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# CHARACTER EXPRESSIONS OF VARIOUS DWARF GENES AT HAPLOID LEVEL

—Genetical studies of rice plant XC—

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## Introduction

Since anther culture has been applied for rice breeding, it has become possible to obtain pure lines both at haploid and diploid levels from any population.<sup>18,20</sup> As a fundamental problem of breeding by the use of haploidy, it is important to know the character expressions of major and minor genes both at haploid and diploid levels.

In this paper, the authors produced a series of near-isogenic dwarf lines at haploid by anther culture and investigated their character expressions in comparison with the corresponding diploid lines.

## Materials and Methods

A series of near-isogenic dwarf lines which were produced by the back-crossings to the recurrent parent, Shiokari in our laboratory<sup>10</sup> are shown in Table 1. For anther culture, flower panicles were excised and pre-treated at 8°C for about one week. Then, anthers at the mid-uninucleate microspore stage were excised and cultured in  $N_6$  medium<sup>3</sup> supplemented with plant hormones, sucrose and agar as shown in Table 2. After callus grew to the diameter of 3–4 mm, pieces of callus were transferred to the redifferentiation medium and kept at 26°C under continuous illumination for plant regeneration. The detailed procedure for anther culture has been described in many papers.<sup>1,2,4,18,20</sup> From 1981 to 1982, the anther-derived plants were transferred individually to pot soil culture in the green house. Ploidy levels were

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TABLE 1. List of the near-isogenic lines used in the experiment

No. of isoline	Gene symbol	Name of dwarf type	Dwarf donor	Number of backcrossing times
ID-S	+	Shiokari		
- 1	<i>d- 1</i>	Daikoku dwarf	H-86	8
- 2	<i>d- 2</i>	Ebisu dwarf	H-85	7
- 3	<i>d-3, d-4, d-5</i> <sup>1)</sup>	Tillering dwarf of Bunketsu-waito	H- 2	4
- 6	<i>d- 6</i>	Ebisumochi dwarf	H-126	6
- 7	<i>d- 7</i>	Cleistogamous dwarf	N- 7	8
-10	<i>d-10</i>	Tillering dwarf of Toyohikari-bunwai	N-70	8
-11	<i>d-11</i>	Norin 28 dwarf	N-58	6
-12	<i>d-12</i>	Yukara dwarf	N-62	5
-13	<i>d-13</i>	Short grained dwarf	M-51	5
-14	<i>d-14</i>	Tillering dwarf of Kamikawa-bunwai	H-147	7
-17	<i>d-17</i>	Slender dwarf	I -71	5
-18 <sup>h</sup>	<i>d-18<sup>h</sup></i> <sup>2)</sup>	Hosetsu dwarf	N-71	8
-18 <sup>k</sup>	<i>d-18<sup>k</sup></i> <sup>2)</sup>	Kotake-tamanishiki dwarf	Fl-26	8
-27	<i>d-27</i>	Tillering dwarf of Bunketsu-to	Fl-86	6
-30	<i>d-30</i>	Waisei-shirasasa dwarf	Fl- 3	5
-47	<i>sd-1(d-47)</i>	Dee-geo-woo-gen dwarf	I -120 (IR-8)	4
-51	<i>d-a(t)</i> <sup>3)</sup>	M-290 dwarf	M-290	4
-52	<i>d-b(t)</i> <sup>3)</sup>	Hiroba dwarf	N-100	4

1) Triplicate genes.

2) Multiple alleles in the order of  $+ < d-18^k < d-18^h$ .

3) Single recessive genes tentatively named.

TABLE 2. Composition of N<sub>6</sub> media used (mg/l)

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	463	KI	0.8
KNO <sub>3</sub>	2,830	Na <sub>2</sub> EDTA	37.3
KH <sub>2</sub> PO <sub>4</sub>	400	FeSO <sub>4</sub> ·7H <sub>2</sub> O	27.9
MgSO <sub>4</sub> ·7H <sub>2</sub> O	185	Glycine	2.0
CaCl <sub>2</sub> ·2H <sub>2</sub> O	166	Thiamine HCl	1.0
MnSO <sub>4</sub> ·4H <sub>2</sub> O	4.4	Pyridoxine HCl	0.5
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	1.5	Nicotinic acid	0.5
H <sub>3</sub> BO <sub>4</sub>	1.6		
For callus induction ;		For plant regeneration ;	
2, 4-D	2	IAA	0.2
Sucrose	30,000	Kinetin	1.0
Agar	7,500	Sucrose	50,000
		Agar	7,500

identified by the morphological features, stoma size and seed sterilities. Morphological traits including culm, panicle, spikelets and stomata in the regenerated plants were preliminary compared with those of the parental dwarf lines. In 1983, haploid plants regenerated by anther culture were propagated vegetatively by division of tillers and used for intensive surveys of the morphological characters. In comparison with the haploids, the diploid plants were grown from the seeds of the dwarf lines in the same plastic house. According to the preliminary experiment, it was confirmed that both plants produced from a tiller and a seed exhibited a similar growth nature. Therefore, the haploid tiller and diploid seed plants from the corresponding dwarf lines were transplanted to pot culture on June 4th, 1983. Compound fertilizer used for rice (N : 7.2 kg,  $P_2O_5$  : 7.2 kg,  $K_2O_5$  : 4.5 kg/10 a) was applied for each pot (2 l) with a quantity of 4 g. Although the heading dates from the tiller plants were variable (from July 28th to August 29th in haploids), the characters at maturation were scarcely affected by the delay of heading time. At maturation, the characters were measured for 8 plants in each plot. A significance of the difference between the characters of haploid and diploid lines were evaluated by t-test.

## Results

### 1. Induction of haploid dwarf lines

Both haploid and diploid plants were induced by anther culture in 14 near-isogenic lines in 1981 and 1982. As shown in Table 3, ID-18<sup>b</sup> showed the highest percentage of callus induction. It is probable that the mid-uninucleate pollens were contained uniformly in the anthers of this extreme dwarf. It is noted that ID-2, ID-7, and ID-30 indicated relatively high frequencies in the regeneration of both albino and green plants. The rate of albino plants was 67.4% throughout the experiment. Regenerated plants were identified as haploid or diploid by the morphological features. Haploid or diploid plants were produced in 14 near-isogenic lines in the first experiment.

Following this, haploid plants of the standard line, Shiokari were produced by the second experiment and used as the standard to the other haploid dwarf lines. Besides that, new variants were found in haploids of ID-7 and ID-11, respectively. Therefore, two haploid types were maintained in both dwarf lines and discriminated as a and b. Conspicuous chimera plants consisting of haploid and diploid parts also appeared in ID-2 and ID-7.

### 2. Preliminary survey of the regenerated plants

R<sub>1</sub> plants regenerated from anther-derived calli were obtained during 1981

TABLE 3. Anther culture response from a sample of 19 near-isogenic lines

Isoline	Number of anthers in culture	Number of calli obtained	% of anthers with callus	Number of plants				
				albino	green	x*	2x* x+2x*	
I D-S	1067	147	13.8	17	6	0	0	0
1	1172	140	11.9	13	10	5	1	0
2	1600	206	12.9	15	23	7	2	1
3	1275	172	13.5	7	6	1	0	0
6	857	111	13.0	0	0	0	0	0
7	1430	275	19.2	16	17	5	3	1
10	1052	144	13.7	6	5	2	0	0
11	1542	97	6.3	10	7	2	3	0
12	1521	185	12.2	16	9	2	0	0
13	273	48	17.6	2	0	0	0	0
14	1325	214	16.2	9	7	1	0	0
17	1334	264	19.8	20	9	1	0	0
18 <sup>h</sup>	957	265	27.6	9	11	3	2	0
18 <sup>k</sup>	743	121	16.3	1	5	2	1	0
27	1484	254	17.1	14	5	0	0	0
30	1484	242	16.3	29	24	5	3	0
47	1531	67	4.4	7	2	2	0	0
51	1326	209	15.8	16	12	4	1	0
52	918	121	13.2	2	4	0	0	0

\* Discrimination at maturity by morphological features.  
A part of green plants died before identification.

TABLE 4. Panicle length and culm length in diploid and haploid plants regenerated by anther culture

Isoline	Panicle length (cm)			Culm length (cm)		
	2x(o)*	2x*	x*	2x(o)*	2x*	x*
I D-S	12.6	10.1 (80)	6.0 (47)	37.0	—	23.3 (63)
- 1	7.5	5.8 (78)	3.4 (45)	13.5	14.5 (107)	10.9 (81)
- 2	13.3	9.8 (74)	6.9 (52)	30.0	31.0 (103)	19.3 (64)
- 3	9.2	—	4.2 (46)			
- 7	9.2	8.2 (89)	5.4 (59)	34.5	34.3 (99)	24.9 (72)
-10	5.2	4.5 (67)	5.3 (101)			
-11	8.1	6.6 (82)	5.1 (63)			
-12	7.1	—	7.1 (101)			
-14	6.4	—	5.9 (91)			
-18 <sup>h</sup>	4.4	3.9 (89)	4.5 (102)			
-18 <sup>k</sup>	8.0	7.7 (96)	5.4 (68)	18.5	18.4 (99)	11.2 (61)
-30	6.5	8.2 (127)	5.8 (89)	24.0	26.6 (111)	19.5 (81)
-47	10.3	—	7.4 (72)	24.5	—	18.5 (76)
-51	9.4	9.0 (96)	6.3 (67)	35.8	—	18.6 (52)

- 1). \* 2x(o);original diploid, 2x; regenerated diploid, x; regenerated haploid.
- 2) Parentheses mean the relative number to the original diploid ( $2x/2x(o) \times 100$  and  $x/2x(o) \times 100$ ), respectively.

TABLE 5. Size of spikelet and stoma in diploid and haploid plants regenerated by anther culture

Isoline	Spikelet length (mm)			Spikelet width (mm)			Ratio <sup>1)</sup>			Stoma length ( $\mu\text{m}$ )			Stoma width ( $\mu\text{m}$ )			Ratio <sup>1)</sup>		
	2x(o)	2x	x	2x(o)	2x	x	2x(o)	2x	x	2x(o)	2x	x	2x(o)	2x	x	2x(o)	2x	x
ID-S	6.4	6.3	4.0	3.7	3.7	3.5	0.59	0.58	0.87	28.9	25.0	14.7	21.9	17.1	19.0	0.68	0.61	1.16
- 1	4.6	4.6	3.0	3.9	3.6	2.7	0.84	0.78	0.90	25.3	28.2	21.4	23.8	20.2	16.7	0.85	0.64	0.70
- 2	6.7	5.4	3.4	3.9	3.5	2.8	0.59	0.64	0.83	22.5	21.2	20.4	18.7	17.6	18.0	0.75	0.74	0.79
- 3	6.3	—	4.4	3.8	—	2.7	0.60	—	0.62	—	—	—	—	—	—	—	—	—
- 7	5.5	5.1	3.7	4.5	3.9	2.5	0.83	0.76	0.68	25.7	24.2	20.4	22.4	20.5	17.6	0.78	0.76	0.77
-10	5.7	6.1	4.2	3.4	3.1	2.6	0.59	0.50	0.61	30.0	29.3	25.4	23.7	24.0	22.1	0.71	0.73	0.78
-11	4.2	5.1	1.9	4.1	3.5	1.6	0.97	0.67	0.82	29.8	26.7	18.1	22.4	21.1	15.3	0.67	0.71	0.76
-12	6.5	—	4.8	3.5	—	3.1	0.55	—	0.64	25.3	—	18.4	17.4	—	15.4	0.62	—	0.75
-14	5.8	—	4.3	3.3	—	2.3	0.57	—	0.55	—	—	—	—	—	—	—	—	—
-18 <sup>a</sup>	6.0	5.8	5.7	3.4	3.3	3.3	0.57	0.58	0.59	28.7	26.2	—	21.2	19.3	—	0.66	0.66	—
-18 <sup>b</sup>	5.7	5.8	4.3	3.1	3.7	2.9	0.54	0.64	0.68	26.4	25.1	17.9	22.5	16.2	16.0	0.76	0.58	0.80
-30	5.9	5.0	3.8	3.6	3.4	2.8	0.61	0.68	0.74	—	—	—	—	—	—	—	—	—
-47	6.1	—	4.5	3.5	—	2.7	0.57	—	0.60	26.5	—	22.9	16.7	—	17.9	0.56	—	0.70
-51	5.1	6.1	4.0	3.4	3.6	2.6	0.67	0.58	0.64	26.5	28.1	20.9	20.5	26.4	19.5	0.69	0.84	0.84

1) Ratio=width/length.

2) 2x(o); original diploid, 2x; regenerated diploid, x; regenerated haploid.

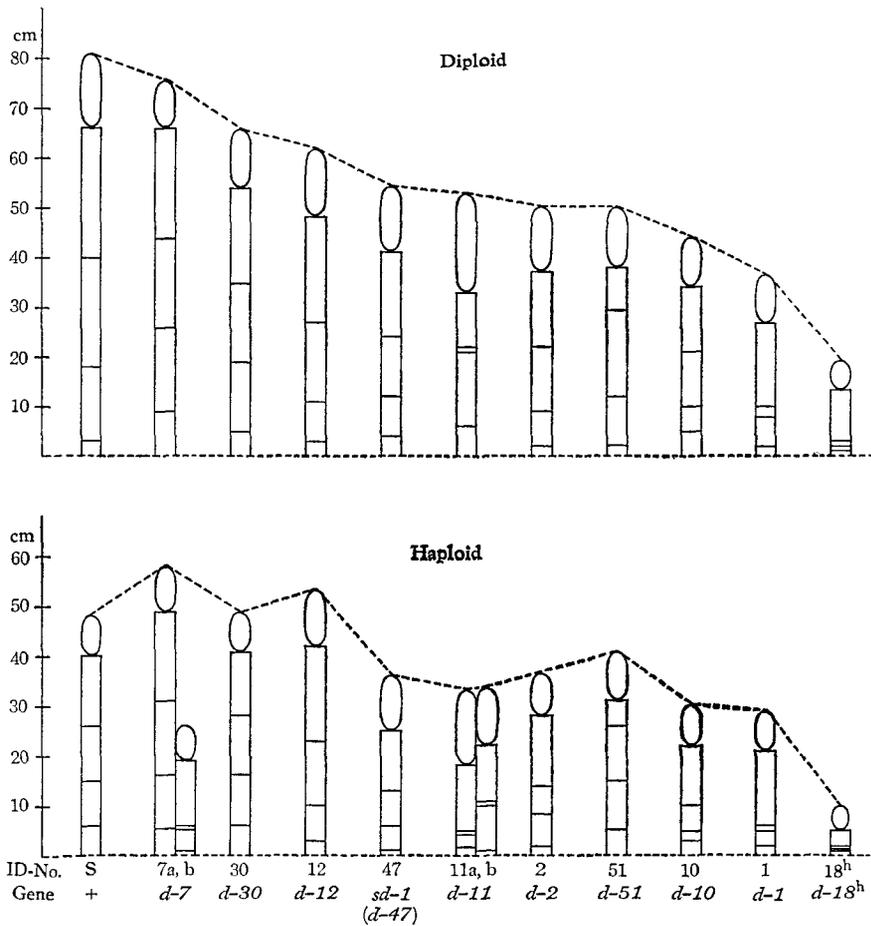
and 1982. As the regenerated plants were individually grown under different conditions in the green house, the characters at maturation were preliminary surveyed. Plants grown from the seeds of the original dwarf lines were also used for comparison with them. Culm and panicle lengths and the sizes of spikelet and stoma are shown in Table 4 and 5. It is noted that the panicle length of diploid  $R_1$  plants differed from those of the original line,  $2x(o)$  in a few cases, while culm length did not show a significant difference between the original line,  $2x(o)$  and the diploid  $R_1$  plant. Although the both lengths of haploid  $R_1$  plants decreased from the diploid lines, the reduction rates were variable among the dwarf lines. Similar reductions in haploids were observed both in length and width of spikelet and stoma, while the ratios in both characters were less variable over the ploidy levels (Table 5).

### 3. Comparison of the characters between haploid and diploid plants in the near-isogenic dwarf lines

Owing to the regeneration of haploids by the anther culture from 11 kinds of near-isogenic lines, it became possible to compare the character expressions of dwarf genes between haploid and diploid levels.

As shown in Fig. 1 and Plates, plant types of haploids at maturity were characterized by the major genes for dwarfness both at haploid and diploid. Idiogram of panicle and internodes were drawn diagrammatically in Fig. 1. It is evident that the reduction of height is variable among the dwarf lines and the variation at haploid was not parallel to that at diploid as to the plant height and culm length. However, most of the internode distribution patterns in the haploids corresponded with the diploid counterpart except for the three variants. Namely, the *dm* type<sup>21)</sup> showing a conspicuous reduction of the second internode was also maintained in the corresponding haploid dwarf types as shown in ID-1 and ID-11a, b respectively. Many tillers in ID-10 and short round grains in ID-1 and ID-11a, b were also expressed at the haploid. As mentioned in the previous paper,<sup>15)</sup> two kinds of indices, namely the percentage of the second internode length to culm length ( $In_2/CL$ ) and the ratio of the first internode length to the third internode length ( $In_1/In_3$ ) expressed in the arctangent (upper internode elongation index) were suitable to express the internode distribution patterns caused by the respective dwarf genes. As seen in both indices in Table 6, the variants such as ID-7b, ID-11a, b showed a typical alteration at haploid. Partial modification was also indicated in the other dwarf lines at haploid. The other characters of culm and leaf both at haploid and diploid are also shown in Tables 6 and 7. The reductions of the characters by haploidization were variable among the dwarf lines at haploid. There was a considerable variation in the actual

values among the different haploid dwarf lines and the reduction rates varied prominently among the haploid lines. An extremely short dwarf, ID-18<sup>b</sup> (*d-18<sup>b</sup>* type) in haploid indicated the smallest value throughout all characters as well as in diploid, while the highest values were replaced by the normal (ID-S) or the dwarf types. Characters related with panicle and spikelet are shown in Table 8. The *size* characters such as panicle length, length of primary branch and length and width of spikelet decreased remarkably by haploidization, while the *number* characters such as number of panicles, number of spikelets in haploid increased more than those of the diploid



**Fig. 1.** Idiogram of panicle and internodes in a series of near-isogenic dwarf lines both at haploid and diploid levels. Panicle (top), first, second, third and lower internodes are shown in each figure.

TABLE 6. Characteristics of culm and internode in 13 haploid lines

Isoline	CL(cm)	In <sub>1</sub> (cm)	In <sub>2</sub> (cm)	In <sub>3</sub> (cm)	In <sub>1</sub> /CL(%)	In <sub>2</sub> /CL(%)	In <sub>3</sub> /CL(%)	PL/PH(%)	UI(In <sub>1</sub> /In <sub>3</sub> )
ID-S	39.8 (60)**	14.0 ( 55)**	10.6 (49)**	8.9 (58)**	34.9 ( 91)	26.6 ( 81)**	22.3 ( 97)	17.2 ( 98)	57.6 ( 98)
- 1	21.4 (79)**	14.5 ( 84)**	1.3 (62)	3.3 (59)	67.8 (106)	6.2 ( 77)	15.5 ( 75)	25.9 ( 98)	77.1 (107)
- 2	27.6 (74)**	13.8 ( 91)	5.8 (45)**	6.1 (93)	49.9 (123)*	20.8 ( 62)*	22.2 (125)	24.9 ( 96)	66.0 ( 99)
- 7a	49.0 (74)**	18.3 ( 84)**	14.8 (83)**	11.0 (65)**	37.5 (113)*	30.3 (113)	22.1 ( 86)	15.5 (117)**	58.9 (113)
- 7b	19.0 (29)**	12.9 ( 59)**	0.3 ( 2)**	3.8 (22)**	67.9 (205)**	1.6 ( 6)**	20.0 ( 77)*	27.2 (206)**	73.6 (142)
-10	21.7 (65)**	11.6 ( 87)	5.0 (45)**	2.4 (47)**	53.5 (134)**	23.0 ( 70)**	11.1 ( 73)**	27.4 (118)**	78.3 (114)
-11a	17.7 (53)**	13.2 (126)	0.3 (77)**	2.5 (17)**	74.6 (253)**	1.8 (152)**	14.1 ( 13)**	45.7 (126)**	79.3 (225)
-11b	22.5 (67)**	11.0 (104)	0.3 (82)	8.6 (57)**	48.7 (180)*	1.5 (131)	38.0 ( 55)	31.8 ( 89)	52.0 (148)
-12	42.4 (89)*	18.9 ( 92)	13.1 (80)*	7.1 (87)	44.5 (102)	30.9 ( 91)	16.7 ( 99)*	19.8 ( 84)**	69.4 (102)
-18 <sup>b</sup>	4.7 (37)**	2.8 ( 28)**	0.7 (48)**	0.4 (77)**	59.6 ( 77)**	13.8 (135)**	8.1 (209)**	47.8 (153)**	82.3 ( 94)
-30	41.0 (70)**	13.3 ( 71)**	12.2 (74)**	9.5 (70)**	32.4 ( 94)	29.8 ( 98)	23.2 ( 92)	16.8 ( 94)*	54.5 (101)
-47	25.4 (62)**	11.5 ( 68)**	6.5 (53)**	4.5 (63)**	45.3 (108)*	25.6 ( 85)*	17.7 ( 98)	28.9 (116)**	68.6 (104)
-51	31.3 (82)**	4.9 ( 58)**	10.8 (62)**	9.7 (99)	15.7 ( 66)**	34.5 ( 76)**	31.0 (121)**	22.9 ( 98)	26.8 ( 66)
Mean	28.0 (64.7)	12.4 (77.5)	6.3 (58.6)	6.0 (62.6)	48.6 (127.1)	19.0 (90.5)	20.2 (93.8)	27.1 (114.8)	65.0 (116.4)
Range	5-49 (29-89)	3-19 (28-126)	0.3-15 (2-83)	0.4-11 (17-99)	16-75 (66-253)	2-35 (6-152)	8-38 (13-209)	16-48 (84-206)	27-82 (66-225)

1) Abbreviation of characters; CL: Culm length, In<sub>1</sub>-In<sub>3</sub>: Length of each internode, PL: Panicle length, PH: Plant height, UI: Upper internode elongation index (arctangent of the ratio; In<sub>1</sub>/In<sub>3</sub>).

2) Parenthesis means the relative number to the corresponding diploid line (x/2x×100).

3) \*, \*\* Signicant at the 5% and the 1% levels, respectively, except UI (no significance test).

TABLE 7. Characteristics of leaf and culm in 13 haploid lines

Isoline	LB <sub>1</sub> (cm)	LB <sub>2</sub> (cm)	LB <sub>3</sub> (cm)	LS <sub>1</sub> (cm)	LS <sub>2</sub> (cm)	LS <sub>3</sub> (cm)	WF (cm)	W2 (cm)	CD (cm)
ID-S	17.0 ( 59)**	28.2 (70)**	28.6 (81)**	15.7 (76)**	14.8 (69)**	16.8 (81)**	1.19 ( 93)*	1.01 (87)**	0.28 (64)**
- 1	13.9 ( 63)**	18.6 (68)**	14.8 (65)**	12.7 (76)**	10.9 (80)**	10.2 (78)**	1.41 ( 80)**	1.15 (74)**	0.28 (60)**
- 2	15.6 ( 86)	18.4 (66)**	14.3 (49)**	13.4 (73)**	11.2 (62)**	10.4 (61)**	1.17 ( 91)*	0.97 (81)**	0.28 (72)**
- 7a	19.8 ( 99)	27.0 (90)	24.5 (76)**	16.6 (90)**	15.7 (83)**	15.0 (80)**	1.18 ( 88)**	1.00 (79)**	0.32 (72)**
- 7b	13.3 ( 67)**	18.9 (63)**	15.2 (47)**	12.5 (68)**	10.5 (56)**	10.7 (56)**	1.36 (102)	1.13 (90)**	0.27 (60)**
-10	15.8 ( 88)	20.9 (77)**	20.1 (69)**	13.2 (93)	12.0 (82)**	11.7 (71)**	0.86 ( 82)**	0.77 (72)**	0.17 (69)**
-11a	16.1 ( 74)**	26.9 (97)	31.8 (90)*	19.4 (93)**	16.7 (89)**	15.7 (89)**	1.03 ( 78)**	1.17 (88)**	0.34 (77)**
-11b	11.0 ( 51)**	20.0 (72)**	27.7 (78)**	18.5 (88)**	16.0 (86)**	16.3 (92)*	0.99 ( 75)**	11.1 (84)**	0.28 (64)**
-12	19.6 (107)	21.5 (68)**	15.5 (41)**	18.2 (84)**	16.6 (81)**	14.3 (73)**	0.90 ( 78)**	0.75 (65)**	0.25 (66)**
-18 <sup>h</sup>	5.8 ( 91)*	7.1 (91)*	6.5 (99)	6.0 (79)**	4.3 (86)*	3.0 (94)	0.82 ( 73)**	0.75 (72)**	0.20 (81)**
-30	14.8 ( 70)**	23.0 (67)**	23.2 (65)**	14.1 (81)**	14.4 (80)**	15.5 (72)**	1.02 ( 82)**	0.93 (78)**	0.30 (66)**
-47	15.8 ( 75)*	19.1 (58)**	17.1 (56)**	16.2 (79)**	14.3 (71)**	12.4 (63)**	1.02 ( 71)**	0.90 (73)**	0.24 (61)**
-51	15.1 ( 75)*	26.8 (80)**	30.0 (89)**	12.2 (81)**	14.6 (82)**	16.4 (92)*	1.18 ( 79)**	1.09 (76)**	0.32 (70)**
Mean	14.9 (77.3)	21.3 (74.4)	26.7 (69.6)	14.5 (81.6)	13.2 (77.5)	13.0 (77.1)	1.09 (82.5)	0.98 (78.4)	0.27 (67.8)
Range	6-20 (51-107)	7-28 (58-97)	7-32 (41-99)	6-19 (68-93)	4-17 (56-89)	3-17 (56-94)	0.8-1.4 (71-102)	0.8-1.2 (65-90)	0.2-0.3 (60-81)

- 1) Abbreviation of characters; LB<sub>1</sub>-LB<sub>3</sub>: Length of blade in the first, second and third leaf, LS<sub>1</sub>-LS<sub>3</sub>: Length of sheath in the first, second and third leaf, WF, W2: Width of blade in the flag and second leaf, CD: Culm diameter measured at the third internode.
- 2) Parenthesis means the relative number to the corresponding diploid line (x/2x×100).
- 3) \*, \*\* Significant at the 5% and the 1% levels, respectively.

TABLE 8. Characteristics of panicle and spikelet in 13 haploid lines

Isoline	PL (cm)	PN	NS	NB <sub>1</sub>	NB <sub>2</sub>	B1L (cm)	SL (mm)	SW (mm)	ED	SI <sub>1</sub>	SI <sub>2</sub>
ID- S	8.3 (58)**	16.9 (104)	82.9 ( 89)*	13.9 (133)**	7.5 ( 58)**	2.4 (42)**	4.5 (64)**	3.1 (90)**	7.8 (166)**	13.9 (58)**	0.70 (137)**
- 1	7.4 (77)**	24.3 (172)**	105.4 (111)	9.9 (124)*	17.9 (113)	2.5 (64)**	3.3 (73)**	2.5 (70)**	10.4 (150)**	8.4 (51)**	0.75 ( 96)
- 2	9.2 (71)**	22.2 (192)**	107.9 (166)**	9.7 (100)	19.1 (318)**	4.0 (88)*	3.6 (59)**	2.5 (74)**	8.3 (223)**	9.1 (43)**	0.69 (125)**
- 7a	8.9 (90)**	21.4 (169)**	137.8 (146)**	12.1 (105)	23.2 (205)**	3.4 (83)**	4.0 (76)**	2.7 (79)**	11.2 (167)**	10.9 (60)**	0.69 (103)*
- 7b	7.1 (71)**	20.8 (164)**	106.0 (112)	9.8 ( 85)*	17.4 (154)**	2.4 (59)**	2.9 (57)**	2.5 (71)**	11.2 (162)**	7.3 (40)**	0.86 (129)**
-10	8.2 (80)**	99.5 (162)**	66.0 (136)**	8.2 (111)*	9.5 (285)**	3.1 (81)*	4.4 (80)**	2.4 (76)**	5.8 (169)**	10.6 (61)**	0.55 ( 94)*
-11a	14.9 (77)**	25.0 (244)**	—	—	—	—	—	—	—	—	—
-11b	10.5 (54)**	14.0 (137)**	—	—	—	—	—	—	—	—	—
-12	10.5 (71)**	23.0 (161)**	103.3 (120)*	9.8 ( 92)	16.7 (172)**	4.3 (73)**	5.0 (75)**	2.8 (80)**	7.0 (167)**	14.0 (60)**	0.56 (198)*
-18 <sup>a</sup>	4.3 (74)**	34.6 (112)	24.2 (115)*	5.2 (118)*	0.2 —	2.1 (68)**	4.8 (79)**	2.6 (79)**	3.0 (159)**	12.5 (62)**	0.54 ( 97)
-30	8.3 (71)**	20.8 (125)**	129.5 (122)*	10.3 (111)	20.7 (123)*	3.5 (74)**	4.0 (72)**	2.6 (81)**	9.0 (139)**	10.4 (58)**	0.65 (108)**
-47	10.1 (76)**	33.8 (289)**	109.3 (150)**	9.7 (112)*	18.2 (227)**	3.9 (75)**	4.7 (73)**	2.7 (75)**	7.8 (198)**	12.7 (55)**	0.57 (105)*
-51	9.3 (80)**	17.0 (155)**	126.0 (156)**	13.2 (123)**	16.6 (268)**	3.6 (78)**	4.7 (78)**	2.7 (79)**	9.8 (193)**	12.9 (61)**	0.58 (102)
Mean	9.0 (73.1)	28.7 (168.2)	99.8 (129.4)	10.2 (110.4)	15.2 (192.3)	3.2 (71.4)	4.2 (71.5)	2.6 (77.6)	8.3 (172.1)	11.2 (55.4)	0.65 (109.5)
Range	4-15 (54-90)	14-100 (104-289)	24-138 (89-166)	5-14 (85-133)	0.2-23 (58-318)	2.1-4.3 (42-88)	2.9-5.0 (57-80)	2.4-3.1 (70-90)	3-11 (139-223)	7-14 (40-62)	0.54-0.86 (94-198)

- 1) Abbreviation of characters; PL: Panicle length, PN: Panicle numbers, NS: Number of spikelets, NB<sub>1</sub> and NB<sub>2</sub>: Number of primary and secondary branches, B1L: Length of primary branches, SL: Spikelet length, SW: Spikelet width, ED: Ear density (NS/PL+B1L), SI<sub>1</sub>: Index of spikelet size calculated by SL×SW, SI<sub>2</sub>: Index of spikelet shape calculated by SW/SL.
- 2) Parenthesis means the relative number to the corresponding diploid line (x/2x×100).
- 3) \*, \*\* Significant at the 5% and the 1% levels, respectively.
- 4) Mature plants of ID-11 a,b were damaged by rat. Relative number in NB<sub>2</sub> of ID-18<sup>a</sup> was not calculated since NB<sub>2</sub> of diploid counterpart was 0.

TABLE 9. Correlation coefficients between haploid and diploid lines

Character	r	Character	r	Character	r
PH	0.743**	LB <sub>1</sub>	0.599*	NS	0.824**
PL	0.878**	LB <sub>2</sub>	0.832**	NB1	0.836**
CL	0.732**	LB <sub>3</sub>	0.703**	NB2	0.284
PL/PH	0.755**	LS <sub>1</sub>	0.918**	B1L	0.529
In <sub>1</sub>	0.713**	LS <sub>2</sub>	0.853**	SL	0.706*
In <sub>2</sub>	0.766**	LS <sub>3</sub>	0.848**	SW	0.432
In <sub>3</sub>	0.584*	WF	0.772**	ED	0.879**
In <sub>1</sub> /CL	0.441	W2	0.825**	SI <sub>1</sub>	0.777**
In <sub>2</sub> /CL	0.817**	CD	0.872**	SI <sub>2</sub>	0.590
In <sub>3</sub> /CL	0.606*	PN	0.944**		

\*, \*\* Significant at the 5% and the 1% levels, respectively.

counterparts. A relatively stable reduction was obtained in the index of spikelet size (SI<sub>1</sub>) through the haploid lines, while the index of spikelet shape (SI<sub>2</sub>) in the haploid dwarf lines indicated variable reduction rates. It is noted that the spikelets of ID-2 (*d*-2 type) at haploid turned out to be small and roundish in comparison with the diploid counterpart.

The correlation coefficients between haploids and diploids by using the 11 near-isogenic lines except the two variants, ID-7b and ID-11b were calculated as shown in Table 9. There were no correlations in the percentage of the first internode, In<sub>1</sub>/CL and the spikelet shape index SI<sub>2</sub>, while PL, In<sub>2</sub>/CL and ED showing the features of dwarf types indicated a high correlation between haploids and diploids. As a whole, a significant correlation was recognized between haploids and diploids throughout most of the characters.

#### 4. Interrelations among morphological characters at haploid level

The morphogenesis at haploid was compared with that at diploid by examining the correlations among the characters related to panicle, culm and leaf. Two haploid variants, ID-7b and ID-11b were excluded from the calculation. As shown in Tables 10 and 11, close correlations found between the characters at haploid, coincided with those at diploid in most of the cases. As a discrepancy, it is noted that the correlation between first internode and first leaf blade lengths found in haploid was not significant in diploid, while the relation between the length and width of spikelet was detected in haploid.

TABLE 10. Correlation matrix of the isogenic lines at diploid (upper diagonal) and haploid (below diagonal) levels-1

	PL	CL	In <sub>1</sub>	In <sub>2</sub>	In <sub>3</sub>	LB <sub>1</sub>	LB <sub>2</sub>	LB <sub>3</sub>	LB <sub>1</sub>	LS <sub>2</sub>	LS <sub>3</sub>	WF
PL		0.31	0.06	0.13	0.55	0.61*	0.58	0.77**	0.81**	0.75**	0.65*	0.057
CL	0.19		0.80**	0.84**	0.83**	0.70*	0.79**	0.73*	0.64*	0.78**	0.81**	-0.037
In <sub>1</sub>	0.42	0.68*		0.60	0.48	0.58	0.58	0.42	0.57	0.55	0.54	-0.009
In <sub>2</sub>	0.03	0.94**	0.44		0.49	0.49	0.73*	0.63*	0.40	0.66*	0.70*	-0.177
In <sub>3</sub>	0.10	0.92**	0.38	0.92**		0.71*	0.69*	0.74**	0.65*	0.72*	0.74**	0.093
LB <sub>1</sub>	0.61*	0.81**	0.82**	0.65*	0.62*		0.92**	0.77**	0.79**	0.83**	0.81**	0.398
LB <sub>2</sub>	0.63*	0.69*	0.47	0.54	0.68*	0.80**		0.90**	0.78**	0.92**	0.94**	0.224
LB <sub>3</sub>	0.63*	0.43	0.16	0.33	0.51	0.53	0.93**		0.85**	0.95**	0.96**	0.035
LS <sub>1</sub>	0.86**	0.57	0.77**	0.36	0.35	0.87**	0.74**	0.58		0.93**	0.84**	0.191
LS <sub>2</sub>	0.79**	0.72*	0.64*	0.58	0.60	0.90**	0.89**	0.75**	0.92**		0.96**	0.125
LS <sub>3</sub>	0.64*	0.74**	0.49	0.61*	0.72*	0.80**	0.97**	0.88**	0.77**	0.94**		0.055
WF	0.03	0.31	0.28	0.11	0.42	0.31	0.42	0.29	0.13	0.21	0.35	

\*, \*\* Significant at the 5% and the 1% levels, respectively.

TABLE 11. Correlation matrix of the isogenic lines both at diploid (upper diagonal) and haploid (below diagonal) levels-2

	CL	UI	PN	NS	NB1	NB2	B1L	SL	SW
CL		-0.43	-0.31	0.75**	0.71*	0.61*	0.66	0.28	0.18
UI	-0.55		0.42	-0.45	-0.76**	-0.22	-0.14	0.32	-0.43
PN	-0.35	0.42		-0.54	-0.57	-0.45	-0.39	-0.03	-0.78**
NS	0.79**	-0.66*	-0.40		0.70*	0.93**	0.62*	-0.01	0.45
NB1	0.74**	-0.74**	-0.34	0.64*		0.41	0.58	0.12	0.52
NB2	0.68*	-0.46	-0.26	0.95**	0.44		0.48	-0.12	0.37
B1L	0.47	-0.30	-0.12	0.57	0.06	0.59		0.68*	0.28
SL	0.17	-0.29	0.14	0.02	0.24	0.05	0.09		-0.21
SW	0.53	-0.45	-0.27	0.35	0.62*	0.32	-0.05	0.73*	

\*, \*\* Significant at the 5% and the 1% levels, respectively.

### 5. Induced variants

Haploid variants were found in the lines, ID-7 and ID-11. One of them, ID-7b is characterized by the extreme shortening of the second internode which was not expressed in the original line at diploid. The other variant, ID-11b possessed a different feature characterized by reduced length of panicle. In addition, the sister line, ID-11a at haploid also showed a modified pattern of the lower internodes in comparison with the original diploid. It is uncertain that these variants are caused by point mutation, chromosome aberration or other causes.

### Discussion

Before the invention of anther culture, KATAYAMA and NEI<sup>8)</sup> made an extensive review on the haploidy of higher plants. They predicted the importance of haploidy method of breeding using pollen culture or other methods.<sup>8,9,17)</sup> They also mentioned using haploidy for the study of morphogenesis.<sup>8)</sup> For this study, the authors produced a series of haploid dwarf lines from the near-isogenic lines of dwarf types at diploid. In anther culture, there was a considerable variation of callus formation among the dwarf lines at diploid. It is supposed that the high percentage of callus formation in ID-18<sup>b</sup> is caused by the synclonization of the uninucleate stage of microspores in the anthers of this extreme dwarf. Albino, mixoploidy and variants were also induced by the culture besides haploids and diploids. A series of haploid

dwarf lines was obtained from 11 kinds of near-isogenic lines.

Haploids in rice were first found by MORINAGA and FUKUSHIMA<sup>14)</sup> and used for cytogenetical studies.<sup>5,7,13,22)</sup> From the studies of haploids from diverse sources, it is generally accepted that haploid plants are characterized by dwarfness, diminution of various organs, awnless and small spikelets and sterility.<sup>11,16,22)</sup> There is a theory that the cell size is basically determined by the ploidy levels.<sup>6)</sup> In haploid rice, the ratio of cell volume to that of diploid was recognized as 1:1.26 or near to  $\sqrt[3]{2}$  in some tissues, but propagation of the cells in respective tissue was much influenced by other factors and produced the deviated ratios.<sup>16,22)</sup> It is plausible that the decrease of the characters shown in the haploids of ID-S (Shiokari) is caused by the effect of a chromosome complement (haploidy). On the other hand, the various dwarf genes show their main and side effects to the various characters related to plant stature at diploid, such as dwarfness, profuse tillers and short round grains. In the haploid regenerated plants, it was possible to discriminate each dwarf type due to the features shown in the diploid level except for the variants induced by the culture. Thus it was demonstrated that the dwarf genes insert their effects at haploid as well as at diploid. However, the variation of plant height and culm length among the various dwarf types at haploid were not parallel with those at diploid. In other words, there was a significant interaction between the haploidy and the dwarf genes in the character expressions of the haploid dwarf lines. There was a considerable variation among the relative numbers of haploid dwarf lines to the corresponding diploids in most of the characters. The characters investigated were arbitrarily classified into the *size*, *number* and *ratio* characters. In the *size* characters, there is a tendency to decrease though the reduction rates of characters varied among the different dwarf lines at haploid. In the *number* characters such as panicle number and number of spikelets, the characters of the haploid dwarf lines increased more than those of diploids due to the luxuriant growth accompanied by complete sterility as shown in haploid tomatoes.<sup>12)</sup> In the various kinds of *ratios*, the relative numbers of the haploids varied considerably depending on the dwarf types. As to the two indices which show the feature of the internode distribution pattern, a prominent fluctuation from the original diploid occurred in the variants induced by the anther culture.

As shown in the correlations between the characters, there was an intimate relation between the character expressions at haploid and diploid. Although the major gene action expressed at haploid level was prominent, the fluctuation of the characters by haploid was not homogeneous through

the various dwarf types. In addition, there is a problem on the dosage effect caused by the respective dwarf genes. According to OKUNO<sup>19</sup>, the amount of amylose was not linearly proportional to the dosage increase of *wx* alleles. Therefore, it is probable that the different dosage effects by the various dwarf genes at haploid are responsible for the variation of the characters causing the different rates of reduction from the corresponding diploid dwarf types. Because the variation of haploid dwarf types was not always parallel with that of the diploid types, it is desirable to carry on the selection of the anther-derived plants after the chromosome doubling in haploid method of breeding.

### Summary

A series of haploid dwarf lines were produced by anther culture from 19 near-isogenic lines having genetic background of cultivar, Shiokari. The morphogenesis of the haploid plants was investigated under comparison with that of the diploid plants. The results obtained are summarized as follows;

1. Callus formation in the different dwarf lines showed a considerable variation. It is supposed that the highest percentage of the callus differentiation in ID-18<sup>b</sup> is caused by the synclonization of microspore stage when excised from the short anther in this extreme dwarf.

2. Plants regenerated from the pollen calli contained albino, mixoploidy and variants besides the haploid and diploid plants. 13 kinds of haploid dwarf lines were established after the vegetative propagation of regenerated plants.

3. The morphological features of dwarf genes were expressed in haploids irrespective of the diminishing effect of haploidy. It was possible to discriminate the respective dwarf types at haploid level as well as at diploid.

4. In the preliminary experiments, the reduction rates in the lengths of panicle and culm in haploids varied among the different dwarf types, while the shape indices of spikelet and stoma was rather consistent between haploids and diploids.

5. Though the features of dwarf types were retained in haploid, the variations of plant height and culm length among the different dwarf types at haploid were not parallel to those at diploid level. There was a significant interaction between the effects of haploidy and dwarf genes.

6. In the panicle number and number of spikelets, haploid plants indicated some increase from the diploid counterparts showing a rather luxuriant growth due to the side effects of complete sterility.

7. As to the indices related to the internode distribution pattern, a stable relation was maintained between haploid and diploid, except in the induced

variants.

8. The correlation coefficients between haploid and diploid dwarf lines were highly significant in most of the characters. Thus, it is indicated that the dwarf genes which were found in diploids insert their similar effects for the morphogenesis of haploids.

9. Most of the character correlations at haploid level coincided with those at diploid level. However, it is noted that the correlation between the lengths of first internode and first leaf blade found in haploid was not significant in diploid, while a relation between the length and the width of spikelet was found in haploid.

10. Three haploid variants were induced by the anther culture. The internode distribution patterns were modified in the variants suggesting the mutation anther culture.

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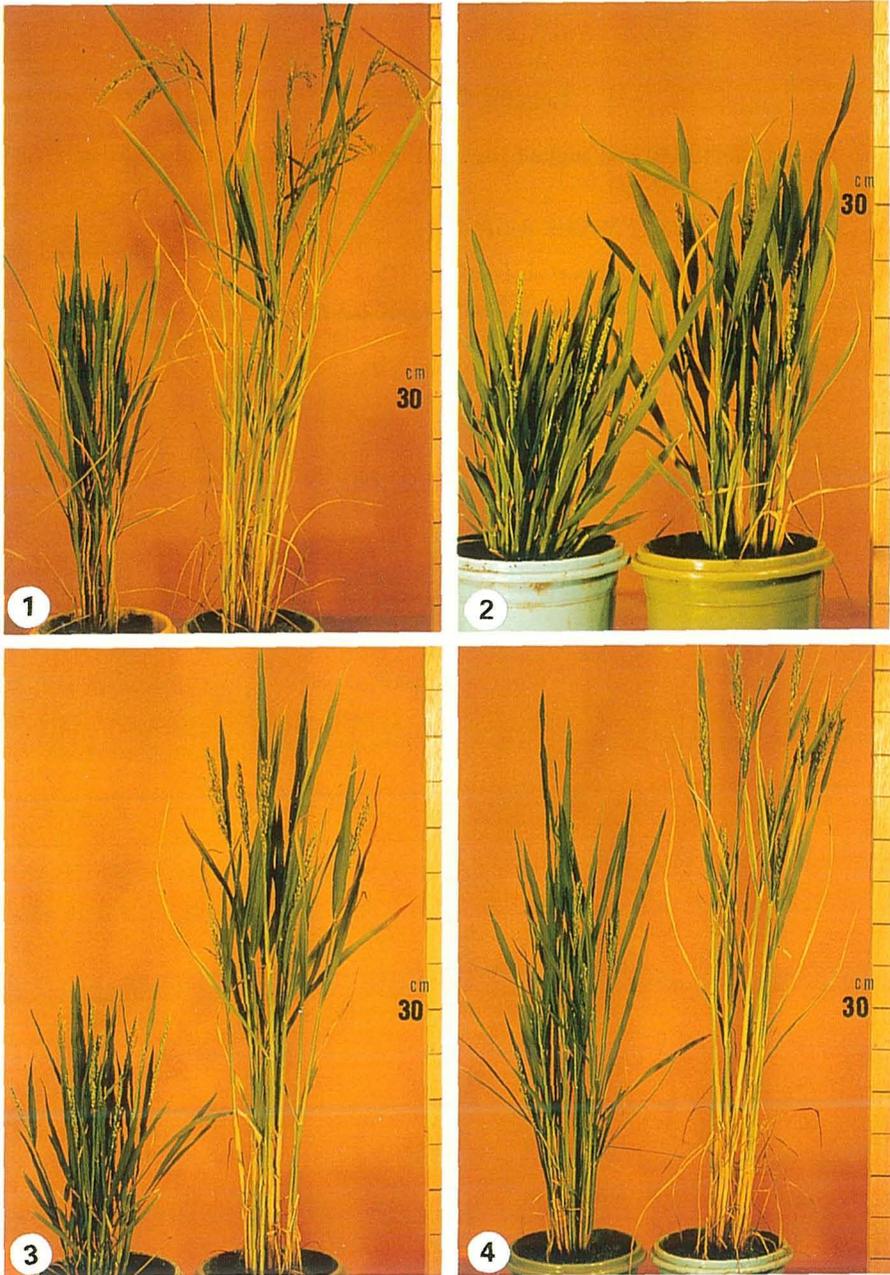
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### **Legend for Plate I**

1. Haploid (left) and diploid (right) of ID-S Shiokari (one scale means 5 mm).
2. Ditto of ID-1 Daikoku dwarf (*d-1*).
3. Ditto of ID-2 Ebisu dwarf (*d-2*).
4. Ditto of ID-7 a Cleistogamous dwarf (*d-3*).



### **Legend for Plate II**

5. Haploid (left) and diploid (right) of ID-10 Toyohikari-bunwai (tillering dwarf, *d-10*).
6. Ditto of ID-12 Yukara dwarf (*d-12*).
7. Ditto of ID-18<sup>h</sup> Hosetsu dwarf (*d-18<sup>h</sup>*).
8. Ditto of ID-47 Dee-geo-woo-gen dwarf (*sd-1*).

