Journal of Agricultural and Crop Research
Vol. 7(7), pp. 119-126, July 2019
doi: 10.33495/jacr_v7i7.19.132
ISSN: 2384-731X
Research Paper

Trials for hybrid seed production and estimation of wheat F_1 hybrids produced by outcrossing using photoperiod-sensitive cytoplasmic male sterile (PCMS) system with elite lines

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Accepted 4th July, 2019.

Abstract. In previous studies, we presented a two-line system for producing hybrid varieties in common wheat (*Triticum aestivum* L.) based on photoperiod-sensitive cytoplasmic male sterility (PCMS) caused by the cytoplasm of relative wild species *Aegilops crassa* L. We have developed several promising elite PCMS lines with the genetic background of Japanese wheat cultivars, which showed high cross-pollination fertility and high male sterility under long-day conditions and high seed fertility under short-day conditions. Here, we performed an F_1 seed production trial between eight elite PCMS lines and the pollinator variety 'Fortunato', which has high combining ability with the PCMS elite lines, under long-day conditions in Hokkaido, Japan. Two of eight PCMS lines showed complete sterility and had more than 50% open pollination fertility. Using the F_1 seeds obtained, the agronomic characters of five F_1 hybrids were examined under short-day conditions in Fukui, Japan. The F_1 hybrids headed 4 to 5 days earlier than the early-heading elite leading cultivar of Fukui, 'Fukukomugi'. Compared with 'Fukukomugi', yield heterosis of F_1 hybrids ranged from 21 to 61% in small plot trials. The results of the present study suggested the practicality of using this PCMS system for hybrid wheat production.

Keywords: Heterosis, hybrid wheat, photoperiod-sensitive cytoplasmic male sterility, wheat.

INTRODUCTION

The generation of high-yielding crops is one of the principal objectives in plant breeding. Breeding programs utilizing hybrid vigor (heterosis) are an effective approach to development of high-yielding varieties of crop species. Recent studies have suggested that genome-wide epigenetic regulation is associated with heterosis (Fujimoto *et al*., 2018). In allogamous species such as maize, sunflower, sorghum, sugar beet and rye, hybrid breeding has been remarkably successful (Duvick *et al*., 2004). Hybrid breeding in rice, an autogamous crop, has also achieved great success especially in China (Xie and Zhang, 2018). However, although hybrid breeding in autogamous cereals such as wheat and barley has long been considered a promising approach, hybrid varieties have generally not been economically successful (Longin *et al*., 2012). For example, in bread wheat (*Triticum aestivum* L.), less than 1% of the total world wheat crop makes use of hybrid varieties. In the case of wheat, the reason for the poor uptake of hybrid breeding worldwide is the lack of effective systems for hybrid seed production, leading to low cost-efficient (Whitford *et al*., 2013).

Currently, four systems are employed for hybrid wheat seed production: 1) chemical hybridizing agent (CHA); 2) cytoplasmic male sterility (CMS); 3) genic male sterility (GMS); and 4) genetic modification (GM). The lack of commercial progress in making use of hybrid wheat is a consequence of the difficulties inherent in these systems (Whitford *et al*., 2013). For example, a CMS system based on *T. timopheevii* cytoplasm is hampered by the lack of effective fertility-restoration (Rf) genes; GMS systems have largely failed due to problems with fertility restoration; and CHA systems suffer from problems of toxicity and selectivity. Recently, the gene for a phospholipid-binding protein (glycosylphosphatidylinositol (GPI) - anchored lipid transfer protein), *Ms1* (*Male sterility 1*), was identified and a GM system using this gene was proposed (Tucker *et al*., 2017; Wang *et al.*, 2017). However, it is not yet known whether this system is practical.

We previously developed an alternative approach for hybrid wheat production using a simple “two-line system” based on photoperiod-sensitive cytoplasmic male sterility (PCMS) produced by the presence of *Aegilops crassa* cytoplasm (Murai, 2001a). In this system, PCMS is induced by long-day conditions of 15 h or longer light period during floret differentiation. PCMS results from the homeotic transformation of stamens into pistilloid (pistil-like) structures, which is mediated by the *Ae. crassa* cytoplasm (Murai *et al*., 2002). Using this PCMS system, hybrid seeds can be produced by outcrossing a PCMS line with a pollinator line under long-day conditions of more than 15 h light, the natural day length for spring-sown wheat in Hokkaido, Japan. In contrast to the “three-line system” that uses *T. timopheevii* cytoplasm (Johnson and Schmidt, 1968), the two-line system does not need a maintainer of male sterility because the PCMS line can grow and multiply by self-pollination under short-day conditions (≦14.5 h light, the natural day length for autumn-sown wheat in Honshu, Shikoku and Kyushu, Japan). A similar two-line system for hybrid wheat production that uses thermo-sensitive cytoplasmic male sterility based on *Ae. kotschyi* cytoplasm has also been developed (Meng *et al*., 2016). This system makes use of temperature change but is comparatively unstable compared to our two-line PCMS system. The practicality of the system is still unknown. The PCMS system also has a further advantage over other CMS systems of a relatively high level of fertility restoration due to the - synergistic effects of short-day environmental conditions and effective fertility-restoring gene(s) (Murai, 1997).

In previous studies, we sought to develop PCMS lines that show high male sterility under long-day conditions and high seed setting rates under short-day conditions. These led to the production of two lines, #6(11)-3 (named as PCMS7 line) and #7(12)-2 (named as PCMS8 line) that fulfilled these criteria; both lines were originated from crossing (cr)-Norin 26/Fujimikomugi//3*Fukuotome (Murai et al., 2008). The alloplasmic line of Japanese wheat cultivar ‘Norin 26’ with *Ae. crassa* cytoplasm ((cr)-Norin 26) shows high seed fertility under short-day conditions, while the alloplasmic line of Fujimikomugi ((cr)-Fujimikomugi) is completely male sterile under long-day conditions. ‘Fukuotome’ is an elite cultivar used for production of Japanese noodles in the western region of Japan. From the progenitors of the PCMS7 and PCMS8 lines, we selected 14 and 18 lines in the BC_2F_8 generation and examined their agronomic characters under long-day conditions at Hokkaido and short-day conditions at Fukui (Murai *et al*., 2016). Based on the data obtained we selected PCMS8#1, #3, #6, #8, #10, #12 and #17 as candidate elite lines with the genetic background of ‘Norin 26’, ‘Fujimikomugi’ and ‘Fukuotome’.

In this study, we firstly performed an F_1 seed production trial by out-crossing (open pollination) between elite PCMS lines and the pollinator line ‘Fortunato’ under long-day conditions in Hokkaido. We used the elite PCMS lines PCMS8#1, #3, #6, #8, #10, #12 and #17 (Murai *et al.*, 2016), together with PCMS8#14 that showed relatively high self-fertility under short-day conditions. F_1 hybrid seeds from PCMS8#3, #6, #12, #14 and #17 were used to examine the performance of F_1 hybrid lines in Fukui, Honshu. Although the present study was the first step and preliminary small plot experiment, the results suggested the commercial practicality of our PCMS system.

MATERIALS AND METHODS

Location of experiment

The study was carried out at the experimental field of HOKUREN Federation of Agricultural Co-operative, Naganuma-cho, Hokkaido, Japan (longitude 142° E latitude 43° N) for the hybrid seeds production and at the experimental field of Fukui Prefectural University, Eiheiji-cho, Fukui, Japan (longitude 136° E latitude 36° N) for the examination of F_1 hybrids.

PLANT MATERIALS

Eight PCMS lines, PCMS8#1, #3, #6, #8, #10, #12, #14 and #17, were used in the F_1 seed production trial in

Hokkaido. These lines are the F_9 generation from crossing (cr)-Norin 26/Fujimikomugi//3*Fukuotome (Murai *et al*., 2016). As a pollinator line, bread wheat cultivar 'Fortunato' was used. 'Fortunato' is a South European hard red spring wheat cultivar which carries unidentified Rf genes that are effective in fertility restoration in our PCMS system (Murai *et al*., 2016).

For the yield performance trial on the experimental field of Fukui Prefectural University, F_1 hybrids derived from PCMS8#3, #6, #12, #14 and #17 were used, because of the sufficiently large number of F_1 hybrid seeds. As controls, wheat cv. 'Fortunato', 'Fukukomugi' and 'Nanbukomugi' were used in the field performance trial in Fukui. 'Fukukomugi' is a leading variety in Fukui Prefecture, and is a multi-purpose soft red wheat cultivar developed by Fukui Prefectural University. 'Nanbukomugi' is the previous leading variety in Fukui Prefecture.

F_1 seed production trial in Hokkaido

In April 2016, crossing blocks for the eight PCMS lines (PCMS8#1, #3, #6, #8, #10, #12, #14 and #17) and the pollinator line 'Fortunato' were set up in the experimental field of Hokkaido. The seeds were sown on April 13. The PCMS lines were planted in two rows, 30 cm apart, at a seeding density of 50 seeds/m in a crossing block of 1 m length; each block was surrounded by two rows, 30 cm apart, of the pollinator line at a seeding density of 100 seeds/m. The PCMS lines were sown with a relatively wider spacing to increase tiller number per plant, because late appearing ears tended to exhibit high and stable male sterility (Murai, 2001b). Just before flowering, three to six ears of the PCMS line in each block were bagged. In August, bagged (3 to 6) and un-bagged (26 to 31) ears were harvested to evaluate self-fertility (%), selfed seed number per spikelet, open pollination fertility (%) and open pollination seed number per spikelet. Fertility (%) was measured as the seed setting rate of the first and second florets of all spikelets. Seed number per spikelet was based on the total number of seeds and spikelets in each ear. Self-fertility (%) and selfed seed number per spikelet were calculated from seed number of bagged ears, and open pollination fertility (%) and open pollination seed number per spikelet were calculated from seed number of un-bagged ears. Hybrid purity (%) of F_1 seeds was estimated by the following formula; [open pollination fertility (%) – self-fertility (%)] / open pollination fertility (%) x 100. In the 'Fortunato' control, open pollination fertility (%) and open pollination seed number per spikelet were measured using 12 un-bagged ears, meaning that they were just fertility and seed number per spikelet because 'Fortunato' is normal wheat cultivar. Heading date, flowering date and culm length (cm) were measured for each PCMS line and for 'Fortunato'.

We harvested all un-bagged ears from each PCMS line. F_1 hybrid seeds were obtained from total un-bagged ears of each crossing block. The thousand kernel weight (g) and volume weight (g/L) were measured for F_1 hybrid seeds; three technical replications for each block were performed. These characters were calculated by measuring the weight and number of kernels of 5 ml using a 10 ml graduated cylinder. The total number of hybrid seeds was calculated by the data of yield and thousand kernel weight.

Field trial to examine agronomic performance of F_1 hybrids in Fukui

Five F_1 hybrids (PCMS8#3 F1, #6 F1, #12 F1, #14 F1 and #17 F1) were grown in season 2016/2017 in the experimental field at Fukui Prefectural University together with control cv. 'Fortunato', 'Fukukomugi' and 'Nanbukomugi'. The F_1 hybrids and control lines were examined by a randomized block design with two each plot. Seeds were sown on October 25 with a seeding density of 300 kernels/m^2 in a plot of 1 m length with five rows, 30 cm apart. The standard amount of fertilizer was provided to the wheat field. Four characters were measured in each F_1 hybrid and control line: heading date, culm length (cm), ear length (cm) and spikelet number per ear. Culm length and spikelet number per ear were measured for nine randomly selected shoots and ears per plot. The wheat plants in two outer rows of plot were excluded from the measurement. Grain yield was determined from the harvested grains from all ears of one row (1 m length) with three replications in each plot. Standard heterosis (%) of F_1 hybrids were calculated using the total means of yield, compared with elite cultivar of Fukui Prefecture, 'Fukukomugi' as a standard variety. Furthermore, Male parent heterosis (%) of F_1 hybrids were calculated using the total means of yield, compared with the pollinator line 'Fortunato' as a male parent. The thousand kernel weight (g) and volume weight (g/L) were measured for the harvested seeds with ten technical replications. These characters were calculated by measuring the weight and number of kernels of 20 ml using a 50 ml graduated cylinder.

Statistical analysis

We tested for significant differences among the F_1 hybrid lines and the controls using Tukey test ($p<0.05$) following an analysis of variance (ANOVA).

RESULTS

Production of F_1 seeds by outcrossing in Hokkaido

The agronomic characters of the eight PCMS lines in the

Table 1. Characteristics of eight PCMS lines with the pollinator line 'Fortunato' in the hybrid seed production field of Hokkaido at long-day conditions.

Line	Heading date	Flowering date	Culm length (cm)[1)]	Self-fertility (%)[2)]	Selfed seed no. per spikelet[2)]	Open pollination fertility (%)[1)]	Open pollination seed no. per spikelet[1)]
Fortunato	16 June	24 June	90.3±0.7^{a}	-	-	92.1±1.5^{a}	2.58±0.11^{a}
PCMS8#1	20 June	25 June	62.3±1.0^{c}	1.2	0.02	29.1±2.6^{e}	0.60±0.06^{d}
PCMS8#3	20 June	25 June	63.1±1.2^{c}	1.2	0.02	44.2±3.2^{cd}	0.98±0.08^{c}
PCMS8#6	21 June	25 June	62.8±1.4^{c}	0.6	0.01	54.3±2.7^{bc}	1.31±0.09^{b}
PCMS8#8	21 June	24 June	63.1±1.2^{c}	0.0	0.00	45.9±2.8^{bcd}	1.01±0.07^{bc}
PCMS8#10	21 June	25 June	70.5±0.9^{b}	2.0	0.04	42.3±3.3^{d}	0.93±0.08^{c}
PCMS8#12	20 June	24 June	63.2±1.9^{c}	2.6	0.05	51.1±1.9^{bcd}	1.17±0.05^{bc}
PCMS8#14	20 June	25 June	64.1±0.6^{c}	0.0	0.00	57.0±2.0^{b}	1.32±0.06^{b}
PCMS8#17	20 June	24 June	65.6±1.3^{bc}	0.0	0.00	50.3±2.8^{bcd}	1.14±0.07^{bc}
ANOVA F-value[3)]	-	-	59.7148**	ns	ns	22.4444**	31.1392**

1) Means ± SE are shown. Different letters indicate significant differences by Tukey test (p<0.05) following ANOVA.
2) Means are shown. Fortunato was not examined.
3) ** indicates significant differences at 1% level in an ANOVA. ns indicates non-significant. Heading date and flowering date were not analyzed by ANOVA.

Table 2. Hybrid seed production performance and agronomic characters in eight PCMS lines open-pollinated by the pollinator line 'Fortunato'.

Line	Yield of hybrid seeds (g)[1)]	Total number of hybrid seeds[1)]	Thousand kernel weight (g)[2)]	Volume weight (g/L)[2)]	Hybrid purity (%)[3)]
PCMS8#1	21.5	636	33.8±1.2^{b}	609±5.8	95.9
PCMS8#3	52.4	1333	39.3±0.9^{a}	606±3.4	97.3
PCMS8#6	57.4	1407	40.8±1.3^{a}	591±9.9	98.9
PCMS8#8	38.5	1013	38.0±0.6^{a}	603±12.7	100
PCMS8#10	41.6	1118	37.2±0.3^{ab}	625±9.4	95.3
PCMS8#12	56.7	1473	38.5±0.9^{a}	611±17.1	94.9
PCMS8#14	70.4	1810	38.9±0.6^{a}	603±1.8	100
PCMS8#17	52.0	1344	38.7±0.4^{a}	597±11.9	100
ANOVA F-value[4)]	-	-	5.9017**	ns	-

1) The PCMS lines were planted as two rows of 1 m lengths.
2) Means ± SE are shown. Different letters indicate significant differences by Tukey test (p<0.05) following ANOVA.
3) Hybrid purity of F_1 seeds was estimated by the following formula; [open pollination fertility (%) – self-fertility (%)] / open pollination fertility (%) × 100.
4) ** indicates significant differences at 1% level in an ANOVA. ns indicates non-significant. Yield of hybrid seeds, total number of hybrid seeds, and hybrid purity were not analyzed by ANOVA.

hybrid seed production field of Hokkaido are shown in Table 1. The heading date of pollinator line 'Fortunato' was 4 to 5 days earlier than the PCMS lines, but flowering time was similar to the PCMS lines. Culm length of 'Fortunato' was significantly longer than the PCMS lines. These characteristics indicated that 'Fortunato' was a suitable pollinator for outcrossing with the PCMS lines.

The PCMS lines PCMS8#8, #14 and #17 showed complete sterility; lines PCMS8#1, #3, #6, #10, #12 and #17 showed a very low level of self-fertility (Table 1). The PCMS lines showed significant difference for open pollination fertility (%) and open pollination seed number per spikelet; PCMS8#6, #12, #14 and #17 had more than 50% open pollination fertility. These analyses indicated that PCMS8#14 and #17 were the best PCMS lines, showing complete self-sterility and high open pollination seed sets.

The yield performances for F_1 seed production in the PCMS lines are shown in Table 2. Our analysis indicated that yield performance for F_1 seed production was associated with open pollination ability: PCMS8#6, #12 and #14 showed the higher F_1 seed yield performances. PCMS8#6 line produced F_1 seeds with highest thousand kernel weight under long-day conditions after cross-pollination. However, their volume weight was very low. By contrast, PCMS8#10 produced F_1 seeds with the highest volume weight but a low thousand kernel weight.

Table 3. Agronomic characters and yield performances in the F_1 hybrids between PCMS lines and the pollinator line 'Fortunato' under short-day conditions at Fukui.

Line	Heading date	Culm length (cm)[1)]	Ear length (cm)[1)]	Spikelet number[1)]	Yield (g/1m length)[1)]	Standard heterosis (%)[2)]	Male parent heterosis (%)[3)]	Thousand kernel weight (g)[1)]	Volume weight (g/L)[1)]
PCMS8#3 F1	8 April	63.8±1.3^{a}	6.5±0.2^{ab}	12.2±0.5^{a}	38.1±1.7^{ab}	34	41	33.5±0.2^{c}	722±6.5^{abc}
PCMS8#6 F1	8 April	58.5±1.1^{bc}	6.7±0.3^{ab}	12.6±0.4^{a}	38.8±1.6^{ab}	37	44	33.3±0.3^{c}	708±3.5^{bc}
PCMS8#12 F1	9 April	63.4±0.9^{a}	7.3±0.3^{a}	13.1±0.4^{a}	45.7±4.6^{a}	61	69	33.4±0.3^{c}	724±4.1^{abc}
PCMS8#14 F1	8 April	58.6±1.0^{bc}	7.0±0.1^{a}	12.9±0.3^{a}	34.4±1.8^{bc}	21	27	32.9±0.2^{c}	717±4.5^{abc}
PCMS8#17 F1	8 April	58.4±0.9^{bc}	7.0±0.2^{a}	12.6±0.3^{a}	35.7±3.6^{abc}	26	32	33.7±0.3^{bc}	725±6.5^{abc}
Fortunato	7 April	55.9±1.1^{c}	5.6±0.2^{c}	10.1±0.4^{b}	27.0±1.6^{cd}	-	MaP	33.2±0.2^{c}	731±2.0^{a}
Fukukomugi	13 April	58.6±0.6^{bc}	5.9±0.1^{bc}	10.2±0.2^{b}	28.4±0.9^{bcd}	StV	-	35.1±0.3^{a}	704±7.2^{c}
Nanbukomugi	26 April	60.1±0.6^{ab}	6.9±0.1^{a}	11.7±0.2^{a}	17.4±1.4^{d}	-	-	34.8±0.4^{ab}	729±5.0^{ab}
ANOVA F-value[4)]	-	8.1498**	9.2946**	11.8214**	12.6627**	-	-	7.6585**	3.4939**

1) Means ± SE are shown. Different letters indicate significant differences by Tukey test ($p<0.05$) following ANOVA.
2) Standard heterosis (%) of F_1 hybrids were calculated using the means of yield, compared with elite cultivar of Fukui Prefecture, 'Fukukomugi' as a standard variety (StV).
3) Male parent heterosis (%) of F_1 hybrids were calculated using the means of yield, compared with the pollinator line 'Fortunato' as a male parent (MaP).
4) ** indicates significant differences at 1% level in an ANOVA. Heading date, standard heterosis and male parent hetorosis were not analyzed by ANOVA.

The inverse correlation of thousand kernel weight and volume weight is characteristic of F_1 hybrid seeds. Since important matter in hybrid seed production is the number of seeds, PCMS8#6, #12 and #14 are excellent PCMS lines (Table 2). Especially, PCMS8#14 produced the largest amount of F_1 seeds with 100% hybrid purity.

Yield performance of the F_1 hybrids

The agronomic characters of five F_1 hybrids were examined in Fukui, along with those of the control cultivars, 'Fortunato', 'Fukukomugi' and 'Nanbukomugi' (Table 3). The F_1 hybrid lines headed 4 to 5 days earlier than the early-heading elite cultivar 'Fukukomugi'. The early-heading trait of the F_1 hybrids may be due to their pollen parent 'Fortunato'. We measured seven agronomic characters in the F_1 hybrid lines and control lines (Table 3). The results indicated that five F_1 hybrid lines had significantly longer ear length and larger spikelet number per ear compared with the pollen parent cultivar 'Fortunato'. These results indicate that hybrid vigor (heterosis) was expressed in these characters. Overall, the yield performances of the F_1 hybrid lines were much higher than those of the control lines. Compared with the standard cultivar 'Fukukomugi', yield heterosis of F_1 hybrid lines ranged from 21 to 61%. The best F_1 hybrid was PCMS8#12 F1. As the yield of 'Fukukomugi' in Fukui ranges 3.5 ton to 4.0 ton per hectare, the yield of F_1 hybrid is estimated to be around 6 ton per hectare. The thousand kernel weight in the F_1 hybrid lines was significantly lower than in 'Fukukomugi', indicating that the F_1 hybrid lines produced smaller grains.

Images of ears and grains of PCMS8#12 F1 and 'Fukukomugi' are shown in Figure 1a and b. The F_1 hybrid lines had a mixture of hard and soft grains, while 'Fukukomugi' only had soft grains (Figure 1b). PCMS lines which have the 'Norin 26', 'Fujimikomugi' and 'Fukuotome' genetic background had a soft wheat nature, while the pollen parent line 'Fortunato' is a hard wheat cultivar. Therefore, hard and soft grains present in the harvested seeds of the F_1 hybrids (Figure 1c), because of segregation for the *Pin* (puroindoline a and b protein) genes, which are associated with grain hardness (Giroux and Morris, 1998). This maybe produces a unique aspectof the F_1 hybrid flours.

DISCUSSION

Japan's food self-sufficiency ratio (on a calorie basis) decreased from 79% in 1960 to 38% in 2017 (Ministry of Agriculture, Forestry and Fisheries of Japan, 2017); this is the largest decrease seen in a major developed country over the last 45 years, and the level of self-sufficiency is now the lowest among countries with a population

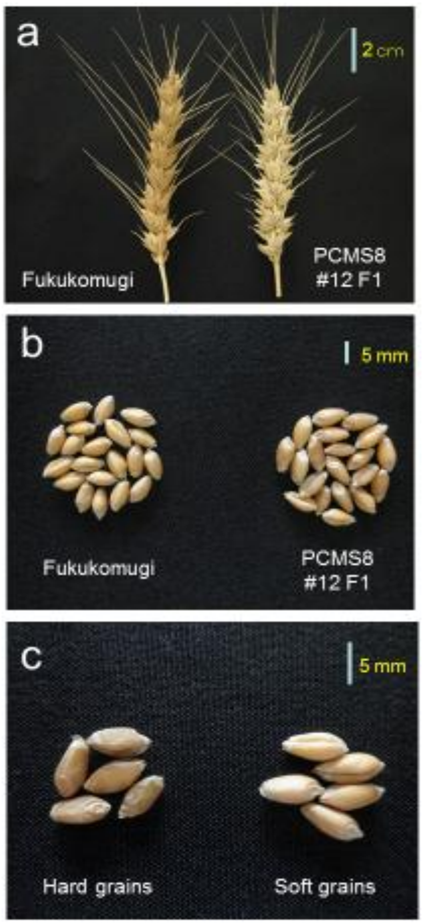

Figure 1. a. Ears of PCMS8#12 F1 hybrid (right) and a leading cultivar 'Fukukomugi' (left). b. Grains of PCMS8#12 F1 hybrid (right) and a leading cultivar 'Fukukomugi' (left). c. Soft grains (right) and hard grains (left) segregated in the grains of PCMS8#12 F1 hybrid.

over 100 million (Kako, 2009). The low rate of food self-sufficiency is related to increases in maize and soybean imports for livestock feeds from countries such as the USA. Furthermore, Japan imports 85% of wheat for bread and noodles from the USA, Australia and Canada. Therefore, increasing domestic wheat production is important for improving food self-sufficiency in Japan.

In Japan, except for the Hokkaido, early-maturing bread wheat cultivars are necessary as they can produce an early harvest. This early maturing habit allows harvesting to occur before the onset of the rainy season. In central to southwestern Japan, autumn-sown early-heading spring wheat cultivars that show reduced photoperiod sensitivity are grown (Tanio *et al*., 2005). Therefore, the growth period of wheat is much shorter in other countries such as UK, resulting in a low yield for wheat production in Japan (Hoshino *et al*., 2001). Currently, wheat yields in central to southwestern Japan are approximately 3.3 tons/hectare (Ministry of Agriculture, Forestry and Fisheries of Japan, 2017).

Compared with breeding new lines, hybrid wheat breeding offers an economically attractive and feasible approach for developing high yield stability (Longin *et al*., 2014; Muhleisen *et al*., 2014). The hybrid wheat approach may be essential to increasing wheat production in Japan; however, the feasibility of this approach depends on the development of hybrid breeding systems suitable for Japanese wheat cultivation with regard to varieties and climate.

In a previous study, we reported that the alloplasmic line cv. 'Norin 26', which carries the cytoplasm of *Ae. crassa*, shows PCMS (Murai and Tsunewaki, 1993). This alloplasmic line was extensively studied in an attempt to utilize it for hybrid wheat breeding (Murai, 2001a) and we developed elite PCMS lines with Japanese wheat genetic background (Murai *et al*., 2008, 2016). PCMS lines can be used as autumn-sown spring wheat and are suitable for sowing in the central to southwestern Japan. In the PCMS system, hybrid seeds can be produced through outcrossing of a PCMS line with a pollinator line under long-day conditions (≧15 h light). Spring-sown wheat in the northern island of Hokkaido encounters long-day conditions. Autumn-sown winter wheat is also grown in Hokkaido. As PCMS lines have a spring growth habit, those sown in spring at Hokkaido are exposed to natural day lengths longer than 15 h from mid-May to mid-July. The floral organ development stage when the photoperiod is crucial for pistillody induction is approximately mid-June (Murai and Tsunewaki, 1993). It is necessary that the pollinator lines have a spring habit; the South European spring cultivar 'Fortunato' fulfills this criterion and was selected as an elite pollinator line (Murai *et al*., 2016). In the present study, we generated F_1 hybrid seeds by outcrossing of spring-sown plants at Hokkaido, and examined the performance of the F_1 hybrids at autumn-sowing in Fukui. The present study is the first step for hybrid wheat breeding using the PCMS system, and just preliminary small scale experiment. Although further validation should be needed in large scale experiments for several years in multiple locations, our results described here indicate the possibility that this PCMS system could be suitable for hybrid wheat production in Japan, i.e. sowing spring wheat in Hokkaido in spring and spring wheat in mid to south Honshu in autumn.

In the present hybrid wheat varieties, 'Fortunato' was used as a pollinator line. 'Fortunato' is an early-heading/flowering cultivar suitable for hybrid wheat breeding with Japanese PCMS lines. It has been reported that semi-dwarf wheat cultivars in Southern and Central Europe have the dwarfing gene *Rht8* and the photoperiodic insensitive gene *Ppd-D1* (Worland *et al*., 1998; Borojevic and Borojevic, 2005). 'Fortunato' is an older cultivar produced in a breeding program in the former state of Yugoslavia. Therefore, we anticipate that other old cultivars in the breeding program are also suitable as pollinator lines for PCMS hybrid wheat breeding. The genome-based prediction system using the 9 k single nucleotide polymorphism (SNP) array could be a powerful tool for identifying elite pollinator lines for high-yielding hybrid wheat breeding (Zhao *et al*., 2013, 2015).

There are additional advantages in hybrid wheat lines produced by the CMS system with cytoplasmic substitution lines as female parental lines. The F_1 hybrids have alien cytoplasm including organellar (mitochondrial and chloroplast) genomes which are derived from the parental CMS lines. The replacement of wheat cytoplasm with cytoplasm of a related species alters nuclear-cytoplasm cross-talk leading to nuclear transcriptome and metabolome alterations (Crosatti *et al*., 2013). Retrograde signaling from mitochondria and/or chloroplasts coordinates nuclear genes and is the main mechanism of nuclear-cytoplasm cross-talk (Woodson and Chory, 2008). A recent study indicated that epigenomic polymorphisms, such as DNA methylation patterns, may be altered by cytoplasm replacement and are associated with growth vigor (Soltani *et al*., 2016). The nuclear-cytoplasmic interaction in hybrid wheat generated by CMS system should be more notable. We are currently examining the potential beneficial effects of *Ae. crassa* cytoplasm in PCMS F_1 hybrid lines on biotic and abiotic stress tolerance.

ACKNOWLEDGEMENTS

We are grateful to the National Bioresource Project – Wheat (NBRP-KOMUGI) for providing wheat materials. This work was supported in part by a Grant-in-Aid (D) from Fukui Prefectural University to K. Murai.

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