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## MALE FERTILITY RESTORATION OF RYE CROSSES IN THE PAMPA CYTOPLASM

### ABSTRACT

Male fertility restoration of  $F_1$  crosses between male sterile lines (P lines) and restorers (R) was studied in 2000. Pollen fertility of 50  $F_1$  crosses derived by crossing 10 P lines to 5 restorers was estimated. The degree of male sterility/fertility was assessed by visual anther score of single plants on a 1–9 scale, restorer indices and visual pollen shedding scores of plots on a 1–9 scale.

Coefficients of correlation between the above fertility traits were calculated.

The analysis of variance showed that the degree of male fertility of  $F_1$  crosses strongly depends on a restorer genotype, a P line genotype as well as on the P × R interaction. The restorer and P line genotypes were a major source of variation.

A considerable range of pollen fertility was evident in the single crosses. The mean restorer index of  $F_1$  crosses ranged from 3,4 to 98,7%. The P lines under study were divided into three groups on the basis of mean restorer indices of their crosses with five restorers: easy, medium and difficult to restore. All the restorers tested, provide a sufficient restoration of male fertility of the  $F_1$  crosses with majority of the P lines. The new restorer, 330 R appeared to be more effective in restoration of pollen fertility than the other currently used lines.

*Key words:* hybrid breeding, male sterility, restoration of male fertility, winter rye

### INTRODUCTION

In rye, hybrid breeding and seed production are based on the cytoplasmic – genic male sterility (CMS) inducing by Pampa (P) cytoplasm. This male sterility source was discovered by Geiger and Schnell (1970) in Argentinian primitive rye. The Pampa male sterility seems to be easy to maintain. In contrast, fertility restoration causes considerable problems due to the scarcity of sufficiently effective restorers in European breeding populations (Geiger and Morgenstern 1975). Moreover inheritance of male fertility restoration appeared to be polygenic (Geiger and Miedaner 1996, Kolasińska 1998b, Miedaner *et al.* 2000). Current pollinator lines developed in breeding programmes are only partial restorers (Kolasińska 1998a, Miedaner *et al.* 2000). The level of fertility

restoration of such partial restorers considerably depends on environmental effects and interaction between seed parent and pollinator (Geiger and Miedaner 1996, Madej *et al.* 1996). An incomplete restoration of male fertility in hybrids favours infections with ergot spores. Therefore, improving of this trait should be the most important task in rye research and breeding.

The objective of this study was to determine pollen fertility of  $F_1$  crosses derived by crossing selected male sterile lines with restorers, currently used in the hybrid rye breeding programme.

#### MATERIAL AND METHODS

Male fertility restoration of  $F_1$  crosses between male sterile lines (P lines) and restorers (R) was studied. 50  $F_1$  crosses were produced by crossing 10 P lines (1 P, 2 P, 5 P, 7 P, 9 P, 10 P, 11 P, 12 P, 13 P, 15 P) with 5 restorers (4 R, 5 R, 330 R, 340 R, 363 R). Both the P lines and the restorers are currently being used in the hybrid breeding programme of Plant Breeding and Acclimatization Institute at Radzików. In 1999  $F_1$  crosses were sown in 2 field trials at Radzików. The first trial was laid out as a completely randomized block design with 3 replicates (hand sowing). The second trial included the same 50  $F_1$  crosses and was sown by machine. In the first trial the level of male fertility was assessed by visual anther scores of single plants (AS) on a 1–9 scale according to Geiger and Morgenstern (1975). Score 1 refers to highly degenerated, non dehiscent, empty anthers. Score 9 refers to full sized, abundantly pollen shedding anthers corresponds to fully male fertile plants in N cytoplasm. Plants rated 1, 2 and 3 were classified as male sterile (ms), 4, 5 and 6 as partially male fertile (pmf), and 7, 8 and 9 as male fertile (mf). Restorer indices (RI–1, RI–2) were calculated by the formulae:

$$[RI-1] = \% mf \text{ plants} + \frac{1}{2} \% pmf \text{ plants}$$

where:

[RI–1]– Restorer index 1 (Geiger and Morgenstern, 1975)

$$[RI-2] = 100 \times \frac{(AS-3)}{6}$$

where:

[RI–2]– Restorer index 2, for AS = 4, for AS = 1, 2, 3 – RI = 0 (Geiger *et al.*, 1995)

In the second trial, visual pollen shedding scores of plots on a 1–9 scale were made according to Morgenstern (1983). Score 1 to 3 refers to plots with all male sterile plants. Score 9 was ascribed to plots with com–

pletely male fertile plants. The scores 4 to 8 mean various proportion of fertile plants of a plot.

Coefficients of correlation between the above mentioned fertility traits were calculated. Analysis of variance were made by the AGRO computer software.

## RESULTS

The analysis of variance of pollen fertility in  $F_1$  crosses showed highly significant effects of restorer, P line and  $P \times R$  interaction (Table 1). However, the restorer and P line genotypes appeared to be a major source of variation. A large variation of pollen fertility was found in  $F_1$  crosses. Restorer index (RI-1) ranged from 0 to 100% (Table 2). Complete or nearly complete fertility restoration was observed in  $F_1$  crosses derived by crossing 12 P, 15 P and 1 P to restorers. The lowest fertility was found in  $F_1$  crosses obtained by crossing 5 P and 10 P to restorers.

Table 1  
F values from analysis of variance of  $F_1$  crosses for anther score, Radzików 2000

Source of variation	Degrees of freedom	F value
P line	9	107.2**
Restorer	4	184.3**
$P \times R$ interaction	36	9.1**

\*\* – Significant at  $\alpha=0.01$

Table 2  
Restorer index [RI-1] of  $F_1$  crosses ( $P \times R$ ) in 2000

P lines	Restorers					Mean
	330	340	4	5	363	
12	97.6	100.0	98	98.1	100.0	98.7
15	88.4	96.0	100	94.4	95.8	94.9
1	99.3	94.4	87	94.2	76.0	90.2
13	96.8	32.7	33.3	46.0	40.5	49.9
7	78.3	56.9	40.5	41.9	30.6	49.6
2	69.3	26.2	50	36.5	54.3	47.3
11	50	60.9	27.8	21.2	38.6	39.7
9	33.3	2.5	22.7	24.1	5.0	17.5
10	32.5	10.2	8	3.4	0.0	10.8
5	3.5	4.5	9.1	0.0	0.0	3.4
Mean	64.9	48.4	47.6	46.0	44.0	50.2

Mean restorer index for P lines based on fertility of their crosses with 5 restorers ranged from 3,3 to 99.4%. The P lines under study were di-

vided into three groups on the basis of their mean restorer indices: easy, medium and difficult to restore. The P lines of the first group (12 P, 15 P and 1P) could be used for creation of hybrid varieties. P lines of the latter group (5 P, 9 P and 10 P) should be used as testers in a restorer breeding programme. Big differences among studied P lines in respect to restorer index of their crosses with the same restorers as well as mean restorer index indicated complicated nature of inheritance of male sterility. It seems that P lines of these groups could have different number of genes responsible for male sterility. Ruebenbauer (1984) stated that male sterility is controlling by the interaction of four pairs of genes  $Ms_1ms_1$   $Ms_2ms_2$   $Ms_3ms_3$   $Ms_4ms_4$  with mutated cytoplasm of the Pampa type, out of which five single genes in a dominant form restore male fertility. Further research is need to elucidate genetic basis of male sterility – pollen fertility restoration system of Pampa type.

All the restorers tested, provide a sufficient restoration of male fertility of the  $F_1$  crosses with P lines of the first and the second groups. The mean restorer index of R lines based on fertility of their crosses with 10 P lines varied from 44.1 to 64.9%. The new restorer, 330 R appeared to be more effective in restoration of pollen fertility than the other currently used R lines.

Coefficients of correlation between fertility traits

Table 3

Fertility traits	RI -1	RI -2	Pollen shedding score ofk plots
Anther score of plants	0.97**	1.00**	0.80**
RI - 1		0.97**	0.81**
RI - 2			0.80**

\*\* – significant at  $\alpha = 0.01$

A high, significant correlation was found between all estimated fertility traits (Table 3). Coefficient of correlation between the anther scores of plants and pollen shedding scores of plots was 0.80\*\*. So, the latter trait appears to be sufficient measure of pollen fertility and could be successfully used in a breeding programme.

#### CONCLUSIONS

1. The level of male fertility of  $F_1$  crosses derived by crossing P lines to restorers depends on a restorer genotype, a male sterile line genotype and the  $P \times R$  interaction.
2. Significant differences among studied male sterile lines were found in respect of their ability to restoration of male fertility. The P lines, in which male fertility could be easily restored should be used for creation of hybrid varieties. Whereas the P lines, in which restora-

tion of male fertility is difficult should be used as testers in a restorer breeding programme.

## REFERENCES

- Geiger H.H., Morgenstern K. 1975. Angewandt-genetische Studien zur cytoplasmatischen Pollensterilität bei Winterroggen. TAG 46: 269–276.
- Geiger H. H., Yuan Y., Miedaner T., Wilde P. 1995. Environmental sensitivity of cytoplasmic-genic male sterility (CMS) in *Secale cereale* L. In: Genetic mechanisms for hybrid breeding. Kück U. and G. Wricke (eds.). Advances in Plant Breeding 18: 7–17.
- Geiger H. H., Miedaner T. 1996. Genetic basis and phenotypic stability of male-fertility restoration in rye. Vortr. Pflanzenzüchtg. 35, 27–38.
- Kolasińska I. 1996. Pollen fertility restoration in topcross hybrids of winter rye. Vortr. Pflanzenzüchtg. 35: 72–74.
- Kolasińska I. 1998 a. Wpływ genotypu komponentów matecznych na płodność pyłku mieszańców żyta (CMS-SC × restorer). Biul. IHAR 205/206: 5–13.
- Kolasińska I. 1998 b. Genetic bases of male fertility restoration in rye. J.Appl. Genet. 39A: 116–117.
- Madej L., Osiński R., Jagodziński J. 1996. Ocena płodności mieszańców żyta. Biul. IHAR 195/196: 283–290.
- Morgenstern K. 1983. Ausprägung der cytoplasmatisch-genischen Pollensterilität bei Roggen in Abhängigkeit von Plasmotyp und Genotyp. Dissertation, Univ. Hohenheim, Stuttgart.
- Miedaner T., Glass C., Dreyer F., Wilde P., Wortmann, Geiger H.H. 2000. Mapping of genes for male-fertility restoration in “Pampa” CMS winter rye (*Secale cereale* L.). Theor. Appl. Genet. 101: 1226–1233.
- Ruebenbauer T., Kubara-Szpunar Ł., Pająk K. 1984. Testing of a hypothesis concerning interaction of genes with mutated cytoplasm controlling male sterility of the Pampa type in rye (*Secale cereale* L.). Genet. Pol. 25, 1:1–16.