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Breeding of Malting Barley Using the Haploid Method

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Abstract
Production of haploid plants followed by chromosome doubling can shorten the duration of plant breeding programs. Barley haploid plants can be obtained by interspecific crossing between barley (*Hordeum vulgare L.*) and *Hordeum bulbosum* L. This paper describes the haploid breeding method applied for malting barley. There were varietal differences in embryo formation and haploid production among the barley genotypes. The varietal differences in embryo formation were due to the difference in the pollen tube growth and the rate of chromosome elimination 3 to 5 days after pollination. To improve the efficiency of haploid production, superior clones of *H. bulbosum* were selected. Haploid production method using intergeneric crossing was newly developed. F_1 plants derived from two cross-combinations of barley cultivars were crossed with *H. bulbosum*. As a result, 209 doubled haploid plants were obtained and 4 elite lines of malting barley were selected. These lines were superior to the check cultivar in agronomic performance and malting quality.

Discipline: Biotechnology/ Crop production
Additional key words: agronomic performance, chromosome elimination, doubled haploid, *Hordeum bulbosum* L., *intergeneric* cross

Introduction

The production of doubled haploid barley is considered to be a useful means for shortening the duration of malting barley breeding programs. Barley haploid plants can be obtained from interspecific crossing between barley (*Hordeum vulgare* L.) and *Hordeum bulbosum* L. Since the chromosomes of *H. bulbosum* are usually eliminated from the hybrid embryos after fertilization during subsequent cell divisions, haploid barley embryos are produced.

Studies on the haploid breeding method of malting barley and the results obtained have been published by several authors[3-8]. In the present paper, the author describes varietal differences and factors in the crossability between barley and *H. bulbosum*, selection of superior clones of *H. bulbosum* to facilitate haploid production, development of other methods of haploid production, and agronomic performance and malting quality of doubled haploid lines obtained by the *bulbosum* method.

Varietal differences and factors in crossability between Japanese two-rowed barley and *H. bulbosum*

The production of barley haploid plants through interspecific crossing between diploid barley (*Hordeum vulgare*) and diploid *H. bulbosum* was first reported in 1970[10]. Thereafter, many researchers have reported genotypic differences in the seed setting and haploid production from barley plants crossed with *H. bulbosum*[1,9,12,13]. Six cultivars were pollinated with the pollen of the diploid *H. bulbosum* clone Cb2920 in a preliminary experiment in 1988. Thirteen leading cultivars and breeding lines of Japanese two-rowed barley were used in 1989. Percentage of embryo formation (embryos obtained/florets pollinated) and percentage of haploid production (haploid plants obtained/florets pollinated) were used to assess the crossability with *H. bulbosum*.

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1) Varietal difference in crossability between barley and H. bulbosum

Percentages of embryo formation and haploid production ranged from 11.1 to 59.8% and from 3.4 to 29.5%, respectively in 1988 and from 1.7 to 72.7% and from 0.6 to 26.5%, respectively in 1989 (Table 1). Embryos which did not grow well developed only poor shoots and roots, and then ceased to grow. Generally, cultivars with a high percentage of embryo formation showed a high haploid production. Six of the 13 cultivars used in 1989 were the same as those in 1988. The ranking of the crossability of these six cultivars was similar in both years. Kanto Nijo 25 showed a remarkably high crossability compared with the other cultivars in both years (Table 1). As the cultivars with a high percentage of embryo formation showed a high haploid production, it was possible to estimate the crossability with *H. bulbosum* based on embryo formation and haploid production. As mentioned above, there were varietal differences in the crossability with *H. bulbosum* in Japanese two-rowed barley.

2) Pollen tube growth and chromosome elimination after pollination

In the cultivar with a high percentage of embryo formation (Kanto Nijo 25), the rate of pistils in which the pollen tube of *H. bulbosum* reached the ovules was high. In contrast, in the cultivar with a low percentage of embryo formation (Yoshikei 15) the growth rate of the pollen tube was low (Table 2). The mean chromosome numbers of embryos were 12.7 in Kanto Nijo 25 and 13.0 in Yoshikei 15 at 3 days after pollination, and 9.6 in the former and 10.5 in the latter 5 days after pollination. At 7 days after pollination, both cultivars examined had 7 chromosomes, a value equal to the number of chromosomes of haploid barley (Table 3). There were no varietal differences in the mean number of chromosomes both 3 and 5 days after pollination. However, the number of cells with 7 to 10 chromosomes increased in the cultivar with a high percentage of embryo formation compared with the cultivar with a low percentage of embryo formation. Conversely, a higher proportion of cells with more than 11 chromosomes was observed in the cultivar with a low percentage of embryo formation. It was suggested that the varietal differences in the efficiency of embryo formation were due to the difference in the growth rate of the pollen tube and rapidity of chromosome elimination 3 to 5 days after pollination.

Table 1. Varietal difference of Japanese two-rowed barleys in crossability with *H. bulbosum*

Year	Cultivar	No. of florets pollinated	No. of embryos obtained/No. of florets pollinated (%)	No. of haploid plants obtained/No. of florets pollinated (%)
1988	Kanto Nijo 25	400	59.8 a[1)]	29.5 a[1)]
	Nishinochikara	159	48.4 b	15.1 b
	Yoshikei 21	340	22.1 c	8.2 c
	Kyushu Nijo 10	273	23.4 c	6.6 c
	Yoshikei 19	290	18.3 c	5.9 c
	Yoshikei 15	386	11.1 d	3.4 d
1989	Kanto Nijo 25	392	72.7 a	26.5 a
	Nishinochikara	149	41.6 b	17.4 b
	Yoshikei 21	190	38.4 bc	12.6 bc
	Tsuyushirazu	249	29.7 cd	12.4 bc
	Nishino Gold	351	20.8 e	10.8 bc
	Kyushu Nijo 10	105	39.0 bc	10.5 bc
	Misato Golden	98	27.6 de	10.2 bc
	Haruna Nijo	89	19.1 e	7.9 cd
	Kyushu Nijo 9	528	24.8 de	5.1 d
	Yoshikei 19	206	18.4 e	3.9 d
	Amagi Nijo	52	5.8 f	3.8 d
	Chikukei 7565	402	1.7 g	0.7 e
	Yoshikei 15	491	7.7 f	0.6 e

1): Means followed by the same letter (within a column) are not significantly different at $P \leq 0.05$, as determined by Duncan's multiple range test of arcsine-transformed data.

Table 2. Growth of pollen tube of *H. bulbosum* to the ovules on barley and embryo formation by crossing between barley and *H. bulbosum*

Cultivar		Pollen tube reaching ovule (%) Time after pollination (h) 6	12	24	Mean	No. of florets pollinated	No. of embryos obtained/No. of florets pollinated (%)
Kanto Nijo 25	A[1)]	81	79	79	79.7	168	81.0
	B	79	79	78	78.7		
	Mean	80.0	79.0	78.5	79.2		
Yoshikei 15	A	14	15	14	14.3	164	15.0
	B	13	14	15	14.0		
	Mean	13.5	14.5	14.5	14.2		

1): A, B: Replication.

Table 3. Chromosome elimination process in embryos derived from barley × *H. bulbosum*

Days after pollination	Cultivar[1)]	No. of embryos scored	No. of cells containing 7–14 chromosomes 7	8	9	10	11	12	13	14	Total	Mean chromosome number
3	Kanto Nijo 25	15	15	2	0	16	2	24	0	117	176	12.7
	Yoshikei 15	15	12	1	0	9	0	13	1	124	160	13.0
5	Kanto Nijo 25	13	26	10	3	20	1	4	0	15	79	9.6
	Yoshikei 15	13	20	8	0	15	0	11	3	20	77	10.5
7	Kanto Nijo 25	10	54								54	7.0
	Yoshikei 15	10	55								55	7.0
9	Kanto Nijo 25	10	56								56	7.0
	Yoshikei 15	10	51								51	7.0
11	Kanto Nijo 25	8	35								35	7.0
	Yoshikei 15	8	33								33	7.0

1): Kanto Nijo 25 and Yoshikei 15 are cultivars with a high and a low percentage of embryo formation, respectively.

H. bulbosum with spring habit and haploid production from barley × maize and barley × Italian ryegrass crosses

H. bulbosum with a winter habit requires vernalization for the induction of flowering[11]. If a *H. bulbosum* clone with a spring habit could be obtained, handling for obtaining pollen from *H. bulbosum* may be simplified. Development of a new method for haploid production may widen the range of application of the haploid breeding method in barley. In this section, selection for clones of *H. bulbosum* with a spring habit to facilitate the crossing of barley with *H. bulbosum* and the production of barley haploid plants using barley × maize and barley × Italian ryegrass crosses are described.

1) Selection for spring habit clone of H. bulbosum

About 200 seeds formed by natural outcrossing among 16 *H. bulbosum* clones (2n = 2x = 14) were harvested at the Fukuoka Agricultural Research Center in 1989. The original clones were obtained from seeds introduced from Morocco in 1987 by one of the authors[2]. It remained to be determined whether these clones displayed a spring or winter habit. Twenty-three out of the 183 clones flowered without vernalization treatment. They were considered to be *H. bulbosum* clones with a spring habit. Other clones tested failed to head or flower. Fifteen out of 23 clones with a spring habit were tested for the efficiency of embryo formation and haploid production in crosses with the barley cultivar Kanto Nijo 25. Embryos and regenerated plants were obtained from crosses between barley and *H. bulbosum*

Table 4. Embryo formation and haploid production of barley cultivar Kanto Nijo 25 crossed with 16 clones of *H. bulbosum*

Clone No.	No. of florets pollinated	No. of embryos obtained/No. of florets pollinated (%)	No. of haploid plants obtained/No. of florets pollinated (%)
16	74	86.5 o[1)]	41.9 o[1)]
Cb2920	88	84.1 no	27.3 mn
2	100	83.0 mno	26.0 lmn
12	94	79.8 lmno	17.0 fghi
9	90	78.9 klmno	25.6 klm
13	88	78.4 jklmno	23.9 jklm
17	86	73.3 ijklmno	32.6 n
14	70	71.4 hijklmn	22.9 ijklm
15	72	65.3 ghijkl	18.1 ghij
23	82	64.6 fghijkl	20.7 hijklm
3	98	44.9 e	8.2 abcd
21	78	35.9 de	10.3 abcde
22	76	32.9 cde	13.2 defg
18	80	28.8 bcd	12.5 cdefg
20	80	28.8 abcd	13.8 efg
19	76	26.3 abcd	11.8 bcdef

1): Means followed by the same letter (within a column) are not significantly different at $P \leq 0.05$, as determined by Duncan's multiple range test of arcsine-transformed data.

clones that were selected as spring type. The mean values of embryo formation and haploid production efficiency ranged from 26.3 to 86.5% and from 8.2 to 41.9%, respectively. Among the selected clones, clone No. 16 showed the highest percentage of embryo formation and haploid production efficiency. Haploid production efficiency of this clone was significantly higher than that of the check clone, Cb2920 (Table 4). Identification of *H. bulbosum* clone with a spring habit may facilitate the utilization of the *bulbosum* technique and contribute to the improvement of barley haploid production programs.

2) Haploid production from 2 intergeneric crosses

Maize F_1 hybrid, B 14 × CI 64 ($2n = 2x = 20$) and vernalized plants of Italian ryegrass cv. Waseaoba ($2n = 2x = 14$) were grown under a 23°C and 16-h daylength regime. Emasculated spikes of barley were covered with polyethylene bags and pollinated with freshly collected pollen of maize and Italian ryegrass at the time of barley anthesis. About 0.3 ml in 0.5 ml of a 75 ppm 2,4-dichlorophenoxyacetic acid (2,4-D) solution was injected into the uppermost internodes of the barley stems immediately after pollination. The spikes were sprayed with 75 ppm gibberellic acid (GA_3) 1 day after pollination. Fourteen barley cultivars were crossed with maize and 3 cultivars with Italian ryegrass. In the barley × maize crosses, regenerated plants were obtained. When 2,4-D was not applied, the ovaries failed to grow and most of them were shrunken. Table 5 shows the varietal differences in embryo formation and plant regeneration in the barley × maize crosses. Embryos were obtained from 9 out of 14 cultivars examined, and plants from 7 out of 14 cultivars. Among the barley cultivars, the efficiency of embryo formation and plant regeneration ranged from 0.0 to 19.6% and 0.0 to 6.9%, respectively. Chikukei 7565 showed a high rate of embryo formation (15.7%) and plant regeneration (6.9%). In the crosses of barley × Italian ryegrass, regenerated plants were obtained. Embryos were obtained from 2 out of 3 cultivars examined and plants from 1 cultivar (Table 6). When 2,4-D was not applied, the ovaries failed to grow and most of them were shrunken. Chikukei 7565 showed a high rate of embryo formation (16.7%) and plant regeneration (10.4%). These values were higher than those (15.7 and 6.9%, respectively) in the barley × maize crosses. All the 24 regenerated plants which were randomly sampled from barley × maize and barley × Italian ryegrass crosses had 7 chromosomes, and since the other regenerated plants showed the same morphological characteristics as the plants examined for the chromosome number, all the plants regenerated were considered to be haploids.

These techniques of haploid production from

Table 5. Varietal difference in embryo formation and haploid production through barley × maize crosses

Cultivar	No. of florets pollinated	No. of embryos obtained	No. of haploid plants obtained
Chikukei 7565	204	32 (15.7)[1)]	14 (6.9)[2)]
Yoshikei 15	64	6 (9.4)	1 (1.6)
Yoshikei 19	88	0 (0.0)	0 (0.0)
Yoshikei 21	104	3 (2.9)	3 (2.9)
Yoshikei 28	42	1 (2.4)	0 (0.0)
Kyushu Nijo 9	101	8 (7.9)	3 (3.0)
Kyushu Nijo 10	74	0 (0.0)	0 (0.0)
Nishino Gold	122	0 (0.0)	0 (0.0)
Kanto Nijo 25	104	1 (1.0)	1 (1.0)
Misato Golden	117	0 (0.0)	0 (0.0)
Amagi Nijo	93	1 (1.1)	0 (0.0)
Haruna Nijo	56	11 (19.6)	2 (3.6)
Tsuyushirazu	127	3 (2.4)	3 (2.4)
Nishinochikara	119	0 (0.0)	0 (0.0)

1): Figures in parentheses indicate the percentage of embryo formation (No. of embryos obtained/No. of florets pollinated).
2): Figures in parentheses indicate the percentage of haploid production (No. of haploid plants obtained/No. of florets pollinated).

Table 6. Varietal difference in embryo formation and haploid production through barley × Italian ryegrass crosses

Cultivar	No. of florets pollinated	No. of embryos obtained	No. of haploid plants obtained
Chikukei 7565	336	56 (16.7)[1)]	35 (10.4)[2)]
Yoshikei 15	84	0 (0.0)	0 (0.0)
Kanto Nijo 25	126	5 (4.0)	0 (0.0)

1), 2): See notes of Table 5.

crosses of barley with maize and with Italian ryegrass offer a new possibility for barley haploid breeding programs. A proper use of the three techniques (barley × *H. bulbosum*, maize and Italian ryegrass crosses) may widen the range of application of the haploid breeding method in barley.

Agronomic performance and malting quality of doubled haploid barley lines developed by the *bulbosum* method

F_1 plants derived from two cross-combinations of barley cultivars were crossed with a clone of *H. bulbosum* (Cb2920), and derived haploid plants were treated with a 0.05% colchicine solution to produce the doubled haploid plants. As a result, 209 doubled haploid plants were obtained from $2F_1$'s (DH1, DH2) by the *bulbosum* method. Ranges for grain yield, 1000 grain weight and plump grain percentage of 143 lines tested in 1988 were 22.8–46.2 kg/a, 41.2–50.6 g, 65.0–96.6% in DH1 and 25.6–53.2 kg/a, 44.0–54.8 g, 75.5–96.4% in DH2, respectively and 56 lines were selected. Ranges for grain yield, 1000 grain weight, plump grain percentage and malting quality of the 56 lines tested in 1989 were 35.2–50.5 kg/a, 44.0–50.9 g, 65.0–95.7%, −14.2 –15.7 in DH1 and 39.6–57.5 kg/a, 43.9–51.5 g, 70.8–93.4% −7.5–13.3 in DH2, respectively. Four superior lines (Yoshikei 30, 31, 32, 33) were selected on the basis of the experimental results obtained in 1988 and 1989, and their local adaptability was tested. These lines were superior to the check cultivars in agronomic performance and malting quality (Tables 7 and 8), and were resistant to powdery mildew and barley yellow mosaic virus (BaYMV). The tests conducted at 5 experiment stations in 1990 indicated that the agronomic performance of the 4 lines was moderately superior to that of the check cultivar Amagi Nijo which shows a good local adaptability. The *bulbosum* method enabled to save 2 years in

Table 7. Agronomic performance of doubled haploid lines (Yoshikei 30, 31, 32 and 33) and check cultivars

Year	Cultivar	Maturing date	Length		No. of spikes/m²	Lodging[1]	Grain yield	1000 grain weight	Plump grain	Plump grain yield
			Culm (cm)	Spike (cm)			(kg/a)	(g)	(%)	(kg/a)
1989	Yoshikei 30	May 22	83.2	6.5	414	0	41.9 (103)[2]	45.1	88.3	37.0 (122)[2]
	Yoshikei 31	May 22	93.8	7.3	551	1.0	50.5 (124)	48.7	88.5	44.7 (147)
	Yoshikei 32	May 22	90.0	6.8	533	0	48.8 (120)	46.7	83.8	40.9 (135)
	Yoshikei 33	May 22	87.2	6.4	529	0	53.0 (130)	47.2	85.2	45.2 (149)
	Nishino Gold	May 22	93.4	6.1	492	0	40.8 (100)	43.5	74.4	30.4 (100)
1990	Yoshikei 30	May 24	83.5*	5.9*	539ns	0.0**	50.0 (106)ns	42.3ns	82.3*	41.1 (119)**
	Yoshikei 31	May 25	87.5ns	6.3ns	529ns	0.5*	53.7 (115)**	46.2**	78.5ns	42.2 (122)**
	Yoshikei 32	May 25	84.5*	5.8**	619**	0.0**	53.5 (114)**	44.9**	84.5**	45.2 (131)**
	Yoshikei 33	May 24	84.0*	5.3**	620**	0.3**	52.6 (112)*	46.2**	81.5*	42.8 (124)**
	Nishino Gold	May 23	89.0ns	5.4**	534ns	1.0ns	42.0 (90)*	42.7ns	78.3ns	32.9 (95)ns
	Amagi Nijo	May 25	88.0-	6.2-	517-	1.5-	46.9 (100)-	43.0-	73.6-	34.5 (100)-
	LSD (0.05)[3]		3.1	0.2	61	0.8	4.1	1.0	6.3	3.5
	(0.01)		4.7	0.3	93	1.2	6.2	1.4	9.6	5.4

1): Lodging evaluated by observed values as 0:none, 1:1-10%, 2:10-30%, 3:30-50%, 4:50-70%, 5:70-100% lodging per plot.
2): Figures in parentheses indicate the percentage compared with the check cultivar.
3): ANOVA was performed for the test in 1990. * and ** show that the differences from the check cultivar Amagi Nijo are significant at the 0.05 and 0.01 probability levels, respectively.
All doubled haploid lines were resistant to barley yellow mosaic virus (BaYMV) and powdery mildew.

Table 8. Malting qualities of doubled haploid lines (Yoshikei 30, 31, 32 and 33) and check cultivars[1]

Year	Cultivar	Malt extract (%)	Extract yield (%)	Nitrogen		Kolbach index (%)	Diastatic power		Examination marks
				Total (%)	Soluble (%)		(°WK)	(°WK/TN)	
1988	Yoshikei 30	85.0	79.4	1.52	0.84	55.3	194	127	74.0 (15.7)[2]
	Nishino Gold	84.6	80.7	1.64	0.76	46.5	161	98	58.3
	Yoshikei 31	84.9	80.2	1.37	0.86	62.6	192	140	79.7 (0.0)
	Nishino Gold	86.1	80.7	1.42	0.86	60.4	204	143	79.7
	Yoshikei 32	85.4	79.2	1.64	0.85	52.0	201	123	69.9 (7.9)
	Yoshikei 33	84.2	79.3	1.54	0.86	56.1	201	131	75.3 (13.3)
	Nishino Gold	84.1	79.8	1.81	0.81	44.7	237	131	62.0
1989	Yoshikei 30	85.1	79.5	1.64	0.80	48.8	219	133	66.5 (1.0)
	Yoshikei 31	82.7	77.2	1.57	0.79	50.6	222	142	64.7 (- 0.8)
	Amagi Nijo	81.6	75.8	1.75	0.90	51.6	256	147	61.3 (- 4.2)
	Nishino Gold	83.6	78.7	1.78	0.85	47.5	238	134	65.5
	Yoshikei 32	83.4	77.1	1.62	0.78	48.3	209	129	63.0 (6.5)
	Yoshikei 33	82.7	77.1	1.58	0.83	52.2	224	141	68.3 (11.8)
	Amagi Nijo	81.3	75.0	1.77	0.85	48.2	226	128	54.3 (- 2.2)
	Nishino Gold	82.5	75.8	1.87	0.85	45.5	218	117	56.5

1): Malting qualities were tested at the Tochigi Branch, Tochigi Agricultural Experiment Station.
2): Figures in parentheses indicate the differences in examination marks from the check cultivar Nishino Gold.

the breeding period compared with the conventional pedigree method employed at the Fukuoka Agricultural Research Center. In conclusion, highly promising lines were obtained in a short period of time by the *bulbosum* method, suggesting that the method could be applied for malting barley breeding.

Conclusion

An efficient method of haploid production was developed for malting barley breeding. The mechanism and factors involved in the varietal differences in crossability with *H. bulbosum* were clarified. Superior *H. bulbosum* clones were selected and new methods of haploid production using intergeneric crosses were developed. Highly promising doubled haploid lines of malting barley were obtained in a short period of time by the *bulbosum* method.

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