewing (Nagato and Ebata, 1959; Koura, 1972; Hanamoto, 1976; Yanagiuchi et al., 1996; Yoshii, 2000; Yoshii and Aramaki, 2001).

Recently, various kinds of sake, such as Ginjoshu and Daiginjoshu, have been developed, and the polishing characteristic concerning high-degree polishing, in addition to water absorptivity and digestibility, has become an important factor for sake brewing. The polishing rate of common rice for cooking is around 90%, but that of rice for sake brewing is usually around 70%, and that for Ginjoshu and Daiginjoshu is less than 60% and 50-30%, respectively. Hitherto, rice grains with a large white-core have been considered to be suitable for sake brewing, and the breeding of rice for sake brewing has been aimed at enlarging the white-core (Akiyama et al., 1997; Ikegami, 1997). However, the white-core tissue has many gaps and lacks physical hardness. Thus, the grains are easily broken during polishing and the rate of broken grain increases. Since broken and unbroken grains differ in grain weight and differently polished, the rate of steam water absorption is not uniform in the grains mixed with broken grains. This has an inferior effect on the solubility of unrefined sake and quality of Koji

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Table 1. Date of heading and maturity, and degrees of loading at maturity in Hattan-type varieties.

Variety	Heading date	Maturity date	Degree of lodging*
Hattanso	Aug. 11	Sep. 24	5
Hattan No. 10	Jul. 31	Sep. 5	3
Hattan No. 35	Jul. 30	Sep. 5	3
Hattan No. 40	Aug. 2	Sep. 10	4
Hattan-nishiki No. 1	Jul. 29	Sep. 5	2
Hattan-nishiki No. 2	Jul. 28	Sep. 5	1

*1 ; no lodging, 2; inclination of plant was 0-45°, 3 ; inclination of plant was almost 45°, 4; inclination of plant was 45-90°, 5 ; complete lodging.

(malted rice). Thus, the presence of broken grains is not suitable for brewing (Mizuma et al., 2000).

In addition to cultivars Yamada-nishiki and Omachi, Kamenoo-type varieties, Gohyakumangoku, and Miyama-nishiki, etc. are cultivated as rice cultivars suitable for sake brewing in Japan. In Hiroshima Prefecture, however, quite different cultivars, Hattan No.35, Hattan-nishiki No.1 and Hatan-nishiki No.2 are mainly cultivated. These cultivars are cultivated only in Hiroshima Prefecture but are notable as the rice suitable for common sake brewing, and were sold to sake brewers in 32 prefectures in 2003.

The history of breeding of Hattan-type varieties started from private breeding of Hattanso, the origin of Hattan-type varieties, in 1875. Since 1907, the Hiroshima Prefecture Agriculture Experiment Station (the present Hiroshima Prefecture Agriculature Research Center) tried to improve these varieties so as to develop the white-core, which is required for rice suitable for sake brewing in addition to disease resistance, lodging resistance and yielding ability. As a result, Hattan No.35 (Takei et al., 1968) was bred in 1962, and Hattan-nishiki No. 1 (Maeshige et al., 1984a) and Hattan-nishiki No.2 (Maeshige et al., 1984b) in 1984. Hattan-nishiki No.1 was bred as a variety for the hilly area (250-350m) and Hattan-nishiki No.2 for a higher altitude area (400m). These two varieties have high yielding ability and lodging resistance similar to those of common nonglutinous rice, and have improved suitability for sake brewing.

Sake brewers have pointed out that grains of Hattan-nishiki No.1 and No.2 have large white tissue and are easily broken by high-degree polishing. Thus, Hattan No.35, whose kernels have smaller white-core tissues and are not easily broken by high-degree polishing, is being used for brewing Ginjoshu and Danginjoshu (Maeshige and Kobayashi, 2000).

The aim of this study is to clarify the changes in the suitability for sake brewing and polishing characteristics of six Hattan-type varieties originating from Hattanso, and to search for genetic characters useful for breeding the varieties that can endure high-degree polishing, keeping suitability for sake brewing of Hattan-type varieties.

Materials and Methods

Six Hattan-type varieties bred from Hattanso in Hiroshima Prefecture (Hattanso, Hattan No.10, No.35, No.40, Hattan-nishiki No.1 and No.2) were used. The seeds were sown on April 25, 2002, at the Hiroshima Prefecture Agriculture Research Center, transplanted on May 21 and harvested at maturity. Chemical fertilizers were applied at the rate of 3.0 g N, P_2O_5, K_2O per m^2 as basal dressing and 2.0g N , P_2O_5, K_2O per m^2 as top dressing on June 25, 2002. After harvest, water content of brown rice and polished rice (apparent polishing rate, 70%) was adjusted to 13.8 and 13.5%, respectively (Research association for brewer's rice, 1996), and used for the following experiments.

The present study was conducted only in 2002; however, Ohdoi et al. (2000) and Mizuma et al. (2001) reported that the growth and yield of rice suitable for sake brewing varied with the year, while the characters related to the suitability for sake brewing didn't vary with the year. Therefore, we presumed that the suitability for sake brewing is inherent in the variety, and we can discuss about the varietal differences based on the results of a single year experiment.

1. Evaluation of the degrees of loading

The degrees of loading were evaluated at maturity on a scale of 1 to 5; 1 = no lodging and 5 = complete lodging.

2. Examination of the characters of brown rice

One hundred brown rice randomly selected were cut in vertical and longitudinal directions, and the cut surface was observed to classify the white-cores into white-bellied-core, ellipsoidal-white-core, lined-white-core, dotted-white-core and non-white-core (Ebata and Nagato, 1960; Takahashi et al., 1999). Percentage of grains with a white-core (% of white-core grains) was determined from the observation of 100 brown-rice grains. Using 200 brown rice grains randomly selected, length, width, and thickness of grains and white-core rate (percentage of white tissue area (white-core) to

Table 2. Varietal difference in the white-core shape in Hattan-type varieties.

Variety	Non-white-core (%)	White-bellied-core (%)	Ellipsoidal-white-core (%)	Lined-white-core (%)	Dotted-white-core (%)	White-core grains (%)
Hattanso	34	29	24	8	5	66
Hattan No. 10	20	33	23	20	4	80
Hattan No. 35	11	19	36	30	4	89
Hattan No. 40	11	37	47	4	1	89
Hattan-nishiki No. 1	3	25	59	12	1	97
Hattan-nishiki No. 2	5	32	50	10	3	95

Table 3. Varietal difference in the shape of brown rice and white-core rate in Hattan-type varieties.

Variety	Length (mm)	Width (mm)	Thickness (mm)	White-core rate (%)
Hattanso	5.38a	3.05b	2.07b	47b
Hattan No. 10	5.14bc	3.15a	2.12ab	56ab
Hattan No. 35	5.00c	3.16a	2.19ab	60a
Hattan No. 40	5.27ab	3.14a	2.13ab	63a
Hattan-nishiki No. 1	5.22ab	3.18a	2.26a	67a
Hattan-nishiki No. 2	5.20abc	3.18a	2.23a	65a

Values with same letters are not significantly different ($P<0.05$ by LSD).

the cut surface area of the grains) were measured using a shape analyzer (RIA-1A, Satake Co. Ltd., Japan). The hardness of rice grains was measured with a durometer (Kiya Co. Ltd., Japan) using 50 grains randomly selected. The experiment was repeated three times.

3. Test of suitability for sake brewing

Based on the National Standard Analysis Method of Materials for Sake Brewing (Research Association for Brewer's Rice, 1996), 1000-grain weight, polishing rate (apparent polishing rate, net polishing rate, void polishing rate), broken grain rate, water absorptivity (20-min absorption rate, 120-min absorption rate), digestibility (absorbed water after steaming, Brix and formorl-N), crude protein and potassium contents were analyzed. The experiment was repeated three times.

Results and Discussion

Table 1 shows the date of heading and maturity, and lodging rate at maturity of each variety. The date of heading and maturity were the latest in Hattanso followed by Hattan No.40, and were nearly the same in the other four varieties. The degrees of loading were the highest in Hattanso and was low in Hattan-nishiki No.1 and No.2.

Table 2 shows the varietal difference in the white-core shape in Hattan-type varieties. The appearance of the white-core in the rice grain varies with the variety, and the white-core is classified into white-bellied-core, ellipsoidal-white-core, lined-white-core, dotted-white-core and non-white-core (Ebata and Nagato, 1960; Takahashi et al., 1999). In Hattanso, the percentage of grains with a white-core (% of white-core grains) was 66%, but it increased in the process of breeding. In Hattan No.40, Hattan-nishiki No.1 and Hattan-nishiki No.2, the rate of ellipsoidal-white-core, the round large white tissue on the cut surface, was relatively high, and in Hattan No.10 and Hattan No.35, the rate of lined-white-core, lined white tissue on the cut surface, was relatively high.

Table 3 shows the varietal difference in the shape of brown rice and white-core rate in Hattan-type varieties. The size of brown rice and white-core tissue tended to be enlarged in the process of breeding. In particular, the width and thickness of grains were increased in Hattan No.35, Hattan-nishiki No.1 and Hattan-nishiki No.2. Ikegami (1997) reported a positive correlation between the grain width and % of white-core grains. In the present experiment with Hattan-type varieties also, a significant correlation ($r = 0.94^{**}$) was observed between them (Fig. 1). A significant correlation was also observed between the grain thickness and % of white-core grains ($r = 0.91^{**}$), but no significant correlation between the grain length and % of white-core grains. Thus, grain enlargement seemed to contribute to the increase in white-core rate, and

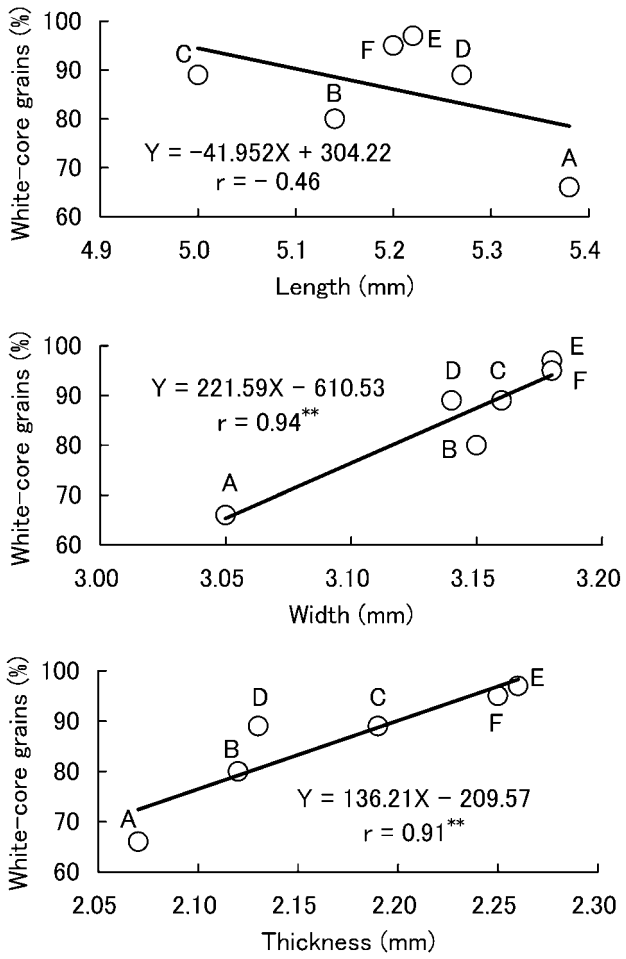

Fig. 1. Relationship between grain length, width, and thickness and the percentage of white-core grains in Hattan-type varieties.
Hattanso : A, Hattan No.10 : B, Hattan No.35 : C, Hattan No.40 : D, Hattan-nishiki No.1 : E, and Hattan-nishiki No.2 : F.
** : Significant at 1% level.

also in % of white-core grains. In the process of the breeding, the lined-white-core in Hattan No.10 and Hattan No.35 seemed to remain unexpectedly.

Table 4 shows the varietal difference in the 1000-grain weight, polishing characteristics and grain hardness in Hattan-type varieties. Since the grain size tended to increase in the process of breeding (Table 3), 1000-grain weight also tended to increase in the process. The polishing characteristics are superior in the variety with a low void polishing rate and low broken grain rate, but the void polishing rate was between 1.4 and 3.0% in all varieties examined, showing no significant varietal difference. Hattan No.35 had a low broken-grain rate, and had superior polishing characteristics. Hattanso, Hattan No.10, Hattan-nishiki No.1 and Hattan-nishiki No.2 showed a broken-grain rate of more than 12%, and are inferior in polishing characteristics. This is probably because these varieties have been bred to increase grain size, white-core size and % of white-core grains, except Hattanso. Tamaki et al. (2002) reported that the distribution of hardness in the grain is different between the grains with an ellipsoidal-white-core that has large white tissue and the grains with a lined-white-core that has small white tissue, because the cellular structure of the white-core of them differs. They also suggested that the grains with ellipsoidal-white-core, which have a large positional difference in the hardness of endosperm tissue, are easily deformed by polishing load and easily broken. Therefore, grains of Hattan-nishiki No.1 and No.2 with a high % of ellipsoidal-white-core may be more easily broken during polishing than grains of Hattan No.35 with a relatively high % of lined-white-core. This may be why the Hattan-nishiki No.1 and No.2 were inferior in polishing characteristics. Generally, the characteristics of rice suitable for sake brewing are examined using the rice grains at an apparent polishing rate of 70% according to the national standard analysis method of materials for sake brewing (Research association for brewer's rice, 1996). In this study also, we used rice grains at an apparent polishing rate of 70% to compare with the data already reported. Preparation of uniform grains at a higher-than-70% polishing rate is difficult due to the limited ability of the person in charge and adjustability of the machine. Therefore, the examination of the varietal differences using such grains is difficult. However, since the aim of this study is to find varieties that can endure high-degree polishing, further studies with grains at a higher-than-70% polishing rate are necessary.

The grain hardness was low in Hattan-nishiki No.1 and No.2 (4.3 and 4.4 kg, respectively), which shows that grains of Hattan-nishiki No.1 and No.2 are fragile. Hattan No.10 had a low percentage of ellipsoidal-white-core grains and had as high as 5.5 kg hardness of grains. Nevertheless, it had poor polishing characteristics. The reason is uncertain, and further examination on the cellular structure of the endosperm will be necessary.

Table 5 shows the varietal difference in the suitability for sake brewing in Hattan-type varieties. Water absorptivity shown by the 20-min water absorption rate was as high as 28% in Hattan No.35, Hattan No.40, Hattan-nishiki No. 1 and Hattan-nishiki No.2 with a high % of white-core grains but was low (23%) in Hattanso with a low % of white-core grains. In white-core grains, many long and slender cylindrical cells are developed in the endosperm (Aramaki et al., 2004), and starch grains are roughly packed in endosperm cells (Nagato and Ebata, 1958; Del Rosario et al., 1968; Ando and Ichikawa, 1974; Yanagiuchi et al., 1996; Takahashi et al., 1999; Yoshii, 2000). Therefore, varieties with a high % of white-core grains crack easily during water absorption, and absorb water rapidly. Soga et al. (1976) also reported that white-core grains

Table 4. Varietal difference in the 1000-grain weight, polishing characteristics and grain hardness in Hattan-type varieties.

Variety	1000-grain weight (g)	Polishing rate			Broken garin rate (%)	Grain hardness (Kg)
		Apparent (%)	Net (%)	Void (%)		
Hattanso	26.1bc	70.0	71.4	1.4	12.6ab	4.7
Hattan No. 10	27.0b	70.0	72.5	2.5	14.6a	5.5
Hattan No. 35	25.1c	70.0	73.0	3.0	7.0c	4.8
Hattan No. 40	27.4b	70.0	72.9	2.9	10.0b	4.9
Hattan-nishiki No. 1	29.2a	69.9	72.9	3.0	12.3ab	4.3
Hattan-nishiki No. 2	27.6b	70.0	72.6	2.6	14.6a	4.4

Values with same letters are not significantly different (P<0.05 by LSD).

Table 5. Varietal difference in the suitability for sake brewing in Hattan-type varieties.

Variety	Water absorption		Absorbed water after steaming (%)	Digestibility		Crude protein (% /D.W)	K (ppm/D.W)
	20 min (%)	120 min (%)		Brix (%)	F-N[1] (ml)		
Hattanso	23.0b	27.8	32.1	8.7b	0.6	4.9	420a
Hattan No. 10	26.4ab	28.2	33.6	9.7a	0.7	5.2	430a
Hattan No. 35	28.3a	28.9	31.7	9.9a	0.7	5.0	357ab
Hattan No. 40	28.1a	29.0	31.5	10.3a	0.7	5.4	437a
Hattan-nishiki No. 1	28.5a	30.8	33.8	10.1a	0.7	5.2	313b
Hattan-nishiki No. 2	27.6a	29.3	33.6	9.8a	0.7	5.3	311b

Values with same letters are not significantly different (P<0.05 by LSD).
[1] F-N ; Formol-N.

have longitudinally developed endosperm cells and absorb water rapidly. The varietal difference in the maximum water absorption shown by a 120-min water absorption was similar to that shown by a 20-min water absorption, though the varietal difference was small. The rate of absorbed water after streaming was slightly higher (about 34%) in Hattan No.10, Hattan-nishiki No.1 and Hattan-nishiki No.2, but its varietal difference was similar to that shown by the 120-min water absorption rate.

The Brix value that shows the solubility of unrefined rice (moromi) was as high as about 10% in Hattan No.35, Hattan No.40, Hattan-nishiski No.1 and Hattan-nishiki No.2, and its varietal difference was similar to that of water absorptivity. The formol-N content that shows the amino acid content of unrefined rice was similar in all varieties. Protein is essential because its amino acid composition gives the taste and flavor of sake, but a high protein content deteriorates the sake quality, and a low protein content is preferable (Maeshige and Kobayashi, 2000). The crude protein content was slightly low in Hattanso (4.9%) and slightly high in Hattan No.40 (5.4%), but the varietal difference was negligible. The potassium content greatly varied with the variety; it was high (about 430 ppm) in Hattanso, Hattan No.10 and Hattan No.40, but low (about 310 ppm) in Hattan-nishiki No.1 and No.2. Aramaki et al. (2004) reported that, in the endosperm mutant of rice with a thick subaleurone layer, inorganic components are not sufficiently removed by polishing, and polished rice grains of the mutant contained a large amount of inorganic components. Since the potassium content did not correlate with the date of heading and maturity (Table 1, 5), potassium might not be sufficiently removed by polishing in Hattanso, Hattan No.10 and Hattan No.40, due to thicker subaleurone layers than others.

Hattanso had been selected from early varieties having large grains with white-cores. It had characteristics adapted to hilly and mountainous area in Hiroshima Prefecture, and had been cultivated for more than 50 years (Maeshige, 1987). Thereafter, the Hiroshima Prefecture Agriculture Experiment Station (the present Hiroshima Prefecture Agriculature Research Center) began breeding new cultivars and isolated Hattan No.10 from Hattanso in 1921. Hattan No.10 had some inferior characteristics such as a long culm, easiness to lodge, susceptibility to rice blast, and low yielding ability. However, it was used as a basic material for breeding varieties suitable for sake brewing in Hiroshima Prefecture (Maeshige, 1993). In 1962, Hattan No.35 was bred from Hattan No.10 by

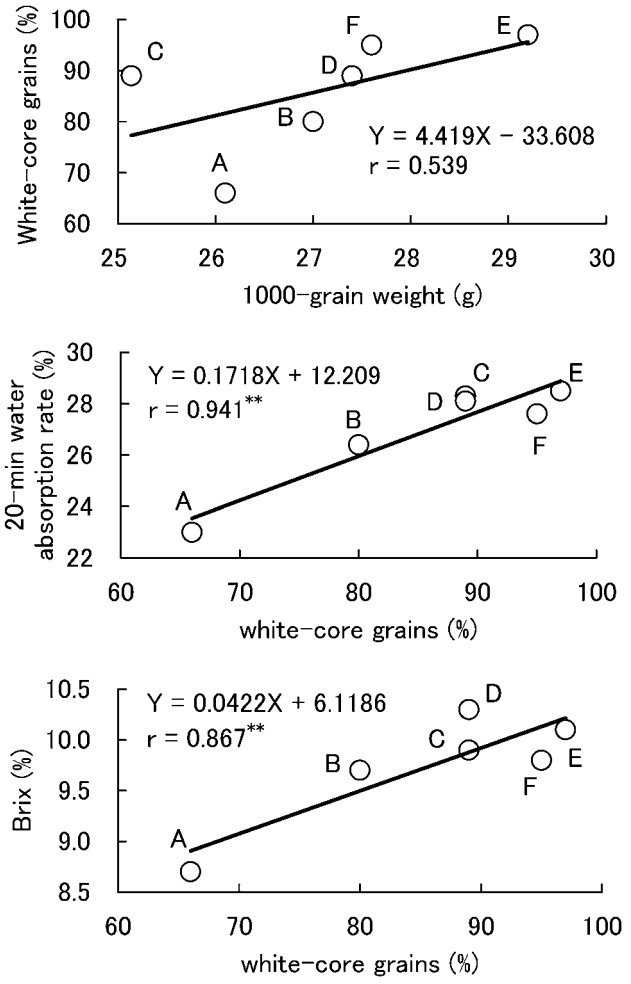

Fig. 2. Correlation of white-core grains (%) with 1000-grain weight, water absorptivity, and digestibility (Brix) in Hattan-type varieties.
Hattanso : A, Hattan No.10 : B, Hattan No.35 : C, Hattan No.40 : D, Hattan-nishiki No.1 : E, and Hattan-nishiki No.2 : F.
** : Significant at 1% level.

introducing rice blast resistance (Takei et al., 1968), and Hattan No.40 was bred in 1965. Then, stable high-yielding ability was introduced to Hattan No.35, and Hattan-nishiki No.1 (Maeshige et al., 1984a) and Hattan-nishiki No.2 (Maeshguge et al., 1984b) were bred in 1984. Hattan-nishiki No.1 was bred as a variety for a hilly area (250-350m) and Hattan-nishiki No.2 for a higher latitude area (400m). These varieties have yielding ability and lodging resistance similar to those of common nonglutinous rice, and improved suitability for sake brewing. Thus, the varieties suitable for sake brewing have been bred aiming to enlarge the white-core and to increase the rate of grains with a white-core. These attempts at breeding have been mostly to improve the cultivation properties.

Since Hattan-type varieties have been bred by selecting for large grain, large white-core and high % of white-core grains, the water absorptivity and digestibility increased (Fig. 2). These characters may affect the invasion of Koji yeast and the amount of brewed sake. However, for brewing Ginjoshu and Daiginjoshu, high-degree polishing is indispensable, and rice varieties that are not easily broken during polishing are desirable. Hattan-nishiki No.1 and No.2 have superior suitability for sake brewing but inferior polishing characteristics, and are not suitable for brewing Ginjoshu and Daiginjoshu.

The present results are summarized as follows. (I) Hattanso has inferior suitability for sake brewing. It is not superior in polishing characteristics and has inferior cultivation characteristics. (II) Hattan No.10 and No.40 have superior suitability for sake brewing but inferior polishing and cultivation characteristics. (III) Hattan No.35 has superior polishing characteristics and suitability for sake brewing but inferior cultivation characteristics. (IV) Hattan-nishiki No.1 and No.2 have large grains and are superior in cultivation and suitability for sake brewing, but inferior in polishing characteristics. These results show that Hattan-type varieties bred from Hattanso in Hiroshima Prefecture had been bred aiming to increase grain size, white-core rate and % of white-core grains. Therefore, the suitability for sake brewing, such as water absorptivity and digestibility, increased. Hattan No.10 and No.35, in particular Hattan No.35, have high % lined-white-core grains, which are not easily broken and can endure high-degree polishing. On the other hand, Hattan-nishiki No.1 and No.2 had a relatively high % of ellipsoidal-white-core grains; and, grains can not endure high-degree polishing.

The shape and size of white-core tissue largely influence the quality of sake (Yanagiuchi et al., 1996). Yamada-nishiki, which is used for brewing high-grade sake such as Ginjoshu and Daiginjoshu, has a lined-white-core in grains, and sake brewers consider that the endurance to high-degree polishing is due to this type of a white-core, "lined-white-core". There is a possibility that Hattan No.10 and Hattan No.35 still have a genetic trait related to a lined-white-core. Therefore, by crossing with these varieties, rather than with other varieties, it may be possible to breed a variety suitable for brewing high-grade sake with the superb taste of Hattan-nishiki No.1 and No.2.

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