(2)

BASIC STUDIES ON HYBRID WHEAT BREEDING UTILIZING *AEGILOPS CRASSA* CYTOPLASM

1992

KOJI MURAI

CONTENTS

I. INTRODUCTION

The successful development of hybrid corn in the 1940s gave a great impetus to breeders of other crops, including self-pollinating cereals such as wheat, rice and barley. The genetic basis for the advantage of hybrids is hybrid vigor (heterosis), the tendency of the hybrids to have greater productivity than the parental varieties.

Heterosis in wheat was first reported when Freeman (1919) found that the F_1 plants averaged taller than the taller parent in some crosses. Two years later, Griffee (1921) indicated that the F_1 hybrids yielded 4 to 32 % more than the parental average. A first comprehensive survey of the heterosis in wheat was made by Briggle (1963). Among the 25 publications surveyed by him, the magnitude of the heterosis for yield reported ranged from 0 to more than 100 % over the mean of the parents. However, a common problem in many of the earlier reports was the limited scope, i. e., a small number of plants were space-planted, and non-commercial and unproductive varieties were frequently used. One of the earliest evidences of recognized heterosis in wheat using commercial varieties were reported by Livers and Heyne (1968). They determined the heterosis by intercrossing nine cultivars of well-adapted winter wheats at Kansas, the U. S. A. The 36 hybrids collectively exceeded parental varieties by 20, 37, 37 and 35 % in the four years (1964-1967), respectively, and the best hybrid has been consistently 30 % better than the best variety for the area.

Wheat is a self-pollinating crop species. Individual wheat

flowers possess both male and female organs. The anthers within a flower normally provide pollen grains to fertilize the ovary within the same flower. Therefore, the methods for hybrid seed production require the introduction of male sterility into a maternal parent, so that the maternal parent can be pollinated by a different pollen parent. Male sterility is induced by factors under nucleo-cytoplasmic or nuclear control, or by chemicals.

The first instance of cytoplasmic male sterility in wheat (Triticum aestivum) was reported by Kihara in 1951. He substituted the nucleus of common wheat into the cytoplasm of Aegilops caudata. Some backcross progenies resembled wheat, but were only partially fertile. Later, Fukasawa (1953) transferred T. durum nucleus to Ae. ovata cytoplasm, and obtained completely male sterile plants. He attributed the sterility to a disharmony between the cytoplasm and the nucleus. Fukasawa (1959) also observed male sterility in backcross generations in which common wheat nucleus was transferred to the Ae. ovata cytoplasm. Beside the male sterility induction, the nucleo-cytoplasmic interaction of the wheat nucleus with the Ae. caudata or Ae. ovata cytoplasm produced other largely undesirable effects. The Ae. caudata cytoplasm induced germless seeds, haploid and twin seedlings and reduced female fertility when associated with the nuclei of various hexaploid wheats, and pistillody with hexaploid and tetraploid wheat nuclei (Kihara 1951, Kihara and Tsunewaki 1967). The Ae. ovata cytoplasm exhibited significantly delayed maturity compared with their euplasmic counter-

parts (Wilson and Ross 1961, Kihara and Tsunewaki 1967).

Soon afterward, Wilson and Ross (1962) showed that a common wheat variety "Bison" showed complete sterility when its nucleus was introduced into the cytoplasm of T. timopheevi, and that there were no apparent deleterious side effects. The first report of fertility-restoring genes for the T. timopheevi cytoplasm was made by Schmidt et. al. (1962) in the same year. They identified derivatives of T. aestivum/T. timopheevi crosses, which appeared to be capable of completely restoring fertility in male sterile plants with the T. timopheevi cytoplasm. These studies prompted the extensive research on hybrid wheat production in the United States and in other countries. Consequently, some hybrid wheat varieties were released commercially in limited quantities both in the United States and Australia in the 1970's. These first hybrids, while being more advantageous than lower yielding but widely grown varieties, have never become widely used because of its poor performance. The relatively poor performance of the hybrids from the T. timopheevi system, when compared with the hybrids by hand-pollination with normal cytoplasm, may be due to one or more of the following factors (Wilson and Driscoll 1983):

a) the influence of the T. timopheevi cytoplasm
b) incomplete fertility restoration
c) the relatively low yield of the restorer lines
d) the influence of the (alien) restorer genes
e) inadequate sampling of the gene pool and assessment of combining ability
f) the low level of heterosis in wheat.

It seems possible that the introduction of both the T. timopheevi cytoplasm and the (alien) restorer genes has somewhat disturbed the normal growth of common wheat. Moreover, the system of the T. timopheevi cytoplasm commonly suffers from several other disadvantages such as kernel shriveling (Schmidt et. al. 1970, Miller et. al. 1975) and pre-harvest sprouting (Doig et. al. 1975, Ellis and Clayton 1976) together with large reduction in seed germinability.

These difficulties have stimulated to find new systems for the male sterility-fertility restoration in common wheat. Franckowiak et. al. (1976) presented an another proposal for hybrid wheat production using the Ae. squarrosa cytoplasm, which seeks to avoid the breeding of restorer lines. The D genome of common wheat contains genetic factor(s) for the restoration of fertility of T. aestivum with the Ae. squarrosa cytoplasm. The nucleus of T. aestivum was substituted into the Ae. squarrosa cytoplasm, and the seeds were treated with a mutagenic agent (ethyl methanesulfonate, EMS) to inactivate the critical gene(s) which causes fertility. This system has the advantage that common wheat varieties would serve as a restorer line. However, no suitable male sterile genes functioning specifically in the Ae. squarrosa cytoplasm were found. Tsunewaki et. al. (1978) proposed a system using the cytoplasms of Ae. kotschyi or Ae. variabilis, which were found to induce complete male sterility in three common wheats, T. aestivum strain Salmon, T. spelta var. duhamelianum and T. macha var. subletschchumicum, while they gave normal fertility to all other wheats. Mukai and Tsunewaki (1979) showed that a common wheat cultivar, Chinese

Spring, carries a gene for fertility restoration designated Rfv1 on the short arm of chromosome 1B. Salmon is known to have an arm of chromosome 1R of rye (Secale cereale) translocated onto the short arm of chromosome 1B, and presumably this rye chromosome does not carry a fertility-restoring gene. The system has the advantages that no restorer lines need to be developed and easier fertility restoration in F_1 hybrids. Several hybrid programs are currently evaluating this system. Panayotov (1980) aimed to search for new sources of cytoplasmic male sterility, and found that Ae. mutica cytoplasm appeared most interesting for hybrid wheat breeding. This is because its side effects are very small and the male sterility induced is very stable, though this cytoplasm lacks any effective fertility restorers. Panayotov et. al. (1988) identified the critical chromosome with fertility-restoring gene in the restorer lines for the T. timopheevi cytoplasm against the Ae. mutica cytoplasm, and suggested a hybrid seed production system using the Ae. mutica cytoplasm and the restorers for the T. timopheevi cytoplasm.

Driscoll (1972) proposed a system of utilizing a nuclear male sterility factors in common wheat. The system involves three lines, referred to as X, Y and Z lines, all of which are homozygous for a recessive allele for male sterility. In addition to this, the X and Y lines carry an extra chromosome, in two and one dose, respectively. The extra chromosome has two main characteristics: first, it carries a gene(s) for male fertility, and secondly, this chromosome does not pair with the normal wheat chromosomes. Fertility restoration of the hybrid

is effected by the normal wheat chromosome complement of the male parent. Therefore, there is absolutely no genetic modification of male parents for hybrid varieties. The Cornerstone male-sterility mutant (Driscoll 1977) is suitable for hybrid wheat production. The alien fertility factor is now available in two forms, one derived from rye (Hossain and Driscoll 1983) and the other from barley (Islam and Driscoll 1984). The advantages of using this system for hybrid wheat production are that the cytoplasm of the hybrid is normal and that the development of restorer lines is not need.

A more recent and alternative system for producing hybrid wheat is through the use of chemical hybridizing agents (CHAs). The CHAs, which can selectively sterilize the stamen without affecting the normal functioning of the rest of plant development, can be used to induce male-sterile parents for hybrid wheat production. The advantages of such chemicals are obvious, because no special developments of sterile and restorer lines are needed, and the increase of parental seeds is simple. Chopra et. al. (1960) reported complete pollen sterility in wheat with a high degree of female fertility for plants treated with maleic hydrazide. The complete male sterility unaccompanied by female sterility firstly occurred by 1,500-3,000 ppm ethrel applications at the early booting stage (Rowell and Miller 1971). After that, several chemical companies have promoted the programs to synthesize, identify, and evaluate the performance of potential CHAs (McRae 1985). Although several chemicals have been tested, none appeared to have the properties

required for commercial hybrid seed production. The major problem associated with chemicals concerns the development of the CHAs which will give adequate sterility without interfering with the normal function of the rest of the plant. An alternative problem on the CHAs is that there is a genotypic variation of wheat cultivars for the response to a CHA. The nature of this variation is not known. However, it is understood that several chemical companies have recently developed for more efficient CHAs which are currently under test.

The objectives of this thesis are to study the effects of Ae. crassa cytoplasm on common wheat and to search for the utilization of the Ae. crassa cytoplasm in hybrid wheat breeding. Chapter II of this thesis deals with the effects of photoperiod on male fertility of two common wheat cultivars with the Ae. crassa (D^2 type) cytoplasm, i. e., photoperiod-sensitive cytoplasmic male sterility (PCMS) and proposes a two-line system for hybrid wheat production using the PCMS. Chapter III summarizes the results of developing the alloplasmic lines of wheat cultivars with the Ae. crassa cytoplasm and PCMS induction in them. Chapter IV deals with the genetic analyses of fertility-restoring genes in Chinese Spring and Norin 61 against the Ae. crassa cytoplasm. Chapter V deals with the effects of the Ae. crassa cytoplasm on agronomic characters of PCMS lines and F_1 hybrids, and the results of preliminary field trial for the production of F_1 seeds and the estimation of the F_1 hybrids. In Chapter VI, I mention briefly my conclusion. The last chapter VII is the summary of this thesis.

II. PHOTOPERIOD-SENSITIVE CYTOPLASMIC MALE STERILITY (PCMS) CAUSED BY THE D^2 TYPE CYTOPLASM

1. Introduction

Tsunewaki et. al. (1976a) have carried out a series of comparative studies on the genetic properties of cytoplasms present in different species of Triticum and Aegilops. The cytoplasms from 23 species have been introduced into 12 strains of common wheat by repeated backcrosses. From the results of the genetic characterization of the cytoplasms, these cytoplasms were classified into eight plasma types. The cytoplasms of Ae. crassa 4x and 6x, Ae. juvenalis and Ae. vavilovii were placed in the D plasma type, the representative of which is the Ae. squarrosa cytoplasm, while they were differentiated from Ae. squarrosa in an aspect, that is prominent pistillody caused by these cytoplasms. According to Sasakuma and Ohtsuka (1979), these cytoplasms induced almost 100 % pistillody and consequently complete male sterility specifically in a Japanese wheat cultivar, Norin 26, when grown in Hokkaido, a northern island of Japan. On the other hand, the Ae. squarrosa cytoplasm did not induce male sterility. Considering these data, the cytoplasms of Ae. crassa 4x and 6x, Ae. juvenalis and Ae. vavilovii were considered as a subtype of the D plasma type, and renamed D^2 (Tsunewaki 1980a).

In this chapter, I will demonstrate that the male sterility of the alloplasmic Norin 26 with a D^2 type cytoplasm is induced by long-day treatment, that the critical day length is 14.5-15 hours, and that the sensitive stage to photoperiod is in the

floret differentiation stage. Based on these findings. I mention a new means for hybrid wheat production, named the "two-line system" which has been taken out a patent by Sumitomo Chemical Co. (Patent No. 19651 (Japan), 4,680,888 (US)). This system requires only male sterility and pollinator lines for producing hybrid wheat.

2. Materials and Methods

1) Plant materials

The cytoplasm of Ae. crassa (both 4x and 6x), Ae. juvenalis and Ae. vavilovii classified as the D^2 type cytoplasm have been transferred to T. aestivum cv. Norin 26 (abbreviated, N26) and cv. Chinese Spring (CS) (Tsunewaki 1980a). The following alloplasmic lines derived from these cytoplasm substitutions were used in the present investigation; Ae. crassa/11*N26, Ae. juvenalis/11*N26, Ae. vavilovii/11*N26, Ae. crassa/11*CS, Ae. juvenalis/12*CS and Ae. vavilovii/11*CS. The Ae. crassa employed was the one extracted from a 6x accession.

2) Studies on the effects of photoperiod and temperature on male fertility

The alloplasmic lines of N26 and CS having the above three D^2 type cytoplasms and their euplasmic lines as controls were studied. They were individually planted in pots of 10 cm diameter and 13 cm height, and grown in growth rooms, in which photoperiod and temperature could be controlled.

Five different photoperiods were tested; these are 13.5,

14, 14.5, 15 and 17 hours light periods for every 24 hours period at 20,000 lux. Temperature condition set was 15 °C in the dark and 18 °C in the light periods for all but the 14 hours light period treatments, while six different temperature conditions, i. e. 9 (dark) - 12 °C (light), 12-15 °C, 15-18 °C, 18-21 °C, 5-18 °C and 10-18 °C, were tested with the 14 hours light period treatment.

Selfed seed fertility (%) was used as a parameter of male fertility, because it is known that all the D^2 type cytoplasms little affect female fertility (Tsunewaki 1980a), and was estimated by the seed setting rate of the first and second florets of all spikelets in three bagged ears per plant. Two to five plants were studied in each line, and their average values were used for analysis.

3) Pollen fertility and pistillody expression

Only an alloplasmic line of N26 having Ae. crassa cytoplasm and its euplasmic line as a control were studied. Their pollen fertilities (%) were estimated by the percentage of normal pollen from the plants grown under the 16 hours light period. Pistillody was examined with the material grown under the 17 hours light period.

To determine the growth stage when a long-day treatment exerts the critical effect on plants to transform stamens toward the pistillate structure, the alloplasmic as well as the euplasmic lines of N26 with the Ae. crass cytoplasm were grown under 13 hours light period, and transferred to 17 hours light period condition in due order of the growth stages. The growth stage

of the plants was determined by the Feekes' scale from FS1 to FS10 (Feekes 1941, see footnote of Table 5). The stages were actually divided into five classes from FS1-2 to FS9-10 in this experiment. Two to nine plants at each growth class were transferred. They remained under the same condition until maturity, and selfed seed fertility (%) of individual plants was estimated.

For histological observation, ears at the flowering stage were fixed with Carnoy's fluid (one part glacial acetic acid and three parts 99 % ethanol). After passing a Randolph's series of ethanol-N-butanol mixtures differing in their concentration, these ears were embedded in paraffin. Serial paraffin sections of the florets were stained with safranine and Fastgreen FCF.

4) Studies on the effects of natural photoperiodic conditions on male fertility

The alloplasmic lines of N26 and CS with the Ae. crassa cytoplasm and their euplasmic lines as controls were grown at the Kasai Experimental Farm, Sumitomo Chemical Co., Hyogo, Japan (lat. 34°55'N) and the experimental field of Tanno Agricultural Cooperative, Hokkaido, Japan (lat. 43°50'N). Fig. 1 indicates the locations of Kasai and Tanno. The materials were sown in November and harvested in next June at Kasai, and sown in May and harvested in August at Tanno. The observations were made for five years (1986/87, 87/88, 88/89, 89/90 and 90/91) at Kasai and for three years (1989, 1990 and 1991) at Tanno. The natural day length at Kasai during the wheat-growing season ranges from 10 to 14.5 hours, whereas that at Tanno varies from 14 to 15.5

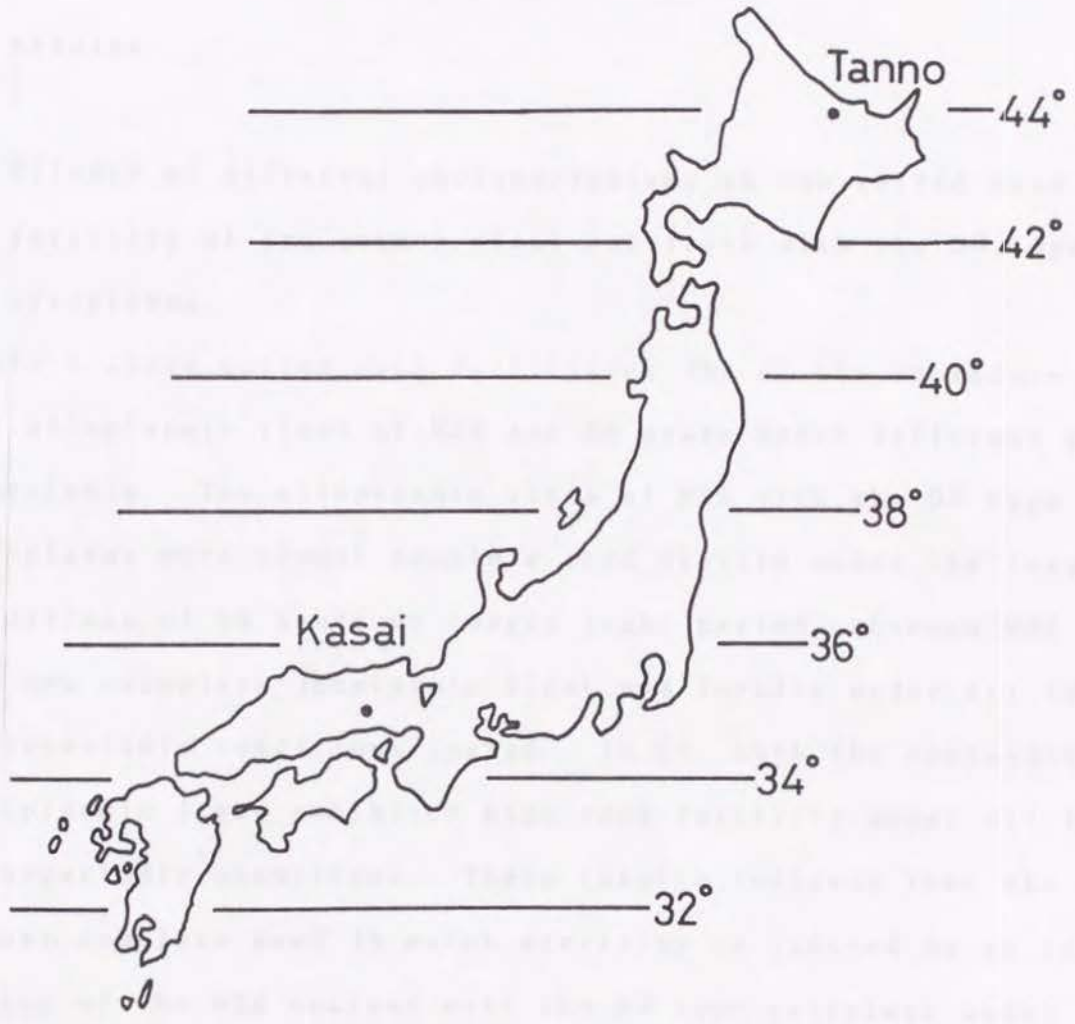

Figure 1. Locations of the Kasai Experimental Farm of Sumitomo Chemical Co., Hyogo and the experimental field of Tanno Agricultural Cooperative, Hokkaido.

hours. The materials tested were space-planted at a 10 cm distance, and the ears of main shoot of three to 14 plants per line were bagged before flowering, in order to estimate their selfed seed fertilities (%).

3. Results

1) Effects of different photoperiodisms on the selfed seed fertility of two common wheat cultivars with the D^2 type cytoplasms

Table 1 shows selfed seed fertilities (%) of the euplasmic and the alloplasmic lines of N26 and CS grown under different photoperiodic. The alloplasmic lines of N26 with the D^2 type cytoplasms were almost complete seed sterile under the long-day conditions of 15 hours or longer light period, whereas N26 with its own cytoplasm (euplasmic line) was fertile under all the photoperiodic conditions tested. In CS, both the euplasmic and alloplasmic lines exhibited high seed fertility under all the photoperiodic conditions. These results indicate that the almost complete seed (≒ male) sterility is induced by an interaction of the N26 nucleus with the D^2 type cytoplasm under the long-day condition of 15 hours or longer light period, and that the CS nucleus has some genetic factors which prevent the reduction in fertility under these long-day conditions.

Effects of different temperatures on seed fertility of common wheats having the D^2 type cytoplasm were investigated with a 14 hours light period (Table 2). No remarkable reduction in seed fertility was found under any temperature conditions,

Table 1. Selfed seed fertilities (%) of the euplasmic and alloplasmic lines of common wheat cultivars, Norin 26 and Chinese Spring, grown under various photoperiodic conditions[1]

Line[2]	Light period (hours)			
	13.5	14.5	15	17
N26	82.7	60.3	71.5	45.5
(crassa)-N26	57.1	40.6	0.5	0.0
(juvenalis)-N26	58.2	37.7	0.0	2.1
(vavilovii)-N26	60.4	34.1	0.7	0.3
CS	54.6	51.6	59.9	74.1
(crassa)-CS	71.6	46.5	58.2	33.2
(juvenalis)-CS	55.0	63.3	52.8	28.2
(vavilovii)-CS	55.8	64.0	40.2	22.0

1) Temperature condition was 15 °C in the dark and 18 °C in the light periods.
2) N26: Norin 26, CS: Chinese Spring
(crassa)-, (juvenalis)- and (vavilovii)-:
Alloplasmic lines with the Ae. crassa, Ae. juvenalis and Ae. vavilovii cytoplasm, respectively.

Table 2. Selfed seed fertilities (%) of the euplasmic and alloplasmic lines of common wheat cultivars, Norin 26 and Chinese Spring, grown under various temperature conditions[1]

Line[2]	Temperature (°C) (dark-light periods)					
	(9-12)	(12-15)	(15-18)	(18-21)	(5-18)	(10-18)
N26	73.4	76.3	82.7	47.5	83.7	73.8
(crassa)-N26	32.8	28.7	25.7	28.8	56.9	29.0
(juvenalis)-N26	37.3	40.5	45.6	20.2	59.0	36.2
(vavilovii)-N26	23.1	22.9	22.5	26.3	50.1	43.5
CS	80.5	48.1	51.7	36.7	66.9	58.1
(crassa)-CS	64.9	42.6	44.1	33.9	59.0	62.1
(juvenalis)-CS	62.9	48.3	52.0	15.5	63.9	52.6
(vavilovii)-CS	70.2	58.7	48.3	21.7	53.6	61.0

1) Photoperiod was 14 hours light and 10 hours dark.
2) N26: Norin 26, CS: Chinese Spring
(crassa)-, (juvenalis)- and (vavilovii)-: Alloplasmic lines with the Ae. crassa, Ae. juvenalis and Ae. vavilovii cytoplasm, respectively.

indicating that male sterility is not affected by temperatures within the present range.

2) Morphological and histological studies on the male sterility expression

More than 90 % of pistillody frequency was observed for the stamens of alloplasmic line of N26 with Ae. crassa cytoplasm under the long-day (17 hours) conditions (Table 3). The remaining normal stamens of the alloplasmic line were found to show the same degree of pollen fertility as that of N26 with normal cytoplasm under the long-day (16 hours) condition (Table 4). Pollen grains of the euplasmic and alloplasmic lines of N26 under the long-day (16 hours) condition were shown in Fig. 2. These results indicate that male sterility with the Ae. crassa cytoplasm is due to pistillody of stamens induced by the long-day conditions. Fig. 3 shows floral organs of the alloplasmic as well as the euplasmic N26 at the flowering stage grown under the long-day conditions. Each of pistilated stamens of alloplasmic N26 looks like a normal pistil. However, they have only one stigma in contrast to two stigmas of the normal one. Transverse sections revealed that there were incomplete ovule-like structures but neither tapetal cells nor pollen grain in the pistilated stamen (Fig. 4). These findings suggest that pistilated stamen has functions of neither stamen nor pistil. Fig. 4 also shows that the pistil of the alloplasmic N26 is normal.

3) Plant growth stage when the male sterility is effected under the long-day conditions

Table 3. Pistillody frequencies (%) of the euplasmic and alloplasmic lines of Norin 26 under the long-day condition of 17 hours artificial photoperiod

Line[1]	No. of stamens		Pistillody frequency (%)
	Examined	Pistilated	
N26	438	0	0
(*crassa*)-N26	492	459	93.3

1) N26: Norin 26, (*crassa*)-N26: Alloplasmic line of Norin 26 with the *Ae. crassa* cytoplasm.

Table 4. Pollen fertilities (%) of the euplasmic and alloplasmic Norin 26 under the long-day condition of 16 hours artificial photoperiod

Line[1]	No. of pollen		Pollen fertility (%)
	Examined	Normal	
N26	146	139	95.4
(*crassa*)-N26	99	95	96.1

1) N26: Norin 26, (*crassa*)-N26: Alloplasmic line of Norin 26 with the *Ae. crassa* cytoplasm.

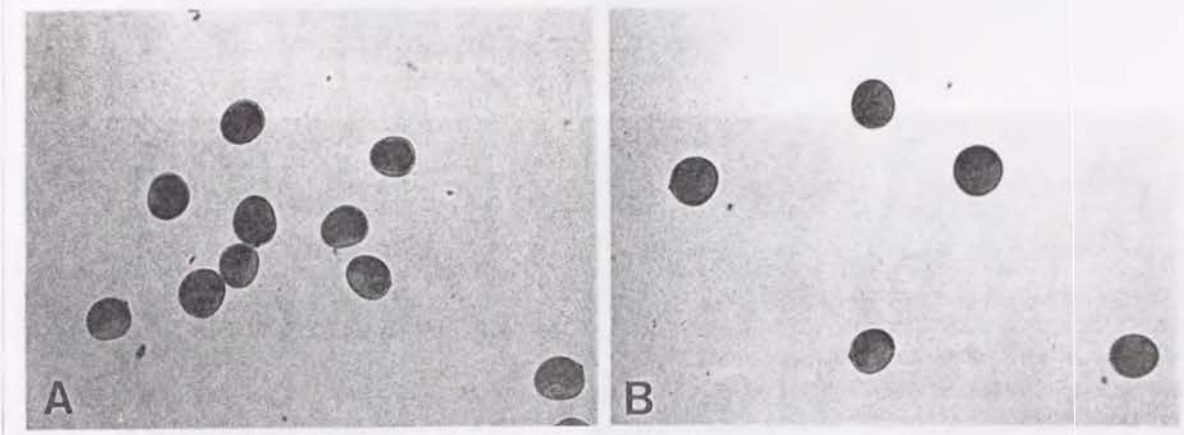

Figure 2. Pollen grains of the euplasmic and alloplasmic lines of T. aestivum cv. Norin 26 (N26) grown under the long-day (16 hours) condition.
A: Pollen grains of the euplasmic N26, B: Pollen grains of the normal stamens of the alloplasmic N26 with the Ae. crassa cytoplasm.

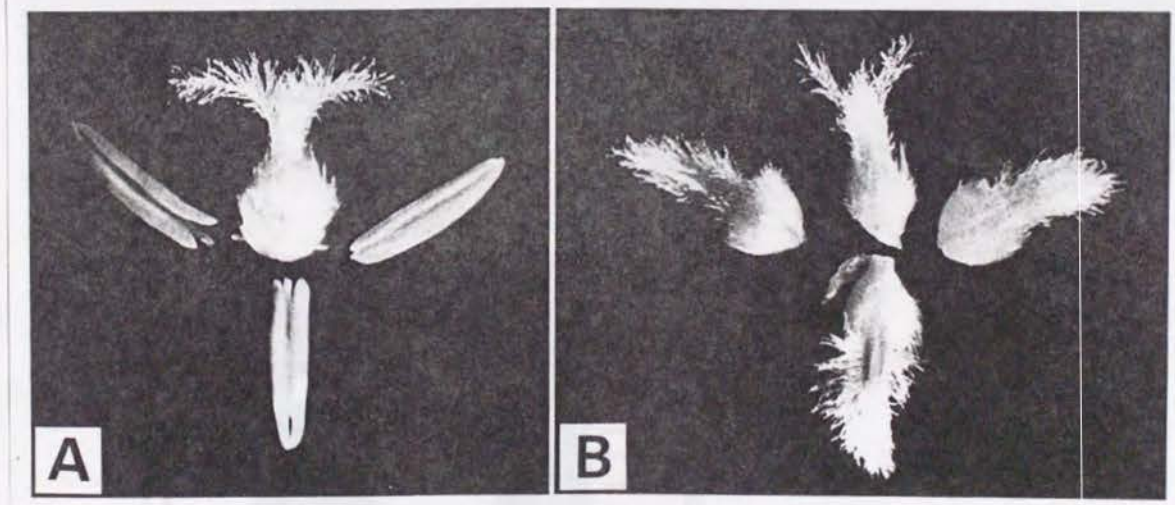

Figure 3. Floral organs of the euplasmic and alloplasmic lines of T. aestivum cv. Norin 26 (N26) grown under the long-day conditions.
A: Euplasmic N26, B: Alloplasmic N26 with the Ae. crassa cytoplasm.

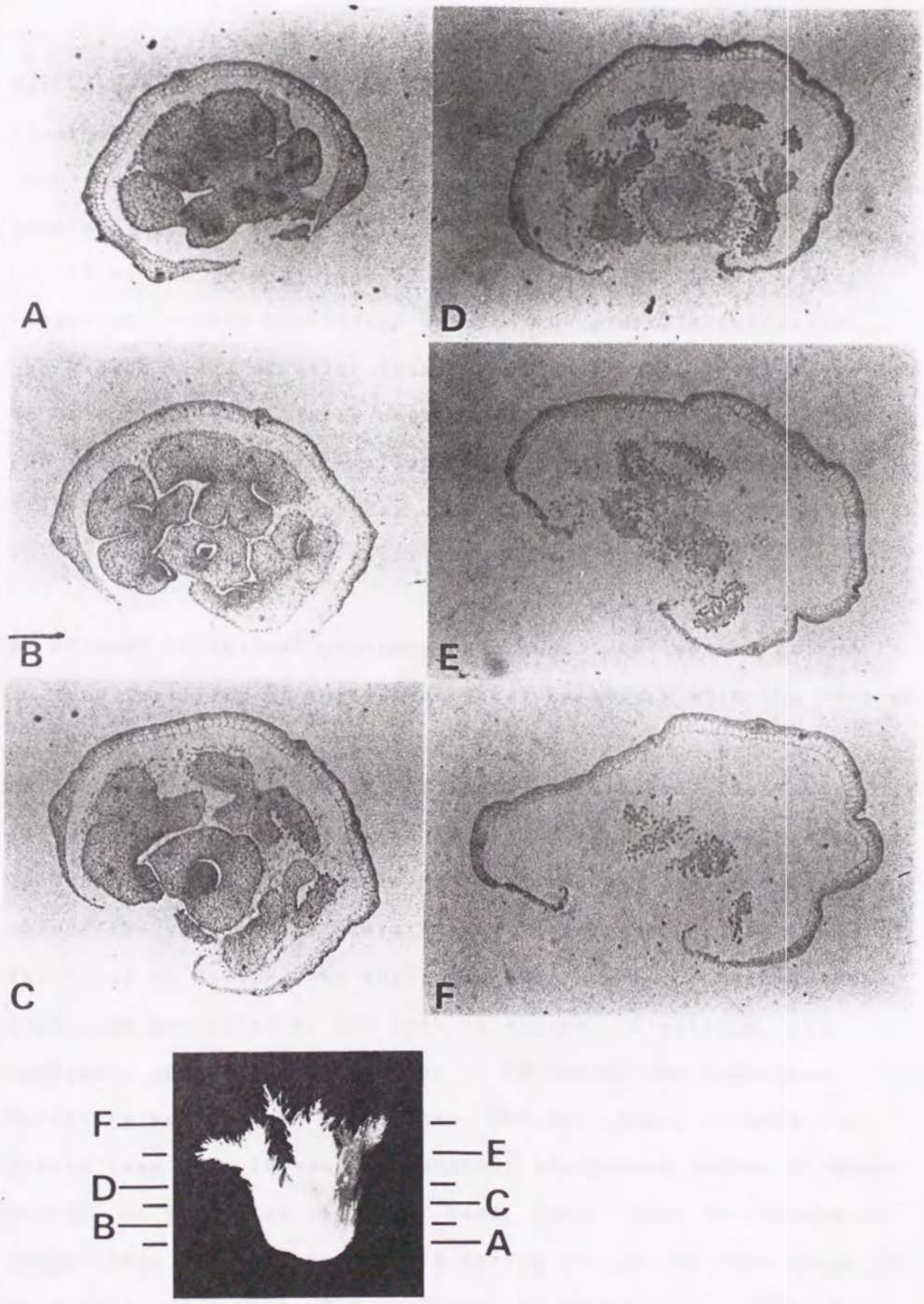

Figure 4. Transverse sections of a floret of the alloplasmic line of T. aestivum cv. Norin 26 with the Ae. crassa cytoplasm. A-F: Basal to apical of the floret.

Selfed seed fertilities (%) of the euplasmic and alloplasmic lines of N26 transferred to the long-day (17 hours) condition from the short-day (13 hours) condition in due order of plant growth stage are shown in Table 5. The alloplasmic N26 transferred at the growth stage of earlier than FS6 showed a clear reduction in male fertility, whereas the plants transferred at the growth stage of later than FS7 did not exhibit any reduction in male fertility. These results imply that the growth stage FS6-7 is crucial for the alloplasmic plant to exhibit male sterility under the long-day condition. The growth stage FS6 to FS7 is the floret differentiation stage.

4) Effects of natural photoperiodic conditions on the selfed seed fertility of two common wheat cultivars with the D^2 type cytoplasm

Selfed seed fertilities (%) of the euplasmic and alloplasmic lines of N26 and CS estimated at both Kasai and Tanno are shown in Table 6. The alloplasmic N26 with the Ae. crassa cytoplasm showed the severe male sterility at Tanno, whereas the high fertility at Kasai. The euplasmic N26, however, exhibited the high male fertility at the both locations. Similarly, the euplasmic and alloplasmic lines of CS showed the high male fertility at the both locations. The day length of Kasai is always less than 15 hours throughout the growth season of wheat plant. At Tanno, on the other hand, wheat plant is exposed to longer than 15 hours day length during the period from about 40 days before to about 10 days after the heading time (Fig. 5). These findings indicate that the alloplasmic N26 results in male

Table 5. Selfed seed fertilities (%) of the euplasmic and alloplasmic Norin 26 transferred to the long-day (17 hours) condition from the short-day (13 hours) condition of artificial photoperiod in due order of plant growth stage

Line[1]	Growth stage[2] when the plants are transferred to the long-day condition				
	FS1-2	FS3-4	FS5-6	FS7-8	FS9-10
N26	63.3	39.8	29.8	37.5	68.5
(*crassa*)-N26	8.7	12.1	12.1	48.3	58.6

1) N26: Norin 26, (*crassa*)-N26: Alloplasmic Norin 26 with the *Ae. crassa* cytoplasm.
2) FS1: One shoot
FS2: Tillering begins
FS3: Tillers formed
FS4: Leaf-sheaths elongate
FS5: Leaf-sheaths strongly erected
FS6: First node of stem becomes visible
FS7: Second node becomes visible
FS8: Last leaf just becomes visible
FS9: Ligule of last leaf just becomes visible
FS10: In "boot"
(Feekes 1941)

Table 6. Selfed seed fertilities (%) of the euplasmic and alloplasmic lines of common wheat cultivars, Norin 26 and Chinese Spring, grown under the natural day length conditions at two different locations

	Kasai					Tanno		
Line[1)]	1986/87	87/88	88/89	89/90	90/91	1989	1990	1991
N26	99.0	98.9	99.7	93.1	94.1	97.5	98.9	97.2
(*crassa*)-N26	56.8	53.3	72.8	60.6	63.1	1.0	6.5	5.2
CS	-	85.0	85.3	65.7	72.7	84.5	79.1	75.2
(*crassa*)-CS	-	70.7	69.2	69.7	68.3	66.2	61.3	56.6

1) N26: Norin 26, CS: Chinese Spring, (*crassa*)-N26 and CS: Alloplasmic lines of Norin 26 and Chinese Spring with the *Ae. crassa* cytoplasm.

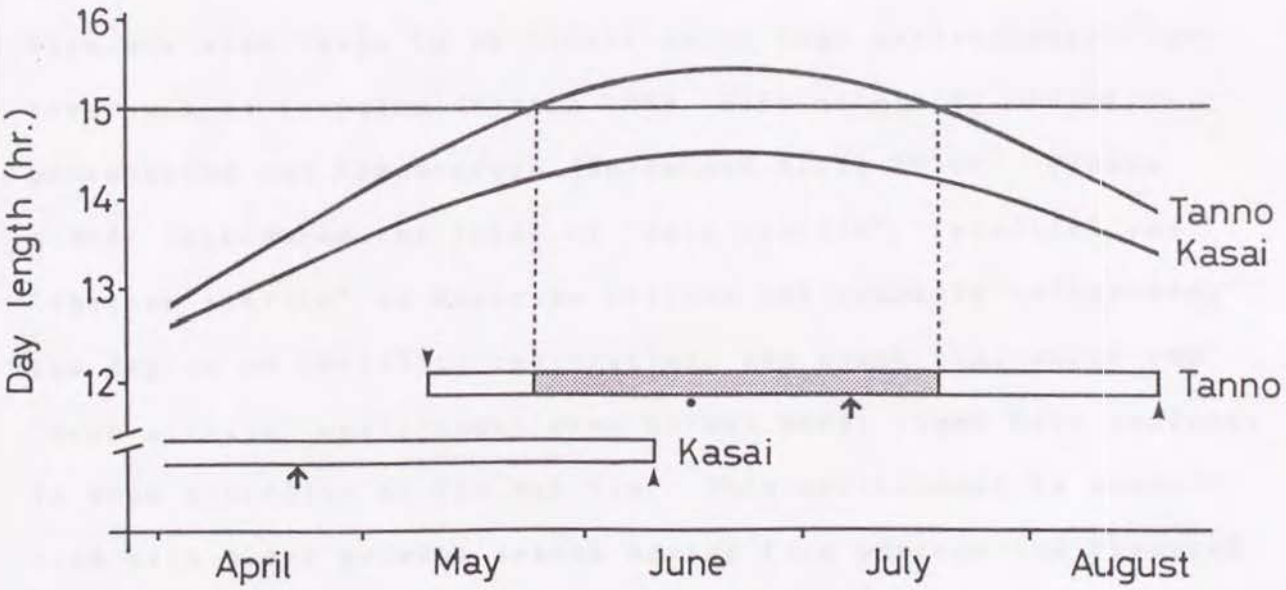

Figure 5. Day length and wheat growth season at Kasai and Tanno. ▼: seeding, ▲: harvesting, ↑: heading, and ●: start of the floret differentiation stage.

sterility under the long-day conditions of longer than 15 hours. The tests were repeated for five seasons at Kasai and three seasons at Tanno (Table 6). It can be said that the male sterility induction at Tanno and the male fertility restoration at Kasai are stable phenomena for the alloplasmic N26.

4. Discussion

1) Photoperiod-sensitive cytoplasmic male sterility (PCMS)

In euplasmic lines of common wheat cultivars, male sterility is induced by a number of environmental factors such as drought (Bingham 1966), shortened photoperiod (Fisher 1972), copper deficiency (Graham 1975) and extreme temperature (Saini and Aspinall 1982). Cytoplasmic male sterility-fertility restoration was also found to be sensitive to some environmental factors such as location (Wilson 1968, Schmidt et. al. 1970), photoperiod and temperature (Welsh and Klatt 1971). Wilson (1968) introduced the terms of "deep sterile", "sterile" and "shallow sterile" to describe various environments influencing the degree of fertility-restoration, and noted that under the "deep sterile" environment even normal wheat lines have tendency to show sterility at the ear tip. This environment is associated with short growing season having long photoperiod resulted from the high north latitude. The cytoplasmic male sterility studied in this thesis, however, can not be explained in terms of the "deep sterile" environment. Namely, I have found the drastic reduction in male fertility induced by the long-day treatments of 15 hours or longer light period on the alloplasmic

lines of Norin 26 having the D^2 type cytoplasms. This is the first case of male sterility caused by a nucleo-cytoplasmic interaction involved in an environmental factor in wheat. Sasakuma and Ohtsuka (1979) suggested that the male sterility of the alloplasmic Norin 26 might be induced by either long-day length or great temperature difference between day and night at Hokkaido during growth season of wheat. My results clearly demonstrate that the male sterility is solely caused by the long-day length and that it is not influenced by temperature. This male sterility may be thus called "photoperiod-sensitive cytoplasmic male sterility (abbreviated, PCMS)". All the D^2 type cytoplasms of Ae. crassa, Ae. juvenalis and Ae. vavilovii similarly induce PCMS when substituted into Norin 26. This fact indicates that the three Aegilops species have the identical plasmon, which has been suggested by the morphological and physiological studies of the alloplasmic lines (Tsunewaki 1980a) and the investigation on the diversity of chloroplast DNAs (Ogihara and Tsunewaki 1988).

PCMS is morphologically expressed as pistillody of stamens. Pistillody induced by photoperiodic treatments was reported by Fisher (1972) in a wheat cultivar, Opal. He found that the transformation of stamens to pistils was caused by transferring plants from 16 hours to 10 hours photoperiods when stamen primordia were visible on the first floret of the most advanced spikelet of the main shoot. Pistillody of stamens has been known to be also induced by alien cytoplasms in wheat since forty years ago. Kihara (1951) observed partial pistillody induction in T. aestivum var. erythrospermum by Ae. caudata

cytoplasm, and the degree of the pistillody was greatly influenced by environmental conditions, especially day length, i. e., long-day conditions enhanced the pistillody of stamens. The Ae. caudata cytoplasm was also reported to cause complete anther malformation, half of which was pistillody of stamens on the alloplasmic line of Norin 26 (Tsunewaki 1980a). The D^2 type cytoplasms induce partial pistillody in Norin 26 under the short-day conditions, and the pistillody induction is affected by day length. However, the D^2 type cytoplasms are distinguished from the Ae. caudata cytoplasm in the following two points: the residual stamens are normal, and the complete pistillody is expressed under the long-day conditions. Kihara and Tsunewaki (1961) reported that an alloplasmic line of T. durum with the Ae. caudata cytoplasm induced pistillody of all stamens under any natural conditions, and that the pistilated stamen had no ovule but only a cavity instead. The pistilated stamens of the PCMS has a different structure. My morphological and histological studies demonstrated that a basal part of them looks like ovary morphologically as was suggested by Sasakuma and Ohtsuka (1979), and they have an incomplete ovule-like structure, without any tapetal cells and pollen grains.

2) Two-line system for hybrid wheat production using the PCMS

A photoperiod-sensitive genic male sterility has been discovered in rice (Shi 1981). It seems to be controlled mainly by a recessive gene of which effect is expressed under long-day conditions (Zhu and Yu 1989). The new system for hybrid rice production using this phenomenon has been commercialized in

China and called "two-line system" for hybrid rice production.

The PCMS proposes a "two-line system" for hybrid wheat production as shown in Fig. 6. The PCMS line can be maintained by self-pollination under the short-day conditions of shorter than 14.5 hours. Hybrid seeds can be produced through the out-crossing of a PCMS line with a pollinator line under the long-day conditions longer than 15 hours. In contrast to the system of hybrid wheat production using T. timopheevi cytoplasm, the present system requires only the PCMS and pollinator lines. No need for the development of maintainer lines leads to the following advantages;

a) ease to maintain the PCMS line, and

b) shortened period required for developing the PCMS line.

Since the PCMS line is partially fertile under the short-day conditions, some fertility-restoring gene(s) is necessary for the pollinator line.

As for in Japan, the long-day conditions were found in Hokkaido, and the short-day conditions in southern part of Honshu, Kyushu and Shikoku islands. Actually, the alloplasmic line of Norin 26 with the Ae. crassa cytoplasm can be maintained in Honshu by self-pollination, and is used as a PCMS line in Hokkaido where male sterility is fully expressed.

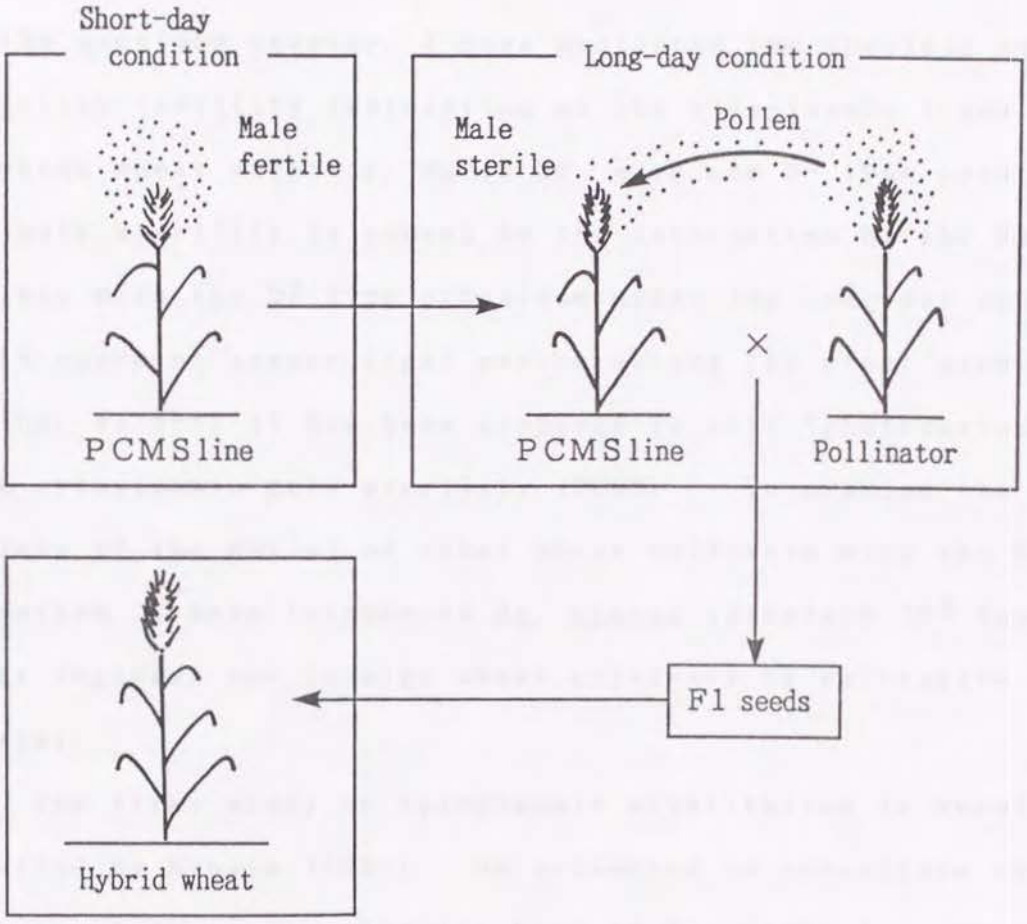

Figure 6. Two-line system for hybrid wheat production using photoperiod-sensitive cytoplasmic male sterility (PCMS). Long-day and short-day indicate photoperiod of longer than 15 hours and shorter than 14.5 hours, respectively.

III. PCMS INDUCTION BY *AEGILOPS CRASSA* CYTOPLASM IN WHEAT CULTIVARS

1. Introduction

In the previous chapter, I have mentioned the specific male sterility-fertility restoration of the alloplasmic lines of a Japanese wheat cultivar, Norin 26, with the D^2 type cytoplasms. The male sterility is caused by the interaction of the Norin 26 nucleus with the D^2 type cytoplasm under the long-day conditions of 15 hours or longer light period during the plant growth period, so that it has been proposed to call "photoperiod-sensitive cytoplasmic male sterility (PCMS)". To examine the interactions of the nuclei of other wheat cultivars with the D^2 type cytoplasm, I have introduced *Ae. crassa* cytoplasm (D^2 type) into other Japanese and foreign wheat cultivars by successive backcrosses.

The first study on cytoplasmic substitution in wheat were reported by Kihara (1951). He attempted to substitute the nucleus of *Ae. longissima* for that of *Ae. aucheri*, starting from a cross made in 1940 between *Ae. longissima* as female and *Ae. aucheri* as male. *Ae. longissima* and *Ae. aucheri* have semihomologous genome, S^lS^l and SS, respectively. In the F_1, the number of bivalents varied from 5 to 7. The chromosome paring improved rapidly with the increasing number of backcrosses, and seven normal bivalents were counted in B_4. The morphology of the ears in the successive backcross generations indicated that in the advanced generations the nucleus of *Ae. longissima* had been

replaced by that of Ae. aucheri in the Ae. longissima cytoplasm. He also introduced the cytoplasm of Ae. caudata into T. aestivum var. erythrospermum (Tve). The two species, a diploid Ae. caudata with genome constitution CC and a hexaploid Tve with AABBDD, have no genome in common. One of the B_2 plants with the chromosome configuration of $21^{II} + 2^{I}$ gave 14 descendants from open pollination, and two of them were Tve-like plants. Characteristic effects of the alien cytoplasm expressed in alloplasmic lines are empirically known not to be altered after many backcrosses (Michaelis 1954, Kihara 1959). To demonstrate this empirical knowledge, Endo and Tsunewaki (1975) used synthetic Ae. triuncialis (syn-triuncialis) derived from a cross of Ae. caudata (female) with Ae. umbellulata (male) (Kondo 1941). Ae. caudata and Kondo's syn-triuncialis were backcrossed to recurrent pollen parents of common wheat to produce alloplasmic lines. Essentially, the Ae. caudata and syn-triuncialis cytoplasms exerted very similar effects in the alloplasmic lines of common wheat, suggesting that the plasma genes of Ae. caudata have persisted unaltered since the time of amphidiploidization in Kondo's syn-triuncialis and through the following substitution backcrosses to common wheat. Later, Ogihara and Tsunewaki (1982) revealed that the chloroplast DNA of the syn-triuncialis was identical to that of Ae. caudata, and suggested that the base sequence of chloroplast DNA was not altered by the presence of alien nuclear genomes.

In this chapter, I will report the results of production of alloplasmic lines of common wheat cultivars with the Ae. crassa cytoplasm and estimation of the PCMS induction in them. More-

over, the distribution of fertility-restoring gene(s) against the Ae. crassa cytoplasm in Japanese and CIMMYT wheat cultivars is considered.

2. Materials and Methods

1) Plant materials

Nucleus donors: In total 45 Japanese cultivars shown in Table 7 and 31 foreign cultivars shown in Table 8 were used as the nucleus donors, and have been backcrossed as the recurrent pollen parents to develop the alloplasmic lines. I selected spring habit cultivars as the nucleus donors, because the estimation for the PCMS induction in the alloplasmic lines is performed by spring sowing cultivation at the experimental field of Tanno Agricultural Cooperative, Hokkaido, Japan. Among 31 foreign cultivars, 20 cultivars were bred in the CIMMYT (International Maize and Wheat Improvement Center), eight in the U. S. A., two in Australia and one in China.

Cytoplasm donor: Alloplasmic line of Norin 26 having the Ae. crass cytoplasm (Ae. crassa/11*Norin 26) was used as the cytoplasm donor in the production of the alloplasmic lines.

Alloplasmic lines: Backcrosses were performed in one or two ears per line at the Kasai Experimental Farm, Sumitomo Chemical Co., Hyogo, Japan, and crossed seed fertilities (%) were estimated for each backcross generation. Backcross generations of the alloplasmic lines are shown in Table 7 for Japanese and Table 8 for foreign cultivars.

Table 7. Japanese wheat cultivars which were used as the nucleus donor in the production of alloplasmic lines in the present investigation

Code no.	Cultivar	Generation
01	Norin 61	B_5
02	Chikushi-komugi	B_5
03	Shirasagi-komugi	B_5
04	Junrei-komugi	B_5
05	Nichirin-komugi	B_5
06	Fujimi-komugi	B_5
07	Yutaka-komugi	B_5
08	Ushio-komugi	B_5
09	Omase-komugi	B_5
10	Kobushi-komugi	B_5
11	Zenkoji-komugi	B_5
12	Danchi-komugi	B_5
13	Asakaze-komugi	B_4
14	Sakigake-komugi	B_4
15	Seto-komugi	B_4
16	Gogatsu-komugi	B_4
17	Iyo-komugi	B_4
18	Hatsuho-komugi	B_4
19	Kairyohaya-komugi	B_4
20	Norin 52	B_3
21	Daruma 2	B_3
22	Hatada-komugi	B_3
23	Haruhikari	B_3
24	Norin 75	B_3
25	Haruminori	B_3
26	Norin 53	B_3
27	Shirogane-komugi	B_3
28	Hayato-komugi	B_3
29	Hiyoku-komugi	B_3
30	Hatamasari	B_3
31	Shinchunaga	B_3
32	Fukuho-komugi	B_2
33	Norin 29	B_2
34	Ejima-shinriki	B_2
35	Fukuwase-komugi	B_2
36	Nishikaze-komugi	B_2
37	Minamino-komugi	B_2
38	Shirowase-komugi	B_2
39	Shinriki	B_2
40	Toyoho-komugi	B_1
41	Haya-komugi 1	F_1
42	Akabozu	F_1
43	Fukuoka-komugi 18	F_1
44	Saitama 27	F_1
45	Ejima	F_1

Table 8. Foreign wheat cultivars which were used as the nucleus donor in the production of alloplasmic lines in the present investigation

Code no.	Cultivars	Origin	Generation
46	Orofen	China	B_5
47	Penjamo 62	CIMMYT	B_5
48	Anza	USA (CIMMYT)	B_3
49	Olaf	USA	B_3
50	White Federation 54	USA	B_3
51	Wheaton	USA	B_3
52	Owens	USA	B_3
53	Sterling	USA	B_3
54	Inia 66R	CIMMYT	B_3
55	Super X	CIMMYT	B_2
56	Yecora Rojo	USA (CIMMYT)	B_2
57	Cranbrook	Australia	B_1
58	Condor	Australia	B_1
59	Ramona 50	USA	F_1
60	Sonora 64	CIMMYT	F_1
61	Tobari	CIMMYT	F_1
62	Torim 73	CIMMYT	F_1
63	Lerma Rojo 64A	CIMMYT	F_1
64	Jaral 66	CIMMYT	F_1
65	Tanori 71	CIMMYT	F_1
66	Lerma Rojo (Amber)	CIMMYT	F_1
67	Yecora 70	CIMMYT	F_1
68	Yecora	CIMMYT	F_1
69	Kalyansona SE-2	CIMMYT	F_1
70	Azteca	CIMMYT	F_1
71	Mexipak	Pakistan (CIMMYT)	F_1
72	Ciano	CIMMYT	F_1
73	Zaragoza 75	CIMMYT	F_1
74	NacozariF76 (BluejayS)	CIMMYT	F_1
75	Kalyansona	India (CIMMYT)	F_1
76	D6301	USA	F_1

CIMMYT: International Maize and Wheat Improvement Center.

2) Estimation of the PCMS induction in wheat cultivars

Selfed seed fertilities (%) observed in successive generations of wheat cultivars during repeated backcrosses for introducing the Ae. crassa cytoplasm into them were estimated at the Kasai Experiment Farm of Sumitomo Chemical Co. and at the experimental field of Tanno Agricultural Cooperative. The materials were sown in November and harvested in next June at Kasai, and sown in May and harvested in August at Tanno. The experiments were performed for six years (1985/86-90/91) at Kasai and for three years (1989-91) at Tanno. The plants tested were space-planted at a 10 cm distance. The ears of main shoot of two to three plants per line were bagged before flowering, and their selfed seed fertilities (%) were estimated by the seed setting rate of the first and second florets of all spikelets.

As for the alloplasmic lines of Norin 61, Shirasagi-komugi, Junrei-komugi, Nichirin-komugi, Fujimi-komugi and Ushio-komugi, their selfed seed fertilities in the B_3 generation were also estimated under an artificial photoperiodic condition of 17 hours light period for 24 hours period at 20,000 lux. Temperature condition set was 15 °C in the dark and 18 °C in the light period. Materials were individually planted in pots of 10 cm diameter and 13 cm height. Selfed seed fertility was estimated by the seed setting rate of the first and second florets of all spikelets in three bagged ears per plant. Two plants were studied in each line, and their average values were used for analysis.

3. Results

1) Production of the alloplasmic lines with the Ae. crassa cytoplasm

Tables 9 and 10 show crossed seed fertilities (%) observed in successive backcross generations of Japanese and foreign cultivars introducing the Ae. crassa cytoplasm into them, respectively. On the average of all backcross generations, the lowest crossed seed fertility observed among 45 Japanese cultivars was 53.3 % of Norin 52 in improved cultivars and 46.6 % of Ejima in native cultivars. As for foreign cultivars, 45.9 % of Wheaton is the lowest crossed seed fertility observed. Since the crossed seed fertility was obtained by artificial pollination which was not always carried out under an ideal condition, these figures indicate almost normal female fertility of the alloplasmic lines of all cultivars.

2) The PCMS induction in Japanese wheat cultivars

Tables 11 and 12 show selfed seed fertilities (%) in successive generations of Japanese cultivars during repeated backcrosses for introducing the Ae. crassa cytoplasm, which were observed under the natural condition at Kasai and Tanno, respectively. In Fig. 7, 12 lines of B_5 generations and seven lines of B_4 generations were plotted to illustrate the relationships between their averaged selfed seed fertilities at Kasai and Tanno. These 19 alloplasmic lines were clearly divided into two groups on the basis of selfed seed fertilities at Kasai and Tanno. The first group consists of nine alloplasmic lines of Chikushi-komugi, Shirasagi-komugi, Junrei-komugi, Fujimi-komugi, Omase-komugi, Kobushi-komugi, Asakaze-komugi, Gogatsu-komugi and Iyo-

Table 9. Crossed seed fertilities (%) observed in successive backcross generations of Japanese cultivars for introducing the *Ae. crassa* cytoplasm into them

Cultivar	Generation						Average
	F_1	B_1	B_2	B_3	B_4	B_5	
Norin 61	60.7	71.7	72.2	83.8	81.9	92.5	77.1
Chikushi-komugi	38.5	50.0	70.1	80.9	78.2	90.0	68.0
Shirasagi-komugi	46.2	26.0	82.5	84.7	86.4	85.0	68.5
Junrei-komugi	65.4	40.5	76.0	94.5	72.8	80.0	71.5
Nichirin-komugi	61.5	62.4	70.2	70.0	85.9	78.4	71.4
Fujimi-komugi	69.2	13.3	82.2	62.5	84.8	66.8	63.1
Yutaka-komugi	38.5	63.7	65.9	88.2	51.9	75.0	63.9
Ushio-komugi	69.2	79.2	63.3	55.6	85.0	92.5	74.1
Omase-komugi	46.4	64.6	61.4	75.0	67.5	62.5	62.9
Kobushi-komugi	63.6	70.8	49.2	80.5	63.1	70.0	66.2
Zenkoji-komugi	31.8	44.4	88.4	67.5	74.5	68.4	62.5
Danchi-komugi	38.5	33.9	88.0	87.8	66.9	63.9	63.2
Asakaze-komugi	29.2	45.0	52.7	86.7	77.5	30.0	53.5
Sakigake-komugi	41.3	67.5	52.5	87.0	60.0	76.1	64.1
Seto-komugi	25.0	85.0	61.5	57.5	35.0	63.4	54.6
Gogatsu-komugi	35.3	81.3	61.1	87.5	60.0	57.5	63.8
Iyo-komugi	27.6	75.0	79.2	80.0	70.0	61.2	65.5
Hatsuho-komugi	57.1	55.9	87.5	100.0	87.5	89.7	79.6
Kairyohaya-komugi	59.6	71.4	86.1	93.2	37.5	89.7	72.9
Norin 52	21.3	54.7	70.5	67.7	52.5	-	53.3
Daruma 2	50.0	85.0	58.0	80.0	88.9	-	72.4
Hatada-komugi	60.0	60.0	75.9	75.0	34.7	-	61.1
Haruhikari	-	53.3	87.5	92.5	83.4	-	79.2
Norin 75	-	25.0	90.0	72.5	72.3	-	65.0
Haruminori	-	35.0	75.0	92.5	97.2	-	74.9
Norin 53	44.7	80.0	78.6	85.0	85.0	-	74.7
Shirogane-komugi	56.6	87.8	84.5	77.5	82.5	-	77.8
Hayato-komugi	51.6	82.5	74.2	52.5	92.5	-	70.7
Hiyoku-komugi	56.4	72.2	69.8	52.5	69.1	-	64.0
Hatamasari	60.5	78.7	92.2	61.8	89.5	-	76.5
Shinchunaga	36.5	89.4	68.2	72.5	100.0	-	73.3
Fukuho-komugi	47.4	65.0	77.5	82.5	-	-	68.1
Norin 29	50.0	86.4	85.0	75.4	-	-	74.2
Ejima-shinriki	47.6	62.5	43.0	77.5	-	-	57.7
Fukuwase-komugi	74.3	60.0	22.5	80.0	-	-	59.2
Nishikaze-komugi	65.9	90.7	82.5	85.0	-	-	81.0
Minamino-komugi	84.0	90.7	95.0	90.0	-	-	89.9
Shirowase-komugi	56.1	91.1	43.7	75.0	-	-	66.5
Shinriki	77.8	90.9	82.5	50.1	-	-	75.3
Toyoho-komugi	44.4	87.5	87.5	-	-	-	73.1
Haya-komugi 1	44.0	78.9	-	-	-	-	61.5
Akabozu	68.2	43.8	-	-	-	-	56.0
Fukuoka-komugi 18	85.0	77.8	-	-	-	-	81.4
Saitama 27	80.0	91.7	-	-	-	-	85.9
Ejima	23.1	70.0	-	-	-	-	46.6

Table 10. Crossed seed fertilities (%) observed in successive backcross generations of foreign cultivars for introducing the Ae. crassa cytoplasm into them

Cultivar	Generation						Average
	F_1	B_1	B_2	B_3	B_4	B_5	
Orofen	81.8	60.4	76.3	94.5	87.5	90.0	81.8
Penjamo 62	38.5	64.5	64.6	18.1	80.5	27.5	49.0
Anza	15.9	67.5	72.5	52.5	73.7	-	56.4
Olaf	14.6	36.7	50.0	52.5	92.5	-	49.3
White Federation 54	41.7	52.8	75.0	32.5	58.8	-	52.2
Wheaton	4.2	60.3	62.5	22.5	80.0	-	45.9
Owens	54.6	86.3	35.1	54.3	88.9	-	63.8
Sterling	68.6	76.4	67.5	77.5	66.4	-	71.3
Inia 66R	46.7	67.0	87.5	45.0	66.0	-	56.4
Super X	65.8	56.7	70.0	72.5	-	-	66.3
Yecora Rojo	36.5	84.6	75.0	57.0	-	-	63.3
Cranbrook	35.0	67.5	80.9	-	-	-	61.1
Condor	55.0	88.7	55.6	-	-	-	66.4
Ramona 50	31.8	81.6	-	-	-	-	56.7
Sonora 64	70.8	94.1	-	-	-	-	82.5
Tobari	54.5	72.2	-	-	-	-	63.4
Torim 73	50.0	56.3	-	-	-	-	53.2
Lerma Rojo 64A	37.5	78.2	-	-	-	-	57.9
Jaral 66	60.0	47.3	-	-	-	-	53.7
Tanori 71	85.0	65.0	-	-	-	-	75.0
Lerma Rojo (Amber)	100.0	82.7	-	-	-	-	91.4
Yecora 70	68.2	83.4	-	-	-	-	75.8
Yecora	75.0	58.3	-	-	-	-	66.7
Kalyansona SE-2	40.0	55.6	-	-	-	-	47.8
Azteca	72.7	71.9	-	-	-	-	72.3
Mexipak	60.0	57.8	-	-	-	-	58.9
Ciano	42.3	71.9	-	-	-	-	57.1
Zaragoza 75	90.0	47.2	-	-	-	-	68.6
NacozariF76(BluejayS)	68.2	71.9	-	-	-	-	70.1
Kalyansona	66.7	75.9	-	-	-	-	71.3
D6301	50.0	84.4	-	-	-	-	67.2

Table 11. Selfed seed fertilities (%) observed at Kasai in successive generations of Japanese cultivars during repeated backcrosses for introducing the Ae. crassa cytoplasm into them

Cultivar	Generation						Average
	F_1	B_1	B_2	B_3	B_4	B_5	
Norin 61	77.7	94.0	88.8	90.1	83.3	86.8	86.8
Chikushi-komugi	61.4	76.9	69.3	81.1	74.3	71.5	72.4
Shirasagi-komugi	50.0	63.8	77.6	62.2	39.5	63.8	59.5
Junrei-komugi	58.7	61.4	56.0	66.7	61.5	60.8	60.9
Nichirin-komugi	71.8	84.6	71.6	93.9	93.0	88.7	83.9
Fujimi-komugi	51.7	68.4	35.0	68.9	42.5	51.6	53.0
Yutaka-komugi	66.2	53.2	63.7	78.1	77.7	79.9	69.8
Ushio-komugi	79.8	86.1	89.6	80.6	85.3	86.4	84.6
Omase-komugi	63.0	59.6	65.2	79.6	64.3	56.1	64.6
Kobushi-komugi	-	58.5	61.7	61.1	55.3	52.9	57.9
Zenkoji-komugi	62.9	71.4	54.4	74.5	83.6	68.8	69.3
Danchi-komugi	60.4	53.6	64.0	75.5	83.6	69.0	67.7
Asakaze-komugi	58.5	62.3	62.5	60.5	49.8	-	58.7
Sakigake-komugi	79.6	70.8	84.4	92.4	89.0	-	83.2
Seto-komugi	64.2	54.7	66.1	69.3	73.0	-	65.5
Gogatsu-komugi	67.8	60.0	93.8	77.1	59.7	-	71.7
Iyo-komugi	35.8	53.3	69.3	59.1	63.9	-	56.3
Hatsuho-komugi	62.9	66.4	89.1	95.8	81.2	-	79.1
Kairyohaya-komugi	64.7	71.3	81.2	76.3	90.4	-	76.8
Norin 52	63.8	63.3	77.0	70.2	-	-	68.6
Daruma 2	61.5	46.8	65.5	61.0	-	-	58.7
Hatada-komugi	78.0	80.9	78.6	72.0	-	-	77.4
Haruhikari	14.8	46.1	90.3	78.8	-	-	57.5
Norin 75	100.0	85.7	83.3	72.8	-	-	85.5
Haruminori	100.0	89.0	63.3	76.5	-	-	82.2
Norin 53	54.5	64.6	73.5	64.9	-	-	64.4
Shirogane-komugi	61.1	61.2	59.5	52.9	-	-	58.7
Hayato-komugi	50.0	60.9	97.7	58.6	-	-	59.3
Hiyoku-komugi	51.4	74.2	54.0	66.2	-	-	61.5
Hatamasari	62.3	68.3	63.4	73.4	-	-	66.9
Shinchunaga	64.1	77.2	72.7	76.1	-	-	72.5
Fukuho-komugi	80.3	62.9	67.1	-	-	-	70.1
Norin 29	89.4	81.8	77.2	-	-	-	82.8
Ejima-shinriki	83.5	63.9	48.4	-	-	-	65.3
Fukuwase-komugi	66.9	43.2	56.1	-	-	-	55.4
Nishikaze-komugi	62.5	79.6	83.1	-	-	-	75.1
Minamino-komugi	87.2	64.6	82.6	-	-	-	78.1
Shirowase-komugi	60.6	43.5	63.9	-	-	-	56.0
Shinriki	70.1	82.6	77.6	-	-	-	76.8
Toyoho-komugi	83.0	87.9	-	-	-	-	85.5
Haya-komugi 1	70.0	-	-	-	-	-	70.0
Akabozu	40.6	-	-	-	-	-	40.6
Fukuoka-komugi 18	68.3	-	-	-	-	-	68.3
Saitama 27	67.5	-	-	-	-	-	67.5
Ejima	76.1	-	-	-	-	-	76.1

Table 12. Selfed seed fertilities (%) observed at Tanno in successive generations of Japanese cultivars during repeated backcrosses for introducing the Ae. crassa cytoplasm into them

Cultivar	Generation						Average
	F_1	B_1	B_2	B_3	B_4	B_5	
Norin 61	-	-	-	38.5	82.0	47.1	55.9
Chikushi-komugi	-	-	-	1.1	11.2	0.0	4.1
Shirasagi-komugi	-	-	-	0.0	27.2	0.0	9.1
Junrei-komugi	-	-	-	0.0	15.2	0.0	5.1
Nichirin-komugi	-	-	-	51.8	45.3	44.1	47.1
Fujimi-komugi	-	-	-	0.0	0.0	0.0	0.0
Yutaka-komugi	-	-	-	34.8	60.2	34.6	43.2
Ushio-komugi	-	-	-	53.4	85.1	17.6	52.0
Omase-komugi	-	-	-	5.6	10.3	19.3	11.7
Kobushi-komugi	-	-	-	7.8	17.6	0.0	8.5
Zenkoji-komugi	-	-	-	0.0	51.1	33.2	28.1
Danchi-komugi	-	-	-	26.5	57.0	14.4	32.6
Asakaze-komugi	-	-	0.0	8.3	0.0	-	2.8
Sakigake-komugi	-	-	41.5	38.3	32.2	-	37.3
Seto-komugi	-	-	8.4	49.0	20.6	-	26.0
Gogatsu-komugi	-	-	1.2	3.8	0.0	-	1.7
Iyo-komugi	-	-	0.0	1.1	0.0	-	0.4
Hatsuho-komugi	-	-	9.1	55.5	30.1	-	31.6
Kairyohaya-komugi	-	-	16.4	46.3	13.3	-	25.3
Norin 52	-	-	5.5	33.1	-	-	19.3
Daruma 2	-	37.0	-	27.5	-	-	32.3
Hatada-komugi	-	16.7	48.2	0.8	-	-	21.9
Haruhikari	-	-	42.0	65.3	-	-	53.7
Norin 75	-	91.1	89.3	64.9	-	-	81.8
Haruminori	-	69.6	91.2	74.8	-	-	78.5
Norin 53	-	-	41.5	15.6	-	-	28.6
Shirogane-komugi	-	0.0	13.0	2.1	-	-	5.0
Hayato-komugi	-	6.7	40.9	0.0	-	-	15.9
Hiyoku-komugi	-	-	13.5	0.0	-	-	5.2
Hatamasari	-	0.0	53.0	37.3	-	-	30.1
Shinchunaga	-	8.3	51.2	38.2	-	-	32.6
Fukuho-komugi	-	37.5	13.3	-	-	-	25.4
Norin 29	-	82.3	74.0	-	-	-	78.2
Ejima-shinriki	-	33.8	21.7	-	-	-	27.8
Fukuwase-komugi	-	4.5	0.0	-	-	-	2.3
Nishikaze-komugi	-	6.9	10.4	-	-	-	8.7
Minamino-komugi	-	31.5	-	-	-	-	31.5
Shirowase-komugi	-	22.5	1.4	-	-	-	12.0
Shinriki	-	69.1	78.3	-	-	-	73.7
Toyoho-komugi	59.0	64.6	-	-	-	-	61.8
Haya-komugi 1	16.6	-	-	-	-	-	16.6
Akabozu	0.0	-	-	-	-	-	0.0
Fukuoka-komugi 18	2.3	-	-	-	-	-	2.3
Saitama 27	0.0	-	-	-	-	-	0.0
Ejima	60.7	-	-	-	-	-	60.7

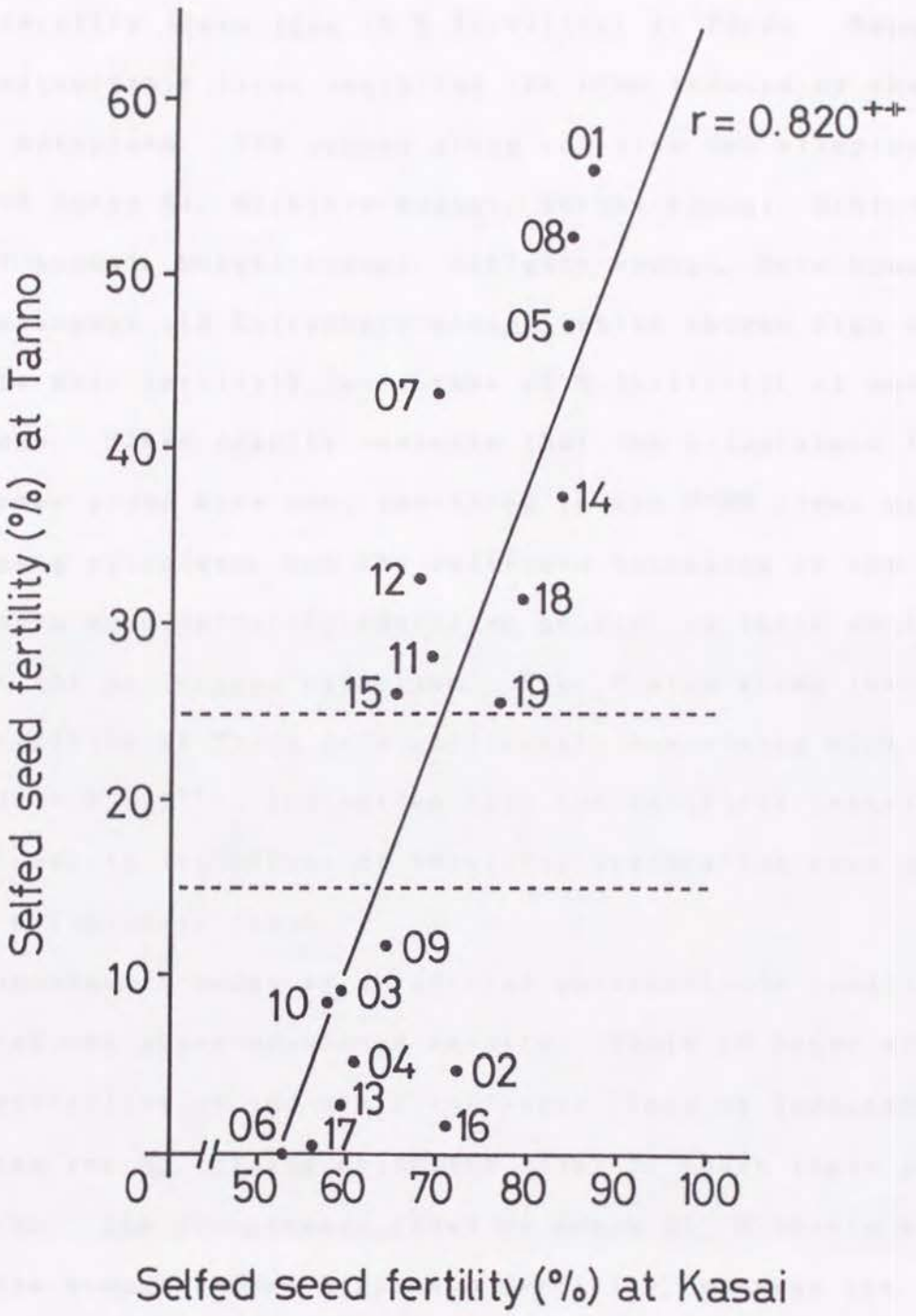

Figure 7. The relationships between selfed seed fertilities at Kasai and Tanno observed in the alloplasmic lines of 19 Japanese wheat cultivars having the Ae. crassa cytoplasm; each line is indicated with its code number given in Table 7.

komugi, which showed high male fertility at Kasai but severe male sterility (less than 15 % fertility) at Tanno. Namely, their alloplasmic lines exhibited the PCMS induced by the Ae. crassa cytoplasm. The second group contains ten alloplasmic lines of Norin 61, Nichirin-komugi, Yutaka-komugi, Ushio-komugi, Zenkoji-komugi, Danchi-komugi, Sakigake-komugi, Seto-komugi, Hatsuho-komugi and Kairyohaya-komugi, which showed high to moderate male fertility (more than 25 % fertility) at both Kasai and Tanno. These results indicate that the alloplasmic lines of the former group have been converted to the PCMS lines with the Ae. crassa cytoplasm, and the cultivars belonging to the latter group have some fertility-restoring gene(s) in their nuclei against the Ae. crassa cytoplasm. Fig. 7 also shows that the male fertility at Tanno is significantly correlated with that at Kasai ($r = 0.820^{**}$), indicating that the fertility-restoring gene(s) exerts its effect of fertility restoration even at Kasai on the alloplasmic lines.

Experiments under an artificial photoperiodic condition supported the above-mentioned results. Table 13 shows selfed seed fertilities of the six alloplasmic lines of Japanese cultivars with the Ae. crassa cytoplasm under 17 hours light period condition. The alloplasmic lines of Norin 61, Nichirin-komugi and Ushio-komugi showed high male fertility, whereas the alloplasmic lines of Shirasagi-komugi, Junrei-komugi and Fujimi-komugi showed severe male sterility (less than 15 % fertility), indicating that the former three cultivars have some fertility-restoring gene(s). Ears of the six alloplasmic lines are shown in Fig. 8.

Table 13. Selfed seed fertilities (%) under the long-day (17 hours) photoperiodic condition observed in B_3 generation of six Japanese cultivars during repeated backcrosses for introducing the Ae. crassa cytoplasm into them

Code no.	Cultivar	Selfed seed fertility (%)
01	Norin 61	50.1
03	Shirasagi-komugi	13.9
04	Junrei-komugi	14.6
05	Nichirin-komugi	58.0
06	Fujimi-komugi	6.7
08	Ushio-komugi	58.8

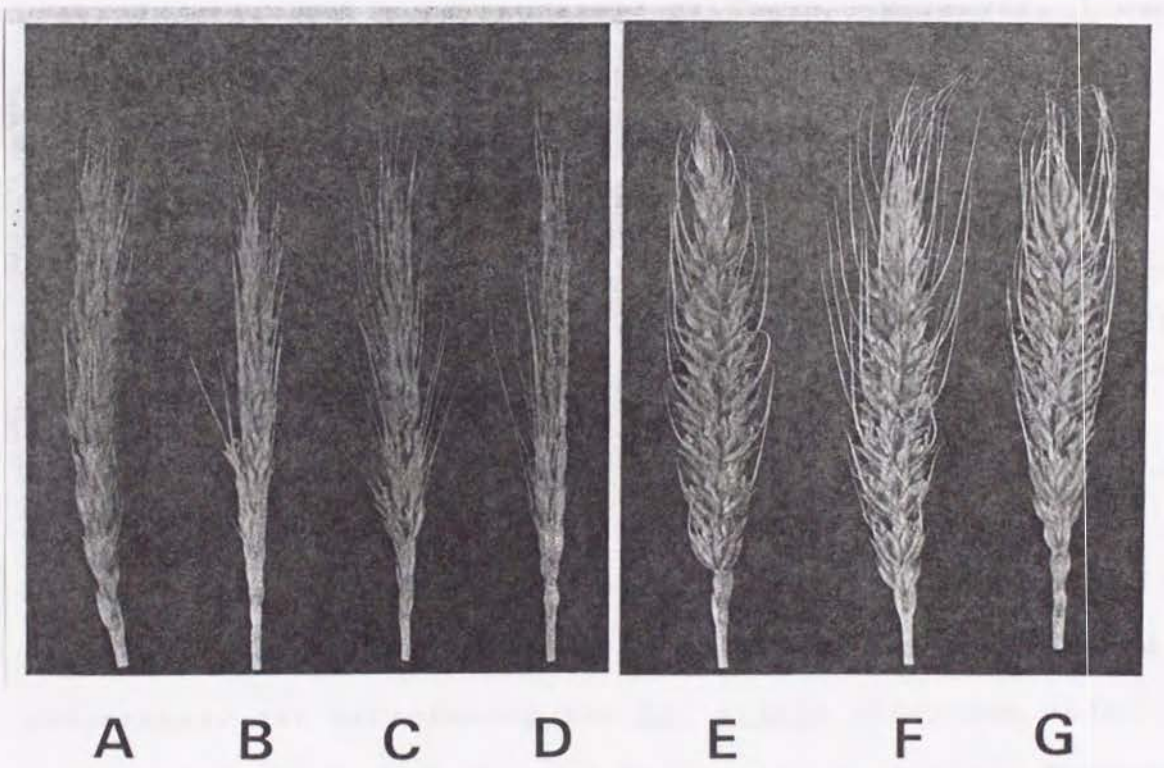

Figure 8. Ears of the seven alloplasmic lines of Japanese cultivars having the Ae. crassa cytoplasm grown under the long-day (17 hours) photoperiodic condition.
A: Norin 26, B: Shirasagi-komugi, C: Junrei-komugi, D: Fujimi-komugi, E: Norin 61, F: Nichirin-komugi and G: Ushio-komugi.

As for other cultivars, it may be said that the alloplasmic lines of Shirogane-komugi, Hiyoku-komugi, Fukuwase-komugi, Nishikaze-komugi and Shirowase-komugi show the PCMS under the presence of the Ae. crassa cytoplasm, because they showed severe male sterility (less than 15 % fertility on the average for two or three backcross generations) at Tanno. On the other hand, Daruma 2, Haruhikari, Norin 75, Haruminori, Norin 53, Hatamasari, Shinchunaga, Fukuho-komugi, Norin 29, Ejima-shinriki, Minamino-komugi, Shinriki and Toyoho-komugi seemed to have some fertility-restoring gene(s), since the moderate male fertility (more than 25 % fertility on the average for two or three backcross generations) were expressed at Tanno, Moreover, I can say that Norin 75, Haruminori, Norin 29 and Shinriki have relatively strong fertility-restoring gene(s) because their alloplasmic lines showed higher fertility than 70 % at Tanno. Among five cultivars examined their male fertility in F_1 generation, Ejima seemed to have some fertility-restoring gene(s) which is effective with the single dose.

3) The PCMS induction in foreign wheat cultivars

Tables 14 and 15 show selfed seed fertilities (%) observed in successive generations of foreign cultivars during repeated backcrosses for introducing the Ae. crassa cytoplasm under the natural condition at Kasai and Tanno, respectively. From the results of the backcross generations of 13 cultivars, it may be said that the alloplasmic line of White Federation 54 exhibits the PCMS and nine cultivars, Orofen, Anza, Olaf, Wheaton, Owens, Starling, Super X, Yecora Rojo, and Condor have some fertility-

Table 14. Selfed seed fertilities (%) observed at Kasai in successive generations of foreign cultivars during repeated backcrosses for introducing the Ae. crassa cytoplasm into them

Cultivar	Generation						Average
	F_1	B_1	B_2	B_3	B_4	B_5	
Orofen	-	74.1	65.2	86.0	75.5	82.0	76.6
Penjamo 62	43.6	43.6	37.5	66.4	61.5	63.0	52.6
Anza	71.2	47.3	65.8	58.7	-	-	60.8
Olaf	64.9	68.5	64.6	59.8	-	-	64.5
White Federation 54	49.2	60.6	49.2	66.3	-	-	56.3
Wheaton	49.9	54.4	62.6	81.6	-	-	62.1
Owens	55.5	63.5	60.6	82.5	-	-	65.5
Sterling	51.2	57.4	65.7	52.3	-	-	56.7
Inia 66R	58.7	62.9	74.8	70.1	-	-	66.6
Super X	61.3	71.9	76.4	-	-	-	69.9
Yecora Rojo	74.6	76.3	70.7	-	-	-	73.8
Cranbrook	91.4	79.9	-	-	-	-	85.7
Condor	84.6	45.0	-	-	-	-	64.8
Ramona 50	62.3	-	-	-	-	-	62.3
Sonora 64	83.2	-	-	-	-	-	82.3
Tobari	89.3	-	-	-	-	-	89.3
Torim 73	84.5	-	-	-	-	-	84.5
Lerma Rojo 64A	78.2	-	-	-	-	-	78.2
Jaral 66	81.9	-	-	-	-	-	81.9
Tanori 71	85.2	-	-	-	-	-	85.2
Lerma Rojo (Amber)	81.7	-	-	-	-	-	81.7
Yecora 70	69.3	-	-	-	-	-	69.3
Yecora	78.0	-	-	-	-	-	78.0
Kalyansona SE-2	65.5	-	-	-	-	-	65.5
Azteca	77.8	-	-	-	-	-	77.8
Mexipak	67.1	-	-	-	-	-	67.1
Ciano	78.3	-	-	-	-	-	78.3
Zaragoza 75	66.2	-	-	-	-	-	66.2
NacozariF76(BluejayS)	85.2	-	-	-	-	-	85.2
Kalyansona	64.8	-	-	-	-	-	64.8
D6301	71.3	-	-	-	-	-	71.3

Table 15. Selfed seed fertilities (%) observed at Tanno in successive generations of foreign cultivars during repeated backcrosses for introducing the Ae. crassa cytoplasm into them

Cultivar	Generation						Average
	F_1	B_1	B_2	B_3	B_4	B_5	
Orofen	-	-	-	74.8	77.1	28.1	60.0
Penjamo 62	-	-	-	-	43.1	2.1	22.6
Anza	-	-	44.6	33.4	-	-	39.0
Olaf	-	-	65.9	37.4	-	-	51.7
White Federation 54	-	-	2.4	0.0	-	-	1.2
Wheaton	-	-	84.4	57.8	-	-	71.1
Owens	-	-	76.2	20.0	-	-	48.1
Sterling	-	-	48.8	11.8	-	-	30.3
Inia 66R	-	6.7	31.7	8.3	-	-	15.6
Super X	-	53.1	8.4	-	-	-	30.8
Yecora Rojo	-	59.2	32.9	-	-	-	46.1
Cranbrook	-	15.6	-	-	-	-	15.6
Condor	60.7	13.6	-	-	-	-	37.2
Ramona 50	5.0	-	-	-	-	-	5.0
Sonora 64	59.4	-	-	-	-	-	59.4
Tobari	47.0	-	-	-	-	-	47.0
Torim 73	31.3	-	-	-	-	-	31.3
Lerma Rojo 64A	9.6	-	-	-	-	-	9.6
Jaral 66	5.6	-	-	-	-	-	5.6
Tanori 71	26.5	-	-	-	-	-	26.5
Lerma Rojo (Amber)	12.2	-	-	-	-	-	12.2
Yecora 70	4.5	-	-	-	-	-	4.5
Yecora	3.6	-	-	-	-	-	3.6
Kalyansona SE-2	5.8	-	-	-	-	-	5.8
Azteca	19.6	-	-	-	-	-	19.6
Mexipak	4.6	-	-	-	-	-	4.6
Ciano	32.7	-	-	-	-	-	32.7
Zaragoza 75	9.9	-	-	-	-	-	9.9
NacozariF76(BluejayS)	50.1	-	-	-	-	-	50.1
Kalyansona	8.2	-	-	-	-	-	8.2
D6301	0.0	-	-	-	-	-	0.0

restoring gene(s). Fig. 9 illustrates the relationships between selfed seed fertilities observed at Kasai and Tanno in F_1 generation of 16 CIMMYT wheat cultivars. This result suggests that nine cultivars, Lerma Rojo 64A, Jaral 66, Lerma Rojo (Amber), Yecora 70, Yecora, Kalyansona SE-2, Mexipak, Zaragoza 75 and Kalyansona have no fertility-restoring gene(s), whereas three cultivars, Sonora 64, Tobari and Nacozari F76 (Bluejay"S"), have some fertility-restoring gene(s) which is effective in its single dose. Moreover, it is clear that two US cultivars, Remona 50 and D6301, have no fertility-restoring gene(s). There are significant relationships between the male fertilities at Tanno and Kasai ($r = 0.683^{**}$), indicating that the fertility-restoring gene(s) exerts its effect of fertility restoration also at Kasai in its single dose.

4. Discussion

1) Production of the PCMS lines of Japanese wheat cultivars

The alloplasmic lines of Chikushi-komugi, Shirasagi-komugi, Junrei-komugi, Fujimi-komugi, Omase-komugi and Kobushi-komugi (all in the B_5 generation), Asakaze-komugi, Gogatsu-komugi and Iyo-komugi (all in the B_4 generation) showed high male fertility at Kasai, while male sterility at Tanno. Consequently, I could successfully produce the PCMS lines of these nine Japanese cultivars of common wheat by introducing the Ae. crassa cytoplasm into them by repeated backcrosses. The fertility restoration of these PCMS lines was stably expressed at Kasai in different generations and years. On the other hand, the sterility

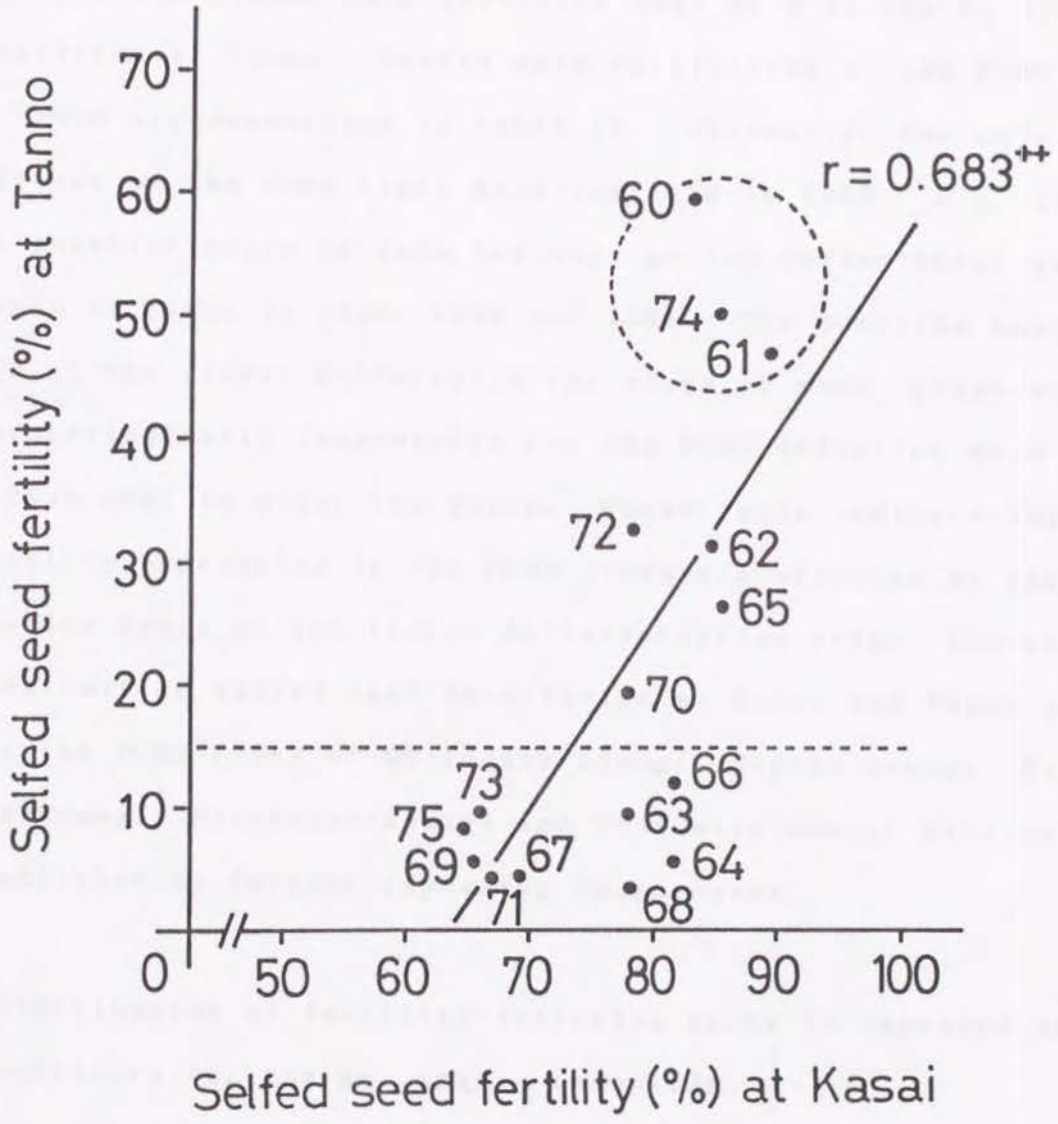

Figure 9. The relationships between selfed seed fertilities of the 16 F_1 hybrids between the alloplasmic Norin 26 having the Ae. crassa cytoplasm and CIMMYT cultivars observed at Kasai and Tanno; each hybrid is indicated with its code number given in Table 8.

expression of the PCMS lines at Tanno has been varied among different years, e. g., the PCMS line of Shirasagi-komugi showed complete male sterility in the B_3 (1989) and B_5 (1991) generations, while higher male fertility than 25 % in the B_4 (1990) generation at Tanno. Selfed seed fertilities of the PCMS lines at Tanno are summarized in Table 16. Obviously, the male fertilities of the PCMS lines were restored in 1990. Fig. 10 shows the sunshine hours of each ten-days period during wheat growing season at Tanno in 1989, 1990 and 1991. The sunshine hours in 1990 at the floret differentiation stage of wheat plant which is photoperiodically responsible for the PCMS induction were shorter than that in other two years. These facts indicate that the sterility expression in the PCMS lines was affected by the sunshine hours at the floret differentiation stage. The results of estimating selfed seed fertilities at Kasai and Tanno suggest that the PCMS lines of Shirogane-komugi, Hiyoku-komugi, Fukuwase-komugi, Nishikaze-komugi and Shirowase-komugi will be established by further repeating backcrosses.

2) Distribution of fertility-restoring genes in Japanese wheat cultivars for the Ae. crassa cytoplasm

I have found that five Japanese cultivars, Norin 61, Nichirin-komugi, Yutaka-komugi, Ushio-komugi and Sakigake-komugi, have relatively strong fertility-restoring gene(s) which can restore higher male fertility than 35 % at Tanno. Also, seven cultivars, Haruhikari, Norin 75, Haruminori, Norin 29, Shinriki, Toyoho-komugi and Ejima, are assumed to have relatively strong fertility-restoring gene(s). The pedigrees of these cultivars

Table 16. Selfed seed fertilities (%) of the PCMS lines of nine Japanese cultivars

Line	Generation[1)]	1989	1990	1991
Chikushi-komugi	B_5	1.1	11.2	0.0
Shirasagi-komugi	B_5	0.0	27.2	0.0
Junrei-komugi	B_5	0.0	15.2	0.0
Fujimi-komugi	B_5	0.0	0.0	0.0
Omase-komugi	B_5	5.6	10.3	19.3
Kobushi-komugi	B_5	7.8	17.6	0.0
Asakaze-komugi	B_4	0.0	8.3	0.0
Gogatsu-komugi	B_4	1.2	3.8	0.0
Iyo-komugi	B_4	0.0	1.1	0.0
Average		1.7	10.5	2.1

1) Backcross generation in 1991.

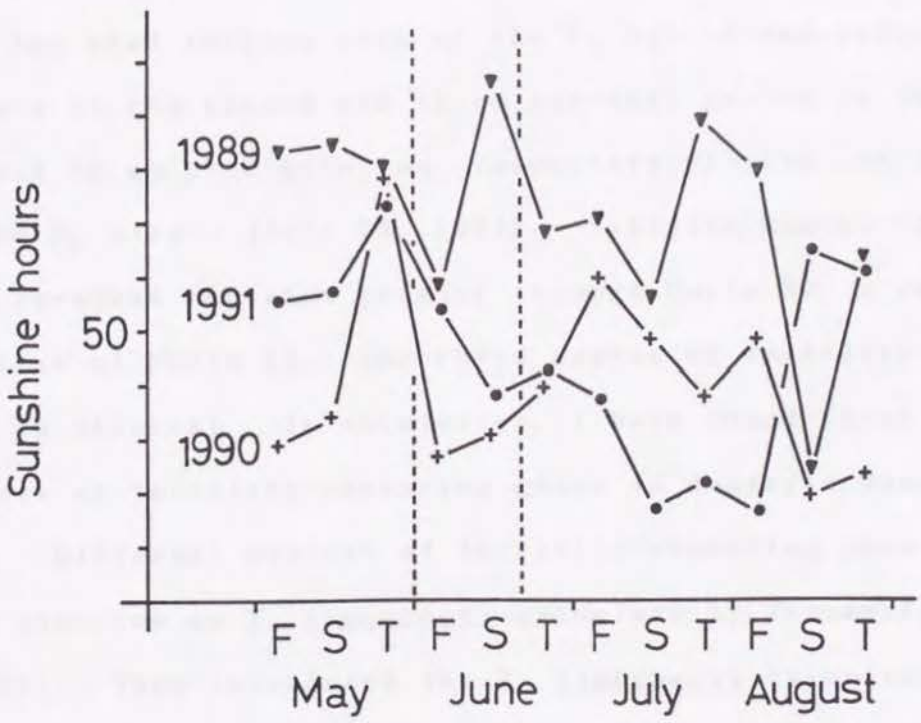

Figure 10. Sunshine hours of each ten-days period during wheat growth season in 1989, 1990 and 1991.
F, S and T: The first, second and third ten-days of a month
¦ ¦ ¦: Spikelet and floret differentiation stage.

are shown in Fig. 11, which indicates that there are three different groups having fertility-restoring gene(s). The first group consists of four cultivars, Haruhikari, Norin 75, Harumi-nori and Norin 29, all of which probably have a common source for their fertility-restoring gene(s), Norin 3. The second group consists of seven cultivars which assume to have received their fertility-restoring gene(s) from Ejima. Among them, Nichirin-komugi, Yutaka-komugi, Ushio-komugi and Toyoho-komugi obviously have an identical gene(s) for fertility restoration with that of Norin 61. The fertility-restoring gene(s) of Norin 61 seems to have been derived from Ejima through Fukuoka-komugi 18, although the F_1 hybrid of the alloplasmic Norin 26 with Fukuoka-komugi 18 showed low fertility at Tanno. It is probable that the seed setting rate of the F_1 hybrid was reduced by a lot of rain at the second and third ten-days period in July, 1991 (30 and 52 mm precipitation, respectively) with the late heading of the F_1 plants (July 13, 1991). Sakigake-komugi is assumed to have received the same gene(s) through Norin 60, a related cultivar of Norin 61. The third source of fertility-restoring gene is Shinriki. In conclusion, I have found three different sources of fertility-restoring genes in Japanese wheat cultivars. Different sources of fertility-restoring gene(s) were also reported on T. timopheevi cytoplasm by Tsunewaki et. al. (1976b). They introduced the T. timopheevi cytoplasm by successive backcrosses into 20 Japanese cultivars of common wheat. In advanced backcross generations, Junrei-komugi, Norin 50, Norin 52 and Norin 69 were found to be carriers of a weak fertility-restoring gene(s). From an investigation of their pedigrees,

Figure 11. Pedigrees of Japanese restorer cultivars to the Ae. crassa cytoplasm. (After Fukunaga and Inagaki 1985) Underlined: Carrier of a fertility-restoring gene(s).

Junrei-komugi, Norin 50 and Norin 52 were assumed to have received their gene(s) from a common ancestor, Shinchunaga, while Norin 69 appeared to have a different gene(s). In the present case, it is interesting to note that relatively strong gene(s) for fertility restoration exists in wheat cultivars.

3) Production of the PCMS lines of foreign wheat cultivars

The PCMS line of White Federation 54 (B_3 generation) was produced by repeated backcrosses. Nine CIMMYT cultivars, Lerma Rojo 64A, Jaral 66, Lerma Rojo (Amber), Yecora 70, Yecora, Kalyansona SE-2, Mexipak, Zaragoza 75, Kalyansona, and two US cultivars, Ramona 50 and D6301, seem to be converted to the PCMS lines by transferring the Ae. crassa cytoplasm to them.

4) Distribution of fertility-restoring genes in CIMMYT cultivars for the Ae. crassa cytoplasm

My results suggest that among the CIMMYT cultivars, Sonora 64, Tobari and Nacozari F76 have some fertility-restoring gene(s) against the Ae. crassa cytoplasm. The pedigrees of these cultivars are shown in Fig. 12, which indicates that the fertility-restoring gene(s) of Nacozari F76 and Tobari is identical with that of Sonora 64. The pedigree also implies that the fertility-restoring gene(s) probably came from Japanese wheat cultivar Norin 10. Although Norin 10 was not studied in this investigation, it seems to have fertility-restoring gene(s) because one of its ancestors, Daruma 2, was found to have weak fertility-restoring gene(s).

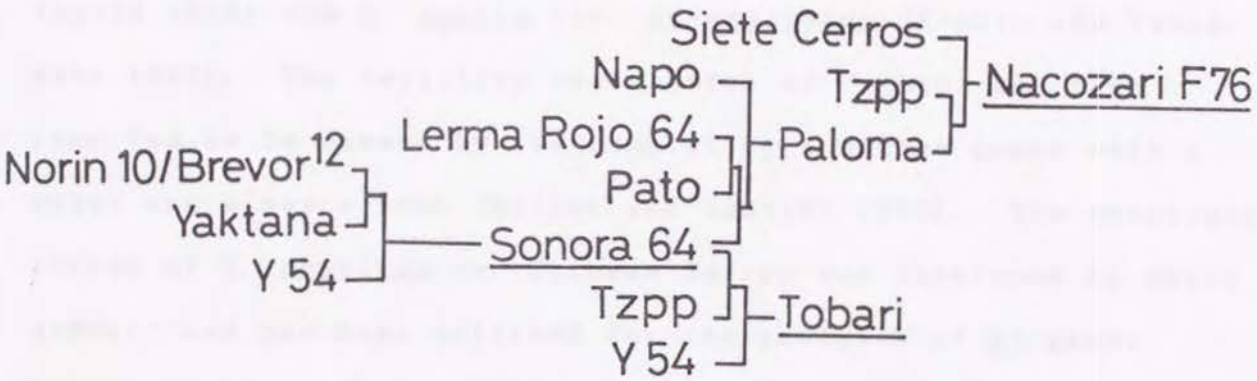

Figure 12. Pedigrees of CIMMYT restorer cultivars to the Ae. crassa cytoplasm. (After Gale et. al. 1981)
Y54: Yaqui 54, Tzpp: Tezanos Pinto Precoz.

IV. GENETIC ANALYSIS OF FERTILITY-RESTORING (Rf) GENE(S) AGAINST *AEGILOPS CRASSA* CYTOPLASM

1. Introduction

Genetic knowledge of cytoplasmic male sterility-fertility restoration mechanisms is helpful in developing parental lines for production of commercial hybrid wheat. Two of the most useful sources of *Rf* gene(s) against *T. timopheevi* cytoplasm found among hexaploid wheats are *T. aestivum* cv. Primepi (Oehler and Ingold 1966) and *T. spelta* var. *duhamelianum* (Kihara and Tsunewaki 1967). The fertility restoration of Primepi has been reported to be caused by two completely dominant genes with a major and minor effect (Miller and Schmidt 1970). The monosomic series of *T. aestivum* cv. Chinese Spring was developed by Sears (1954), and has been utilized for the analysis of *Rf* genes. Using the monosomic analysis, it was shown that the two *Rf* genes of Primepi was shown to be located on chromosomes 1B and 5D (Bahl and Maan 1973). Tahir and Tsunewaki (1969) reported that *T. spelta* var. *duhamelianum* carries a dominant *Rf* gene on its chromosome 1B, and that chromosome 7B exerts a weak suppressing effect to this gene.

In Chapter II, *T. aestivum* cv. Chinese Spring has some *Rf* gene(s) to the *Ae. crassa* cytoplasm. In Chapter III, I have demonstrated that *T. aestivum* cv. Norin 61 is an effective restorer to the *Ae. crassa* cytoplasm, and that its *Rf* gene(s) is distributed among other Japanese cultivars. In this chapter, conventional and chromosome analyses have been carried out to

identify Rf genes in Chinese Spring and Norin 61. From the results obtained, I will discuss genetic mechanisms and utilization of the Rf gene(s).

2. Materials and Methods

A. Genetic analysis of the Rf gene(s) in Chinese Spring

1) Plant materials

Alloplasmic line of Norin 26 having the Ae. crassa cytoplasm (Ae. crassa/11*Norin 26 ((c)-N26)) and Chinese Spring (CS) were used for the genetic analysis of the Rf gene(s). F_1 seeds were produced by hand-pollination between (c)-N26 as female and CS as male parent in greenhouse. N26 x CS F_1 was also examined as a control. F_2 plants were developed by self-pollination of the F_1 plants of cross, (c)-N26 x CS, at a growth room. Two kinds of backcrosses, (c)-N26 x {N26 x CS} and (c)-N26 x {(c)-N26 x CS}, were performed in 1988 at the Kasai Experimental Farm of Sumitomo Chemical Co., Hyogo, Japan.

Ditelosomic lines of CS developed by Sears and Sears (1978) was utilized in this investigation. Since ditelo-2AL, -4AS, -5AS, -5BS, -5DS and -7DL are not available because of male sterility, monotelodisomic-2AL, -4AS, -5AS, -5BS, -5DS, and nulli-7D tetra-7A and nulli-7D tetra-7B produced by Sears (1966) were used for analysis. All the telosomic and nulli-tetrasomic lines used in this investigation are shown in Table 17 with their selfed seed fertilities (%) observed at Kasai in the season of 1987/88.

Table 17. Selfed seed fertilities (%) observed at Kasai of the telosomic and nulli-tetrasomic lines used in this investigation

Line	Fertility (%)	Line	Fertility (%)	Line	Fertility (%)
Ditelo-1AL	66.0	Ditelo-1BL	64.0	Ditelo-1DL	59.6
-1AS	20.0	-1BS	47.7	-1DS	47.9
-2AS	59.8	-2BL	29.5	-2DL	28.3
-3AL	71.2	-2BS	40.5	-2DS	65.5
-3AS	52.4	-3BL	61.4	-3DL	76.1
-4AL	39.6	-3BS	32.0	-3DS	23.8
-5AL	52.3	-4BL	22.7	-4DL	56.5
-6AL	80.0	-4BS	53.5	-4DS	52.2
-6AS	50.0	-5BL	56.3	-5DL	86.0
-7AL	77.1	-6BL	59.3	-6DL	40.9
-7AS	64.3	-6BS	52.3	-6DS	75.0
		-7BL	77.6	-7DS	76.9
		-7BS	65.2		
Monotelo disomic-2AL	72.7	Monotelo disomic-5BS	61.0	Monotelo disomic-5DS	75.9
-4AS	76.9				
-5AS	84.0				
				Nulli-7D tetra-7A	67.3
				Nulli-7D tetra-7B	73.1
Disomic	85.0				

Monotelodisomic plants were identified by cytological examination of mitosis of their selfed progenies. For chromosome checking, seeds were sown in petri dishes, and root-tips were collected two to three days after seeding, pretreated in ice water for 24 hours, and fixed with Farmer's fixative (1:3 acetic alcohol). Cytological preparation was made by squashing a piece of the root-tip stained with acetocarmine for 15 minutes to one hour and warmed in flame about five seconds.

2) Conventional analysis

Parental lines and F_1 hybrids were grown at the Kasai Experimental Farm and the experimental field of the Tanno Agricultural Cooperative, Hokkaido, Japan. The materials were sown in November and harvested in next June at Kasai and sown in May and harvested in August at Tanno. The plants tested were space-planted at a 10 cm distance. Ears of main shoot of two to three plants per line were bagged before flowering, and their selfed seed fertilities (%) were estimated by the seed setting rate of the first and second florets of all spikelets.

The parental lines and F_1 hybrids as well as the plants of F_2 and B_1F_1 generations were also examined in growth rooms in which photoperiod and temperature could be controlled. The plants tested were individually planted in pots of 10 cm diameter and 13 cm height. Photoperiod condition set was 16 hours light period for every 24 hours period at 20,000 lux. and temperature set was 15°C in the dark and 18°C in the light periods for all but the analysis of the F_1 hybrids, for which three different photoperiodic conditions, i. e. 13, 16 and 17 hours

light period were tested. Selfed seed fertility (%) was estimated by the seed setting rate of the first and second florets of all spikelets in three bagged ears per plant. The average of the three ears represented the selfed seed fertility of individual plants. Two to five plants for the parental lines and three plants for the F_1 hybrids were studied. One hundred and thirty plants of the F_2 generation, 65 plants of the B_1F_1 generation of the cross, (c)-N26 x {N26 x CS}, and 63 plants of (c)-N26 x {(c)-N26 x CS}, were investigated for their selfed seed fertilities and examined for their segregations on fertility.

3) Telosomic analysis

Plants studied in telosomic analysis were individually planted in pots of 10 cm diameter and 13 cm height and grown in a growth room, in which photoperiod condition given was 16 hours light period for 24 hours at 20,000 lux. and temperature condition was 15 °C in the dark and 18 °C in the light periods. Selfed seed fertility (%) was estimated by the seed setting rate of the first and second florets of all spikelets in three bagged ears per plant. The average of the three ears represented the selfed seed fertility of individual plants.

(C)-N26 was pollinated with 36 ditelosomic lines of CS at Kasai in 1988 or 1989. In the F_1 generation of each line, selfed seed fertility was estimated using three plants per line and compared with that of disomic F_1 hybrid. From the selfed progenies of five monotelodisomic lines of CS, one or two monotelodisomic plants were selected for each line by checking the chromosome, and were crossed to (c)-N26 with their pollen. In

the F_1 generation, seven to 16 plants were studied on selfed seed fertility and examined for their segregation on fertility in each line. (C)-N26 was also pollinated with the pollen of two nulli-tetrasomic lines, nulli-7D tetra-7A and nulli-7D tetra-7B. In the F_1 generation, three plants were studied on selfed seed fertility.

B. Genetic analysis of the Rf gene(s) in Norin 61

1) Plant materials

Alloplasmic line of Norin 26 having the Ae. crassa cytoplasm (Ae. crassa/11*Norin 26 ((c)-N26)) and Norin 61 (N61) were used for the genetic analysis of the Rf gene(s). F_1 seeds were produced by hand-pollination between (c)-N26 as female and N61 as male at Kasai in 1989 and 1990. The F_1 plants of the crosses, N61 x (c)-N26, N26 x N61, N61 x N26, (c)-N61 (Ae. crassa/5*Norin 61) x N26, were also examined as controls. F_2 plants were developed by self-pollination of the F_1 plants of cross, (c)-N26 x N61, in a growth room. F_3 plants were obtained by self-pollination of the F_2 plants of highly fertile (68.8 % fertility) and severely sterile (21.4 % fertility) in a growth room. Two kinds of backcrosses, (c)-N26 x {N26 x N61} and (c)-N26 x {(c)-N26 x N61}, were performed in 1990 at Kasai.

Since CS carries the Rf gene(s) against the Ae. crassa cytoplasm, monosomic lines of N61 were used for analysis of Rf gene(s) in N61. The monosomic series of N61 were developed by Dr. T. Ryu Endo, Nara University, Japan (personal communication), which were in the B_2 generation of the backcrosses

between monosomic series of CS made by Sears (1954) and N61 as the recurrent parent. They are expected to have about 87 % purity of the nuclear genes of N61 and their monosome entirely come from N61.

2) Conventional analysis

Parental lines and F_1 hybrids were grown at Kasai and Tanno. The materials were sown in November and harvested in next June at Kasai and sown in May and harvested in August at Tanno. The experiments were performed for two years of 1989/90-90/91 at Kasai and 1990-91 at Tanno. The plants tested were space-planted at a 10 cm distance. Ears of main shoot of two to three plants per line were bagged before flowering, and their selfed seed fertilities (%) were estimated by the seed setting rate of the first and second florets of all spikelets.

The parental lines and F_1 hybrids as well as the plants of F_2, F_3 and B_1F_1 generations were also examined in growth rooms. The plants tested were individually planted in pots of 10 cm diameter and 13 cm height. Photoperiodic condition set was 16 hours light period for every 24 hours period at 20,000 lux. and temperature set was 15°C in the dark and 18°C in the light period. Selfed seed fertility (%) was estimated by the seed setting rate of the first and second florets of all spikelets in three bagged ears per plant. The average of the three ears represented the selfed seed fertility of individual plants. Five plants for the parental and F_1 hybrids were examined in growth room tests. One hundred and twenty F_2 plants, 40 and 25 F_3 plants derived from the highly fertile F_2 plant (68.8 %

fertility) and severely sterile (21.4 % fertility), respectively, and each 50 B_1F_1 plants of the crosses, (c)-N26 x {N26 x N61} and (c)-N26 x {(c)-N26 x N61}, were estimated for their selfed seed fertilities.

3) Monosomic analysis

The following procedures were taken for monosomic analysis of the Rf gene(s) in N61: From the selfed progenies of 21 monosomic lines of N61, two monosomic plants were selected for each line by checking the chromosome number in root-tip mitosis, and were pollinated with the pollen of N26 at Kasai in 1990. In the F_1 generation, one monosomic plant for each line was selected by root-tip checking of the chromosomes, and was test-crossed as the pollen parent to (c)-N26 at Kasai in 1991. Seeds from these test-crosses were individually planted in pots of 10 cm diameter and 13 cm height, and grown in a growth room in which photoperiod condition was 16 hours light period for 24 hours at 20,000 lux. and temperature condition was 15°C in the dark and 18°C in the light periods. Selfed seed fertility (%) was estimated by the seed setting rate of the first and second florets of all spikelets in three bagged ears per plant. The average of the three ears represented the selfed seed fertility of individual plants. Five to six plants per line were examined.

3. Results

A. Genetic analysis of the Rf gene(s) in Chinese Spring

1) Fertility of parental lines and F_1 hybrids

Table 18 shows selfed seed fertilities (%) of the parental lines and the F_1 hybrids of the crosses, (c)-N26 x CS and N26 x CS as a control. (C)-CS exhibited normal male fertility at both Kasai and Tanno and under all the artificial photoperiodic conditions tested. On the other hand, (c)-N26 showed almost complete sterility at Tanno and under the long-day conditions of 16 and 17 hours light periods. The F_1 hybrid from (c)-N26 x CS showed relatively high male fertility at Tanno and under the artificial long-day photoperiodic conditions, indicating that CS carries dominant Rf gene(s) against the Ae. crassa cytoplasm.

2) Segregation of fertility in F_2 generation derived from the F_1 hybrid

Segregation of the fertile and sterile plants in the F_2 generation derived from the F_1 hybrid between (c)-N26 as female and CS as male parent, which were grown under the long-day condition of 16.0 hours light period was studied. Fertility distribution of the F_2 plants is shown in Fig. 13. Taking 15 % as a breaking point between the fertile and sterile classes, the fertiles and steriles were segregated in a 3 (fertile) : 1 (sterile) ratio (Table 19). These results indicate that the fertility restoration by CS against the Ae. crassa cytoplasm is mainly controlled by a single dominant gene. Wide distribution (15-80 %) of selfed seed fertility of the fertile F_2 plants suggests that many modifying genes are also involved in the fertility restoration.

Table 18. Selfed seed fertilities (%) of the parental lines and the F_1 hybrids grown under the natural photoperiodic conditions in two locations and the various artificial photoperiodic conditions

Line[1]	Kasai	Tanno	Light period (hours)[2]		
	1987/88	1989	13	16	17
N26	98.9	97.5	89.7	62.8	45.5
(c)-N26	53.3	1.0	41.2	5.8	0.0
CS	85.0	84.5	68.0	74.7	74.1
(c)-CS	70.7	66.2	59.9	59.4	33.2
N26xCS F_1	94.2	99.0	90.4	-	86.6
(c)-N26xCS F_1	52.0	46.1	54.9	61.4	21.4

1) N26: Norin 26, CS: Chinese Spring
(c)-N26 and -CS: Alloplasmic lines of Norin 26 and Chinese Spring with the Ae. crassa cytoplasm, respectively.
2) Temperature condition was 15°C in the dark and 18°C in the light period.

Table 19. Segregation of the fertile and sterile plants in the F_1 and F_2 generations of the cross, (c)-N26 x CS

Material[1]	No. of plants			%	χ^2-value
	Total	Fertile	Sterile	Sterile	(3:1)
(c)-N26 x CS F_1	5	5	0	0	-
" F_2	130	97	33	25.4	0.01ns

1) (c)-N26: Alloplasmic line of Norin 26 with the Ae. crassa cytoplasm, CS: Chinese Spring.

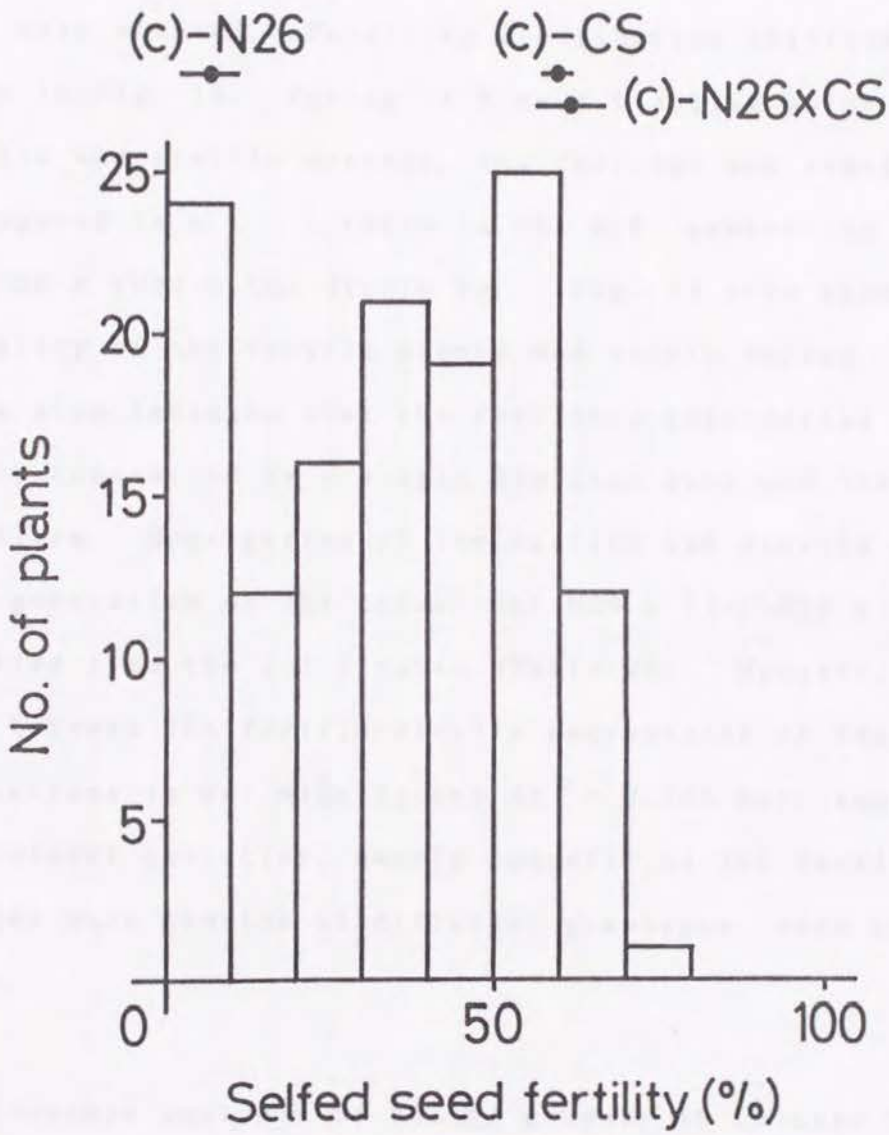

Figure 13. Distribution of selfed seed fertility in the F_2 generation derived from the F_1 hybrid between the alloplasmic Norin 26 having the Ae. crassa cytoplasm and Chinese Spring grown under the long-day (16 hours) condition.

3) Segregation of fertility in B_1F_1 generation

Segregation of the fertile and sterile plants in two kinds of B_1F_1 generation, (c)-N26 x {N26 x CS} and (c)-N26 x {(c)-N26 x CS}, were studied. Fertility distribution observed in them is shown in Fig. 14. Taking 15 % as a breaking point between the fertile and sterile classes, the fertiles and steriles were segregated in a 1 : 1 ratio in the B_1F_1 generation of cross, (c)-N26 x {N26 x CS} (Table 20). Fig. 14 also shows that the fertility of the fertile plants was widely varied. These results also indicate that the fertility restoration by CS is mainly controlled by a single dominant gene and involves many modifiers. Segregation of the fertile and sterile plants in the B_1F_1 generation of the cross, (c)-N26 x {(c)-N26 x CS}, slightly deviated from the 1 : 1 ratio (Table 20). However, the difference between the fertile-sterile segregation of the two B_1F_1 generations is not significant ($\chi^2 = 0.165$ ns), suggesting that a prominent certation, namely competition for fertilization between male gametes of different genotypes, does not takes place.

4) Telosomic analysis of the Rf gene(s) in Chinese Spring

Table 21 shows selfed seed fertilities (%) of the F_1 hybrids from the crosses of (c)-N26 x CS ditelosomics grown under the long-day condition of 16 hours light period. The fertility of each F_1 hybrid was compared with that of the disomics F_1. Among 36 F_1 hybrids from these crosses, 21 were significantly different in the fertility from the disomics. These ditelosomics will be designated the critical ones. Among the 21 critical ditelo-

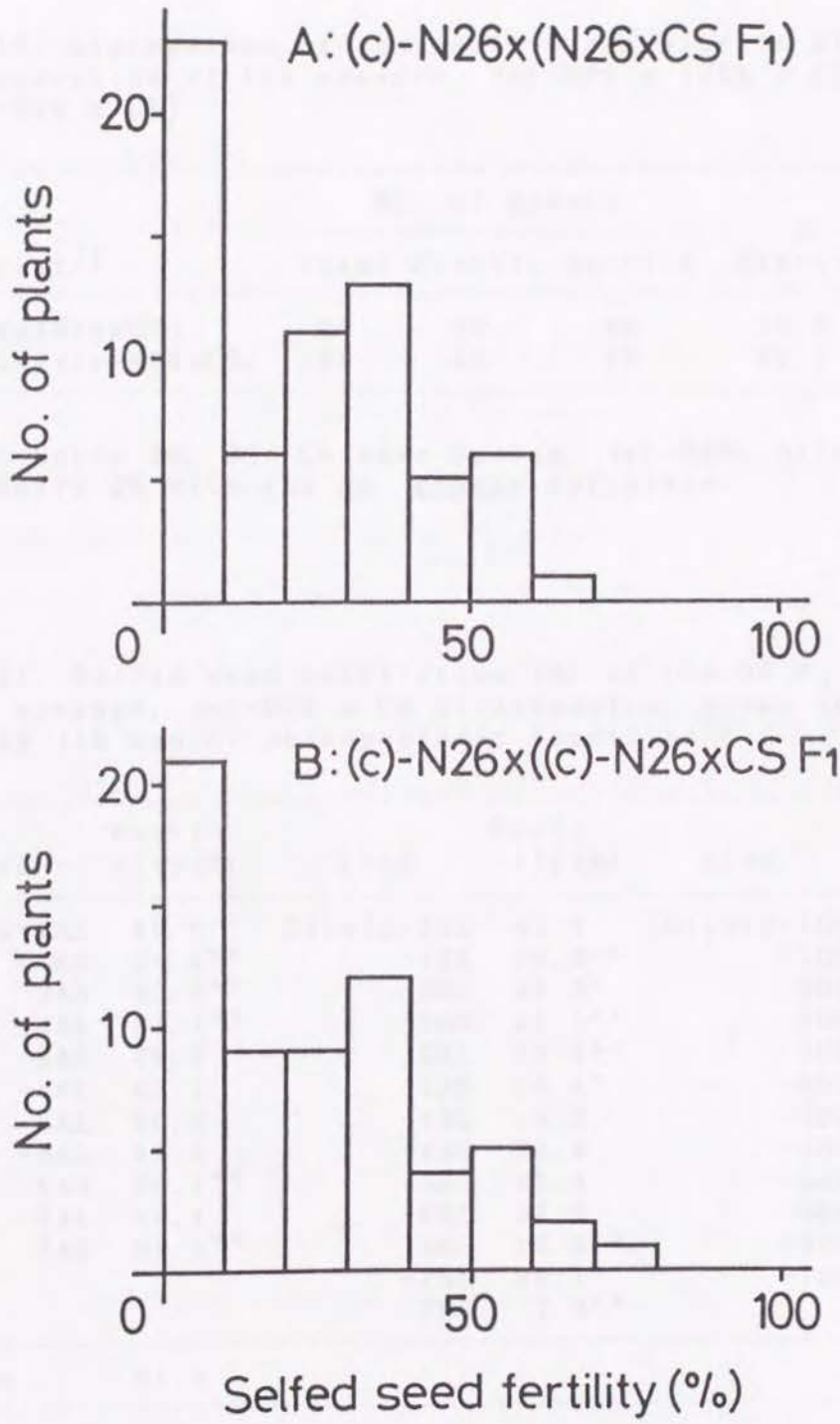

Figure 14. Fertility distribution of the B_1F_1 generation grown under the long-day (16 hours) photoperiodic condition.
A: (c)-N26 x {N26 x CS}, B: (c)-N26 x {(c)-N26 x CS}
N26: Norin 26, CS: Chinese Spring, (c)-N26: Alloplasmic line of Norin 26 having the Ae. crassa cytoplasm.

Table 20. Segregation of the fertile and sterile plants in the B_1F_1 generation of the crosses, (c)-N26 x {N26 x CS} and (c)-N26 x {(c)-N26 x CS}

Material[1)]	No. of plants			%	χ^2-value
	Total	Fertile	Sterile	Sterile	(1:1)
(c)-N26x{N26xCS}	65	39	26	40.0	2.60ns
(c)-N26x{(c)-N26xCS}	63	40	23	36.1	4.59*

1) N26: Norin 26, CS: Chinese Spring, (c)-N26: Alloplasmic line of Norin 26 with the Ae. crassa cytoplasm.

Table 21. Selfed seed fertilities (%) of the 36 F_1 hybrids of the crosses, (c)-N26 x CS ditelosomics, grown under the long-day (16 hours) photoperiodic condition[1)]

Line	Fertility(%)	Line	Fertility(%)	Line	Fertility(%)
Ditelo-1AL	44.8	Ditelo-1BL	41.6	Ditelo-1DL	48.6
-1AS	34.3**	-1BS	14.8**	-1DS	33.4**
-2AS	32.2**	-2BL	35.9*	-2DL	30.5**
-3AL	33.1**	-2BS	27.1**	-2DS	14.2**
-3AS	49.8	-3BL	33.5**	-3DL	39.4*
-4AL	43.1	-3BS	36.6*	-3DS	39.3*
-5AL	50.6	-4BL	49.2	-4DL	19.4**
-6AL	61.2	-4BS	53.6	-4DS	25.5**
-6AS	30.7**	-5BL	53.4	-5DL	56.3
-7AL	46.1	-6BL	56.6	-6DL	43.4
-7AS	31.1**	-6BS	18.5**	-6DS	41.7
		-7BL	39.1*	-7DS	21.6**
		-7BS	2.9**		
Disomic	61.4				

* and **: Significantly different from the disomic line at the 5 % and 1 % level, respectively.

1) Temperature condition was 15°C in the dark and 18°C in the light period.

somic lines, only ditelo-7BS produced the F_1 hybrid showing almost complete sterility. If the dominant major gene of fertility restoration is located on the long arm of chromosome 7B, I expect that the F_1 hybrid between (c)-N26 and CS ditelo-7BS is almost completely sterile. The present result strongly suggests that the major dominant Rf gene is located on the long arm of chromosome 7B. The remaining 20 F_1 hybrids showed a significantly lower fertility than the disomic F_1. It will be assumed that chromosome arms deleted in the corresponding ditelosomic lines have some modifying genes for fertility restoration.

Table 22 shows segregations of the steriles and fertiles in five F_1 families of the crosses, (c)-N26 x CS monotelodisomics grown under the long-day condition of 16 hours light period. Two F_1 families from the crosses of (c)-N26 x CS monotelodisomic-2AL and -5DS segregated the steliles, suggesting that some minor gene(s) for fertility restoration are located on the short arm of chromosome 2A and the long arm of chromosome 5D.

Table 23 shows selfed seed fertilities of two F_1 hybrids from the crosses of (c)-N26 x CS nulli-tetrasomics grown under the long-day condition of 16 hours light period. No reduction in the fertility was found in both F_1 hybrids, suggesting that no major gene(s) is located on chromosome 7D.

B. Genetic analysis of the Rf gene(s) in Norin 61

1) Fertility of parental lines and F_1 hybrids

Selfed seed fertilities (%) of the parental lines and the F_1 hybrids are shown in Table 24. (C)-N26 showed almost complete

Table 22. Segregation of the steriles and fertiles in five F_1 families of the crosses, (c)-N26 x CS monotelodisomics grown under the long-day (16 hours) photoperiodic condition[1)]

Line	Sterile			Fertile		
	No. of plants	Fertility (%)	Range	No. of plants	Fertility (%)	Range
Monotelodisomic-2AL	5	8.7	0-13.0	10	41.3	23.6-59.5
-4AS	0	-	-	7	53.0	40.8-59.7
-5AS	0	-	-	15	47.7	20.4-61.4
-5BS	0	-	-	16	54.8	46.3-63.1
-5DS	5	12.3	6.6-17.7	8	37.1	29.5-44.7

1) Temperature condition was 15°C in the dark and 18°C in the light periods.

Table 23. Selfed seed fertilities (%) of two F_1 hybrids of the crosses, (c)-N26 x CS nulli-tetrasomics grown under the long-day (16 hours) photoperiodic condition[1)]

Line	Fertility (%)
Nulli-7D tetra-7A	39.1
Nulli-7D tetra-7B	48.6

1) Temperature condition was 15°C in the dark and 18°C in the light periods.

Table 24. Selfed seed fertilities (%) of the parental lines and the F_1 hybrids grown under the natural photoperiodic conditions in two locations and the artificial condition of 16 hours light period

Line[1]	Kasai		Tanno		16 hours light period[2]
	1989/90	1990/91	1990	1991	
N26	93.1	94.1	98.9	97.2	62.8
(c)-N26	60.6	63.1	6.5	5.2	5.8
N61	89.1	88.9	100.0	98.9	79.5
(c)-N61	83.3	86.8	82.0	47.1	64.8
N26xN61	98.4	91.6	98.0	100.0	80.9
(c)-N26xN61	78.7	86.2	24.3	44.5	51.1
N61x(c)-N26	-	96.5	-	97.9	-
N61xN26	-	94.9	-	94.9	-
(c)-N61xN26	-	84.4	-	30.7	-

1) N26: Norin 26, N61: Norin 61
(c)-N26 and -N61: Alloplasmic lines of Norin 26 and Norin 61 with the Ae. crassa cytoplasm, respectively.
2) Temperature condition was 15°C in the dark and 18°C in the light periods.

sterility under the natural photoperiod at Tanno and under the long-day condition of 16 hours artificial light period, while (c)-N61 exhibited normal fertility under all the conditions tested. The F_1 hybrids from (c)-N26 x N61 and (c)-N61 x N26 showed moderate male fertility at Tanno and under the long-day artificial condition, indicating that N61 carries incomplete dominant Rf gene(s) against the Ae. crassa cytoplasm.

2) Distribution of fertility in F_2 generation derived from the F_1 hybrid

Fig. 15 illustrates distribution of selfed seed fertility in the F_2 generation derived from the F_1 hybrid between (c)-N26 as female and N61 as male parent, which were grown under the long-day condition of 16 hours light period. No critical segregation of the fertile and sterile plants in the F_2 generation was found, indicating that the fertility restoration by N61 against the Ae. crassa cytoplasm is controlled by many incomplete dominant genes.

3) Distribution of fertility in F_3 generation derived from the F_2 plants

Fig. 16 illustrates distribution of selfed seed fertility in the F_3 generations of a highly fertile F_2 plant and a severely sterile F_2 plant. The F_3 plants derived from the highly fertile F_2 plant were fertile and no sterile plants (under 10 % fertility) were produced. On the other hand, half of the F_3 plants from the severely sterile F_2 plant exhibited complete or almost complete sterility (under 20 % fertility). These results also

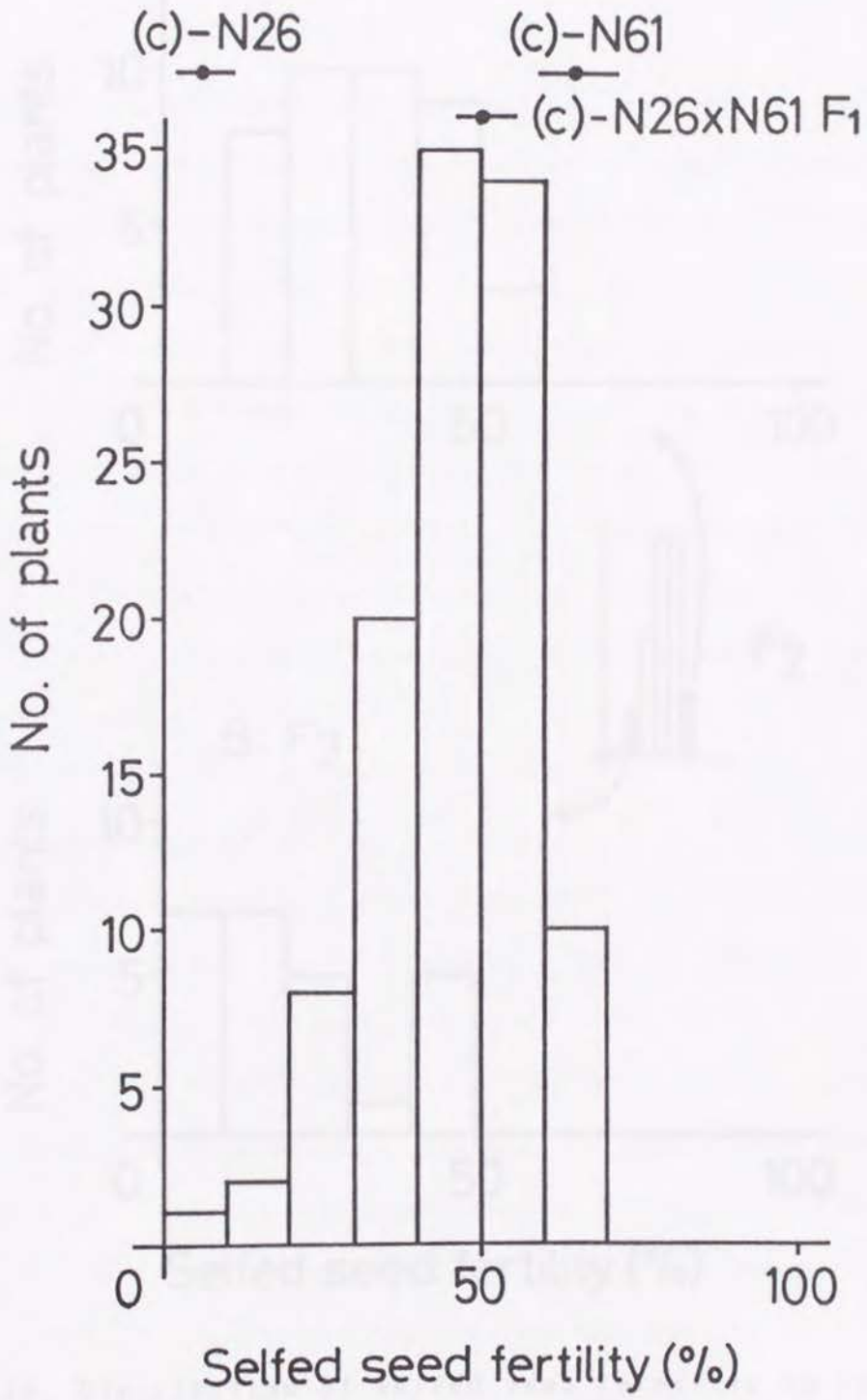

Figure 15. Distribution of selfed seed fertility in the F_2 generation derived from the F_1 hybrid between the alloplasmic Norin 26 having the Ae. crassa cytoplasm and Norin 61 grown under the long-day (16 hours) condition.

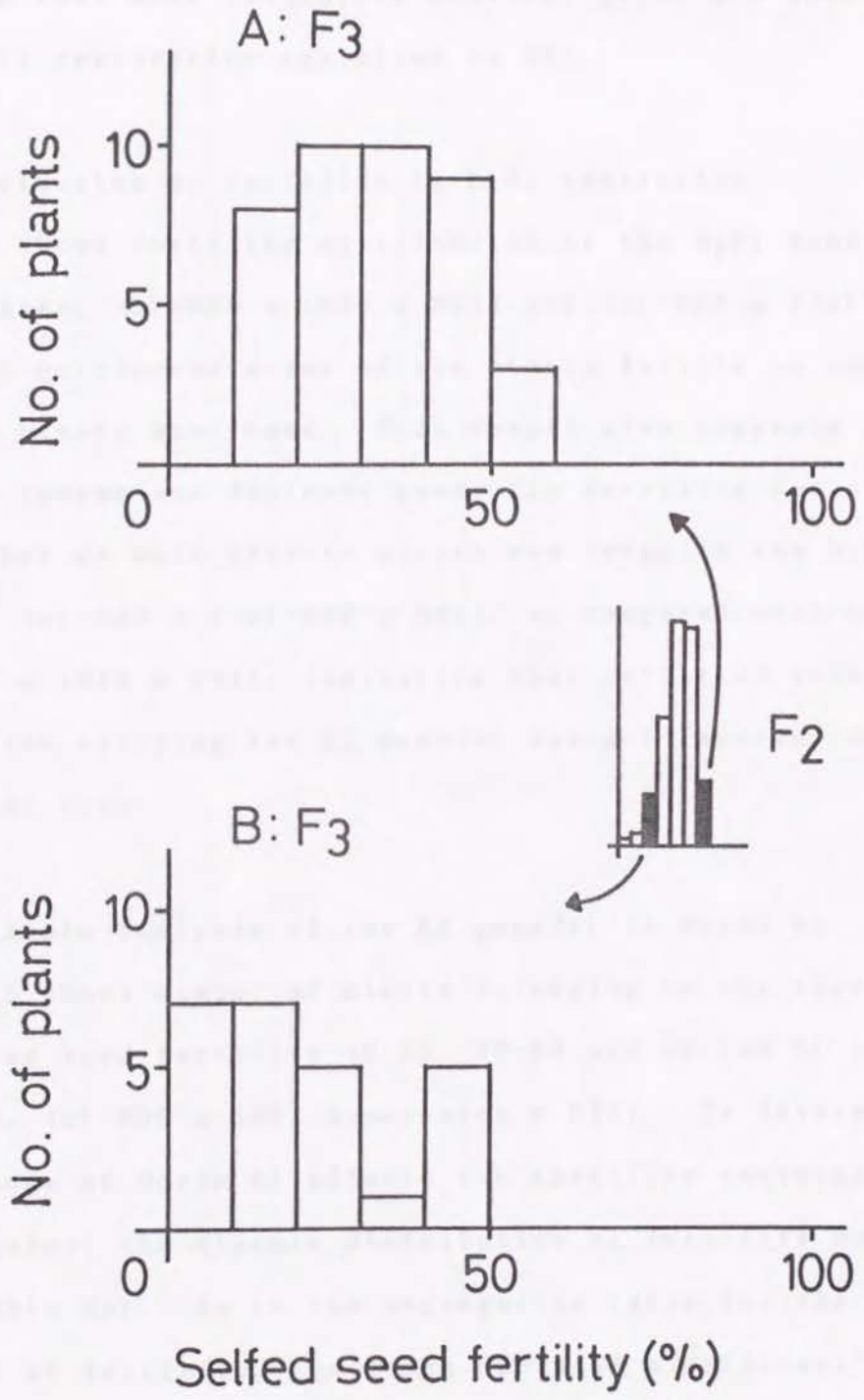

Figure 16. Distribution of selfed seed fertility in the F_3 generation grown under the long-day (16 hours) condition.
A: F_3 generation of a highly fertile (68.8 % fertility) F_2 plant.
B: F_3 generation of a severe sterile (21.4 % fertility) F_2 plant.

indicate that many incomplete dominant genes are involved in fertility restoration exhibited by N61.

4) Distribution of fertility in B_1F_1 generation

Fig. 17 shows fertility distribution of the B_1F_1 generation of the crosses, (c)-N26 x {N26 x N61} and (c)-N26 x {(c)-N26 x N61}. A continuous array of the highly fertile to completely sterile plants was found. This result also suggests existence of many incomplete dominant genes for fertility restoration. The number of male sterile plants was large in the B_1F_1 generation of (c)-N26 x {(c)-N26 x N61}, as compared with that of (c)-N26 x {N26 x N61}, indicating that certation took place and the pollen carrying the Rf gene(s) was not favored, comparing to the other type.

5) Monosomic analysis of the Rf gene(s) in Norin 61

Table 25 shows number of plants belonging to the three classes of selfed seed fertility (0-25, 25-50 and 50-100 %) in the crosses, (c)-N26 x {N61 monosomics x N26}. To determine which chromosome of Norin 61 affects the fertility restoration, a χ^2-test against the disomic distribution of fertility were carried out (Table 25). As to the segregation ratio for the three classes of fertility, ten lines deviated significantly from the disomic ratio, i. e., mono-4A, 1D, 3D, 5D and 7D at the 1 % level, and mono-7A, 1B, 2B, 4B and 4D at the 5 % level. All these ten lines showed excess of the sterile class, indicating the presence of some Rf genes on these chromosomes of Norin 61.

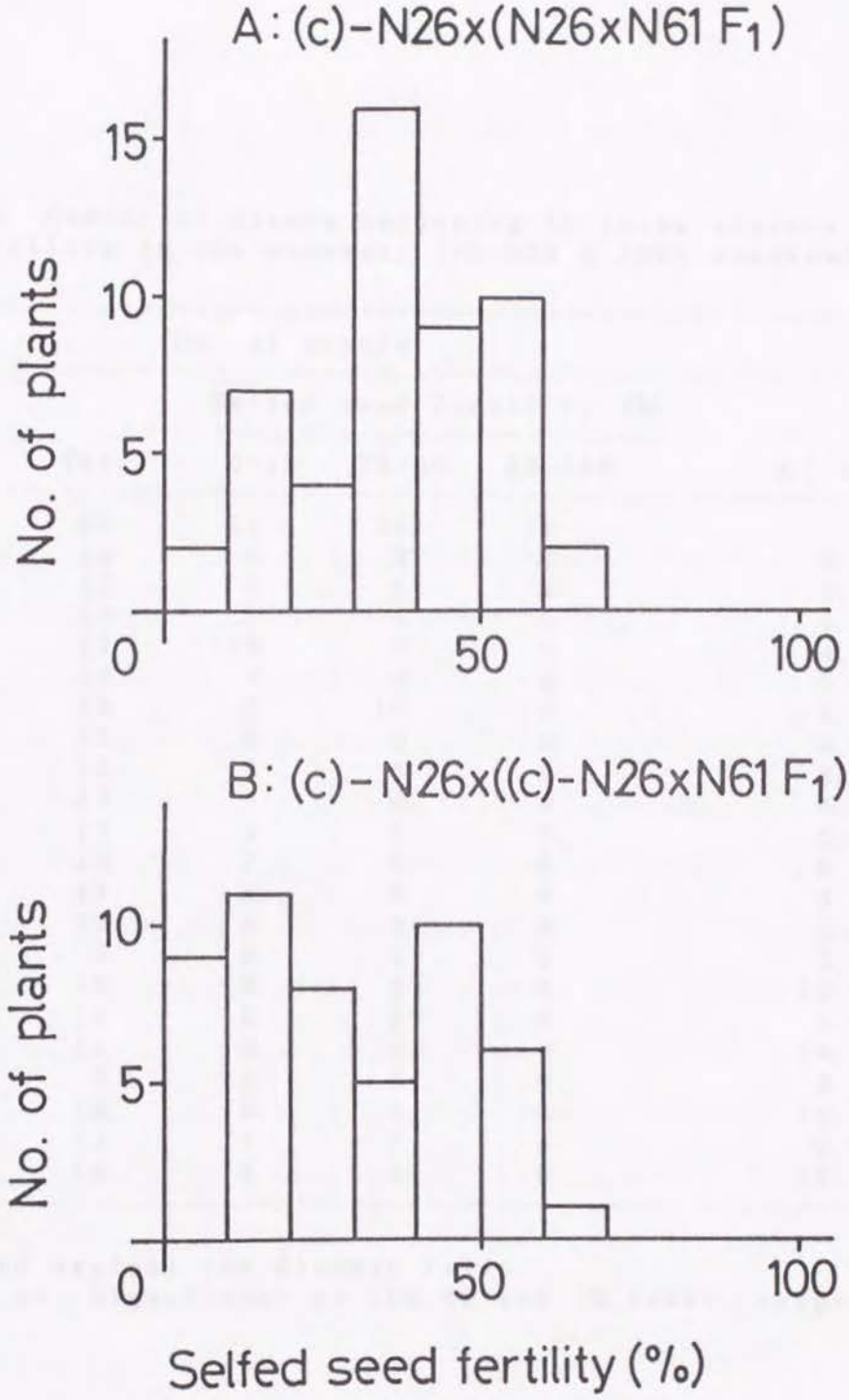

Figure 17. Fertility distribution of the B_1F_1 generation grown under the long-day (16 hours) photoperiodic condition.
A: (c)-N26 x {N26 x N61}, B: (c)-N26 x {(c)-N26 x N61}
N26: Norin 26, N61: Norin 61, (c)-N26: Alloplasmic line of Norin 26 having the Ae. crassa cytoplasm.

Table 25. Number of plants belonging to three classes of selfed seed fertility in the crosses, (c)-N26 x {N61 monosomics x N26}

	No. of plants				
		Selfed seed fertility (%)			
Line	Total	0-25	25-50	50-100	χ^2-value[1]
Disomic	50	11	27	12	-
Mono-1A	13	5	8	0	4.326
-2A	13	6	6	1	3.682
-3A	13	7	5	1	5.482
-4A	13	10	3	0	14.529**
-5A	13	7	4	2	5.138
-6A	13	3	10	0	4.049
-7A	13	8	5	0	8.959*
-1B	13	8	5	0	8.959*
-2B	13	7	6	0	6.904*
-3B	13	6	7	0	5.351
-4B	13	7	6	0	6.904*
-5B	13	4	9	0	3.872
-6B	13	5	8	0	4.326
-7B	5	0	5	0	3.953
-1D	12	9	3	0	12.989**
-2D	12	5	7	0	4.364
-3D	11	9	2	0	14.913**
-4D	9	6	3	0	8.082*
-5D	12	9	3	0	12.989**
-6D	13	3	7	3	0.009
-7D	13	9	4	0	11.502**

1) Tested against the disomic ratio.
* and **: Significant at the 5% and 1% level, respectively.

4. Discussion

1) Genetic mechanism of fertility restoration by the Rf genes in Chinese Spring

The results are summarized as follows:

a) The fertility restoration expressed by Chinese Spring against the Ae. crassa cytoplasm is controlled mainly by a single dominant gene located on the long arm of chromosome 7B.

b) A large number of modifiers (more than 22 loci) improve the level of fertility restoration.

Fertility restoration controlled by a single dominant gene and some (or many) modifying genes has been reported by Tahir and Tsunewaki (1971) in T. aestivum strain P168 against the Ae. ovata cytoplasm and by Mukai and Tsunewaki (1979) in T. aestivum cv. Chinese Spring against Ae. kotschyi and Ae. variabilis cytoplasms. In this study, I have demonstrated that the fertility restoration of Chinese Spring against the Ae. crassa cytoplasm is also controlled by a single dominant gene and many modifying genes. This single dominant gene will be designated by a symbol, Rfp1, which is the first identified gene for fertility restoration for the photoperiod-sensitive cytoplasmic male sterility caused by the Ae. crassa cytoplasm.

From a monosomic analysis, Bahl and Maan (1973) found that one of Rf genes (Rf7) of a fertility-restoring line R5 (T. zhukovskyi/3*T. aestivum cv. Justin) against the T. timopheevi cytoplasm was located on chromosome 7B. The present investigation has revealed that the long arm of the same chromosome of CS

carries a dominant fertility-restoring gene (Rfp1) against the Ae. crassa cytoplasm. A further study is necessary, in order to know the allelic relationship for the two genes, Rf7 and Rfp1.

The chromosomal location of Rf genes for the T. timopheevi and other sterilizing cytoplasms was summarized by Mukai and Tsunewaki (1979). It is interesting to note that all the restorer lines derived from T. timopheevi and its close relatives carry one of their restorer genes on chromosome 1A (Robertson and Curtis 1967, Yen et. al. 1969, Bahl and Maan 1973, Talaat 1973). Other reasonably effective genes for the T. timopheevi, Ae. umbellulata, Ae. variabilis, Ae. kotschyi and Ae. ovata cytoplasms are located on chromosome 1B (Tahir and Tsunewaki 1969, Bahl and Maan 1973, Tsunewaki 1974, Mukai and Tsunewaki 1979, Tsunewaki 1982). The Rfc3 gene from T. compactum against the Ae. caudata cytoplasm is located on chromosome 1D (Tsunewaki 1974). These facts indicate that group 1 chromosomes appear to be important sources for fertility-restoring genes for most cytoplasms studied. However, the Rfp1 gene for fertility restoration with Norin 61 against the Ae. crassa cytoplasm studied in the present investigation is located on chromosome 7B, indicating that the origin of the Rfp1 gene is different from that of other Rf genes mentioned above.

The effects of genes for photoperiodic response in wheat have been studied widely. Wheat is normally classified as a quantitative long-day plant species (Vince-Prue, 1975), of which flowering is accelerated by the transfer to long-day conditions. However, day-length insensitive cultivars exist. Photoperiod insensitivity is controlled by three dominant genes. Keim et.

al. (1973) assigned the symbols Ppd1 and Ppd2 to two of them, and Welsh et. al. (1973) tentatively located them on chromosomes 2D and 2B, respectively. Later, Law et. al. (1978) suggested that the third gene, Ppd3, is located on chromosome 2A, and that all three group 2 chromosomes are involved in photoperiodic response. Therefore, the photoperiodic response controlled by the Ppd genes is not associated with the Rfp1 gene.

Tsuji and Murata (1976) introduced several alien cytoplasms, including the Ae. crassa cytoplasm, into ditelosomic lines of D-genome chromosomes of Chinese Spring, and observed seed fertility and pistillody frequency in the alloplasmic lines produced. They found that ditelo-2DL plants became completely sterile, and ditelo-7DS plants were almost sterile due to pistillody caused by the Ae. crassa cytoplasm. As shown in the male sterile Emmer wheat with the Ae. squarrosa cytoplasm, the male sterility and the pistillody were not necessarily related to each other. Therefore, they concluded that the reduction of fertility in the ditelosomics with the Ae. crassa cytoplasm were not caused by the lack of the fertility-restoring genes, but by the enhanced pistillody by the chromosomal aberrations. However, my results have indicated that the F_1 hybrids from the crosses of (c)-N26 x CS ditelo-2DL and -7DS show the pistillody, resulting in the reduction in fertility under the long-day condition. Thus, I conclude that the fertility reduction is induced by the lack of the fertility-restoring genes (modifiers) located on the short arm of chromosome 2D or the long arm of chromosome 7D.

2) Genetic mechanism of fertility restoration by the Rf genes in Norin 61

The results are summarized as follows:

a) The fertility restoration expressed by Norin 61 against the Ae. crassa cytoplasm is controlled by many incomplete dominant genes.

b) They are located on chromosomes 4A, 7A, 1B, 2B, 4B, 1D, 3D, 4D, 5D and 7D.

Fertility restorations controlled by more than one dominant gene have been reported by several workers. Bahl and Maan (1973) considered that the fertility-restoring lines against T. timopheevi cytoplasm, R1, R2 and R5, carried three genes for fertil-ity restoration, while two other restorer lines, R3 and R4, had two genes. Tsunewaki (1982) demonstrated that four chromosomes of Chinese Spring, 5A, 7A, 1B and 5B, carry some Rf genes against Ae. ovata cytoplasm. My results is the first case of fertility restoration in which a large number (at least 10) of incomplete dominant Rf genes all involved. The Rf genes are located on all chromosomes of D genome except chromosomes 2D and 6D, in addition to two chromosomes of A genome, and three chromosomes of B genome. These facts indicate that these Rf genes were produced after the diversification of A, B and D genome during the evolution of diploid species related common wheat.

Fig. 18 shows the relationships between the selfed seed fertility and the number of days to heading in seven alloplasmic lines with the Ae. crassa cytoplasm under the artificial long-day (17 hours) condition. The seven alloplasmic lines are clearly classified into two groups. The first group consists of

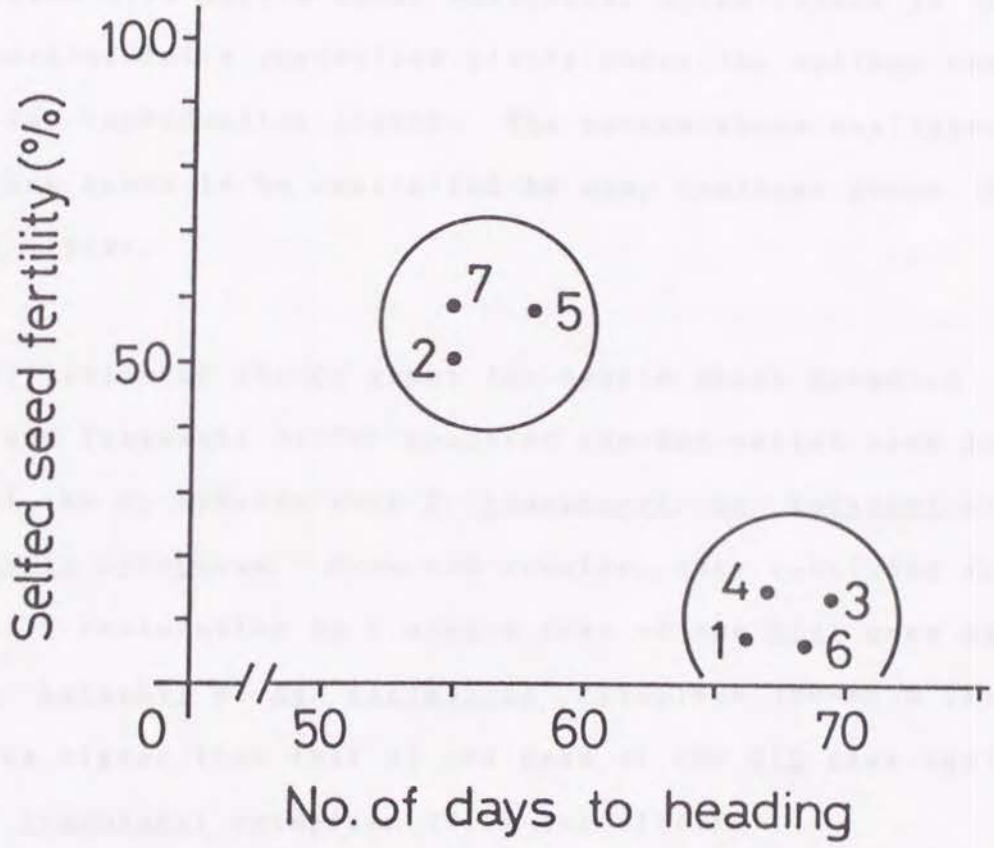

Figure 18. The relationships between selfed seed fertility and number of days to heading in seven alloplasmic lines with the Ae. crassa cytoplasm under the artificial long-day (17 hours) condition.
1: Norin 26, 2: Norin 61, 3: Shirasagi-komugi, 4: Junrei-komugi, 5: Nichirin-komugi, 6: Fujimi-komugi and 7: Ushio-komugi.

four alloplasmic lines of Norin 26, Shirasagi-komugi, Junrei-komugi and Fujimi-komugi. As shown in Chapter III, these four cultivars are known to have no Rf genes against the Ae. crassa cytoplasm. The other group contains the alloplasmic lines of Norin 61, Nichirin-komugi and Ushio-komugi which have the Rf genes. These facts strongly suggest that the Rf genes are associated with narrow-sense earliness, which refers to the earliness of fully vernalized plants under the optimum conditions for reproductive growth. The narrow-sense earliness in wheat are known to be controlled by many dominant genes (Kato et. al. 1989).

3) Utilization of the Rf genes for hybrid wheat breeding

Mukai and Tsunewaki (1979) compared average selfed seed fertilities of the F_1 hybrids with T. timopheevi, Ae. kotschyi or Ae. variabilis cytoplasm. From the results, they concluded that the fertility restoration by a single dose of the Rfv1 gene against the Ae. kotschyi or Ae. variabilis cytoplasm (80-86 % fertility) was higher than that of two dose of the Rf3 gene against the T. timopheevi cytoplasm (71 % fertility).

According to my investigation, the F_1 hybrid from the cross, (c)-N26 x CS, showed 52.0 % fertility at Kasai. These results indicate that a single dose of the Rfp1 gene with other modifiers in heterozygous condition can not restore higher fertility than (c)-N26 (53.3 %) under the short-day condition at Kasai. Therefore, the utilization of the Rfp1 gene in breeding hybrid wheat is greatly limited.

On the other hand, the fertility of the F_1 hybrid from the

cross, (c)-N26 x N61, was restored up to about 80 %, and about 20 % higher fertility than (c)-N26 (60-65 %) under the short-day condition at Kasai, indicating that the fertility restoration by a single dose of the Rf genes of N61 against the Ae. crassa cytoplasm seems to be higher than that of two doses of the Rf3 gene against the T. timopheevi cytoplasm and almost equal to that of a single dose of the Rfv1 gene against the Ae. kotschyi or Ae. variabilis cytoplasm reported by Mukai and Tsunewaki (1979). Consequently, I conclude that the Rf genes of Norin 61 can be utilized for hybrid wheat breeding. However, it is difficult to transfer a complete set of all the Rf genes in Norin 61 to a restorer line. Fortunately, several Japanese cultivars derived from Norin 61, such as Nichirin-komugi, Ushio-komugi, Sakigake-komugi, are considered to have the same set of the Rf genes as Norin 61 dose. I may be able to produce many restorer lines from these cultivars.

V. BASIC STUDIES ON HYBRID WHEAT PRODUCTION

1. Introduction

Before starting the development of parental lines for hybrid wheat breeding utilizing cytoplasmic male sterility, it is necessary to clarify the degree of genetic influence of a male sterile cytoplasm on the characters and performance of common wheat. Tahir (1971) investigated the genetic influence of two male sterile cytoplasms from Ae. ovata and T. timopheevi using the male sterile lines of 11 Pakistani cultivars. The cytoplasm of Ae. ovata delayed heading by 13 days on an average and increased plant height, tiller number and spikelet number/ear, while the T. timopheevi cytoplasm did not affect any greatly on these characters. Jošt et. al. (1975) reported that the male sterile lines of 11 Yugoslavian common wheats with the T. timopheevi cytoplasm had shorter plant height, but they had more tillers, longer leaves and ears and more spikelets per ear than their fertile counterparts. Fujigaki and Tsunewaki (1976) investigated heading date, plant height, ear number/plant, flag leaf length and selfed and open-pollinated seed fertilities of the male sterile lines of 18 Japanese and 11 US cultivars with the T. timopheevi cytoplasm. All the characters analyzed were influenced by the T. timopheevi cytoplasm, to which the Japanese and US cultivars responded differently.

Field production of hybrid wheat using the T. timopheevi cytoplasmic male sterility-fertility restoration system requires out-crossing in the following two procedures: the increase of

the male sterile parent and the production of F_1 seeds. The seeds produced by out-crossing of male sterile line with the T. timopheevi cytoplasm tend to be wrinkled and shriveled (Schmidt et. al. 1970, Miller et. al. 1975) and show pre-harvest sprouting (Doig et. al. 1975, Ellis and Clayton 1976). The seeds also have lower test weight and germination rate, but higher seed weight and protein percentage (Johnson and Lucken 1986).

In Chapter III, I have shown that several PCMS lines of Japanese cultivars have been produced, and that some cultivars are available as the restorer line.

In this chapter, agronomic characters of the PCMS lines were compared with their normal counterparts in order to determine the genetic effects of the Ae. crassa cytoplasm on the PCMS lines. In the "two-line system" using the PCMS, the out-crossing for the maintenance and increase of the male sterile lines is not needed, because the PCMS lines can be multiplied by self-pollination under a short-day condition. Seed quality of the PCMS lines obtained by self-pollination was also studied. Moreover, genetic effects of the Ae. crassa cytoplasm on the F_1 hybrids obtained by hand-pollination between the PCMS and restorer lines were studied, and the degree of heterosis expressed in the F_1 hybrids was estimated.

F_1 seeds were preliminarily obtained by natural cross-pollination of the PCMS with the restorer lines under the long-day condition at Tanno, Hokkaido. Characteristics of the F_1 seeds and performance of the F_1 hybrids were studied.

2. Materials and Methods

A. Characteristics of the PCMS lines and F_1 hybrids

1) Plant materials

PCMS lines: Five PCMS lines of Norin 26, Shirasagi-komugi, Junrei-komugi, Fujimi-komugi and Asakaze-komugi with the Ae. crassa cytoplasm were used in this study. Their backcross generations are shown in Table 26. They are expected to have higher purity than 96 % of the nuclear genes of the cultivars used as the recurrent parents in the substitution backcrosses.

Restorer lines: Five cultivars, Norin 61, Nichirin-komugi, Ushio-komugi, Sakigake-komugi and Orofen were employed (Table 26). They are known to have the genes for fertility restoration against the Ae. crassa cytoplasm as shown in Chapter III.

2) Field test for observing agronomic characters of the PCMS lines and F_1 hybrids

Twenty-three F_1 hybrids were obtained by hand-pollination between five PCMS and five restorer lines in greenhouse under a long-day condition (15 hours light period). Crossed seed fertility (%) was estimated for all cross combinations. The PCMS lines and F_1 hybrids were grown in the growing season of 1990/91 together with their normal counterparts in an experimental field of the Kasai Experimental Farm, Sumitomo Chemical Co., Hyogo, Japan. The split-plot design with two replications was employed, in which the lines were allocated to the main plots and the cytoplasms to the subplots. The plants were space-planted at a 10 cm distance. The following ten characters were observed for the PCMS lines: heading date, plant height (cm), ear length

Table 26. The PCMS and restorer lines of common wheat cultivars used in the present investigation

Line[1)]	Backcross generation
(PCMS line)	
(crassa)-Norin 26	B_{10}
(crassa)-Shirasagi-komugi	B_5
(crassa)-Junrei-komugi	B_5
(crassa)-Fujimi-komugi	B_5
(crassa)-Asakaze-komugi	B_4
(Restorer line)	
Norin 61	-
Nichirin-komugi	-
Ushio-komugi	-
Sakigake-komugi	-
Orofen	-

1) (crassa)-: Alloplasmic line with the Ae. crassa cytoplasm.

(cm), spikelet number/ear, ear number/plant, selfed and open-pollinated seed fertilities (%), 1000-grain weight (g), test weight (g) and germination rate (%). In addition to these characters (except germination rate), the following five yield characters were also studied in the F_1 hybrids: grain weight/plant (g), grain number/ear, harvest index (%), standard and mid-parent heterosis (%) on grain weight/plant. Among these characters, plant height, ear length and spikelet number/ear were measured using the main shoot and its ear of each plant. The ear of the main shoot of each plant was bagged before flowering, and their selfed seed fertility (%) was estimated by the seed setting rate of the first and second florets of all spikelets. Open-pollinated seed fertilities (%) of ears with the second shoot of each plant were also estimated. Grain number/ear (A) was estimated from ear number/plant (B), grain weight/plant (C) and 1000-grain weight (D), using a formula $A = 1000 \times C/[B \times D]$.

The data of these characters were taken from two to three plants in each line, and the average of all the plants was subjected to statistical analyses. Analysis of variance was applied to detect the effects of the nucleus and cytoplasm. In addition, the effect of the male sterile cytoplasm on the interrelationships among yield characters of the F_1 hybrids was investigated. Correlation coefficients (r) were calculated in the F_1 hybrids with the Ae. crassa cytoplasm and normal cytoplasm separately, using the average performance of two replications, and each r was converted to z, where $z = 1/2(\log_e(1+r) - \log_e(1-r))$ (Snedecor and Cochran 1967). Then, the significance

of the difference between the two z's was tested.

Mid-parent heterosis of the F_1 hybrids for the yield components, ear number/plant, grain number/ear, 1000-grain weight and grain weight/plant were calculated by the average performance of two replications. Analyses of correlation coefficient among the degree of heterosis on the yield characters of the F_1 hybrids were carried out.

B. Production of the F_1 hybrids by out-crossing in field

1) Plant materials

PCMS line: Four PCMS lines of Norin 26, Shirasagi-komugi, Junrei-komugi and Fujimi-komugi with the Ae. crassa cytoplasm were used in this study. Their backcross generations are shown in Table 27. They are expected to have higher purity than 96 % of the nuclear genes of the cultivars used as the recurrent parents.

Restorer lines: Two cultivars, Yutaka-komugi and Ushio-komugi, were employed as the restorer lines (Table 27). They are known to have the identical genes for fertility restoration against the Ae. crassa cytoplasm as shown in Chapter III.

F_1 hybrids: Two F_1 hybrids of 75 % purity were obtained by natural out-crossing between the alloplasmic Norin 26 with the Ae. crassa cytoplasm ((c)-N26) and Yutaka-komugi or Ushio-komugi.

2) Preliminary field test for production of the F_1 seeds by out-crossing

Table 27. PCMS and restorer lines of Japanese common wheat cultivars used in the present investigation

Line[1)]	Backcross generation
(PCMS line)	
(*crassa*)-Norin 26	B_{10}
(*crassa*)-Shirasagi-komugi	B_4
(*crassa*)-Junrei-komugi	B_4
(*crassa*)-Fujimi-komugi	B_4
(Restorer line)	
Yutaka-komugi	-
Ushio-komugi	-

1) (*crassa*)-: Alloplasmic line with the *Ae. crassa* cytoplasm.

In May of 1989, the crossing blocks containing (c)-N26 as a PCMS line and a pollinator cultivar, Yutaka-komugi or Ushio-komugi were set up at the experimental field of the Tanno Agricultural Cooperative, Hokkaido, Japan. The euplasmic line of N26 was also grown as a control. The PCMS line was sown in two 30-cm apart rows at a 1 g/m seeding density in a crossing block of 3 m length, that was surrounded by two 30-cm apart rows of pollen parents at a 2 g/m seeding density. The experiment was replicated twice for each combination. Just before flowering, ten ears of the PCMS line at each block were bagged. In August, ten bagged and ten non-bagged ears were sampled to evaluate male sterility (%), out-crossing rate (%) and hybrid purity (%). They were estimated by counting the seed number/spikelet as follows:

Selfed seed fertility (%) = A/C x 100 ..... (1)

Male sterility (%) = 100 - (1)

= (1 - A/C) x 100 ..... (2)

Seed setting rate (%) = B/D x 100 ..... (3)

Out-crossing rate (%) = (3) - (1)

= (B/D - A/C) x 100 ..... (4)

Hybrid purity (%) = (4) / (3) x 100

= (B/D - A/C) / (B/D) x 100 ... (5)

A: Seed no./spikelet of bagged ear of (c)-N26

B: Seed no./spikelet of non-bagged ear of (c)-N26

C: Seed no./spikelet of bagged ear of N26

D: Seed no./spikelet of non-bagged ear of N26

F_1 seeds of each crossing block were harvested and observed for yield (g/m^2), test weight (g), 1000-grain weight (g) and germi-

nation rate (%).

3) Preliminary field test for estimating performance of the F_1 hybrids

Trial was conducted during the season of 1989/90 at the Kasai Experimental Farm of Sumitomo Chemical Co., Hyogo, Japan. The F_1 hybrids and parental lines as controls were examined by a randomized block design with two replications. Seeds were sown in November at a 1 kg/a seeding density in plot of 3 m length containing six rows 30 cm apart. Nitrogen fertilizer was applied in fall and spring at the rate of 6 and 4 kg N/10a, respectively. The following five characters were observed in field; heading date, plant height (cm), ear length (cm), spikelet number/ear and ear number/m^2. Among these characters, plant height, ear length and spikelet number/ear were measured using 20 shoots and their ears per plot. The ear number/m^2 was determined before harvesting by counting the number of ears in two rows of 50 cm length in two replications in each plot. Also just before harvest, the plants in two rows of 50 cm length were pulled out for the determination of harvest index. The weights of grains and straws, including chaff, were measured after about one week drying. The harvest index (%) was obtained by the ratio of grain/(grain+straw). Test weight (g) and 1000-grain weight (%) were measured using these grains. Grain yield was studied using the grains harvested from four 2-m long rows at each plot plus the grains used for the harvest index study.

4) Studies on the effects of seeding density and varietal

difference in the PCMS lines on the degree of male sterility The alloplasmic line of N26 with the Ae. crassa cytoplasm and its euplasmic line as a control were grown at different seeding densities at Tanno in 1991. Three different density levels were tested; these are 1 g/m, 0.5 g/m and 0.25 g/m. The experimental plots were arranged according to a split-plot design with three replications, in which the density levels were allocated to the main plots and the cytoplasms to the subplots. Each subplot consisted of two 3-m long rows.

Four PCMS lines of Norin 26, Shirasagi-komugi, Junrei-komugi and Fujimi-komugi with the Ae. crassa cytoplasm and their normal counterparts as controls were grown at Tanno to study their agronomic characters and the degree of male sterility. The experiment was performed in the growing season of 1991. The PCMS lines were sown at a seeding rate of 1 g/m. The experimental plots had three replications.

The following seven characters were observed; heading date, plant height (cm), ear length (cm), spikelet number/ear, ear number/plant, non-ear bearing tiller number/plant and seed number/spikelet. Among these characters, plant height, ear length and spikelet number/ear were measured using the main shoot or its ear of three plants per plot. These ears were bagged before flowering, and seed number/spikelet were counted. The averages of the three plants was subjected to statistical analyses. Analysis of variance was applied to determine the effects of the seeding density and cultivars. Male sterility (%) of the PCMS lines was estimated by the average seed setting rate of three replications, compared with that of normal coun-

terparts.

3. Results

A. Characteristics of the PCMS lines and F_1 hybrids

1) Effects of the Ae. crassa cytoplasm on agronomic characters of the PCMS lines

Mean values of the ten characters of the PCMS lines are shown in Table 28. The analysis of variance indicates significant difference between the PCMS and normal lines on six characters, heading date, spikelet number/ear, selfed and open-pollinated seed fertilities, test weight and germination rate (Table 29). Average heading date of the PCMS lines was one day later than that of the normal ones. The analysis of variance also indicates that the variation attributable to the cytoplasm was significant at the 5 % level. The PCMS lines had a tendency toward a decreased spikelet number/ear. The variations attributable to both the cytoplasm and cytoplasm x line interaction were highly significant (1 % level). However, the difference between the PCMS and normal lines was negligible from a practical point of view because its mean difference was only 0.5. The averages of the selfed and the open-pollinated seed fertilities of the PCMS lines were 40 % and 25 % lower than those of the normal ones, respectively. Analyses of variance revealed that the effects of the cytoplasm were significant at the 1 % level. These results indicate that the fertility of the PCMS lines was decreased by the Ae. crassa cytoplasm even under

Table 28. Average performance on ten characters of the PCMS and the corresponding normal lines

Cultivar	Generation	Heading date (day)		Plant ht. (cm)		Ear length (cm)		Spikelet no./ear		Ear no./plant		Seed fertility (%) (Selfed)		Seed fertility (%) (Open-poll.)	
		Ms	N	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N
Norin 26	B_{10}	4.18	4.17	82	79	9.7	9.3	19.0	18.5	13.2	10.7	59.5	95.4	69.8	95.0
Shirasagi-komugi	B_5	4.18	4.17	82	85	9.3	9.1	18.2	17.9	12.5	13.5	60.9	94.1	75.0	95.0
Junrei-komugi	B_5	4.16	4.16	82	88	9.9	9.7	18.2	18.4	15.2	15.9	60.5	98.4	80.0	95.4
Fujimi-komugi	B_5	4.16	4.15	72	82	9.4	9.7	16.7	18.5	12.0	14.0	64.0	95.2	70.8	95.2
Asakaze-komugi	B_4	4.13	4.12	61	66	9.6	10.4	16.9	18.0	15.3	15.4	34.8	90.3	50.0	94.3
Average		4.16	4.15	76	80	9.6	9.6	17.8	18.3	13.6	13.9	55.9	94.7	69.1	95.0

1000-grain weight (g)		Test weight (g)		Germination rate (%)	
Ms	N	Ms	N	Ms	N
32.5	35.7	677	708	89.6	97.6
37.5	36.0	703	712	93.8	99.2
37.3	36.0	685	701	95.2	96.2
35.7	37.3	675	722	85.3	94.1
28.8	34.6	632	699	72.2	91.3
34.4	35.9	674	708	87.2	95.7

Note) Ms and N denote the PCMS and normal lines, respectively.

Table 29. The results of analyses of variance for the performance on the ten characters of the PCMS (*Ae. crassa* cytoplasm) and corresponding normal (*T. aestivum* cytoplasm) lines

Source of variation	d.f.	Mean square: Heading date	Plant height	Ear length	Spikelet no./ear	Ear no./plant	Seed fertility (Selfed)	Seed fertility (Open-poll.)	1000-grain weight	Test weight	Germination rate
Main plot											
Rep.	1	4.05	31.25	0.22	0.04	28.08	3.44	6.38	6.84	105.80	0.72
Line (L)	4	17.80**	298.58**	0.38	1.12*	9.91	207.95**	139.63*	19.27	989.55*	145.85*
Error	4	0.55	15.63	0.16	0.10	7.32	4.68	19.97	3.33	105.05	8.71
Subplots											
Cytoplasm (C)	1	4.05*	76.05	0.04	1.10**	0.31	7515.56**	3377.40**	12.01	5848.20**	359.55**
L x C	4	0.30	22.43	0.23	0.96**	2.86	94.51	120.84*	9.63	534.45	44.38
Error	5	0.45	16.55	0.05	0.06	9.04	18.40	21.77	4.38	194.80	10.90

* and **: Significant at the 5 % and 1 % level, respectively.

the short-day condition at Kasai. Among the PCMS lines, the alloplasmic Asakaze-komugi showed lowest seed fertility, i. e., 35 % for selfed and 50 % for open-pollinated seed fertilities. However, since the average open-pollinated seed fertility of the PCMS lines was 70 %, the maintenance and seed increase of the PCMS lines are assumed to be of no problem at Kasai. The PCMS lines also had a tendency toward a decreased test weight and germination rate. This is probably due to the shriveled seeds resulted from low seed fertility in the PCMS lines. Analyses of variance revealed that the effects of the cytoplasm were significant at the 1 % level. Table 29 also indicates that plant height, ear length, ear number/plant and 1000-grain weight were not affected by the Ae. crassa cytoplasm. Ears of the PCMS and corresponding normal lines are shown in Fig. 19. Grains of the alloplasmic and euplasmic lines of Norin 26 with the Ae. crassa cytoplasm are shown in Fig. 20. This figure shows that about half of the grains of alloplasmic Norin 26 are shriveled. Preharvest sprouting was not observed in both the PCMS and corresponding normal lines in this investigation at Kasai.

Table 30 shows crossed seed fertilities (%) of the PCMS lines and their normal counterparts pollinated with the pollen of the restorer lines under the long-day (15 hours) condition. Obviously, the crossed seed fertilities of the PCMS lines were lower than those of the normal lines, indicating that the female fertility was affected by the Ae. crassa cytoplasm. The highest average fertility was 45.6 % for the alloplasmic Norin 26 and the lowest 13.0 % for the alloplasmic Fujimi-komugi. The degree of female sterility of the alloplasmic lines is dependent on its

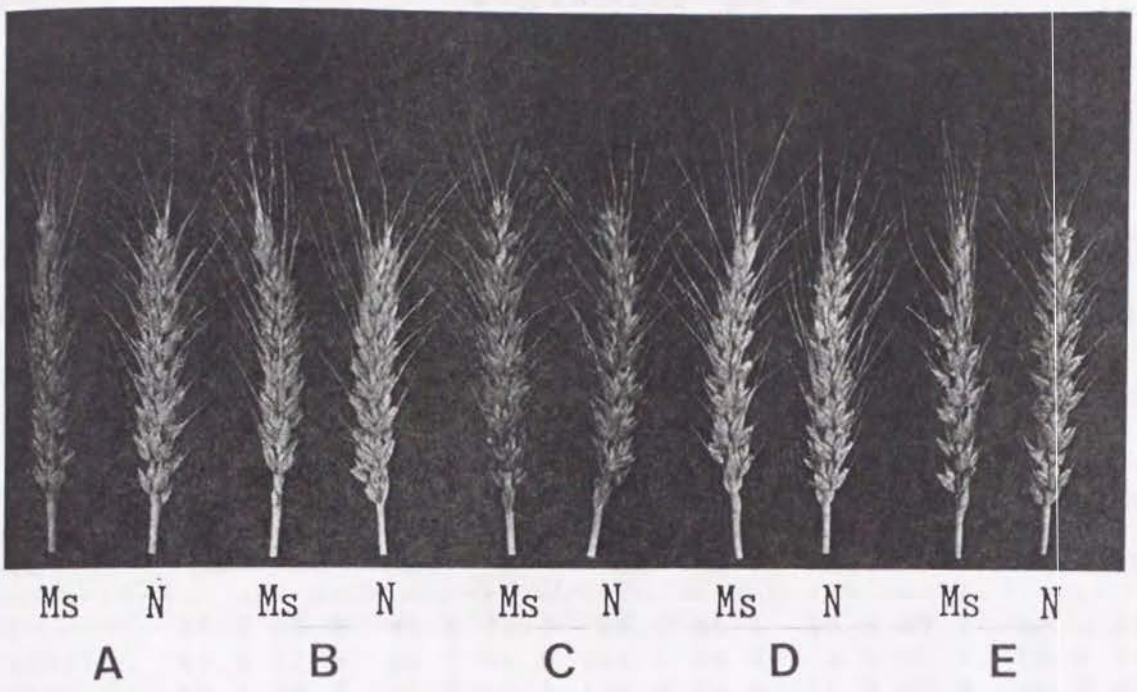

Figure 19. Ears of the PCMS and corresponding normal lines of five cultivars grown at Kasai.
Ms and N: Alloplasmic lines with the Ae. crassa cytoplasm and the corresponding normal line, respectively.
A: Norin 26, B: Shirasagi-komugi, C: Junrei-komugi, D: Fujimi-komugi, E: Asakaze-komugi.

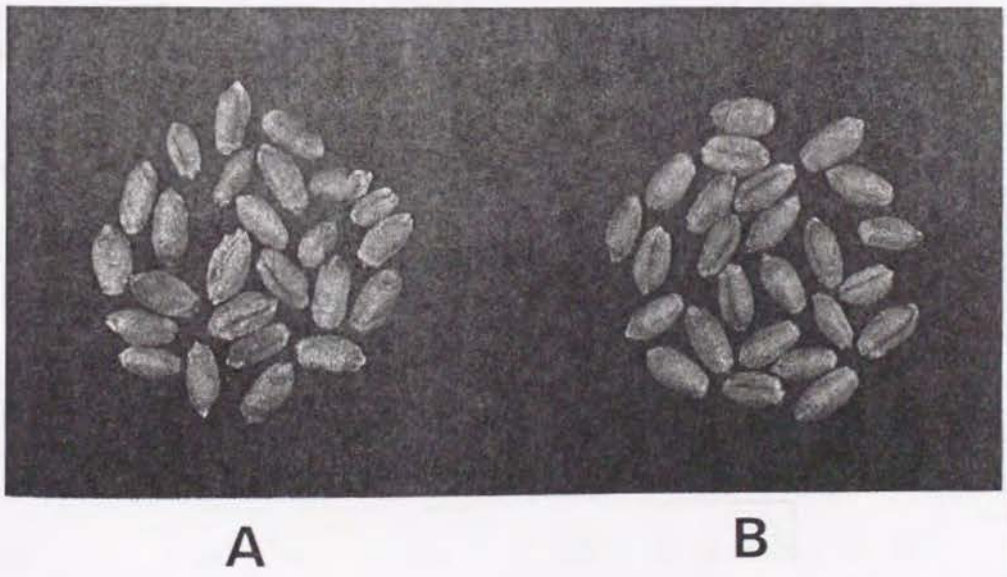

Figure 20. Grains of the alloplasmic line of Norin 26 with the Ae. crassa cytoplasm and the normal line grown at Kasai
A and B: Alloplasmic and euplasmic lines, respectively.

Table 30. Crossed seed fertilities (%) of the PCMS and corresponding normal lines

Restorer line[1]	PCMS line[2] N26 Ms	N26 N	Shirasagi Ms	Shirasagi N	Junrei Ms	Junrei N	Fujimi Ms	Fujimi N	Asakaze Ms	Asakaze N
N61	37.5	66.2	40.6	43.2	39.4	51.1	33.9	27.3	41.1	69.4
Nichirin	41.0	51.9	20.7	56.9	11.8	46.0	4.5	21.7	18.8	53.7
Ushio	55.2	48.6	56.5	50.0	40.4	58.6	11.8	32.9	38.2	33.3
Sakigake	37.5	55.3	41.2	29.7	52.2	61.1	9.3	58.8	35.4	33.0
Orofen	56.6	80.8	34.2	70.0	40.6	60.5	5.3	47.0	33.5	43.8
Average	45.6	60.6	38.6	50.0	36.9	55.5	13.0	37.5	33.4	46.6

Note) Ms and N denote the male sterile and normal lines, respectively.

1) N61: Norin 61, Nichirin: Nichirin-komugi, Ushio: Ushio-komugi, Sakigake: Sakigake-komugi.

2) N26: Norin 26, Shirasagi: Shirasagi-komugi, Junrei: Junrei-komugi, Fujimi: Fujimi-komugi, Asakaze: Asakaze-komugi.

nuclear background.

2) Effects of the Ae. crassa cytoplasm on agronomic and yield characters of the F_1 hybrids

Average performance on the agronomic and yield characters of the 23 F_1 hybrids having the Ae. crassa and T. aestivum (control) cytoplasms are shown in Tables 31 and 32, respectively. Two F_1 hybrids, Junrei-komugi x Sakigake-komugi and Fujimi-komugi x Nichirin-komugi, were not available for this investigation because of their low germinability and a small number of seeds available, respectively. The analyses of variance revealed that the effects of the Ae. crassa cytoplasm were significant on seven characters, ear length, selfed and open-pollinated seed fertilities, 1000-grain weight, grain weight/plant, grain number/ear and harvest index (Tables 33 and 34). The F_1 hybrids with the Ae. crassa cytoplasm had a tendency to have longer ear than those with the wheat cytoplasm. The over-all averages of both the selfed and open-pollinated seed fertilities of the F_1 hybrids with the Ae. crassa cytoplasm were 10 % lower than those of the F_1's with the wheat cytoplasm. Analyses of variance indicated that the effects of the line, cytoplasm and their interaction were all significant at the 1 % level. In spite of the decreased fertility (80 % fertility), the F_1 hybrids with the Ae. crassa cytoplasm showed, on the average, 19 % and 14 % heterosis on grain weight/plant over the standard parent (Norin 61) and the mid-parent, respectively (Table 32). The analyses of variance indicated that the variations attributable to the cytoplasm were significant for grain weight/plant, grain number/

Table 31. Average performance on the seven agronomic characters of the F_1 hybrids having the Ae. crassa (Ms) and T. aestivum (N) cytoplasms

Cross combination[1)]	Heading date (day)		Plant ht. (cm)		Ear length (cm)		Spikelet no./ear		Ear no./plant		Seed fertility (%) Selfed		Seed fertility (%) Open-poll.	
	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N
N26 x N61	4.17	4.18	85	76	10.1	8.9	17.7	17.8	16.4	12.2	85.9	88.4	87.9	85.1
N26 x Nichirin	4.16	4.14	79	79	10.2	8.9	19.7	17.7	20.8	17.5	83.6	95.8	82.4	95.6
N26 x Ushio	4.15	4.16	82	79	9.7	9.6	17.7	16.8	13.4	15.2	86.0	91.9	85.4	88.8
N26 x Sakigake	4.12	4.13	80	80	10.5	9.1	18.2	16.0	16.9	15.2	73.3	92.2	81.1	97.7
N26 x Orofen	4.20	4.17	101	85	10.1	9.7	17.7	16.5	12.7	15.9	83.2	86.2	79.7	87.9
Shirasagi x N61	4.18	4.16	82	90	9.8	10.1	18.3	18.5	19.5	16.5	70.5	91.3	68.0	94.5
Shirasagi x Nichirin	4.15	4.15	84	82	10.1	9.1	19.4	18.0	19.4	12.0	90.7	92.6	88.0	97.5
Shirasagi x Ushio	4.14	4.14	82	85	9.3	9.2	17.5	17.5	15.2	12.5	90.5	96.4	84.6	97.2
Shirasagi x Sakigake	4.21	4.12	75	79	8.5	9.7	17.4	17.2	11.9	14.5	74.1	96.6	73.5	95.6
Shirasagi x Orofen	4.21	4.22	99	96	10.0	10.1	19.0	19.2	12.7	16.4	77.1	80.5	78.9	73.9
Junrei x N61	4.16	4.17	86	86	10.3	10.4	18.5	19.5	15.0	22.2	91.2	96.5	85.6	92.6
Junrei x Nichirin	4.15	4.22	85	73	10.1	7.9	19.5	17.5	24.2	18.0	87.9	94.3	86.6	91.4
Junrei x Ushio	4.12	4.16	89	81	10.7	10.1	18.0	18.5	20.5	19.0	84.0	94.3	86.8	92.3
Junrei x Orofen	4.18	4.21	103	100	10.6	11.1	18.4	20.4	18.0	18.3	70.0	89.7	71.1	87.9
Fujimi x N61	4.16	4.16	84	87	8.9	9.3	16.2	17.0	15.5	16.2	81.1	93.8	87.5	93.0
Fujimi x Ushio	4.15	4.13	76	77	9.3	8.7	17.5	16.7	20.7	13.2	76.8	96.1	78.6	98.6
Fujimi x Sakigake	4.13	4.13	74	78	9.0	9.7	15.9	16.7	17.2	19.4	93.0	96.2	80.9	96.8
Fujimi x Orofen	4.21	4.19	101	91	10.7	10.1	19.5	17.7	12.7	14.0	61.7	86.6	78.1	85.9
Asakaze x N61	4.16	4.15	70	79	9.3	9.9	17.2	17.7	15.2	17.4	80.7	94.1	77.8	93.3
Asakaze x Nichirin	4.14	4.13	68	73	9.8	9.4	17.7	17.7	14.9	14.7	68.9	95.8	81.6	95.6
Asakaze x Ushio	4.14	4.13	70	77	9.3	9.7	16.4	17.2	18.5	17.2	85.3	96.6	80.3	96.1
Asakaze x Sakigake	4.14	4.13	68	68	9.4	9.7	17.5	17.5	12.3	16.3	77.8	90.5	76.0	92.2
Asakaze x Orofen	4.18	4.17	89	84	10.8	10.5	17.7	17.4	13.7	17.4	81.7	85.5	75.9	86.0
Average	4.16	4.16	83	82	9.8	9.6	17.9	17.7	16.4	16.1	80.7	92.3	80.7	92.0

1) N26: Norin 26, Shirasagi: Shirasagi-komugi, Junrei: Junrei-komugi, Fujimi: Fujimi-komugi, Asakaze: Asakaze-komugi, N61: Norin 61, Nichirin: Nichirin-komugi, Ushio: Ushio-komugi, Sakigake: Sakigake-komugi.

Table 32. Average performance on ten yield characters of the F_1 hybrids having the Ae. crassa (Ms) and T. aestivum (N) cytoplasms

Cross combination[1]	1000-grain weight (g)		Test weight (g)		Grain wt. /plant (g)		Grain no. /ear		Harvest index (%)		Heterosis (%)[2] (Standard)		Heterosis (%)[2] (Mid-parent)	
	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N
N26 x N61	35.4	39.9	690	729	18.4	20.1	32.0	39.3	32.6	38.3	5.0	14.7	12.5	23.3
N26 x Nichirin	36.6	34.7	705	707	23.1	23.9	30.8	39.4	32.7	36.6	31.8	36.2	53.0	58.3
N26 x Ushio	39.6	40.3	705	717	18.6	23.3	35.4	38.9	35.3	40.4	6.0	32.9	15.5	45.2
N26 x Sakigake	38.4	38.1	701	710	20.2	22.1	31.4	38.2	34.1	39.9	15.5	26.4	52.1	65.5
N26 x Orofen	40.6	35.6	726	710	18.1	18.6	35.4	32.5	27.1	33.4	3.5	6.4	20.5	23.6
Shirasagi x N61	41.3	39.0	722	704	25.6	27.4	31.7	42.6	33.7	39.1	45.9	56.3	36.2	46.1
Shirasagi x Nichirin	38.4	36.9	714	724	26.4	20.1	35.4	45.3	36.5	39.6	50.5	14.5	50.4	14.5
Shirasagi x Ushio	40.2	41.2	699	714	15.0	21.9	27.4	41.7	26.4	37.2	-14.7	25.0	-19.1	18.4
Shirasagi x Sakigake	40.7	38.2	726	713	15.7	21.5	32.9	39.1	32.1	41.2	-10.5	22.6	-0.6	36.1
Shirasagi x Orofen	44.3	41.7	727	731	17.1	26.6	30.6	37.5	28.4	29.8	-2.5	51.6	-2.6	52.0
Junrei x N61	39.9	36.6	709	705	21.4	35.7	35.9	43.5	35.9	37.8	22.2	103.9	2.9	72.0
Junrei x Nichirin	38.6	33.7	704	709	32.1	21.7	34.6	32.8	35.1	38.8	83.1	24.0	38.1	11.0
Junrei x Ushio	41.9	40.4	703	717	29.6	32.8	34.5	42.7	37.0	41.2	68.9	87.0	44.0	60.0
Junrei x Orofen	45.5	42.2	717	719	27.0	32.0	33.0	41.3	31.0	33.4	53.9	82.6	38.1	64.1
Fujimi x N61	40.1	40.9	715	716	21.6	27.3	34.9	41.2	35.8	38.2	23.3	55.8	4.9	32.8
Fujimi x Ushio	38.6	40.0	698	724	19.6	22.6	22.7	43.1	29.2	40.8	11.9	29.2	-53.3	11.3
Fujimi x Sakigake	40.6	39.4	720	716	18.1	30.8	25.9	40.4	29.9	40.6	3.1	75.7	28.4	75.0
Fujimi x Orofen	44.9	37.9	748	718	19.9	22.9	35.5	41.3	29.3	34.9	13.3	30.6	2.8	18.7
Asakaze x N61	36.6	38.3	710	719	16.3	27.5	29.7	42.0	34.7	40.0	-6.9	56.7	-17.5	39.6
Asakaze x Nichirin	36.5	37.1	714	711	17.4	21.3	32.1	38.5	37.1	41.8	-0.8	21.6	-5.9	15.1
Asakaze x Ushio	38.0	38.6	708	715	23.6	27.4	33.8	40.9	36.8	40.7	34.9	56.1	21.5	40.9
Asakaze x Sakigake	39.0	36.7	713	705	13.9	21.1	28.7	35.8	31.9	39.3	-20.8	20.2	-17.0	26.0
Asakaze x Orofen	44.5	42.9	710	712	20.3	30.2	33.5	40.1	31.2	37.3	16.0	72.4	9.7	63.7
Average	40.0	38.7	712	715	20.8	25.2	32.1	39.9	32.8	38.3	18.8	43.6	13.7	39.7

1) N26: Norin 26, Shirasagi: Shirasagi-komugi, Junrei: Junrei-komugi, Fujimi: Fujimi-komugi, Asakaze: Asakaze-komugi, N61: Norin 61, Nichirin: Nichirin-komugi, Ushio: Ushio-komugi, Sakigake: Sakigake-komugi.

2) Heterosis (%) are given by percentages of grain weight/plant over the standard (Norin 61) and the mid-parent.

Table 33. The results of analyses of variance for the agronomic characters of the F_1 hybrids

Source of variance	d. f.	Mean square: Heading date	Plant height	Ear length	Spikelet no./ear	Ear no./plant	Seed fertility (%) (Selfed)	Seed fertility (%) (Open-poll.)
Main plot								
Rep.	1	2.46	14.88	0.15	2.48	15.37	0.04	37.33
Line (L)	22	24.90**	282.00**	1.07**	2.90*	19.79	108.60**	67.98**
Error	22	2.83	13.68	0.32	1.17	18.22	22.69	21.76
Subplots								
Cytoplasm (C)	1	1.84	30.53	1.39*	1.44	1.51	3098.36**	2914.31**
L x C	22	8.45**	41.78	0.65*	1.24	14.76	62.86**	57.36**
Error	23	2.73	29.25	0.29	0.82	20.83	15.16	13.05

* and **: Significant at the 5 % and 1 % level, respectively.

Table 34. The results of analyses of variance for the yield characters of the F_1 hybrids

Source of variance	d. f.	Mean square: 1000-grain weight	Test weight	Grain wt./plant	Grain no./ear	Harvest index
Main plot						
Rep.	1	3.48	1448.10**	18.90	179.48**	38.61**
Line (L)	22	20.28**	192.32	57.87	19.20	31.20**
Error	22	4.76	96.42	50.45	17.27	4.20
Subplots						
Cytoplasm (C)	1	39.26**	148.79	432.61*	1414.18**	696.85**
L x C	22	6.53	209.75	30.52	23.70	8.04
Error	23	4.27	146.01	57.89	15.51	9.31

* and **: Significant at the 5 % and 1 % level, respectively.

ear and harvest index at the 5 %, 1 % and 1 % level, respectively. The decreases in these characters of the F_1 hybrids with the Ae. crassa cytoplasm was associated with their lower fertility than that of the F_1's with the wheat cytoplasm. A significant increase in 1000-grain weight of the F_1 hybrids with the Ae. crassa cytoplasm is probably due to the compensatory effect of the decreased seed fertility. No significant effect was detected on test weight, indicating that the grains of the F_1 hybrids having the Ae. crassa cytoplasm were not shriveled. Ears and grains of the F_1 hybrids between Norin 26 (PCMS line) and Nichirin-komugi (restorer line), which performed best, are shown in Fig. 21.

The correlation coefficients (r) between four yield characters, grain weight/plant, ear number/plant, grain number/ear and 1000-grain weight were calculated for both the F_1 hybrids with the Ae. crassa and wheat cytoplasm. Each r was converted to z, then the significance of the difference between the two z's was tested (Table 35). The results indicate that the correlation coefficients between grain weight/plant and ear number/plant, and between grain weight/plant and grain number/ear were highly significant in the both F_1 hybrids with the Ae. crassa and normal cytoplasm, while none of the correlation coefficients for other character pairs was significant in both cytoplasm types. These results suggest that grain weight/plant of the F_1 hybrids was more strongly associated with the ear number/plant and grain number/ear than their 1000-grain weight. The difference between the correlation coefficients of the F_1 hybrids with the Ae. crassa and wheat cytoplasm was not significant, indicating that

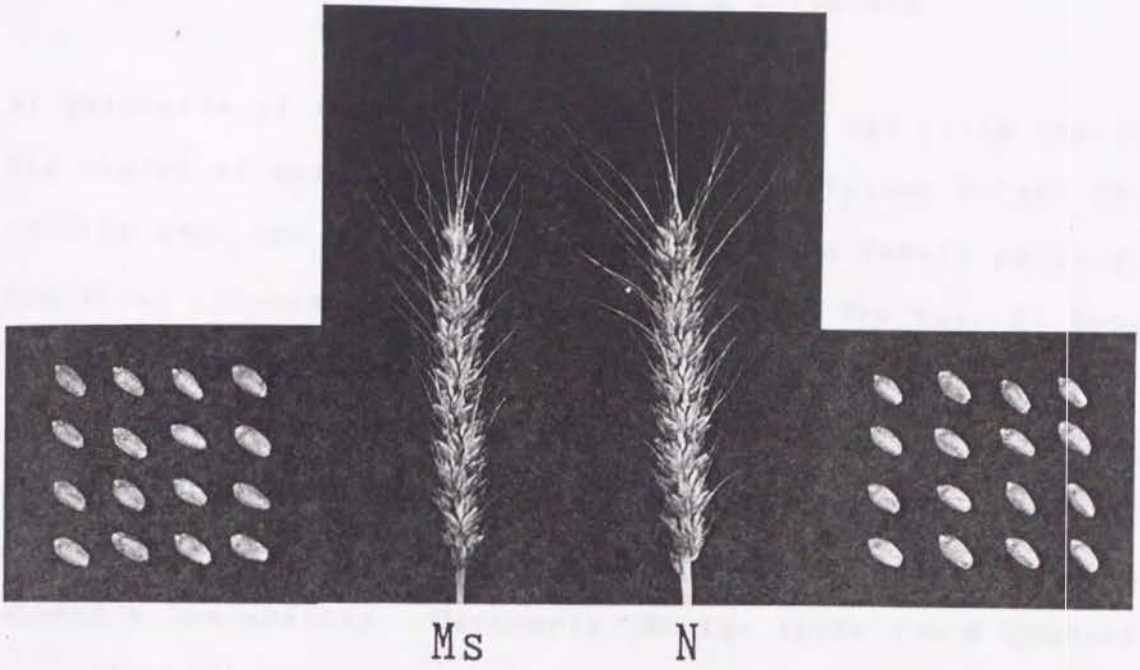

Figure 21. Ears and grains of the F_1 hybrid between Norin 26 (PCMS line) and Nichirin-komugi (restorer line), that performed best at Kasai.
Ms and N: F_1 hybrids having the Ae. crassa and wheat cytoplasm, respectively.

Table 35. Tests on the significance of difference between the F_1 hybrids with the Ae. crassa cytoplasm and their normal counterparts on the correlation coefficients between four yield characters[1)]

	GW/EN	GW/GN	GW/1000	EN/GN	EN/1000	GN/1000
Ms (r1)	0.796**	0.421**	0.128	-0.121	-0.283	0.194
N (r2)	0.804**	0.462**	0.404	-0.041	-0.099	0.403
Difference (Z)[2)]	0.070	0.161	0.946	0.256	0.608	0.731

* and**: Significant at the 5 % and 1 % level, respectively.
Ms and N: F_1 hybrids having the Ae. crassa and wheat cytoplasm, respectively.
1) GW: Grain weight/plant, EN: ear no./plant, GN: Grain no./ear, 1000: 1000-grain weight.
2) $Z = (z1 - z2) / 0.316$
$z = 1/2 [\log_e(1+r) - \log_e(1-r)]$

no interrelationships between different yield characters were not greatly affected by the Ae. crassa cytoplasm.

3) Heterosis of the F_1 hybrids expressed on the yield characters

The degree of heterosis (%) on grain weight/plant of all the F_1 hybrids over the mid-parent for the male and female parents with the wheat cytoplasm are shown in Table 36. The best F_1 hybrid was Norin 26 (PCMS line) x Nichirin-komugi (restorer line), showing 53 % heterosis. As for the PCMS lines, Norin 26 and Junrei-komugi with the Ae. crassa cytoplasm showed a high general combining ability, while Fujimi-komugi and Asakaze-komugi showed a low ability. Heterosis (%) for three yield components, ear number/plant, grain number/ear and 1000-grain weight, are shown in Tables 37, 38 and 39, respectively. The F_1 hybrids of the crosses of Norin 26 or Junrei-komugi as the PCMS parent showed high heterosis on ear number/plant. This indicates that high heterosis expressed by these F_1 hybrids on grain weight/plant is caused by their large ear number/plant. The F_1 hybrids of the crosses having Fujimi-komugi as the PCMS parent showed the lowest heterosis on grain number/ear and 1000-grain weight. The F_1 hybrids of the crosses having Asakaze-komugi as the PCMS parent showed lowest heterosis on ear number/plant. These results indicate that the low heterosis observed on grain weight/plant was caused by small grain number/ear and low 1000-grain weight in the F_1 hybrids of Fujimi-komugi and small ear number/plant in the F_1 hybrids of Asakaze-komugi.

To clarify the relationships between the degrees of heterosis expressed on different yield characters, analysis of corre-

Table 36. Heterosis (%)[1] observed on grain weight/plant of the F_1 hybrids; compared to the mid-parent

Male sterile line[2]	Restorer line[3]					
	N61	Nichirin	Ushio	Sakigake	Orofen	Average
N26	12.5	53.0	15.5	52.1	20.5	30.6
Shirasagi	36.2	50.4	-19.1	-0.6	-2.6	12.9
Junrei	2.9	38.1	44.0	-	38.1	30.8
Fujimi	4.9	-	-53.3	28.4	2.8	-4.3
Asakaze	-17.5	-5.9	21.5	-17.0	9.7	-1.8
Average	7.8	33.9	1.6	15.7	13.7	13.6

1) Performance of the F_1's divided by that of the mid-parent for the male and female parents having the wheat cytoplasm.
2) N26: Norin 26, Shirasagi: Shirasagi-komugi, Junrei: Junrei-komugi, Fujimi: Fujimi-komugi, Asakaze: Asakaze-komugi.
3) N61: Norin 61, Nichirin: Nichirin-komugi, Ushio: Ushio-komugi, Sakigake: Sakigake-komugi.

Table 37. Heterosis (%)[1] observed on ear number/plant of the F_1 hybrids; compared to the mid-parent

Male sterile line[2]	Restorer line[3]					
	N61	Nichirin	Ushio	Sakigake	Orofen	Average
N26	26.6	77.8	2.7	56.5	15.1	35.7
Shirasagi	35.9	48.1	5.2	-2.5	2.8	17.9
Junrei	-3.5	69.9	31.0	-	32.8	39.9
Fujimi	6.2	-	-2.7	71.1	0.8	18.9
Asakaze	-0.7	6.0	20.1	-6.5	3.0	4.4
Average	12.9	50.5	11.3	37.6	10.9	23.4

1) Performance of the F_1's divided by that of the mid-parent for the male and female parents having the wheat cytoplasm.
2) N26: Norin 26, Shirasagi: Shirasagi-komugi, Junrei: Junrei-komugi, Fujimi: Fujimi-komugi, Asakaze: Asakaze-komugi.
3) N61: Norin 61, Nichirin: Nichirin-komugi, Ushio: Ushio-komugi, Sakigake: Sakigake-komugi.

Table 38. Heterosis (%)[1] observed on grain number/ear of the F_1 hybrids; compared to the mid-parent

Male sterile line[2]	Restorer line[3] N61	Nichirin	Ushio	Sakigake	Orofen	Average
N26	-10.6	-20.2	-0.3	-9.4	-1.9	-8.5
Shirasagi	-12.8	-10.3	-24.2	-7.3	-17.2	-14.4
Junrei	-3.1	-29.7	-6.1	-	-12.1	-13.3
Fujimi	-9.5	-	-52.7	-29.9	-9.1	-25.3
Asakaze	-18.6	-18.5	-6.6	-18.8	-9.5	-14.4
Average	-10.9	-19.7	-18.0	-16.1	-10.0	-15.2

1) Performance of the F_1's divided by that of the mid-parent for the male and female parents having the wheat cytoplasm.
2) N26: Norin 26, Shirasagi: Shirasagi-komugi, Junrei: Junrei-komugi, Fujimi: Fujimi-komugi, Asakaze: Asakaze-komugi.
3) N61: Norin 61, Nichirin: Nichirin-komugi, Ushio: Ushio-komugi, Sakigake: Sakigake-komugi.

Table 39. Heterosis (%)[1] observed on 1000-grain weight of the F_1 hybrids; compared to the mid-parent

Male sterile line[2]	Restorer line[3] N61	Nichirin	Ushio	Sakigake	Orofen	Average
N26	-1.3	8.4	11.1	7.6	5.7	6.3
Shirasagi	14.7	13.3	12.3	13.5	14.9	13.7
Junrei	10.8	13.9	17.0	-	18.0	15.6
Fujimi	9.4	-	5.9	11.2	14.5	5.3
Asakaze	3.7	9.9	8.3	11.0	17.6	10.1
Average	7.5	6.2	10.9	12.3	14.1	10.2

1) Performance of the F_1's divided by that of the mid-parent for the male and female parents having the wheat cytoplasm.
2) N26: Norin 26, Shirasagi: Shirasagi-komugi, Junrei: Junrei-komugi, Fujimi: Fujimi-komugi, Asakaze: Asakaze-komugi.
3) N61: Norin 61, Nichirin: Nichirin-komugi, Ushio: Ushio-komugi, Sakigake: Sakigake-komugi.

lation coefficients was carried out (Table 40). Three yield components, ear number/plant, grain number/ear and 1000-grain weight, did not show any significant relationships with each other. Ear number/plant was strongly correlated (at the 1 % level) with grain weight/plant in the F_1 hybrids, indicating that the increase in grain weight/plant contributed to that of ear number/plant.

B. Production of the F_1 hybrids by out-crossing in field

1) Production of the F_1 seeds

The results of out-crossing between the alloplasmic Norin 26 with the Ae. crassa cytoplasm ((c)-N26) and two restorer cultivars are shown in Table 41. Male sterility (%) ranged 85-90 %. Out-crossing rate (%) was 30-40 % and grain yield 80-95 g/m^2. The seeds produced were estimated to be 75 % hybrid purity. Yield of the hybrid seeds seemed to correlate with the out-crossing rate. Fig. 22 shows a selfed (bagged) and out-crossed (non-bagged) ear of (c)-N26 surrounded by Ushio-komugi as the pollinator. The hybrid seeds have a tendency to decrease test weight and germination rate compared with the self-pollinated seeds of Norin 26. This is partly due to the low hybrid purity (75 %), i. e., the remaining 25 % seeds were not the F_1 seeds but the self-pollinated seeds of (c)-N26 which show low test weight and germinability (Table 28).

2) Performance of the F_1 hybrids

Performance of the F_1 hybrids produced by the out-crossing of

Table 40. Correlation coefficients obtained between the degree of heterosis on four yield characters of the F_1 hybrids

	Grain wt. /plant	Ear no. /plant	Grain no. /ear	1000-grain weight
Grain wt. /plant	-	0.821**	0.382	0.340
Ear no. /plant		-	-0.158	0.034
Grain no. /ear			-	0.138
1000-grain wt.				-

* and **: Significant at the 5 % and 1 % level, respectively.

Table 41. Results of field trials for F_1 seeds production

Combination		Heading date		Male sterility 2)	Out-cross. rate 2)	Hybrid purity 2)	Yield 3)	Test weight	1000-kernel weight	Germination rate
PCMS line 1)	Pollinator line 1)	PCMS	Polli.	(%)	(%)	(%)	(g/m2)	(g)	(g)	(%)
(c)-N26	Yutaka	7.08	7.08	89.7	32.4	75.9	81.5	715	29.1	92.9
"	Ushio	7.08	7.08	86.5	40.9	75.2	93.5	720	29.5	89.2
N26								767	30.0	97.0

1) (c)-N26: Alloplasmic line of Norin 26 with the Ae. crassa cytoplasm
Yutaka and Ushio: Yutaka-komugi and Ushio-komugi, respectively.
2) Male sterility (%) = (1 - A/C) x 100
Out-crossing rate (%) = (B/D - A/C) x 100
Hybrid purity (%) = (B/D - A/C) / (B/D) x 100
A and B: Seed no./spikelet of bagged and non-bagged ear of (c)-N26, respectively.
C and D: Seed no./spikelet of bagged and non-bagged ear of N26, respectively.
3) Yield (g/m^2) = gram seeds /m^2 female plants.

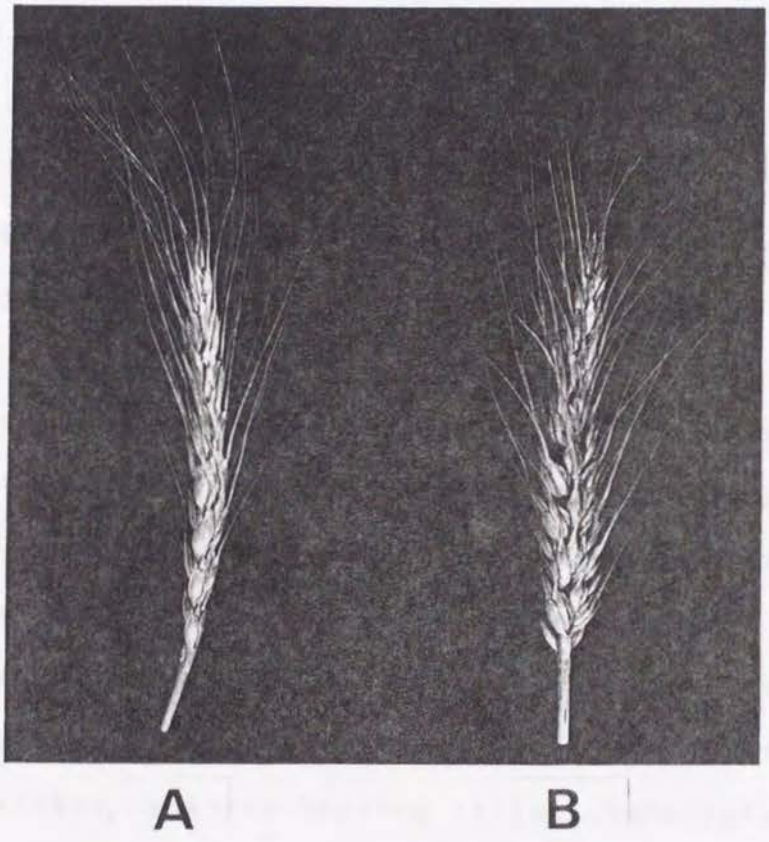

Figure 22. Selfed (bagged) and out-crossed (non-bagged) ears of the alloplasmic line of Norin 26 with the Ae. crassa cytoplasm that was surrounded by Ushio-komugi as the pollinator.
A: Selfed (bagged) ear, B: out-crossed (non-bagged) ear.

(c)-N26 with two restorer cultivars is shown in Table 42. Heading dates of the F_1 hybrids showed were the same as those of the mid-parents. Heterosis was expressed on plant height and ear number/m^2. Grain yields of the F_1 hybrids were, however, not higher than those of the corresponding parents with the wheat cytoplasm, and consequently, harvest indexes of the F_1 hybrids were smaller than those of the parents. The low yields of the F_1 hybrids were probably due to the low hybrid purity (75 %), because about 25 % of the seeds are assumed to have produced (c)-N26 that has low productivity (Table 28). The decreased test weight of the F_1 hybrids also was assumed to have been caused by the same reason.

3) Effects of the seeding density of the performance of alloplasmic Norin 26 with the Ae. crassa cytoplasm

Mean values of the seven characters and male sterility (%) of (c)-N26 at different seeding densities are shown in comparison with those of euplasmic N26 in Table 43. Analysis of variance indicated significant differences among the density levels on two characters, non-ear bearing tiller number/plant and seed number/spikelet (Table 44). The two cytoplasms showed significantly different effects on three characters. Non-ear bearing tiller number/plant was increased with the reduction in seeding density in both the PCMS and normal lines. Seed number/spikelet was decreased in the PCMS line, while increased in the normal line with the decreased seeding density level. This is due to the increase in ear number/plant with the decreased density level and the decrease in seed fertility in the late-produced

Table 42. Performance on nine characters of the F_1 hybrids produced by out-crossing of a PCMS line with Yutaka-komugi and Ushio-komugi

Line[1)]	Hybrid purity (%)	Heading date	Plant height (cm)	Ear length (cm)	Spikelet number /ear	Ear number /m^2	Yield (kg/a)	HI (%)	Test weight (g)	1000-grain wt. (g)
Norin 26	-	4.21	90	7.3	15.6	388	28.8	36.1	699	34.6
(c)-N26	-	4.22	89	7.6	15.5	409	18.7	20.2	681	34.8
Yutaka-komugi	-	4.22	101	7.5	15.9	413	28.3	33.5	715	33.4
Ushio-komugi	-	4.20	82	8.2	16.2	395	27.9	39.4	720	38.6
(c)-N26 x Yutaka F_1	75.9	4.22	101	7.8	15.3	452	21.9	25.4	708	36.0
(c)-N26 x Ushio F_1	75.2	4.21	104	7.9	15.8	598	24.6	23.7	707	36.5

1) (c)-N26: Alloplasmic line of Norin 26 having the Ae. crassa cytoplasm,
Yutaka: Yutaka-komugi, Ushio: Ushio-komugi.

Table 43. Agronomic characters of a PCMS line grown at different seeding densities

Seeding density	Heading date (day)		Plant ht. (cm)		Ear length (cm)		Spikelet no./ear		Ear no./plant		Non-ear tiller no.		Seed no./spikelet		Male sterility 1) (%)
	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N	
1.0 g/m	7.01	7.01	70	70	7.2	6.8	16.9	15.8	5.9	4.8	7.7	1.8	0.08	1.96	95.9
0.5 g/m	7.01	7.01	71	71	7.1	6.8	16.0	15.9	8.8	6.9	11.6	3.2	0.03	2.08	98.6
0.25 g/m	7.01	7.01	70	71	7.1	7.0	15.5	16.0	8.7	9.7	15.0	10.0	0.01	2.34	99.6
Average	7.01	7.01	70	71	7.1	6.9	16.1	15.9	7.8	7.1	11.4	5.0	0.04	2.13	98.0

Note) Ms and N denote the alloplasmic and euplasmic Norin 26, respectively.
1) Male sterility (%) = (1 - A/B) x 100
A: Seed no./spikelet of (c)-N26, B: Seed no./spikelet of euplasmic N26.

Table 44. The results of analyses of variance for the performance of the alloplasmic and euplasmic Norin 26 grown at different seeding densities

Source of variation	d.f.	Mean square						
		Heading date	Plant height	Ear length	Spikelet no./ear	Ear no./plant	Non-ear tiller no.	Seed sets /spikelet
Main plot								
Rep.	2	0.056	33.500	0.116	2.516*	1.184	1.221	0.005
Density (D)	2	0.222	1.167	0.024	0.554	22.722	94.302*	0.041**
Error	4	0.056	7.167	0.031	0.266	3.246	5.487	0.001
Subplots								
Cytoplasm (C)	1	0.00	0.500	0.294*	0.201	2.000	186.245**	19.656**
D x C	2	-0.00	0.500	0.057	0.974	3.327	4.486	0.077**
Error	6	0.167	9.833	0.036	0.206	1.939	7.196	0.005

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

ears of (c)-N26. Consequently, male sterility was increased according to the decrease in seeding density, resulting in higher male sterility in lower density level in the PCMS line.

4) Effects of varietal difference in the PCMS lines on male sterility

Mean value of the seven characters and male sterility (%) of four PCMS lines having different varietal backgrounds are shown in Table 45. Analysis of variance indicated significant differences existed among the PCMS lines on heading date and seed number/spikelet (Table 46). The seed number/spikelet of (c)-N26 was significantly higher than those of other PCMS lines at the 5 % level by Duncan's new multiple-range test, indicating that the male sterility was expressed in Shirasagi-komugi, Junrei-komugi and Fujimi-komugi with the Ae. crassa cytoplasm more strongly than in (c)-N26. Especially, the alloplasmic Fujimi-komugi showed complete male sterility. Fig. 23 shows ears of the PCMS and corresponding normal lines grown at Tanno. These results suggest that the PCMS line with complete male sterility can be developed.

4. Discussion

1) Effects of the Ae. crassa cytoplasm

Tsunewaki (1980a) and his co-workers have carried out a series of comparative studies on genetic properties of the cytoplasms from 22 different species of Triticum and Aegilops by introducing them into 12 common wheats. Morphological and physiological

Table 45. Agronomic characters of the PCMS lines with different varietal backgrounds

Line[1]	Heading date	Plant height (cm)	Ear length (cm)	Spikelet number /ear	Ear number /plant	Non-ear tiller	Seed number /spikelet	Male sterility (%)[2]
(c)-Norin 26	7.01	70	7.2	16.4	5.9	7.7	0.08	95.9
(c)-Shirasagi	7.01	69	6.7	16.2	4.5	7.0	0.01	99.5
(c)-Junrei	6.30	65	7.2	16.0	4.2	5.0	0.02	98.8
(c)-Fujimi	6.30	66	7.1	15.1	4.8	5.1	0.00	100.0

1) (c)-: Alloplasmic line with Ae. crassa cytoplasm, Shirasagi: Shirasagi-komugi, Junrei: Junrei-komugi, Fujimi: Fujimi-komugi.
2) Male sterility (%) = (1 - A/B) x 100
A: Seed no./spikelet of the PCMS line.
B: Seed no./spikelet of the corresponding normal line.

Table 46. The results of analyses of variance for the agronomic characters of four PCMS lines having different varietal backgrounds

Source of variation	d.f.	Mean square: Heading date	Plant height	Ear length	Spikelet no./ear	Ear no. /plant	Non-ear tiller no.	Seed no. /spikelet
Rep.	2	0.167	36.750	0.141	2.786	2.531	7.286	0.0030
Line	3	3.333*	18.750	0.181	1.594	1.678	5.441	0.0034*
Error	6	1.167	13.083	0.058	0.806	1.582	9.179	0.0006

*: Significant at the 5 % level.

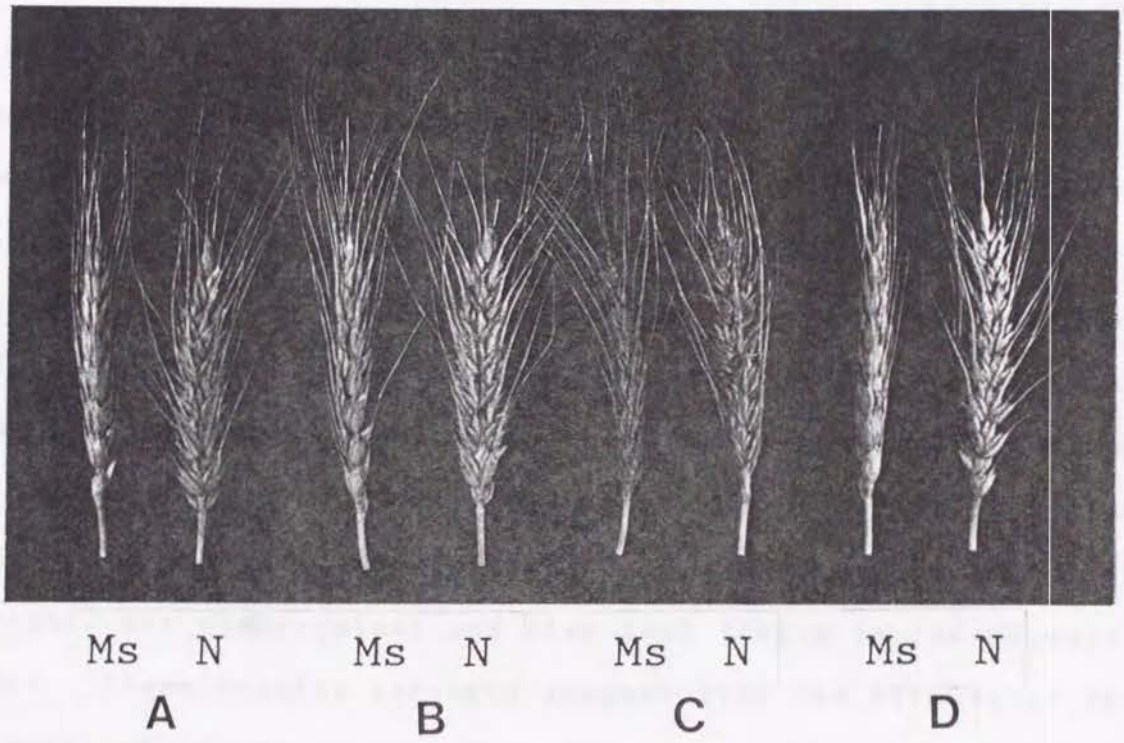

Figure 23. Ears of the PCMS and corresponding normal lines of four cultivars grown at Tanno.
Ms and N: Alloplasmic lines with the Ae. crassa cytoplasm and euplasmic line, respectively.
A: Norin 26, B: Shirasagi-komugi, C: Junrei-komugi, D: Fujimi-komugi.

characters of wheat plant have all been shown to be affected by one or more cytoplasms. From a viewpoint of hybrid wheat breeding, T. timopheevi cytoplasm has been most closely examined on different characteristics other than the male sterility (Hori and Tsunewaki 1969, Tahir 1971, Tsunewaki and Endo 1973, Jošt et. al. 1975, Fujigaki and Tsunewaki 1976). Tahir (1971) reported that the male sterile lines with the T. timopheevi cytoplasm did not deviate in heading date, plant height, tiller number and spikelet number/ear from their normal lines in Pakistani cultivars. However, Jošt et. al. (1975) revealed that the T. timopheevi cytoplasm reduced plant height but increased tiller number and spikelet number/ear in Yugoslavian cultivars of common wheat. Fujigaki and Tsunewaki (1976) reported that the T. timopheevi cytoplasm hastened heading date and increased plant height, ear number/plant and flag leaf length in Japanese cultivars, while delayed heading date and decreased plant height, ear number/plant and flag leaf length in the US cultivars. These results strongly suggest that the effects of the T. timopheevi cytoplasm depend on the genotype of the cultivars in which this cytoplasm has been introduced.

In this chapter, I have investigated the genetic effects of the Ae. crassa cytoplasm in the parental PCMS lines and the F_1 hybrids of Japanese cultivars. My results have indicated that the Ae. crassa cytoplasm delays heading date and reduces spikelet number/ear, and selfed and open-pollinated seed fertilities in the PCMS lines, while increases ear length and reduces selfed and open-pollinated seed fertilities in the F_1 hybrids. From a practical point of view, the deviations of these characters

except the selfed and open-pollinated seed fertilities can be neglected because of small amounts. The open-pollinated seed fertility of the PCMS lines ranged 50-80 % and depended on their genotype. Therefore, it is necessary to utilize the PCMS line having a high seed fertility such as alloplasmic Junrei-komugi (80 % open-pollinated seed fertility) for effective maintenance and seed increase of the PCMS line. Although the open-pollinated fertility of the F_1 hybrids was 80 % on the average, heterosis expressed on grain weight/plant was 19 % over the standard parent (Norin 61) and 14 % over the mid-parent. From these results, I conclude that the Ae. crassa cytoplasm is usable for hybrid wheat production from the point of heterosis.

In the T. timopheevi cytoplasmic male sterility-fertility restoration system, out-crossing of the male sterile line is needed for its maintenance and increase. Johnson and Lucken (1986) reported that the out-crossed seeds had lower test weight and germination rate, but higher seed weight than the self-pollinated seeds of the maintainer line. One of the advantages of the "two-line system" using the Ae. crassa cytoplasm is that this system does not require the out-crossing of the male sterile line for its maintenance and increase. However, my results have indicated that the self-pollinated seeds of the PCMS line had also lower test weight and germination rate than the self-pollinated seeds of the normal counterpart. These facts suggest that the reduced test weight and germination rate are caused by low seed fertility. Grains on the ear of low fertility seem to have a tendency to be shriveled, and consequently show low test weight and germination rate.

Grain quality of hybrid wheat is determined by different characteristics of grains produced by F_1 hybrid. My results have revealed that no significant effects of the Ae. crassa cytoplasm was exerted on test weight of grains set on the F_1 hybrids. The Ae. crassa cytoplasm has a tendency to increase 1000-grain weight in the F_1 hybrids, that is favorable for grain quality.

2) Heterosis expressed in the F_1 hybrids

As mentioned by Virmani and Edwards (1983), a number of workers have observed heterosis on various agronomic characters of the F_1 hybrid wheat. However, published reports on heterosis in Japanese wheat hybrids have been limited. One of the earliest researches on heterosis in the F_1 hybrids using Japanese cultivars was carried out by Akemine and Kumagai (1966). They studied heterosis on yield and its components of the F_1 hybrids using eight cultivars, one of which was Japanese native cultivar and seven were improved cultivars of Japan, India or Italy. Their results suggest that yield level of the F_1 hybrids is expected to be, on an average, about 20 % more than the mid-parent in the space-planted experiments. Tsunewaki (1970) investigated heterosis on heading date, plant height and yield of the F_1 hybrids between 12 Japanese and US cultivars in space-planted plots, and concluded that the F_1 hybrids yielded, on an average, 34 % more than the better parents. These reports demonstrate that practically usable heterosis is expressed in the F_1 hybrids of Japanese cultivars. These investigations, however, were carried out using the F_1 hybrids with wheat cyto-

plasm, and did not take into account the effects of a male sterile cytoplasm. My results have revealed that the F_1 hybrids with normal cytoplasm yield, on an average, 44 and 40 % more than the standard parent (Norin 61) and the mid-parent, respectively, in space-planted plots.

Tsunewaki (1980b) reported that the average grain yield of 30 F_1 hybrids produced by out-crossing between the male sterile lines with the T. timopheevi cytoplasm and restorer lines was only about 58 % of that of the standard cultivars. The yield reduction resulted from partial sterility, because other yield components did not differ from or were even better than those of the standard cultivar. The best F_1 hybrid, Norin 72 (male sterile line) x Nichirin-komugi (restorer line), out-yielded about 14 % over the average of three standard cultivars, and about 4 % over the best standard cultivar. My results have demonstrated that the average heterosis of 23 F_1 hybrids between the PCMS lines and restorer lines is 19 and 14 % over the standard cultivar, Norin 61 and mid-parent, respectively. This heterosis was estimated with the space-planted materials. However, the F_1 hybrids may be expected to show heterosis on yield at normal seeding density, because grain weight/plant is associated with ear number/plant as well as grain number/ear.

Among the PCMS lines with the Ae. crassa cytoplasm, those of Norin 26 and Junrei-komugi show high general combining ability (GCA). As for the restorer lines, Nichirin-komugi shows the highest GCA. The best F_1 hybrid is obtained by the combination between alloplasmic Norin 26 as the PCMS parent (high GCA) and Nichirin-komugi as the restorer parent (high GCA).

Correlation between the degree of heterosis on yield and its components of F_1 hybrids was analyzed by Akemine and Kumagai (1966). The results indicate that heterosis on yield is usually related with that on grain number/ear. My regression analysis of heterosis on yield and its components in the F_1 hybrids with the Ae. crassa cytoplasm indicates that ear number/plant is more strongly correlated with grain weight/plant than grain number/ear. Apparently, ear number/plant is more important than grain number/ear for increasing yield of the F_1 hybrids. Actually, the best F_1 hybrid, Norin 26 x Nichirin-komugi shows that highest heterosis on ear number/plant among all F_1 hybrids.

3) Problems in F_1 seed production

One of the most important factors for the success of hybrid wheat is whether F_1 seeds can be efficiently and economically produced by out-crossing of the male sterile line with a restorer line. Madhukar et. al. (1967) investigated the seed setting rate of the male-sterile, alloplasmic line of T. aestivum cv. Bison with T. timopheevi cytoplasm surrounded by four rows of the pollinator. They used 45 wheat cultivars including important commercial cultivars of Texas, U.S.A., as the pollinators. Seed setting rate of the male sterile plants ranged from 11 to 61 % with an overall average of 31 % for the 45 pollinators. Miller et. al. (1975) studied the seed setting rate with varying ratios of female to male parent. They used one male sterile line with the T. timopheevi cytoplasm and one restorer line carrying the fertility-restoring genes derived from T. timopheevi. The crossing blocks of 1:1, 2:1 and 3:1 ratios of

female to male gave the mean seed setting rates of 64, 51 and 45 %, respectively. As for Japanese cultivar, Tsunewaki (1980b) reported that the seed setting rate of 36 out-crossing combinations ranged from 33 to 90 % at 1:1 ratio of female to male parent. He also indicated that there were clear differences existing among the male sterile lines for their ability of receiving pollen grains and among the restorer lines for their pollination ability.

In my investigation of the field test on F_1 seed production in which female and male parents were in a 1:1 ratio, the out-crossing rates of (c)-N26 with Yutaka-komugi and Ushio-komugi as the restorer lines were 32 and 41 %, from which the seed setting rates were estimated to be 43 and 54 %, respectively. From these results, I conclude that even under the spring sowing condition at Tanno, Hokkaido, a seed setting rate higher than 50 % can be easily attained by the selection of suitable restorer lines such as Ushio-komugi. However, it is also necessary to select a suitable PCMS line for its ability of receiving pollen grains, because the female fertility of the PCMS line depends on its nuclear genotype.

From the field test on hybrid seed production, I could get 80-95 hybrid seeds of 75 % purity per square meter. However, the 75 % purity seems to be insufficient for the yield increase, because the remaining 25 % is self-pollinated seeds of alloplasmic Norin 26 which gives low seed productivity even under the short-day condition at Kasai. Consequently, the yield heterosis was not detected in the present field test. Apparently, hybrid purity is concerned with male sterility rate. Highly purified

hybrid seeds will be produced by out-crossing of a PCMS line showing complete male sterile with the pollen of a restorer line. I have investigated the effects of two factors associated with male sterility of the PCMS line: one is the seeding density for the PCMS lines, and the other is the varietal difference among the PCMS lines. The results on the former factor indicate that the male sterility of the PCMS line is intensified with the decrease in the seeding density. However, it happens that the crossing block of a lower seeding density gave a lower productivity of hybrid seeds. The results on the second factor indicate that the male sterility rate of the PCMS line depends on its genotype. The PCMS line showing complete male sterility such as the alloplasmic Fujimi-komugi will produce the hybrid seeds of a high purity. However, this type of the PCMS line has a tendency to decrease female fertility. For a practical use, it is necessary to produce the PCMS lines having high male sterility with high female fertility.

VI. CONCLUSION

It has been passed 30 years since the research on hybrid wheat breeding was began. However, commercial hybrids which show appreciably high yield have neither been developed nor used on any significant area. Nevertheless, as wheat is the second largest food crop in the world, every effort should and will be made to lead the development of hybrid wheat successful.

The problems encountered in producing hybrid wheat are briefly summarized as follows:

For cytoplasmic system

* undesirable influences of the male sterile cytoplasm and the fertility-restoring genes
* incomplete fertility restoration

For nuclear system

* no promising system developed

For chemical system

* undesirable influences of the chemical on both plant and human
* differences among genotypes in response to the chemical

In this thesis, I described the "two-line system" for producing hybrid wheat using photoperiod-sensitive cytoplasmic male sterility (PCMS) caused by the Ae. crassa cytoplasm. This system has the following advantages in comparison with a "three-line system", the T. timopheevi system:

a) ease to maintain the PCMS line
b) shortened period required for developing the PCMS line
c) low cost for developing the PCMS line

d) no undesirable influences of the Ae. crassa cytoplasm and the fertility-restoring genes on the agronomic characters of the F_1 hybrids and their grain quality

e) sufficient level of fertility restoration in the F_1 hybrids

This system requires only the PCMS and restorer lines. No need for developing the maintainer line leads to the first and second advantages. The third advantage of this system is derived from these two advantages. The present investigation has revealed the forth and fifth advantages of the system utilizing Ae. crassa cytoplasm.

Seeds of the PCMS line produced by self-pollination had a tendency to reduce seed quality, i. e., low test weight and germination rate resulted from seed shriveling. These features are the same as those of the male sterile line with T. timopheevi cytoplasm produced by out-crossing. Hybrid seeds produced by out-crossing of the PCMS line with a restorer line showed low test weight and germination rate. It was mainly due to low purity of the hybrid seeds. It is generally known in the T. timopheevi system that out-crossed seeds show low test weight and germination rate resulted from seed shriveling. The shriveled seeds are not accepted by farmers and millers. The commercialization of hybrid wheat seems to be difficult unless their consensus has been obtained to accept shriveled seeds for industrial use. My results indicated that the F_1 hybrids with the Ae. crassa cytoplasm obtained by hand-pollination showed adequate heterosis on grain weight/plant with the space-planted materials. However, the field test on the performance of the F_1

hybrids using hybrid seeds obtained by out-crossing revealed no heterosis on yield. This is due to low purity of the hybrid seeds produced by out-crossing. This low purity of the F_1 seeds was resulted from incomplete male sterility of the PCMS line. To solve this problem, a PCMS line showing high male sterility must be developed. The PCMS line should also have a good general combining ability. The success of developing commercial hybrid wheat utilizing this system depends on breeding the excellent parental lines.

VII. SUMMARY

The alloplasmic line of the Japanese wheat cultivar, Norin 26, having the Aegilops crassa cytoplasm (abbreviated, (c)-N26) showed almost complete male sterility under the long-day conditions of 15 hours or longer light period, and high male fertility under the short-day conditions of 14.5 hours or shorter. On the other hand, an another wheat cultivar, Chinese Spring, with the Ae. crassa cytoplasm ((c)-CS) exhibited male fertility under all photoperiodic conditions tested. These results imply that the male sterility is induced by an interaction of the N26 nucleus with the Ae. crassa cytoplasm under the long-day conditions of longer than 15 hours, and that the CS nucleus has fertility-restoring (Rf) gene(s) which prevents the male fertility reduction caused by the Ae. crassa cytoplasm under the long-day conditions. No significant influence of temperature on the male fertility of (c)-N26 was observed. Thus, this type of male sterility is called "photoperiod-sensitive cytoplasmic male sterility (PCMS)". The PCMS is expressed in the form of pistillody of stamens. The floret differentiation stage of the plant is sensitive to the photoperiod as to pistillody induction. (C)-N26 shows deep sterility at Tanno, Hokkaido, in which the plants are exposed to longer day length than 15 hours during the floret differentiation stage, and high fertility at Kasai, Hyogo, where day length is always shorter than 15 hours. (C)-CS, however, exhibits male fertility at the both locations.

The PCMS can be used as a new means for hybrid wheat production, named "two-line system". The PCMS line is maintained

and multiplied by self-pollination under the short-day conditions, and the hybrid seeds can be produced by out-crossing of the PCMS line with a pollinator line under the long-day conditions. In contrast to the system of hybrid wheat production using the Triticum timopheevi cytoplasm, the present system requires only PCMS and pollinator lines.

Forty-five Japanese and 31 foreign cultivars of common wheat have been repeatedly backcrossed as the recurrent pollen parent to (c)-N26. Selfed seed fertilities in successive backcross generations were examined at Kasai and Tanno. Among the alloplasmic lines of 19 Japanese cultivars which reached the B_5 (12 lines) or B_4 (nine lines) generation, nine lines of Chikushi-komugi, Shirasagi-komugi, Junrei-komugi, Fujimi-komugi, Omase-komugi, Kobushi-komugi, Asakaze-komugi, Gogatsu-komugi and Iyo-komugi showed the PCMS induced by the Ae. crassa cytoplasm, while ten lines of Norin 61, Nichirin-komugi, Yutaka-komugi, Ushio-komugi, Zenkoji-komugi, Danchi-komugi, Sakigake-komugi, Seto-komugi, Hatsuho-komugi and Kairyohaya-komugi showed high male fertility at Tanno. These results indicate that the former group of cultivars are converted to the PCMS line by transfer of the Ae. crassa cytoplasm, and the latter group having some Rf gene(s) against this cytoplasm can be used as the pollinator line. Pedigrees of the cultivars suggest that three different sources of relatively strong Rf gene(s) exist among Japanese cultivars. Two of them are Japanese native cultivars, Ejima and Shinriki, and the third one is Norin 3. Among 16 CIMMYT cultivars, three i. e., Sonora 64, Tobari and Nacazari F76, are seemed to have relatively strong Rf gene(s) against the Ae.

crassa cytoplasm. Their pedigrees suggest that the Rf gene(s) have been derived from a Japanese cultivar, Norin 10.

Genetic analyses on the Rf gene(s) in CS and Norin 61 have been conducted. The results of conventional and telosomic or monosomic analysis have indicated that the fertility restoration by CS against the Ae. crassa cytoplasm is mainly controlled by a single dominant gene (Rfp1) located on the long arm of chromosome 7B. In addition, a large number of modifiers are involved in improving the level of fertility restoration. As for the fertility restoration by Norin 61, incomplete dominant genes located on chromosomes 4A, 7A, 1B, 2B, 4B, 1D, 3D, 4D, 5D and 7D have been identified. A single dose of the Rfp1 gene in CS can not restore the fertility above 50-55 % under the short-day condition at Kasai, while a set of the Rf genes of Norin 61 restore the fertility to about 80 %, suggesting that the Rf genes of Norin 61 are preferred to the Rfp1 gene for a practical use.

Twenty-three F_1 hybrids were produced by hand-pollination between five PCMS lines of Norin 26, Shirasagi-komugi, Junrei-komugi, Fujimi-komugi and Asakaze-komugi with the Ae. crassa cytoplasm and five restorer lines of Norin 61, Nichirin-komugi, Ushio-komugi, Sakigake-komugi and Orofen. The PCMS lines and the F_1 hybrids were tested for their agronomic characters at Kasai. Analyses of variance revealed that the effects of the Ae. crassa cytoplasm were significant on six characters, heading date, spikelet number/ear, selfed and open-pollinated seed fertilities, test weight and germination rate of the PCMS lines, and on seven characters, ear length, selfed and open-pollinated

seed fertilities, 1000-grain weight, grain weight/plant, grain number/ear and harvest index of the F_1 hybrids. No effects of the cytoplasm were significant on test weight of the grains produced by the F_1 hybrids, indicating that the grain shriveling not was caused by the Ae. crassa cytoplasm. Instead of the reduction in male fertility, the F_1 hybrids with the Ae. crassa cytoplasm showed on an average 14 % heterosis on grain weight/plant over the mid-parent of the female and male parents with the wheat cytoplasm. A field test for the production of F_1 seeds by out-crossing of (c)-N26 with Yutaka-komugi or Ushio-komugi, that has been conducted at Tanno, provided hybrid seeds with 75 % purity and 90 % germination rate. A field test on the performance of the F_1 hybrids, however, showed no heterosis on yield because of low purity of the hybrid seeds, that resulted from partial male fertility of the PCMS lines. The degree of male sterility was increased with the decrease in seeding density of the PCMS line, that was dependent upon the genotype of the PCMS line.

ACKNOWLEDGEMENTS

I wish to express my deepest appreciation to Professor Dr. K. Tsunewaki, Laboratory of Genetics, Faculty of Agriculture, Kyoto University, for his continuous kind-hearted guidance for this work.

I also thank Dr. S. Tsuji, Takarazuka Research Center, Sumitomo Chemical Co., for his kindness in reading of the manuscript. Thanks are also due to Dr. H. Hirohara and Mr. A. Nishikawa, Takarazuka Research Center, Sumitomo Chemical Co., for their kind encouragement and help given during the present investigation. I am greatly indebted to Mr. Y. Sakai, Mr. M. Nishimura and Mr. K. Yuasa, Tanno Agricultural Cooperative, Hokkaido, for providing facilities for field tests. I am also grateful to Dr. K. Nishikawa and Dr. Y. Furuta, Faculty of Agriculture, Gifu University, and Dr. T. Ryu Endo, Nara University, for supplying the aneuploids for genetic analysis and helpful information. Thanks are also due to Mr. H. Yogai and Ms. K. Nakatsu, Takarazuka Research Center, Sumitomo Chemical Co., for their technical assistance for histological studies.

Finally, I especially thank Mr. M. Miyamoto, my partner in Takarazuka Research Center, Sumitomo Chemical Co,, for his continued general and technical assistance all way through this study.

REFERENCES

Akemine, H. and K. Kumagai, 1966. Heterosis in wheat hybrids. Seiken Ziho 18:64-75.

Bahl, P. N. and S. S. Maan, 1973. Chromosomal location of male fertility restoring genes in six lines of common wheat. Crop Sci 13:317-320.

Bingham, J., 1966. Varietal response in wheat to water supply in the field and male sterility caused by a period of drought in a glasshouse experiment. Ann Appl Biol 57:365-77.

Briggle, L. W., 1963. Heterosis in wheat -- a review. Crop Sci 3:407-412.

Chopra, V. L., S. K. Jain and M. S. Swaminanthan, 1960. Studies on the chemical induction of pollen sterility in some crop plants. Indian J Genet Plant Breed 20:188-199.

Doig, R. I., A. A. Done and D. F. Rogers, 1975. Pre-harvest sprouting in bread wheat (Triticum aestivum) as influenced by cytoplasmic male-sterility derived from T. timopheevi. Euphytica 24:229-232.

Driscoll, C. J., 1972. XYZ system of producing hybrid wheat. Crop Sci 12:516-517.

Driscoll, C. J., 1977. Registration of Cornerstone male-sterile wheat germplasm. Crop Sci 17:190.

Ellis, J. R. S. and P. B. Clayton, 1976. The influence of cross-pollination on pre-harvest sprouting in F_1 hybrid wheat seed. Cereal Res Comm 4:127-132.

Endo, T. Ryu and K. Tsunewaki, 1975. Genetic diversity of the cytoplasm in Triticum and Aegilops. I. On the origin of the

cytoplasm of Aegilops triuncialis L. Seiken Ziho 25-26: 55-66.

Feekes, W., 1941. De Tarwe en haar milieu. Vers XVII Tech Tarwe Comm, Groningen, pp. 560-561.

Fisher, J. E., 1972. The transformation of stamens to ovaries and of ovaries to inflorescences in T. aestivum L. under short day treatment. Bot Gaz 133:78-85.

Franckowiak, J. D., S. S. Mann and N. D. Williams, 1976. A proposal for hybrid wheat utilizing Aegilops squarrosa L. cytoplasm. Crop Sci 16:725-728.

Freeman, G. F., 1919. Heredity of quantitative characters in wheat. Genetics 4:1-93.

Fujigaki, J. and K. Tsunewaki, 1976. Basic studies on hybrid wheat breeding. VII. Characteristics of the male sterile lines of common wheat cultivars. Jpn J Breed 26:179-186.

Fukasawa, H., 1953. Studies on restoration and substitution of nucleus in Aegilotricum. I. Appearance of male sterile durum in substitution crosses. Cytologia 18:167-175.

Fukasawa, H., 1959. Nucleus substitution and restoration by means of successive backcrosses in wheat and its related genus Aegilops. Jpn J Bot 17:55-91.

Fukunaga, K. and M. Inagaki, 1985. Genealogical pedigrees of Japanese wheat cultivars. Jpn J Breed 35:89-92.

Gale, M. D., G. A. Marshall and M. V. Rao, 1981. A classification of the Norin 10 and Tom Thumb dwarfing genes in British, Mexican, Indian and other hexaploid bread wheat varieties. Euphytica 30:355-361.

Graham, R. D., 1975. Male sterility in wheat plants deficient in

copper. Nature 254:514-515.

Griffee, F., 1921. Comparative vigor of F_1 wheat crosses and their parents. J Agr Res 22:53-63.

Hori, T. and K. Tsunewaki, 1969. Basic studies on hybrid wheat breeding. II. Effects of three alien cytoplasms on main quantitative characters of hexaploid wheats. Jpn J Breed 19:425-430.

Hossain, M. A. and C. J. Driscoll, 1983. Fertility compensation of Cornerstone male sterility of wheat by rye. Genetics 104:181-189.

Islam, A. K. M. R. and C. J. Driscoll, 1984. Latent male fertility in 'Cornerstone' chromosome 4A. Can J Genet Cytol 26:98-99.

Johnson, K. M. and K. A. Lucken, 1986. Characteristics and performance of male-sterile and hybrid seed production by cross-pollination in hard red spring wheat. Crop Sci 26:55-57.

Jośt, M., M. Glathi-Jost and V. Hrust, 1975. Influence of T. timopheevi cytoplasm on characters of male sterile common wheat. 3. The plant morphology and kernel characters. Cereal Res Comm 3:15-56.

Kato, K, T. Wada and K. Hayashi, 1989. Varietal difference in narrow-sense earliness of wheat and the genetic analysis (in Japanese). Jpn J Breed 39(Suppl) 1:406-407.

Keim. D. L., J. R. Welsh and R. L. McConnell, 1973. Inheritance of photoperiodic heading response in winter and spring cultivars of bread wheat. Can J Pl Sci 53:247-250.

Kihara, H., 1951. Substitution of nucleus and its effects on

genome manifestations. Cytologia 16:177-193.

Kihara, H. 1959. Fertility and morphological variation in the substitution backcrosses of the hybrid Triticum vulgare x Aegilops caudata. Proc 10th Int Congr Genet 1:142-171.

Kihara, H. and K. Tsunewaki, 1961. Pistillody of Triticum durum induced by an alien cytoplasm. Seiken Ziho 12:1-10.

Kihara, H. and K. Tsunewaki, 1967. Genetic principles applied to the breeding of crop plants. In: Brink, R. A. (Ed.), Heritage from Mendel. Univ Wisconsin Press, Madison, Wisconsin, pp. 403-418.

Kondo, N., 1941. Chromosome doubling in Secale, Haynaldia and Aegilops by colchicine treatment. Jpn J Genet 17:46-54.

Livers, R. W. and E. G. Heyne, 1968. Hybrid vigor in hard red winter wheat. Proc 3rd Int Wheat Genet Symp, pp. 431-436.

Law, C. N., J. Sutka and A. J. Worland, 1978. A genetic study of day-length response in wheat. Heredity 41:185-191.

Madhukar, K. K., I. M. Atkins, O. G. Merkle and K. B. Porter, 1967. Cross pollination studies with male sterile wheat of three cytoplasms, seed size on F_1 plants, and seed and anther size of 45 pollinators. Crop Sci 7:389-394.

McRae, D. H., 1985. Advances in chemical hybridization. Plant Breed Rev 3:169-191.

Michaelis, P., 1954. cytoplasmic inheritance in Epilobium and its theoretical significance. Adv Genet 6:288-401.

Miller, J. F. and J. W. Schmidt, 1970. Inheritance of genes controlling male fertility in two different lines of wheat. Agron Abstr 1970:23.

Miller, J. F., K. J. Rogers and K. A. Lucken, 1975. Effects of

field production techniques on hybrid wheat seed quality. Crop Sci 15:329-332.

Mukai, Y. and K. Tsunewaki, 1979. Basic studies on hybrid wheat breeding. VIII. A new male sterility-fertility restoration system in common wheat utilizing the cytoplasms of Aegilops kotschyi and Ae. variabilis. Theor Appl Genet 54:153-160.

Oehler, E. and M. Ingold, 1966. New cases of male-sterility and new restorer source in T. aestivum. Wheat Inf Serv 22:1-3.

Ogihara, Y. and K. Tsunewaki, 1982. Molecular basis of the genetic diversity of the cytoplasm in Triticum and Aegilops. I. Diversity of the chloroplast genome and its lineage revealed by the restriction pattern of ct-DNAs. Jpn J Genet 57:371-396.

Ogihara, Y. and K. Tsunewaki, 1988. Diversity and evolution of chloroplast DNA in Triticum and Aegilops as revealed by restriction fragment analysis. Theor Appl Genet 76:321-332.

Panayotov, I., 1980. New cytoplasmic male sterility sources in common wheat: their genetical and breeding considerations. Theor Appl Genet 56:153-160.

Panayotov, I., D. Gotsova and M. Savov, 1988. Chromosome location of Rf genes for Aegilops mutica and Triticum timopheevi cytoplasms. Proc 7th Int Wheat Genet Symp, pp. 609-613.

Robertson, L. D. and B. C. Curtis, 1967. Monosomic analysis of fertility restoration in common wheat (Triticum aestivum L.). Crop Sci 7:493-495.

Rowell, P. L. and D. G. Miller, 1971. Induction of male sterility in wheat with 2-chloroethylphosphonic acid

(Ethrel). Crop Sci 11:629-631.

Saini, H. S. and D. Aspinall, 1982. Abnormal sporogenesis in wheat (Triticum aestivum L.) induced by short periods of high temperature. Ann Bot 49:835-846.

Sasakuma, T. and I. Ohtsuka, 1979. Cytoplasmic effects of Aegilops species having D genome in wheat. I. Cytoplasmic differentiation among five species regarding pistillody induction. Seiken Ziho 27-28:59-65.

Schmidt, J. W., V. A. Johnson and S. S. Maan, 1962. Hybrid wheat. Nebraska Exp Stn Quart Rept Fall, pp. 9.

Schmidt, J. W., V. A. Johnson, M. R. Morris and P. J. Mattern, 1970. Cytoplasmic male sterility and fertility restoration. Seiken Ziho 22:113-118.

Sears, E. R., 1954. The aneuploids of common wheat. Mo Agr Exp Stat Res Bull 572:1-59.

Sears, E. R., 1966. Nulli-tetrasomic combinations in hexaploid wheat. In: Riley, R. and K. R. Lewis (Eds.), Chromosome Manipulation and Plant Genetics, pp. 29-45.

Sears, E. R. and L. M. S. Sears, 1978. The telocentric chromosomes of common wheat. Proc 5th Int Wheat Genet Symp 1:389-407.

Shi, M. S., 1981. Preliminary report of later Japonica natural 2-lines and application. Hubei Agric Sci 7:1-3.

Snedecor, G. W. and W. G. Cochran, 1967. Statistical Methods. Iowa State Univ Press, Ames, Iowa, pp. 177-180.

Tahir, C. M., 1971. Genetic influence of male sterile cytoplasms and fertility-restoring genes on performance of common wheat varieties. Jpn J Breed 21:189-194.

Tahir, C. M. and K. Tsunewaki, 1969. Monosomic analysis of Triticum spelta var. duhamelianum, a fertility-restorer for T. timopheevi cytoplasm. Jpn J Genet 44:1-9.

Tahir, C. M. and K. Tsunewaki, 1971. Monosomic analysis of fertility restoring genes in Triticum aestivum strain P168. Can J Genet Cytol 13:14-19.

Talaat, E. H., S. S. Maan and K. A. Lucken, 1973. Chromosomal location of genes controlling pollen fertility restoration in three restorer lines of wheat. Egypt J Genet Cytol 2:195-205.

Tsuji, S. and M. Murata, 1976. Specific interactions between the D genome and the three alien cytoplasms in wheat. II. Seed inviability induced by the alien cytoplasms. Jpn J Genet 51:327-336.

Tsunewaki, K., 1970. Basic studies on hybrid wheat breeding. III. Heterosis in F_1 hybrid between Japanese and U. S. A. varieties. Jpn J Breed 20:69-74.

Tsunewaki, K., 1974. Monosomic analysis of two restorers to Ae. caudata and Ae. umbellulata cytoplasms. Jpn J Genet 49: 425-433.

Tsunewaki, K., 1980a. Genetic diversity of the cytoplasm in Triticum and Aegilops. Japan Society for the Promotion of Science, Tokyo.

Tsunewaki, K., 1980b. Basic studies on hybrid wheat breeding utilizing the timopheevi cytoplasm and Rf3 gene - Summary of the results. Seiken Ziho 29:40-56.

Tsunewaki, K., 1982. Monosomic analysis on the fertility restoration by Triticum aestivum cv. Chinese Spring against

Aegilops ovata cytoplasm. Jpn J Genet 57:513-525.

Tsunewaki, K. and T. Ryu Endo, 1973. Genetic relatedness among five cytoplasms in Triticum and Aegilops. Proc 4th Int Wheat Genet Symp, pp. 391-397.

Tsunewaki, K., Y. Mukai and T. Ryu Endo, 1978. On the descent of the cytoplasms of polyploid species in Triticum and Aegilops. Proc 5th Int Wheat Genet Symp, pp. 261-281.

Tsunewaki, K., Y. Mukai, T. Ryu Endo, S. Tsuji and M. Murata, 1976a. Genetic diversity of the cytoplasm in Triticum and Aegilops. V. Classification of 23 cytoplasm into eight plasma types. Jpn J. Genet 51:175-191.

Tsunewaki, K., Y. Nakai and J. Fujigaki, 1976b. Basic studies on hybrid wheat breeding. VI. Production of male sterile lines of common wheat cultivars. Jpn J Breed 26:25-31.

Vince-Prue, D., 1975. Photoperiodism in Plants. McGram Hill, London.

Virmani, S. S. and I. B. Edwards, 1983. Current status and future prospects for breeding hybrid rice and wheat. Adv Agr 36:145-214.

Welsh, J. R. and A. R. Klatt, 1971. Effects of temperature and photoperiod on spring wheat pollen viability. Crop Sci 11:864-865.

Welsh, J. R., D. L. Keim, B. Pirasteh and R. D. Richards, 1973. Genetic control of photoperiod response in wheat. Proc 4th Int Wheat Genet Symp, pp. 879-884.

Wilson, J. A., 1968. Problems in hybrid wheat breeding. Euphytica (Suppl) 1:13-33.

Wilson, P and C. J. Driscoll, 1983. Hybrid wheat. In: Frankel,

R. (Ed.), Monographs on Theor Appl Genet Vol. 6 Heterosis, pp. 94-123.

Wilson, J. A. and W. M. Ross, 1961. Cross-breeding in wheat, Triticum aestivum. I. Frequency of the pollen-restoring character in hybrid wheats having Aegilops ovata cytoplasm. Crop Sci 1:191-193.

Wilson, J. A. and W. M. Ross, 1962. Male sterility interaction of the Triticum aestivum nucleus and Triticum timopheevi cytoplasm. Wheat Inf Serv 14:29-30.

Yen, F. S., L. E. Evans and E. N. Larter, 1969. Monosomic analysis of fertility restoration in three restorer lines of wheat. Can J Genet Cytol 11:531-546.

Zhu Y.-G. and J. H. Yu, 1989. Stability and inheritance of photoperiod-sensitive genic male sterility in rice. Proc 6th Int Congr SABRAO, pp. 745-748.