Breeding of Naked Barley for Protein Improvement

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Area planted to naked barley, once accounted for 590,000 ha at the maximum (1950) in Japan, had decreased to only 15,000 ha in 1977, due to decreased demand for food use and decreased winter croppings on paddy fields in general. In response to such a situation, naked barley breeding laboratories located at several places have been gradually abolished, leaving only one, i.e. the First Crop Laboratory, Agronomy Division, Shikoku National Agricultural Experiment Station.

Major breeding objectives in this laboratory are high yielding, strong culm and better quality. However, the better quality mentioned here implies only milly endosperm, whiteness, and high pearling grade. Lines having such characters have so far been selected. In other words, almost no attention has been paid to nutritional quality as a human food and animal feed.

It has been known that the first limiting amino acid in cereal protein in general is lysine, and the addition of lysine improves nutritional value of cereal protein. An approach to this problem from plant breeding was initiated by the discovery of a high lysine maize, Opaque-2, in 1964. In barley, Hiproly with high protein and high lysine contents was discovered in Sweden in 1968, and high lysine Risø 1508 was also discovered in Denmark in 1972. Since then, barley breeding for protein improvement has been initiated in many countries of the world.

To improve the nutritional value of Japanese naked barley, a breeding program was started by using high protein and high lysine lines in 1971, in the Shikoku National Agricultural Experiment Station. The present authors have carried out the program, and the results obtained will be presented in this paper.

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Gene sources and their characteristics

Several characteristics of Hiproly and Risø 1508 grown in the field of Shikoku National Agricultural Experiment Station are shown in Table 1. Both Hiproly, 2-rowed naked barley, and Risø 1508, 2-rowed covered barley, are late-maturing varieties with medium culm length. Yield potential of Hiproly is extremely low, while that of Risø 1508 is relatively high. Weight of 1,000 grains of them was heavier than 6-rowed varieties, Nanpu-hadaka and Kikai-hadaka, but lighter than a 2-rowed covered variety, Daisen-gold (43.8 g for 1,000 grains), and their grains are of shrunken type. Hiproly shows less whiteness of the grain, and Risø 1508 shows a quite high viviparity. Hiproly has high protein content, while Risø 1508 showed the highest value of Std DBCp, followed by Hiproly, and Nanpu-hadaka, and Kikai-hadaka showed low values. DBCp (dye binding capacity per unit quantity of protein) indicates lysine content, and Std DBCp expresses standardized DBCp.

Hiproly crosses

In the combination of Hiproly (vitreous

Table 1. Characters of parental varieties and lines

	Varieties and lines			
	Hiproly	Nampu-hadaka	Kikai-hadaka	Risφ 1508
Heading date	Apr. 18	Apr. 8	Apr. 15	Apr. 17
Maturity date	May 27	May 23	May 24	May 29
Culm length, cm	92	86	95	88
Spike length, cm	5.6	6.0	5.9	9.4
No. spikes/m²	537	594	468	792
Lodging degree	1.8	1.3	1.0	3.5
Grain yield, kg/a	30.2	51.5	53.4	56.5
Weight of 1 *l* grains, g	712	781	784	551
Weight of 1000 grains, g	39.4	25.9	25.5	34.4
Grain whiteness, %	10.1	17.1	15.4	—
Viviparous germination, %	56.5	19.6	10.8	100.0
Protein content, %	13.0	11.3	10.7	10.4
Std DBCp mM	1.093	0.980	0.964	1.123

grain) and Nanpu-hadaka or Kikai-hadaka (both normal grain), segregation of vitreous and normal grains was observed on spikes of BC_2F_1 and BC_1F_1 6-rowed plants. Length, width, thickness and weight of the vitreous grains were 96, 92, 87, and 75%, respectively, of those of the normal grains, showing that the vitreous grains are small in size.

Protein content and DBCp were compared between both grains. Although the protein content differed with different plants, no difference was observed between both grains produced on the same plants. On the other hand, the vitreous grains showed significantly higher values of Std DBCp, indicating higher lysine content of vitreous grains than normal grains.

Furthermore, the vitreous grains and normal grains segregated on BC_2F_1 and BC_1F_1 plants were sown as pair lines, and several characteristics were examined in the subsequent generation. Karlsson (1972) reported that the high lysine gene is located on the 7th chromosome, being linked with a marker gene, short rachilla hair. As shown in Table 2, number of plants with short rachilla hairs, derived from vitreous grains, were more than expected, clearly indicating the linkage relationship. Among agronomic characters and protein-related characters, difference between populations originated from vitreous and normal grains was recognized with the following 10 characters. The population from vitreous grains are characterized by short spikes, more number of sterile spikelets in the central row, lighter weight each of 1,000 grains, a spike, and grains per plant, dark grain color, high protein content, and high DBCs (DBC per sample) but low protein yield due to smaller weight of grains per plant, and high Std DBCp. Namely, plants originated from the vitreous grains are superior with the characters related to protein, but inferior with agronomic characters.

Table 2. The rachilla hair length of F_2 plants

Crosses	Vitreous F_2 seed		Normal F_2 seed	
	S	*s*	*S*	*s*
H × N³	27	300	319	11
H × K³	9	51	43	1
H × N²	4	116	116	4
H × K²	36	204	234	6

H : Hiproly
N : Nanpu-hadaka
K : Kikai-hadaka
S : Long rachilla hair
s : Short rachilla hair

Risø 1508 crosses

In BC_1F_1 plants of naked grain type, in 5 combination of Risø 1508 to Nanpu-hadaka,

Table 3. Comparison between shrunken grains and normal grains

Rows	Grain character	1000 grain weight g	Protein content %	Std DBCp mM
2 rowed	Shrunken	29.9	10.6	1.256
	Normal	38.3	9.5	1.086
6 rowed	Shrunken	23.5	8.4	1.241
	Normal	27.5	7.9	1.101

Kikai-hadaka, Yunagi-hadaka, Shiratama-hadaka, and Beni-hadaka, plants bearing only normal grains and plants showing the segregation of normal grains and shrunken grains were observed. The ratio in number of both plant groups coincides the segregating ratio of grain shrinkage as monofactorial recessive gene.

The shrunken grains showed lighter weight of 1,000 grains, and higher protein contents and Std DBCp (Table 3). This result is well consistent with the report that the high lysine gene of Risø 1508 is monofactorial recessive and linked with shrunken grains. As the linkage is very strong, it has not succeeded yet to get a high lysine line with normal grains.

Crosses of Risø 1508 × Hiproly

From the F_2 population of Risø 1508 × Hiproly, 213 plants of naked grain type were selected, and based on the variance of the F_2 plants and the parents the broad sense heritability for several characters was estimated. Heritability of spike density and Std DBGp, both determined by each major gene, was very high, as a matter of course, while that of protein content was fairly low, being intermediate between culm length and grain weight per plant. A total of 150 F_3 plants was classified into 4 groups according to lysine level, i.e. HR (transgressive type with lysine level higher than Risø 1508), R (lysine level similar to Risø 1508), H (lysine level similar to Hiproly), and L (low lysine level), and their protein contents and weight of 1,000 grains were examined, as shown in Table 4. The result showed that the higher the lysine level, the higher was the protein contents, but the lower the weight of 1,000 grains.

Table 4. Protein contents of grains and weight of 1000 grains in F_3 population

		Protein content %	1000 grain weight g
Risφ 1508		9.3±0.8 (10.4*)	43.4±2.1 (39.0*)
Hiproly		13.5±1.0	41.6±1.6
Lysine level	HR	16.0±1.6	29.2±3.5
	R	12.9±1.1	39.4±3.0
	H	12.2±1.5	42.1±4.6
	L	10.8±1.3	49.0±5.5

* Figures in parenthesis: Converted into hull-less weight, assuming the hull weight as 10%

Selected lines from the Hiproly crosses

Of the lines, which were submitted to the preliminary yield test of 1977, 38 lines derived from the crosses with Hiproly were judged to be of high lysine, and they were compared with low lysine 55 lines from the conventional crosses. The high lysine lines showed shorter spikes, more spikes, lower grain yields, and lower values for litre-weight and 1,000-grain weight. Visual grade of grains, grain whiteness, and stand evaluation were also low. On the other hand, they showed higher protein contents, DBCs, and Std DBCp. Therefore, it can be said that the high lysine lines, as a whole need to be improved as for agronomic characters, although they have better protein-related characters.

However, some of the high lysine lines, listed in Table 5, which were selected for productivity, showed higher or almost equal grain yield as compared to Kikai-hadaka, taken as a control, with higher protein yields and lysine yields. Thus, substantial improvement in yielding was recognized as compared to the parent Hiproly. The lysine was analyzed by ion-exchange chromatography.

At present, the combination of protein

Table 5. Contents and yields of protein and lysine in selected lines

Varieties and selected lines	Protein content %	Lysine content %	Grain yield kg/a	Grain yield index %	Protein yield index %	Lysine yield index %
Nanpu-hadaka	12.7	3.4	44.6	109	114	121
Kikai-hadaka	12.1	3.2	41.0	100	100	100
Hashiri-hadaka	14.1	3.0	44.6	109	127	120
Yunagi-hadaka	12.3	3.4	41.9	102	104	110
Hiproly	14.0	4.5	28.3	69	80	112
Hiproly	14.0	4.0	35.7	87	101	127
Yonkei-8389	13.0	3.8	42.0	102	110	133
-8382	14.6	3.9	38.0	93	112	138
-8421	13.7	4.4	43.5	106	120	165
-8422	15.5	3.6	41.7	102	130	148
-8358	13.5	3.9	47.2	115	128	158
-8361	14.8	4.0	41.2	100	123	154
-8362	14.0	3.9	42.8	104	121	150
-8427	13.3	4.2	45.7	111	123	160
-8341-1	13.1	4.1	46.7	114	123	161
-8355	13.3	3.9	40.0	98	107	132

analysis by the use of auto-analyzer and DBC measurement by orange 12 is being used for the first step of analysis aiming at screening as many plants and lines as possible. The lines which were evaluated to be high lysine by the first step of analysis and showed better agronomic characters such as yielding in the yield test were selected, and then submitted to the second step of lysine analysis by the ion-exchange chromatography. This system is considered to be good for breeding laboratories.

By this method, efforts have been made to develop high lysine lines with protein contents equal to or 1-2% higher than those of currently used varieties, with the result shown in Table 5. Further improvement of agronomic characters and protein-related characters will be attempted by using these lines as parental lines.

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