Korean J. Crop Sci. 45(5): 347~351(2000)

Investigation of Cytoplasmic Male Sterility and Development of Maintainer and Restorer Lines in Rye (*Secale cereale* L.)

Hwa-Young Heo*†, Byung-Hee Hong**, Rak-Chun Seong** and Moon-Woong Park*

*National Crop Experiment Station, RDA, Suwon 441-100, Korea
**College of Natural Resources, Korea University, Seoul, 100-100, Korea

ABSTRACT : Rye has been a major winter forage crop in Korea. Varietal improvement of rye has been practiced either by hybrid or population breeding systems. Hybrid breeding offers important advantages over population breeding since it is normally a cross-pollinated crop. The hybrid breeding in rye has been possible since cytoplasmically inherited forms of male sterility (CMS) and corresponding nuclear restorer genes were found. The objectives of this research were to develop the maintainer and restorer lines of Korean inbred lines and to estimate the effect of 'Pampa' type of CMS cytoplasm on yield and its related characteristics. For easy discrimination of male-sterile status of plants, anther scoring and the restore index system in which seed-setting and pollen quantity of viability were taken into account were established. High significant correlation between pollen quantity and pollen viability was found. For "Pampa" cytoplasm, four of 14 Korean inbred lines tested turned out to be a maintainer but no restorer was found. But for "235b" CMS cytoplasm, seven inbred lines acted as complete restorers. The Korean inbred rye lines acted mainly as maintainers in "Pampa" cytoplasm but acted mainly as restorer in "235b" cytoplasm. The 'Pampa' cytoplasm inducing male sterility reduced culm length and plant height and increased the number of tiller, so forage yield and grain yield were enhanced. However, heading date was slightly delayed compared to the normal cytoplasm.

Keywords **:** rye, cytoplasmic male sterility (CMS), maintainer, restorer, 'Pampa', '235b', inbred line, hybrid.

Among the crop plants, cytoplasmic male sterility (CMS) was first found in flax (Bateson & Gairdner, 1921; Chittenden & Pellew, 1927), maize (Rhoades, 1931), and some years later, Jones and Clarke (1943) and Owen (1945) found it in onion and sugar beets, respectively.

Cytoplasmic male sterility in rye was discovered about 30 years ago (Geiger & Schnell, 1970). Putt (1954) studied the cytogenetics of pollen abortion in several families and Schnell (1959) investigated several male-sterile forms of winter rye. Chekhovskaja (1965) and Kobyljanskij (1968 and 1969) also found male-sterile plants in various populations of winter rye. Some of the male-sterile plants could be caused by cytoplasmic factors. In certain progenies, sterility could be ascribed to a single recessive gene, *rf* (Kobyljanskij, 1969). Hybrid breeding in rye became feasible in 1970 after the CMS inducing cytoplasm 'Pampa' had detected in a rye population of Argentine origin (Geiger & Schnell, 1970).

Side effects of CMS inducing cytoplasm have frequently been observed in maize, sorghum and other species (Oraby, 1970). Some studies revealed remarkable influence of genotype and environment on cytoplasmic effect. Marker *et al.* (1985) reported 'Pampa' cytoplasm reduced plant height and had some decreased effect on yield component except 1,000 kernel weight in rye.

Morgenstern and Geiger (1982) described that to diminish genetic vulnerability due to cytoplasmic uniformity, utilization of two or more different male sterility inducing cytoplasm would be desirable. Differences in plasmotype can be identified by inducing a number of genotype in each of different cytoplasm. Several sources of CMS and corrresponding restorer genes have been reported (Geiger & schnell, 1970; Kljucko & Belousov, 1972; Kobyljanskij, 1971; Madej, 1975). They can be classified into two major groups, the P(Pampa) and the V(Vavilov) type. Maintainers of P type of CMS were found at sufficient frequency in all rye materials. Restorers occur considerably less frequently than maintainers. The V type of CMS was known to be difficult to be maintained (Madej, 1976; Winkel *et al.*, 1979).

In rye, the restorers occur considerably less frequently than maintainers. Most crosses between P (Pampa) male steriles and European rye cultivars display low to intermediate pollen fertility. The V (Vavilov) type of CMS was very difficult to be maintained (Madej, 1976; Winkel *et al.*, 1979). Most pollinator genotypes acted as partial to complete restores. Geiger (1982) reported that the maintainer of the P type sterility completely restored fertility to the V type, and the maintainers of the V type partially restored fertility to the P type.

Fertility restoration is controlled by at least two major genes and an unknown number of modifiers (Geiger & Morgernstern, 1975; Madej, 1976). Environmental and gen-

†Corresponding author: (Phone) +82-31-290-6669 (E-mail) heohy.rda.go.kr
<Received November 20, 2000>

otypeenvironment interaction effects may contribute to the sterilityfertility expression, especially in semi-steriles (Morgenstern & Geiger, 1982).

MATERIALS AND METHODS

Detection of male sterility

For the detection of pollen sterility, the cytoplasmic male sterile line, 304-P, and fertility restore line, 18-R were planted after six weeks vernalization to 1/5,000 a pot in glass house in January 15, 1992. Both lines were introduced from Germany. The offsprings were sown in November 10, 1992 in rows of 40 cm with 2 m length, in polyethylene film house.

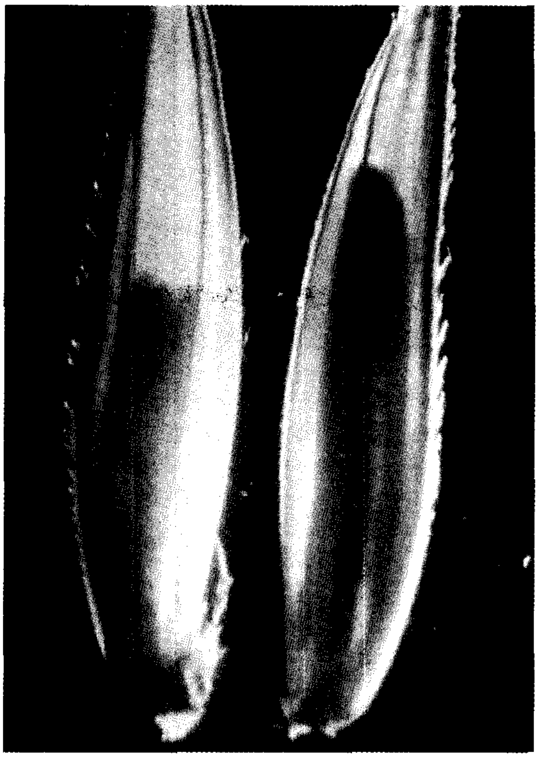
"Anther score" (1-9) were detected (Table 1) by the method of Geiger and Morgenstern (1975). Completely male sterile (ms) plants were graded as 1, 2, or 3, partially male sterile (pms) plants as 4, 5, or 6, and male-fertile (mf) plants as 7, 8, or 9. The three scores within each sterility class were taken into account the various degrees (Fig. 1) in degenerated and not emerging (score 1) to normal size anthers with abundant pollen shedding (score 9).

Pollen quantity and viability were determined from four preparations for each plant. Each preparation included the pollen sample derived from all anthers of one spike. Two florets were chosen from each of two spikes. Pollen quantity was scored (1-9) and subsequently converted into a percentage scale (1=0%, 2=12.5%, ..., 9=100%). Pollen viability was determined by staining with acetocarmine. Pollen grains with good staining and morphology were classified as "viable" and the remainders as "unviable".

Four spikes per plant were chosen and enveloped with isolation paper bag. The ratio of seed set was calculated as follow; Seed set ratio (%) = (number of ripened grainnumber of floret per spike)100

Table 1. Designation of anther score related with degree of male sterility and their description in rye.

Group	Anther score	Anther		
		Anthesis	Size of anther	Emergence of anther from floret
Male sterile	1	No	Small(degenerated)	No(all in floret)
	2	No	Middle	A little
	3	No	Normal	Normal
Partial male sterile	4	Partial	Small	Normal
	5	Partial	Middle	Normal
	6	Partial	Normal	Normal
Male fertile	7	Full	Small	Normal
	8	Full	Middle	Normal
	9	Full	Normal	Normal



Male-sterile† Male-fertile

† Male-sterile : transparent and small anther
Male-fertile : dark and big anther

Fig. 1. Stamens in floret classified as male-sterile and male-fertile in rye.

Screening of maintainer and restorer to CMS lines

Two types of CMS cytoplasms, Pampa and 235b used in this study were both derived from *Secale cereale* L. The P type originated from Argentinian Pampa and the 235b type from Iranian primitive rye. Both cytoplasms were introduced from Germany.

Fourteen inbred lines used in this experiment. were bred true over five generations of selfing in National Crop Experiment Station.

To evaluate maintaining and restoring ability of Korean inbred lines, crosses between CMS sources and inbred lines were made. Cytoplasmic male sterile lines and inbred lines were grown the winter season of 1996/1997 at 1/5,000 a pot in a greenhouse and two CMS lines (Pampa and 235b) used as female parents, were crossed, with 14 inbred lines.

The F_1 plants were planted in two different environmental conditions, field and transparent vinyl house at Suwon, National Crop Experiment Station in October 27, 1997. The detection of the maintainer and restorer to the CMS lines was made for each plant by anther scoring system, ranging from 1 to 9.

Effect of "Pampa" cytoplasm on agronomic characteristics

To investigate which traits, besides male sterility, are affected by "Pampa" cytoplasm, 314-P, which has pampa CMS cytoplasm, was backcrossed five times with normal cytoplasm, 307-N from 1992 to 1995 in field and greenhouse for accelerating the generation.

In 1995 the two single crosses on "Pampa" and normal cytoplasm were made. For production of the male fertile line, the female were emasculated by clipping the glumes. The male sterile lines were established without emasculation. In 1996 the two cytoplasmic versions of each F_1 cross were grown together in transparent vinyl house to obtain F_2 seeds.

The resulting CMS and male fertile F_2 plants were tested in 1997 at National Crop Experiment Station, Suwon. Data were recorded on heading date, plant height, culm length, spike length, number of tillers per plant, number of grains per spike, forage yield per plant and grain yield per plant.

RESULTS AND DISCUSSION

Measures of pollen sterility

The average pollen quantity, pollen viability and seed set according to the anther score are given in Table 2. Plants graded as 1 and 2, produced only traces of pollen and almost all of pollen were unviable and seed set ratio was near zero. Although plants scored as 3 showed less pollen sterility than grade 1 and 2, seed set was zero under most bag. A steady increase in pollen quantity, pollen viability and seed set was found for another scores 4 to 9. Even in most fertile class, the plants still produced about 40% less pollen than plants with "normal" cytoplasm. Pollen viability and seed set of fertile classes were also reached 62.1% and 75.5% to those of 'normal' cytoplasm, respectively.

Close relationships between different pollen sterile measures of individual plants were found (Table 3). There was close relationship between pollen quantity, viability, and seed set. The microscopic sterility measures were more closely related to the restore index than to the seed set.

The anther scores, microscopic pollen analysis data and the seed set value were closely related with one another. Thus, the laborious microscopic pollen analysis does not seem to provide more accurate and reliable information on the degree of male sterility than appling the visual inspection of anthers only.

Table 2. Anther scoring and its related characteristics in rye with pollen and seed set ratio in the cross between CMS line, 304-P, and fertile line, 18-R.

Anther score	Pollen quantity	Pollen viability	Seed† set	Classification
	%			
1	0.1	0.1	0.1	Male sterile
2	0.4	0.3	0.2	
3	2.1	1.2	0.8	
4	10.4	9.4	8.4	Partial male sterile
5	19.2	20.2	15.5	
6	38.2	40.1	42.2	
7	50.4	48.8	51.4	Male fertile
8	59.2	58.1	70.4	
9	64.4	62.1	75.5	
Check variety (Paldangomil)	100	82.5	87.9‡	

†Pollination in paper bag, ‡Open pollination

Table 3. Correlations of restorer index with pollen characteristics and seed set ratio in the offsprings obtained from the cross between 304-P and 18-R in rye.

	Pollen quantity	Pollen viability	Seed set
Restorer index	0.91**	0.82**	0.85**
Pollen quantity		0.94**	0.81**
Pollen viability			0.84**

**Significant at the 0.01 level of probability.

Screening of maintainer and restorer

Male-sterile readings (number of male fertile : partial male

Table 4. Distribution of plant sterility based on anther scoring of F_1 plants from the crossed of 'Pampa' CMS line with 14 inbred lines in rye.

Cross combination		Field			Greenhouse		
		mf†	pms	ms	mf	pms	ms
Pampa ×	SF1‡	0	2	10	0	4	20
	SF2	0	4	8	0	2	11
	SF3	0	7	18	0	3	14
	SF4	0	0	12	0	0	6
	SF5	0	3	10	0	0	12
	SF6	0	0	21	0	0	13
	SF7	0	5	13	0	2	12
	SF8	0	0	11	0	4	21
	SF9	0	0	12	0	0	6
	SF10	0	0	19	0	0	10
	SF11	1	8	4	2	6	5
	SF12	0	10	2	1	3	3
	SF13	0	4	7	1	2	10
	SF14	0	2	11	0	4	8

†mf: male fertile, pms: partial male sterile, ms: male sterile.
‡Korean inbred rye lines.

Table 5. Duncan's multiple range test for the percentage of each pollen sterility in 'Pampa' cytoplasm of rye according to the growing conditions.

Growing condition	mf†	pms	ms
	------------	%	------------
Field	0.5b‡	22.1a	77.4a
Greenhouse	2.2a	16.2b	81.6a

†mf: male fertile, pms: partial male sterile, ms: male sterile.
‡Means with the same letter are not significantly different at the 0.05 probability level within the pollen sterility.

sterile : male sterile plants) of F_1s obtained from crosses between Pampa CMS line and 14 inbred lines showed on Table 4. In "Pampa" cytoplasm, four inbred lines, SF4, SF6, SF9, SF10 turned to be a good maintainer showing complete sterility in field and greenhouse. No restorer was found to "Pampa" cytoplasm in 14 inbred lines but only SF11 line produced a little fertile offsprings. The SF8 line showed as a complete maintainer in field but some partial male sterile plants were detected in greenhouse.

The percentage of male fertility was significantly higher in greenhouse than field but the percentage of partial male sterility was higher in field. In male sterile, there was no significant difference in two different growing conditions (Table 5). Therefore, the different results of fertility mighty be influenced by environmental condition, such as temperature and humidity.

In "235b" CMS cytoplasm, there is no male sterile plants in field and greenhouse when the "235b" was crossed with 14 inbred lines.

Table 6. Distribution of plant sterility based on the anther scoring of F_1 plants from the crosses of '235b' CMS line with 14 inbred lines in rye.

Cross combination		Field			Greenhouse		
		mf†	pms	ms	mf	pms	ms
235b ×	SF1‡	14	4	0	36	5	0
	SF2	11	8	0	24	7	0
	SF3	21	0	0	17	0	0
	SF4	7	3	0	18	0	0
	SF5	6	1	0	12	3	0
	SF6	14	0	0	17	0	0
	SF7	17	0	0	20	0	0
	SF8	11	4	0	9	7	0
	SF9	8	7	0	12	0	0
	SF10	22	0	0	7	0	0
	SF11	14	0	0	14	0	0
	SF12	5	7	0	11	0	0
	SF13	13	0	0	11	0	0
	SF14	11	0	0	13	0	0

†mf: male fertile, pms: partial male sterile, ms: male sterile.
‡Korean inbred rye lines.

Table 7. Proportion of each pollen sterility in '235b' cytoplasm of rye according to the growing conditions.

Growing condition	mf†	pms	ms
	------------	%	------------
Field	83.7b‡	16.3a	0
Greenhouse	90.9a	9.1b	0

†mf: male fertile, pms: partial male sterile, ms: male sterile.
‡Means with the same letter are not significantly different at the 0.05 probability level within a column by Duncan's multiple range test.

Table 8. Proportion of each pollen sterility according to the cytoplasmic male sterile types in rye.

CMS	mf†	pms	ms
	------------	%	------------
Pampa	1.3b‡	19.3a	73.4a
235b	87.6a	12.4b	0.0b

†mf: male fertile, pms: partial male sterile, ms: male sterile.
‡Means with the same letter are not significantly different at the 0.05 probability level within a column by duncan's multiple range test.

Seven inbred lines including SF6, acted as complete restorer and other lines produced some partial male sterile plants (Table 6).

The pattern of distribution in percentage of each male sterility was as same as the pattern of distribution in field and greenhouse for Pampa cytoplasm (Table 7).

The percentage of male fertility was significantly higher in 235b cytoplasm than Pampa but the percentage of partial male sterility and male sterility were higher in field (Table 8). The Korean rye inbred lines acted mainly as maintainer in "Pampa" cytoplasm but as restorer in "235b" cytoplasm. This results agreed with previous results obtained by Geiger studies using European rye lines (Geiger, 1982).

The two cytoplams were quite different from each other. Therefore, in order to reduce the genetic vulnerability of hybrid rye, 235b cytoplasm could be used as a second source of CMS.

Effect of "Pampa" cytoplasm

Significant cytoplasmic differences were observed for all traits except spike length and grain number (Table 9). Generally, the direction of change was in favor of the breeding goal. Since, for enhanced forage and grain yield, culm length and plant height were considerably reduced, so lodging was markedly improved and the number of tillers was also increased. But heading date was a little delayed compared to normal cytoplasm. Therefore, it would be necessary to use early heading male parents when making hybrid using

Table 9. Effect of 'Pampa' cytoplasm on major agronomic characters in rye.

Characters	Normal cytoplasm	Pampa cytoplasm
Heading date	May 7	May 9*
Plant height† (cm)	110	88**
Culm length (cm)	142	121**
Spike length (cm)	10.4	10.6ns
Number of tillers per plant	6	11**
No. of grains per spike	44	46ns
Forage yield† (g/plant)	71	98**
Grain yield (g/plant)	4.9	8.5**

†Measured at May 2.
‡ns, * and ** indicate nonsignificance and significance at the 0.05 and 0.01 probability levels, respectively, as determined by a *t*-test.

"Pampa" cytoplasm as a female.

The tendency of "Pampa" cytoplasm to reduce the culm length, increase the number of tiller and delay the heading date agree with previous results obtained by Geiger (1982).

REFERENCES

Bateson, W., and A. E. Gairdner. 1921. Male-sterility in flex, subject to two types of segregation. *J. Genet.* 11 : 269-275.

Chekhovskja, E. S. 1965. Sterile forms of winter rye. *Selekcija I Semenovodstvo* 30 : 53-54.

Chittenden, R. J., and C. Pellew. 1927. A suggested interpretation of certain cases of anisogeny. *Nature* 119 : 10-12.

Geiger, H. H. and K. Morgenstern. 1975. Angewandt-genetische Studien zur cytoplasmatischen Plooensterilit t bei Winterroggen. *Theoret. and Appl. Genetics* 46 : 269-276.

Geiger, H. H. and F. W. Schnell. 1970. Cytoplasmic male sterility in rye (*Secale cereale* L.). *Crop Sci.* 10 : 590-593.

Geiger, H. H. 1982. Breeding methods in diploid rye (*Secale cereale* L.). *Tag.-Ber., Akad. Landwirtsch.-Wiss. DDR, Berlin,* 198 : 305-332.

Jones, H. A., and A. E. Clarke. 1943. Inheritance of male sterility in the onion and the production of hybrid seed. *Proc. Amer. Soc. Hort. Sci.* 43 : 189-194.

Kljucko, P. F. and A. A. Belousov. 1972. Ergebnisse genetischer Untersuchungen mit zytoplasmtisch sterilen Roggenformen. *Genetika* 8(7) : 9-15.

Kobyljanskij, V. D. 1971. Die Schaffung von sterilen Analogsorten des Winterroggens, von Fixatoren der Sterilit t und Restorern der Fertilit t. *Trud. Priklad. Bot. Genet. Seleke.* 44(1) : 76-85.

Kobyljanskij, V. D. 1968. Rye. *Trud. Priklad. Bot. Genet. Seleke.* 39 : 80-91.

Kobyljanskij, V. D. 1969. Cytoplasmic male sterility in diploid rye. *Vestnik Selskokhoz. Nauki* 6 : 18-22.

Madej, L. 1975. Research on male sterility in rye. *Hodowla Rosl. Aklim. Nasienn* 19 : 421-422.

Madej, L. 1976. The genetical characteristics of three sources of male sterility in rye (*Secale cereale* L.). *Hodowla. Rosl. Aklim. Nasienn* 20 : 157-174.

Marker, R. M., H. H. Geiger and P. Wilde. 1985. Influence of 'Pampa' cytoplasm on agronomic characters of F_2 crosses among inbred lines of winter rye. *Eucarpia meeting of the cereal section on rye. Svalov, sweden. Proc. Part* I : 279-289.

Morgenstern, K, and H. H. Geiger. 1982. Plasmotype/genotype interaction with regard to male sterility in rye (*Secale cereale* L.). *Tag-Ber., Akad. Landwirtsch.-Wiss. DDR, Berlin* 1982.

Oraby, F. T. 1970. Yields and other agronomic characteristics of cytoplasmically pollen sterile maize hybrids compared to their normal and restored counterparts. *Some methological achievements of the Hungarian hybrid maize breeding.*

Owen, F. V. 1945. Cytoplasmically inherited male-sterility in sugarbeets. *J. Agric. Res.* 71 : 423-440.

Putt, E. D. 1954. Cytogenetic studies of sterility in rye. *Canad. J. Agric. Sci.* 34 : 81-119.

Rhoades, M. M. 1931. Cytoplasmic inheritance of male sterility in *Zea mays*. *Science* 73 : 340-341.

Schnell, F. W. 1959. Roggen. W. Rudorf. *Dreissig Jahre Zuchtungsforschung* : 135-139.

Winkel, A., H. W. Muller and G. Grabow. 1979. Uber die gegenw rtigen M glichkeiten zur Schaffung von Hybridsorten und zur Nutzung von Sterilit tssystemen in der Roggenz chtung. *Tag-Ber., Akad. Landwirtsch.-Wiss. DDR, Berlin* 168 : 171-177.