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Photoperiod-sensitive cytoplasmic male sterile elite lines for hybrid wheat breeding, showing high cross-pollination fertility under long-day conditions

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Abstract Photoperiod-sensitive cytoplasmic male sterility (PCMS) caused by *Aegilops crassa* cytoplasm under long-day (LD) photoperiods (≧15 h) has been proposed as a two-line system for producing hybrid varieties in common wheat (*Triticum aestivum*). The PCMS line is maintained by self-pollination under short-day (SD) conditions, and hybrid seeds can be produced through outcrossing of the PCMS line with a pollinator line under LD conditions. Maintainer lines of the PCMS lines are not necessary in this system. In our previous study, we developed two PCMS lines with the genetic background of the Japanese wheat cultivar 'Fukuotome', which showed high male sterility under LD conditions and high seed fertility under SD conditions. These PCMS lines were from a BC_2 generation and were not genetically pure. Therefore, we screened the progeny of the BC_2 lines, and identified BC_2F_8 lines that showed high male sterility and high cross-pollination fertility under LD conditions and high seed fertility under SD conditions. Furthermore, we screened the pollinator lines suitable for the PCMS system. These PCMS elite lines and selected pollinator lines will be useful for developing hybrid wheat varieties.

Keywords *Aegilops crassa* · CMS · Hybrid wheat · Photoperiod-sensitive cytoplasmic male sterility · *Triticum aestivum* · Wheat

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Abbreviation

PCMS Photoperiod-sensitive cytoplasmic male sterility

Introduction

Although bread wheat (*Triticum aestivum*) is an autogamous plant species, heterosis (hybrid vigor) has been observed in hybrid wheat varieties. For example, in a previous report using Japanese wheat cultivars, we demonstrated that 23 F_1 hybrids produced an average of 40 % more than the mid-parent yield (Murai 1997a). Recently, Longin et al. (2012)

summarized a number of reports and found that heterosis in wheat could range from 3.5 to 15 % in commercial hybrid wheat varieties. A report from the International Maize and Wheat Improvement Center (CIMMYT) in 2011 concluded that hybrid wheat is a promising approach for improving yield potential and stability and for pyramiding stress tolerances (CIMMYT 2011). They suggested that by 2030 a minimum of 15 million hectares of hybrid wheat will be grown in developing countries with a 15 % yield increase over a 4 ton per hectare average yield. They also pointed out that development of cost-effective hybrid wheat systems is necessary.

The main hybrid wheat-growing countries in Europe are France (160,000 ha) and Germany (25,000 ha) that use chemical hybridization agents (CHAs) to produce hybrids (Longin et al. 2012). In Asia, hybrid wheat is mainly cultivated in China (30,000 ha) and India (35,000 ha). Cytoplasmic male sterility (CMS) systems based on *Triticum timopheevii* cytoplasm is utilized in these countries. In China, genic male sterility (GMS) systems are also used for hybrid seed production. Although hybrid wheat breeding programs have been established for several decades, hybrid wheats still represent only a small fraction of the total wheat sown. Whitford et al. (2013) identified various factors that have limited the use of hybrid wheat: (1) CMS systems are difficult to use due to a lack of effective fertility-restoration genes, (2) GMS systems have largely failed due to problems with fertility restoration, and (3) CHA systems still suffer from problems of toxicity and selectivity.

We proposed an alternative approach for hybrid wheat production using a simple "two-line system" based on photoperiod-sensitive cytoplasmic male sterility (PCMS) resulting from the presence of *Aegilops crassa* cytoplasm (Murai 2001a). PCMS is induced by long-day (LD) conditions of 15 h or longer light periods during the floret differentiation stage. PCMS is expressed in the form of homeotic transformation of stamens into pistilloid (pistil-like) structures that is mediated by the *A. crassa* cytoplasm (Murai et al. 2002). Using the PCMS system, hybrid seeds can be produced through outcrossing of a PCMS line with a pollinator line under long-day conditions ($\geqq$15 h light, the natural day length for spring-sown wheat in Hokkaido, Japan). In contrast to the "three-line system" using *T. timopheevii* cytoplasm (Johnson and Schmidt 1968), the two-line system does not need a maintainer of male sterility because the PCMS line will grow and multiply by self-pollination under short-day (SD) conditions ($\leqq$14.5 h light, natural day length for autumn-sown wheat in Honshu, Shikoku and Kyushu, Japan). The PCMS system has the further advantage over other CMS systems of a relatively high level of fertility restoration due to the synergistic effects of SD environmental conditions and effective fertility-restoring gene(s) (Murai 1997a).

We sought to develop practical PCMS lines that show high male sterility under LD conditions and high seed setting rates under SD conditions and produced two lines, #6(11)-3 (PCMS 7 line) and #7(12)-2 (PCMS 8 line); (cr)-Norin 26/Fujimikomugi//3*Fukuotome, that fulfilled these criteria (Murai et al. 2008). The alloplasmic line of the Japanese wheat cultivar 'Norin 26' with *A. crassa* cytoplasm ((cr)-Norin 26) shows high seed fertility under SD conditions, whereas the alloplasmic line of Fujimikomugi ((cr)-Fujimikomugi) is completely male sterile under LD conditions. 'Fukuotome' is an elite cultivar used for production of Japanese noodles in the western region of Japan. These PCMS lines were the BC_2 generation and were not genetically homogenous lines (Murai et al. 2008). From the progenitors of the PCMS 7 and PCMS 8 lines, we selected 14 and 18 lines in the BC_2F_8 generation and examined their agronomic characters under LD conditions at Hokkaido and SD conditions at Fukui. Based on the obtained data, we identified eight promising elite PCMS lines that showed high cross pollination fertility and high male sterility under LD conditions and high seed fertility under SD conditions.

Materials and methods

Plant materials

Thirty-two PCMS lines were used in this study. These lines have a 'Fukuotome' cultivar genetic background, namely (cr)-Norin 26/Fujimikomugi//9*Fukuotome, and were developed from the BC_2 lines #6(11)-3 (PCMS 7 line) and #7(12)-2 (PCMS 8 line) (Murai et al. 2008). As controls, cv. 'Fortunato I. Bo. 219' and 'Fukuotome' were used in Hokkaido and Fukui, respectively. 'Fortunato I. Bo. 219' is an Italian hard red spring wheat cultivar and a candidate of the

pollinator lines for the PCMS system; this line was also used as a pollinator line in crosses to examine the cross-pollination ability in the PCMS lines under LD conditions at Hokkaido.

For screening of pollinator lines suitable for the PCMS system, five alloplasmic lines of hard red spring wheat cultivars were used: (cr)-Norin 26/2*Yumeshiho, (cr)-Norin 26/3*Kitami-haru 63, (cr)-Norin 26/3*Fortunato I. Bo. 219, (cr)-Norin 26/3*Aroora, and (cr)-Norin 26/3*Wildcat, which are described as (cr)-Yumeshiho, (cr)-Kitami-haru 63, (cr)-Fortunato I. Bo. 219, (cr)-Aroora, and (cr)-Wildcat, respectively, in the text. The alloplasmic line of Fukuotome, (cr)-Norin 26/6*Fukuotome BC_5F_3 (described as (cr)-Fukuotome), which has no *Rf* gene was used as a comparison of fertility restoration under SD conditions in the alloplasmic lines of pollinator candidates.

Field trials in Hokkaido to examine agronomic characters of the PCMS lines

In April 2015, crossing blocks for the 32 PCMS lines (PCMS 7#1-#14, PCMS 8#1-#18) and a pollinator line, 'Fortunato I. Bo. 219', were set up at the experimental field of Kitami Agricultural Experimental Station, Hokkaido. The PCMS lines were planted at a 3.5 cm spacing in a single row which was surrounded on each side by a row of pollinators planted at a 2 cm spacing.

At the heading stage, the frequency of pistillody was determined in one ear from each of the PCMS 7 and PCMS 8 lines. The frequency of pistillody (%) was measured as the proportion of pistilloid stamens in the total stamens of the first and second florets in all spikelets in an ear. Culm length (cm) was also measured using the main shoot of three plants in each PCMS 7 and PCMS 8 line.

Five characters were studied in the ear of the main shoot of three plants in each PCMS 8 line (PCMS 8#1-#18): ear length (cm), spikelet number per ear, grain number per ear, grain number per spikelet, and open pollination fertility (%). Grain number per spikelet was based on the total number of seeds and spikelets in each ear. Open pollination fertility (%) was measured as the seed setting rate of the first and second florets of all spikelets. Three ears in each PCMS 8 line were bagged before flowering, and self-fertility (%) was measured as the seed setting rate. The data for each character were obtained from three plants in each line; the means for each character were used in the analyses of variance.

The 1000-grain weight (g) and volume weight (g/L) were estimated from the grain number, grain weight and grain volume of five ears in PCMS 8#12 and 'Fortunato I. Bo. 219'.

Field trials for examining agronomic characters of the PCMS lines in Fukui

Eighteen PCMS 8 lines (PCMS 8#1-#18) were grown in the season 2014/2015 at the experimental field in Fukui Prefectural University. The plants were grown with a spacing of 10 cm in one row. Six characters were measured in each PCMS line and in 'Fukuotome': culm length (cm), ear length (cm), spikelet number per ear, grain number per ear, grain number per spikelet and self-fertility (%). With the exception of self-fertility, the characters were measured using the main shoot and its ear in each plant. The ear of the second shoot of each plant was bagged before flowering, and self-fertility was estimated by the seed setting rate of the first and second florets of all spikelets. The data for each character were obtained from five plants in each line; the means for each character were used in the analyses of variance (ANOVAs).

Screening of pollinator lines suitable for the PCMS system

Six alloplasmic lines ((cr)-Yumeshiho, (cr)-Kitami-haru 63, (cr)-Fortunato I. Bo. 219, (cr)-Aroora, (cr)-Wildcat, and (cr)-Fukuotome) and the corresponding euplasmic lines were grown in the season 2010/2011 at the experimental field in Fukui Prefectural University. Using ears bagged before flowering, self-fertility (%) and grain number per spikelet were measured. The means for them were used in the analyses of variance (ANOVA).

Statistical analyses

We screened for significant differences among the PCMS lines and the controls using an analysis of variance (ANOVA). Differences at the $P < 0.05$ level were considered significant. We also used a *t* test to compare seed characters in PCMS 8#12 and the control.

Results

Culm length and frequency of pistillody in the PCMS lines under LD conditions

The mean culm lengths in 32 PCMS lines (PCMS 7#1-14, PCMS 8#1-18) and in the 'Fortunato I. Bo. 219' pollen parent, grown under LD conditions in Hokkaido, are shown in Table 1. PCMS 7 lines were consistently taller than 'Fortunato I. Bo. 219', and in 8 lines the difference was significant. PCMS 8 lines were shorter than 'Fortunato I. Bo. 219', indicating that they were more suitable for outcrossing as it is better that PCMS lines are shorter than the pollinator line.

The PCMS 7 lines exhibited lower frequencies of pistillody than the PCMS 8 lines (Table 1). Based on plant heights and frequency of pistillody, we excluded PCMS 7 lines as candidates for the elite PCMS lines. Among PCMS 8 lines, PCMS 8#4, #5, #7, and #13 had a frequency of pistillody of <55 % and were also excluded. The pistilloid stamen and pistil of a PCMS line together with the pistil of a normal line ('Fortunato I. Bo. 219') are shown in Fig. 1a–c.

Table 1 Culm length and frequency of pistillody in PCMS 7 and PCMS 8 lines under long-day conditions at Hokkaido

Line	Culm length (cm)		Pistillody (%)
Fortunato	78.4 ± 0.93		0.0
PCMS 7#1	81.0 ± 1.53		22.2
PCMS 7#2	79.3 ± 1.67		40.9
PCMS 7#3	86.7 ± 2.33**	High	15.9
PCMS 7#4	82.3 ± 1.45		30.0
PCMS 7#5	80.7 ± 1.20		27.8
PCMS 7#6	84.7 ± 1.76**	High	25.3
PCMS 7#7	85.3 ± 2.33**	High	13.6
PCMS 7#8	81.3 ± 0.88		22.2
PCMS 7#9	86.3 ± 1.45**	High	21.2
PCMS 7#10	89.3 ± 0.88**	High	19.2
PCMS 7#11	83.7 ± 1.33*	High	28.9
PCMS 7#12	88.3 ± 1.67**	High	19.4
PCMS 7#13	83.0 ± 1.73*	High	14.8
PCMS 7#14	81.0 ± 1.00		25.3
PCMS 8#1	74.3 ± 2.03		95.7
PCMS 8#2	70.3 ± 1.67**	Low	63.3
PCMS 8#3	77.7 ± 0.88		77.8
PCMS 8#4	72.7 ± 1.86**	Low	33.3
PCMS 8#5	77.0 ± 2.52		52.7
PCMS 8#6	74.7 ± 0.33		58.9
PCMS 8#7	74.3 ± 2.40		50.0
PCMS 8#8	76.0 ± 2.52		57.5
PCMS 8#9	73.7 ± 1.20*	Low	67.8
PCMS 8#10	73.0 ± 0.56*	Low	75.0
PCMS 8#11	79.0 ± 2.52		57.4
PCMS 8#12	75.3 ± 2.03		79.8
PCMS 8#13	74.7 ± 1.45		47.9
PCMS 8#14	73.0 ± 1.53*	Low	67.8
PCMS 8#15	74.7 ± 0.33		77.4
PCMS 8#16	72.7 ± 2.33**	Low	90.8
PCMS 8#17	74.7 ± 1.76		94.0
PCMS 8#18	76.3 ± 0.67		77.1

* and ** Significantly different at 5 and 1 % level, respectively, compared with 'Fortunato I. Bo. 219' in an ANOVA

Agronomic characters of the PCMS 8 lines under LD conditions

We measured five agronomic characters in the PCMS 8 lines and 'Fortunato I. Bo. 219' (Table 2). The data was analyzed by an analysis of variance, which indicated that 10 PCMS lines had significantly shorter ears and 8 lines had a smaller spikelet number per ear than 'Fortunato I. Bo. 219'. All PCMS lines showed no self-fertility (Table 2), although the frequencies of pistillody were not 100 % (Table 1). This indicated that the PCMS lines produced non-pistilloid stamens together with pistilloid stamens, and that the non-pistilloid stamens did not have functional pollen, i.e., they were male sterile. As the PCMS lines were completely male sterile, then any grains in the ears of PCMS lines must be the result of out-crossing to the pollinator line. Therefore, cross-pollination ability was estimated from the data on grain number per spikelet and open pollination fertility (Table 2). An ANOVA identified significant differences among PCMS lines for cross-pollination ability. As a result, PCMS 8#2 and #16, which had <20 grains per ear, were excluded as candidates for elite PCMS lines.

Agronomic characters of the PCMS 8 lines under SD conditions

We measured six agronomic characters in PCMS 8 lines and 'Fukuotome', as a control, grown under SD conditions in Fukui (Table 3). The PCMS lines had a

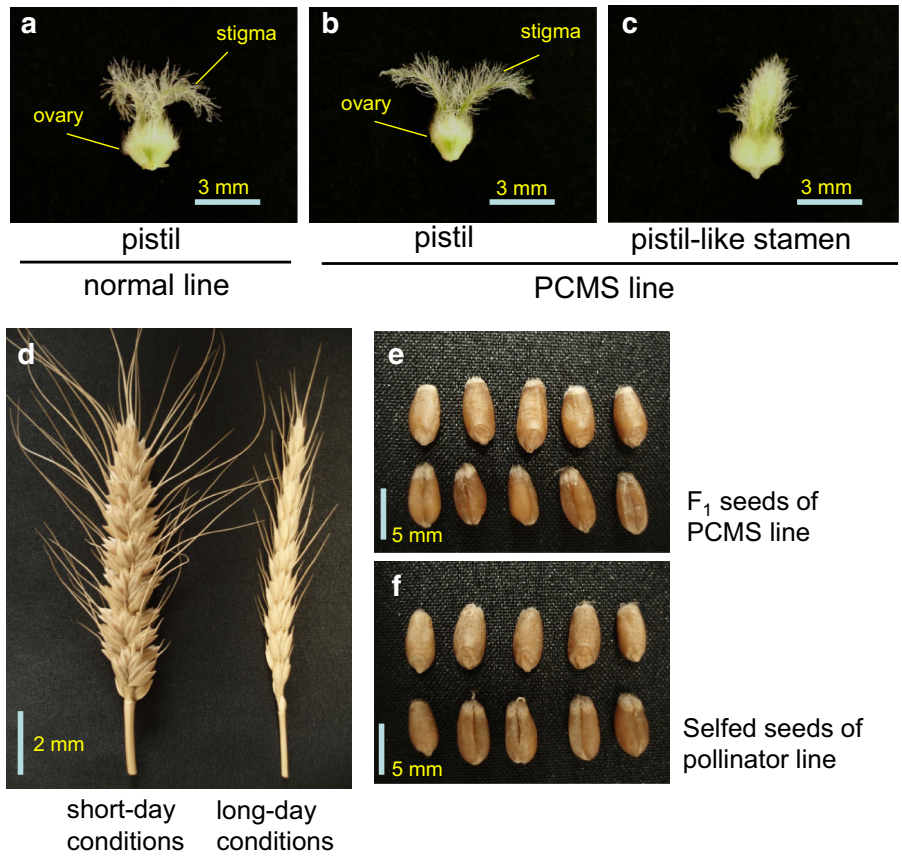

Fig. 1 **a** Pistil of normal line. **b** and **c** Pistil and pistilloid (pistil-like) stamen of PCMS line. **d** Ears of PCMS line grown under short-day and long-day conditions. **e** F_1 seeds obtained by cross-pollination between PCMS line and pollinator line under long-day conditions. **f** Selfed seeds of pollinator line under long-day conditions

'Fukuotome' genetic background. Six PCMS lines had significantly shorter ears and two lines had a smaller spikelet number per ear than 'Fukuotome'. Elite PCMS lines are expected to have high self-fertility under SD conditions because of efficient seed formation. We excluded PCMS 8#4, #5, #7, #9, #11, #13, #14, #15, #16 and #18 as candidate elite lines as they showed significantly smaller grain numbers per ear compared with the highest value of 54.7 seen in PCMS 8#3 (Table 3).

Screening candidate elite PCMS lines

Based on the data obtained under LD conditions at Hokkaido and SD conditions at Fukui, our selection procedure left PCMS 8#1, #3, #6, #8, #10, #12 and #17 as candidate elite lines. The characteristics of a selfed ear in a PCMS line under LD or SD conditions are shown in Fig. 1d. Among the selected seven lines, PCMS 8#12 was most promising as it showed the highest open pollination fertility (cross-pollination ability) under LD conditions (Table 2) and had a high level of self-fertility under SD conditions (Table 3). PCMS 8#12 produced good quality F_1 seeds under LD conditions after cross-pollination; these seeds were similar to the selfed seeds of a normal line (Fig. 1e, f). The grains of PCMS 8#12 exhibited higher 1000-grain and volume weights than the pollinator cultivar 'Fortunato I. Bo. 219' (Table 4).

Screening pollinator lines suitable for the PCMS system

Comparison of self-fertility (%) and grain number per spikelet between euplasmic and alloplasmic lines of Fukuotome under SD conditions were shown in Fig. 2. The alloplasmic (cr)-Fukuotome exhibited significantly lower fertility (<60 %) and smaller grain number per spikelet (<1.5) compared with the euplasmic Fukuotome. As Fukuotome has no *Rf* gene(s) against the PCMS, it was fertile but showed low fertility under SD conditions. Contrary to Fukuotome, differences of self-fertility between euplasmic and alloplasmic lines in five cultivars (Yumeshiho,

Table 2 Means ± SE of agronomic characters in the PCMS 8 lines open pollinated by the pollinator line 'Fortunato I. Bo. 219' under long-day conditions at Hokkaido

Line	Ear length (cm)[a]	Spikelet no. per ear[a]	Grain no. per ear[b]	Grain no. per spikelet[b]	Open pollination fertility (%)[b]	Cross-pollination ability	Self-fertility (%)
Fortunato	7.9 ± 0.30	16.6 ± 0.25	48.2 ± 2.25	2.90 ± 0.10	98.2 ± 0.74	nd	nd
PCMS 8#1	6.4 ± 0.17**	15.6 ± 0.25	22.6 ± 0.51**	1.45 ± 0.04**	60.9 ± 1.97**	Medium	0
PCMS 8#2	6.8 ± 0.29**	13.8 ± 0.58**	17.4 ± 1.21**	1.27 ± 0.09**	56.9 ± 2.98**	Low	0
PCMS 8#3	6.5 ± 0.10**	14.6 ± 0.51**	20.8 ± 0.66**	1.43 ± 0.01**	63.8 ± 1.75*	Medium	0
PCMS 8#4	7.8 ± 0.08	15.6 ± 0.25	22.2 ± 0.80**	1.42 ± 0.04**	60.2 ± 2.31**	Medium	0
PCMS 8#5	7.6 ± 0.18	16.2 ± 0.2	22.2 ± 0.80**	1.37 ± 0.06**	61.2 ± 2.37**	Low	0
PCMS 8#6	7.0 ± 0.08**	15.4 ± 0.25*	27.0 ± 0.63[ST]	1.75 ± 0.04[ST]	70.2 ± 2.09	High	0
PCMS 8#7	7.2 ± 0.17*	15.6 ± 0.25	22.8 ± 1.16**	1.46 ± 0.08**	62.2 ± 2.82**	Medium	0
PCMS 8#8	8.4 ± 0.08	16.0 ± 0.00	25.4 ± 1.17	1.59 ± 0.07	64.4 ± 3.20*	High	0
PCMS 8#9	7.9 ± 0.22	16.0 ± 0.32	21.0 ± 1.64**	1.44 ± 0.08**	58.7 ± 2.70**	Medium	0
PCMS 8#10	6.9 ± 0.25**	15.4 ± 0.60*	26.2 ± 1.28	1.70 ± 0.07	68.0 ± 3.44	High	0
PCMS 8#11	7.0 ± 0.67**	16.2 ± 0.37	25.6 ± 1.12	1.58 ± 0.06	65.3 ± 1.42*	High	0
PCMS 8#12	7.3 ± 0.26	14.2 ± 0.58**	23.6 ± 0.93*	1.67 ± 0.07	72.6 ± 2.86[ST]	High	0
PCMS 8#13	7.5 ± 0.19	16.0 ± 0.00	25.2 ± 1.02	1.57 ± 0.06	64.4 ± 2.53*	High	0
PCMS 8#14	7.9 ± 0.10	15.2 ± 0.37*	21.8 ± 0.74**	1.44 ± 0.07**	62.6 ± 2.44**	Medium	0
PCMS 8#15	6.6 ± 0.36**	14.4 ± 0.51**	20.2 ± 1.91**	1.41 ± 0.15**	58.5 ± 2.88**	Medium	0
PCMS 8#16	7.1 ± 0.24**	14.0 ± 0.55**	13.6 ± 0.51**	0.98 ± 0.04**	44.5 ± 2.36**	Low	0
PCMS 8#17	7.7 ± 0.20	15.6 ± 0.25	21.8 ± 0.86**	1.40 ± 0.06**	62.8 ± 2.39**	Medium	0
PCMS 8#18	6.7 ± 0.12**	15.6 ± 0.25	23.6 ± 1.03*	1.52 ± 0.07*	64.7 ± 2.06*	High	0

[a] * and ** Significantly different at 5 and 1 % level, respectively, compared with Fortunato I. Bo. 219 in an ANOVA

[b] * and ** Significantly different at 5 and 1 % level, respectively, compared with highest value (ST) in an ANOVA

nd indicates no data

Table 3 Means ± SE for agronomic characters in the PCMS 8 lines self-pollinated under short-day conditions at Fukui

Line	Culm length (cm)[b]	Ear length (cm)[a]	Spikelet no. per ear[a]	Grain no. per ear[b]	Grain no. per spikelet[b]	Self-fertility (%)[b]
Fukuotome	nd	8.4 ± 0.24	16.7 ± 0.42	61.5 ± 3.27	3.68 ± 0.13	98.4 ± 1.12
PCMS 8#1	63 ± 1.2	7.5 ± 0.09**	15.4 ± 0.30*	44.3 ± 3.05	2.81 ± 0.18	83.1 ± 3.69
PCMS 8#2	64 ± 2.3	8.7 ± 0.16	16.6 ± 0.20	43.9 ± 5.87	2.91 ± 0.35	80.3 ± 6.71
PCMS 8#3	63 ± 1.4	8.3 ± 0.26	16.2 ± 0.48	54.7 ± 6.29ST	3.22 ± 0.34ST	87.0 ± 6.08
PCMS 8#4	55 ± 1.9**	7.6 ± 0.35*	15.7 ± 0.57	28.7 ± 5.42**	1.97 ± 0.37**	63.6 ± 8.88**
PCMS 8#5	66 ± 1.3	8.1 ± 0.17	15.9 ± 0.34	32.4 ± 5.59**	2.02 ± 0.34**	61.3 ± 10.78**
PCMS 8#6	65 ± 1.3	7.9 ± 0.19	16.3 ± 0.33	42.5 ± 2.46	2.73 ± 0.20	81.2 ± 4.76
PCMS 8#7	65 ± 1.5	7.6 ± 0.17*	15.7 ± 0.18	40.3 ± 4.92*	2.62 ± 0.32	81.0 ± 7.32
PCMS 8#8	61 ± 2.1*	8.3 ± 0.24	16.2 ± 0.37	50.8 ± 4.26	3.13 ± 0.21	85.6 ± 4.16
PCMS 8#9	65 ± 1.9	8.6 ± 0.28	16.5 ± 0.29	36.3 ± 4.21*	2.25 ± 0.22*	71.8 ± 4.89
PCMS 8#10	62 ± 0.9*	7.7 ± 0.19*	16.0 ± 0.19	47.8 ± 2.09	3.02 ± 0.15	89.6 ± 2.96ST
PCMS 8#11	62 ± 2.2*	7.7 ± 0.19*	16.6 ± 0.37	38.0 ± 4.73**	2.36 ± 0.27*	77.3 ± 6.30
PCMS 8#12	67 ± 0.9ST	8.4 ± 0.16	16.3 ± 0.25	49.8 ± 3.28	3.00 ± 0.16	86.0 ± 2.94
PCMS 8#13	63 ± 1.7	7.8 ± 0.17	16.0 ± 0.22	38.9 ± 4.22*	2.40 ± 0.27*	74.4 ± 6.29
PCMS 8#14	62 ± 0.9*	8.0 ± 0.28	16.0 ± 0.38	41.1 ± 3.58*	2.52 ± 0.19	81.4 ± 4.78
PCMS 8#15	62 ± 0.7*	7.2 ± 0.13**	15.7 ± 0.18	38.3 ± 3.38*	2.36 ± 0.20*	78.9 ± 5.17
PCMS 8#16	65 ± 1.0	8.0 ± 0.15	15.7 ± 0.29	40.1 ± 4.31*	2.46 ± 0.28*	78.0 ± 5.37
PCMS 8#17	62 ± 1.1*	7.9 ± 0.16	15.3 ± 0.37**	48.9 ± 3.26	2.78 ± 0.16	86.9 ± 2.97
PCMS 8#18	57 ± 2.0**	8.0 ± 0.53	16.0 ± 0.68	28.2 ± 4.07**	1.72 ± 0.25**	63.5 ± 8.77**

Data for 'Fukuotome' were obtained from open-pollinated ears

[a] * and ** Significantly different at 5 and 1 % level, respectively, compared with 'Fukuotome' in an ANOVA

[b] * and ** Significantly different at 5 and 1 % level, respectively, compared with highest value (ST) in an ANOVA

nd indicates no data

Table 4 Means ± SE for seed characters of the PCMS 8#12 line compared with the normal line 'Fortunato I. Bo. 219'

Line	1000 grain weight (g)	Volume weight (g/L)
PCMS 8#12	40.4 ± 0.93	623 ± 13.6
Fortunato I. Bo. 219	38.3 ± 1.31	510 ± 18.6
t value	1.2924 ns	4.8412**

** Significantly different at 1 % level in a *t* test

Kitami-haru 63, Fortunato I. Bo. 219, Aroora, and Wildcat) were not significant (Fig. 2). Three of them, (cr)-Yumeshiho, (cr)-Forutunato I. Bo. 219 and (cr)-Aroora, showed the large grain number per spikelet as much as those in the corresponding euplasmic lines, suggesting that they contain strong *Rf* gene(s) against the PCMS lines. Heading date of Fortunato I. Bo. 219 was closest to that of (cr)-Fukuotome (Fig. 2). This suggests that Fortunato I. Bo. 219 is a most suitable as a pollinator line for the PCMS lines with Fukuotome genetic background.

Discussion

Hybrid wheat breeding systems are classified into four categories based on the method of fertility control: chemical hybridizing agents (CHAs); cytoplasmic male sterility (CMS); genic male sterility (GMS); and, genetic modification (GM) systems. The CHA system is a comparatively easy approach for developing hybrid wheat varieties because it does not require the development of three parental lines (a male sterile line with CMS cytoplasm, a maintainer line with

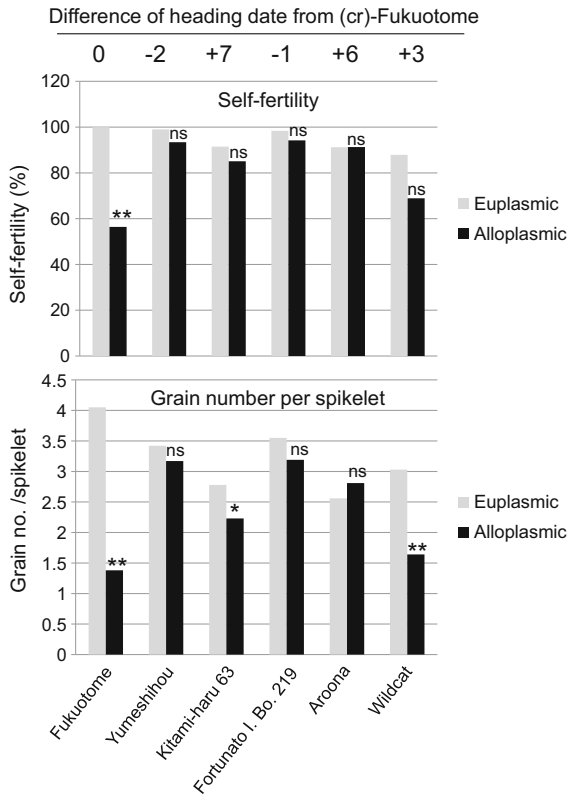

Fig. 2 Self-fertility and grain number per spikelet in the euplasmic and alloplasmic lines of Yumeshiho, Kitami-haru 63, Fortunato I. Bo. 219, Aroora, and Wildcat, compared with those of Fukuotome. * and ** indicate significantly different at 5 % and 1 %, respectively, between euplasmic and alloplasmic lines. The differences of heading date compared with (cr)-Fukuotome are indicated on the *top* of figure

normal cytoplasm, and a restorer line with an *Rf* gene). There is, however, still a problem of toxicity of CHAs; Croisor 100, provided by Saaten-Union, is the only CHA currently used for commercial hybrid seed production (Longin et al. 2012; Whitford et al. 2013). The CMS system is superior to the CHA system in seed production costs. However, the standard CMS system uses the cytoplasm of *T. timopheevii*, which has deleterious effects (Pickett 1993; Edwards 2001). Other CMS systems using *A. kotschyi*, *A. mutica* or *A. uniaristata* cytoplasm have been proposed and recently the *Rf* genes against these cytoplasms were identified in 1BS-1RS recombinant lines (Tsunewaki 2015); however, the utility of these alternative CMS systems is still unknown. A photo-thermo-sensitive GMS variety has been developed in China, but there are limited fertility restoring lines (Sun et al. 2001). A cytogenetic method using an additional chromosome 4E from *Elytrigia elongate* (Zhou et al. 2006) and an XYZ system using a non-conditional GMS mutant (Driscoll 1972) have been proposed but not used in practice. As detailed in the CIMMYT report, there is a need for the development of cost-effective and simple systems to allow world-wide expansion of hybrid wheat cultivation. Recently, several systems using genetic modification (GM) for hybrid breeding have been proposed (Whitford et al. 2013). Although molecular genetic-based technologies might improve hybrid production systems and reduce hybrid seed production costs, it is still not known whether GM systems for hybrid breeding would be accepted by consumers.

In a previous study, we reported that an alloplasmic line of the Japanese wheat cv. 'Norin 26', which carried the cytoplasm of *A. crassa*, showed PCMS (Murai and Tsunewaki 1993). This alloplasmic line was extensively studied in an attempt to utilize it for hybrid wheat breeding (Murai 2001a). Japanese wheat cultivars were classified into two groups based on the effect of *A. crassa* cytoplasm: carriers or non-carriers of the *Rf* gene against this cytoplasm (Murai and Tsunewaki 1995). For example, 'Norin 26' and 'Fujimikomugi' are *Rf* non-carrier cultivars, and 'Norin 61' and 'Ushiokomugi' are *Rf* carriers. Genetic analysis indicated that 'Norin 61' has multiple *Rf* genes located on at least four chromosomes, namely, 4A, 1D, 3D, and 5D (Murai 1997b). By recurrent back-crossing, we produced several alloplasmic lines of *Rf* non-carrier cultivars as PCMS lines (Murai and Tsunewaki 1995). F_1 hybrids between the PCMS lines and *Rf* carrier cultivars showed high restoration of fertility, indicating that the *Rf* genes were effective in the PCMS system for hybrid wheat breeding (Murai 1997a). We also reported two other types of *Rf* gene against PCMS. The first was a single dominant *Rf* gene in the cultivar 'Chinese Spring' located on the long arm of chromosome 7B; however, additional modifiers were present (Murai and Tsunewaki 1994; Murai et al. 2002). The second was a recessive *Rf* gene in a fertility-restoring mutant of alloplasmic 'Norin 26' (Murai et al. 1995). These findings suggest that PCMS induction and fertility restoration is a complicated phenomenon.

In this study, we developed several PCMS lines with Fukuotome genetic background. The PCMS lines are soft red spring wheat. As the pollinator lines for the

PCMS system, we screened hard red spring wheat cultivars. Hybrid wheat varieties between soft red wheat and hard red wheat produce wheat flour with middle property of soft wheat and hard wheat. Such hybrid wheat flour should be suitable for Japanese thin noodle, somen. In a screening of pollinator lines for the PCMS system, we found that Italian hard red wheat cultivar 'Fortunato I. Bo. 219' contained *Rf* gene(s) that was/were effective in fertility restoration against the PCMS; this cultivar was used as a pollinator line in this study.

Our previous studies revealed that the levels of male sterility under LD conditions and male fertility under SD conditions in PCMS lines are determined by the genotype of the nuclear donor. For example, the PCMS line cv. 'Norin 26' ((cr)-Norin 26) shows partial male sterility under LD conditions (Murai and Tsunewaki 1993; Murai 1998, 2001b), whereas that of cv. 'Fujimikomugi' ((cr)-Fujimikomugi) is completely male sterile under LD conditions (Murai and Tsunewaki 1995; Murai 2001b). However, PCMS cultivar 'Fujimikomugi' shows lower female fertility under LD conditions (Murai 2001b), and lower seed setting under SD conditions (Murai and Tsunewaki 1995).

PCMS lines that show high male sterility under LD conditions and high seed setting under SD conditions were developed in a previous study by screening the progeny of the F_1 between '(cr)-Norin 26' and 'Fujimikomugi' after backcrossing to the elite wheat cultivar 'Fukuotome' (Murai et al. 2008). This screen identified two PCMS lines, namely, (cr)-Norin 26/Fujimikomugi//3*Fukuotome #6(11)-3 and #7(12)-2, which were renamed here as PCMS 7 line and PCMS 8 line, respectively. These PCMS lines were from a BC_2 generation and were not genetically pure lines. By selfing and screening the progenies from lines PCMS 7 and PCMS 8 in the experimental field of Fukui Prefectural University, we developed 32 BC_2F_8 lines, PCMS 7#1-14 and PCMS 8#1-18. An important factor that contributes to the success of a hybrid wheat-breeding program is the efficient production of F_1 seeds by crossing a male sterile line with a pollen parent. In this study, we examined the agronomic characters of the 32 BC_2F_8 lines under LD conditions at Hokkaido and under SD conditions at Fukui. Based on the data, we selected eight promising PCMS lines that showed high cross-pollination ability under LD conditions, high male sterility under LD conditions and high seed setting under SD conditions. These PCMS elite lines will be useful for developing hybrid wheat varieties. In the next step, combining ability of the PCMS lines with the efficient pollinator lines such as 'Fortunato I. Bo. 219' will be examined.

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